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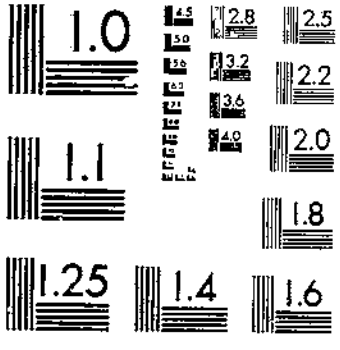

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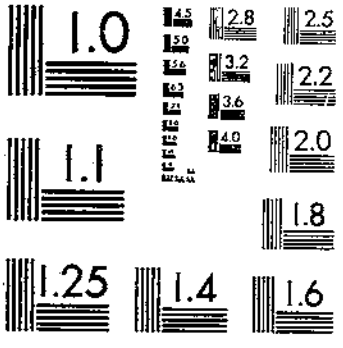

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DEVELOPMENT OF THE SWINE NEMATODE STRONGYLOIDES RANSOMI AND THE BEHAVIOR OF ITS INFECTIVE LARVAE

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CONTENTS

INTRODUCTION

In view of the fact that several observers, notably Perroncito (*19*),[1] Reisinger (*22*), and Marotel (*16*), have shown, on the basis of clinical studies, that *Strongyloides* is pathogenic for pigs, the writer undertook a study of the life cycle and behavior of the infective larvae of *S. ransomi*, the species of common occurrence in pigs in the United States, for the purpose of discovering facts that might be of value in connection with the control of this parasite. Schwartz and Alicata (*25*) have already briefly described some of the essential features in the preparasitic life history of *S. ransomi*. However, these writers were primarily concerned in establishing the validity of *S. ransomi* as a new species, and their observations

[1] Italic numbers in parentheses refer to Literature Cited, p. 29.

on the life cycle were only incidental to the main thesis of their paper. In the course of the writer's study, numerous additional details concerning the preparasitic development of *S. ransomi* and many facts relating to the development of the parasite in its host have been brought to light which, together with the data already supplied by Schwartz and Alicata, make possible the presentation of a virtually complete account of the life history of this species.

SCOPE AND METHODS OF INVESTIGATION

The investigation reported in this bulletin was conducted at Beltsville, Md., from October 1932 to May 1933. The program of the work was as follows: (1) The intermediate stages in the heterogeneous preparasitic development of *Strongyloides ransomi* were studied in feces and charcoal cultures and in water cultures to which helminthologically sterile fecal extract was added at the time the larvae issued from the eggs; (2) the effects of various environmental conditions on the survival of infective larvae were determined; (3) the reactions of the infective larvae to stimuli and environment were observed; (4) the modes of infection were established experimentally; and (5) the development of the parasite and the clinical symptoms produced in pigs, the normal hosts, and in other hosts were studied.

Larvae for all experimental purposes were obtained from cultures prepared from feces passed by pigs which harbored no parasites other than *S. ransomi*. All cultures were incubated at room temperature (20° to 24° C.). With two exceptions, the pigs used for oral and skin-infection experiments were raised parasite free. All experimental animals were kept in individual cages under conditions of strict sanitation designed to prevent extraneous infection or reinfection with *S. ransomi* or other worm parasites. A thermostatically controlled electric refrigerator was used for experiments involving low temperatures. The Baermann isolation apparatus was routinely used for the purpose of obtaining larvae from cultures, and the failure to obtain larvae from a culture by means of this apparatus was accepted as indicative of the absence of living larvae in the culture. The Baermann apparatus was also used for the isolation of parasitic larvae from organs and tissues.

PREPARASITIC DEVELOPMENT OF STRONGYLOIDES RANSOMI

MODES OF DEVELOPMENT

Schwartz and Alicata have shown that both the direct and indirect modes of development occur in the life cycle of *Strongyloides ransomi*, as they do in the life cycle of other species of the genus *Strongyloides*. Sandground (*24*), who studied the quantitative relationship between direct and indirect development in species of *Strongyloides* from different hosts, showed this relationship to be subject to constant changes. The writer has observed that cultures consisting of a mixture of animal charcoal and feces from young pigs harboring *S. ransomi* often showed predominantly direct development early in the history of an infestation and later, as the infestation waned, usually showed mainly indirect development. Both modes of development usually occur in a single fecal culture. However, records of

two cultures, prepared from the feces of two infested pigs, indicate that only unisexual adults of *S. ransomi* were present about 48 hours after the cultures had been prepared. Schwartz and Alicata found that the number of males present in 48-hour cultures was small compared with the number of females. The writer has found this to be the usual occurrence in cultures. In a few cultures no males were seen, whereas in one 50-hour culture 79 males and only 40 females were found. Hence it appears that cultures prepared from feces containing eggs of *S. ransomi* may exhibit practically every possible relationship between the two modes of development and that the proportion of males to females, when indirect development occurs, is variable.

DEVELOPMENT OF INFECTIVE LARVAE FROM EGGS OF THE PARASITIC FEMALE

EGGS

Schwartz and Alicata have described the size and morphology of eggs of *Strongyloides ransomi* which are passed in the feces of infested pigs. They state that the eggs are from 45μ to 55μ long and from 26μ to 35μ wide. Occasionally, eggs slightly longer or wider than indicated above have been found by the writer. The eggs are embryonated when oviposited.

The time required for larvae to hatch from the eggs eliminated with the feces of infested pigs is variable. Young larvae were found in the water used to flush the small intestine of infested animals at post-mortem examination about 4 hours after the water and intestinal contents were mixed. The vast majority of eggs in fecal or water cultures hatch in from 12 to 18 hours at room temperature (20° to 24° C.).

FIRST-STAGE LARVAE

Newly hatched "rhabditiform" larvae are from 220μ to 240μ long. Just after hatching most larvae show a separation of the cuticle at the anterior end, which is early evidence of the approach of the first molt (fig. 1, *A* and *B*). A newly hatched larva, cultured in water, showed the following size relationships: Length, 230μ; width in region of esophageal bulb, 18μ; length of pharynx, 5.5μ; distance from nerve ring to anterior end, 55μ; length of esophagus, 69μ; length of genital primordium, 10.5μ; length of tail, 39μ. In first-stage larvae, a short region of the esophagus immediately posterior to the pharynx or mouth cavity differs in structure from the remainder of the corpus. The genital primordium, which appears to contain two small circular germinal cells, is slightly anterior of the midpoint between the anus and the junction of the esophagus and intestine. It remains small throughout the first stage, retains its characteristic oval shape, and at the time of the first molt is from 10μ to 15μ long.

As the first-stage larvae grow, their morphology is not perceptibly modified. Increasing evidence of the separation of the cuticle from the body of the larva is noted in the course of the first 12 hours of its existence. The cuticle first loosens from the body in the head and tail regions (fig. 1, *C*). Usually complete separation of the cuticle occurs when the larvae are from 275μ to 325μ long (fig. 1, *D*).

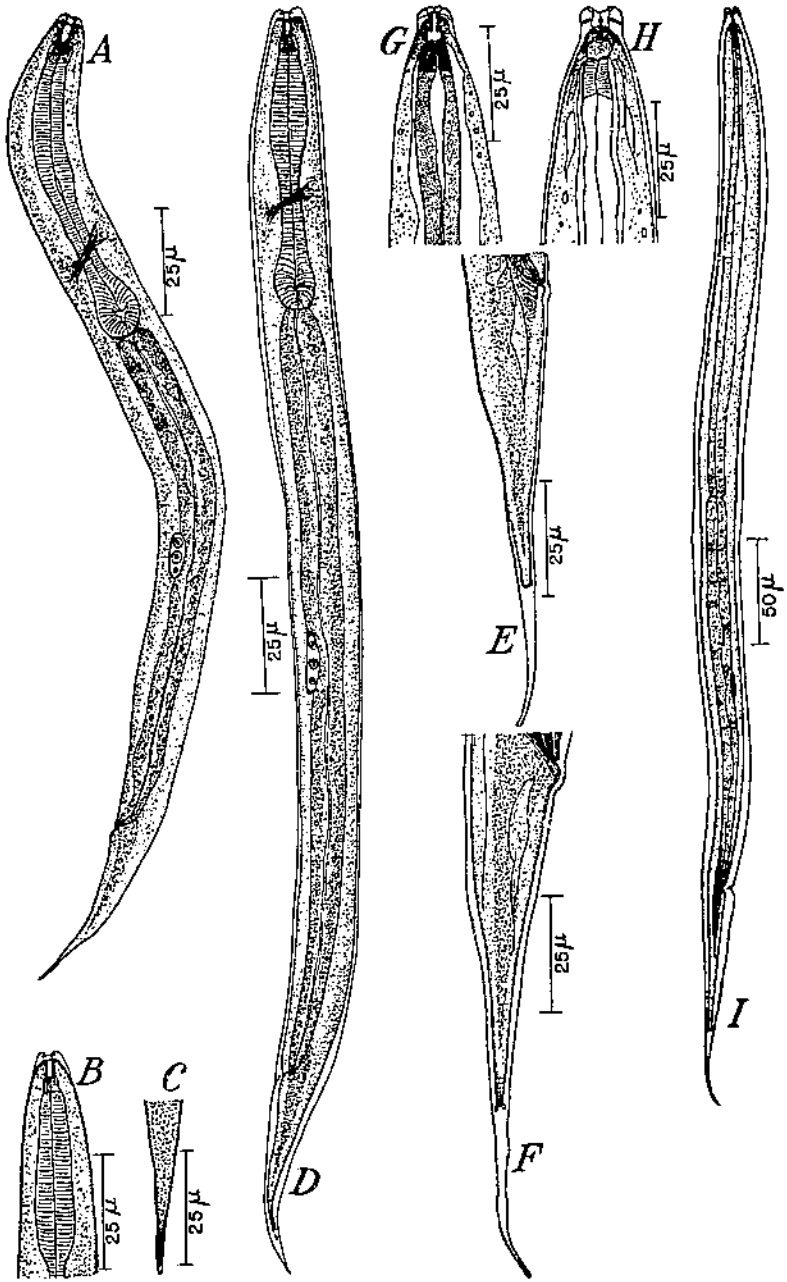

FIGURE 1.—Various stages in the development of strongyliform larvae of *Strongyloides ransomi* (direct cycle): *A*, First-stage larva (newly hatched); *B*, anterior end of first-stage larva; *C*, posterior end of first-stage larva; *D*, larva in first ecdysis; *E*, posterior end of second-stage larva, showing early phase of second ecdysis; *F*, posterior end of second-stage larva, showing buds at tip of tail; *G* and *H*, anterior ends of second-stage larvae; *I*, larva in late phase of second ecdysis.

The first molt occurs from 12 to 18 hours after the larvae hatch. The casting of the sheath was observed in a larvae only 290μ long, but as a rule, larvae about 325μ long have not yet molted. At the time the cuticle is cast, the larvae again show evidence in the head region of the approach of another molt.

SECOND-STAGE LARVAE

Immediately following the first molt, second-stage larvae are not distinguishable from first-stage larvae by any definite structural differences except for the absence of appreciable separation of the cuticle in the former. However, in 36-hour cultures, larvae were found undergoing transition to the so-called "filariform", more properly, strongyliform, state. The writer's observations on the morphological changes during the second ecdysis, by virtue of which the larvae assume the strongyliform characteristics, are essentially similar to those of Leuckart (*12*) on larvae of *Strongyloides stercoralis*.

When the larvae have reached a length of from 375μ to 400μ, the tail has increased considerably in length and fineness, and the second cuticle has already become separated to some extent from the body in the head and tail regions. A larva 420μ long exhibited the first transitional step in the process of the formation of the tail characteristic of third-stage or strongyliform larvae. A shorter, blunt, slightly oblique tail is formed through the separation and disintegration of the fine filamentous tip (fig. 1, *E*). Subsequently two very small lateral oval processes or buds appear at the tip of this newly formed tail (fig. 1, *F*); later these fuse completely with the basic tail process to form the characteristic notched tail. Sandground's observation (*23*) based on a study of the development of several species of *Strongyloides*, to the effect that "* * * the notch in the tail is formed by the rupture of the tail at the time that the filariform larvae emerged from the old cuticula * * *" has not been confirmed by the writer.

Ordinarily the esophagus begins to lengthen and lose its "rhabditiform" character before the changes in the morphology of the tip of the tail are completed. Some larvae in which the tail is already notched still retain the esophageal valve and show no conspicuous increase in the length of the esophagus. However, before the second molt is completed, the esophagus attains a completely strongyliform structure. Complicated changes appear in the structure of the buccal cavity and head. These changes result in the loss of the mouth-cavity characteristic of first-stage and early second-stage larvae. The head structure of larvae which were undergoing the transition to the strongyliform stage is illustrated in figure 1, *G* and *H*. During the second stage the genital primordium is not noticeably modified in structure except for a more pronounced slenderness; it is from 10μ to 17μ long when the transitional changes described above are completed.

The size attained by the larvae during the second stage is variable and depends to some degree on environmental conditions during the period of feeding and growth. Ordinarily larvae from 450μ to 550μ long have completed their development to the strongyliform stage,

and the second molt (fig. 1, *I*) occurs in the manner usual among larval nematodes. In charcoal and feces cultures, the kind used throughout this investigation for the most part, the second molt may occur at room temperature in less than 48 hours after the preparation of the cultures.

THIRD-STAGE OR INFECTIVE STRONGYLIFORM LARVAE

Schwartz and Alicata have given a description and figure of the strongyliform larva of *Strongyloides ransomi*, and it is unnecessary, therefore, to redescribe this stage. In the writer's experience, larvae obtained from eggs cultured in water, a procedure which was occasionally followed, tend to be smaller than those which develop in feces and charcoal cultures. From both sources, strongyliform larvae less than 500μ long have been frequently obtained. The genital primordium of third-stage larvae varies from 10μ to 17μ in length, usually from 12μ to 14μ.

DEVELOPMENT OF FREE-LIVING UNISEXUAL ADULTS FROM EGGS OF THE PARASITIC FEMALE

FIRST-STAGE LARVAE

The time required for the larvae to hatch from the eggs at room temperature has been given previously. Newly hatched larvae of this cycle are from 230μ to 250μ long and are distinguishable from first-stage larvae of the direct cycle, already described, only by the fact that the former possess a larger genital primordium, within which, particularly as growth proceeds, several rounded cells are visible (fig. 2, *A*). In four young first-stage larvae 230μ, 245μ, 250μ, and 265μ long, the genital primordium was 17μ, 15.5μ, 20μ, and 23μ long, respectively. A newly hatched larva showed the following typical size relationships: Length, 255μ; width in region of esophageal bulb, 16μ; distance from nerve ring to anterior end, 50μ; length of esophagus, 75μ; length of genital primordium, 19.5μ; length of tail, 50μ.

The larvae show evidence of the impending first molt immediately after hatching, when it is already possible to discern the separation of the cuticle from the body of the larva in the head region. The separation of the cuticle progresses as the larvae grow, and is next observed in the tail region. When the larvae have attained a length of from 300μ to 350μ, the separation of the cuticle is usually complete and the genital primordium, which has become irregularly elongate oval in outline, has reached a length of from 21μ to 26μ (fig. 2, *B*).

First-stage larvae were observed in the act of casting the first cuticle when they were from 300μ to 350μ long. It was noted, however, that some larvae slightly more than 350μ long had not yet cast their sheaths, although the cuticle was completely separated from the body. The first molt occurs about 10 hours after the larvae hatch.

SECOND-STAGE LARVAE

Since the molting and development of an individual larva were not followed in sequence from the first stage to the free-living adult

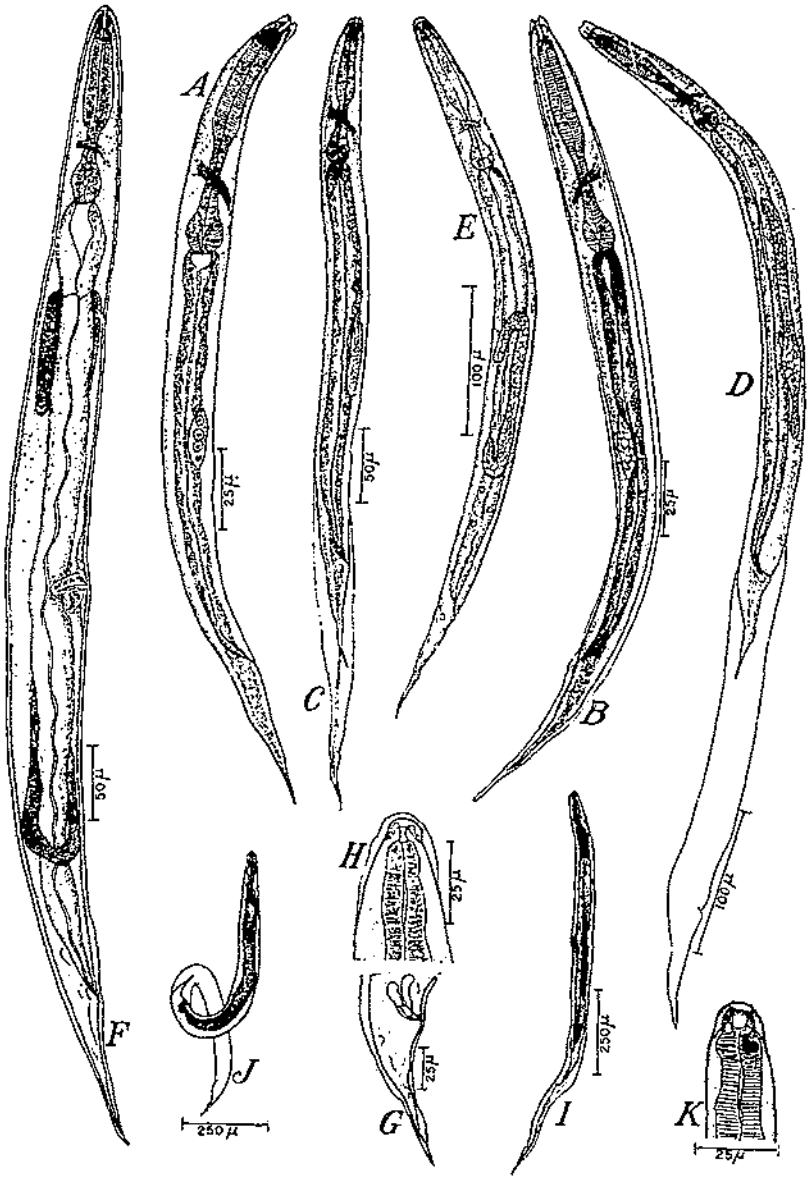

FIGURE 2.—Various stages in the development to free-living unisexual adults of *Strongyloides ransomi*: *A*, First-stage larva (newly hatched); *B*, larva in first ecdysis; *C*, second molt; *D*, third molt; *E*, larva in early fourth stage; *F*, larva (female) in fourth ecdysis; *G*, posterior end of male larva in fourth ecdysis; *H*, anterior end of male larva in fourth ecdysis; *I*, female larva undergoing fourth molt; *J*, male larva undergoing fourth molt; *K*, anterior end of adult female.

state, separation into "rhabditiform" second and third stages is based on the fact that larvae isolated from cultures about 24 to 36 hours old and which were observed in the act of casting their sheaths, are of two groups so far as size and the degree of development of the genital primordium are concerned. A final molt occurs when the larvae have attained sexual characters, following which they become adults.

Larvae which have been tentatively determined as of the second stage showed the following size relationships: Length, 300μ to 450μ; width of body in region of esophageal bulb, 20μ to 25μ; length of esophagus, 75μ to 106μ; length of genital primordium, 25μ to 70μ; length of tail, 60μ to 78μ. Except for the continued growth and elongation of the genital primordium and the multiplication of the cells within it, the morphology is not noticeably modified during this stage. Larvae about 375μ long usually afford very slight evidence of the separation of the newly formed cuticle from the body; larvae from 400μ to 450μ long usually show complete separation of the cuticle. Larvae from 415μ to 435μ long were found which had already molted and to which the cast-off sheath still adhered in the tail region (fig. 2, *C*). The second molt occurs from 22 to 24 hours after the larvae hatch.

THIRD-STAGE LARVAE

The only morphological changes which occur as further growth proceeds are confined to the genital primordium.

So far as was determined, larvae which have been referred to the third stage did not show sex differentiation. Apparently the genital primordium of larvae which later transform into males develops less rapidly than that of individuals which later become females. Larvae referred to this stage showed the following size relationships: Length, 420μ to 550μ; width of body in region of esophageal bulb, 25μ to 31μ; distance from nerve ring to anterior end, 70μ to 90μ; length of esophagus, 92μ to 110μ; length of genital primordium, 60μ to 160μ; length of tail, 70μ to 80μ. Larvae from 480μ to 550μ long which had already molted were found. The cast-off cuticle still adhered to the bodies of these larvae (fig. 2, *D*), and the genital primordium had reached a length of from 60μ to 150μ.

FOURTH-STAGE LARVAE

During the fourth, or final, stage of their development the larvae assume the morphology of the adults of the two sexes. A larva in an early phase of the final stage is illustrated in figure 2, *E*. The genital primordium has become slightly reflected anteriorly and posteriorly. As will be noted in the figure, the structure of the head simulates the condition found in adults. The smallest specimen in which the formation of the musculature of the vagina and the slit of the vulva was indicated was 500μ long. The formation of the vulva and growth of the ovaries and uterus, as well as the formation of the testis and spicular apparatus of the male, may be followed in detail during this stage. The cuticle, which can first be observed as separate from the body of the larva at the head end, can also be distinctly seen in the vulvar region of female larvae from 600μ to 700μ long. Female larvae 750μ long usually show more or less com-

plete separation of the cuticle (fig. 2, *F*). The anterior and posterior extremities of a male larva enveloped in its sheath, just preceding the final molt, are illustrated in figure 2, *G* and *H*.

Female larvae from 750μ to 875μ long were observed in the act of casting the fourth-stage cuticle (fig. 2, *I*). At this stage the uterus does not contain eggs. The ovaries appear to be completely formed. Male larvae about 750μ long were seen casting the sheath of the final molt. The spicular apparatus is completely formed at this time (fig. 2, *J*).

ADULT MALES AND FEMALES

Adults were found in cultures maintained at room temperature after incubation for from 36 to 48 hours. A description of the adults is unnecessary, since Schwartz and Alicata have described and figured both sexes. Figure 2, *K* illustrates the structure of the anterior end of a female in more detail than is shown in the figures of these authors. Schwartz and Alicata state that the spicules of "rhabditiform" males of *S. ransomi* are from 26μ to 29μ long. These authors measured the length of the spicules in a straight line from tip to tip. The spicules of six male specimens, which the writer measured along the arc of their curvature by means of the method of projection, were 36μ, 37.5μ, 39.5μ, 37.5μ, 36μ, and 35μ long, respectively.

DEVELOPMENT OF INFECTIVE LARVAE FROM EGGS OF THE FREE-LIVING FEMALE

EGGS

Fully formed eggs, measured while still in the uterus of free-living females, were from 39μ to 50μ long and from 27μ to 32μ wide. Ordinarily, the eggs did not contain a vermiform embryo when they passed out of the vagina of the female.

Larvae hatched from the eggs in water in about 12 hours. In some cases eggs which were within the uteri of dead female worms had hatched, and the liberated larvae were seen feeding within the body of the disintegrating mother worm. This phenomenon was not observed in living females during the active egg-producing period.

FIRST-STAGE LARVAE

The first-stage larvae (fig. 3, *A*) are morphologically similar to those of the direct cycle of development. The separation of the cuticle (fig. 3, *B* and *C*) also progresses as was described in the case of first-stage larvae of this cycle of development. No noticeable morphological changes precede the first molt. The genital primordium remains small and is from 10μ to 12.5μ long when the first molt occurs. As long as 48 hours may be required for the larvae to molt in water, particularly when food is not abundant. Larvae from 310μ to 340μ long which had already molted were noted. The cast-off cuticle still adhered to these larvae (fig. 3, *D*).

SECOND-STAGE LARVAE

The transition to strongyliform larvae, which occurs during the second stage, takes place in the same manner as has been described in the case of larvae of direct, or homogonic, development. The larvae are from 400μ to 450μ long when they begin to undergo this

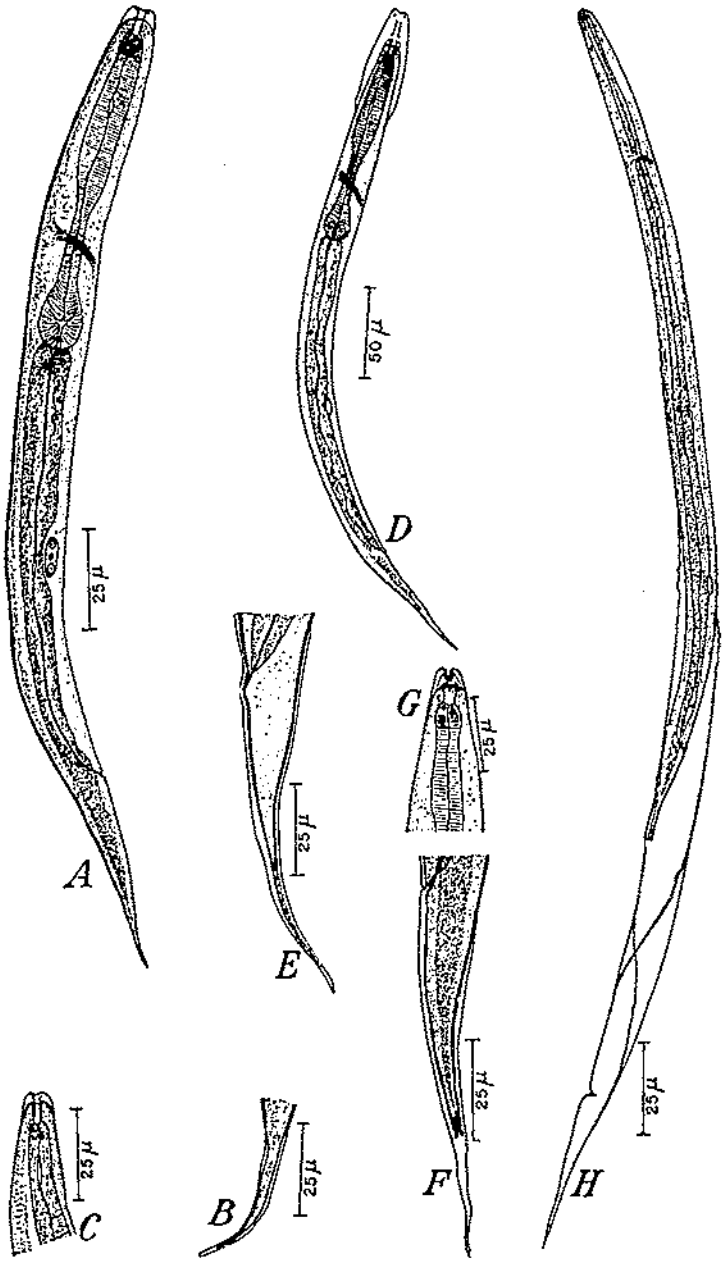

FIGURE 3.—Various stages in the development of strongyliform larvae of *Strongyloides ransomi* (indirect cycle): *A*, First-stage larva (newly hatched); *B*, posterior end of first-stage larva during early first ecdysis; *C*, anterior end of first-stage larva; *D*, second-stage larva immediately following first molt; *E*, posterior end of second-stage larva showing disintegration of tip of tail; *F*, posterior end of a more advanced second-stage larva; *G*, anterior end of second-stage larva during second ecdysis; *H*, strongyliform larva undergoing second molt.

transition (fig. 3, *E*, *F*, and *G*). When the complete strongyliform morphology has been attained, the second molt occurs. In water culture the larvae tend to be somewhat dwarfed in size. A larva only 460μ long was observed in the act of shedding the skin in this medium (fig. 3, *H*).

THIRD-STAGE OR INFECTIVE LARVAE

The strongyliform larvae of the indirect cycle of development are morphologically indistinguishable from those which develop directly from the eggs of the parasitic female. Incubation of feces cultures at room temperature for from 6 to 8 days is ordinarily necessary before all the larvae of the indirect cycle of development attain the infective state. Infective larvae obtained from the eggs of free-living females cultured in water required from 48 to 60 hours for their development.

PATHS OF ENTRY OF INFECTIVE LARVAE INTO HOST

The ability of infective larvae of several species of *Strongyloides* to penetrate the skin of normal and other hosts has been repeatedly demonstrated and is so well known to parasitologists that a review of the extensive literature on this subject is unnecessary. That infection may occur by the oral route is also well known. Fülleborn (*2*), however, has reported that when larvae of *S. stercoralis* are administered to dogs by mouth the vast majority of the larvae are killed by the digestive juices, and that infection occurs only by virtue of the fact that a few of the larvae bore into the stomach wall, pass to the lungs by way of the portal system, and return by way of the trachea and esophagus to the intestine where they develop to maturity. Mönnig (*17*) has reported, on the other hand, that sheep normally become infected with *S. papillosus* by the oral route and that infective larvae of this species are rather poor skin penetrators. Mönnig states also that larvae administered by mouth apparently do not migrate to the lungs. Because of the wide difference of opinion which exists as to the facility with which *Strongyloides* larvae of different species penetrate the skin or establish themselves in the intestine following ingestion, it seemed appropriate to investigate the facts with reference to *S. ransomi*. In view of the facts brought to light also by Looss (*13*) and Fülleborn (*2*) concerning the migration of *Strongyloides* larvae following percutaneous infection, the lungs of all experimental animals so infected were examined.

PENETRATION OF SKIN

In accordance with the technic described by Goodey (*7*), the freshly excised skin of a 3-day-old rat was stretched, hair upward, on a circular cork ring floated in a small glass dish containing physiologic saline solution warmed to a temperature of 37° C., and maintained at this temperature in the incubator. A drop of water containing 400 infective larvae was placed on the rat skin. The water evaporated completely in 15 minutes; 1 hour thereafter the salt solution was removed, centrifuged, and examined. Three larvae were found in it. Sections of the skin prepared in the usual manner were microscopically examined for larvae with negative results.

A second experiment was performed in the same manner. Forty-five minutes were required for the evaporation of the drop of water, which contained 950 larvae. One-half hour later the salt solution was examined and 5 larvae were found in it. The area of skin upon which the larvae had been placed was fixed in hot 70-percent alcohol. The epithelial layer was mechanically removed and cleared in an alcohol-phenol mixture. Microscopic examination showed numerous larvae lying in a horizontal position on the surface of the skin, but none appeared to have penetrated. The dermal layer was stained and sectioned; examination failed to reveal the presence of larvae in this layer.

Thus less than 1 percent of the larvae placed on rat skin in these two experiments were subsequently found in the salt solution, and none were found in the skin sections examined microscopically.

Infective larvae suspended in a few drops of water were applied to the skin of 5 pigs, 1 rabbit, and 2 guinea pigs. After the water had evaporated from the skin, a period of drying, which ranged from 10 to 180 minutes, was allowed. Usually the skin area was then washed with water or soap and water and was wiped off with absorbent cotton. In most cases 70-percent alcohol was also used to wash the skin. After the skin area had been washed, a further period of drying was allowed before the animal was returned to its cage. Control animals were not used, since fecal examinations or post-mortem findings were deemed adequate proof, under the conditions of the experiments, as to the occurrence or nonoccurrence of penetration. In the case of the pigs, the preliminary fecal examinations were made by a modification of the Lane technic. Following is an account of the experiments involving the rabbit and the guinea pigs:

Since natural infestations with *Strongyloides* do not occur in laboratory-raised rabbits, a preliminary examination of the feces of rabbit 2 was omitted. On May 4, 6, and 10, respectively, a few drops of water, in which a very large number of infective larvae of *S. ransomi* were suspended, were placed on separate shaved areas of the skin of this animal. On May 13 the rabbit was killed. The contents of the small intestine were flushed out with warm water, and several adults and numerous larvae of *S. ransomi* were recovered. The larvae ranged from 525μ to 2.5 mm in length. The lungs were cut up and placed in the Baermann apparatus. Twenty strongyliform larvae of *S. ransomi* were recovered from these organs in this way.

A large number of larvae suspended in a small quantity of water were placed on a shaved area of the skin of guinea pig 1 on April 29. The animal was killed on May 1, and this area of skin was excised and examined. Several living larvae were found in the skin and subcutaneous tissue. In a similar manner, large numbers of infective larvae were applied to the skin of guinea pig 2 on May 3 and 5. The animal was killed on May 8, and several living larvae were found in the skin and subcutaneous tissue which was excised from the areas where larvae had been applied. No larvae were recovered from the lungs or small intestine of either of these two guinea pigs.

TABLE 1.—*Results of skin-penetration experiments with Strongyloides ransomi larvae on 5 pigs*

Pig no.	Age	Results of preliminary fecal examinations by modification of Lane technic	Date of application of larvae to skin	Larvae applied	Ante-mortem evidence of infection (microscopic examination of feces)	Date of first appearance of eggs in feces	Date of post-mortem examination	Results of post-mortem examination of—	
								Small intestine	Lungs
	Weeks			*Number*					
2-A	20	Few *Strongyloides* eggs present.	Nov. 11	14,000	Increase in egg count.	(1)	Jan. 3	Several mature and immature adults; several strongyliform larvae 500μ to 600μ long.	No larvae.
			Dec. 9	18,500					
			Dec. 22	5,000					
			Dec. 30	8,500					
3	8	Negative to repeated examinations.	Jan. 25	3,000	Eggs found [2]	Feb. 2	Mar. 14	Several adults; numerous larvae 550μ to 2.5 mm long.	Several strongyliform larvae.
			Mar. 7	25,000					
			Mar. 11	38,000					
4	10	do	Mar. 18	16,000	do [3]	Mar. 26	Mar. 27	No adults found, but numerous eggs present; several larvae 500μ to 2.5 mm long.	Several strongyliform larvae; 1 from trachea.
			Mar. 20	26,000					
			Mar. 22	28,000					
			Mar. 24	19,000					
			Mar. 25	17,000					
			Mar. 26	10,000					
5	10	do	Mar. 18	16,000	do [3]	Mar. 26	(4)		
6	15	do	Apr. 24	11,500	None [5]		Apr. 28	No adults or larvae	1 strongyliform larva.
			Apr. 25	20,000					
			Apr. 26	34,000					
			Apr. 27	24,000					

[1] Undetermined.
[2] Feces examined by the salt flotation method.
[3] Feces examined by a modification of the Lane technic.
[4] This animal was not killed during the experiment. See data in table 2.
[5] This pig was killed on the fourth day following the first application of larvae to the skin.

The results of the experiments involving the five pigs are given in table 1. The data in the table are supplemented by figure 4, which is a graphic presentation of egg counts obtained on samples of feces from one of the pigs, no. 2–A.

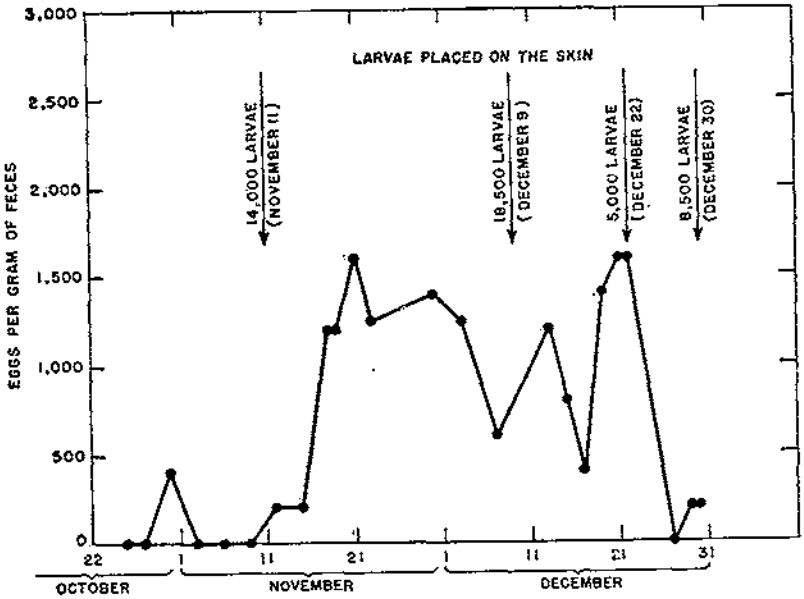

FIGURE 4.—Egg counts obtained on samples of feces from pig 2–A exposed to infection, as indicated, during the period October 26 to December 30.

The data given show that infective larvae of *S. ransomi* were capable of penetrating the skin of pigs and a rabbit, and that intestinal infestations resulted from the percutaneous entry of the larvae in all cases except that of pig 6, in which animal one larva was found in the lungs. The larvae also penetrated beneath the skin of two guinea pigs, but had not reached the lungs or intestine of guinea pig 2, 5 days after the first infection.

INFECTION BY ORAL ROUTE

Experiments on infection by the oral route involved 5 pigs, 1 of which was a control, and 1 rabbit. An account of the experiment with the rabbit follows:

Rabbit 1, a laboratory-raised animal, was given large oral doses of infective larvae of *Strongyloides ransomi* on April 27 and 29. On post-mortem examination May 3, the sixth day following the first administration of larvae, the animal was found to harbor adult and larval *S. ransomi* in its small intestine. The lungs were finely cut up with a scissors. Examination by means of the Baermann apparatus did not reveal the presence of larvae.

The experiments involving the pigs are summarized in table 2. Pig 5 is the same animal which was subjected to a single percutaneous

TABLE 2.—*Results of administering Strongyloides ransomi larvae orally to pigs*

Pig no.	Age	Results of preliminary fecal examinations by Lane technic or egg count	Date of administrations of infective larvae	Larvae fed	Ante-mortem evidence of infection (microscopic examination of feces)	Date of first appearance of eggs in feces	Date of post-mortem examination	Results of post-mortem examination of—	
								Small intestine	Lungs
	Weeks			*Number*					
1-A	22	No eggs	Dec. 21	5,000	None; egg count remained zero		Jan. 22	1 adult	No larvae found.
			Jan. 5	11,000					
			Jan. 9	3,400					
			Jan. 17	6,600					
			Jan. 19	7,700					
			Jan. 20	1,000					
1-B	5	do	(2)	0	None [3]		(1)		
2-B	5	do	Mar. 15	2,900	Eggs found [3]	Mar. 22	(1)		
3-B	5	do	do	5,000	do.[3]	Mar. 21	Apr. 11	12 adults	(4).
5	13	do	Apr. 6	23,000	do.[3]	Apr. 13	Apr. 30	More than 250 adults	(4).

[1] Animal not killed.
[2] This animal was a control; consequently, no larvae were administered.
[3] Feces examined by the salt flotation method.
[4] Not examined.

infection. Although the feces of this pig were positive for eggs of *S. ransomi* on the eighth day following infection by the percutaneous route, subsequent examinations failed to reveal the presence of eggs in the feces. Pig 1–A had been given by mouth 5,600 larvae of *S. papillosus* on October 25 of the preceding year, and 9,500 larvae of this species were placed on its skin on October 26. During the following 8 weeks, 20 egg counts on the feces of this pig were made, no eggs being found. However, the failure of *S. papillosus* to develop in this animal, as judged by the subsequent failure to find eggs by the Stoll egg-counting method, is rendered inconclusive by the fact that the egg production remained zero despite repeated feedings of infective larvae of *S. ransomi*.

The data given indicate that the four pigs to which larvae were fed and the rabbit acquired infestation following the administration by the oral route of infective larvae of *S. ransomi*, as indicated by the appearance of eggs in the feces or by the post-mortem finding of adults of this species. Eggs appeared in the feces of pigs 3–B and 2–B on the sixth and seventh days, respectively, following the feeding of larvae. Pig 1–A was almost entirely resistant to infestation. Eggs appeared in the feces of pig 5 on the seventh day after infection. The feces of the control pig 1–B were repeatedly examined and remained negative for helminth eggs.

DEVELOPMENT OF STRONGYLOIDES RANSOMI IN NORMAL AND OTHER HOSTS

IN LUNGS AND SKIN

Larvae were recovered from the lungs of pigs 3, 4, and 6 and rabbit 2. The dates of percutaneous administrations of larvae to these animals and the dates of the respective necropsies have been previously given. The larvae from the lungs were morphologically similar to infective third-stage larvae except for slightly greater thickness, greater length and width of the genital primordium, slightly more pronounced striation of the cuticle, and the greater prominence of the nerve ring and its associated structures (fig. 5, A). Measurements obtained from 2 larvae from the lungs and 1 from the trachea of pig 4 appear in table 3. The tails of all larvae obtained from the lungs were notched as in preparasitic third-stage larvae. None of the larvae from the lungs of the pigs or rabbit were undergoing a molt or showed any evidence of the approach of a molt.

TABLE 3.—*Principal measurements (in microns) of representative parasitic larvae of Strongyloides ransomi found in pigs and a rabbit*

Measurement	Pigs							Rabbit intestine (2 larvae)	
	Lungs (2 larvae)		Trachea (1 larva)	Intestine (4 larvae)					
Length	507	540	555	470	555	483	530	725	2,050
Width in region of esophagus	20	22	26	21	25	18	24	22	48
Distance from nerve ring to anterior end	120	89	91	63	80	93	80	112	247
Length of esophagus	260	250	260	277	275	272	275	345	648
Length of genital primordium	19	17	20	14	28	17	15	43	----
Length of tail	60	72	62	56	60	66	63	73	70

Some of the larvae removed from the skin and subcutaneous tissue of guinea pig 2, 5 days after the animal was subjected to a percutaneous infection, had become slightly modified in morphology as compared with free-living infective third-stage larvae. The genital primordium had increased in size, particularly in width, and the larvae resembled those found in the lungs of pigs. None of the larvae from the skin of this guinea pig, removed 3 and 5 days, respectively, after 2 infections, nor from the skin of guinea pig 1, 2 days after infection, were undergoing a molt or showed evidence of the approach of a molt.

IN SMALL INTESTINE

The smallest and morphologically least advanced larvae (fig. 5, *B*) recovered from the small intestines of the above-mentioned pigs and rabbit and also from the intestine of pig 2–A were indistinguishable from the larvae obtained from the lungs. The tail presented the notched appearance characteristic of third-stage larvae. Four of the smallest larvae found in the intestines of percutaneously infected pigs showed the size relationships given in table 3. In specimens of about this size, the distance from the genital primordium to the anus was found to be noticeably greater than the distance from the primordium to the bulb of the esophagus. About four cells were visible in the primordium at this stage. Morphologically similar larvae were found in the small intestine of rabbit 1, which was given infective larvae by mouth 4 and 6 days, respectively, previous to necropsy.

The structure of a somewhat larger larva from the intestine of rabbit 2 indicated that the first changes which appear, as growth takes place, are a further increase in the size of the genital primordium and a multiplication of the number of cells within it. Measurements obtained from this larva are given in the next to the last column of table 3. It is noteworthy that the tail of this larva was still notched at the tip. A slightly larger larva was found in which the tail was beginning to undergo the transition in shape from a notched tip to the rounded tip found in more advanced larvae. Slight separation of the cuticle had occurred at the tip of the tail and the tip of the sheath was definitely notched, the loss of the notched-tail termination evidently occurring at the very commencement of the final ecdysis.

Numerous larvae from 900μ to 2.5 mm long were removed from the intestines of pigs and rabbits following either percutaneous or oral entry of the larvae. Larvae about 1 mm long exhibited the beginning of the formation of the vulva and vagina. The genital primordium was seen, in lateral view, to have extended both posteriorly and anteriorly from the primordium of the vulva, and was also visible anteriorly in a position dorsal to the intestine (fig. 5, *C*). The formation of the vulva is well indicated before the ovaries are recognizable as such. Larvae about 1.5 mm long showed the reflection of the primordium anteriorly and posteriorly (fig. 5, *D*). All larvae falling within the size range mentioned above exhibited a progressive separation of the cuticle from the body. Especial

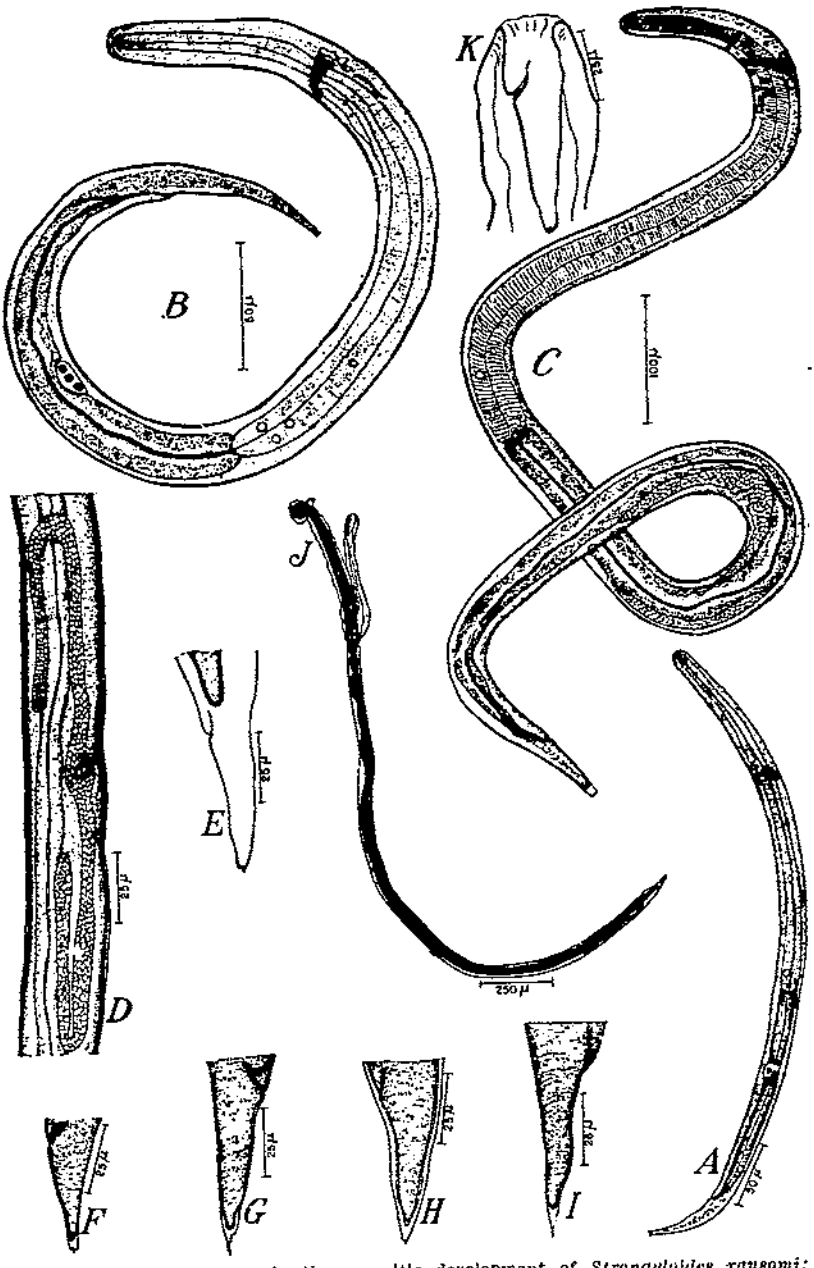

FIGURE 5.—Various stages in the parasitic development of *Strongyloides ransomi*: *A*, Strongyliform larva from lungs of a pig about 3 days after percutaneous infection; *B*, larva from small intestine of pig about 4 days after percutaneous infection; *C*, larva from intestine of pig during early final ecdysis; *D*, vulvar region of more advanced larva from intestine of pig; *E* to *I*, posterior ends of larvae during final ecdysis, showing variation in shape of tip of sheath; *J*, preadult female from intestine of rabbit 6 days after percutaneous infection, undergoing final molt; *K*, posterior end of cast sheath of final molt.

attention was given to a study of the shape of the posterior tip of the sheath, and although much variation was noted, owing possibly to some extent to the pressure of the coverslip but also unquestionably to normal variation, the tip was found to be definitely notched in many instances. In some cases the tip of the sheath appeared to terminate in three processes or evaginations, whereas in other specimens it tapered to a rather sharp point. The variations found are illustrated in figure 5, *E* to *I*. In all these larvae the shape of the tail resembled that of the adult female.

Larvae which had attained a length of about 2.5 mm were found to have attained the morphology of the adult female. Individuals of this size were found which were enveloped in the sheath of the final molt or to which the cast-off cuticle still adhered. A larva (fig. 5, *J*) from the intestine of rabbit 1, which was in the act of casting the final cuticle, had the measurements shown in the last column of table 3. Additional measurements of this larva were: Distance from vulva to anterior end, 1,660μ; distance from vulva to posterior end, 938μ; width in region of vulva, 49μ. The posterior tip of the sheath of this specimen was notched, as illustrated in figure 5, *K*.

DISCUSSION OF PREPARASITIC AND PARASITIC DEVELOPMENT OF STRONGYLOIDES RANSOMI

The writer's observations, indicating that two molts occur in the development of strongyliform infective larvae from the eggs of parasitic or "rhabditiform" females of *Strongyloides ransomi*, are in agreement with those of Looss (*14*, *15*), who, for certain other species of *Strongyloides*, including *S. stercoralis*, reported two molts in the course of the development of filariform (i.e., strongyliform) larvae of the direct and indirect cycles. The writer's results are at variance with the reports of Grassi and Parona (*8*), Perroncito (*18*), Leuckart (*12*), Grassi and Segrè (*9*), Golgi and Monti (*5*), Zinn (*27*), Leichtenstern (*11*), Gonder (*6*), and Kreis (*10*), who mention only one molt during the course of the development of filariform larvae of *S. stercoralis* and other species. Four molts have been described by the writer in the course of the development of "rhabditiform" unisexual adults from the eggs of the parasitic female of *S. ransomi*. This is in disagreement with the findings of Looss (*15*) and other investigators of the life history of *S. stercoralis*, all of whom observed only one molt (i.e., the final molt) in the development of free-living adults.

Third-stage larvae of *S. ransomi* which gain entry to the body of the host, either through the oral or percutaneous route, have been observed to molt once in the intestine of the host animal. This molt occurs about 6 days after infection, when the larvae have attained the morphology of the adult female. As has been mentioned, when the larvae first arrive in the intestine they differ from free-living infective larvae only in minor particulars, and still possess a notched tail. Moreover, the posterior tip of the sheath of molting larvae in progressive states of development is usually also definitely notched. These facts are a strong indication that the entire development from the smallest larvae found in the intestine to the adult form occurs in

connection with but one molt. It cannot, however, be concluded with certainty that only one molt occurs during the parasitic development of *S. ransomi*, since it is possible that following percutaneous entry to the host the larvae may molt while they are in the lungs. However, none of the larvae recovered from the lungs of experimental animals were molting. None of the larvae removed from beneath the skin of a guinea pig showed evidence of a molt, despite the fact that some of these larvae had undergone morphological advancement comparable to that attained by larvae during their passage through the lungs. In the case of orally infected animals no evidence of the occurrence of an earlier molt was discovered.

In this connection, it is of interest that only one molt in the course of the parasitic development of *Strongyloides* was reported by Looss (*15*) who, on the basis of observations on the development of three unnamed species, one of which unquestionably was *S. stercoralis*, states that filariform (i.e., strongyliform) larvae cast their skins a third time and thereby assume the adult condition. Fülleborn (*2*), on the other hand, apparently considers that two molts occur in the development of *S. stercoralis* in dogs, the larvae concerned having been removed by him from the trachea of the host. Although the occurrence of two molts in the lungs seems rather unusual in the development of an intestinal helminth, Fülleborn's discovery that *S. stercoralis* can reach sexual maturity in the trachea of dogs may explain this finding. It is of further interest that Fülleborn (*4*) found that infective *S. stercoralis* larvae maintained beneath the skin of rabbits from 6 to 17 days could attain a structure comparable to that usually reached in 3 or 4 days by larvae in the trachea, but he presents no information as to whether a molt accompanied the attainment of this more advanced developmental stage.

While with respect to paths of entry and migration within the body, it cannot be definitely stated that strongyliform larvae of *S. ransomi* do not migrate to the lungs following entry into the body of the host by the oral route, the available evidence is negative. No larvae were found in the lungs of a pig and a rabbit to which oral infections had been given 2 and 4 days, respectively, previous to autopsy. Significant infestations were readily produced in pigs and rabbits by either the percutaneous or oral route; hence it must be concluded, on the basis of the experiments performed, that both modes of infection take place and are of importance in nature.

EFFECTS OF ENVIRONMENTAL FACTORS ON VIABILITY OF EGGS AND SURVIVAL OF LARVAE

EFFECT OF COLD ON EGGS

Freshly prepared cultures from the feces of pigs infested with *Strongyloides ransomi* were subjected to low temperatures for various periods, and the effects were studied in a number of experiments. The cultures were contained in Petri dishes and following the desired exposure to cold were examined after they had incubated for a suitable time at room temperature to permit infective larvae to develop from any eggs which might have been able to withstand the exposure. The amount of moisture in the cultures and the proportion of charcoal to feces, when charcoal was employed, were so regulated as

to provide optimum conditions for development. In each experiment a control culture was used. This was prepared in the same manner from the same fecal sample as the experimental culture, but was kept at room temperature. In all cases the control cultures yielded very large numbers of infective larvae when examined after incubation for periods of from 7 to 9 days.

The data relating to the cultures which were subjected to low temperatures are given in table 4.

TABLE 4.—*Effects of low temperatures on viability of eggs of Strongyloides ransomi in types of cultures indicated*

Experiment no.	Nature of culture	Temperature at which culture was exposed	Duration of exposure	Duration of subsequent incubation at 20°–24° C.	Infective larvae found in culture upon examination
		° C.	*Hours*	*Days*	
1	Feces [1] and charcoal	−4 to −8	168	9	None.
2	do.[1]	−4 to −8	46	6	Do.
3	do.[1]	−4 to −8	27	7	Few.
4	do.[1]	−4 to −8	24	4	None.
5	Moist feces [2]	−4 to −8	46	6	Do.
6	do.[2]	−4 to −8	23	8	Few.
7	Feces [3] and charcoal	2 to 6	147	5	None.
8	do.[3]	2 to 6	48	7	Several.
9	Moist feces [4]	2 to 6	50	5	None.

[1] Feces were 24-hour accumulation from 2 infested pigs in outdoor pen in December; no preinfective larvae present.
[2] Feces were 24-hour accumulation from 1 infested pig in animal room at about 24° C; not examined for preinfective larvae.
[3] Freshly voided feces; no preinfective larvae present.
[4] Freshly voided feces; not examined for preinfective larvae.

The data given in table 4 indicate that two cultures kept at temperatures from −4° to −8° C. for approximately 24 hours yielded few larvae when examined after subsequent incubation of the cultures for a suitable time at room temperature. In one case no larvae were found in a culture subjected to similar treatment. Each of two cultures exposed to this range of temperatures for 46 hours failed to yield larvae subsequently, as did also one culture exposed for 168 hours. Of two cultures exposed for about 48 hours to temperatures slightly above freezing, only one subsequently yielded infective larvae. A culture kept at these temperatures for 147 hours did not yield larvae when examined after incubation at room temperature. The time required to effect equivalent inhibition or the total suppression of larval development by brief exposures to low temperatures was independent of the composition of the two types of cultures used.

EFFECT OF COLD ON INFECTIVE LARVAE

The effect of low temperatures on the survival of infective larvae was studied according to the procedure used by Alicata (*1*) with larvae of *Obeliscoides cuniculi*. Larvae were placed upon moist charcoal contained in small stoppered glass vials which were placed in an electric refrigerator. One group of vials was kept for various periods at subfreezing temperatures, whereas a second group was kept at temperatures slightly above freezing. After a given exposure, the vials were permitted to remain at room temperature for

an hour or more before examination was made by repeatedly washing the charcoal with water and removing the wash water to a small dish prepared to facilitate the counts of living and dead larvae. In this way the vast majority of the larvae placed in the vials were recovered.

Infective larvae of *Strongyloides ransomi* do not lend themselves well to this type of experimentation, since under optimum conditions deaths continually occur, and the larvae do not live in cultures at room temperatures much longer than 3 weeks. Thus many of the larvae placed in similar vials kept at room temperature as controls died during the experiments. Six experiments were performed at subfreezing temperatures. A majority of the larvae from the control vial for experiment 1 were still alive after 190 hours; about 40 percent of the larvae from the control vial for experiment 5 were alive after 45 hours; and many of the larvae in the two control vials for experiment 6 were still alive after 262 hours. Control vials were not prepared for experiments 2, 3, and 4. The data pertaining to the experiments at subfreezing temperatures are summarized in table 5.

TABLE 5.—*Effect of subfreezing temperatures on survival of infective larvae of Strongyloides ransomi*

Experiment no.	Vial no.	Larvae used	Duration of exposure at −4° to −8° C.	Duration of subsequent maintenance at room temperature		Larvae recovered alive
		Number	*Hours*	*Hours*	*Minutes*	*Percent*
1	1	500	17	1		0
1	2	500	40	1		0
2	3	500	6	1	15	10.0
3	4	1,000	17	2		5.5
3	5	1,000	17	2		2.8
4	6	1,000	26	2		3.4
4	7	1,000	41	2		.4
5	8	1,000	18	2	15	1.6
5	9	1,000	41	2	15	0
6	10	1,000	12	2	15	12.0

Examination of table 5 shows that from 88 to 100 percent of the larvae recovered from the vials after exposure to temperatures from −4° to −8° C. for periods ranging from 6 to 26 hours were dead. About 40 hours' exposure to these temperatures killed all the larvae recovered from 2 preparations, and 99.6 percent of those from a third preparation.

Owing to the inconsistent behavior of larvae maintained at room temperature as controls, a detailed account of the results of tests made at temperatures from 2° to 6° C. is omitted. From 12 to 24 percent of the larvae recovered from vials exposed from 64 to 116 hours at this range of temperature showed evidence of life. A few larvae were feebly active in a preparation exposed to these temperatures for 185 hours.

EFFECT OF DIRECT SUNLIGHT ON INFECTIVE LARVAE

To test the effect of direct sunlight, 750 active infective larvae, suspended in water, were placed in a shallow dish out of doors on a brownish background in such a way that direct rays of the sun reached the larvae without first passing through glass. Under these conditions the larvae were exposed to December sunlight for 15

minutes at a temperature of 9° C. Immediate examination revealed most of the larvae to be slightly active. The preparation was subjected to a further 15 minutes' exposure to sunlight, making a total exposure of 30 minutes. Immediate examination revealed that 180 larvae were slightly motile; 1½ hours later, during which time the larvae were kept at room temperature, 50 larvae were feebly motile. The remaining larvae appeared to be dead.

In a second test, 500 larvae were similarly exposed to sunlight on the same day at a temperature of 6° C. for 45 minutes. Immediate examination showed but 1 larva to be slightly active; 1½ hours later, during which time the larvae were kept at room temperature, 3 larvae exhibited feeble motility. Since the remaining larvae did not revive, it was concluded that they were killed by sunlight.

As a control on the effect of brief exposure to the temperatures mentioned, a similar glass dish containing larvae suspended in water was placed in the electric refrigerator for 30 minutes at 6° C. Immediate examination showed most of the larvae to be inactive. However, after a brief period at room temperature, it was found that the vast majority of the larvae regained their normal activity.

Since in the above experiments a few larvae survived exposure to sunlight for 45 minutes another experiment was later performed. A water suspension of 250 active larvae was placed in each of 4 shallow dishes. The larvae contained in 3 of the dishes were exposed to direct April sunlight at a temperature of from 29° to 30° C. for the intervals shown in table 6. Water of this temperature was added to the dishes from time to time to compensate for evaporation. The fourth preparation was kept in the shade in the laboratory as a control. When examined after 2 hours, the vast majority of the larvae in the control dish showed normal activity.

TABLE 6.—*Results of exposure of 250 Strongyloides ransomi larvae to direct April sunlight, at a temperature of 29°–30° C.*

Time of exposure (minutes)	Larvae active—	
	On immediate examination	1 hour later
	Number	*Number*
15	230	237
30	229	100
60	0	0

From the data presented, it may be concluded that infective larvae of *Strongyloides ransomi* exposed to direct sunlight at nonlethal temperatures are killed in from 45 to 60 minutes of exposure.

EFFECT OF DESICCATION ON INFECTIVE LARVAE

A drop of water containing a small number of active larvae was placed on each of 11 glass slides. The contents of the slides were permitted to dry in air at room temperature, and the instant at which complete evaporation of the water occurrred, as determined by examination under a binocular microscope, was noted. When the desired air desiccation was accomplished, a drop of water was placed

on each slide in the area where the larvae were present, and the subsequent behavior of the larvae was noted, as indicated in table 7. Larvae from two similarly prepared cultures were used in making the test.

TABLE 7.—*Effect of desiccation, in air (temperature 24° C.), on infective Strongyloides ransomi larvae*

Culture and slide no.	Larvae used	Period of desiccation	Larvae active, after addition of water, at expiration of—		
			10 minutes	30 minutes	60 minutes
	Number	*Minutes*	*Number*	*Number*	*Number*
Culture 1:					
1	25	3	12	16	[1] 11
2	23	7	5	7	[1] 3
3	35	10	0	0	0
4	21	12	0	0	0
6	20	14	0	0	0
Culture 2:					
1-A	31	2	27	[1] 27	----
2-A	35	3	7	[1] 12	----
3-A	30	4	0	[1] 6	----
4-A	37	5	0	[1] 8	----
5-A	31	8	0	[1] 3	----
6-A	32	9	0	[1] 0	----

[1] Heat applied to determine vitality of larvae.

The results given in table 7 warrant the conclusion that infective larvae of *Strongyloides ransomi*, present on a nonabsorptive surface, are killed by 9 or 10 minutes of air drying at room temperature. Under these conditions many larvae are killed or their vitality is impaired by an exposure of from 3 to 5 minutes.

EFFECT OF CHEMICALS ON INFECTIVE LARVAE

Larvae were transferred, by means of a wire loop, from clusters present on a Petri-dish lid into a drop of the desired chemical solution, and viewed through a binocular microscope in order to note the time when all the larvae became inactive. Under these conditions, larvae were rendered instantaneously inactive in 95- and 70-percent alcohol. The drop was greatly diluted with water, but only a few larvae had revived an hour later. On other occasions it was noted, in agreement with the findings of Fülleborn (*2*), that a large percentage of the larvae, contained in a drop of water, which had been rendered inactive by their introduction into 70-percent alcohol, regained normal activity when the alcohol was greatly diluted.

Larvae also were introduced into 1-percent copper sulphate solution. A few were able to survive for 30 minutes or more, but most of them were killed within 30 minutes.

The majority of larvae introduced into a drop of 1-percent phenol solution were immediately killed, although a few larvae showed total cessation of activity only after 9 minutes of exposure to this chemical. Subsequently the drop was greatly diluted, but none of the larvae revived during the ensuing 18 hours.

An ordinary cresol disinfectant, in the concentration recommended for the spraying of outbuildings, killed the larvae quickly. All larvae ceased movement within 2½ minutes after their introduction into this chemical solution, and none revived during the ensuing 18 hours despite great dilution with water.

REACTIONS OF INFECTIVE LARVAE TO STIMULI AND ENVIRONMENT

REACTION TO HEAT

A number of larvae were placed in water contained in a shallow glass dish. A fairly equal distribution of the larvae in the dish was effected in such a manner that none were visible in a particular field of a binocular microscope, when the dish was so placed on the microscope stage that its center was above the aperture in the center of the stage. A warmed glass rod was applied to the bottom of the dish in the center of the field. In a few moments larvae were seen migrating from the periphery toward the source of heat, and a considerable number soon became concentrated at the point where the rod had been applied. The experiment was repeated with similar results.

It may, therefore, be concluded that infective larvae of *Strongyloides ransomi* are attracted to a source of moderate heat. Larvae present on a glass slide are stimulated to great activity when heat is applied to the under surface of the slide. Apparently very slight changes in temperature are detected by the larvae, since the proximity of a finger to a slide is sufficient quickly to cause movement of the larvae present in a drop of water on the slide toward this source of heat.

REACTION TO STRONG ARTIFICIAL LIGHT

The water contained in a small glass dish was agitated to produce an equal distribution of about 500 larvae present. The dish was placed on the stage of a binocular microscope in such a manner that one-half of the bottom of the dish was shaded by a black background impermeable to light. The same portion of the top and sides was shaded with black paper. A strong beam of light from a covered and shaded microscope lamp in a dark room was reflected by the microscope mirror through the unshaded half of the dish. After 30 minutes' exposure to the light, it was found that 115 larvae were present in the unshaded half of the dish and 383 in the shaded half.

In an essentially similar experiment a 100-watt electric lamp was used as a source of light. It was placed in front of and above the dish, arranged as before, at a distance of 4 inches from the center of the latter. Thermometers indicated a differential of less than 1° C. in the temperature at the shaded and unshaded sides of the dish. The contents of the dish were agitated as before. Examination after 10 minutes' exposure to the light showed that the vast majority of the larvae were present in the shaded half of the dish. It may, therefore, be concluded that infective larvae of *Strongyloides ransomi* avoid strong artificial light.

REACTION TO DIFFUSE DAYLIGHT

One-half of a Petri dish, including the cover, sides, and bottom, was painted with two coats of india ink. A large number of infective larvae, rather equally distributed in water, were put in the dish, which was so placed on a black background that diffuse daylight from northern and eastern windows reached it. Two hours later

the majority of the larvae were present in the unshaded portion of the dish.

Except for a small circular area in the center of the cover and bottom, a Petri dish was entirely painted with two coats of india ink. A homogeneous suspension of larvae in water was introduced into the assembled dish, which was so placed on the stage of a binocular microscope that the central unshaded area of the cover and bottom was directly above a small aperture in the center of the stage. This arrangement permitted the passage of a beam of diffuse daylight from the microscope mirror through the small unshaded central area of the dish. The approximate number of larvae visible in the low-power field was noted. Fifteen minutes later a noticeable increase in the number of larvae visible had occurred, and within 45 minutes the visible larvae were so numerous that they could not be counted with accuracy. This experiment was repeated by substituting a much less concentrated suspension of larvae. Seventy larvae were visible in the field at the beginning of the experiment. After 30 minutes, the visible larvae were too numerous to permit an accurate count. It is evident that infective larvae of *Strongyloides ransomi* respond positively to diffuse daylight.

REACTION TO EXCLUSION OF AIR

Fülleborn (*3*) has shown that larvae of *Strongyloides stercoralis* are somewhat attracted to oxygen and are repelled by carbon dioxide. He showed that larvae rendered inactive through the exclusion of oxygen from their environment by means of an atmosphere of hydrogen revived when air was again made available to them.

A type of behavior, which it is believed may be explained on the basis of Fülleborn's results, has been noted in the case of larvae of *S. ransomi*. A culture containing infective larvae was placed in the Baermann apparatus. About 18 hours later, a few cubic centimeters of liquid was withdrawn from the rubber tube of the apparatus into a shallow dish. Inactive, apparently dead, larvae were present in the fluid. After a few minutes' exposure of the liquid to air, a number of larvae exhibited feeble movements. Air was bubbled through the liquid for a few moments, and immediately a large percentage of the larvae began to move about actively. Apparently the initial inactivity was due to the exclusion of the oxygen of the air from the larvae by the column of water in the Baermann apparatus, which was about 15 inches deep.

MIGRATION

The lid was removed from a Petri dish which contained a rich culture of larvae and was replaced by a clean lid not in contact with the culture. Within 1 hour the new lid was found to harbor so many larvae that they were readily visible to the naked eye. It was noted on repeated occasions that larvae migrated from within culture dishes which provided optimum favorable conditions to the exposed inside surface of the sides or edges of Petri-dish covers, where they gathered in macroscopically visible clusters and where they soon perished as a result of desiccation. Infective larvae of *Strongyloides ransomi* were, therefore, energetic migrants under the conditions described.

CLINICAL SYMPTOMS OF PATHOGENICITY

A special study to determine whether intestinal or pulmonary lesions result in pigs from infection with *Strongyloides ransomi* was not within the scope of the present investigation. However, certain observations, incidental to the experiments performed, were made in connection with the clinical symptomatology produced in the host by this parasite.

It was noted in the case of pigs 3, 4, and 5 that when larvae were placed on the skin there was almost immediate reddening of the skin in the areas where larvae were applied. This reaction took the form of pimplelike eruptions and often involved the hair follicles. After several hours, the eruptions usually became pale and vesicular. In two other pigs no reaction of the skin occurred following the application of larvae to it. In the case of a rabbit, when larvae were applied to the skin, there was no pronounced immediate reaction, but after several hours reddened, scaly, indurated elevations appeared. In guinea pigs, immediate congestion of the skin in areas where larvae were placed was noted. Later indurated elevations appeared.

A few days after the oral administration of larvae to pigs 1–A, 2–B, and 3–B, diarrhea occurred. In pig 3–B, the anus became prolapsed about 10 days after the larvae were given and following a period of rather severe diarrhea. In rabbit 1, to which larvae were administered orally, marked diarrhea, lassitude, and emaciation occurred. Rabbit 2, infected percutaneously, was similarly affected. Pig 5 was given a large oral dose of larvae, and it was noted that, beginning about 16 days later, the fecal pellets were often heavily coated with a foul-smelling mucus, suggesting a catarrhal condition of the intestine. About 1 week later the animal died, and postmortem examination revealed the presence in the small intestine of large numbers of *S. ransomi*. The necropsy showed the animal to be suffering from a pronounced anemia.

It is believed that infection and parasitism with *S. ransomi* were responsible for the conditions which have been described, but other causes cannot be excluded with absolute certainty. There were, however, no changes in diet or other factors, and it is difficult to explain the injury observed as due to anything other than parasitism with *S. ransomi*. The symptoms observed are similar to those noted by European investigators in pigs infested with *Strongyloides*.

SUGGESTIONS FOR THE CONTROL OF THE PARASITE

The following suggestions for the control of *Strongyloides ransomi* are based on experimental data presented in this bulletin.

The McLean swine sanitation system developed by workers in the Bureau of Animal Industry and described by Raffensperger and Connelly (*20*) should be used. Owing to the rapidity with which infective larvae develop directly from the eggs of *S. ransomi* outside the host under favorable conditions, it is essential to keep the farrowing house dry and to remove all litter daily.

In order to take advantage of the destructive effects of drying and sunlight upon the infective larvae, farrowing houses, feeding pens, and watering troughs should be located on well-drained, unshaded areas of soil free from vegetation or debris which might

provide shade or moisture. The lot selected should not have been occupied by swine during the previous 3 months. In addition, to prevent infection with other parasites the lot selected should have had a crop grown on it since its previous occupation by swine, as recommended by Ransom (*21*) under the swine sanitation system for the control of ascarids, and by Spindler (*26*) for the control of kidney worms.

Since the life span of infective *S. ransomi* larvae under laboratory conditions is brief, and presumably would be brief even under favorable natural conditions, and since the larvae are readily killed by subfreezing temperatures, the precautions necessary in the selection of a clean pasture to which pigs may be hauled should be governed in part by these considerations.

SUMMARY AND CONCLUSIONS

Two molts take place in the course of the development from the eggs of the parasitic or free-living females to the infective strongyliform larvae of *Strongyloides ransomi*.

Four molts apparently occur in the course of the development from the egg of the parasitic female to the free-living adult stage.

The infective larvae can infect percutaneously and by the oral route; in the former case the larvae go through the lungs. The larvae can penetrate the skin of rabbits and guinea pigs as well as that of swine. In rabbits the worms have been reared to fertile maturity.

Only one molt has been observed to occur in the course of the development of *S. ransomi* in its normal host and in an experimental host. This molt takes place in the intestine about 6 days after infection.

The development of infective larvae was prevented by exposure of cultures for 46 hours to temperatures from −4° to −8° C.; virtually all infective larvae were killed by an exposure of 40 hours at these temperatures.

Infective larvae are attracted to a source of moderate heat, they avoid strong artificial light, and they are attracted by diffuse daylight. Direct sunlight killed the larvae at nonlethal temperatures in from 45 to 60 minutes.

Air drying for from 3 to 5 minutes at room temperature is fatal to many larvae; an exposure of from 9 to 10 minutes destroys all larvae.

A 1-percent solution of copper sulphate or phenol and an ordinary dilute cresol disinfectant were each destructive to the larvae. Of these, cresol was the most potent and destructive.

Lack of air renders the larvae inactive; activity is renewed by some larvae upon readmission of air within a suitable time.

The larvae migrate actively and may reach situations where, owing to lack of moisture, they perish.

Skin lesions have been observed following the penetration of the larvae into experimental hosts; diarrhea, anemia, and general lassitude are symptoms which may accompany intestinal infestation with *S. ransomi*.

Measures suggested for the control of *S. ransomi* infestation in pigs include thorough sanitation and the selection of a dry, unshaded area as a permanent lot.

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