1937

Translation of Nakamura, Yoshitaka. 1937. Development of *Trichostrongylus instabilis*, section II: The larva. *Keio Igaku*, Tokyo 17(4): 797-814

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TRANSLATION NO. 11

Translated from Japanese by Guang-Tsan Wang

Nakamura, Yoshitaka
1937. Development of Trichostrongylus instabilis*. Section II. The larva. Keio Igaku, Tokyo. 17(4):797-814.

Introduction:

This is the continuation of Section I, "Development within the Egg Shell and Hatching." It contains a detailed description of the development and the structure from the first stage to the infective stage of the larvae.

Chapter I MATERIALS AND METHODS

Feces from a goat with a pure infection of T. instabilis were used in this experiment, just as had been done in the previous experiment. Fresh fecal pellets, collected immediately after defecation, were diluted ten times with tap water and sifted through a wire mesh covered by a layer of gauze. The filtrate was allowed to settle in a sediment cup for several hours, and then the clear supernatant fluid was discarded. The sediment was pipetted onto a culture tile which was placed in a Petri dish containing tap water, and was then incubated at 28°C.

Applying the principle that only the infective larvae can move into the culture water, whereas the first and the second stages cannot, I selected a few samples of the fecal culture and diluted them with water to look for the first, second and early third stage larvae, and I examined the culture water for infective larvae. After the diluted samples were passed over a flame three or four times, the detailed structures of the partly-dead larvae were studied under a microscope. A longer time over the flame was needed to straighten out the larval bodies for measurement. The amount of heat needed to render the larvae inactive neither changed nor injured any of the structures. Moreover, the structures remained well-defined for quite a long time.

Looss divided the free living stages of hookworms into first, second and third stages. Matsuzaki designated the period from the hatching of the egg to first ecdysis as post-hatched, granule-deposited, and ensheathed stages; the period from the second stage to second ecdysis as rapid-growing, stoma-partially-sealed, and stoma-completely-sealed stages; and the period after the second ecdysis as the third, or infective, stage. His seven-stage method of division was followed in the present study to describe the characteristics of development in each stage of T. instabilis larvae as follows:

*Note: The presently accepted name for this species is Trichostrongylus colubriformis. Translator.

Post-hatched stage: the body was transparent, thick, short, and moved inactively. (Fig. A).

Granule-deposited stage: food granules were stored in the intestinal cells, especially in the anterior parts. The larvae were light greenish-yellow in color; they were growing longer and moving actively. The Y-shaped valve of the esophagus moved frequently. (Fig. B).

Ensheathed stage: the larvae were growing bigger and longer. The sheath had inner and outer layers, and its anterior end resembled a cap. Transverse and lateral striations could be seen clearly. (Fig. C).

Rapid-growing stage: the intestinal cells overflowed with food granules. The larvae were greenish-yellow in color; they moved actively and reached their maximum size. (Fig. D).

Stoma-sealed stage: the anterior end of the buccal cavity was closed, while the anterior part of the posterior buccal cavity became narrow. The esophagus began to shrink. (Fig. E).

Exsheathed stage: the posterior part of the buccal cavity became inconspicuous. The inner and outer layers of the sheath had separated. Sixteen typical, well-defined intestinal cells could be seen. The tip of the larval tail was separated from that of the sheath.

Infective stage*: the head was covered with a thin cap. The sheath showed rather refractile folds. The tip of the tail was separated internally even more from the tail of the sheath. The esophagus involuted so that the crop, the bulb and the Y-shaped valve simultaneously became inconspicuous, whereas the excretory duct and sac became conspicuous. (Fig. G).

Chapter II DEVELOPMENT AND MORPHOLOGY OF THE FIRST STAGE LARVA

The eggs in fresh feces, cultivated as in the previous method, began to hatch as early as 17 hours after inoculation, but the majority took 20 to 24 hours. The larvae of the post-hatched stage moved sluggishly and were almost transparent in appearance. Food granules were few and limited to the anterior half of the intestine. As soon as the larvae began to feed, the intestinal cells accumulated more food granules. The size of the larvae increased, and subsequently the larvae developed into the granule-deposited stage.

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*This stage is now known as the third stage, infective ensheathed larva. Translator.

I. Post-hatched Stage Larva

(See: Figs. A, H, O, 10, 11; Figs. 1, 12, 19, 20, 21, 22, 23, 24, 27, 29)

The width of the body increased slowly from the head, reaching its maximum at the esophageal bulb, decreasing gradually to the anus, and then tapering very rapidly to a short, sharp point. Although the head end appeared round immediately after hatching, in most cases it gradually became angular. The body was generally transparent because only a few granules were scattered in the intestinal cells. The esophagus was clearly separated into three parts. In the center of the esophageal bulb lay a Y-shaped valve. The larvae slowly coiled and uncoiled. This kind of movement was repeated, but the larvae did not change location at all.

Immediately after hatching, the larvae were short (maximum 0.426 mm., minimum 0.298 mm., average 0.386 mm.) but gradually became longer. Their width at the esophageal bulb was 0.0208 mm. maximum, 0.0114 mm. minimum, 0.0186 mm. average; at the genital primordium, maximum 0.0198 mm., minimum 0.0128 mm., average 0.0177 mm.; and at the anus, maximum 0.0134 mm., minimum 0.009 mm., average 0.0119 mm.

The cuticle of the body was very thin and faintly revealed light black circular striations under which lay a greyish-white subcuticular layer. Usually, these two layers adhered closely together. Regularly and successively the cuticle presented numerous, fine, transverse lines, which although they could be clearly seen immediately after hatching, usually became inconspicuous when the larva approached the granule-deposited stage. The two longitudinal, lateral lines were still hard to recognize. Beneath the subcuticular layer was a loose, greyish-white muscular layer. The ellipsoidal muscle nuclei were lined up along the intestine, from the posterior end of the esophageal bulb to the anus. Although the number of nuclei in each line varied from ten to sixteen, there were usually twelve.

During the vermiform stage in the egg shell, the stoma was triangular in en face view, and was surrounded by three distinct, round, thick, soft, milk-white lips. Immediately after the hatching, the lips of the head shortened and turned outwards, resembling a rough-edged circle.

The anterior part of the buccal cavity was rather perpendicular to the extremely short end of the head. The face of the head, originating from the stoma, ran a short course vertical to the body axis, then bent outwards and backwards forming an obtuse angle which bent back further. After following a short course parallel to the body axis, it curved outwards and backwards to the swollen side of the body. The lips simultaneously kept growing longer and more prominent until the time immediately preceding the granule-deposited stage. At the same time, the anterior part of the buccal cavity increased its depth. On each side of the head was a small papilla which visibly lengthened or shortened depending on the extent it projected out of the cuticle. Because of the feeding process, the larval lips protruded and moved in a general left-right direction.

The funnel-shaped buccal cavity consisted of an anterior part which led to the outside from the stoma, and a cylindrical posterior part. Soon after hatching, the anterior part was very shallow and lined with chitin; afterwards it gradually developed and increased in length. During the time immediately before the granule-deposited stage, the stoma became a little narrower and the lips thickened. Further inside from the stoma, the cavity enlarged, narrowed immediately, enlarged again, and then suddenly constricted at the posterior part of the buccal cavity. The anterior part was 0.0016 mm. long, and the posterior part, which appeared as two parallel lines under microscope, was 0.0112 to 0.0125 mm. Immediately after hatching these lines were inconspicuous, but they gradually increased in size and light refractibility until they became distinct black lines in contrast to the light colored anterior part of the buccal cavity. If carefully examined under the microscope, the lines showed a few pairs of dark black spots symmetrically arranged on both sides. One pair of spots, on the junction between the anterior part and the posterior part of the buccal cavity, was called first points. Another pair, on the anterior third portion of the posterior part of the buccal cavity, was called second points. The last two and the clearest pairs, located at the posterior end of the buccal cavity, were called third, and fourth points. There did not appear to be any connection between the third and the fourth points; however, a thin membrane between them could be detected upon close observation. The first points indicated where the anterior and posterior parts of the buccal cavity joined, the second points where the esophageal wall and the buccal cavity were attached, the third points where the buccal cavity terminated, and the fourth points where the esophageal lumen began. The opening of the esophageal gland was embedded between the third and the fourth points, which gradually became hazy during the second ecdysis. Between the second and the third points, about two-thirds of the posterior part of the buccal cavity was encased by a thin tendonoid membrane which resembled the esophageal ligament in the adult worm. The second points showed where the membrane attached at its anterior extremity, while the third points did the same at the posterior end.

In considering the length of the posterior part of the buccal cavity, Kitamura and Noya claimed that in Trichostrongylus orientalis this part could not extend. However, Matsuzaki noted that in hookworms it changed in accordance with development, being slightly extended during the time of growth but contracted and inconspicuous once the ensheathed stage had been reached. He pointed out that in the second stage it became thicker and longer, but in the stoma-sealed stage an interesting change occurred which lasted until the third stage. The author observed that in Trichostrongylus instabilis it thickened slightly and lasted into the second stage during larval development.

The esophagus measured about one quarter of the body length and appeared greyish-white in color. The anterior end was a thin membrane which attached to the second points of the posterior part of the buccal cavity. The posterior part with a range of 0.067 mm. to 0.0992 mm. in length connected with the esophageal pylorus and was distinctly separated into three portions--corpus, isthmus, and bulb. The corpus, about two-thirds or less of the esophagus, measured 0.0368 to 0.0624 mm. in length, and was spindle-shaped.

The isthmus measured 0.0162 to 0.0202 mm. in length and 0.058 mm. in width. The bulb, which contained a Y-shaped valve resembling an anchor, measured 0.014 to 0.0166 mm. in length and 0.0112 to 0.0128 mm. in width, and was leek-shaped. At the anterior end of the esophagus, which connected with the posterior part of the buccal cavity, were three muscle fibers surrounding the anterior end of the corpus and forming the narrow part of the funnel-shaped esophageal canal. According to Matsuzaki, in hookworm larvae, the Y-shaped valve which was surrounded by the esophageal pylorus, served as a tool for smashing food granules, while the bulb corresponded to the stomach. Even though the morphology of the Y-shaped valve had been studied by Leichtenstern, Looss, Stekhoven and Noya, the real structure has not yet been well defined. As to its function, no definite description has been accepted. Some claimed that it was a chewing organ; others, however, thought that it served as a sucking organ to prevent the ingested food from flowing back. By closely observing the larvae of each free-living stage, the Y-shaped valve was seen to be composed of three complicated valves, each of which was formed of four chitinous valvettes with the function of each valvette illustrated.

According to my own observation, each of these valves originated from the internal wall of the esophageal canal and developed remarkably by forming a thick muscular hump coming to a tip. These three valves joined together and moved up and down, opening and closing by means of the contraction of the bulbous muscles, to chew the ingested food and prevent it from flowing back. In other words, the place that resembled the base of an anchor was the location of the margin of the well-developed muscles, while the direction of the stem of the anchor was the place where the two lateral wall joined. [The description here is confused; one cannot be certain what view the author has taken.] In regard to the middle of the corpus, the author took special note of the strongest, refractive light portion which might have controlled the action of the Y-shaped valve, while the muscles in this portion controlled the movement of the esophageal canal. In all of the free-living stages, the bulb was always slightly to one side of the dorsal position, so that the distance between the ventral body wall and the ventral surface of the bulb was 1.5 times more than that between the dorsal body wall and the dorsal surface of the bulb. The excretory sac and the gland were embedded in the former space (Figs. 21, 22, A).

The esophageal pylorus was slightly enlarged and had a greyish-white, structureless appearance. The cylindrical pylorus was 0.006 to 0.008 mm. long and 0.006 to 0.01 mm. wide and consisted of muscular fibers which could contract and extend voluntarily. The anterior end connected with the esophageal bulb, while the posterior end was surrounded by the intestinal cells. A slender duct went through the middle.

The length of the intestine, occupying about a half of the body, increased as the larva grew, the rate of increase being much faster than that of any other organs. In the post-hatched stage it was 0.128 to 0.224 mm. long, with an average of 0.192 mm., and was 0.0128 mm. wide. The intestine was composed of dorsal and ventral rows of spindle-shaped cells. During this stage, the food granules scattered in the intestinal cells were so few that the cells had a relatively transparent appearance. Although the intestinal

cells were not as well-defined as in the third stage larva, sixteen could be counted. These were arranged alternately with 8 dorsal and 8 ventral cells, and the cells in each row appeared to be swallowing those opposite. There was a relatively large nucleus situated in or near the middle of each intestinal cell. According to Looss, hookworms have 16 intestinal cells in the post-hatched stage--sometimes 15 or 17 or even 18 cells. However, T. instabilis always has 16 cells. In profile, the intestinal lumen had a wavy appearance with varying diameter, dilating at the junctions of the cells, especially at the junctions of the esophageal pylorus and of the rectum. It formed a wavy curve on both ends of the intestine, becoming the pylorus-intestinal lumen at one end and the intestine-rectal lumen at the other. In most cases the intestinal lumen was transparent. The rectum was a narrow canal, with a dorsal convex curve leading ventrally to the posterior end and thereby reaching the anus. Sometimes, there was a swollen portion on the rectum. The anus opened ventrally at a point about five-sixths of the distance from the anterior end of the larva. The body suddenly narrowed posteriorly beyond the anus, coming to a sharp point. The invaginated cuticle of the body wall formed the linings of the rectum and extended to the end of the intestino-rectal lumen; it was highly refractile. At the junction of the intestine with the rectum, there were three large peach-shaped or semilunar, nucleated cells. These cells surrounded the posterior end of the intestine, appeared greyish-white, and served as the ligamental cells of the rectum. (Noya noticed that there was a highly refractile, Y-shaped valve forming three "teeth" resembling an anchor around the rectal ligamental cells of Trichostrongylus orientalis. However, the present author did not find anything like a Y-shaped valve.) The cuticle linings gradually disappeared from the granule-deposited stage to the ensheathed stage.

The body width decreased acutely at the anus and was about half of the width at the level of the esophageal bulb. The length from the anus to the tip of the tail was about one-sixth that of the body. In this stage, the body was 0.0576 to 0.0736 mm. long and 0.0093 to 0.014 mm. wide at the level of the anus, whence the body acutely tapered, especially on the ventral side to the tip of the tail.

The esophageal gland opened on the third and fourth points of the posterior buccal cavity, namely, the junction of the posterior buccal cavity with the esophageal lumen. A group of glandular cells, which lay ventral to the esophageal bulb, became completely unidentified in the exsheathed stage.

Excretory duct: In the same stage larva of Trichostrongylus orientalis, Kitamura found light, refractile, oval particles (excretory sac) lying ventral to the beginning of the esophageal bulb, but he failed to locate the cuticular opening of the excretory duct. However, Noya noted that the excretory duct could still not be completely identified in this stage. In hookworm larvae, Matsuzaki observed that in this stage the excretory pore distinctly opened ventrally in the area between the level of the center and the anterior end of the esophageal bulb. In the early post-hatched stage of Trichostrongylus instabilis, this author found it hard to recognize these structures; however, the only structure similar to the visible opening was the slightly prominent cuticle on the ventral surface. In the later period of this stage, although the esophageal duct showed a hazy opening on the ventral wall related to the

anterior end of the esophageal bulb, as it continued inwards, it appeared as a curve just in front of the esophageal bulb. It ran a short course backwards and outwards, and then paralleled the body axis. Finally it became lost to view behind a group of fluorescent green, oval cells which lay ventrally between the posterior end of the esophagus and the anterior end of the esophageal pylorus.

The genital primordium lay ventral to the fifth intestinal cell, about midway along its course, and appeared semi-transparent and greyish-white. It was 0.005 to 0.0065 mm. long and 0.004 to 0.005 mm. wide. Kitamura and Noya found two cells in Trichostrongylus orientalis, whereas Matsuzaki observed three in the hookworm. In Trichostrongylus instabilis, this author found the largest oval cell on the left side of the ventral part; there were also two others of different sizes, the larger one anterior to and the smaller one posterior to the largest cell on their left. In left profile, they looked like a big "lens" with two overlapped heads in front and a semi-transparent body. The location of the genital primordium in relation to the middle of the intestine changed with the development of the larva; in the post-hatched stage it was slightly anterior to, in the ensheathed stage it was usually at, in the rapid-growing stage and the stoma-sealed stages it was slightly posterior to, and in the infective stage (in most cases) it again moved anterior to the middle of the intestine.

The fibrous nerve ring encircled the anterior end of the esophageal isthmus. In profile, the dorsal part of the ring, located more anteriorly, was wider than the ventral part, which gradually narrowed as it extended inwards and backwards. From the dorsal surface, the curve faced anteriorly and was crescent shaped. The ring belt, 0.0045 to 0.006 mm. wide, was located 0.055 to 0.072 mm. from the anterior end. The nerve cells were scattered almost throughout the body, from the superficial nerve cells beneath the cuticle to the deeper groups in the vicinity of the circumesophageal nerve ring, the esophageal bulb, and the area from the rectal ligament to the region around the rectum. The tip of the tail contained scattered small nerve cells.

II. Granule-Deposited Stage Larva

(See: Figs. 2, 13, B, I, O; Figs. 19, 20, 21, 22, 23, 24, 27, 29)

The larvae sometimes reached the granule-deposited stage as early as 20 hours, but usually 21 to 24 hours after cultivation of the fresh eggs on the culture tile. Although the duration of the post-hatched stage was relatively short, the granule-deposited stage lasted about 13 to 14 hours, the longest period in the first stage. Because of the feeding process, the intestinal cells were filled full with food granules, and these cells darkened in color until the whole intestine had a yellowish-green appearance. During this stage, the larvae grew rapidly, gradually completing their body shape, and increased in length to two-thirds or more of the length of the infective larvae in the late period of this stage. Although the body systems did not change very much from those in the post-hatched stage, the outlines of every organ became conspicuous; the mouth and head were especially well developed.

The larvae, which had taken a considerable amount of food in the previous stage, continued to feed actively, to store more food granules, and to develop rapidly. Only ten or more hours elapsed between the early and the late period of this stage, during which time the body length and width changed greatly. In the late stage, the maximum body length was 0.579 mm., and the maximum width was 0.0224 mm. at the esophageal bulb, 0.0218 mm. at the genital primordium, and 0.0135 mm. at the anus. The previous stage larvae, which swelled out at the esophageal part and rapidly tapered from the middle of the body, increased the width of the posterior half of the body as growth proceeded along the body. The width at the esophageal bulb was not much different from that at the genital primordium. The anal portion also widened. The final appearance of the whole body was slender.

The body wall increased in thickness and appeared as a thick, dark line under rather high magnification. The subcuticular layer developed later. In the late stage, the transverse lines could be clearly seen between the two layers, and the lateral lines also increased in visibility. By about the ensheathed stage, a space gradually appeared between the tip of the tail and the tip of the sheath (Fig. 2).

During this stage, the larval lips protruded. The anterior part of the buccal cavity increased in length and appeared greyish-white under the microscope. Behind the anterior end of the buccal cavity, a second-stage larval head slowly appeared. The stoma was thicker than that in the previous stage. Behind the stoma, the anterior part of the buccal cavity promptly dilated, and then narrowed at the bottom before connecting with the posterior part of the buccal cavity. The stoma and the anterior part of the buccal cavity, which looked like an ampulla, were lined with cuticle which ended between the first and second points of the posterior part of the buccal cavity. This portion was rather thick and markedly refractile, and corresponded to the second points of the hookworm larva described by Matsuzaki. In the middle, left, right, and posterio-lateral directions of the anterior part of the buccal cavity were curved, yellowish-green, highly refractile outlines. These curves, which gradually became distinct, later formed the anterior end of the second stage larva. In the late stage, the stoma became smaller, while the chitin which lined the buccal cavity became thicker. The anterior dilated part of the buccal cavity decreased in width, and the place where it connected with the posterior part of the buccal cavity gradually narrowed and closed in the next stage. The posterior part of the buccal cavity thickened as it developed. Under the microscope, the black lines became larger and, altho they reached a maximum length of 0.0134 mm., they shrank slightly and appeared as two slenderer lines in the late stage.

The esophagus increased in length and width, attaining a maximum length of 0.144 mm. The esophageal bulb was 0.0176 mm. long and 0.0128 mm. wide, and its Y-shaped valve moved actively. The bulb lay a little bit dorsally, and the position of the Y-shaped valve, located anteriorly in it, was the same as in the previous stage. Owing to its rapid development, the intestine, which had averaged 0.192 mm. in length in the previous stage, reached an average of 0.275 mm. in this stage, with a maximum of 0.358 mm. Because the intestinal cells were filled with numerous food granules, the intestine became wider and its yellowish-green tinge darkened. Since most of the food granules

had been deposited in the anterior intestinal cells (located in front of the genital primordium), the intestine was darker anteriorly and lighter posteriorly. The intestinal cells increased in length, but they had not yet reached their typical spindle shape. The pyloro-intestinal lumen was narrower than in the previous stage, sometimes having a peculiar pot shape or being linear. The intestino-rectal lumen, wider in some parts and narrower in others, appeared wavy in profile and as a straight line in dorso-ventral view.

The rectum and anus were not much different from those in the previous stage, except that the cuticle lining of the anus was becoming thicker and that in the terminal rectum was becoming distinct and Y-shaped. Describing Trichostrongylus orientalis, Noya observed that, although we might say that the Y-shaped cuticle in the rectum of this stage looked like the Y-shaped valve in the esophageal bulb, we still did not know whether it could move.

The tail increased in length and width, but did not seem much different from that of the previous stage, except that the space between the cuticle and the subcuticular layer was particularly distinct in this stage.

The excretory organs were slightly clearer than those of the previous stage. Although the opening of the excretory duct moved anteriorly as time passed, it still opened on the ventral wall between the anterior and the middle part of the esophageal bulb, and did not seem very different from that in the previous stage.

The genital primordium, in addition to being slightly larger, had a conspicuous outline. It was located a little more posteriorly than that in the previous stage; however, in most of the larvae, it still lay on the anterior half, and in an extremely few cases on the posterior half, of the intestine.

III. Ensheathed Stage Larva

(See: Figs. C, J, O, 3, 14; Figs. 19, 20, 21, 22, 23, 24, 27, 29)

The larvae reached this stage 32 to 40 hours, in most cases 35 to 36 hours, after cultivation of the eggs. This stage lasted 4 to 5 hours, and then advanced into the second stage. In the late granule-deposited stage, the stoma narrowed, and anterior part of the buccal cavity became thinner, the lips were longer, and the head of the second stage larva appeared as a distinct, brimless cap. The space between the internal and external layers of cuticle extended, especially on the tail, the tip of which could be clearly differentiated from the internal side of the cuticle. The cuticle lining the anus and the buccal cavity began to separate. The larvae moved freely within the old cuticle. The esophagus began to shrink, and the Y-shaped valve stopped its activity.

During the granule-deposited stage, the larvae had ingested much food and had developed rapidly. When the larvae entered this present stage, they increased in width from the well-developed esophagus to the bulky intestinal cells, and thereby acquired a plump appearance. The body width at the esophageal bulb was 0.0198 to 0.0256 mm. with an average of 0.0228 mm.;

at the genital primordium, it was 0.0192 to 0.0256 mm. with an average of 0.0226 mm.; and at the anus, it was 0.0121 to 0.0256 mm. with an average of 0.0161 mm.

The subcuticular layer, which had developed distinctly and had become a new cuticle late in the previous stage, was thickening and appeared much darker than the old cuticle. The space between the two layers widened to as much as 0.0032 mm., and appeared greyish-white under the microscope. Beneath the new cuticle was a greyish-white subcuticular layer. At the esophageal bulb, the width between the two old cuticles was 0.0256 mm. at the maximum, while that between the two new cuticles was only 0.0192 mm. The old cuticle was much thinner than the new one, and was separated from the new larva from the anus, along the internal wall of the rectum, to the recto-intestinal extremity. The old cuticle, which had many wrinkles, was gradually cast off from the anus, and then also from the stoma along the anterior buccal cavity to the internal wall of the posterior buccal cavity. The larvae, and especially their heads, moved actively within the old cuticle. Sooner or later the larvae emerged from the anterior end of the old cuticle and advanced into the second stage. In this stage, the transverse lines were conspicuous and there was no question about the lateral lines. The parallel lateral lines became inconspicuous in the region of the esophageal bulb. The intestine, because of its dark tinge, was unclear; however, the portion reaching the anus was conspicuous and that behind the anus again became inconspicuous. The anterior buccal cavity, the terminal opening of which had been closed in the late granule-deposited stage, gradually became narrow in this stage. The joining points of anterior and posterior buccal cavity consequently separated from the first points in order to cast off the old cuticle which lined the wall of the posterior buccal cavity, and the separated space was connected with fine fibers. The head of the new larva gradually showed up at the junction of the anterior and posterior buccal cavity. The anterior buccal cavity of the new larva was shallow and appeared at the portion between the cast cuticle and the first points of the posterior buccal cavity. At this time, the posterior buccal cavity shrank and also became inconspicuous. The cast-off cuticle, after narrowing gradually, blocking the buccal cavity, casting off from the anterior buccal cavity, connecting the old stoma and the new anterior buccal cavity, closing the old stoma which appeared somewhat protrusive, and separating completely from the new head end, bent at the space between the old and new head ends. Traces of two papillae on both sides of the old cuticle head end could be seen, and the chitin (the bat-shaped old cuticle) which lined the excretory duct, was pulled out but still connected with the lining of the excretory opening. At this time, the posterior buccal cavity became conspicuous again, and sooner or later the larva molted.

The esophagus gradually shrank, the esophageal corpus became rather inconspicuous, and the bulb also shrank somewhat. The Y-shaped valve continued its motion during the time when the stoma of the old cuticle and the new head end was still connected with the chitin substance, but it stopped when they separated. At this stage, the esophageal bulb became inconspicuous and decreased slightly in width. The intestine, which was 0.294 to 0.454 mm. long, contained numerous stored food granules which intensified its yellowish-green tinge.

Although the intestine would consume a few food granules and become more transparent, it was still full of food granules and retained almost its original shape. The intestinal lumen was slightly wider than that of the granule-deposited stage, and appeared in profile as a distinct wavy line between the dorsal and the ventral cells. During this time, the genital primordium was located at the middle of the intestine, and gradually appeared as a distinct, shiny, greyish-white body. The newly formed cuticle, extending from the rectum to the anus, was separated from the old cuticle. The space gradually widened as the old twisted cuticle was cast off from the anus until the Y-shaped cuticle shadow in the terminal intestine became completely indistinct. In the late stage, the new larva moved actively in the sheath, and the old cast-off cuticle of the anus was frequently seen in reverse side.

Although the excretory organ was the same as that in the granule-deposited stage, it opened slightly more anteriorly at the ventral side of the anterior end of the esophageal bulb, and its terminus connected with the opening of the old cuticle.

The esophageal gland was so inconspicuous that it could not be distinguished at all.

IV. First Ecdysis

As the ecdysis occurred, the esophagus again became conspicuous, and the esophageal bulbular muscles and the Y-shaped valve began to move. Looss and Noya observed that molting occurred very rapidly; however, Matsuzaki said that it took considerable time. Wuhehnosatzu observed that in Haemonchus contortus molting required quite a long time. Carefully examining the 38 to 48 hour culture, this author found many pre-exsheathed, few exsheathing, and many post-exsheathed larvae. The larvae, which had finished their first-stage development and had cast off the cuticle of the buccal cavity, the anus, the papillae and the excretory duct, were still enclosed within the old sheath. Moreover, the larvae continued to move quickly or slowly as though they would have suffocated if they had not exsheathed. Many other researchers and I have been vexed at being unable to discern the very moment when the larva was breaking its sheath. The few larvae which I did observe had all exsheathed near the anterior end. The posterior part of the broken sheath slipped from the esophageal or the intestinal portion to the tail, appearing to be attached by a long, white, transparent membrane. The head and the tail moved so as to complete exsheathment quickly. Fragment in anticipation of exsheathment was never once found. Perhaps it was typical of the exsheathment of T. instabilis that the larva widened the opening of the stoma and exsheathed from it. Exsheathment in T. instabilis did not progress very rapidly. Under the microscope, it took a little more than ten minutes for a larva whose sheath had already slipped down to the middle of the body to finish the process. Another larva, which took several times more than ten minutes for exsheathment, began to exsheath gradually after the cover glass was moved back and forth. This phenomenon might be due to the fact that there was only a small amount of debris inside on the smooth surfaces of the glass dish and the cover glass for proper exsheathment. Another ensheathed-stage larva, which was hindered from moving out from under a large piece of debris, came out as a completely-exsheathed

larva after the cover glass was compressed two or three times by the tips of a pair of forceps. The cast-off sheath twisted and appeared transparent white with greyish-white outlines under the microscope.

Chapter III DEVELOPMENT AND MORPHOLOGY OF THE SECOND STAGE LARVA

I. Rapid-growing Stage Larva

(See: Figs. D, K, P, 4, 15; Figs. 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30)

This stage lasted a long time, usually 16 to 17 hours. The larvae reached this stage in 38 to 42 hours (sometimes as early as 34 hours, but in most cases 41 to 42 hours) after cultivation of the eggs. As the larvae exsheathed, they began to move actively and to ingest numerous food granules. As early as 52 hours (in most cases 56 to 57 hours) after cultivation of the eggs, they reached the stoma-sealed stage. The rate of development differed greatly between the early and the late periods of this stage.

In this stage, the length ranged from 0.509 to 0.688 mm. with an average of 0.605 mm. The body width at the esophageal bulb was 0.0192 to 0.024 mm., with an average of 0.0222 mm.; at the genital primordium it was 0.0189 to 0.024 mm. with an average of 0.0222 mm.; at the anus it was 0.0112 to 0.016 mm. with an average of 0.0139 mm. The range between the maximum and the minimum widths was obviously becoming greater. This suggested that development was rapid during this stage. Because of an unusual development of the intestine in this stage, the middle portion of the body increased rapidly until the whole larva had a slender appearance.

The larvae which had cast off their sheaths had a thicker cuticle than the first stage larvae. The subcuticle was distinct and becoming thicker and thicker. The cuticle bore fine distinct, transverse striations at regular intervals, and two parallel lateral lines along the body were particularly distinct in the region between the esophagus and the anus.

In dorsal or ventral view, the head was round. Internally, behind the two papillae which had grown distinct since the post-hatched stage, were a pair of short papillae with wide spherical bases on the dorsal wall parallel to the joining points of the anterior and posterior buccal cavity--namely, the first points of the posterior buccal cavity. The spindle-shaped protuberances were present on both sides behind the papillae. In other words, they lay on the dorsal wall of the head between the second points of the posterior buccal cavity and the anterior half of the third points, and between the lateral wall and the posterior buccal cavity. They appeared lens-shaped, with a long wide base.

The anterior buccal cavity was shallow and the lips were short. In dorsal view, the latter was inconspicuous; however, in lateral view, the dorsal stoma-lip portion was thicker and protruded rather anteriorly, whereas the ventral stoma-lip portion was thinner and located rather posteriorly. Although the stoma-lip portion would extend a little in the late stage, it never became as long as that of the first stage larva. In the early stage, the buccal

cavity, resembling that in the post-hatched stage larva, was composed of an anterior buccal cavity lined with a thicker cuticle than that of the first stage, and a well-developed posterior buccal cavity. The posterior buccal cavity, through the development of the previous three stages, became most distinct in this stage, ranged in length from 0.0128 to 0.0144 mm., and was wider than before. In the early stage, the first and second points on the posterior buccal cavity were very distinct, whereas, the intermediate points to which the lining cuticle was attached could not be identified. Near the stoma-sealed stage, the connection of the anterior buccal cavity with the posterior buccal cavity separated slightly from the first points of the posterior buccal cavity, and gradually narrowed. The cuticle of the anterior end gradually increased in thickness, and two lines of the posterior buccal cavity extended forward from the second to the first points, resembling the pointed metal head of a spear.

Immediately after ecdysis, the esophagus, which had shrunk slightly during the previous stage, began to develop so that the esophageal corpus and bulb became very distinct. The maximum length of the esophagus was 0.153 mm. The maximum width of the well-developed esophageal bulb was 0.0144 mm. As the muscular fibers in the bulb became well-developed, the Y-shaped valve increased in size and moved actively.

In the early period of this stage, because most of the food granules were used up during the ensheathed stage and the first ecdysis, the intestinal cells as well as the pyloro-intestinal lumen and the rectal lumen lightened so that the intestine appeared light yellowish-green. Then, since the larva in this stage fed very actively, the intestinal cells overflowed with food granules and the original yellowish-green layer became dark bluish-green. Each intestinal cell increased its length so that the intestine extended and measured 0.668 mm. at the maximum. Also, the pyloro-intestinal lumen and the rectal lumen usually became narrower. The esophageal pylorus appeared narrow, in contrast with the large, bulging intestinal cells. The recto-ligamental cells which surrounded the rectal lumen were not greatly different from what they had been in the first stage. At the same time that the intestine was becoming well-developed, the anus increased in length to 0.0162 mm.

The tail, which was not greatly different from that in the first stage, ranged in length from 0.079 to 0.089 mm. In the late stage, a greyish-white space between the cuticle and the subcuticle separated the two layers, but these layers still remained slightly in contact with each other. The site where the knob of the tail proper would appear (namely, at a part of two-thirds of the length of the tail) became slightly distinct. From this site downwards, the tail suddenly tapered in appearance. Owing to the excellent development of the tail, the knob which had been located at the middle of the tail in the first stage moved down to its final site about two-thirds of the distance between the anus and the posterior end.

The esophageal glands, which had been inconspicuous in the ensheathed stage, appeared again in this stage. The excretory organs were much clearer than they had been in the first stage. The excretory duct extruding from the anterior end of the two excretory cells, which were always located behind the esophageal bulb and beneath the esophageal pylorus, passed anteriorly inwards along the ventral side of the esophageal bulb, curved ventrally at the site

immediately in front of the bulb, then divided into two terminal branches, and finally opened on the ventral wall at the site about 0.0103 to 0.00928 mm. from the head end, right under the nerve ring. The space between the ventral wall and the two branches looked like a delta. The genital primordium was also well-developed. In profile, it appeared like two pieces of lens located in the concavity of the intestine and was 0.009 to 0.013 mm. long.

II. Stoma-Sealed Stage Larva

(See: Figs. E, L, P, 5, 6; Figs. 19, 20, 21, 22, 23, 24, 27, 29)

In the late previous stage, the connection of the anterior buccal cavity to the posterior buccal cavity was narrowed. As the cuticle of the stoma portion gradually increased in thickness and the anterior part of the posterior buccal cavity became narrowed, the larvae reached this stage as early as 52 to 53 hours, but usually 58 hours after cultivation of the eggs on the culture tile. After this stage had lasted 10 to 13 hours, the larvae advanced into the exsheathed stage. In this stage, the larvae still moved actively, but the Y-shaped valve had completely stopped moving.

The larvae of both this stage and the next ensheathed stage which had already passed seven stages were larger than the others, ranging from 0.5614 to 0.7008 mm., with an average of 0.6464 mm. The body width at the esophageal bulb was 0.02048 to 0.02272 mm. with an average of 0.02221 mm.; at the genital primordium, it was 0.0192 to 0.02272 mm. with an average of 0.02195 mm.; at the anus it was 0.0128 to 0.01632 mm. with an average of 0.01552 mm. Matsuzaki in his description of hookworm larvae pointed out that in this stage the width at the genital primordium was usually greater than that at the esophageal bulb. The larvae of T. instabilis, which were not as bulgy as hookworm larvae, were slender in appearance.

The subcuticular layer, which had been well-developed in the late period of the rapid-growing stage, gradually increased in thickness; the greyish-white thin space between the cuticle and the subcuticle, which finally became a new cuticle, could be clearly seen. The transverse striations and the lateral lines gradually became conspicuous as the latter extended slightly forward to the anterior part of the esophageal isthmus and backward to a site 0.022 mm. behind the anus.

The buccal cavity changed greatly and the head end slightly. The head end was longer than that of the previous stage. The cuticle on the stoma portion gradually thickened and took on a yellow tinge. The anterior buccal cavity became wine-glass shaped. The junction points of the anterior and posterior buccal cavities gradually approached each other to form a point, and the buccal cavity gradually narrowed until the new, round head end on the anterior border of the posterior buccal cavity could be clearly recognized. The two lines extending anteriorly from the posterior buccal cavity moved closer together and joined at the site of the first points. The joining parts of the anterior and posterior buccal cavities moved slightly forward, then enlarged slightly so that they appeared to be cut from the anterior end of the

posterior buccal cavity, and ultimately enlarged greatly, appearing connected by a thin thread-like membrane. The esophageal ligament which had surrounded the portion between the second and the third points began to shrink. After the above sequence of changes, the larvae advanced into the exsheathed stage.

Because the larvae in this stage no longer ingested nutriment, the esophagus began to involute. As the posterior buccal cavity degenerated, the esophagus became somewhat inconspicuous; the esophageal bulb stopped developing and began to shrink, and the Y-shaped valve also stopped moving and simultaneously decreased in width until it gradually degenerated. The intestinal cells were as full as in the previous stage. The intestinal lumen was wavy from the side, and straight on dorsal view. The yellowish-green intestine generally appeared shiny dark brown. The rectum was not greatly different from that of the previous stage. The subcuticle layer gradually developed and separated from the cuticle. The cuticle which lined the rectal lumen gradually appeared so distinct that it could be clearly seen as a Y-shape. The anus was not greatly different from that of the previous stage.

On the tail, the subcuticle developed and the greyish-white space between the new and the old cuticles increased so that a new rather inconspicuous tail appeared. In the late period of this stage, as the posterior buccal cavity involuted, the esophageal lines also became inconspicuous; however, the shrunken excretory organs gradually became conspicuous. The other parts were not greatly different from those in the previous stage. Furthermore, the excretory cells came to be located beneath the ventral side of the esophageal bulb. The genital primordium ultimately became located more posteriorly.

III. Exsheathed Stage Larva

(See: Figs. F, M, P, 6, 17; Figs. 19, 20, 21, 22, 23, 24, 28, 30)

The larvae reached this stage as early as 56 to 60 hours--but in most cases, 68 to 70 hours--after cultivation of the eggs on the culture tile. In comparison with hookworm larvae, most of which reached this stage in 60 hours, the development of T. instabilis larvae was quite slow, although it was almost the same as that of T. orientalis larva. The terminal end of the anterior buccal cavity had already closed in the previous stage, and the slightly-separated posterior buccal cavity gradually became inconspicuous in this stage. After the inner and the outer membranes had completely separated, the larvae advanced into the third stage.

The morphology, the body length, and the width were not greatly different from those of the previous stage. The maximum length was 0.7008 mm., the minimum 0.557 mm., and the average 0.6531 mm. The width at the esophageal bulb was 0.0208 to 0.024 mm. with an average of 0.02227 mm.; at the genital primordium it was 0.0192 to 0.024 mm. with an average of 0.02204 mm.; at the anus it was 0.01376 to 0.01664 mm. with an average of 0.01587 mm. The width at the esophageal bulb was the greatest in all stages, and the width at the genital primordium was usually the same as or narrower than that at the esophageal bulb.

The new cuticle gradually increased in thickness and visibility. In the early stage, the body wall from the posterior half of the esophagus backwards appeared as two distinct layers, and old cuticle formed wrinkles on the flexed side. The distance between the transverse striations became very short, and the lateral lines became very distinct between 0.016 mm. from the anterior end and 0.026 mm. from the tip of the tail.

The buccal cavity and the esophagus changed markedly. The cuticle on the head end increased in thickness, especially in the middle portion of the anterior buccal cavity, and appeared shiny yellow or dark black. The stoma became quite small. The new round head end appeared at the site between the anterior and the posterior buccal cavities, and could be seen clearly beneath the old cuticle. Ever since the late stoma-sealed stage, the outlines of the posterior buccal cavity had been so indistinct that only two lines could be seen. The portion anterior to the second points was relatively distinct and appeared pyramid-shaped. The posterior end which connected with the esophagus was inconspicuous, and the third points nearly disappeared. The esophagus began to shrink slowly and its outlines were inconspicuous; but the widths of the corpus, isthmus, and bulb were not greatly different from those in the previous stage. The Y-shaped valve was so inconspicuous that it could be seen only under the oil immersion lens. The cuticle surrounding the stoma and buccal cavity gradually thickened, thereby decreasing the size of the latter. The outlines of the new anterior end gradually appeared. The posterior buccal cavity nearly disappeared behind the second points, and that in front of the second points appeared as a short dorso-lateral line. The connection of the anterior end of the esophagus with the posterior buccal cavity had a hazy outline. The esophageal ligament which surrounded the second and the third points nearly disappeared. In contrast, as the larva developed further, the old cuticle which enclosed the stoma became thin and looked firm. The new anterior end gradually became conspicuous, broad and round, and gradually curved on both sides. Greyish-white lips also appeared. The space between the anterior buccal cavity of the old cuticle and that of the new anterior end was connected by the old cuticle. Each part of the esophagus narrowed. Its width varied little between the corpus and the isthmus, and it varied even less between the isthmus and the bulb. The intestinal cells, filled completely with food granules, were not greatly different from those of the previous stage. The fat granules in the space between the intestine and the muscular layer gradually decreased so that the outlines of the intestinal cells became distinct.

The space between the new and the old cuticles of the tail gradually widened. In the late stage, the new tail gradually appeared, and the tip of the new tail finally shrank back to a position about two-thirds of the length of the old tail from the posterior end and was located at a point about 0.03 mm. anterior to the tip of the old cuticle tail. After that, the old cuticle which surrounded the surface of the rectum and the anus separated from the new cuticle until the two membranes could be clearly seen, it was shed from the anus in the form of a rod, and the larva moved into the third stage. The excretory pore, which had been located on the ventral wall immediately in front of the esophageal bulb, moved anteriorly to a point about two-thirds from the anterior end of the esophagus (namely, a point behind the nerve ring about

0.1 mm. from the head end). The terminal end of the excretory duct bifurcated near the opening of the cuticle, and the space between the two branches gradually widened and ultimately formed a delta as in the previous stage.

In this stage, the genital primordium was located at the middle of the intestine. In the late stage, the largest cell sloped inward and lay at an angle to the body axis (Figs. 28, 30).

The nerve ring moved slightly backwards. As the esophageal isthmus shrank and decreased in width, the nerve ring decreased slightly in diameter and increased in visibility.

Chapter IV MORPHOLOGY AND DEVELOPMENT OF THIRD STAGE LARVA

I. Infective Stage Larva

(See: Figs. G, N, Q, R, 7, 8, 9, 18, 19, 20, 21, 22, 23, 24)

The third stage larvae, which had shed the stringy, old cuticle from the anus and had separated from the anterior head portion of the old cuticle in which they had moved freely, moved quite actively and then became infective larvae. The larvae reached this stage as early as 65 hours--but, in most cases, about 80 hours--after cultivation of the eggs.

The body length ranged from 0.5824 to 0.72 mm. with an average of 0.6576 mm. The width at the esophageal bulb was 0.0217 to 0.024 mm. with an average of 0.0227 mm.; at the thick base of the genital primordium it was 0.0198 to 0.024 mm. with an average of 0.02234 mm.; at the anus it was 0.0153 to 0.0176 mm. with an average of 0.01645 mm. The body appeared slender.

Beneath the cuticle were a thin subcuticle and a muscular layer. Being similar in color, they were hard to distinguish from each other. The transverse striations on the body surface were thin and rather indistinct. The lateral lines gradually became distinct from a point about 0.035 mm. from the head end and disappeared at a point 0.02 mm. anterior to the tip of the tail or 0.06 mm. behind the anus. Attached to the sheath behind its anterior end was the old cuticle shed from the anterior buccal cavity, and at the anus was the long, double-contoured, stringy, old cuticle shed from the rectum. In addition, the extremely thin, stringy old cuticle shed from the two papillae was also attached to the sheath at the head end. The stringy cuticle shed from the excretory duct could also become attached to the sheath at the location of the excretory pore.

The distance between the round anterior end and the anterior head end of the old cuticle was extremely short, measuring 0.003 mm. in most cases. It was even shorter in the ensheathed stage. Internally, behind the slightly protrusive, left and right papillae which were located near the end of the stoma-lip portion, appeared two papillae which had larger bases than the former ones had had. Furthermore, just as in the previous stage, there was also a spindle-shaped swelling which lay parallel to the body axis on the left and the right dorsal walls of the head at the level of the anterior half of the posterior buccal cavity.

The slight concavity at the center of the anterior end was the stoma. The anterior buccal cavity was narrow, about 0.0025 mm. to 0.0027 mm. long. The posterior buccal cavity was 0.01 to 0.0128 mm. long, and its lines gradually became inconspicuous. The first points joined together, whereas the second points remained at their original location, forming an acute angle at the anterior end of the posterior buccal cavity. Although the two lines became inconspicuous, the dorsal one between the first and the second points was comparatively distinct and dark. Both of these two lines shrank and became inconspicuous from the third points backwards.

The beginning of the esophageal lumen became inconspicuous; and the esophagus changed very distinctly, becoming narrower and inconspicuous. Because the corpus became slender, its border connecting the isthmus became inconspicuous. The portions anterior to the esophageal bulb looked like a slender column, and the bulb, also slender, resembled a small leek. The wide portion was about 0.009 mm. in diameter. As the sheath formed, the Y-shaped valve disappeared so that only a trace could be seen.

The nerve ring was a little narrower and clearer than it had been in the late period of the previous stage. Although the nerve cells located on the esophageal portion were not different from what they had been in the previous stage, they became rather indistinct. In this stage, many highly refractile glandular granules appeared on the esophagus. The esophageal pylorus shrank. The intestinal cells, which had been rather inconspicuous before the formation of the sheath became distinctly demarcated and arranged themselves in a regular formation. The total of 16 cells formed a dorsal row of eight and a ventral row of eight cells, and the intestinal lumen was zig-zag. The first cell was located in the dorsal row, and the last one in the ventral row. They were filled with food granules and appeared shiny greenish-black. The dorsal intestinal cells of a representative larva from the first to the eighth were 0.032 mm., 0.032 mm., (? mm.; print too poor to read), 0.032 mm., 0.288 [sic] mm., 0.288 [sic] mm., 0.032 mm., and 0.048 mm. long; the ventral cells were 0.0416 mm., 0.0352 mm., 0.032 mm., 0.032 mm., 0.0256 mm. (the genital primordium lay behind this cell), 0.032 mm., 0.048 mm., and 0.0448 mm. long. The cell where the genital primordium was located was the shortest, whereas the last cell of the dorsal row and the last two cells of the ventral row were the longest. Because of the pyloro-intestinal lumen, the first two cells in each row appeared narrower than the others. The intestine was about 0.0128 mm. wide. The three cells surrounding the rectal lumen shrank until they formed an ellipsoid with its long axis slightly parallel to the body axis.

The tail shortened and its tip became pointed, angular, or round. The nerve cells, on the ventral side of the rectum and in the region of the anus, plus the cellular groups on the dorsal parts of the rectum, as well as the neural groups distributed on the ventral side behind the rectum toward the tip of the tail could be seen; however, they were not as distinct as they had been in the second stage. Because the tail tapered rapidly from this part backward, the anus looked somewhat prominent.

The excretory pore formed a delta and opened on the ventral wall about two-thirds of the length from the anterior end of the esophagus. After it extended inward about 0.007 mm., it turned slightly outward to form a curve, and extended posteriorly 0.008 mm. along the lateral side of the esophagus. In the description of hookworm larvae by Matsuzaki, this site was swollen and exerted distending and contracting motions. In the description of the hookworm larvae by Stekhower, this site was the excretory ampulla. However, this site was unclear in the larva of T. instabilis. The two excretory cells which surrounded this site were long, spindle-shaped. The posterior end of the excretory cells extend to the end of the esophageal bulb.

Because the posterior intestinal cells had filled with granules and increased in length, the genital primordium moved forward to the anterior half of the intestine; however, since the esophagus had become longer, it, needless to say, was located in the posterior half of the body. The genital primordium usually lay ventrally at the middle or posterior part of the fifth intestinal cell or between the fifth and the sixth intestinal cells. Among the three cells, the smallest one, which lay posteriorly, became inconspicuous, and the largest one, which was located behind the middle-sized cell, enlarged obliquely to the body axis until it became a large ellipsoidal body. The largest cell, about 0.0128 mm. long, could be distinctly seen. When the largest cell was located parallel to the body axis, its surface was smooth; however, in this stage, numerous, superficially-divided striations appeared on the surface, and as time passed they became more distinct.

II. Migration of the Infective Larva out of the Culture

The infective larvae came to the water of the Petri dish and moved quite actively. The larvae which had just migrated into the water were filled with both food and fat granules; therefore, their bodies, particularly in the intestinal region, were usually dark or yellowish-brown. However, after a few days the larvae became more transparent because of a reduction in number of granules. Noya noted that the larvae of the hookworm and T. orientalis could be separated by means of the different dates when they began to migrate into the water. In T. instabilis, about 90% of the larvae migrated into the water on the 5th day after cultivation. They started to migrate as early as the 4th day, and almost all of them had done so by the 7th day. The larvae of T. instabilis migrated several hours later than the hookworm larvae, which moved into the water on about the 4th day; but they migrated one day earlier than those of T. orientalis.

The infective larvae in the culture water did not exsheath at all for several months. Of the larvae cultivated on January 25, only about half had exsheathed by July 13.

Before laying aside his pen, the author would like to express his acknowledgement to Mr. Kozumi for his revision.

3/23/64sj

MEASUREMENTS OF THE FREE-LIVING LARVAE OF TRICHOSTRONGYLUS INSTABILIS (MM.)

(MEANS IN PARENTHESES)

		FIRST STAGE			SECOND STAGE			THIRD STAGE
		Post-hatched Stage	Granule-Deposited Stage	Ensheathed Stage	Rapid-growing Stage	Stoma-Sealed Stage	Exsheathed Stage	Infective Stage
Body length	Length	0.298-0.426 (0.386)	-0.579		0.509-0.688 (0.605)	0.5664-0.7008 (0.6464)	0.557-0.7008 (0.6531)	0.5824-.072 (0.6576)
	Esophageal Bulb	0.0114-0.0208 (0.0186)	-0.0224	0.0198-0.0256 (0.0228)	0.0192-0.024 (0.0222)	0.02048-0.02272 (0.02221)	0.0208-0.024 (0.02227)	0.0217-0.024 (0.0227)
Body width at	Genital Primordium	0.0128-0.0198 (0.0177)	-0.218	0.0192-0.0256 (0.0226)	0.0189-0.024 (0.0222)	0.0192-0.02272 (0.02195)	0.0192-0.024 (0.02204)	0.0198-0.024 (0.02234)
	Anus	0.009-0.0134 (0.0119)	-0.0135	0.0128-0.256 (0.0161)	0.0112-0.016 (0.0139)	0.0128-0.01632 (0.01552)	0.01376-0.01664 (0.01587)	0.0153-0.0176 (0.01645)
Anterior buccal cavity	Length	0.0016						0.0025-0.0027
Posterior buccal cavity	Length	0.0112-0.0125	-0.0134		0.0128-0.0144			0.01-0.0128
Esophageal corpus	Length	0.0368-0.0624						
	Width							
Esophageal isthmus	Length	0.0162-0.0202	0.144		0.153			
	Width	0.058						
Esophageal bulb	Length	0.014-0.0166	0.0176					
	Width	0.0112-0.0128	0.0128		0.0144			
Esophageal Pylorus	Length	0.006-0.008						
	Width	0.006-0.01						

Table (continued)

Intestine	Length Width	0.128-0.224 (0.192) 0.0128	(0.275)	0.294-0.454				0.0128
Tail	Length				0.079-0.089			
Tip of Tail to Tip of Sheath							0.03	