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CONTRIBUTIONS TO THE LIFE HISTORIES OF *GORDIUS ROBUSTUS* LEIDY AND *PARAGORDIUS VARIUS* (LEIDY)

WITH TWENTY-ONE PLATES

BY

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Contributions from the
Zoological Laboratory of the University of Illinois
under the direction of Henry B. Ward, No. 144

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
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INTRODUCTION

The Gordiacea seem to have escaped the observation of the earlier writers or else to have been mistaken for filariae. Meissner (1856) and Villot (1874) who review the older literature, agree that the first reference to the group was made by Albert the Great. Linnaeus introduced the term Gordius on account of the resemblance of a mass of the worms to the Gordian knot. He included in the genus the three species *G. aquaticus*, *argillaceus* and *medinensis*, representing respectively the present families of Gordiidae, Mermithidae and Filariidae, only the first of which is today retained in the order Gordiacea.

Dujardin was the first to give detailed descriptions of two species, *Gordius aquaticus* and *Gordius tolosanus*, and to point out the difference between Gordius on the one hand and Mermis on the other.

Von Siebold and following him Meissner (1855) placed Gordius and Mermis together to form the order Gordiacea. Meissner's work on the anatomy and physiology of Gordius and Mermis is in many respects an excellent production and it is inconceivable how, after such observations, he could still regard the two groups as closely related. The work of Grenacher (1868) on the anatomy of Gordius was a step in the right direction. He emphasized again the difference between Gordius and Mermis and stated that the two could not possibly belong to the same family.

Villot was the first to take up the study of museum specimens and living material in larger quantities. His investigations were carried on for a long series of years and with an earnest desire to solve the problems of taxonomy, physiology and life history; but unfortunately did not contribute much to a clearer knowledge of the group. The problem was too great for the methods he employed.

In England Baird described several species and in Germany von Linstow added a large number of names without giving descriptions adequate for identification.

The greatest contributions to the taxonomy of the group have undoubtedly been made by Camerano, chiefly because he had at his disposal more material than has ever been available to any other writer. He not only described a large number of species, but subdivided the group into several genera. Creplin (1847) had already established the genus Chordodes. Camerano (1897) carried the division farther in separating the genera Paragordius and Parachordodes from the genus Gordius. This separation was made purely on external characters. Montgomery in a paper coming out somewhat later also established a genus Paragordius which, so far as the anatomy of the forms is known, includes the same species as does that of

Camerano, but is founded on much more essential characters. That the genus has found universal acceptance is perhaps due more to Montgomery's characterization than to that of Camerano. The genus Parachordodes has not been universally accepted; but here again the evidence presented in this report shows that the characters given by Camerano are accompanied by others which indicate a natural division of the group. He characterizes Gordius by the presence of a postcloacal ridge in the male and the absence of true areoles on the cuticula, Parachordodes by the absence of postcloacal ridge and the presence of areoles.

In America several species were described by Leidy, but most of the systematic work was done later by Montgomery. Here as well as in Europe little more than pioneer work has been done. Descriptions and identifications have been made chiefly from preserved material and isolated specimens and only in a few cases from living material collected in abundance.

The confusion that still exists in the group is due in a large measure to the fact that the variations within a species are very great while the differences between species are relatively small. When isolated and often poorly preserved specimens are studied it is natural that essential characters are often overlooked and variations are taken for specific characters. This tends on the one hand to throw species together and on the other to separate members of a single species.

The two characters causing the most confusion are size and color. Nearly all of von Linstow's descriptions include besides these only those that are common to nearly all Gordiacea. Such descriptions are useless. I have in my own collection specimens of a single species ranging in length from 10 to 50 cm. and in color from light brown to nearly black and others that are an iridescent gray. Even as late as 1910 Wesenberg-Lund identified specimens as *Gordius aquaticus* on account of their size and made the errors that I shall point out later.

The light spots in the cuticula and the postcloacal ridge in the male have been taken as specific characters, and the species bearing either has usually without hesitation been assigned to *Gordius aquaticus*. The ridge certainly is possessed by more than one species and may be a generic character while the white spots, as Montgomery suspected, are due to physiological conditions in the American species but may be due to structures in the hypoderm in some of the European species.

Altho the structure of the cuticula is one of the best specific characters, its use has led to confusion because different authors have studied it under different conditions and have made different interpretations of what they saw. The erroneous theory that size, color and cuticular structures change with the age of the free living specimen has also contributed to the tangle.

The habits of different species are practically unknown. Most of the material has been found accidentally as isolated specimens and observations on behavior have been made mostly on animals in captivity as Wesenberg-Lund has already pointed out. The scant observations made on animals in nature are mostly referred to the group as a whole and not to any particular species. But there is no reason for supposing that the habits of different species are the same, and they are not the same in the species forming the subject of this paper.

The problem of the life history of the group has attracted much attention. Villot at one time thought he had the complete cycle, but found later that he had to modify his theories. He observed the embryological development, the encystment of the larvae in a large number of animals, and the presence of nearly adult worms in beetles. After holding for a long time the view that the animals harboring the encysted larvae were intermediate hosts he finally concluded that the encysted larvae perish and that the life cycle is completed in some other way. Camerano independently arrived at the same conclusion. Blunck (1915) again speaks of an intermediate host. He states that the larvae of *Gordius tolosanus* penetrate soft-bodied animals and these in turn are devoured by Dytiscus larvae. Tadpoles form for the most part the intermediate host. Development is completed in Dytiscus and the worms escape soon after the beetle emerges from the puparium. The facts upon which these deductions are based are not given. Nothing has been published on the metamorphosis or the structure of the early parasite. In regard to the later organogeny Villot (1891) and Vejdovsky (1894) have supplied the only information.

The adult organization is better known. If the knowledge of it is still incomplete, that is due chiefly to the fact that the material at hand has often been scarce and the methods employed have given only poor results. Here as elsewhere the greatest confusion has arisen because of the belief that what is true of one species must be true for all. Writers have not hesitated to denounce their fellow workers when their particular species failed to show what others had found in very different species.

This very brief discussion of the literature on Gordiacea has been given not with the purpose of presenting an historical account of the subject but with the object of pointing out the need for further investigation and some of the difficulties that have presented themselves.

The present investigation was undertaken with the object of increasing the knowledge of some of the common American Gordiacea. The chief purpose was to trace out if possible the complete life cycle in one or the other of the two species most easily available; special attention being given to the host succession and the organogeny.

The work was suggested in the fall of 1913 by Professor Henry B. Ward, under whose direction it was carried on. To him I wish also to express my sincere appreciation for a keen interest in the work and for many helpful suggestions. Professor Ward also placed at my disposal his library and his collection.

Other material was obtained for study from Harvard University, the University of California and the University of Pennsylvania. Doctor Minnie Watson Kamm kindly donated an infected host collected in the vicinity of Urbana and containing valuable material of *Paragordius varius*.

The early collections were made under the direction of Professor Frank Smith, while the work at Douglas Lake, Michigan, was made possible thru the kindness of the director of the station, Doctor H. A. Gleason, and was carried on under the direction of Doctor W. W. Cort.

Many helpful suggestions were obtained from Doctor T. B. Magath and other workers in Professor Ward's laboratory.

MATERIAL AND METHODS

The two species studied are *Gordius robustus* Leidy 1851 and *Paragordius varius* (Leidy 1851).

Gordius robustus is well known in America; its range extends from the Atlantic to the Pacific. It is by far the most abundant species in the streams near Urbana, Illinois, and is occasionally picked up in collections made for the zoological laboratory of the University of Illinois. Eight males and one female were collected among dead grass at the water's edge in a small stream on March 25, 1914. Then, for nearly a month, diligent searches in similar localities were fruitless. On April 18 several more specimens were found.

It was noted at the time that both localities were at the edges of rapids. This led to the investigation of other rapids with the result that hundreds of specimens were collected. It was possible to walk along the bank of a stream and descend at rapids with grassy borders and collect Gordius in masses containing sometimes as many as 25 or 50 worms. Collections were made at short intervals until the middle of June. More material was collected in 1915 and 1916.

The material in the collection of Professor Henry B. Ward at the University of Illinois was available for study as well as Leidy's specimens of 1879 and the material from the collections at Harvard University and the University of California.

Eggs were found thruout the month of May and the larvae hatched the latter part of May and during June.

Parasitic stages were obtained in large numbers in the fall of 1914 and again in 1916. Earlier stages were obtained by infecting the hosts in the laboratory. About 500 specimens in different stages of development were obtained for the investigation.

Paragordius varius is even more abundant than the previous species and is the one most commonly collected in this country.

Several adults of this species were collected early in June of 1914. Hundreds of specimens were obtained at Douglas Lake, Michigan, during June, July and August, 1915, and a few more at Urbana in the spring of 1916. Material from the collections mentioned under *Gordius robustus* was also available.

Eggs and larvae were obtained in large numbers wherever adults were found.

Parasitic stages were obtained in the fall of 1914 from one host given to me by Minnie Watson Kamm, who was working on gregarines in this laboratory at that time. Abundant material was obtained in all but the very youngest stages in the summer of 1915 at Douglas Lake. Over 500 specimens were available for study.

The ordinary methods used in anatomical and histological study were found to be almost useless when applied to the study of the Gordiacea and special methods had to be adapted and devised at nearly all stages of the investigations.

The study of living material was confined mostly to field observations on adults and hosts and to the study of the embryonic development and larval structure. Nothing is gained by the study of the parasitic forms in the living condition.

For the removal of parasites from the hosts it was necessary to use a normal salt solution of full strength (0.75%). Even in this a slight injury usually caused a flowing out of part of the body contents. In pure water the specimens rupture at short intervals all along the body almost as soon as immersed. This applies of course only to the younger stages and not to those that have already formed the adult cuticula. The specimens were usually removed by tearing away the host tissues in salt solution by means of fine forceps or needles. For smaller specimens the host tissues were teased out in a watch glass and the contents examined under the low power of a microscope at a magnification of about 100 diameters.

The problem of the proper killing fluid was one of the most difficult to solve, and in part has not yet been solved. On account of the special methods of dehydration and imbedding it was impossible to test out quickly the action of any particular killing fluid and on account of the short seasons at which material was available such testing could usually not be done during the collecting season. It was necessary under those conditions to use the rapidity with which the killing fluid acts and the general appearance of the killed material as criteria. Most of the earlier material was killed in a saturated solution of corrosive sublimate to which from five to ten per cent of glacial acetic acid had been added. Later this solution was saturated with picric acid because with that modification it killed specimens more quickly and prevented to a great extent the rupturing of the parasitic forms in the killing fluid. But histological preparations show that this fluid is inferior to plain corrosive acetic. Other killing fluids tried were Flemming's solution, Zenker's fluid, Kleinenberg's picro-sulphuric, formalin and other less known fluids. The best preparations so far have been obtained with corrosive acetic when the solution was used at a temperature of from 40 to 60° C. The glycerol-alcohol mixture recommended by Looss for killing nematodes yields specimens as flat as ribbons and bearing no resemblance to Gordiacea.

For killing infected hosts the solution of Carnoy and Lebrun consisting of equal parts of absolute alcohol, chloroform and glacial acetic acid saturated with corrosive sublimate was found to give very excellent results. It could not be used for killing Gordiacea because it made the material collapse nearly as badly as did the glycerol-alcohol mixture.

The methods of preparing the material for microscopic study were more easily devised as it was possible to take up this problem at convenience. The ordinary methods of dehydration, clearing and imbedding were found to yield nothing but flattened, torn and distorted preparations. In delicate specimens at certain stages a sudden increase of one per cent in the concentration of the alcohol caused excessive flattening and distortion. It was therefore necessary to use an apparatus for insuring the gradual changing of the liquids in dehydrating and clearing. Several devices for this purpose have been introduced by European workers. The one best known in this country is the differentiator introduced by N. A. Cobb for making microscopic preparations of free living nematodes. This apparatus is made possible by the fact that successive layers of alcohol of increasing strengths can be introduced into a narrow glass tube without mixing. By stirring up the tube a little it is possible to obtain a column of alcohol gradually increasing in strength from the bottom upward. If the specimen is placed in the bottom of the tube and the alcohol permitted to ooze out thru a capillary point, it is possible to draw over this specimen a stream of alcohol of gradually increasing strength.

The apparatus used for this work depends upon a slightly different principle. When alcohol is introduced at the bottom of a broad tube filled with alcohol of a lower strength there is a certain amount of mixing of the two liquids. Such a tube can be used as a mixing chamber. The essential parts of the apparatus used consist of a reservoir, the mixing chamber and the specimen chamber. The reservoir is a tube about 2 cm. in diameter and 25 cm. long. It is supplied at the bottom with a rubber stopper thru which a piece of small glass tubing leads nearly to the bottom of the mixing chamber. The best results are obtained when this glass tube is drawn out so as to leave an opening of not more than 2 mm. at the bottom. The mixing chamber consists of a piece of glass tubing about 1.5 cm. wide and 5 cm. long supplied at each end with a perforated rubber stopper. From the bottom of this chamber a piece of narrow glass tubing leads to the top of the specimen chamber. This tube must be bent in the shape of an S to raise the specimen chamber to a point where the outlet is above the lowest part of the mixing chamber to keep the apparatus from running dry. The same result can of course be obtained by inserting the specimen chamber under the mixing chamber and bending up the outlet tube to a point above the top of the specimen chamber.

This apparatus was for this work preferred to the Cobb type because it permitted the dehydration of a large amount of material at one time and so saved an infinite amount of labor.

When the specimens were dehydrated they were removed from the chamber into a small stendor dish and cleared in xylene by means of the string differentiator described by Magath. This consists essentially of three dishes placed one above the other like steps in a stairway. The upper dish contains the liquid to be introduced, the middle dish contains the specimens, and the lower one the waste. The liquid is transferred from dish to dish by means of string siphons. The string drawing the liquid from the specimen dish does not reach the bottom of that dish and so prevents the removal of all the liquid from the specimens when the upper dish goes dry. The whole apparatus is covered by a bell jar sealed at the bottom to prevent the alcohol from absorbing moisture from the air.

This differentiator was later adapted also for dehydrations. The chief objection to the use of the apparatus described by Magath for dehydration lies in the fact that the stronger alcohol introduced into the specimen dish tends to form a layer at the top and is drawn off again by the second string, increasing only very slowly the strength of the lower alcohol, causing an enormous waste of liquid, and usually ruining the specimens. It is possible to withdraw the alcohol from the bottom of the specimen dish by means of a capillary glass tube bent in the shape of a U. This capillary tube must widen out rather suddenly at the outlet to an inside diameter of about two millimeters and this end must be bent outward so that the liquid drops freely from the tube without touching the dish. If this tube is not widened at the outlet it is impossible to keep the alcohol in the specimen dish at the proper level on account of the different effect of capillarity on the different grades of alcohol. An ordinary capillary tube that will keep water at the proper level will drain the dish completely when the higher grades of alcohol are used. The level at which the liquid will be maintained is determined by the position of the outlet of the capillary tube as the liquid will stop flowing when the level tends to become lower and will start automatically when that level is raised. For clearing specimens dehydrated in this differentiator the capillary tube is replaced by a string. This apparatus is by far the most convenient and the safest for delicate specimens as they can be kept in the same dish thruout the entire process of dehydration and infiltration.

Specimens transferred from xylene to a solution of paraffin in xylene or from a saturated solution of paraffin in xylene to melted paraffin are usually injured by the appearance of gas bubbles in the tissues and consequent tearing and distortion. To avoid this the following method of infiltration was introduced. Pieces of solid paraffin are successively added to the xylene containing the specimens until the solution becomes saturated. When the solution is saturated at room temperature the dish is placed in a warming oven or on the top of the paraffin bath and the process continued till saturation is again reached. The infiltration to this point usually requires two to three days. The dish is now placed in the paraffin bath and the process continued rather rapidly until the solution becomes practically pure paraffin. The specimens are then transferred to pure paraffin and imbedded after about two hours. The entire period during which the specimens are left in the paraffin bath is usually not more than four hours.

For imbedding Gruebler's best paraffin with a melting point of 56 to 58° C. was found to give the best results. The ordinary paraffins were found to be either too soft or too brittle for this work. Parowax, the Standard Oil product on sale at nearly all groceries, would be an excellent medium if the temperature of the sectioning room could be kept down to about 15° C.

For sectioning specimens imbedded in the hard paraffin at ordinary room temperature it was necessary to dip the trimmed block into melted soft paraffin. After cooling the soft paraffin was removed from all sides except the lower. Blocks treated in that way, when sectioned 7 to 10μ thick, produce beautiful ribbons in which the sections show no evident shrinkage or rolling.

All cross sections and most of the longitudinal sections were cut 7μ thick. Longitudinal sections of specimens in which the adult cuticula had been formed had to be cut 10μ and even then gave only poor results on account of the unequal expansion and contraction of different parts when the sections were cut. Cross sections of such specimens also showed distortions from the same causes. In younger specimens in which the cuticula was still soft the sections could be flattened out fairly well by placing them on water on a slide and warming the slide suddenly to the point where the paraffin began to become clear but did not become completely melted. Even with that treatment the cuticula often retained a wavy outline not normal to the living specimen. For later stages the warming usually had to be continued until the paraffin was completely melted. Good results were obtained with such specimens when the slide was placed in the paraffin bath for half an hour or more until the water had evaporated from under the ribbons. For that purpose the bath must be warm enough to melt the paraffin as otherwise blistering takes place and the sections may be ruined.

In all cases the sections were fixed to the slide by means of Meyer's albumen fixative. Slides containing older specimens had further to be treated with a very thin solution of celloiden while transferring from absolute alcohol to 95% before staining.

In sectioning adults the friction between the specimen and the knife often caused the ribbon to become highly charged with static electricity. This was especially true of specimens that had become excessively hardened in the process of dehydration and infiltration. The only remedy was to trim the block less closely and make sure that it was properly treated with soft paraffin. The same rules had to be followed in sectioning crickets and grasshoppers.

The best stain was found to be iron hematoxylin. Unna's polychromatic methylene blue method with orcein as a counter stain gave fair results, Mallory's connective tissue stain was useful for demonstrating basement membranes; Delafield's hematoxylin, Ehrlich's hematoxylin and the carmine stains gave very mediocre results.

The iron hematoxylin method had to be modified according to the developmental stage and condition of the material, and the structures to be shown. For mordanting a 4% solution of iron ammonia alum was used and for staining a 0.5% solution of hematoxylin in water. Sections were usually mordanted about twice as long as they were stained except in case of very short staining periods when they were mordanted about half an hour.

Older parasitic stages were usually stained from half an hour to one hour, adults from one to two hours or for nerve structures from six to twelve hours, younger stages for very short periods, sometimes not more than thirty seconds. Destaining was nearly always done in a saturated solution of picric acid in water. This was found to take the stain out more uniformly than did the iron alum.

For counterstaining the slides were left for from twelve to twenty-four hours in xylene to every fifty cubic centimeters of which had been added three to five drops of a saturated solution of eosin in absolute alcohol. Fresh eosin must be added from time to time as it precipitates very rapidly unless there is a large amount of alcohol in the xylene.

OBSERVATIONS ON GORDIUS ROBUSTUS

Since the investigation was begun on *Gordius robustus* Leidy and since the series of observations is most complete in this species, it is but natural that it should form the first part of the discussion.

DETERMINATION OF SPECIES

On account of the confusion existing in the literature in regard to this and related species it is necessary to take up at this point a precise characterization of the species and a determination of its position in the group. The species was named by Leidy in 1851 and specimens were again referred to it in 1879. The single female of 1851 has not been preserved but the specimens of 1879 have fortunately been kept in fair condition. Leidy's early characterizations are not sufficient for identification but his description of 1879 is fairly complete and the material is available for study. Montgomery has given a somewhat detailed description of the species, but he overlooked one of the most essential characters, the dorsal and ventral bands. Only a general description of the species will be given at this point, details of the structure being left for the discussion of the adult morphology.

Dimensions. Of the specimens collected near Urbana the females vary in length from 100 to 470 mm. and the males from 120 to 420 mm. The diameter of the females ranges from 0.5 to 1.25 mm., that of the males from 0.3 to 0.75 mm. Some of Montgomery's specimens from California are considerably larger. The females in Leidy's collection are very short and thick.

Form. Both males and females are cylindrical, decreasing very slightly in diameter toward the ends, the females more than the males. The shape of the anterior end is essentially the same in both sexes. In the average specimen the end is rounded in the shape of a hemisphere, separated from the body by a very slight constriction and of the same diameter as the body just behind the constriction (Fig. 27). In very stout-bodied females there is no trace of a constriction and the body becomes distinctly attenuated just before the end. This condition is at its extreme in case of the short, thick females in Leidy's collection (Fig. 28).

The posterior extremity of the female is slightly enlarged in the shape of a bulb and abruptly truncated at the end (Figs. 25, 26). The cloacal aperture is located at the center of the truncated area and there is no trace of a dorso-ventral furrow (Fig. 34).

The posterior end of the male bears two short, stout prongs (Figs. 8, 32). The length of the prongs varies somewhat, but is usually not much more than half the diameter of the body. Each prong is of conical shape with a slight flattening on the inner side. The body attenuates rapidly dorsoventrally at the base of the prongs so that the diameter of the latter is less than half that of the body (Fig. 24). On the ventral side, a short distance anterior to the bifurcation is a crescent-shaped ridge with the ends of the crescent passing slightly onto the bases of the prongs. I have usually found this ridge to be a broad, open crescent and not U or V-shaped as represented in most of Montgomery's figures. The anus is located a short distance anterior to the middle of the crescent, almost at its very base.

Color. The usual color is light brown, but specimens vary from nearly white to nearly black and females that have deposited their eggs are gray. When light is reflected in the proper way the cuticula shows a distinct iridescence, very pronounced in females after deposition of the eggs. Such females examined in the sunlight present brilliantly all the colors of the rainbow. Ordinarily the iridescence gives the body of the worm the appearance of being longitudinally corrugated. The anterior end is clear white followed by a ring of dark brown which passes rather abruptly into the normal brown of the body. At the center of the anterior white area is often found a black spot indicating the position of the mouth (Fig. 33). Passing backward from the dark ring are two bands, one ventral and one dorsal, slightly darker than the rest of the body (Figs. 26, 27, 28). These bands can usually be traced to the posterior end of the female, but are more difficult to trace in the male. Even there the dorsal line can often be traced to the base of the fork while the ventral line disappears a short distance before the anus. Montgomery does not mention these lines or bands in his description of the species, but I have found them present in all of his material that I have examined as well as in Leidy's material. They are mentioned by Leidy in his description of 1879. In the female the cloacal opening is situated at the center of a dark area, which itself is surrounded by an area slightly lighter than the body color, and around that is a brown circle, the dark color of which passes more or less gradually into the general body color (Fig. 34). In the male the crescent is dark brown, almost black, and there is a small dark area surrounding the anus (Fig. 32). Lighter spots scattered over the body may or may not be present. I have found them on many specimens at Urbana (Fig. 16), but never as pronounced as in Montgomery's specimens from California.

Cuticula. The cuticula never shows any traces of areoles. Under low magnification it appears to be divided into rhomboidal areas, while higher magnification shows a system of finer intersecting lines (Figs. 4, 5). The white area at the anterior end is of more homogeneous structure. Bristles or hairs are present over the entire body. Montgomery says they are branching, but I have been unable to find any branching forms and, from the manner in which they develop, I do not believe that branching forms occur in this species. The branching forms probably occur on some other species that Montgomery regarded as identical with this. The bristles are more abundant at the two ends. In the males on the outside of the prongs they are slightly longer than elsewhere and usually curved, while on the inner surfaces there are very short, conical setae (Figs. 121, 122, 123). No special bristles are located around the anus of the male (Figs. 96, 97, 98).

Eggs and larvae. In nature the eggs are laid in thick cords which break up into pieces seldom more than 10 to 15 mm. long and of a diameter nearly equal to that of the worm. The larva belongs to the type with a single terminal spine at the posterior end. In the newly hatched larva (Fig. 20) the body is about twice as long as the proboscis but later becomes much reduced (Fig. 14).

Montgomery at first referred this species to *Gordius aquaticus* var. *robustus*. Later he regarded it as *Gordius villoti* and eliminated the variety. It belongs to the group known by most European writers as *Gordius aquaticus*. The identification is usually based on Villot's description of what he regarded to be Dujardin's *Gordius aquaticus*. Rosa regarded the identity of the two species as impossible or at least highly improbable and called Villot's species *Gordius villoti*. At the same time he redescribed the species, basing his description on a male and two females. He found on the surface irregularly polygonal areolae which it is difficult to interpret as the rhomboidal areas in the cuticula. Villot later called them pseudoareolae and he as well as Camerano included them in the description of *Gordius aquaticus*, stating that they are not present in all specimens. Of Rosa's specimens only the male possessed the dorsal and ventral bands and the character is not included in the original description of Villot's species. It is however mentioned in the description of Dujardin and Villot's later descriptions. I have never found the bands absent on any specimen of *Gordius robustus* or *Paragordius varius* and believe the character is not variable within a species. On account of this and other differences between the male and females described by Rosa it seems certain that he included at least two species in his description, and it is possible that neither of them was identical with Villot's species.

Rosa believes that Villot's species can not be identical with that of Dujardin because Dujardin's description mentions pores 0.006mm. in diameter in the fibrous cuticula which Villot does not mention. On the other hand Villot mentions a dark collar behind the white anterior end, a postcloacal crescent in the male and clear spots in the cuticula not mentioned by Dujardin. Villot in 1886, however, does describe pores in the fibrous cuticula which he claims may attain the diameter of 0.006mm. On the other hand Dujardin describes clear areas 0.06mm. in diameter which he regarded as openings in the homogeneous cuticula. Furthermore, since the dark collar is almost universally present in Gordiacea of this group, it is probable that it was not actually lacking in the specimen of Dujardin; and the fact that he overlooked this leads also to the possibility that he overlooked a crescent that may have been present. The latter assumption is moreover justified because there is at present no form known with a cuticula that is devoid of true areoles and in which the male possesses no crescent. Indeed Camerano makes the combination of presence of crescent and absence of areoles a generic character. The use of the name *Gordius aquaticus* by Villot seems justified in the light of these considerations. But his description probably includes a group of closely related species which some future investigator in Europe may be able to separate.

Since there are several characters ascribed by various authors to the European species that are not present in *Gordius robustus* the two species can not be combined. Among the characters not present in *Gordius robustus* are pseudoareolae, pores in the cuticula, a dorsoventral furrow at the posterior end of the female, and groups of cells extending from the hypoderm into the cuticula as described by Camerano (1888) and Rauther (1905). The larva, also, of the European form appears to have a shorter body than that of the American species.

It is difficult to see why Montgomery assigned the American species to *Gordius villoti*, since he himself states that Leidy's descriptions are sufficient to establish the identity of the species and he had Leidy's material at hand for additional information if necessary; furthermore, he certainly was aware of the fact that Leidy's first description was given in 1851 and his second description in 1879, while that of Rosa did not appear until 1882.

HABITS OF ADULTS

Gordius robustus emerges from its host during September and October and possibly the latter part of August. Specimens may then be found swimming freely in the streams or stranded at the water's edge. It is at this time that they are most easily obtained in general collections. But the period of migration does not last very long as the specimens soon become entangled in the grass and debris along the edge of the water.

During November and December I have still succeeded in finding specimens in the grass just below the level of the water in small brooks. Even at this time they tend to accumulate at or just below rapids. During January and February I have made no collections, but the latter part of March, when the ice has gone, specimens are again found in the grass. At that time I have usually found them deeper down, entangled in the roots of the grass even several inches in the ground.

During April and early May there seems to be another migration on the part of some of the worms, but I have never found them free in the water. Since worms in captivity will usually remain quiet during the day but become active during the early part of the night it is probable that migrations in the streams occur at that time. By the middle of May all the worms seem to have accumulated among the roots of grass in or at the edges of rapids.

It is likely that copulation does not ordinarily take place during the fall migration, as specimens at that time are usually found isolated and seem to remain more or less isolated during the winter. During the spring migration, however, they gather together in large numbers and I have several times found females that still retained the mass of spermatozoa at the posterior end, showing that copulation had taken place not more than three days before. It is soon after this migration that egg-laying begins.

The process of copulation is not difficult to observe. If two fresh worms, male and female, are placed in a glass cylinder about 10 cm. in diameter, filled with water, copulation takes place in a short time and may be observed thru the walls of the cylinder. When specimens are placed in a large open dish they swim about actively for a long time and copulation usually does not take place until the latter part of the night, when they become more quiet, and sometimes not until the second night. For the observations for this report I used specimens that had just emerged from their hosts, but in early spring collections most of the females copulate after being brought to the laboratory and observations could easily be made on them. In spite of the fact that egg-laying does not seem to take place in the fall, the specimens are mature for copulation when they emerge. I have kept males and females that had just emerged or had been removed from the hosts in open dishes and had copulation take place within 48 hours after emergence.

There seems to be no definitely directed effort on the part of either male or female to seek its mate. There is of course the usual tendency on the part of both to become entangled with the other, but the solid knot that makes observation impossible is usually not formed by two specimens until they have been together for a long time. The two worms merely become intertwined at places and then again disentangled, only to become reentangled again. That process is continued until finally the body of the female comes to lie within the spiral coil formed by the posterior end of the male. This coil soon tightens, the prongs are spread over the body of the female (Fig. 30), and the posterior end of the male with a rotary motion passes backward over the body of the female. The male does not seem to choose the direction in which it is to move except that it tends to move contrary to the motion of the female. The direction taken is nearly as frequently toward the anterior end of the female as toward the posterior end. Usually after several trials the posterior end of the male passes over the posterior end of the female. When the prongs have already passed over the end (Fig. 31) a discharge of sperm takes place. The cloacal openings of the two specimens are not superimposed at this time and the sperm mass does not enter the body of the female but adheres to the outside. The discharge usually lasts not more than thirty seconds and during this time the male continues to rotate on the body of the female. The sperm seems to be fluid when it leaves the body of the male but soon solidifies. Some is lost in the water.

The sperm mass (Figs. 10, 113) disappears from the female within two or three days. Most of the spermatozoa pass into the seminal receptacle. The mass is so tough that it is almost impossible to crush it in order to make a microscopic preparation and it does not seem possible that many spermatozoa are brushed off. The migration into the seminal receptacle is probably passive, as the spermatozoa show no movement when placed on a slide.

The first eggs appear the latter part of April and laying continues until early June. The eggs are deposited while the worms remain entangled in masses among the roots of grass. They are laid in thick cords about 0.5mm. in diameter and break up into short pieces from 5 to 30mm. long. When fresh they are pure white, but soon become discolored by the surrounding earth. They do not adhere very strongly to each other and are easily crushed under a cover glass for microscopic examination.

After the deposition of the eggs the adults soon become inactive and begin to die and disintegrate in parts. One may actually find females with the anterior end dead and disintegrated so that nothing but the cuticula is left while the posterior end is still depositing eggs. More commonly, however, disintegration does not appear until all the reproductive products have been discharged and it may begin at any part of the body or the whole specimen may die at once. Males usually die a week or two earlier than do the females. Most of the specimens are dead by the middle of June.

EARLY DEVELOPMENT

Since Montgomery in 1904 gave a detailed account of the development of the larva of *Paragordius varius* it seemed only of minor importance to repeat his work on some other species and consequently little attention was at first given to the embryology of this species. The observations that were made, however, show that not only the larval development of *Gordius robustus* but also that of *Paragordius varius* requires further investigation. It has not been possible to undertake that investigation for the present report.

To fill out the gap I shall give a very general account of the larval development of *Paragordius varius* as described by Montgomery. The eggs are fertilized in the cloaca and the two polar bodies are given off soon afterwards. The cleavage is total and adequal and soon forms a coeloblastula which early passes over into a typical gastrula. Mesenchyme is formed by the separation of cells from the invaginated entoderm. At the end opposite the blastopore the ectoderm thickens and forms a second invagination, that of the proboscis. The entire proboscis develops from ectoderm except for a few mesenchyme cells which have migrated into it to form the muscles. The blastopore becomes nearly closed and the anterior end of the intestine does not communicate with the cavity of the proboscis during the embryological stages.

The description of the larva also can be given only in the most general terms at this time. The larva of *Gordius robustus* differs greatly in form from that of *Paragordius varius*, but in essential structures the two do not seem to differ much. The newly hatched larva (Fig. 20) is very much elongated, but after a week or so it has become shrunken to about half its original length (Fig. 14) and has increased slightly in diameter. Like all other known Gordius larvae it consists of proboscis and body. The proboscis is armed in front with three retractile stylets and at the sides with three circles of hooks which point backward when the proboscis is extended, but are withdrawn into the proboscis when this is retracted. A set of retractor muscles is inserted at the base of the stylets and protractors lie close to the outer wall of the proboscis. The body in the newly hatched larva is at least twice as long as the proboscis and of a slightly smaller diameter. Both body and proboscis are covered by external, more or less irregular cuticular rings which do not seem to be in any way related to the deeper structures of the larva. The posterior end of the body runs out to a point resembling a heavy spine. Between the proboscis and the body there appears to lie a partition separating the end of the intestine on one side from a cord of cells coming from the base of the stylets on the other. Just behind this partition is a mass of cells belonging to the intestine which farther back has very thin walls and encloses within its lumen two elongated masses of a homogeneous, highly refractive substance. Montgomery figures similar masses in the intestine of *Paragordius varius* and regards them as excretory waste. In *Gordius robustus* these masses are later absorbed as large cells invade this region. The intestine opens at the posterior end on the ventral side somewhat anterior to the spinelike elongation.

Beginning near the proboscis and extending backward two-thirds of the length of the body on the ventral side are two rows of nuclei indicating the rudiments of the ventral nerve cord. Only longitudinal muscles appear to be present in the body and they adhere so closely to the outer wall that it is difficult to detect them.

When fully developed the larva ruptures the egg-membrane and escapes from the egg-string by means of the armature of its proboscis. Quite active at first, it becomes more and more sluggish as it grows older. Larvae picked up with a pipette from the bottom of the dish containing the eggs usually show only a few active forms. If among a number of larvae in an open drop on a slide about ten per cent are active, then when a coverglass is placed over the drop about half of the specimens will become active within five or ten minutes. The reaction is not due to pressure as the larvae can easily stand on end in the ordinary film of water. It may be a reaction to the lack of oxygen or the presence of carbon dioxide in the water.

The larvae bore their way into any animal tissue that happens to be accessible at the time. Villot was the first to discover this, and since he regarded all these animals as true hosts of the parasite, he stated that Gordius has no specific hosts. Later Villot himself, Montgomery and others found that the larvae merely encyst in most of these animals and are unable to undergo further development.

PARASITISM

With the entrance of the larva into the proper host begins one of the most important phases of the life cycle, the period that leads thru growth and differentiation to the formation of the mature worm.

The final and perhaps the only hosts of *Gordius robustus* I have found to be members of the grasshopper family Locustidae. The most common host around Urbana is *Orchelimum vulgare* Harris, but *Orchelimum nigripes* Scudder and *Xiphidium nemorale* Scudder seem to be equally heavily infected tho less common, and I have obtained two adult parasites from a female of *Scudderia furcata* Brunner. Over 100 specimens of *Xiphidium fasciatum* (DeGeer) from localities in which *Orchelimum vulgare* was heavily infected were examined but no infection was found. Over 200 specimens each of *Melanoplus differentialis* and *Melanoplus femur-rubrum* from similar localities also proved to be not infected. Large numbers of *Gryllus assimilis* and *Nemobius fasciatus* examined in the investigation on *Paragordius varius* were also not infected with *Gordius robustus.* Many aquatic insect larvae were also examined, but no infected specimens were found. From two to three percent of the crickets and grasshoppers examined were found to be infected with Mermithidae.

An intermediate host is not necessary. If one occurs in nature it can be nothing more than a carrier in which the larva undergoes no change. Evidence presented later shows that a larva that has begun to change into the parasitic form can not undergo a change of hosts without being destroyed in the process. Furthermore, I have succeeded in producing in the laboratory an infection of at least fifty per cent in Locustidae collected in a locality in which later collections showed that no infection occurred in nature.

I 1. On July 6, 1916 forty-one young Locustidae, mostly *Xiphidium fasciatum* and *Orchelimum vulgare* were infected by injecting a drop of water in which larvae of *Gordius robustus* were suspended into the abdomen by means of a capillary pipette made of hard glass. From counts made on similar drops of the suspension placed on a slide under the microscope it was estimated that from five to ten larvae were injected into each host. On account of the unfavorable conditions under which they were kept most of the hosts died in the next few days. On the ninth, six of the infected hosts were killed, the tissues teased out in salt solution and the sediment examined. Two active larvae of *Gordius robustus* were found, both showing signs of having begun their development. Both were lost in an attempt to stain and mount them. On July 11 all hosts were dead except five. When these were examined as before, four somewhat older, but still active larvae were found. They also were lost by accident.

I 2. Suspensions of larvae of *Gordius robustus* were injected into the mouths of eleven young Locustidae on July 6. All died within a few days.

I 4. Four young specimens of *Orchelimum vulgare* were infected as in I 1 on July 9. On July 15 three of the specimens were examined and all were found to be infected, yielding four small parasites (Figs. 9, 11). The other host died.

I 7. On July 11 fifty-three Locustidae mostly by *Orchelimum vulgare*, which had become easily recognizable by this time, were infected as before. Specimens of this lot were killed for sectioning, at first every day and later every two days. On July 15 an adult was examined but nothing found. Another adult was killed and examined on July 22 and found to contain three young worms, the longest being about 10mm. in length and the smallest one about 5mm. On the following day the last three hosts were examined and all were found to be infected, yielding 11 parasites ranging from 3 to 10mm. in length.

I 8. On July 14 seventy-eight Locustidae, mostly young *Orchelimum vulgare* were infected. Some of these were again killed for sectioning and staining as were those of I 7. One adult host was examined on July 22 and found to be infected with one young parasite (Fig. 15). An examination of ten more hosts on the following day yielded only a single parasite about 2mm. long in the coiled stage. On August 11 the last two specimens were examined. Only one was infected and contained two parasites 25 and 30mm. long respectively.

Later infection experiments proved less successful. Of 45 control hosts not one was found to be infected.

Of the specimens preserved for sectioning only seven from I 7 have been thoroughly examined and six were found to be infected. Sections of some of the specimens found are shown in figures 47-49 and 50-55.

The results of these infection experiments show conclusively that an intermediate host is not necessary for *Gordius robustus*.

Infection in nature must begin in late June or early July and end the latter part of July or in August. The young of *Orchelimum vulgare* collected early in July were still small and could not have hatched more than a week or so before they were collected. By the middle of September most of the parasites are well along in their development.

TABLE I

RESULTS OF INFECTING *Orchelimum vulgare* WITH LARVAE OF *Gordius robustus* IN THE LABORATORY

Exp. No.	Date in 1916	No. Inocu-lated	Examined							
			Fresh				Slides			
			No.	Inf.	Par.	Not Inf.	No.	Inf.	Par.	Not Inf.
I 1	Jy. 6	41	11	?	6	?				
I 4	9	4	3	3	4	0				
I 7	11	53	5	4	14	1	7	6	7	1
I 8	14	78	13	3	4	10				
I 10	22	16	16	0	0	16				
I 11	23	15	9	1	1	8				
Control			45	0	0	45				

Infection takes place at or near the place where the larvae have hatched. In the spring of 1914 Gordius was very abundant in all of the streams at which collections were made. Later all specimens of *Orchelimum vulgare* collected near these streams were equally and heavily infected. During the summer of 1915 the streams were continually flooded and, judging from the scarcity of Gordius in the fall of that year and the following spring the infection must have been very light. The only place where any appreciable infection can have taken place is a stream north of the city which ordinarily goes dry during the summer months. It was in that locality that adult worms were found in the spring of 1916 and the Locustidae were found to be heavily infected later in the summer. The stream west of Champaign which had supplied the major part of the material in 1914 is bounded by narrow, steep banks and the high water must have prevented the grass from growing in the bed of the stream as usual. This stream is also becoming more and more polluted with sewage and other refuse. Of 453 specimens of *Orchelimum vulgare* collected along the bed of this stream August 28 to September 4, 1916, only six specimens or 1.5 per cent were found to be infected. I had not succeeded in finding any specimens of Gordius in the stream the previous spring. Of 555 specimens of Locustidae collected in the bed of the stream north of Urbana along the distance showing the heaviest infection 110 specimens or about 20 per cent were infected and yielded 164 parasites while in the other collection no host had more than one parasite. A collection of 143 hosts along the same stream, but starting where the previous collection had left off, contained 8 infected specimens or 6 per cent and only a single parasite was found in each host.

These data show that infection is local to a high degree and consequently that infection must take place at the point where the larvae occur; in other words, that the larvae are not widely distributed by an intermediate carrier. The data also show that the host itself does not ordinarily migrate very much during the summer. The migration of the average host seems to be limited to a radius of about half a mile, perhaps less.

Just how the larva enters the host I have not been able to discover. In the spring of 1914 when the infection was so heavy I had not yet discovered the host and in the two succeeding years the infection was ordinarily so low that an attempt to work out that particular phase of the problem by observation in nature would have been a waste of time.

It will be sufficient here to give a brief account of the habits of the hosts, leaving the discussion of the possible modes of infection to be taken up later. Altho the species of Orchelimum and Xiphidium found to be the hosts of *Gordius robustus* are the common meadow Locustidae, they are much more abundant in the tall grass near the water's edge than elsewhere. *Orchelimum vulgare* and *Orchelimum nigripes* are found most abundant right at the water's edge while *Xiphidium nemorale* is more abundant on the taller weeds or on the banks of the streams.

The species are omnivorous but feed chiefly on grass and weeds. Several times I have found *Orchelimum vulgare* feeding on other insects. In captivity all of the species are cannibals, feeding on each other even when in larger cages. The collected grasshoppers had to be brought home in tightly stoppered bottles in which the oxygen supply soon became so low that the insects became quiescent, and even then they often injured each other severely before they could be brought to the laboratory and examined.

Tho the species are so abundant on the grass near the border I have never seen them deliberately entering the water. But their behavior differs so greatly during the different times of the day that it is possible for them to enter the water regularly at some time during the night or early morning but never do so during the time at which my collections were made. The specimens are most active during the early part of the day, especially when the sun shines. Since all specimens were collected by hand, simply by approaching slowly and grasping them suddenly, it was almost impossible to get specimens during the forenoon or early afternoon of a hot, sunny day. Altho on such days the males are singing everywhere, they become aware of the approach and drop to the ground long before they are in reach. Contrary to the habits of the Acrididae, these Locustidae never leap from place to place to escape an enemy, but drop down almost perpendicularly to a lower level in the grass or even to the ground. They either remain quiet where they drop or run along for some distance and then remain quiet so that it is almost impossible to find them. When situated on the grass over the water they do not hesitate in the least to drop down into the water and are in no haste to get back to land. On cloudy days the specimens often remain at the bottom of the grass and can not be obtained at all, but on bright days, when it is just cloudy enough for the sun to be shaded, many of them come up and are easily obtained because they are less active than on sunny days. I have been able to obtain them most easily at twilight just after sunset. At that time they come out on the grass and weeds and do not easily become aware of approach, and even when disturbed they often merely run along to a new position without making any serious attempt to escape.

The larvae at first penetrate the adipose tissues of the hosts, making their way not only between the cells but also thru them (Figs. 47, 54).

In later stages they come to lie free in the body cavity of the host (Fig. 55). There is usually a difference in the location in the host between the Mermithidae and Gordiacea. While the Gordiacea occupy exclusively the body cavity, the Mermithidae usually penetrate the tissues surrounding the body cavity and sew themselves thru between the muscle bundles of the thorax. Whenever Gordius becomes too crowded in the abdominal cavity and is forced into the thorax it passes between the alimentary canal and the thoracic muscles, but never between the muscle bundles.

The parasite does not appear to impair very greatly the health of the host, for, unless the infection is very heavy, the infected specimens appear to be just as active as those that are not infected. In this respect the infection differs from an infection with Mermis. I have several times found specimens infected with Mermithidae to be sluggish.

Observations made during 1914 seemed to indicate that the infection was confined to the females and that the parasites prevented the development of the ova. No attention was paid to the sexes of the hosts during the early part of the season, but during October only females were found to be infected.

During the infection experiments in 1916 it became evident that males as well as females became infected, and later when hosts were collected in the field for examination males were found to be almost as heavily infected as females. Of 711 males examined 64 or 9 per cent were infected and of 440 females examined 59 or 14 per cent were infected. Of the infected females many had eggs, but usually the number of eggs was smaller than in normal females, and in heavy infections with older worms there were usually no eggs present. In males no effect on the reproductive organs was noted, perhaps mainly because it is more difficult to detect a diminution of the testes than of the ovaries.

The records of 1914 showing no infection in males remained a puzzle until a collection was made on October 14. Of 10 males collected not one was infected, while in 11 females seven were infected. It is probable, then, that the earlier collections of 1914 did not show the difference in infection between males and females that was noticed later. Whether the later difference is due to the fact that infected males die early or to the fact that males mature and lose their parasites earlier than do the females has not been determined.

No actual observations on the length of the parasitic period have been made, and since the data bearing on this subject are given elsewhere, this topic may be left for later discussion.

The period during which parasites become mature and emerge from their hosts lasts from early September until late October. In 1914 no collections were made before September 21 and only a few hosts were collected before September 23. On the latter date 78 hosts were collected and several parasites escaped before the collection was brought to the laboratory. The first large collection in 1916 was made on September 5 and yielded 64 parasites of which three were developing the brown color. Of 8 parasites obtained on September 6 one was developing color, 20 obtained September 7 were all white, but 80 collected September 8 included 5 adults ready to emerge. The other limit to the time for emergence is set by the death of the last host. In 1914 the last big collection containing 126 hosts, was made on October 3 and yielded 17 parasites. The record contains a note stating that one of the specimens was the youngest obtained to that time and that the material should be good for study as it contained specimens of all ages. After that *Orchelimum vulgare* became more and more scarce. On October 17, 54 hosts were collected and were found to contain mostly mature parasites, but a few that were not fully developed. In three further collections made respectively on October 24, 29, and 31, only that of October 29 contained a single female of *Orchelimum vulgare*. In 1916 the latest collection was made on October 14 and according to the records one parasite was still quite young. At that time the hosts were so extremely scarce that no further collections were made.

Without exception I have found the parasites escaping with the anterior end first from a region near the anus of the host. In all I have seen more than two dozen specimens of *Gordius robustus* escape from their hosts.

The mature parasites in the hosts react definitely and quickly to water. On September 24, 1914 one specimen of *Orchelimum vulgare* when caught was found to have the anterior end of a Gordius protruded at its posterior end. When the host was dropped into the bottle and thus the pressure on the abdomen released the parasite withdrew. After a short time the bottle was partly filled with water and the parasite emerged within one minute. On the same date two other hosts with parasites in the same stage were collected and placed in a dry bottle. During the remainder of the trip which lasted about two more hours the parasites remained in the hosts. In the laboratory the hosts were placed in water and in about one minute the parasites, two from one host and one from the other, had escaped. On October 19, 1914, a specimen of *Orchelimum vulgare* which had been collected on October 15 and kept in a cage in the meantime, was placed in water. Within less than five minutes six specimens of *Gordius robustus* escaped. Similar observations were made several times after that. These observations show that *Gordius robustus* may remain for a long time in the body of the host after the adult state has been reached and that it escapes only under favorable conditions.

ORGANOGENY

On account of the large amount of material available, including specimens of nearly all ages, it has been possible to follow out the essential changes that take place from the time the larva enters the host to the time the adult emerges. The following discussion is not in any sense to be the final word on the organogeny of *Gordius robustus*, but on account of the size of the field to be covered it seems best to present at this time a general account of the changes involved and to leave certain particulars for further study by means of special methods.

Metamorphosis

The changes that take place soon after the larva has entered its host, as development is initiated, hardly justify the name of metamorphosis. There is no encystment, the larva remaining active even a short time after development has begun. The evidence for this has already been given in the discussion of the infection experiments. Growth begins at about the same time in all of the tissues of the body and parts of the proboscis (Figs. 9, 11, 15, 50–53). Development in the proboscis is at first slower than in the body, but later the difference disappears and it is impossible to locate the division between body and proboscis except from the location of the partition that exists between the two in the larva (compare Figs. 15 and 67). The parts of the proboscis that do not enter into later development are the cuticula bearing the hooks and stylets with the underlying hypoderm, the muscles, and the column of cells connecting the stylets with the partition between body and proboscis (Figs. 57, 67). The cells found in the larva at the anterior end of the intestine (Fig. 20), increase very rapidly in size at first (Figs. 11, and 15,) but later are frequently found to have disintegrated (Figs. 67, 68, 73) and are probably to be regarded as a special organ of the early developmental stages and possibly also of the larva.

Derivation of tissues

Most of the tissues are already outlined in the larva and merely undergo further development in the parasitic stage.

Ectodermal derivatives. The derivatives of the ectoderm of the embryo are the cuticula, hypoderm and nervous system.

The larval cuticula is retained.

The hypoderm is derived directly from that of the larva. In the larva it is a very thin layer lying immediately beneath the cuticula and having only very few nuclei except in the rudiment of the nerve cord (Figs. 21, 49). Figures 14 and 20 are somewhat misleading in this respect as it was impossible to determine the division line between the hypoderm and the underlying muscles. Even in the young parasitic forms the hypoderm is still comparatively thin (Figs. 15, 51, 65, 66).

The nerve cord arises as a thickening in the hypoderm (Figs. 15, 55, 58) along the ventral side as indicated by the two rows of nuclei in the larva (Figs. 14, 20, 56) and may therefore be regarded as derived directly from the nerve rudiments of the latter.

The derivation of the brain is more difficult to trace and an exact determination will have to be postponed until the larva can be studied more thoroly. It arises in the posterior end of the proboscis rather late in development. In the five day parasite (Figs. 50–53) and also in the six day form (Fig. 11) its location is not yet definitely indicated. In the nine day form (Fig. 15) it is indicated by a slight enlargement of a ring of cells around the proboscis just in front of the division between the proboscis and body. In the 12 day stage the cells have become enormously enlarged, are located just outside of the muscle strands of the larval proboscis (Fig. 57) and remain connected with the hypoderm only at the extreme anterior end and on the ventral side (Figs. 67–69).

Entodermal derivatives. The young larval parasite possesses no entodermal derivative except the intestine and this develops directly from that of the larva. Its development is at first very rapid, so that in the five and six day stages (Figs. 11, 50–52) it makes up a large part of the bulk of the parasite and even in the nine and twelve day stages (Figs. 15, 66) it is relatively enormous.

Mesodermal derivatives. On account of the minuteness of all the cells and the indefinite staining reactions it has been impossible to connect the mesodermal derivatives definitely with larval structures. It is possible, however, to outline their appearance in the early parasitic stages.

In the five and seven day stages the muscles appear as minute cells between the intestine and the hypoderm (Figs. 50, 51, 54). In the nine day stage they are clearly outlined as a continuous layer of elongated cells lying just inside of the hypoderm (Fig. 55).

Since the parenchyma appears very late its origin will be taken up in the discussion of the later development.

The reproductive organs appear in the five day stage as a double row of cells on each side of the intestine, slightly dorsal in position, along the main part of the body (Figs. 50, 51). In the nine day stage (Fig. 55) and more clearly in the twelve day stage (Fig. 66) they appear as two definite ridges, just inside of the muscle layer, dorso-lateral to the intestine, and extend almost the entire length of the body.

Later development

From the nine day stage (Fig. 15) the parasite passes over into a spiral form and this soon straightens out into a loose spiral and finally a straight, cylindrical form with rounded ends. The straight form is often reached in twelve days. After that the parasites, tho usually coiled in the body of the host, are straight when relaxed in salt solution. In this they differ from parasitic Mermithidae which when relaxed, take the form of a helix. To the 28 day stage and beyond, the parasites are so transparent that it requires a dark background to see them. Later stages are white until the adult color develops.

Development takes place uniformly thruout the length of the body. This is shown in the stage of development of the nerve cord in figurers 29 and 72, taken from the middle and posterior end of the same specimen and in the development of the cuticula in figures 108 and 107 taken respectively from the anterior end and posterior end of a male in which the fibrous layer of the adult cuticula was in the process of formation.

In the following discussions the different tissues and organs will be taken up separately and their development traced to the adult structure. Comparisons with the results obtained by other authors will be taken up in a separate division of this report after the description of the different structures has been completed.

Cuticula. Earlier stages are covered only by the larval cuticula since the adult cuticula appears very late in development.

Larval cuticula. With the initiation of development there comes a decided increase in the permeability of the cuticula. While it is almost impossible to dehydrate and mount free living larvae without having them collapse, the early parasitic stages may be mounted with comparative ease. Together with this increase in the permeability comes an increase in the thickness of the cuticula. While in the larva and the very young form it appears only as a very fine line when magnified 800 times, in the twelve day stage it appears as a much heavier line at a magnification of only 500 times. Actual measurements give values of about 0.35μ foı the larva, 0.55μ for the five day stage, and 0.7μ for the twelve day stage. This increase in thickness continues at about the same rate until the time the nerve cord separates from the hypoderm and growth has already taken place in part of the germ cells. At that time the diameter is nearly two micra. Soon after that there appears directly beneath the cuticula a finely granular layer, stainable with iron hematoxylin and alkaline methylene blue. There now appears between the larval cuticula and the granular layer a more homogeneous layer (Figs. 85, 112) not stainable in iron hematoxylin (Figs. 108, 117) but heavily stained by aniline blue in Mallory's connective tissue stain. The larval cuticula remains connected with the granular layer by very fine strands (Figs. 38). At times there appear in this layer larger amorphous bodies which are probably due to the action of the killing agent. This homogeneous layer, as it may be called, attains a diameter of about 10μ at the time the tissues of the parasite have reached their full development and then begins to disintegrate. By the disintegration of this layer the larval cuticula becomes loosened from the underlying granular layer (Fig. 39), soon becomes torn, and is sloughed off from the body of the parasite when it is ready to leave the host. When fully developed parasites are taken from their hosts pieces of the larval cuticula are often seen trailing from one or both ends like transparent threads. The larval proboscis which has been lying just beneath the larval cuticula (Fig. 73) is also shed at this time. In some cases the deeper part of the intervening layer does not become disintegrated by the time the larval cuticula is shed and remains for a short time attached to the granular layer, but ultimately it becomes entirely removed. The structure of the larval cuticula is homogeneous thruout.

Adult cuticula. The earliest beginning of the adult cuticula is the formation of the granular layer under the larval cuticula as described in the previous section. This granular layer, which increases somewhat in thickness, but never has any very definite boundaries, forms the layer known as the homogeneous cuticula of the adult. The granules become crowded closer together so that they are not easily distinguishable.

The fibrous cuticula of the adult appears as a differentiation of the cytoplasm of the hypoderm some time after the formation of the granular layer, when the intervening homogeneous layer has already reached nearly half its final diameter (Figs. 112, 41, 35, 116). Thruout development the fibrous cuticula consists of fibrous strands connecting the granular layer with the hypoderm and of an intervening matrix (Fig. 43, 119). The intervening matrix is under the most favorable conditions resolvable into layers of fibers perpendicular to the radiating strands and forming nodules at the intersections with those strands (Fig. 43). The fibers composing these layers in the matrix are the rudiments of the ultimate diagonally intersecting fibers of the adult cuticula. The nodules at first appear to produce a thickening of the radiating fibers but later fuse along the diagonal fibers and separate from each other along the radiating fibers to form the heavy fibers of the adult cuticula. The layers of diagonal fibers are not formed in a regularly alternating series, but two layers in one direction alternate with one in the other (Fig. 38). This fact can be determined only on diagonal sections made parallel to the fibers of one of the layers. The number of layers of intersecting fibers is variable, but near the middle of the body it is about 45 (Fig. 38) and even on the prongs of the fork of the male it is seldom less than 30 (Figs. 121–123). Since in two adjacent layers of parallel fibers the fibers of one are of the same diameter as those of the alternating layer, but those of the other are much thinner, low magnification shows the two adjacent layers of heavy fibers as a single dark layer and between two such dark layers the thinner fibers as a lighter layer, thus giving the cuticula the appearance of being composed of ten to fifteen dark layers (Fig. 124).

The adult cuticula, macerated in nitric acid and separated into thin layers, shows clearly under low magnification the rhomboids formed by the intersection of the coarser lines (Fig. 5) and under high magnification the finer intersecting fibers (Fig. 4). The coarser lines enclosing the rhomboids are due to a slight increase in the thickness of the fibers as well as in the pigmentation.

The bristles of the adult cuticula, when they first become evident, are heavy radiating strands connecting the larval cuticula with the hypoderm (Figs. 38, 40). At first they are thick and translucent, but later they become shrunken and opaque, and it is impossible to trace them beneath the first layer of the fibrous cuticula. At the time of moulting they become detached from the larval cuticula and remain attached to the surface of the adult cuticula. The bristles pass thru the granular layer (Fig. 44) and consequently are not covered by the homogeneous cuticula of the adult.

The postcloacal ridge of the male is formed by elongated cells in the hypoderm (Figs. 37, 60, 61) and appears as a thickening in the granular layer. In the adult it appears to be continuous with the homogeneous cuticula and on cross section has the appearance of a stout hook set upon a projecting base of the fibrous cuticula and curved slightly backward and inward (Fig. 98).

Over the anterior end the fibrous cuticula develops in the normal way, but is not so thick as elsewhere. Later the fibres become more closely packed together, all granular substances disappear, and the cuticula under the white area becomes nearly homogeneous and transparent.

During the entire period of development the layers of the cuticula are pure white. Pigmentation begins when the homogeneous layer underlying the larval cuticula has already begun to disintegrate. Pigmentation begins first in the dark ring behind the anterior white area. It next delineates the dorsal and ventral bands, beginning at the anterior end and passing backward. By the time these bands are shown on about the anterior fourth of the body, pigmentation of the rest of the cuticula begins at the anterior end and slightly later also at the posterior end. At this end also the dark bands appear first, but are never so clearly outlined as at the anterior end. The bands from the two ends soon come together slightly posterior to the middle of the body and the pigmentation of the rest of the cuticula proceeds in the same order. In case of all specimens observed leaving their hosts the pigmentation could not be distinguished from that of free living specimens. If specimens in which the pigmentation is not complete are removed from their hosts the intensity of the pigmentation does not appreciably increase in the free state. Several such specimens were observed for short periods and one female with little pigmentation except the dark ring and bands was removed from the host on September 6, 1916, and kept alive in an aquarium until the beginning of March, 1917, with no appreciable increase in pigmentation. The specimen at that time died from an attack of fungus.

Hypoderm. In the specimens five day old the hypoderm is already clearly distinguishable as a layer of flattened cells, slightly thickened in the region of the nerve cord, and lying just beneath the cuticula (Figs. 50, 51). Partly by the thickening of the layer, but chiefly by the rapid multiplication of the cells, the latter have become cuboidal when the nine day stage has been reached (Fig. 55). By a continuation of the multiplication and the increase of the thickness of the layer the cells soon come to be columnar in character. This condition is clearly shown at the ends of the specimens in the twelve day stage, and appears over the entire body at slightly later stages (Figs. 84, 86). Multiplication of the cells appears to be complete by the time growth begins in the germ cells (Fig. 86) and further development depends upon growth. In some cases there is a secondary flattening of the cells before the development of the adult cuticula is initiated (Fig. 87), but whether this secondary flattering occurs or the cells remain columnar (Fig. 99), the small, round nuclei (Figs. 71, 73) become enlarged and flattened in a direction parallel to the surface of the specimen (Figs. 42, 99). The enlargement and flattening occur by the flowing together of several chromophil centers into one nucleus, the accumulation of achromatic substance around these centers, and the development of a definite nuclear membrane surrounding both (Figs. 70, 127). The chromatic centers remain as distinct nucleoli within the larger nuclei. As the adult stage is approached the nucleoli increase in size and become more diffused so as to occupy more or less completely the entire space within the nuclear membrane. At the same time the nucleus shrinks and becomes excessively flattened, crowding together the nucleolar matter into a dense mass (Fig. 124).

Altho in cross sections of the hypoderm the cells appear to form a syncytium with the cell boundaries merely indicated here and there (Figs. 41, 73), tangetial sections and preparations of separated pieces of hypoderm show distinctly the cell outlines (Figs. 127, 128). Such preparations, however, show that the cell boundaries are not complete, the cells remaining connected with each other by numerous protoplasmic strands. During the earliest stages of the formation of the fibrous cuticula there appears within the outer part of the hypoderm a system of canals surrounding the cells (Fig. 41) and, at the intersections, sending out branches to their bases.

The hypoderm is always of greater diameter at the ends of the body than in the middle. The elongated cells forming the postcloacal ridge of the male have already been mentioned. Other modifications of the hypoderm will be taken up in the next topic.

Nervous system. The nervous system consists of brain, ventral cord, cloacal ganglion, peripheral fibers and nerve cells located in various parts of the hypoderm.

Central nervous system. The very early appearance of the central nervous system has already been described. In the description of the later development each part will be taken up separately.

As stated in a previous section, the brain is outlined at first as a ring of cells in the hypoderm of the proboscis. It soon separates from the hypoderm, remaining connected with it only at the anterior end and the ventral side (Figs. 57, 67–69). It consists at this time of a few large cells situated just in front of the partition between the region developed from the larval proboscis and that developed from the body of the larva. The cells completely surround the larval muscles and the strand that connects the stilets with the partition in the larva. These large cells remain permanently in that position (Figs. 73, 74) while the rest of the brain develops in front and around them, most of the later cells appearing antero-ventrad to the original group. By the growth of the anterior region the larval connecting strand becomes stretched out and torn, the major part of it usually remaining in the base of the brain, while the armature of the proboscis is carried forward and pushed out of the hypoderm at the anterior end (Figs. 73, 22, 23). The strand in the base of the brain later disintegrates, leaving an open space (Fig. 74). At the time the cells have reached their full development the original group forms the postero-dorsal part of the brain (Figs. 81–83), while the other cells, mostly smaller in size, surround the rest of the brain and form a heavy mass at the ventral side continuous with the cells of the ventral cord (Figs. 73, 81–83). The central core of the brain is occupied chiefly by fibres with a few scattered cells. From the ventral cell mass a group of cells projects dorsad into the anterior part of the fibre mass and tends to become separated by intervening fibres from the underlying cells. Ventral and slightly posterior to this group of cells is a large, definitely outlined cross commissure (Fig. 74) dividing at each end into a ventral and a dorsal branch. The cells of the brain appear to be multipolar but that fact has not been definitely established.

The fibres in the brain pass in all conceivable directions, and many of them are directly continuous with those of the cord. At the anterior end under the white area and part of the dark ring the hypoderm is very much thickened, most of the cells are modified into bipolar nerve cells like those which connect the ventral cord to the hypoderm in the rest of the body, and on the ventral side these fibres pass directly over into the connecting fibres of the cord.

The ventral cord arises as a thickening in the hypoderm, as has already been shown, but later becomes separated from it, passing inward even beyond the muscle layer and remaining connected with the hypoderm only by a single row of cells (Figs. 58, 105).

The cells that later make up the nerve cord at first appear as two rows of larger cells in the hypoderm (Fig. 56) corresponding to the two rows of nuclei on the ventral side of the larva. Even in later stages these two rows of cells remain clearly distinguishable altho they crowd each other so that they come to lie alternately one behind the other and do not usually show in a single section. Between these two rows of cells and on each side of them appear very early in development three longitudinal fiber tracts (Figs. 56, 58), which are the rudiments of the three main fiber tracts of the nerve cord. Nerve cells later appear under these fibre tracts and on the two sides of them (Fig. 88), separating the tracts entirely from the rest of the hypoderm. By the growth of the cells under the median tract, that is pushed out beyond the two lateral tracts, and the division between the two rows of cells becomes nearly obliterated. The cord, after separating from the hypoderm, has in cross section the shape of a loop or fan with rounded corners, the cells forming the base and projecting far into the interior.

It has been impossible to determine the structure of the smaller cells. The larger cells, where their structure could be made out, have been found to be bipolar, giving off one fibre to the longitudinal tract and one to the dorsal border of the cord (Fig. 139). The longitudinal fibres as well as the radiating fibres stain very deeply with iron hematoxylin (Figs. 105, 106, 114) but the structures shown throw little light on the physiology of the nerve cord. The longitudinal fibres have been traced only for short distances (Fig. 102). In a number of cases fibres have been found to pass over crosswise from one part of the cord to the other (Fig. 114). Both the radiating fibres and the crossing fibres enlarge slightly toward the periphery and end abruptly at the edge of the cord.

In later stages the connection between the nerve cord and hypoderm consists of spindle-shaped, bipolar cells placed in close succession one behind the other in a single row, with nowhere an indication of a ganglion (Fig. 45).

In the male the ventral cord separates into two branches at the posterior end, a branch passing into each prong of the fork (Fig. 98) and ultimately disappearing in the hypoderm (Fig. 36). Beginning at about the point where the connection between hypoderm and cord becomes divided and passing backward to the cloacal musculature there is an enlargement of the cord, the cloacal ganglion (Figs. 101, 102). It consists chiefly of an increase of the fibrous part of the cord and hardly deserves the name of ganglion.

In the female an enlargement of the cord occurs near the posterior end, opposite the cloacal musculature (Fig. 92), while the cord is passing into the hypoderm. This appears as an enlargement of the cellular part of the cord with fibres passing into the musculature and the hypoderm surrounding the cloacal aperture.

Peripheral nervous system. The ordinary methods of technic show very little of the peripheral nervous system and consequently it will have to be dismissed at this time with a few passing remarks. In a few adults stained with iron hematoxylin nerve fibres were shown passing from the nerve cord into the hypoderm and could also be traced for some distance in the hypoderm (Figs. 115, 118, 120). The fibres pass directly into the hypoderm and, some distance from the cord, are seen to lie well within the hypoderm mesad from the nuclei. At the time of the formation of the cuticula it is usually possible to detect flask-shaped cells in the inner part of the hypoderm (Figs. 42, 129) and in a few cases fibres passing outward from these cells or parallel to the surface of the hypoderm.

Digestive system. The digestive system consists only of a straight tube beginning near the anterior end and opening at the posterior end. Neither mouth nor esophagus is present in this species at any stage of development. The structures that might be mistaken for mouth and esophagus have already, in the discussion of the brain, been shown to be merely the spaces previously occupied by parts of the larval proboscis.

As in the larva (Fig. 20) so in the young parasitic forms, the anterior part of the intestine consists of a solid mass of cells (Figs. 11, 15), and the lumen begins behind this cell mass. Later this cell mass disintegrates, as is shown in some specimens of the twelve day stage (Figs. 67, 68). By the time the twenty-eight day stage has been reached the space left by the disintegration of those cells has been filled by mesenchyme, thus the brain and intestine have become distinctly separated and the lumen of the intestine is closed by a single layer of cells. In some cases, however, the mass of cells does not disintegrate completely until a much later stage is reached (Fig. 73). In either case the mesenchyme cells soon invade the region between the intestine and the brain, so that in the later stages the two come to be separated by a solid mass of parenchyma equal in length to more than half the diameter of the body (Fig. 74).

Whether or not there is in the larva and young parasite an outlet of the cell mass thru the proboscis, can not be determined from the material at hand. A tube can be traced from the anterior end to the base of the stylets (Fig. 47) and in some cases appears to be indicated in the connecting strand behind the stylets, but on account of the extreme minuteness of the structures as compared with the thickness of the sections it is impossible to make a definite determination. But, even if there is a tube leading from the end of the stylets to the cell mass, it never passes thru the cell mass to the lumen of the intestine.

From the early stages until the adult cuticula has been nearly completed the walls of the intestine consist of a syncytium of heavy cells with large nuclei and with the cell boundaries only very faintly indicated (Figs. 50, 72, 73, 75, 79, 84, 86, 106). Around the outside of the intestine is a heavy membrane (Fig. 77), easily demonstrated when stained with Mallory's connective tissue stain. The inner edge of the wall is frequently differentiated into a loose, porous or spongy structure, with no definite membrane on the inner surface (Fig. 106). During the early part of the formation of the fibrous cuticula the cells of the intestine begin to decrease in size, in the adults they are excessively shrunken, and by the time the reproductive products have been discharged there is little left but the skeletal structure of the cells (Fig. 46).

At the posterior end the intestine in the very young forms opens slightly ventral (Figs. 11, 15), but by the twelve day stage has become nearly terminal (Figs. 63, 64). At that stage diverticula are formed at the points where the seminal receptacle and oviducts in the female and the sperm ducts in the male later enter the cloaca (Figs. 64, 65). The part of the intestine behind these diverticula is in the young stages lined by a heavy membrane and in the adult condition lined by the homogeneous cuticula (Fig. 78) and must be regarded as consisting of invaginated hypoderm. In the female the opening of the intestine remains terminal, but in the male it is again shifted to the ventral side. The lobes of the fork first grow out beyond the cloacal aperture (Fig. 59) and then by an overgrowth of the dorsal wall the aperture is turned to the ventral side (Figs. 60, 61). The larval cuticula does not enter the space between the prongs, but leaves this space to be filled out by a substance similar to the homogeneous intermediate layer, the intestine opening terminally thru a passage in this substance (Figs. 36, 60).

Excretory system At no stage in the development is there present any trace of an excretory system corresponding in any way to the excretory systems found in other animals.

Circulatory system. There exists no definite circulatory system, but there are present at all stages in development spaces in different parts of the body that undoubtedly aid in the distribution of the body fluids. Very early the intestine becomes surrounded by an open space, remaining attached to the other tissue only on the ventral side. This space is later invaded to a great extent by the parenchyma but is seldom entirely eliminated. It is the only space that is usually present. In the females a second space later appears on the dorsal side between the points of attachment of the ovaries. These spaces usually do not approach the ends of the body which are filled with parenchyma.

Muscles. In the very young forms the muscles appear as a layer of longitudinally arranged, spindle-shaped cells, lining the hypoderm (Fig. 67). The cells are at first rounded in cross section but soon become flattened by crowding against each other so that they appear as columnar cells in cross section with the nucleus lying close to the inner edge. As development goes on the cells become more and more flattened and elongated (Figs. 112, 117). When viewed from the edge the cells take on the appearance of very much elongated spindles with the two ends running out into fine points. From the side they appear as long blades with one edge straight and the other rounded at the ends (Fig. 126). When in position the blades are placed with the straight edge against the hypoderm and the nucleus is located in the middle of the opposite edge. The ends can easily be detected in cross section lying in the outer half of the muscle layer (Fig. 112). The nucleus, at first nearly round, later comes to be a very much elongated, flattened, oval body, lying either at the inner edge or near the inner edge of the cell, and occupying nearly the whole diameter of the cell at that point (Fig. 107).

The cytoplasm of the cell at first does not appear different from that of other cells but later there is formed a deeply staining granular substance extending from the nucleus to the outer edge of the cell; this substance finally forms longitudinal fibrils which arrange themselves in a continuous layer around the inner, spongy cytoplasm and the nucleus (Figs. 43, 105, 112, 124, 126). The fibrils are not of homogeneous structure, but are composed of serially arranged granules.

The cells at first are contiguous, but at the time the heavy walls appear in the parenchyma a substance having the same appearance and staining reactions as those walls surrounds each cell, so that the cells become separated from each other, from the hypoderm and from the parenchyma (Fig. 116). At the ends of the body the muscles gradually lose their characteristic structure and pass over into the parenchyma.

At the time of the discharge of the reproductive products the muscles begin to disintegrate slowly from the inner edge. In some specimens sectioned this process had consumed nearly the whole muscle cells (Figs. 46, 124).

The cloacal musculature of the male consists of radiating fibres around the cloaca and circular fibres surrounding the sperm ducts just before they enter the cloaca (Figs. 96, 97). In the early stages the cells are not differentiated from parenchyma cells, but later they become very much elongated and in the adults lack the heavy cell walls that are found in the parenchyma. The radiating fibers arise from the dorsal and lateral walls of the cloaca and extend for the main part in a dorso-lateral direction.

The cloacal musculature of the female consists of a heavy circular muscle forming the constriction between the cloaca and the sperm receptacle (Fig. 94) and weaker circular muscles around the oviducts. The fibers are similar to those of the male cloacal musculature. There is also present a heavy group of circular fibers around the posterior end of the cloaca, and a sheet of longitudinal fibers surrounding the glandular part of the cloaca. These fibers are intermediate in structure between muscle fibers and parenchyma cells. They are elongated, closely compact, but do not stain as deeply as muscle cells and possess walls nearly as heavy as those of the parenchyma.

Parenchyma and mesenteries. The parenchyma arises first as spindle-shaped or multipolar mesenchyme cells (Figs. 63, 67–69). Most of the cells appear to arise at the ends of the body, but a few cells are also found in the intermediate region at a very early stage and may arise there. In the twelve day stage many of the cells are not distinguishable from muscle cells between which they are frequently inserted, and even in much later stages the distinction between the two kinds of cells is not always clear. The cells remain generalized for a long time and are scarcely distinguishable from cells that form the rudiments of other organs (Fig.109).

The multiplication of the cells takes place very rapidly. In the male they often fill completely the spaces between the organs. Except at the ends, where they form a solid mass from the beginning, they first form an irregular layer lining the muscles and the nerve cord and forming triradiate septa which enclose the germ cells. Ventral to the germ cells the cavity still remains and the intestine is attached to the layer of cells over the nerve cord (Fig. 75). By further multiplication of the cells the germ cells become farther removed from the muscles and all or nearly all of the space becomes invaded (Figs. 107, 108).

In the females the multiplication of the cells is not so prolific. The layer lining the muscles and nerve cord and holding the intestine in place is formed as in the males, but it passes between the muscles and the ovaries only at the end of the body, leaving the ovaries in contact with the muscles thruout nearly their whole length. A few mesenchyma cells enter between the ovaries and others are scattered thruout the body at different places, but there are no definite layers enclosing the germ cells (Figs. 76, 79, 86). By the later growth of the eggs nearly all of the spaces in the body are eliminated.

A short time before the adult cuticula is formed the cells become surrounded by heavy layers of a hyaline substance that is stained with aniline blue in Mallory's connective tissue stain. The cells then lie in cuboidal, rounded, or polygonal chambers completely isolated from each other (Figs. 112, 116). Very soon the cells become shriveled, leaving only the heavy walls with here and there a fragment of a nucleus or of cytoplasm.

The layer of parenchyma immediately surrounding the nerve cord deserves special mention on account of its peculiar mode of development. In early stages, when the nerve cord is merely a thickening in the hypoderm, this layer is continuous with the muscle layer lining the rest of the hypoderm and can at first not be distinguished from that layer (Fig. 84). Even when fully developed these parenchyma cells are narrow and very much elongated and in that respect resemble muscle cells (Fig. 102). These cells are themselves covered by the layer of mesenchyme cells that later lines the muscles. Ultimately, however, there is no difference between the prenchyma cells that originally covered the nerve cord and those that later migrated over them, the one passing gradually into the other (Fig. 106).

At times the intestine becomes separated from the underlying layer and remains attached to it by a longitudinal sheet of cells of single thickness, the ventral mesentery. The intestine adheres to a broadened surface of the terminal cell, but its outer membrane is in no way continuous with the covering of that cell. The structures by which the egg masses appear to be suspended in the older females are not mesenteries but the remains of the ovaries and will be discussed in the next section.

Reproductive organs. In the early stages one can find no difference between males and females. The first difference appears in the character of the diverticula formed at the posterior end of the intestine. In the female three diverticula are formed (Fig. 109), one for the seminal receptacle and two for the oviducts, while in the male only two appear for the sperm ducts (Fig. 29). These diverticula have made their appearance before the 28 day stage (Figs. 64, 65). Somewhat later the ovaries become differentiated from the testes by the formation of buds along the ventral sides (Figs. 76, 84, 86). These buds are not always opposite in the two ovaries nor are they of uniform size (Fig. 62).

Each ovary at first is enclosed by a definite, heavy membrane, but later the membrane becomes thin at the buds and the eggs pass into the body cavity soon after they have begun the growth period (Figs. 76, 84, 86). The eggs, however do not lie loose in the body cavity, but continue to be enclosed in thinner membranes of the ovary. By the time the first eggs have reached their full size nearly all of the germ cells have left the ovarian tubes and have in masses, strands, or sheets become distributed among the developed oocytes which tend to form layers around them. As the increase in the diameter of the body continues, the ovarian tubes become broadened dorso-ventrally, and by the discharge of the germ cells they become flattened, so that they take the form of broad, thin sheets suspended from the dorsal muscles (Fig. 79). In later stages they remain as double membranes still helping to support the masses of eggs in the body cavity. The extensions of the membranes at the buds have become thrown into many folds and have been thickened in places to help in the support of the ovarian mass. At the time of the discharge of the eggs the membranes become ruptured and the heavy parts remain attached to the dorsal muscles (Fig. 46).

The growth period in the oocytes begins somewhat before the homogeneous layer is formed under the larval cuticula and continues until the formation of the adult cuticula is well under way (Figs. 76, 86, 77). It does not occur simultaneously in all of the germ cells but progresses from the buds toward the dorsal part of the ovary. The full-grown oocyte is about 0.023 mm. in diameter and is surrounded by a definite membrane. At the center of the cell is found a large nucleus, while the cytoplasm is filled with yolk granules. The nucleus has a diameter of about 0.006 mm. and consists of a large, central chromophil mass surrounded consecutively by an achromatic and a chromatic layer (Fig. 106).

Toward the anterior end the ovaries gradually decrease in size, the space surrounding them, even on the dorsal side, becoming filled with parenchyma. In some cases the invading parenchyma interrupts the ovaries at places, forming bead-like masses following each other in longitudinal series. The formation of these masses is even less regular than the location of the buds on the ventral sides of the ovaries.

At the posterior end the oviducts are formed by continuations of the ovaries and they unite to form the cloacal gland and the seminal receptacle (Figs. 109–11). It has been impossible in the very early stages to recognize the germ cells in this region, but at the time the ovaries are still round they pass without interruption back to the point of union and form a mass of cells, the rudiment of the cloacal gland, around the ventral side of the intestine. At that stage the lateral diverticula connect with these cells near the latero-posterior margins of the mass while the ventral diverticulum joins them somewhat anterior to those points. The seminal receptacle exists as an anterior lobe of the cell mass. The membrane surrounding those structures is continuous with those surrounding the ovaries and no difference is apparent in the character of the cells of the cloacal structures and the ovaries, either in staining reaction or in structure.

Later the cells become modified so that it is impossible to distinguish between those that have been derived from the intestine and those derived from the germ glands. The oviducts become indistinguishable behind the points where they enter the cloacal gland. Anterior to those points the cells in the oviducts rearrange themselves so as to form definite walls around the central ducts (Figs. 88–90, 93–95). Even at that stage the change from oviducts to ovaries is a gradual one, the oviducal walls continuing farther anteriad on the ventral side than on the dorsal.

The cloacal gland takes up a median position beginning somewhat posterior to the points at which the ovaries were previously inserted into the intestine and extending anteriad to the seminal receptacle, into which it opens broadly (Figs. 78, 93–95, 99, 100). The intestine opens into the dorsal side of the gland near its posterior end, the oviducts open into the latero-ventral sides near the anterior end. The cells of the gland become closely packed and form finger-like projections that extend inward and forward into the seminal receptacle. The large muscle surrounding the posterior part of the gland has been described under the topic of cloacal musculature.

The seminal receptacle extends forward as an elongated sac with definite walls similar to those of the oviducts at their posterior ends. It is empty and distended, occupying in cross section fully one half the diameter of the body and extending lengthwise over a space equal to three or four times that diameter. The intestine passes around to the ventral side of the body before the end of the seminal receptacle has been reached.

The testes in the males remain as cylindrical or somewhat triangular tubes extending nearly the whole length of the body (Figs. 72, 80, 107, 108). They are enclosed in heavy membranes and very early become completely surrounded by parenchyma cells. As in the ovaries so in the testes there are never any traces of cellular walls.

At the anterior end the testes are subject to bead formation similar to that in the ovaries. The sperm ducts are posterior extensions of the testes, opening into the intestine a short distance anterior to the anus, and in the early stages in no way distinguishable from the testes (Figs. 29, 70–71). Cellular walls for the sperm ducts are formed only within the cloacal musculature, anterior to that all of the cells develop into spermatozoa. The intestinal diverticula as in the case of the females consist of nothing more than a turning out of the walls of the intestine at those points.

The transformation from spermatocytes to spermatozoa begins at a slightly later stage in development than does the growth of the oocytes in the female. It takes place almost simultaneously in all parts of the testes and is completed before the adult cuticula has been fully formed.

The chromatin rod appears as a semicircle at one side of a rounded cell, but later becomes straightened and takes up a median position while the cell becomes spindle-shaped. At the same time the cytoplasmic contents pass to one end leaving the rod at the other end surrounded by only a thin layer of cytoplasm, the thickest part of the spindle being either at or even beyond the end of the rod (Figs. 12, 13, 18, 19). In this form the spermatozoa leave the male. Before they pass into the body of the female they become somewhat elongated (Fig. 10). In the seminal receptacle of the female the cytoplasmic part of the spermatozoon elongates into a heavy flagellum of uniform diameter while the rod remains as a slightly thickened head at one end (Fig. 17).

OBSERVATIONS ON PARAGORDIUS VARIUS

On account of the excellent description of the adult organization of this species by Montgomery in 1903 and on account of the general similarity in the development of this species and the one just described, the following description will be made as brief as possible.

DETERMINATION OF THE SPECIES

Nothing needs to be added to the descriptions of this species given by Montgomery (1898, 1903) except that here also, as in *Gordius robustus*, there are present two longitudinal, darker bands; a broader dorsal and a narrower ventral band. They are even more distinct than in the previous species.

HABITS OF THE ADULTS

This species prefers quiet water to rapids and more frequently inhabits lakes than streams. It is not very abundant in the waters about Urbana, but is the common species reported from the Great Lakes region. Nothing has been observed in regard to the winter habitat. The earliest specimens were taken near Urbana the latter part of May, 1914. At Douglas Lake large numbers were emerging from their hosts the latter part of June, 1915. While at Urbana both males and females were found in the grass at the water's edge, only males were found in similar positions at Douglas Lake. Females that had emerged during the night could still be found swimming near the shore, but during the entire summer of 1915 only a few females were found that had wound themselves around grass and had laid eggs.

Females that have just emerged, while swimming near the shore, soon encounter males and copulation takes place. The deposition of eggs begins the following day. On June 27, 1915, an adult female was removed from a host. It was kept alone in an aquarium until the 29th, when it was placed with a male in a large vial and mating was observed. The next morning it was found to have laid a string of eggs. Another female removed from the host on June 30 and mated on the same day laid eggs July 1. Other similar cases were observed.

Mating was observed in several cases. The process is in every respect similar to that described for *Gordius robustus*. The male more actively responds to the stimulus from the female and the discharge of sperm is almost instantaneous. There is again no choice of direction and discharges of sperm may in some cases take place at other parts of the body of the female than the posterior end. There is no interlocking of the lobes at the posterior ends of the two specimens, and after copulation the spermatozoa remain in a large mass enveloping the lobes of the female, but pass into the seminal receptacle in less than a day.

On July 9 a mutilated male was obtained from the lake. It consisted of the posterior end of the body with more than a third of the body removed from the other end. A female was placed with this male and after a few hours showed a mass of spermatozoa at the posterior end.

The eggs are deposited in long strings about 0.2 mm. in diameter, and adhere very tenaciously to each other. Females will deposite eggs in aquaria just as freely as in nature. When grass or other objects are present the strings are wound around them, otherwise they are deposited in large, tangled masses.

Males and females that have discharged their reproductive products die and disintegrate in the manner described for the previous species.

EARLY DEVELOPMENT

The observations made on the development and structure of the larva are, for reasons stated before, only fragmentary and can not be included in this report.

PARASITISM

Like *Gordius robustus,* this species enters a host as larva and undergoes its entire development in the parasitic stage.

Hosts. Both at Douglas Lake and Urbana parasitic stages were found in adults or older nymphs of *Gryllus assimilis* (Fabricius) as defined by Rahn and Hebard (1915). At Douglas Lake they were also found in *Nemobius fasciatus* (DeGeer). The specimen staken from Nemobius were as a rule smaller and shorter in proportion to their diameters than were those taken from Gryllus.

Altho the larvae penetrate the tissues of various species of aquatic animals it has been impossible to determine if any or all of these animals may serve as intermediate hosts. If some of them do serve as intermediate hosts they must serve merely as carriers. The delicate tissues that appear as soon as the larva begins to change into the parasitic form make it impossible for a further change of hosts to take place without causing the destruction of the parasite. Attempts to infect the hosts artificially proved unsuccessful.

Infection. Only two infected hosts were taken at Urbana. At Douglas Lake in 1915 adults were emerging from the hosts in large numbers the latter part of June and young parasitic stages were still found by the middle of August. Early parasitic stages were obtained thruout the entire summer.

Infection in this species also was extremely local and slightly heavier in females than in males. Of 125 males of *Gordius assimilis* collected on the hill just above the laboratory and within half a mile of the lake 6, or 5 per-cent, were infected, yielding 7 parasites. Of 152 females collected at the same place 24 were infected, making an infection of about 17 per cent and yielding 31 parasites. In collections of the same species made near the shore 154 males yielded 35 infected specimens or an infection of 23 per cent and contained 52 parasites, 276 females contained 135 infected specimens or an infection of 49 per cent and yielded 377 parasites, averaging more than two parasites to each infected host. During the latter part of the summer 37 large nymphs were collected on the hill about two miles from the lake and not a single one was found to be infected. The specimens of *Nemobius fasciatus* were all collected near the shore. Of 15 males 8 were found to be infected, yielding 16 parasites, and of 24 females only 6 were infected containing 12 parasites.

The habits of this host also make an intermediate carrier not necessary. I have several times found crickets accumulated in large numbers about pools of water at night. The fact that many unmated females of *Paragordius varius* were found in the shallow water of the lake during the early forenoon, but disappeared later, indicates that infected hosts get into the water and lose their parasites during the early morning. The local character of the infections shows that infection takes place at or near the water's edge.

Location in the host. Early developmental stages of *Paragordius varius* have not been found in sections of hosts. In later stages the parasites lie free in the body cavity (Fig. 134). In dissections the location in the host is found to be in every respect similar to that of *Gordius robustus*.

Practically no effect of the parasite on the host was found except in cases of very heavy infection. In those cases a diminution in the size of the reproductive organs could be detected.

Length of parasitic period. Since experiments were unsuccessful, no direct observations on the developmental period could be made, and since infection took place almost uniformly thruout the summer, the time of infection and time of emergence could also not be taken as criteria. The earliest appearance of adult parasites in nymphs, however, produced some valuable evidence on the subject. Most of the nymphs of Gryllus appeared during the last week in June and Nemobius did not hatch until about a week later. By the middle of August adult parasites were found in the nymphs of both species. The first adult *Paragordius varius* from *Nemobius fasciatus* was obtained on August 14 while the host was still a nymph. The developmental period can not possibly have been more than six weeks. Some of the first infected specimens of *Nemobius fasciatus* were obtained on August 2, and the parasites at that time were mostly younger than the 28 day stage in *Gordius robustus*. On August 12 most of the parasites were of nearly adult size.

Emergence in this species occurs in a manner similar to that in *Gordius robustus*. About six specimens were seen leaving their hosts. In all cases the parasites emerged with the anterior end first from near the anus of the host. The definite reaction toward the presence of water was again observed. On July 12 a male of *Gryllus assimilis* was found to have the anterior end of a female of *Paragordius varius* protruding from the posterior end when it was caught. The cricket was placed in a dry vial and the parasite withdrew and remained in the host until the latter was placed in water in the laboratory two hours later. In the water the parasite left the host in less than five minutes. The emergence was witnessed by Dr. W. W. Cort and Mr. A. C. Conger. Other similar cases were observed during the summer.

ORGANOGENY

On account of the lack of material in the early stages little can be said about the metamorphosis and derivation of tissues in this species. Only two young specimens were obtained and they were mounted as totos (Figs. 130, 131).

Cuticula. Stages in its development in this species are even more difficult to obtain than in *Gordius robustus* indicating that the cuticula develops with extreme rapidity.

Larval cuticula. It was in this species that the shedding of the larval cuticula was first observed. Nevertheless, its presence is difficult to demonstrate during the development of the adult cuticula. It is clearly evident in younger stages and changes in size and thickness during development just as does that of the previous species. But no homogeneous layer appears under it before the beginning of the development of the adult cuticula. Much later, when the fibrous cuticula has almost reached its full development, a very thin, homogeneous layer appears under the larval cuticula (Fig. 167) but is difficult to distinguish because it adheres closely and has nearly the same density and staining reactions. The larval cuticula later separates from this layer (Fig. 163) and comes off in large sheets, being retained longest at the ends. Adult specimens removed from their hosts frequently show these pieces of the larval cuticula trailing from the two ends. When such sheets of larval cuticula are mounted and stained they show a perfectly homogeneous structure.

Adult cuticula. The development of the adult cuticula commences with the formation of a rather indefinite granular layer under the larval cuticula, but almost simultaneously there appears under this a lighter layer which is the beginning of the fibrous cuticula. The granular layer is the rudiment of the homogeneous or non-fibrous cuticula and areolar structures of the adult. Fibrous cuticula and areolar structures develop simultaneously (Figs. 161, 169). The stains employed failed to bring out any structures in the fibrous cuticula during its development. The hypoderm cells often give off conical projections into the developing cuticula and the apices of the cones can sometimes be seen to extend to the granular layer (Figs. 166, 167). At the points where the apices reach the granular layer the latter becomes thickened and in it are developed hyaline bodies, usually two at each point (Figs. 104, 161, 169). These hyaline bodies later become oval in outline and give the cuticula its areolated appearance (Fig. 160). The homogeneous cuticula, which always remains more or less granular, is formed from the granular layer chiefly over these bodies, but also between and under them. The connections between the granular layer and the hypoderm remain as definite protoplasmic strands, coming to the surface usually between the oval bodies (Figs. 1, 160, 164). At intervals heavier protoplasmic strands pierce the fibrous cuticula, and over them the granular layer thickens to form the short cuticular tubercles or bristles which are found especially in two ventral rows, but also sparingly scattered over the rest of the body (Figs. 140, 164). Where the heavier strands pass thru the cuticula the fibers remain separated to form a cross (Fig. 1). At the sides of the male cloacal aperture the bristles in the two ventral rows are very much elongated. Anteriad from that point they gradually shorten, posteriad they remain high to the bases of the prongs and then become shorter and are scattered over the inner ventral surfaces of the prongs (Fig. 133). The oval bodies are absent on these surfaces.

The outer surface of the homogeneous or non-fibrous cuticula is usually hyaline in appearance, while the granules remain evident in a lower layer which indefinitely grades into the hyaline layer, and at places may be absent or at others may reach the surface. The bristles are composed chiefly of the hyaline substance, but usually show at the base a cone of the granular material. Since the granular substance stains deeply it has been impossible to trace the protoplasmic strands farther than to the bases of the bristles.

Fibers do not appear in the cuticula until it has reached nearly its full diameter. They are wound spirally around the body in two directions so that they cross each other forming antero-posterior angles of about 175 degrees and lateral angles of about 65 degrees (Fig. 1). Since the fibers are all of the same size and the layers alternate regularly, cross sections of the cuticula do not present the appearance of stratification so obvious in the cuticula of *Gordius robustus* unless the sections are made nearly parallel to one set of fibres. The number of layers of fibers is variable, but seldom exceeds 24. Montgomery reports only 11, but his figures show that he made his counts on sections parallel to one series, and counted only alternate layers. Over the white surface at the anterior end the fibers become more closely packed and tend to form a homogeneous mass.

The color appears in the manner described for *Gordius robustus* and reaches its full intensity before the parasite leaves its host. On June 30 a female was removed from the host when it was still incompletely colored. The dark ring and the dorsal and ventral bands were clearly outlined, color had begun to appear over the rest of the cuticula at the two ends, but the middle region of the body was still white. The specimen was kept alive until July 26, when it was attacked by fungi and had to be killed. It had been mated and had laid some eggs, and had been kept in an open glass dish in the window where the sun shone on it part of the day; but the color had not noticeably changed except that the white had become soiled.

Hypoderm. The hypoderm in this species develops very much as in *Gordius robustus.* The cells at first are flattened, very early become columnar, and after the completion of the adult cuticula become flattened again over most of the surface of the body. They remain higher at the two ends. At the anterior end, under the white surface, they project far into the interior, becoming rod-shaped or almost fibrous in nature. The nuclei remain in the outer halves of the cells while the lower parts become clear and form a distinct mass just anterior to the supraesophageal ganglion. These hyaline bases of the cells later disintegrate or else form a substance that is dissolved during the preparation of the mount (Figs. 153; 154).

Protoplasmic connections between the hypoderm cells are present in this species as in the previous one but no canal system has been found. The cells are in cross section more easily distinguishable than in *Gordius robustus.*

The nuclei from the first are more distinct than in *Gordius robustus* and each consists of a large central nucleolus surrounded by an achromatic sphere and a somewhat indefinite membrane (Figs. 165,170). Later the membranes become more distinct, but the nuclei do not become so definitely outlined as in *Gordius robustus* and the fusion of chromatic spheres occurs only in very few instances. During the formationof the adult cuticula the chromatic substance increases in quantity and becomes scattered thruout the nucleus (Fig. 167). In the adult stage the nucleus shrinks and the chromatic substance forms one or two discs, almost completely filling the membrane.

Nervous system This system is built on the same fundamental principle as in the previous species, but while some parts stand out clearer, the struc ture of the others is not so easily brought out.

Central nervous system. The brain in this species also appears in the posterior part of the proboscis, but does not begin its development until even later than in *Gordius robustus.* The rudiment of the brain appears as a group of deeper staining cells around the connecting strand between the stylets and the base of the proboscis (Fig. 162) but the cells soon lose their staining properties and become indistinguishable from the mesoderm cells which surround them (Fig. 151). The bundles of nerve fibres leading from them can, however, be distinguished. Even at a much later stage, when the nerve cord is beginning to separate from the hypoderm, the ganglion cells are difficult to distinguish from the rest.

At that time the main group lies in an indefinite mass over the esophagus, just anterior to the end of the nerve cord, and is connected with the cord by two large commissures passing around the esophagus. Under the esophagus, at the very end of the cord, a smaller group of ganglion cells is more easily distinguishable.

In further development the dorsal group becomes completely isolated from other tissues, remaining connected only with the nerve cord by the commissures and in some cases also with the anterior hypoderm by scattered, half disintegrated fibres (Fig. 153). No marked change occurs in the ventral group. At the sides of this group fibres pass anteriad to the anterior hypoderm cells. The fibres usually become more or less definitely separated into two ventral and two lateral tracts.

The ventral cord arises and develops as described for *Gordius robustus* (Figs. 135, 146, 147, 158). The cellular elements do not all remain under the fibre tracts, pushing up into them as they did in *Gordius robustus*, but grow up over the sides, partly enclosing the fibres and leaving them in contact with the parenchyma over only about one third of the circumference, in later stages even less (Fig. 141). On account of this overgrowth of the cells the cross fibres do not present a radiating appearance but cross each other at various angles within the cord. There are, how ever, two longitudinal rows of heavier fibres originating from the two primary cell rows and passing to the dorsal side of the cord, dividing the longitudinal fibres into three main tracts (Fig. 159).

The structure of the large cells in the two primary rows is more easily demonstrated in this species than in the previous one. The cells are bipolar, giving off one fibre to the dorsal wall of the cord and another to the longitudinal fibre tract (Fig. 139). The body of the cell is rounded or flask-shaped and the two fibres are given off at one side. Some smaller cells were also found to be bipolar and of similar structure, but in case of the majority of the smaller cells it is impossible to make out the exact structure.

No cell bodies can be distinguished in the connection between the nerve cord and hypoderm after the two have separated, but fibres can be traced thru it from the cord to the hypoderm. At the point where the fibres from the cord enter the hypoderm longitudinal fibres are frequently found, and these fibres in some cases separate to form a longitudinal canal, the subneural canal of European workers.

In the male the nerve cord ends at the posterior end as it does in the previous species. The cloacal ganglion consists of a slight thickening of the cord beginning somewhat anterior to the musculature of the sperm ducts and extending back to the point of bifurcation of the cord (Fig. 164). It is intimately connected with the musculature of the vasa deferentia.

The cloacal ganglion in the female is similar to that found in *Gordius robustus* but presents some modifications on account of the posterior extension of the dorsal and lateral lobes. The nerve cord ends at the beginning of the lateral lobes and is partly inturned with the hypoderm that lines the cloaca. On account of this inturning the cord passes into the hypoderm not on the external body surface as it does in *Gordius robustus*, but in the ventral wall of the cloaca. Here the longitudinal fibers pass into the hypoderm and to a less extent than in the previous species they also pass around to the dorsal side of the cloaca.

Peripheral nervous system. Just as in the previous species the peripheral nervous system consists of fibers and cells in the hypoderm. Since these structures were clearly described by Montgomery, no detailed account of them will be given here.

Digestive system. This system is like that found in *Gordius robustus*, but in the adult condition does open to the exterior at the anterior end.

Mouth and esophagus. In this species the strand of cells which connects the stylets to the base of the proboscis remains attached to the anterior end of the intestine and is not only retained in the tissue, but actually undergoes development. At first it is a very short connection between the part of the proboscis that does not develop and the anterior end of the intestine, but as development proceeds it elongates and thickens, forming a bulb-like enlargement in front of the intestine with an elongation projecting dorsally over the end of the latter (Figs. 151, 153–4). It passes thru between the dorsal and ventral cell groups of the cephalic ganglion and between the two commissures. In the young stages it has been impossible to demonstrate definitely the presence of a tube in this strand. Altho some sections give the appearance of the presence of a capillary tube, this tube, if present, is so small and its walls so indefinite that it cannot be traced. At about the time the adult cuticula is formed most of the cells composing the strand disintegrate, forming a tube about which the parenchyma cells form fairly definite walls. Some of the outer cells of the strand usually also remain intact and take part in the formation of the walls (Figs. 153–4). By the loss of the proboscis, when the larval cuticula is shed, the tube is opened at its anterior end.

Intestine. The development of the intestine proceeds in this species much as it does in *Gordius robustus*. Here also diverticula are formed to receive the oviducts and sperm ducts (Figs. 148, 165). The cells of the intestinal walls are more easily distinguished than they were in the previous species, and an inner, vascular zone is never differentiated. The modifications at the posterior end will be taken up in the discussion of the reproductive system.

Excretory system. This species also presents no trace of an excretory system

Circulatory system. No vessels are present, but longitudinal cavities are present in this species as they were in *Gordius robustus*. Here also the main cavity lies around the dorsal part of the intestine and may be divided by the gonads into a median canal over the intestine and two lateral canals, or the median canal may be absent (Figs. 145, 158). In the males frequently the parenchyma fills the entire space, leaving no cavities around the intestine. In the females there is usually also a cavity on the dorsal side between the ovaries. This is seldom present in the males. In later stages, when part or all of the reproductive products have been discharged, other cavities appear in both males and females.

Muscles. As in the previous species, these consist of a cylinder of longitudinal fibers just beneath the hypoderm and of the cloacal musculatures. The cylinder of longitudinal muscles is interrupted only on the ventral side by the connection between the nerve cord and the hypoderm, and is lost in the parenchyma before the extreme ends of the body are reached.

Longitudinal muscles. The longitudinal muscles begin their differentiation slightly later than they do in *Gordius robustus*. In the youngest specimens sectioned they are still similar to mesenchyma cells, and appear rounded or cuboidal in cross section, but have the shape of short spindles when viewed from the side (Figs. 147, 155, 156, 165). Later the ends elongate, and the main bodies of the cells become crowded inward by the intercalation of the elongating ends near the hypoderm. Soon, however, the bodies of the cells also elongate and the diameters become nearly equal at the inner and the outer edges. The ultimate shape of the muscle cell is essentially the same as in the previous species, but the cell is even more elongated and the nucleus is very much elongated so that it extends even into the narrower parts of the cell (Figs. 6, 158, 174). The adult muscle cell shows a layer of longitudinal fibrils, similar to that of the previous species, lying just inside the cell membrane and completely surrounding the remainder of the cell. Some of the cells that are crowded inward by the nerve cord and come to lie at the side of the connecting lamella develop into muscle cells, so that some of the muscles appear to be inserted on the lamella.

Cloacal musculature. The radiating cloacal musculature of the male, so prominent in the previous species, is lacking in *Paragordius varius* except for a few longitudinal fibres at the posterior side of the cloaca which are clearly continuous with the longitudinal body muscles.

The circular muscles around the sperm ducts are located a short distance anterior to the cloaca and are more highly developed than in the previous species (Fig. 164). They develop from mesenchyme cells (Fig. 142).

As in the female of *Gordius robustus*, so in the female of *Paragordius varius*, circular fibers are found chiefly around the duct connecting the cloacal gland with the seminal receptacle, but also surrounding the gland itself and in a thin sheet even surrounding the cloaca behind the gland. Very thin layers of these fibers also surround the oviducts. The fibers develop from mesenchyme and in the later stages have heavy walls similar to those of the parenchyma.

Parenchyma and mesenteries. The parenchyma arises as in the preceding species, but fills the body cavity more completely in the early stages. It not only forms the lining for the muscles and surrounds the testes in the males, but it also surrounds the ovaries in the females. As the ventral buds in the ovaries appear and the eggs fill the body cavity, the inner walls of the ovaries become extended around the intestine to the sides of the nerve cord, and in that way the two mesenteries are formed (Fig. 158). The outer walls of the ovaries are turned back upon themselves at the points where the buds arise, and thus double lamellae are established reaching from those points to the muscles on the dorsal side of the body. There the layer of each lamella that lies next to the original ovarian tube is continuous over that tube with its inner wall, while the outer layer of the lamella is continuous with the outer covering of the ovarian bud, passing close to the lining of the muscles, around to the ventral side to join the ventral edge of the mesentery, or inner wall of the bud. As development proceeds, the germ cells leave the primary ovarian tubes, just as they do in *Gordius robustus*, and the outer walls of these tubes appear as parts of the mesenteries. As a result the mesenteries in later stages appear to be composed of three layers of cells in the dorsal part of the body, but of a single layer in the ventral part (Fig. 159). Even in the males the parenchyma forms about the gonads more definite layers than were found in *Gordius robustus* (Fig. 174).

During the formation of the adult cuticula heavy walls appear about the parenchyma cells, in many cases much heavier than in the previous species (Fig. 164). The cells are normally somewhat elongated, polyhedral or barrel-shaped (Figs. 3, 164), but may in other cases have the irregular polyhedral form found in *Gordius robustus*. The cells in this species remain more intact and more completely fill the spaces.

In the anterior end, behind the dorsal group of ganglion cells, the mesenchyme cells become crowded together very closely and form a capsule which encloses the ganglion cells on all sides except that covered by the flattened anterior surface (Figs. 153–4). This capsule is formed just before the heavy cell walls appear. Its adult structure has been adequately described by Montgomery.

Reproductive organs. The germ cells arise as in *Gordius robustus*, and in early stages can not be distinguished from mesenchyme cells in other parts of the body (Fig. 136). In the middle of the body the gonads assume a definite shape before they become surrounded by mesenchyme cells, which at that time have become easily distinguishable (Figs. 147, 156, 157). After that the mesenchyme proliferates very rapidly and completely envelopes the gonads with definite layers. The gonads of the two sexes can not be distinguished in the early stages.

Development of the testes in the male proceeds as described for *Gordius robustus*. The membranes enclosing the testes are usually not so heavy as in that species and the cellular parts of the sperm ducts extend both anterior and posterior to the short muscular areas.

The spermatozoa develop as in the previous species except that the head and cytoplasmic parts become more definitely separated before the axis of the cell becomes straightened, causing the spermatozoon to be doubled upon itself when it is first formed (Fig. 137). Head and cytoplasmic part are definitely separated from each other. In the seminal receptacle of the female the cytoplasm elongates into the heavy flagellum of uniform diameter (Fig. 138).

The reproductive organs in the female develop very much as they do in *Gordius robustus*. The membranes forming the primary ovarian tubes are not so heavy as in that species. The cloacal structures arise essentially as in *Gordius robustus*, but show modifications in certain details.

The part of the cloaca lined by the invaginated hypoderm forms a tube at the posterior end of the body equal in length to at least twenty times the diameter of the body at that point. The intestinal diverticula arise a short distance anterior to the point of union between ectoderm and entoderm and are less distinct than in *Gordius robustus*, appearing only as the points at which the intestinal wall begins to become modified. The oviducts develop from the posterior ends of the primary ovarian tubes and unite to form the rudiment of the cloacal gland and seminal receptacle The receptaculum seminis becomes distinctly separated from the gland, remaining connected by a narrow neck (Fig. 168). The ovaries open at the sides into the anterior end of the gland and the intestine opens into it on the dorsal side at the posterior end, or perhaps it would be more correct to say in this case that the gland opens into the ventral side of the intestine. (Figs. 168, 171).

At the point of union between entoderm and ectoderm a constriction or valve appears in later stages (Fig. 171). The inner ends of the cells lining the cloaca between the valve and the cloacal gland secrete a clear substance that almost completely fills the lumen of the tube. A similar substance is secreted by the cells lining the oviducts. Radiating from the cells are thin membranes apparently enclosing the substance, and a heavier layer surrounds the remnant of the lumen. In the adult stage the inner part of the secretion is swept away and little remains except the bases of the membranes which Montgomery regarded as cilia. The hypoderm of the posterior region of the cloaca also secretes a hyaline substance which passes in long threads thru cuticular pores into the lumen (Figs. 7, 171). This substance disappears at the time of the entrance of the spermatozoa and may aid in their migration into the seminal receptacle, but the secretion of the substance continues after insemination.

The epithelium of the cloacal gland at the time of the formation of the adult cuticula develops projections in which the cells appear like buds on central stalks. (Fig. 163.)

In specimens that have deposited their eggs the cells become shriveled and the body cavity contains only the nerve cord, a very small intestine, and the parenchyma membranes, which now have all taken more or less a dorso-ventral position and tend to flatten the body in that direction (Fig. 103).

DISCUSSION

In the following discussion I shall compare briefly the results obtained in the present investigations with those obtained by other authors and give interpretations of some of the facts observed. In the comparisons I shall confine myself almost exclusively to the more recent literature. The lack of proper methods of investigation makes the reports of the older writers of little value except as historical documents.

BIOLOGY

Altho the behavior of the Gordiacea has attracted the attention of all workers who have obtained living material, the observations have for the most part been fragmentary and have yielded little that is of scientific value. Even the present report does not pretend to be more than the mere beginning of a systematic study of the behavior of certain species during the different stages of their life cycle.

Occurrence and behavior of adults

Various workers have reported that among the Gordiacea there is a predominance of males over females. The most recent statement to that effect was made by Meyer (1913), who reported that he collected 201 specimens and found only 6 to be females. Von Linstow (1891) found that the proportion of males to females was 7:3. More recently Mühldorf (1914) stated that in his collections, which were perhaps larger than any previous collections made, he failed to find any consistent difference in the number of males and females.

With the additional information presented in this paper it is possible to explain the previous observations and to show that the differences observed were apparent and not real.

Von Linstow gives a table of the specimens collected by him, including both free living and parasitic forms. His conclusion is based on the entire collection which included 31 females and 74 males, and does not hold true either for the free living forms or the parasitic forms when considered separately. In case of the parasitic forms he actually found three males and five females, or a predominance of females.

Camerano in the following year published several tables showing the parasites obtained from *Blaps mucronata* in the neighborhood of Turin. These tables show that he also found no predominance of males over females.

In the parasitic forms obtained during the present investigations I have been unable to find any difference in the number of specimens of the two sexes.

So far as the parasitic forms are concerned, then, there is in literature no evidence of any real difference in the number of males and females produced. It is merely necessary to explain the difference observed in case of free living specimens.

The results of Mühldorf have already been mentioned. In the collections of *Gordius robustus* made during these investigations I have usually obtained a slight predominance of females over males, only in a few small collections was a predominance of males present. In the collection of *Paragordius varius* made at Urbana the females were far more numerous than the males. At Douglas Lake, however, the reverse was true. Most of the collections made along the shore of the lake contained very few females.

The explanation must be sought in the behavior of the animals and not in any real difference in the numbers of the two sexes. The specimens in the older collections were obtained mostly accidentally and were either specimens that had just left their hosts, or were in the act of migration, or specimens that had not found a normal resting place. Since males are as a rule more active than females and more seldom come to rest in secluded places, as do females during the egg-laying period, it is but natural that they were the ones most commonly obtained in random collections. The results of Meyer are easily explained on this basis as he obtained his specimens by collecting in open water or dredging at the bottom of ponds. In those locations he would get nothing but migrating specimens, chiefly males. Mühldorf made most of his collections in small bodies of water where the females could not seclude themselves and he obtained no real difference in numbers. My own collections of *Gordius robustus* were made chiefly at the egg-laying habitats of the females and consequently there was a slight predominance of females. Since in this species the males have a habit of remaining for the greater part with the females the predominance was not very large. This also explains why very few specimens of *Gordius robustus* are obtained in general collections. The males of *Paragordius varius* are more active in nature and consequently very few of them were taken at Urbana, but they are more frequently obtained in general collections. At Douglas Lake I did not succeed in finding the habitats of the egg-laying females and as a result the females obtained were chiefly those that had just escaped from their hosts. A few were obtained that had settled down on grass near the shore to lay eggs.

Nothing very definite can be said about the seasonal variations of the Gordiacea as reported by previous workers. The present investigations indicate that the seasonal distribution depends more on the life cycle of the host than on the habits of the Gordiacea themselves.

The egg-laying habits of the females and the possible protection of the eggs by the adults require some further explanation. Villot (1874) describes to some extent the egg-laying habits of several species. Other workers have made smaller contributions to the knowledge of that subject, but have usually assumed that the observations obtained on the particular species at hand must hold true for all members of the group. Since in most cases also the identifications of the material at hand were obviously erroneous, such reports have done little more than add to the confusion that exists. One of the most recent of such reports is that of Wesenberg-Lund (1910). Some of its errors have already been pointed out by Mühldorf. This writer, however, does not himself distinguish clearly between the habits of the different species under his observation. From my own observations it is clear that Wesenberg-Lund observed two different species, that he described the egg strings of one species, which he did not identify, and based his conclusions in regard to the protection of the eggs on what he observed in another species, which he identified as *Gordius aquaticus*.

Of the first species he obtained a single specimen with a long string of eggs wound around the stem of a plant. The male is absent and from the illustration given it is evident that the female has no protective instinct, as the eggs are uncovered and partly deserted by the female. From the character of the egg string and the habits of the specimen it is evident that the latter belonged to Chordodes, Parachordodes, or Paragordius.

In the case of the second species several masses were observed, but the egg strings were not described. Both males and females were present in the mass that was examined and pieces of egg strings were found when the mass was separated later in the season. Had Wesenberg-Lund separated a mass earlier in the season he would also have found nothing but pieces of egg strings. These specimens belonged to *Gordius aquaticus* or a closely related species which never lay long strings of eggs.

In regard to the supposed protective instinct of the parent Gordiacea my observations confirm those of Villot and Mühldorf, who were unable to find any evident attempt on the part of the parents to protect their eggs. In nature the specimens of *Gordius robustus* usually remain with their eggs as in the case of the specimens observed by Wesenberg-Lund. In captivity this species seldom lays eggs and if it does it pays no attention to them, but allows them to drop to the bottom of the aquarium in small fragments. When disturbed in nature it does not hesitate to abandon the eggs. It remains with the eggs not because it tries to protect them, but merely because there is no stimulus to cause it to move on. In case of *Paragordius varius* the male does not remain with the female even when undisturbed in nature. The female usually remains with the eggs, but I have also found cases in which the eggs were deserted. Even when the female remains with the eggs it usually does not surround them, but merely remains in the same vicinity because it has become sluggish. This type of behavior is indicated by the figure of the first specimen described by Wesenberg-Lund. In neither case can one speak of a true protection of eggs or young by the parents. Indeed, in case of the specimens observed by Wesenberg-Lund in the close masses it is very likely that most of the eggs had dropped to the bottom of the pond long before the larvae were ready to hatch.

Behavior of larvae

Little is known to the present time in regard to the habits of the larvae, except that they penetrate the tissues of a great many animals and in most of them become encysted and perish later. Cort (1915) even found such encysted larvae in trematodes. Villot (1891) and Camerano (1897) conclude from their own observations that such encysted forms are invariably lost and can not undergo further development. My observations on *Gordius robustus* show that an encysted stage in that species is not necessary and also that larvae that have lived free in the water for some time are usually incapable of development.

Infection and intermediate host

It is possible in the light of the present evidence to show that some of the former theories of infection are not tenable. The most commonly accepted theory in Europe has been that which assumes an active migration of the Gordius larvae into the larvae of aquatic insects or into other soft bodied aquatic animals and a consequent passive migration into a second host, usually an aquatic insect, which devours the first host. This theory finds its support again in the recent preliminary account of the life history of *Gordius tolosanus* published by Hans Blunck. He differs from the older views in that they assume that the adult Dytiscus, to mention a specific case, devoured the infected first host while he claims that the larval Dytiscus ingests the first host and becomes infected. The older theories seemed very logical in cases where the final host was an aquatic carnivore, but were difficult to apply where it was a supposed herbivore like a grasshopper or a cricket. Montgomery supposed in such cases that the first hosts were perhaps Mayfly larvae, that the encysted parasites were carried out of the water by the emergence of the Mayflies, and that they were liberated at the death of the insect, remained for a time on grass or leaves, and were taken into the final host with the vegetable food. Even as late as 1904 he had not discovered that *Gryllus abreviatus* (*assimilis*), which he knew to be the host of *Paragordius varius* is not only an omnivore but a cannibal and that it is fond of its nightly bath. Assuming that the host is truly terrestrial, he went so far as to undertake experiments on desiccation of worms that had just emerged and to formulate theories in regard to the chances a worm deposited on dry land had for getting back to the water. Observations made during the present investigations show that all hosts of both species of Gordiacea here considered are neither truly terrestrial nor truly herbivorous. There is then, so far as the habits of the hosts are concerned, no reason why the infection should not take place as indicated in the earlier papers of Villot and Camerano and in the reports of most other writers. But the later conclusions of Villot and Camerano did not agree with the older views, neither do the results of the present investigation bear them out. Those views have obtained their greatest support in the report of Blunck. But since he does not submit the facts upon which his conclusions are based it is impossible to know at present whether the larvae that developed in the young stages of Dytiscus were encysted forms that the insect had devoured with prey or whether they were larvae that had merely adhered to the food or had even bored thru the external covering of the insects and thus actively migrated into them. The mere fact that he observed encysted larvae in the prey of the larval beetles would in no way constitute a proof of the fact that these encysted forms were identical with the parasitic stages found later in the beetles. The actual evidence presented up to the present time indicates that there is in the life history of the Gordiacea no encysted stage and no change of hosts. However, different species of the group may differ in this respect.

Developmental period

The conclusion of Švábeník, that the young forms must live several years in the bodies of the insects, is not confirmed by the present report. Blunck also does not indicate that the developmental period is very long, as he states that the parasites usually emerge soon after the beetle has attained its adult form.

The theory of Villot, Camerano and others that the Gordiacea frequently leave their hosts before the cuticula is completely formed has in no way been confirmed in the present work. On the contrary, the observations show that in the species investigated no essential change takes place in the cuticula after the specimens have emerged. The theory was not based upon observations made on the same specimens but merely upon the fact that certain specimens presented slightly different cuticular structures than did others. These differences may have been due to the fact that the specimens developed in different host species or in the same host species under different conditions. The placing of small or light colored specimens in the category of young individuals is based upon no scientifically established facts. The present observations show that the statement of Villot that young specimens of *Gordius villoti* have a smooth cuticula and that the bristles develop later is founded upon error. Either the smooth individuals belong to a different species from the ones with bristles or the European species is variable in regard to that character.

ORGANOGENY

In regard to the organogeny the work of Vejdovsky (1894) stands almost alone. The contributions of Villot on that subject are of little scientific value. His specimens were rendered unfit for histological investigation by the methods he employed in removing and killing the parasites. As proof it is merely necessary to consider his figures and interpretations of the hypoderm. I have obtained essentially the same results in specimens that were removed in water and were not properly killed. In that case the cells shrink and appear as small, deeply staining bodies with the intercellular bridges forming radiations from these centres. The whole mass appears as a network such as Villot (1874) has figured.

Even Vejdovsky's work is not free from similar defects. The figures of the degenerating nuclei are certainly nothing more than those of poorly preserved nuclei in which parts of the nuclear structures had been macerated out. Naturally different stages of such a process would be found. He supplies part of the evidence for that himself in stating that the specimens obtained from Camerano, which were preserved in alcohol and were otherwise in very poor condition, gave him the best results in the study of these stages in the nuclear changes. Vejdovsky was handicapped in many ways. He was unable to cut sections less than 20 μ thick and had at his disposal no better stains than the carmins.

In regard to the morphology of the adults all the papers except those of Montgomery, Vejdovsky, Rauther and Švábeník are of little scientific value because the investigations were either too fragmentary or they were carried out under conditions that could produce no accurate results. The present investigations show that all conclusions based purely upon adult structures are subject to verification. It is impossible to interpret properly the adult structures of the Gordiacea without knowing something about their development.

Metamorphosis

Altho the present work gives some information in regard to the metamorphosis of the two species studied, there are many questions that still remain unanswered. There exists no previous literature on this subject. Montgomery (1904) and after him Mühldorf assumed that the proboscis of the larva is a precephalon and does not take part in the development. The present investigations show that this is not the case, but that the brain of the adult in both of the species examined develops from the posterior part of the proboscis, and that even the cord of tissue connecting the stylets with the partition between proboscis and body, representing possibly the larval esophagus, develops in one of the species.

The present investigations have also for the first time revealed the fact that the larval cuticula is shed when the parasite attains its full development and that the remnants of the larval proboscis which do not take part in development are lost with the larval cuticula.

Later development

The later development in both species consists of uninterrupted growth and differentiation from the time the first rudiments of the organs of the adult appear to the time the parasites are ready to leave their hosts.

The question of what constitutes a larval stage in the Gordiacea has become even more complicated thru the present studies. Villot regarded the larva as an embryo and designated the parasitic stage as larva. Nearly all other writers have considered the stage that is free living after leaving the egg as larva and the later stages as developmental, young or juvenile. The application of the name larva to the parasitic stage was regarded as incorrect because it had no remnant of the earlier larva except the degenerated proboscis and there was no definite change that marked the transition between this stage and the adult. The discovery of the larval cuticula and the fact that it is shed at the time the parasite becomes ready to leave its host removes to a great extent the objections to the application of the term to the parasitic stage, and the term larva used to designate all the stages from the time the free living form emerges from the egg to the time the parasite is ready to leave the host would certainly be justified. But since the free living and the parasitic stages are in many ways completely different, I have used the term larva in this paper to designate only the free living form and have used for the other the term parasite or parasitic stage. The term embryo is incorrectly used when applied to the free living form. The other terms for the parasitic stage have for the most part been avoided in this paper because they are misleading. The term juvenile can be applied to any other stage except the adult. The term developmental can just as correctly be applied to the embryological stage as to the parasitic.

Cuticula. During the entire developmental period the larval cuticula expands and also increases in thickness. In that respect it differs from the cuticula of arthropods.

The fibrous cuticula of the adult is in neither of the species a secretion of the hypoderm, but a differentiation of the upper parts of the cells, as was already pointed out by Rauther. In other respects the cuticula in the two species is formed very differently. In *Gordius robustus* there is an intermediate layer formed between larval and the adult cuticula, the non-fibrous cuticula is laid down before the fibrous cuticula begins to be formed, the bristles are projections of the fibrous cuticula, and there are no evident protoplasmic connections between the non-fibrous cuticula and the hypoderm. In *Paragordius varius* the larval cuticula remains in contact with the non-fibrous cuticula, the structures of the non-fibrous cuticula are laid down by protoplasmic strands that extend up to it from the hypoderm and are present even in the adult, and the bristles or tubercles are structures of the non-fibrous cuticula. The larger radiating fibers that form the bristles in *Gordius robustus* may be homologous with the protoplasmic strands in *Paragordius varius*, but they do not appear to retain living substance in the adult condition.

Montgomery states that he failed to find any strands of protoplasm passing thru the fibrous cuticula of *Paragordius varius* but found granules in the fibrous cuticula of the male in the region where the tubercles are present. He also figures branching roots for the tubercles. His method of staining for a long time with iron hematoxylin would not readily bring out continuous fibers. What he figures as granules in the fibrous cuticula are undoubtedly nothing more than the ends of some of the protoplasmic strands which in that locality are very large. The roots of the tubercles can be nothing else than several pieces of strands which he could not trace accurately and consequently regarded as passing to the same tubercle. The protoplasmic strands in the cuticula have been figured for other species of Gordiacea, and Vejdovsky (1894) shows them in definite relation to the areolae.

Hypoderm and nervous system. It is impossible within the space of this paper to discuss the minor differences that exist in the descriptions of the hypoderm. Some of the artifacts in the figures of Vejdovsky have already been pointed out.

The development of the nervous system requires no further discussion. In the structure of the nerve cord a minor difference appears in the two species studied, in *Gordius robustus* the neural lamella consists of a series of cells while in *Paragordius varius* all the cells are located in the cord itself and only fibers connect the cord with the hypoderm. The subneural canal of Vejdovsky was probably an artifact due to the separation of the hypoderm cells at the point where the fibers from the cord enter. Rauther regarded most of the large cells of the nervous system as belonging to the supporting tissue. That is certainly an error.

The mass of cells in *Paragordius varius* designated by Montgomery as retina must be regarded as the major part of the cephalic ganglion.

Alimentary canal. The favorite textbook doctrine that the alimentary canal of the Gordiacea is well developed and functional in the parasitic stages must be regarded as disproved. Vejdovsky has already pointed out that there is no difference in the essential structure of the alimentary canal in the young forms and in the adults. He, however, was unable to trace the origin of the anterior part of the tract, as the youngest specimens examined by him were at the stage where the adult cuticula begins its formation and were in such miserable state of preservation that he was unable to locate the gonads in them. Evidently the entire interior had become disintegrated. He found the larval proboscis at the point where the mouth should have been, but in spite of that regarded the mouth as open. In his forms the larval esophagus underwent even more development than in *Paragordius varius*. The brown gland which he found in the region of the esophagus is either homologous with the part of the larval esophagus of *Paragordius varius* that grows over the intestine or else is homologous with the anterior glandular part of the intestine itself. The latter has been the opinion of Montgomery and others who have studied the larva. From the present investigations it is evident that the mouth does not become open until the adult stage is reached. In Gordius it never becomes open; for the connection between the larval esophagus and the intestine is severed at the very beginning of the parasitic stage and no opening can be present after that. It was upon examination of sections of *Gordius robustus* that Ward (1892) made the positive assertion that in the specimen examined there was no trace of an esophagus. Others have obtained similar results in this and related species. Thus Rauther in the form he designated as *Gordius aquaticus* states that he still finds the mouth opening as a thin chitinous tube, but fails to find any trace of an esophagus. Švábeník states that in *Gordius montenegrinus* the alimentary canal is very degenerate. Both of these species are very closely related to *Gordius robustus*.

There is a regression in the cells of the alimentary canal when the adult stage is reached, but it is not much more pronounced than the regression that begins at the same time in the other tissues.

The cilia mentioned in the cloaca and genital tubes in the reports of Montgomery and Rauther have been explained in the description of *Paragordius varius*. Rauther also figures cilia for the intestine of *Gordius tolosanus*. His description, however, explains his error. He states: "In der kaudalen Darmregion von *G. tolosanus* war auch deutlich zu beobachten, dass die freie innere Oberfläche des Epithels einen sehr regelmässigen fibrillär struirten Saum trägt, der offenbar aus kurzen Cilien besteht." What he observed was nothing more than the inner differentiated zone that was in some cases found in *Gordius robustus* in the present investigation. It is this type of theoretical interpretation found everywhere in Rauther's paper that makes his conclusions almost worthless. Fortunately he has usually given his actual observations before interpreting them. I have found no cilia in the intestine of either of the species studied in any stage of development.

Another case of Rauther's interpretation is his defense of Vejdovsky's statement that the male cloaca is evertable and serves as a bursa copulatrix. He defended Vejdovsky's statement in an attempt to explain the bristles around the anus of the male, in spite of the fact that he knew that it had been contradicted by Camerano, von Linstow and Villot, and that he had observed no evidence to prove its correctness. But, the figure given by Vejdovsky (1886, Fig. 31) shows conclusively that the structure at the anal opening can not possibly be the everted cloaca. He shows the cloaca still in place and the cellular part ending at the anus. The part extruded was evidently a mass of spermatozoa.

Muscles and parenchyma. The very close relationship between muscle and parenchyma cells is evident from the descriptions of both of the forms studied. In the very earliest stages observed there is no distinction between muscle cells and mesenchyma cells that later make up the parenchyma. The longitudinal muscle cells can of course from their position be distinguished in a general way from mesenchyme cells; but in many cases cells appear that are partly within and partly without the muscle layer, and on account of the existence of all degrees of intercalation, must be interpreted as belonging to the mesenchyma. Furthermore, the cells that line the hypoderm which later forms the nerve cord are at the earliest stages not distinguishable from the muscle cells lining the rest of the hypoderm. In case of the cloacal muscles all gradations can be found between unmistakable muscle cells and regular parenchyma cells. All of these muscles except the radiating muscles surrounding the posterior part of the cloaca of the male develop from mesenchyme. The radiating muscles develop from the muscle cells lining the hypoderm that is inturned at the cloaca. From these facts it seems probable that both muscle and parenchyma cells develop from mesenchyme and that the position rather than any inherent properties of the cells determines whether they are to form muscle or parenchyma cells.

The fibrils in the longitudinal muscles do not appear until the adult stage has nearly been reached. Normally they arrange themselves in a row completely surrounding the rest of the cell, but in cases of excessive flattening they may appear to be interrupted at the outer edge. Such cells formed the basis of Vejdovsky's contention that the muscles of the Gordiacea are open toward the hypoderm.

In the light of the present investigations the descriptions of the peritoneal linings of epithelial nature must be regarded as resting upon misinterpretations. Vejdovsky was the most positive advocate of the theory that the parenchyma layers are to be interpreted as true epithelium. Villot (1881, 1887) discovered the true origin of the parenchyma layers, but his interpretation was not universally accepted because it lacked conclusive proof. Von Linstow (1889) also believed that no true epithelium was present. Most other workers were not inclined to give any positive statements, except Švábeník, who followed the footsteps of Vejdovsky, and, altho his figures show nothing that contributes in any way to the knowledge of the subject, asserted in the most positive terms the existence not only of true epithelium, primary and secondary body cavities, but also a rudimentary segmentation of the body cavity.

The present investigations show that in the early stages there are no epithelial layers except the hypoderm and the intestine, that the muscles and parenchyma arise as mesenchyme, and that the mesenteries and peritoneal linings are nothing more than layers of parenchyma. The cavities present are not true coelomic cavities, but remnants of the blastocoel or primary body cavity. The intestine adheres to one side of the cavity, but is not covered by the peritoneal lining. In the early stages the gonads also are not covered by parenchyma.

The two species investigated differ widely in the distribution of the mesenchyme in the female. In *Gordius robustus* the mesenchyme does not surround the ovaries while in *Paragordius varius* it completely surrounds them.

Vejdovsky was unfortunate in his investigations in that the earliest stages at his disposal were those in which the mesenchyme had just completed the formation of the lining of the muscles and the covering for the gonads. At that stage it appears more like true epithelium than at any other. In his specimens the appearance was even more that of true epithelium because the looser mesenchyme cells were not preserved.

No true circulatory system is present, but the body cavities may be regarded as chambers that aid in the distribution of liquids in the body.

No trace of a special organ for excretion is present. Montgomery found in one female an elongated organ passing along the dorsal side of the anterior part of the intestine and at intervals giving off branches. He regarded this as the vestige of an excretory organ and described and figured it in detail. Indeed he described it so well that his error in interpretation is easily detected. The structure was nothing more than the mycelium of a fungus, such as are often found in older specimens. Montgomery himself states that "it is most remarkable that this organ appears to possess no nuclei of its own. Small deep-staining nuclei are found in it (about 29 in number), but from the close resemblance of these to the nuclei of the parasitic organisms found in the lumen of the medio-ventral canal, they certainly belong to such parasites which have penetrated the walls of the organ."

Reproductive organs. The origin of the germ cells must still remain a mystery. That the bodies found by Schepotieff in the larval stage at the sides of the intestine are really the primordial germ cells is very doubtful both from the appearance of similar bodies in other species and from his own description. In the larvae of *Paragordius varius* examined by me similar bodies were present. Montgomery figures two bodies, a smaller one anterior and a larger one posterior, but states that more may be present. In the specimens examined by me there were present invariably the larger posterior body, which undoubtedly is a part of the intestine filled with a substance of nearly homogeneous nature, and two smaller, spherical bodies with deeply staining centers, attached to the antero-lateral edges of the larger body. Montgomery regards the substance included in the bodies as excretory in nature. In *Gordius robustus*, where two bodies appear that answer more closely to the descriptions and figures of Montgomery, these bodies disappear in the later stages and the intestinal wall in that region becomes built up of large cells. In *Paragordius varius* I have found no trace of the disappearance of the bodies in larvae that had lived in the free condition for a long time.

Schepotieff in his description says that the bodies are formed from a vesicle which arises from the dorsal side of the intestine. He states that they are vesicles composed of walls filled with a gelatinous, feebly staining, homogeneous mass. Each wall he believes to contain two flattened nuclei. He thought this structure was similar to that of the reproductive tubes of the adults. The present investigation, however, has revealed that the gonads in the early stages are composed of cells of an undifferentiated nature and consequently it is doubtful that they are derived from larval structure of such specialized character.

The reproductive organs in the two sexes arise in the same manner and are differentiated only later in development. The term germinal epithelium is hardly applicable to the early rudiments of the gonads. Even in later stages, with the exception of the efferent ducts, no part of the reproductive columns contains a structure that in any way resembles epithelium. The resemblance of the walls of the oviducts and sperm ducts to epithelium has been described by others. Rauther assumed from analogy with similar structures in other animals that the gonads in this group must arise as evaginations of some epithelial structure, and believed that the epithelial remnants presented by the oviducts indicated that the evagination had taken place at the point where the oviducts enter the cloaca. The results of the present investigation show that this theory does not hold. The gonads appear some time before there is any trace of an evagination in the region that later forms the cloaca. The connection between gonads and intestine is only secondarily acquired. Moreover, the epithelial structure of the oviducts is not a remnant of a previous epithelial covering of the gonads, but a secondary structure formed by the rearrangement of cells that in the early stages show no indication of an epithelial nature. It is possible, of course, that the gonads do arise from the posterior end of the intestine, but in that case they are at first completely cut off, their origin being more like that of mesenchyme than of mesothelium, and are later reunited.

In later stages the ovaries are still essentially like the testes, except that the lower walls have become distended at intervals to allow for the growth of the ova. The entire ovarian contents are transformed into germ cells and consequently the membranes containing them have usually been regarded as egg reservoirs or uteri. Even Montgomery, who was aware of the true nature of the dorsal tubes, retained the name uterus for them. He believed that the eggs at the time of laying pass back into those tubes and backward along them to the oviducts. That, however, is an error. In

Paragordius varius some of the eggs are retained in these tubes and later pass back along them to the oviducts, but the majority of eggs are contained in the ovarian buds and are liberated by the rupture of the membranes, pass back along the tubes formed by the parenchymatous walls which form the mesenteries, and enter the oviducts when they reach the posterior end. In *Gordius robustus* no eggs are retained in the primary ovarian tubes and all pass back along the body cavity. It is evident from the descriptions given in this report that the only name applicable to either a primary ovarian tube or the ovarian buds or both together is ovary.

The budding of the ovaries, which by Vejdovsky and Švábeník was regarded as rudimentary body metamerism, was found in the species examined to be highly irregular and not opposite in the two ovaries. Consequently it can be regarded as no more of an indication of true metamerism than the branching of the intestine in the polyclads or of the uterus in some of the larger cestodes.

PHYSIOLOGY

The functions of the organs in the Gordiacea are practically unknown. Interpretations have usually been made from analogy with similar organs in other groups, rather than from actual observations. But in an isolated group like that of the Gordiacea such interpretations are very unreliable. In the present investigations certain observations have some bearing on the possible functions of some of the organs and the interpretations are given here more as possibilities than as certainties.

Nutrition. The absorption of nutritive substances seems to be carried on by the entire outer body surface. In the younger stages of *Gordius robustus* the hypoderm cells appear also to secrete a digestive substance that attacks the cells of the surrounding tissues of the host. Thus the young specimens enclosed in the tissues of the hosts in the later stages are always found in larger pockets formed by the digestion of the cells immediately surrounding the parasite. The digestion does not appear to take place at any one point of the body of the parasite, but occurs simultaneously at all points. In later stages even in *Paragordius varius* it seems impossible that any capillary tube that may form the anterior opening of the intestine could supply a large enough quantity of fluid from the body cavity of the host to feed the developing parasite. In *Gordius robustus* that is entirely out of the question, as in that species there is clearly no anterior opening to the intestine.

Excretion. The conclusion that the hypoderm and not the intestine carries out digestive functions in the parasite leaves the high stage of development of the intestine entirely unexplained. It is, however, not necessary to look far for the probable function of the intestine. It fulfils every requirement of an excretory tube. The resemblance of its structure to that of the Malpighian tubules in insects is very close, as I had sufficient opportunity to observe in the search for young parasitic forms in sections of hosts. The tube is either nearly or entirely closed at the anterior end and widely open at the posterior end.

Since the fluid in the body cavity of the insect is itself partly excretory in nature it is difficult to understand how a large parasite can live in that fluid and possess no trace of an excretory system.

Meissner already regarded the intestine as an excretory tube, but did not recognize its true ontogenetic position as a part of the alimentary canal. Montgomery regarded the bodies enclosed in the intestine of the larva as excretory in nature.

Functions of the nervous system. All reactions in the two species observed are of a very low nature. The most definite response observed is the grasping reaction of the male when in contact with the body of the female. All other responses consist merely of motion, the degree of motion depending both upon the magnitude of the stimulus and the state of activity of the specimen. In case of specimens at rest it usually requires several successive stimuli to produce any great irritation. There is no direct response to light in case of free specimens, but the difference in the activity of the specimens at different times of the day may be partly due to the difference in the light intensity. The most definite case of orientation to light is the orientation of the developed specimens in the abdomen of the host so that the anterior end is always at the point of exit. This orientation may, however, be in part due to some other agent. The emerging reaction on contact with water is next to the grasping reaction of the male the most definite response to stimuli, but the reaction here is also nothing more than motion.

RELATIONSHIPS

The results obtained in the present investigations afford new evidence both in regard to the interrelationships of the group and their relations to other groups.

From the descriptions given in this report it is evident that the two species studied differ widely in regard to the form and structure of the larva, the participation of the proboscis in later development, the arrangement of the parenchyma in the female, and the development of the adult cuticula. An examination of the literature shows that these differences are not confined to the species studied, but appear in the same grouping in all cases where sufficient information is at hand to permit comparison. I have myself examined larvae and also sections of adults of Chordodes sp. and find an essential agreement in structure with ***Paragordius varius***. The larva of Chordodes is even more abbreviated than that of Paragordius, the cuticular structures and the mesenteries are more pronounced, and the esophagus is present in the adult.

In view of these differences I believe that there exist in the present family of Gordiidae two well defined, natural groups, one of which is represented by the genus Gordius and the other by the three genera Chordodes, Paragordius and Parachordodes. For that reason I propose to retain the family Gordiidae for the genus Gordius and to establish a new family, Chordodidae, for the other three genera.

Since the position of the present family of Nectonemidae is not definitely established, and since from the descriptions given Nectonema resembles the nematodes and especially the Mermithidae in the structure of the muscle cells, the alimentary canal, the hypoderm with its longitudinal thickenings and lack of cell boundaries, the structure of the cephalic ganglion, and in the location of the nerve cords within the thickening of the hypoderm, it is not possible to retain the Nectonemidae in the order Gordiacea. The family may for the present be assigned to an independent position in the vicinity of the Nematoda.

The limits of the old family Gordiidae then become the limits of the order Gordiacea.

The description of the proposed family Gordiidae may be given as follows: Gordiacea with a smooth cuticula, presenting no true areoles. Bristles on the body arising from the fibrous cuticula. Mouth, when cavity is present, not connected with the intestine. Ovaries not enclosed by mesenchyme and consequently no double mesenteries in the female.

Posterior end of male provided with two projecting lobes or prongs arising a short distance behind the anus. A post-anal crescent is present and has its tips directed toward the prongs. Posterior end of female entire. Larva with elongated body and pointed posterior end. Only genus in family: Gordius.

The limits of the new family Chordodidae are the following: Gordiacea with rough cuticula, presenting true areoles. Tubercles and bristles arising from the non-fibrous cuticula. Ovaries enclosed by mesenchyme, consequently double mesenteries present in the female. Posterior end of male forked or provided with a dorso-ventral groove. Post-anal crescent absent. Posterior end of female entire or provided with three lobes. Larva with short body, rounded at posterior end and provided with postero-lateral spines. Genera included in family: Chordodes, Paragordius, Parachordodes.

The evidence presented in this paper shows clearly that the supposed relationship to the Annelida does not exist. True coelom and segmentation are absent. Other workers have already shown that there is in the development of the Gordiacea no trace of the trochophore larva of the Annelida. Almost the only thing in common for the two is the ventral position of the nerve cord and its passage around the esophagus.

The evidence for a possible relationship to the Nematoda is strengthened by the discovery of a moult in the development of the Gordiacea and by the establishment of the absence of cilia or a true coelom in that group. The absence of a complicated metamorphosis and the fact that the proboscis is not exclusively a larval organ remove some of the objections to such a relationship.

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EXPLANATION OF PLATES

Plate I consists of photomicrographs made from slides. The other figures were drawn either from slides by means of a camera lucida or from photographs by means of a copying lens. Magnifications were obtained in case of drawings by the projection of the stage micrometer scale on the paper and in case of photographs by projecting the stage micrometer scale on the focussing screen.

The original thesis deposited in the library of the University of Illinois contains 656 figures, of which all but twelve are photomicrographs. Those photographs illustrate many points in the description that could not be shown in the drawings.

The author is indebted to the University of Illinois for special services from an artist of the university to draw a number of the figures. He is likewise indebted to the Zoological Division of the Bureau of Animal Industry at Washington, D. C. for permitting the artist of the division to draw a large number of the figures.

The abbreviations I 4, I 7, and I 8, as used in the descriptions of figures, are the serial numbers of the infection experiments and are described in the section on parasitism in *Gordius robustus*.

PLATE I

EXPLANATION OF PLATE

PHOTOMICROGRAPHS OF MOUNTS

Fig. 1.—*Paragordius varius*, tangential section of fibrous cuticula of adult; shows fibres, ends of radiating strands, and cross formed by the passage thru the cuticula of a large strand to a tubercle; × 800.

Fig. 2.—*P. varius*, longitudinal section of young female; shows that buds of ovaries are not opposite; × 50.

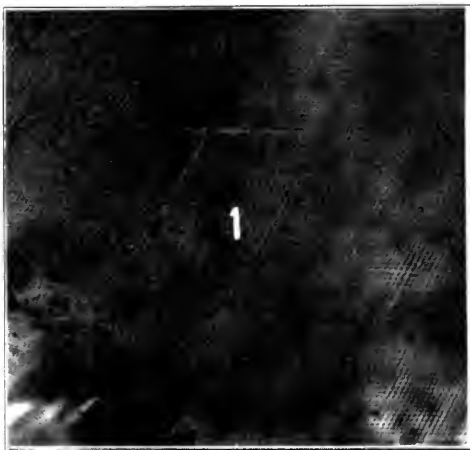
Fig. 3.—*P. varius*, longitudinal section thru anterior end of adult, showing large parenchyma cells; × 100.

Fig. 4.—*Gordius robustus*, thin layer of fibrous cuticula of adult after maceration with nitric acid; × 1240.

Fig. 5.—Same as Fig. 4; × 175.

Fig. 6.—*P. varius*, longitudinal section perpendicular to surface of muscle cells of adult; shows elongated condition of cells and nuclei; × 150.

Fig. 7.—*P. varius*, tangential section thru wall of cloaca, showing pores; × 475.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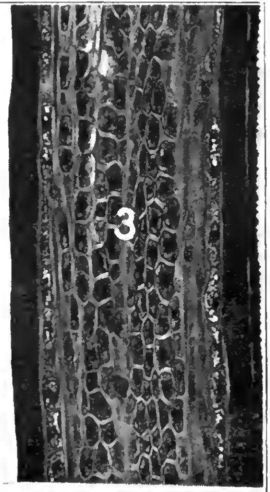
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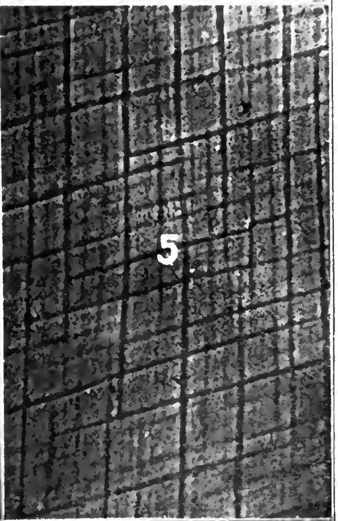
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MAY GORDIUS AND PARAGORDIUS PLATE I

PLATE II

EXPLANATION OF PLATE

Gordius robustus

Fig. 8.—Posterior end of male; end view, slightly ventral; free hand sketch from alcoholic specimen; × 20.

Fig. 9.—Six day specimen from I 4; posterior end turned up and not shown; × 250.

Fig. 10.—Section thru sperm mass just posterior to anal opening of female; shows free area where spermatozoa have migrated into cloaca; × 250.

Fig. 11.—Six day specimen from I 4; large nuclei in anterior end of intestine; × 385.

Fig. 12.—Spermatozoa in anterior end of male in which the adult cuticula is not fully developed; × 800.

Fig. 13.—Spermatozoa from posterior end of same specimen as in Fig. 12; × 800.

Fig. 14.—Larva after prolonged free existence; side view; drawn in optical section from stained specimen cleared in oil of wintergreen; × 1200.

Fig. 15.—Eight day specimen from I 8; intestine with large nuclei at anterior end; cells in cephalic ganglion somewhat enlarged; × 190.

Fig. 16.—Adult cuticula; surface view, showing intersecting lines and light spots; drawn to scale from living specimen obtained from Mt. Vernon, Illinois; × 30.

Fig. 17.—Spermatozoa from seminal receptacle of female; × 800.

Fig. 18.—Mature spermatozoa from male; × 800.

Fig. 19.—Spermatozoa in section of adult male; × 800.

Fig. 20.—Larva just hatched; side view; drawn in optical section from stained specimen cleared in oil of wintergreen; × 1325.

Fig. 21.—Proboscis of free living larva; section; × 800.

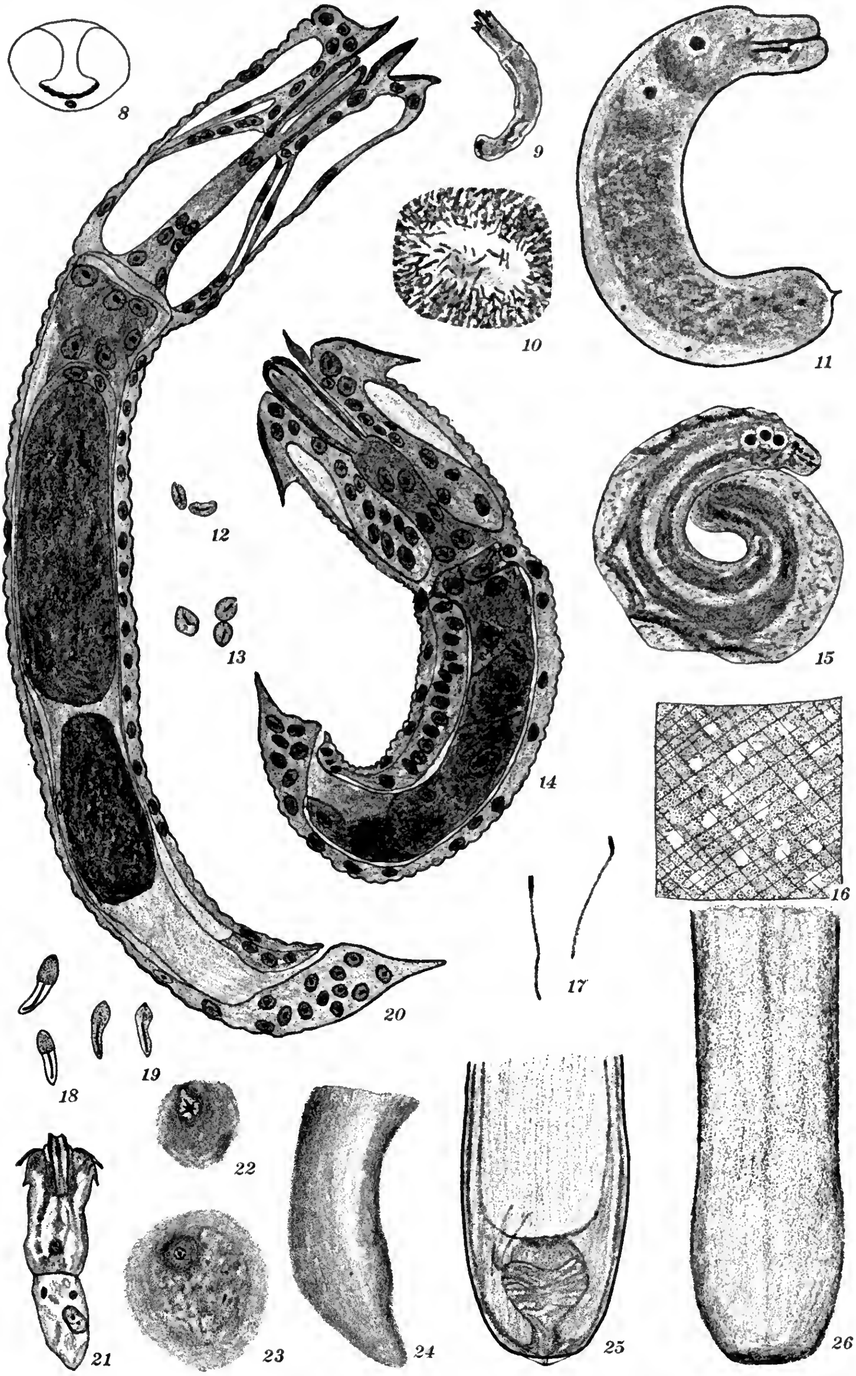
Fig. 22.—Anterior tip of parasitic form, showing larval hooks; × 240.

Fig. 23.—Section just posterior to preceding; × 240.

Fig. 24.—Posterior end of white male, nearly adult; side view; × 25.

Fig. 25.—Posterior end of white female, nearly adult; semitransparent; side view; × 25.

Fig. 26.—Posterior end of adult female; dorsal view; drawn from alcoholic specimen; × 25.



MAY GORDIUS AND PARAGORDIUS PLATE II

PLATE III

EXPLANATION OF PLATE

Gordius robustus

Fig. 27.—Anterior end of adult male; dorsal view, drawn from alcoholic specimen; × 25.

Fig. 28.—Anterior end of female, Leidy collection of 1879; dorsal view; drawn from alcoholic specimen; × 25.

Fig. 29.—Section thru posterior end of young male, showing junction of sperm ducts and intestine; × 360.

Fig. 30.—Male and female; early stage in process of mating; sketch from living specimens; × 10.

Fig. 31.—Male and female; just before discharge of sperm; × 10.

Fig. 32.—Posterior end of adult male; ventral view; drawn from alcoholic specimen; × 40.

Fig. 33.—End view of anterior end; drawn from alcoholic specimen; × 50.

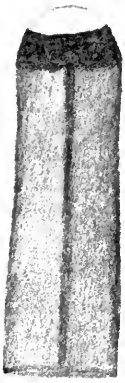
Fig. 34.—Posterior end of adult female; end view; drawn from alcoholic specimen; × 50.

Fig. 35.—Early stage in the development of adult cuticula; × 480.

Fig. 36.—Cross section thru base of fork of male; adult cuticula not completely formed; shows canal passing from end of intestine to outlet in larval cuticula at tip of fork; × 120.

Fig. 37.—Sagital section thru postcloacal ridge of young male; × 140.

Fig. 38.—Structure of adult cuticula; × 440.



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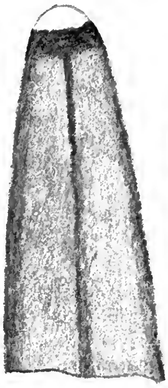

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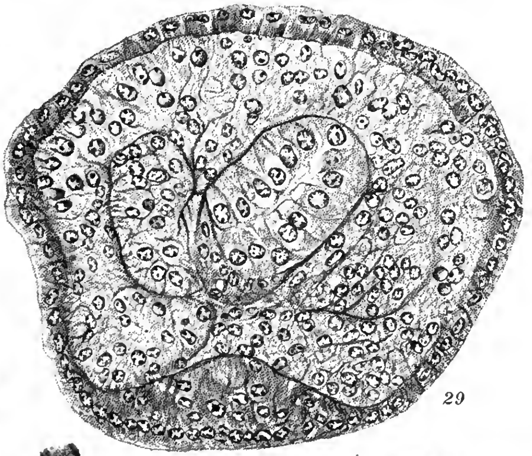

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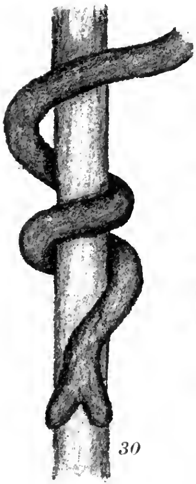

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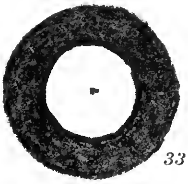

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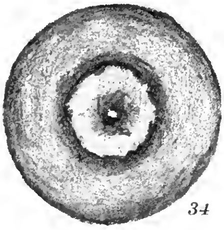

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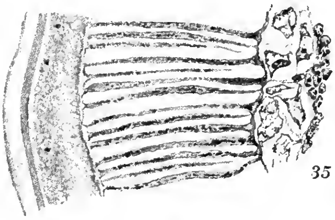

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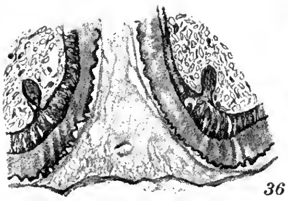

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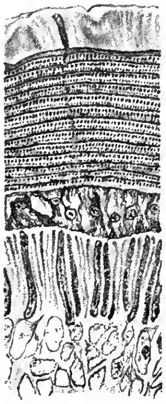

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MAY GORDIUS AND PARAGORDIUS PLATE III

PLATE IV

EXPLANATION OF PLATE

Gordius robustus

Fig. 39.—Cross section in front of cephalic ganglion in specimen shedding the larval cuticula; × 90.

Fig. 40.—Longitudinal section of specimen with adult cuticula nearly completed; shows spine in early development with minute fibre barely traceable to hypoderm; × 870.

Fig. 41.—Hypoderm and the formation of granular layer under larval cuticula; × 500.

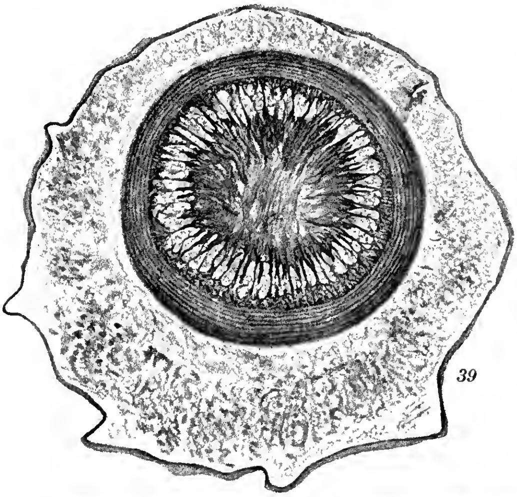
Fig. 42.—Cross section of specimen with developing cuticula; shows nerve cell and fibre in hypoderm; × 450.

Fig. 43.—Stage in the development of adult cuticula later than that shown in Fig. 35; cuticula has attained about half its final diameter; × 450.

Fig. 44.—Late stage in development of adult cuticula, longitudinal section; shows bristle passing thru granular layer; × 665.

Fig. 45.—Section parallel to neural lamella in specimen nearly mature; shows bipolar cells; × 320.

Fig. 46.—Cross section thru body of female that has deposited its eggs; shows tissues partly degenerated; × 135.



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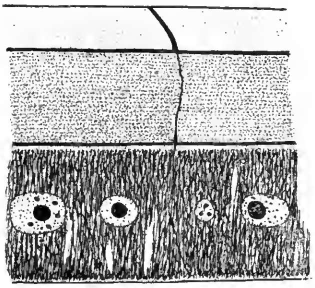

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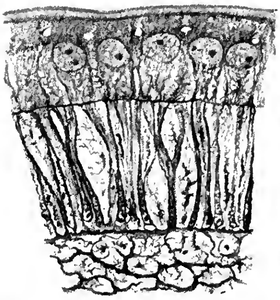

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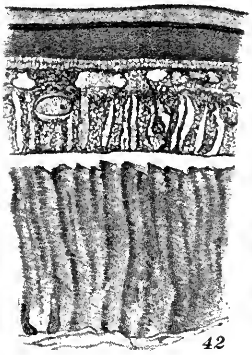

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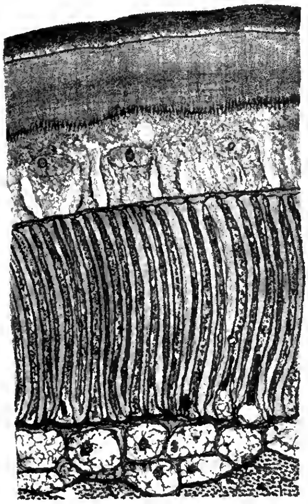

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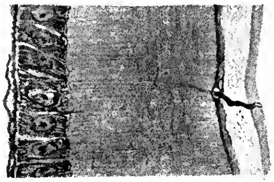

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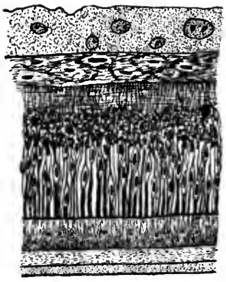

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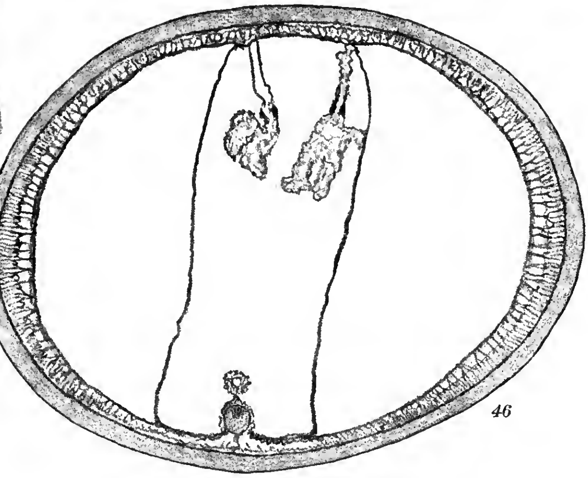

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PLATE V

EXPLANATION OF PLATE

Gordius robustus

Fig. 47.—Three day specimen from I 7; cross section of stylets in tissues of host; × 640.

Fig. 48.—Adjacent section posterior to Fig. 47; cross section of hooks of proboscis in tissues of host; × 640.

Fig. 49.—Adjacent section posterior to Fig. 48; muscles in proboscis; × 640.

Fig. 50.—Five day specimen of I 7; section thru anterior end and middle of body in tissues of host; × 480.

Fig. 51.—Section adjacent to Fig. 50; × 480.

Fig. 52.—Section adjacent to Fig. 51; × 480.

Fig. 53.—Section thru extreme ends of same specimen; hooks in proboscis; × 480.

Fig. 54.—Section of seven day specimen of I 7 in tissues of host; × 225.

Fig. 55.—Section of nine day specimen of I 7 in tissues of host; × 225.

Fig. 56.—Twenty-eight day specimen of I 8; tangential section thru hypoderm, showing the two rows of nerve cells; × 220.

Fig. 57.—Twelve day specimen of I 7; cross section thru anterior end, showing proboscis muscles; × 400.

Fig. 58.—Twenty-eight day specimen of I 8; cross section near anterior end; × 220.

Fig. 59.—Sagittal section thru posterior end of young male; anus still opens terminally; × 70.


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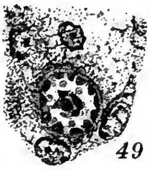
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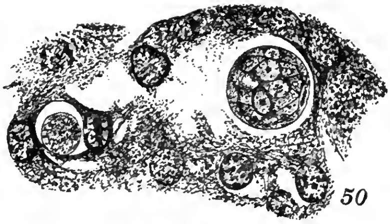
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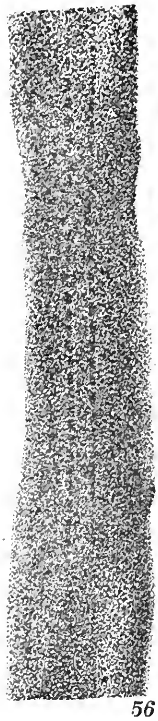
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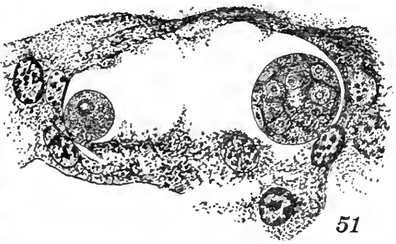
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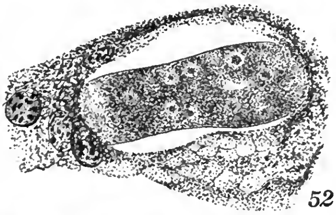
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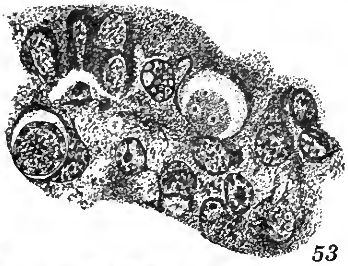
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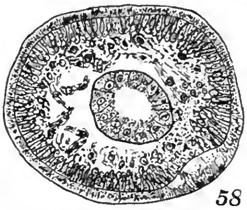
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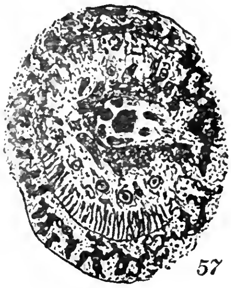
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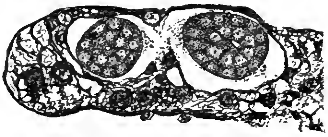
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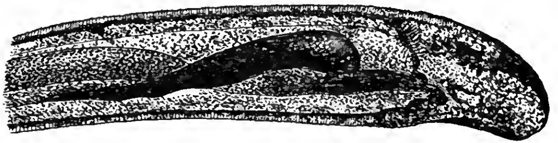
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PLATE VI

EXPLANATION OF PLATE

Gordius robustus

Fig. 60.—Sagittal section thru posterior end of young male; slightly older than that of Fig. 59; × 70.

Fig. 61.—Similar section thru still older specimen; × 70.

Fig. 62.—Young female; longitudinal section, showing budding of ovaries; buds not opposite; × 55.

Fig. 63.—Twelve day specimen of I 7; oblique section of posterior end, showing early intestinal diverticula where reproductive organs later join; × 400.

Fig. 64.—Similar section thru another specimen of the same lot; × 400.

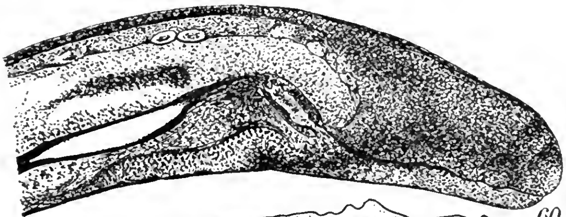
Fig. 65.—Twenty-eight day specimen of I 8; cross section of posterior end, showing intestinal diverticula; × 400.

Fig. 66.—Twelve day specimen of I 7; cross section; × 400.

Fig. 67.—Twelve day specimen of I 7; longitudinal section of anterior end; shows disintegration of cells in anterior part of intestine, cephalic ganglion in early stage, and tissue growing in between intestine and cephalic ganglion; × 400.

Fig. 68.—Similar section of another specimen of the same lot; × 400.

Fig. 69.—Similar section of specimen from same lot; × 400.



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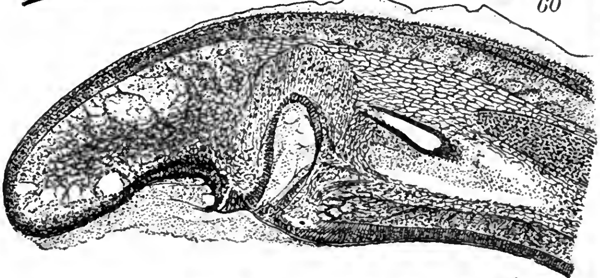

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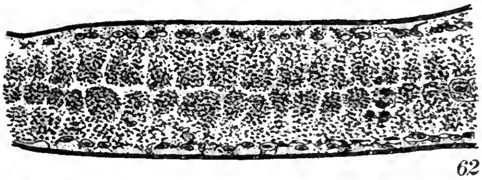

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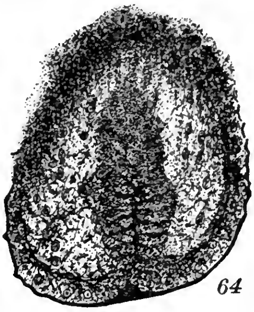

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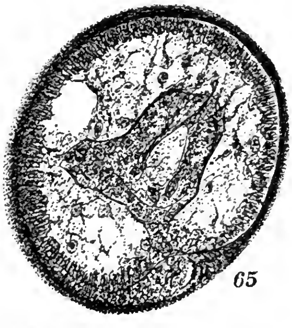

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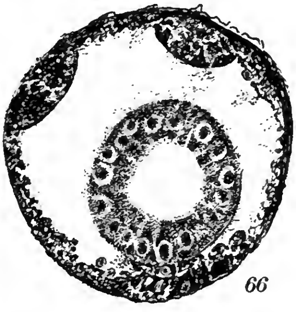

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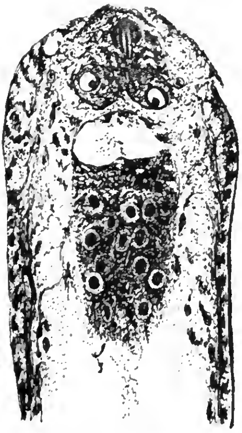

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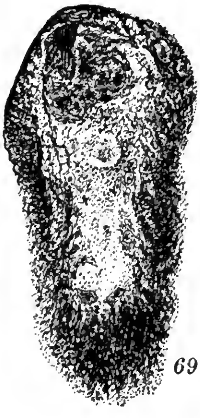

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PLATE VII

EXPLANATION OF PLATE

Gordius robustus

Fig. 70.—Young male; section near posterior end; junction of intestine and sperm ducts; × 380.

Fig. 71.—Cross section posterior to that of Fig. 70; × 380.

Fig. 72.—Young male; same specimen as Fig. 29; section thru middle of body; × 380.

Fig. 73.—Anterior end of young specimen; longitudinal section; shows intestine ending considerably posterior to cephalic ganglion, remnants of proboscis, and cord connecting stylets with partition between body and proboscis; × 220.

Fig. 74.—Similar section of specimen almost fully developed; × 105.

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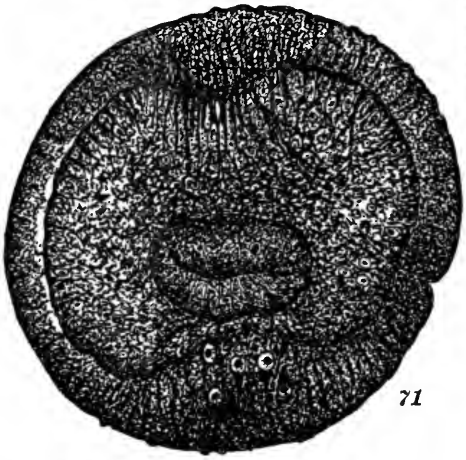

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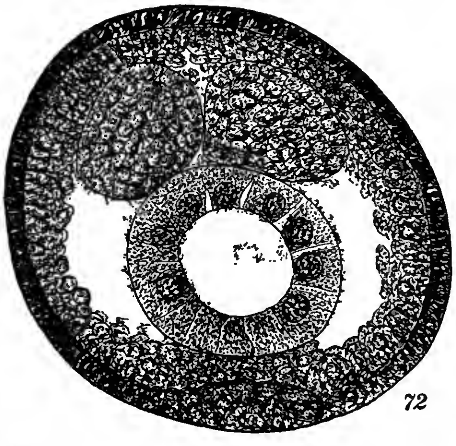

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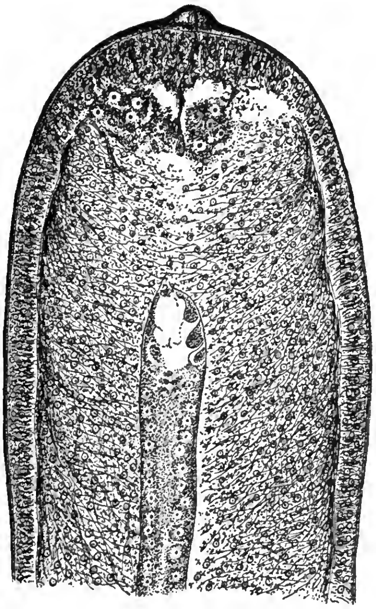

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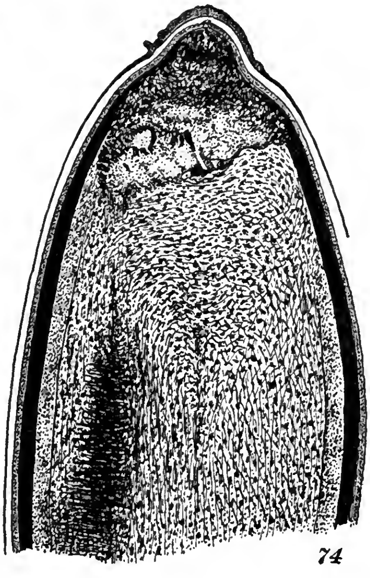

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MAY GORDIUS AND PARAGORDIUS PLATE VII

PLATE VIII

EXPLANATION OF PLATE

Gordius robustus

Fig. 75.—Cross section of male somewhat older than that of Fig. 72; × 195.

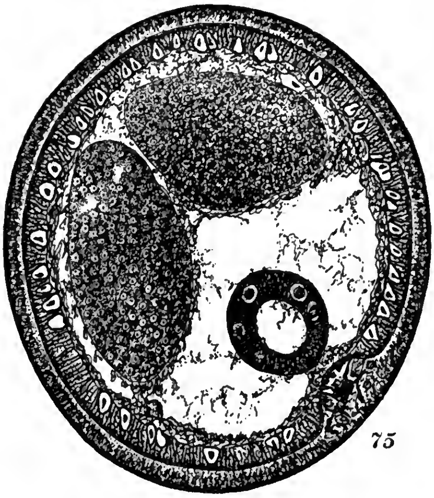
Fig. 76.—Cross section thru young female of about the same age; × 195.

Fig. 77.—Cross section thru female with adult cuticula nearly complete; × 60.

Fig. 78.—Sagittal section thru posterior end of female, nearly adult; × 55.

Fig. 79.—Female at beginning of formation of adult cuticula; cross section near anterior end of body; × 130.

Fig. 80.—Cross section of male in same stage of development as female of Fig. 77; × 80.



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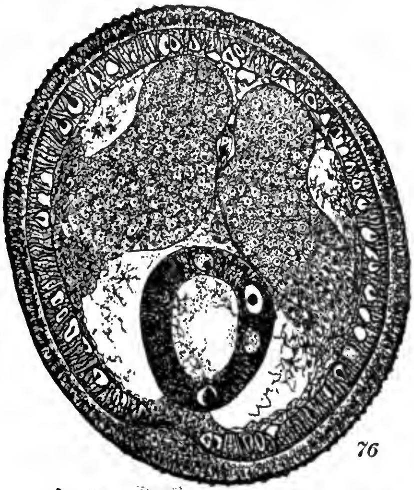

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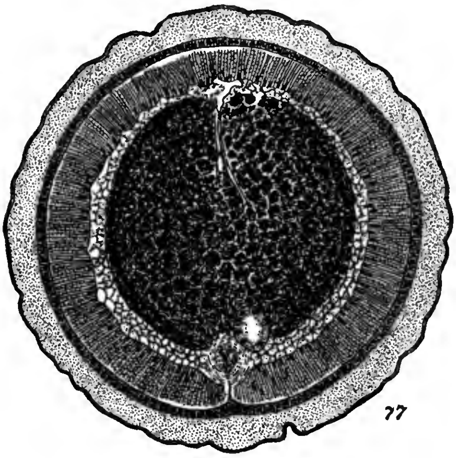

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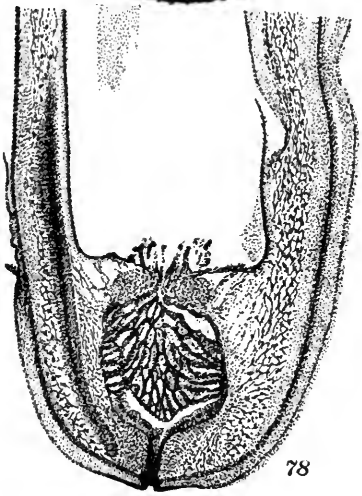

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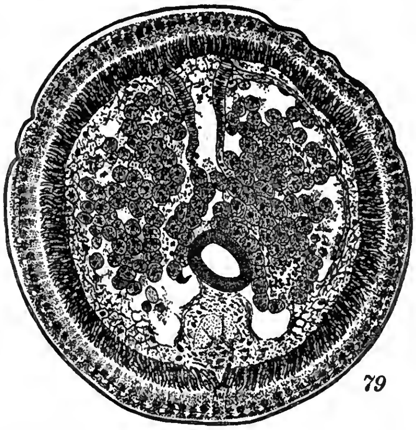

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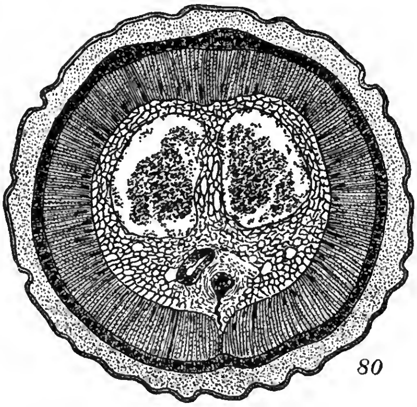

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PLATE IX

EXPLANATION OF PLATE

Gordius robustus

Fig. 81.—Cross section thru cephalic ganglion just before formation of adult cuticula; early large cells near middle of section; × 160.

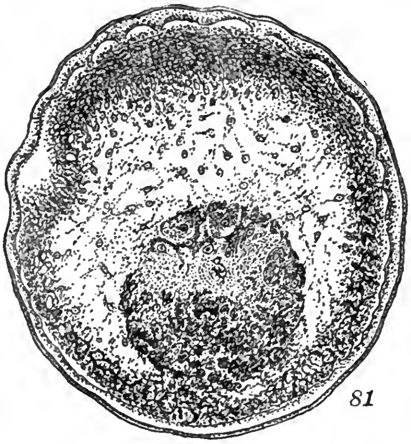
Fig. 82.—Adjacent section just posterior to that of Fig. 81; × 160.

Fig. 83.—Adjacent section posterior to that of Fig. 82; × 160.

Fig. 84.—Young female; buds forming on ovarian tubes; × 270.

Fig. 85.—Early stage in development of adult cuticula; formation of hyaline layer outside of adult cuticula; × 640.

Fig. 86.—Young female; cross section showing early growth period in oocytes; × 170.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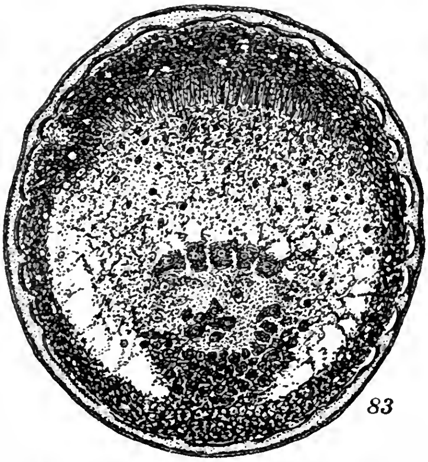
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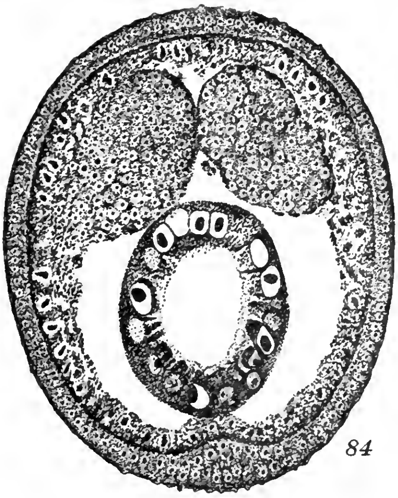
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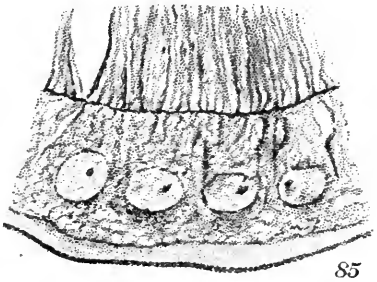
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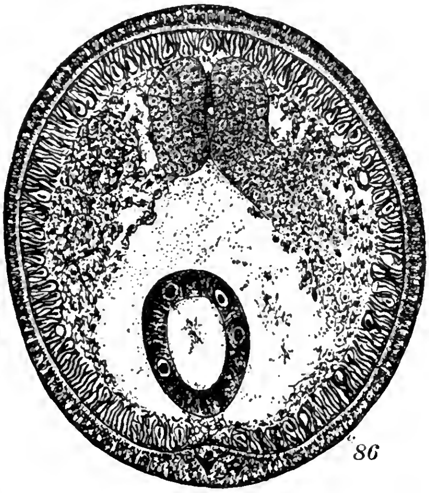
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PLATE X

EXPLANATION OF PLATE

Gordius robustus

Fig. 87.—Female at beginning of formation of adult cuticula; cross section near middle of body; × 95.

Fig. 88.—Section near posterior end of same female; seminal receptacle and ovaries just before they pass over into the oviducts; × 105.

Fig. 89.—Section slightly posterior to that of Fig. 88; × 105.

Fig. 90.—Section slightly posterior to that of Fig. 89; × 105.

Fig. 91.—Section thru posterior end of cloacal gland of same female; × 105.

Fig. 92.—Section behind cloacal gland of same female; cloacal ganglion; × 140.

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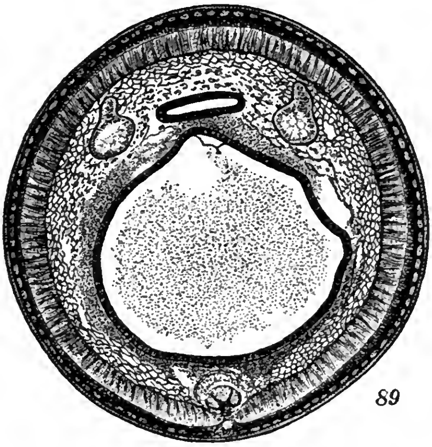

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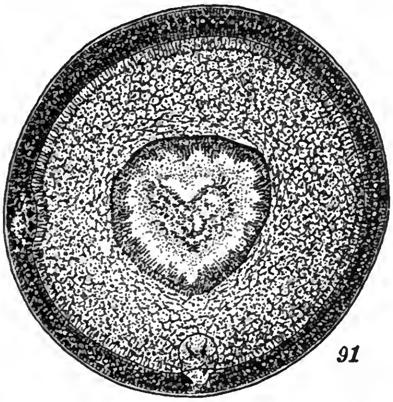

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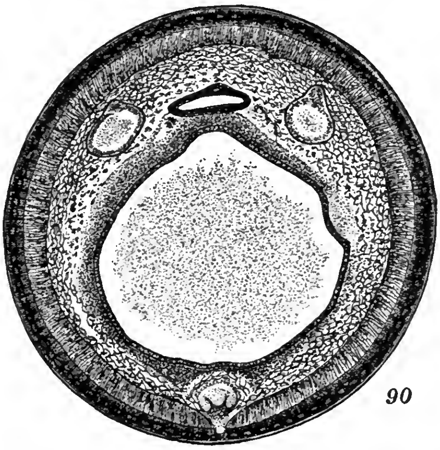

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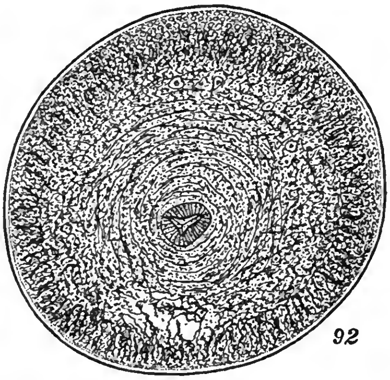

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EXPLANATION OF PLATE

Gordius robustus

Fig. 93.—Section thru anterior end of cloacal gland of female shown in Plate 10; oviducts, intestine, and posterior end of seminal receptacle; × 105.

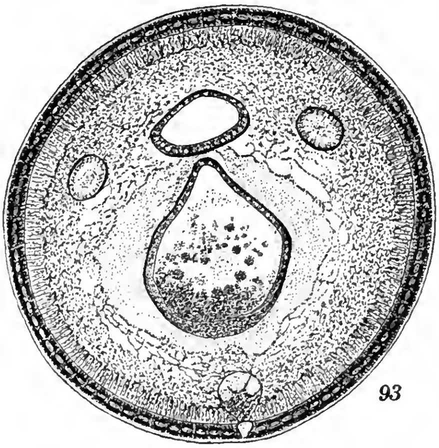
Fig. 94.—Section thru constriction between cloacal gland and seminal receptacle; circular musculature surrounding constriction; × 105.

Fig. 95.—Section posterior to that of Fig. 94; entrance of oviducts into cloaca; × 105.

Fig. 96.—Cross section thru cloacal musculature of male, nearly adult; just behind anus; × 90.

Fig. 97.—Section a short distance behind that of Fig. 96; × 90.

Fig. 98.—Section thru crescent of same male; × 90.



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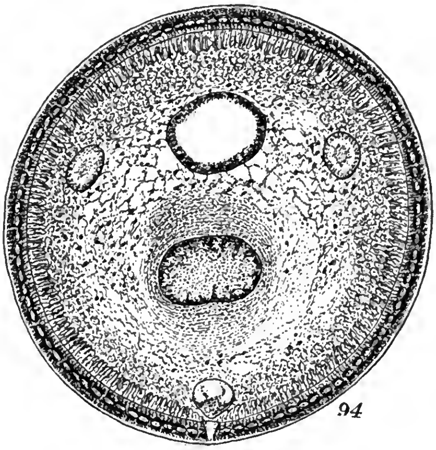

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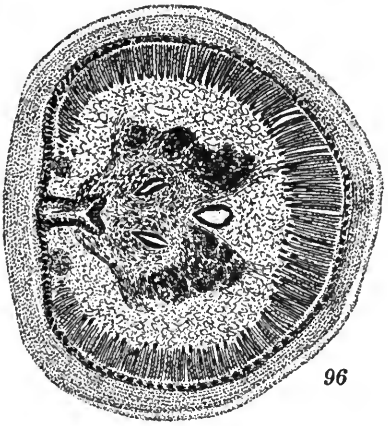

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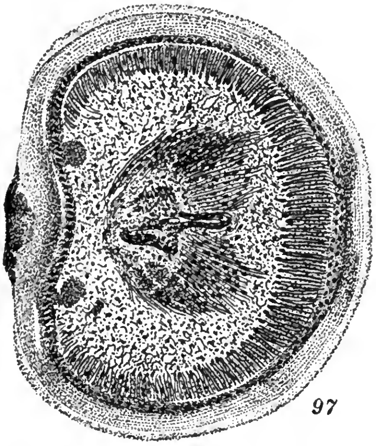

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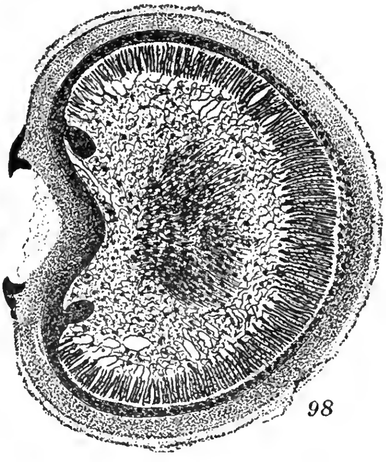

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PLATE XII

EXPLANATION OF PLATE

Fig. 99.—*Gordius robustus*, section thru cloacal ganglion of same female as Fig. 95; × 105.

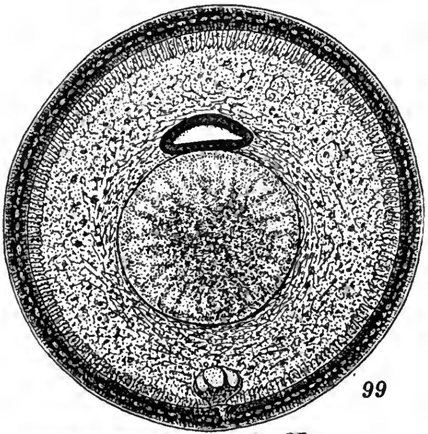
Fig. 100.—*G. robustus*, section slightly posterior to that of Fig. 99; intestine enters cloaca; × 105.

Fig. 101.—*G. robustus*, sagittal section thru cloacal ganglion of male; crescent in process of formation; × 105.

Fig. 102.—*G. robustus*, same section as Fig. 101; × 340.

Fig. 103.—*Paragordius varius*, cross section thru female that has deposited its eggs; × 70.

Fig. 104.—*P. varius*, tangential section thru developing adult cuticula; areolae in the process of formation; × 400.



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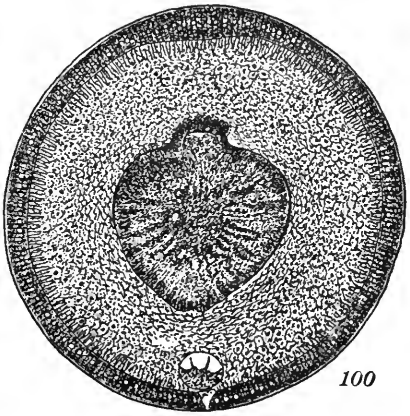

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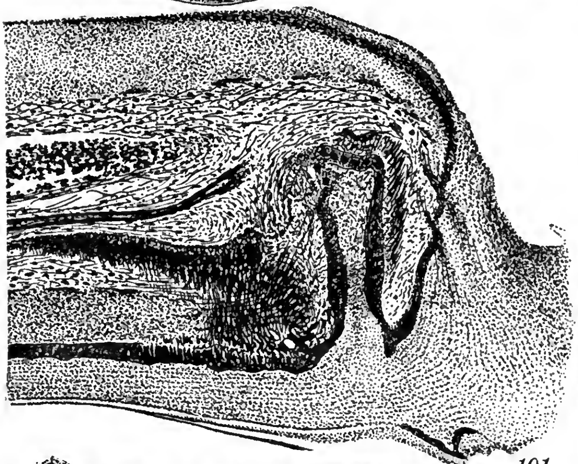

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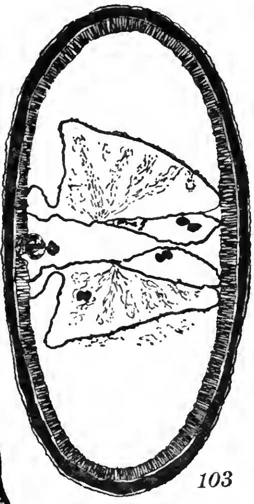

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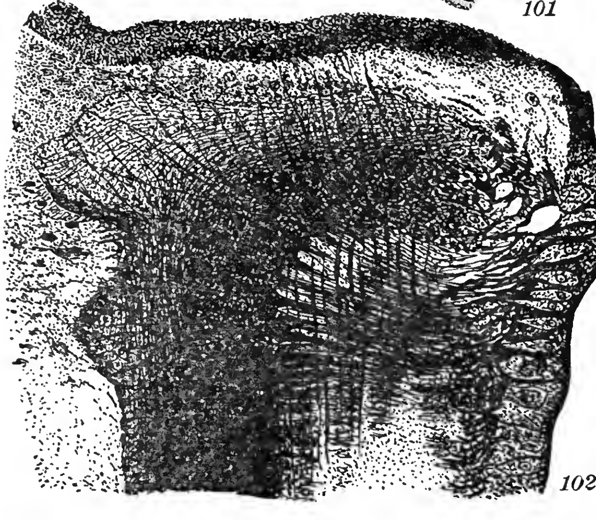

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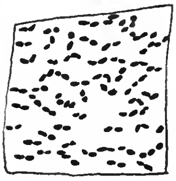

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PLATE XIII

EXPLANATION OF PLATE

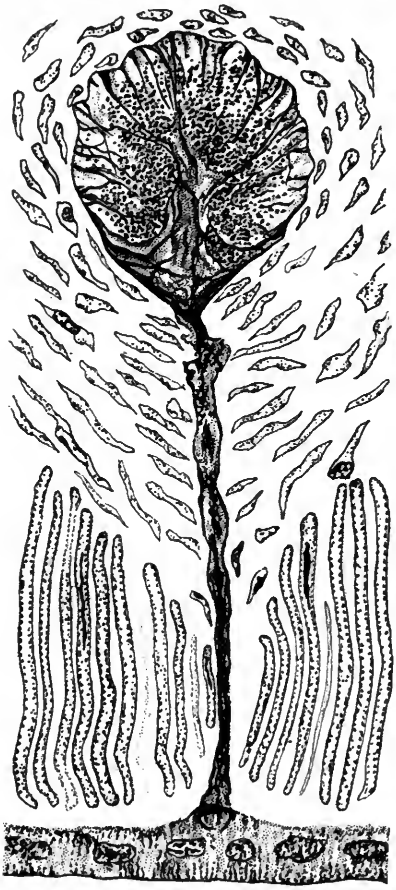
Gordius robustus

Fig. 105.—Cross section of nerve cord of adult; × 700.

Fig. 106.—Nerve cord in specimen with adult cuticula nearly complete; × 340.

Fig. 107.—Section near posterior end of male with fibrous cuticula nearly complete; × 120.

Fig. 108.—Section thru anterior end of same specimen as Fig. 107; × 120.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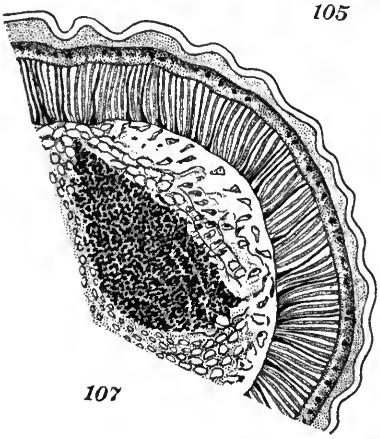
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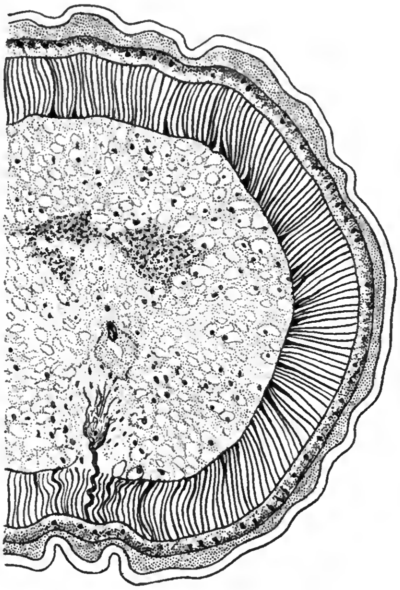
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PLATE XIV

EXPLANATION OF PLATE

Gordius robustus

Fig. 109.—Young female; cross section near posterior end; early stages of oviducts, seminal receptacle, and cloacal gland; × 250.

Fig. 110.—Section slightly posterior to that of Fig. 109; × 250.

Fig. 111.—Section thru posterior end of cloacal gland of same female; × 250.

Fig. 112.—Development of cuticula and muscles; slightly later stage than Fig. 85; × 720.

Fig. 113.—Cross section thru posterior region of female showing mass of spermatozoa adhering to outside; × 60.

Fig. 114.—Cross section of nerve cord of adult; × 530.

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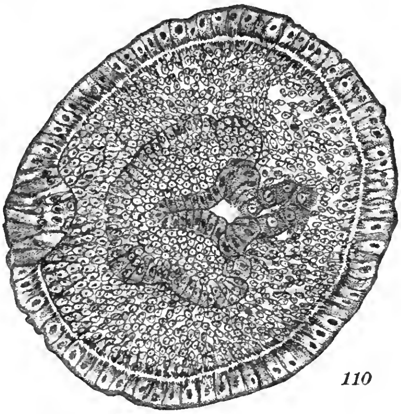

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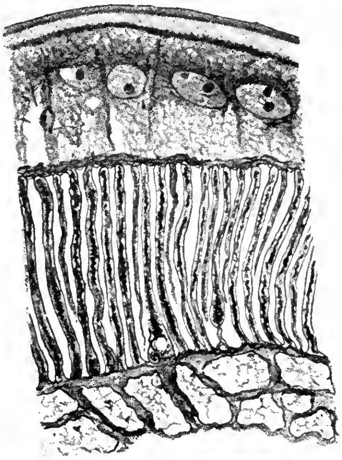

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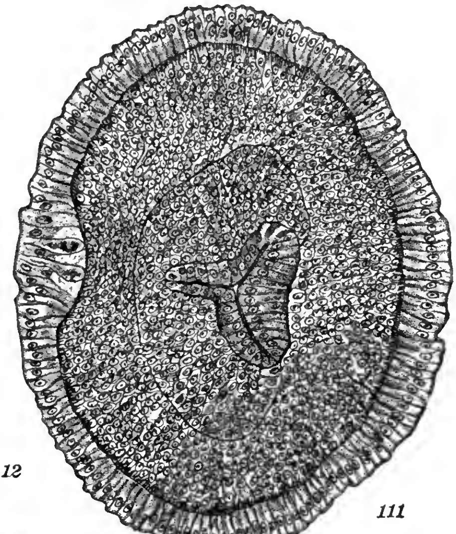

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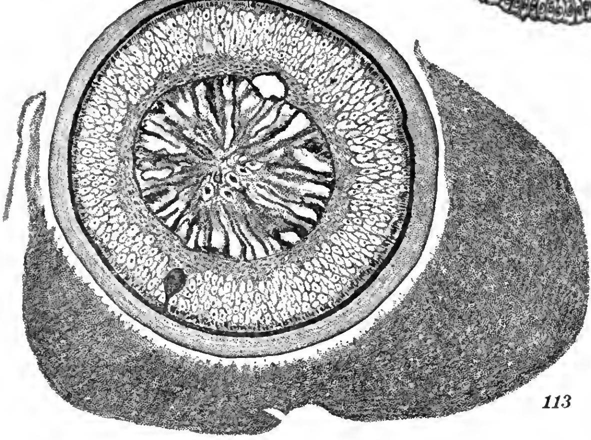

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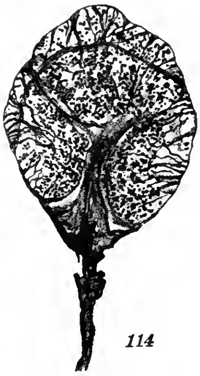

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MAY GORDIUS AND PARAGORDIUS PLATE XIV

PLATE XV

EXPLANATION OF PLATE

Gordius robustus

Fig. 115.—Nerve fibre entering hypoderm of adult; × 475.

Fig. 116.—Early stage in development of fibrous cuticula; slightly later stage than Fig. 35; × 800.

Fig. 117.—Fibrous cuticula about half developed; × 430.

Fig. 118.—Adult; nerve fibres passing from neural lamella into hypoderm; × 450.

Fig. 119.—Fibrous cuticula; development almost complete; × 625.

Fig. 120.—Nerve fibres in hypoderm of adult; × 435.

Fig. 121.—Cross section thru inner, ventral wall of prong of male; shows stout bristle; × 625.

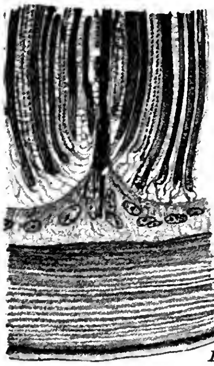
Fig. 122.—Section slightly outward from that of Fig. 121; × 625.

Fig. 123.—Section thru outer wall of prong of same specimen; × 625.

Fig. 124.—Old specimen; section showing extreme degeneration of muscles; × 430.

Fig. 125.—Section thru old specimen; shows beginning of degeneration of muscle fibres; × 430.

Fig. 126.—End of muscle cell of adult; isolated after maceration with nitric acid; × 625.



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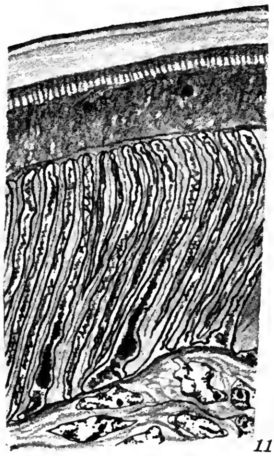

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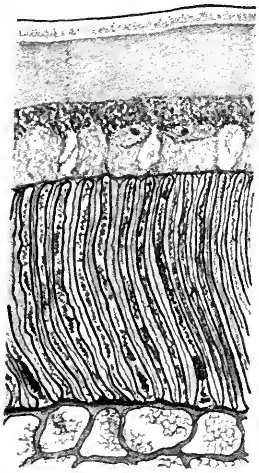

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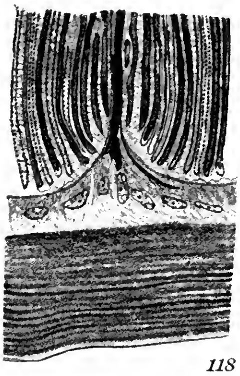

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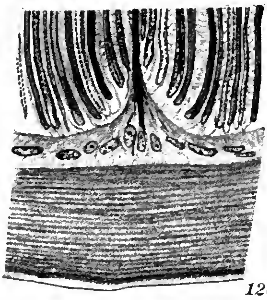

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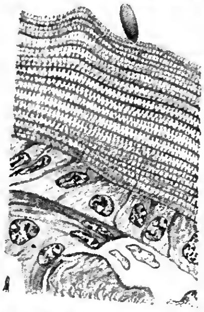

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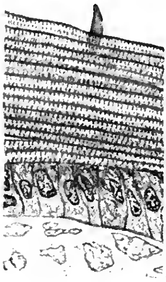

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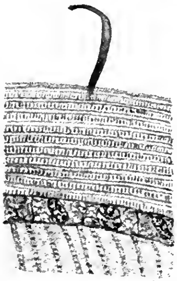

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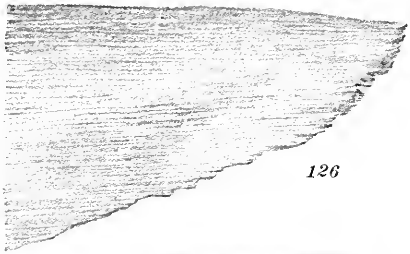

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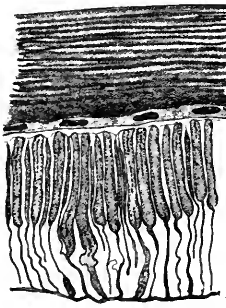

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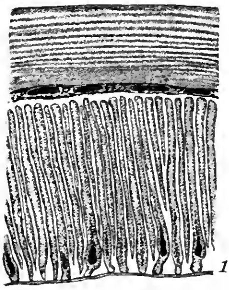

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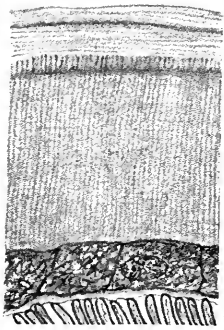

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PLATE XVI

EXPLANATION OF PLATE

Fig. 127.—*Gordius robustus*, tangential section of hypoderm of young specimen; shows nuclei and nucleoli as well as radiating canals; × 800.

Fig. 128.—*G. robustus*, tangential section thru hypoderm of specimen in which fibrous cuticula is forming; intercellular bridges; × 400.

Fig. 129.—*G. robustus*, section similar to that of Fig. 128; nerve fibre in hypoderm; × 475.

Fig. 130.—*Paragordius varius*, young parasite in the coiled stage; × 190.

Fig. 131.—*P. varius*, very young parasite; side view; specimen somewhat flattened; × 250.

Fig. 132.—*P. varius*, anterior end of adult; side view; semi-transparent; × 70.

Fig. 133.—*P. varius*, posterior end of adult male; ventral view, shows rows of bristles; × 70.

Fig. 134.—*P. varius*, cross section of host containing young parasite; × 37.

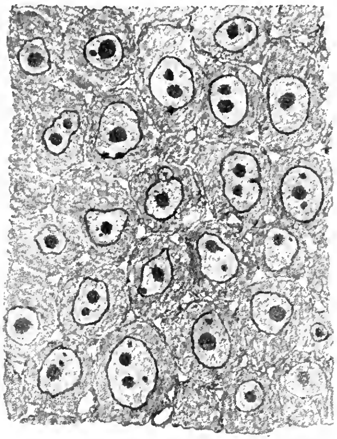
Fig. 135.—*P. varius*, section of parasite from Fig. 134; × 130.

Fig. 136.—*P. varius*, cross section thru middle of body of young specimen; early development of gonads; × 500.

Fig. 137.—*P. varius*, spermatozoa; smear made from male; × 800.

Fig. 138.—*P. varius*, spermatozoa; smear made from seminal receptacle of female; × 800.

Fig. 139.—*P. varius*, longitudinal section parallel to large nerve cells in ventral cord; shows bipolar cells; × 250.



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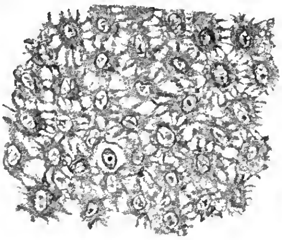

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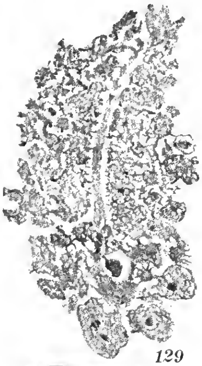

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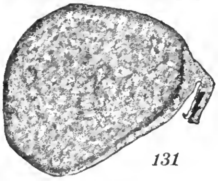

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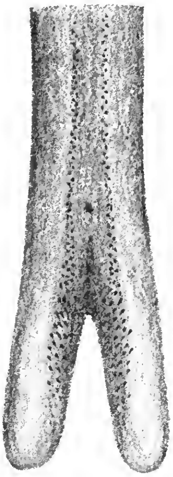

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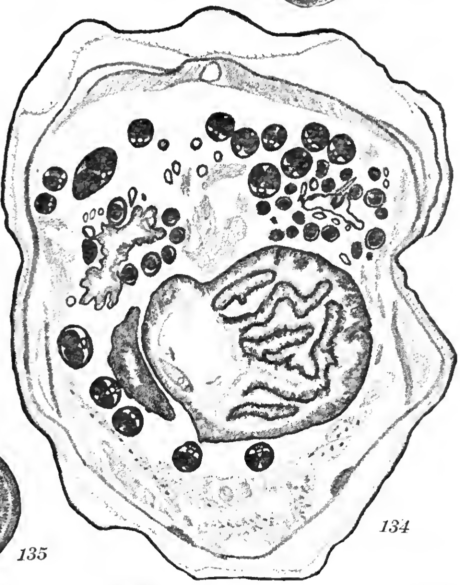

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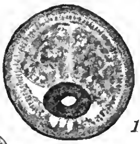

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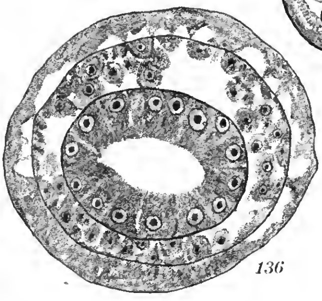

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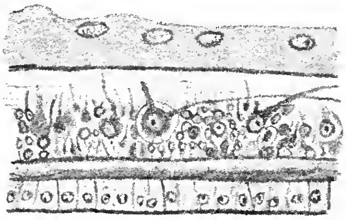

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PLATE XVII

EXPLANATION OF PLATE

Paragordius varius

Fig. 140.—Cross section thru posterior end of male at time of formation of adult cuticula; × 365.

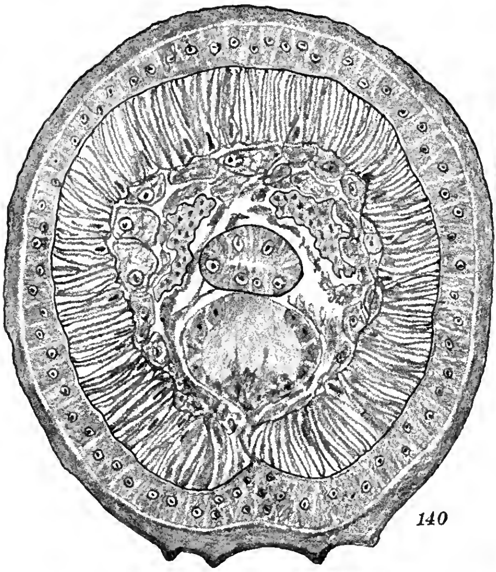
Fig. 141.—Section thru posterior region of young male; × 375.

Fig. 142.—Section of same specimen; shows sperm ducts entering cloaca; × 375.

Fig. 143.—Section thru cloaca of same specimen; × 375.

Fig. 144.—Section thru same specimen at base of prongs; × 375.

Fig. 145.—Young parasite; cross section; × 500.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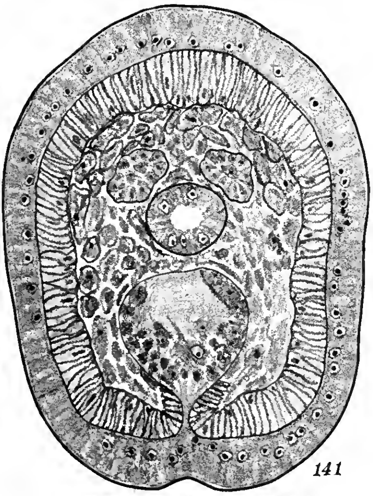
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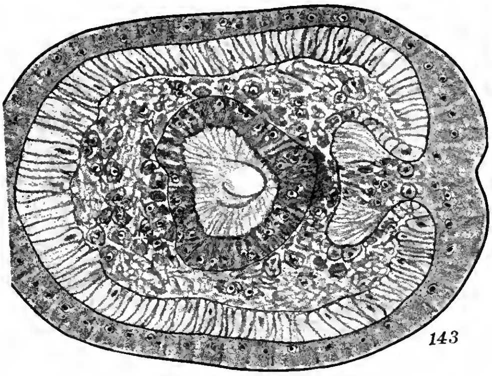
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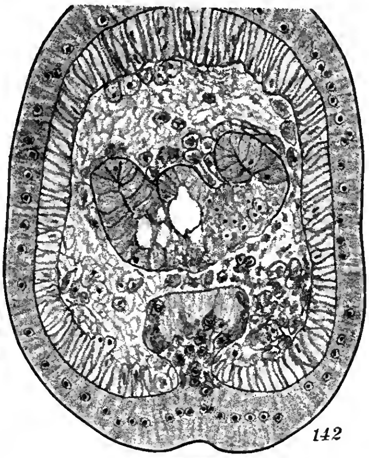
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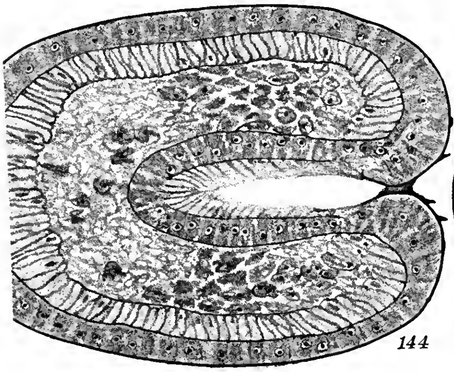
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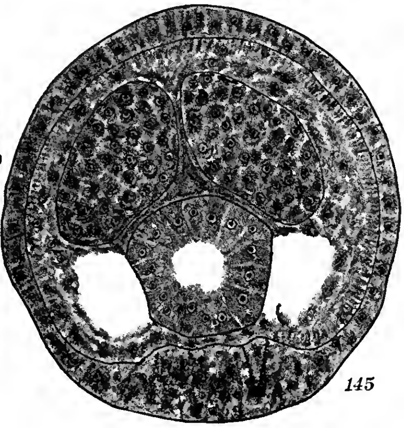
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PLATE XVIII

EXPLANATION OF PLATE

Paragordius varius

Fig. 146.—Cross section near anterior end of young specimen; × 500.

Fig. 147.—Section near middle of body of same specimen; × 500.

Fig. 148.—Section near posterior end of young male; intestinal diverticula for entrance of sperm ducts; × 500.

Fig. 149.—Section thru anal region of same male; × 500.

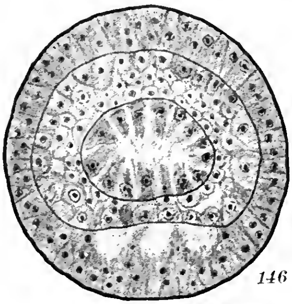
Fig. 150.—Young specimen; longitudinal section thru anterior end; × 115.

Fig. 151.—Part of Fig. 150; shows connection between intestine and proboscis; × 500.

Fig. 152.—Longitudinal section thru anterior region of female; shows ovarian pockets which appear to be placed irregularly not indicating segmentation; × 25.

Fig. 153.—Sagittal section thru anterior end of young specimen; shows esophagus; × 150.

Fig. 154.—Section adjacent to that of Fig. 153; × 150.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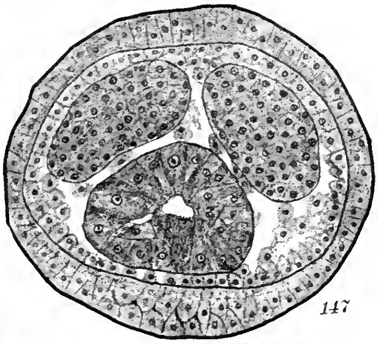
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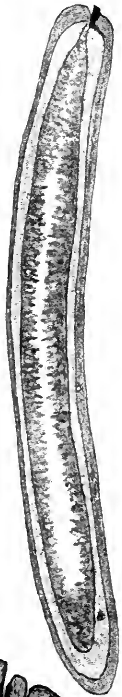
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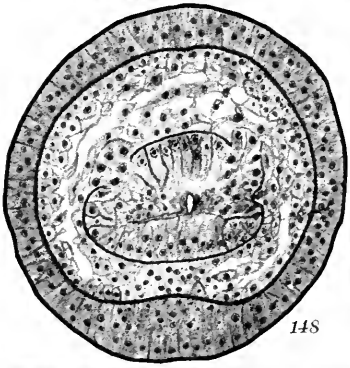
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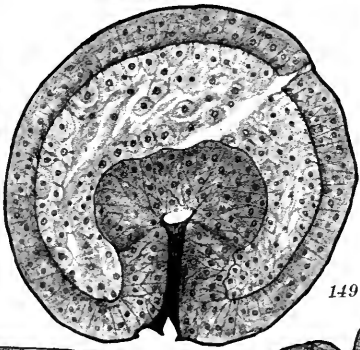
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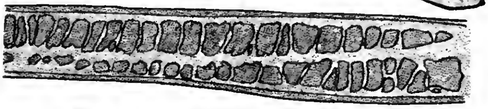
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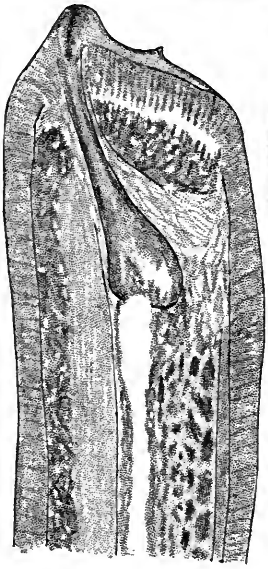
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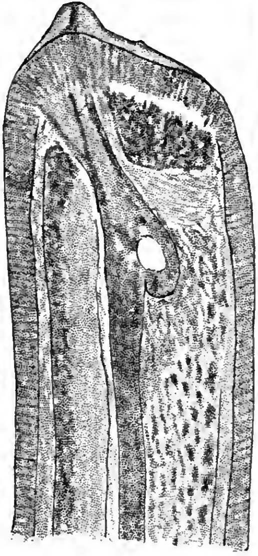
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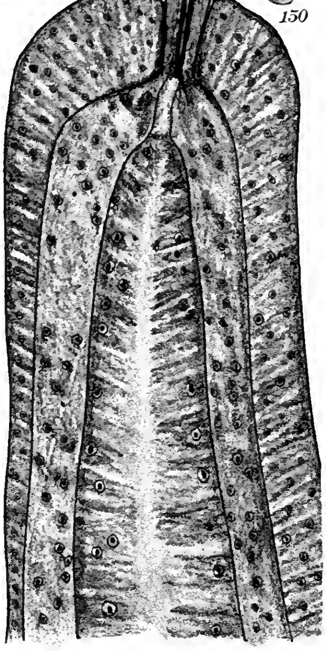
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MAY GORDIUS AND PARAGORDIUS PLATE XVIII

PLATE XIX

EXPLANATION OF PLATE

Paragordius varius

Fig. 155.—Section thru young parasite; × 500.

Fig. 156.—Section near middle of body of young parasite; × 500.

Fig. 157.—Section near middle of body of slightly older parasite; × 500.

Fig. 158.—Section thru middle of body of young female; just before formation of adult cuticula; shows formation of mesenteries; × 400.

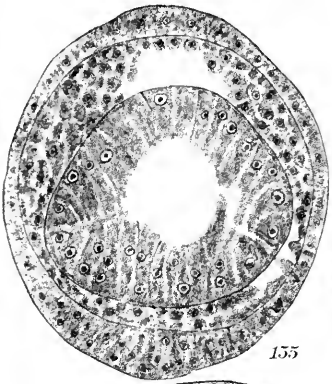
Fig. 159.—Section thru female at beginning of formation of adult cuticula; mesenteries formed; × 200.

Fig. 160.—Longitudinal section thru cuticula; shows areola and protoplasmic strand; × 400.

Fig. 161.—Ventral part of female during formation of adult cuticula; cross section; formation of areolae; × 500.

Fig. 162.—Slightly oblique section thru anterior end of young parasite; beginning of cephalic ganglion; × 500.

Fig. 163.—Cross section thru cloacal gland of female at the time the larval cuticula is shed; larval cuticula partly loose; × 150.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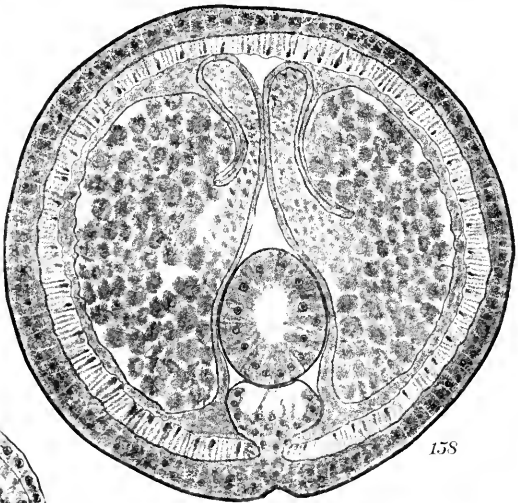
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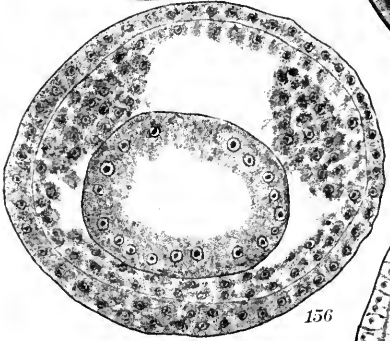
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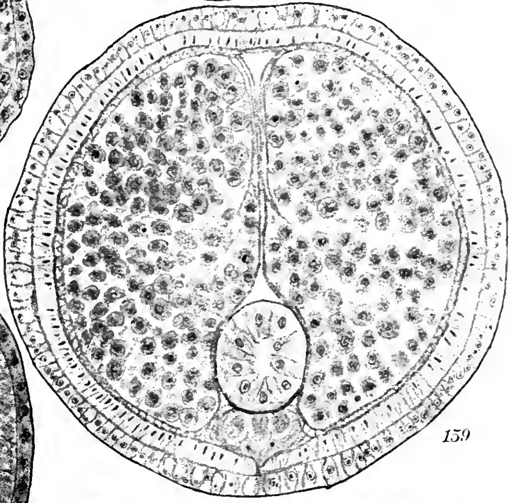
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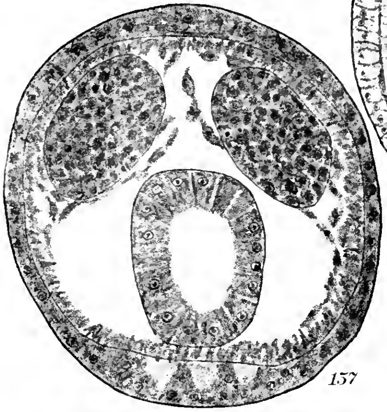
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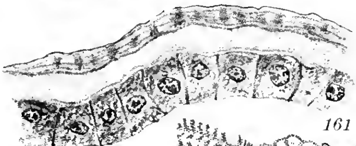
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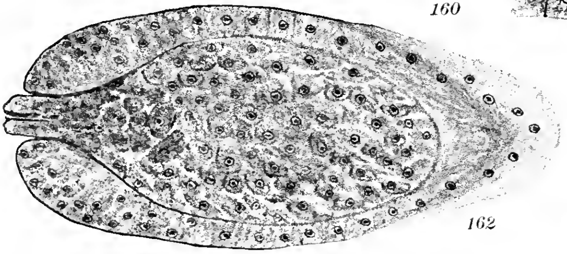
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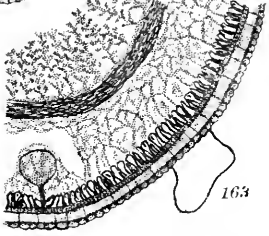
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PLATE XX

EXPLANATION OF PLATE

Paragordius varius

Fig. 164.—Section thru posterior end of adult male; cloacal ganglion and circular muscles surrounding the sperm ducts; × 275.

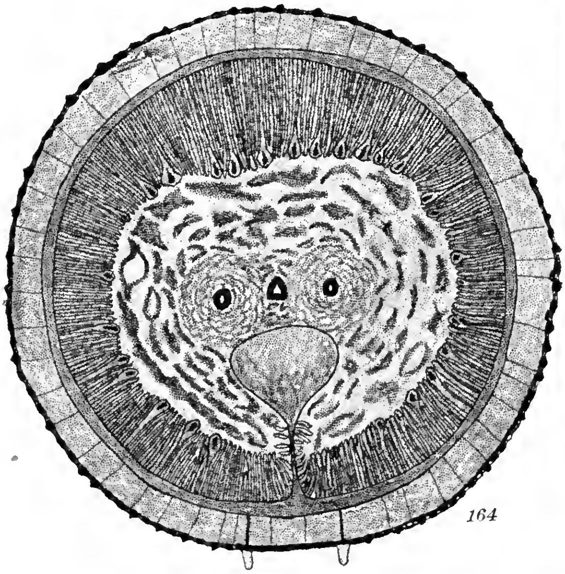
Fig. 165.—Longitudinal section thru posterior end of young parasite; shows invaginated ectoderm forming intestinal diverticula; × 500.

Fig. 166.—Cross section thru anterior region of specimen with adult cuticula nearly complete; shows cellular projections into cuticula; × 275.

Fig. 167.—Cross section of female with adult cuticula nearly formed; cellular projections and formation of areolae; × 500.

Fig. 168.—Sagittal section thru upper end of cloaca of female; × 100.

Fig. 169.—Early stage in formation of cuticula; areolae very small; × 500.



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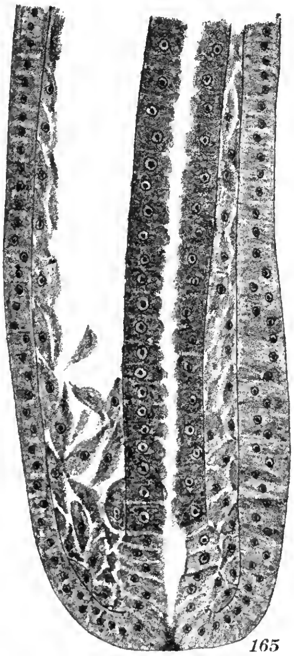

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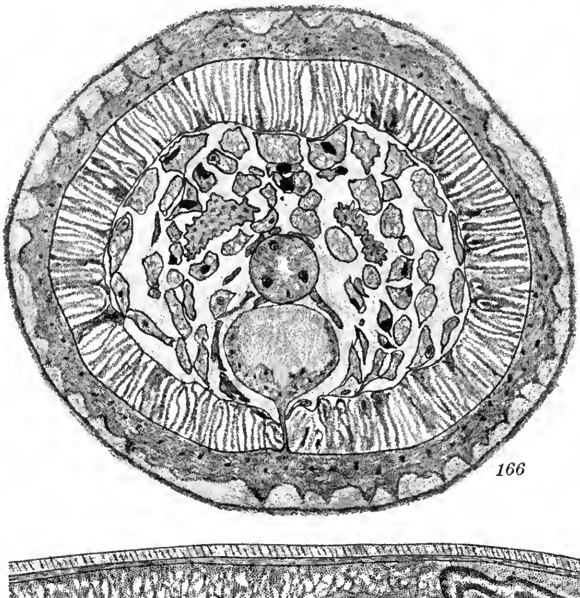

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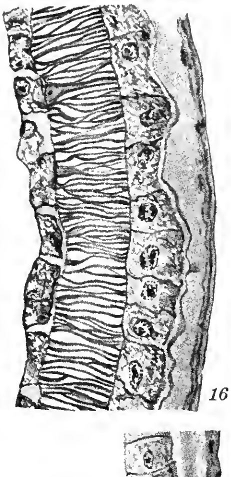

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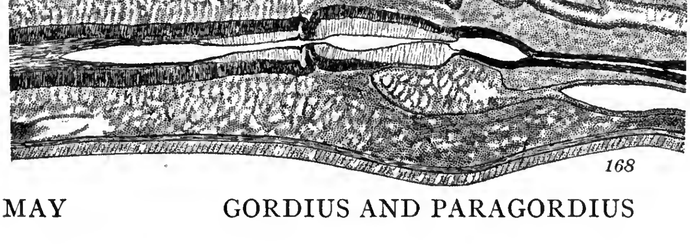

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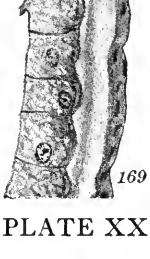

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PLATE XXI

EXPLANATION OF PLATE

Paragordius varius

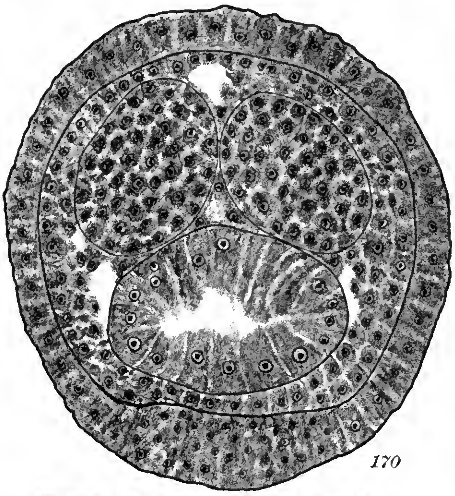
Fig. 170.—Cross section thru body of young female; ovarian buds not yet formed; × 500.

Fig. 171.—Frontal section thru upper end of cloaca of young female; × 115.

Fig. 172.—Cross section thru body of female; dorsal part of section; beginning of formation of adult cuticula; structure of dorsal part of mesenteries; ×500.

Fig. 173.—Ventral part of same section as Fig. 172; × 500.

Fig. 174.—Cross section of male at beginning of formation of adult cuticula; × 375.



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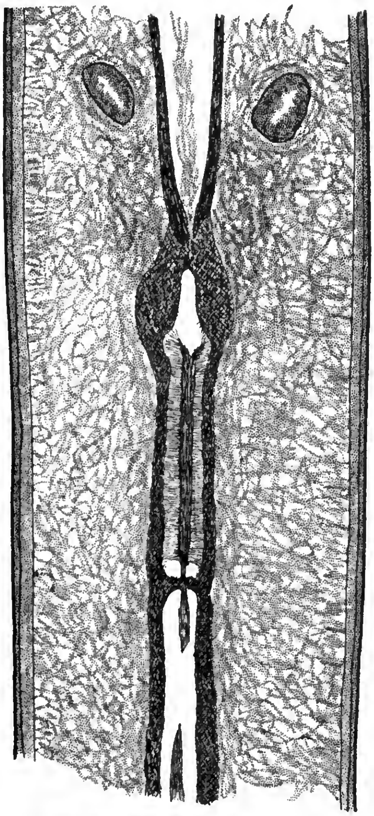

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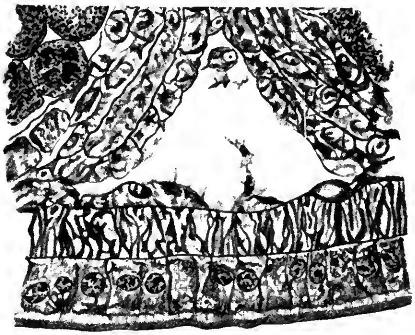

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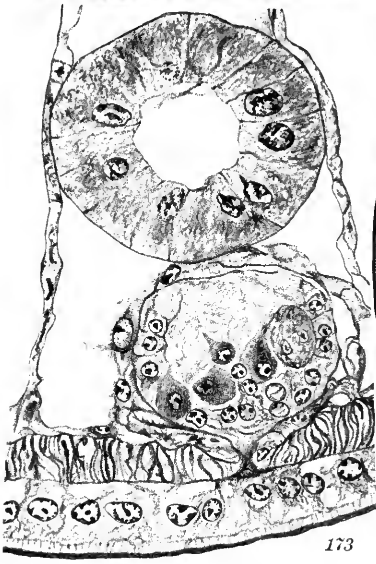

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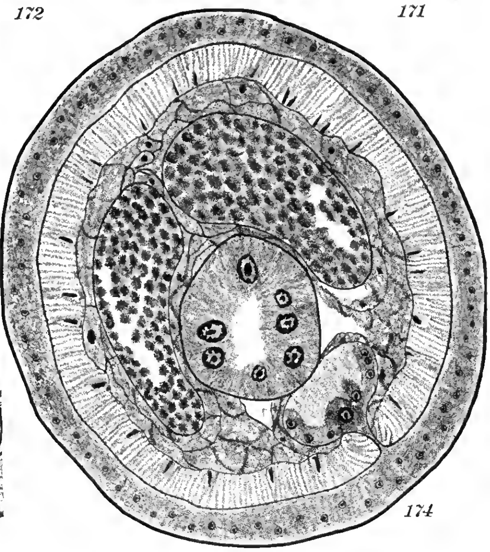

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