

ON THE STRUCTURE OF CLINOSTOMUM MARGINATUM, A TREMATODE PARASITE OF THE FROG, BASS AND HERON

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SEVENTEEN FIGURES

PREFATORY NOTE

In a previous article an account was given of the distribution in this country and Canada of *Clinostomum marginatum* together with some notes on its habits. A short time after the publication of that article (Osborn, '11) Professor Linton informed me that he had recently found specimens of *Clinostomum marginatum* in brook trout which were taken from Alder Lake, a private preserve in the Catskill Mountains in New York. The conditions under which the trout live are well described by Linton ('10). "It is a lake of forty acres in the heart of the mountains. The owner maintains a well equipped hatchery on the stream below the outlet and allows no other fish than trout in the lake." It is thus clear that the infection takes place in the lake, or, in other words, that the first stages of this worm and its primary host are to be found there. The lake is visited by fish-eating birds and thus we can readily account for the introduction of the parasite. As pointed out in my previous article, we possess no information as to the early stages in the life history of *Clinostomum*. We do not know its first host nor anything about its development. It is evident from the facts now known as to the occurrence of the parasite at Alder Lake that the infection must come from some form living in that lake, very likely some invertebrate serving as food to the trout. Occurrence in a small lake narrows down the problem of discovering this missing

chapter in the life history of our subject to very workable limits. More favorable conditions for a study of the point could hardly be imagined. Professor Linton's communication also adds a new host and a new locality to our knowledge of the distribution of this animal.

The present paper gives an account of the organization of *Clinostomum marginatum*. In justification of this when two accounts are already extant I may plead the fact that neither of them are fully adequate and in some points both are erroneous. *Clinostomum* is a parasite of some of our most desirable game and food fishes and it is especially obnoxious because it is lodged in the edible portion of its host. In order to keep the paper within reasonable size I have left out many histological items and it is hoped that later, when certain points have received additional study, a further account of the histology may be published.

EXTERNAL FORM AND DIMENSIONS

The outer form of this, as of most trematodes, is extremely changeable. It has therefore seemed best to give a description of the form and measurements of worms after fixation. There is little difference in form and proportions of body between the late immature stages from cysts in the fish and frog and mature worms from the heron. The encysted worms appear to average very little smaller. Figs. 1 to 4 enable one to obtain an idea of the form of the animal. Fig. 1 is from a worm killed under compression, which, after carmine staining, has been mounted entire. Figs. 2 and 4 are from horizontal and transverse series; they show parts which are on different planes as if they were on the same level and need to be checked by the transverse sections shown in fig. 3. Fig. 3 shows transsections from seven levels, all drawn to the same scale. They are from a series of about one thousand sections and the accompanying number is that of the section in the series, and shows, though only very roughly, the distance of the sections from each other.

The body is subdivided into two regions separated at the level of the ventral sucker. Anteriorly it is almost cylindrical, its cross section being an ellipse, posteriorly it broadens considerably

and is frequently somewhat concave on the ventral surface. In living animals the posterior region of the body at times becomes momentarily flattened and may thin out to a sharp lateral fin but this is a merely momentary form followed by the thicker form seen in the sections. The constriction of the body at the level of the ventral sucker is shown in Wright's figure ('79, fig. 1) and in that of Linton ('98, pl. 44, fig. 6). It is also found in the other species of the genus as can be seen in several of the forms figured by Braun ('00).

The dimensions of thirty-nine individuals were obtained from worms mounted whole in balsam and drawn in outline with the camera lucida. The measurements were taken both from mature heron material and from bass worms.

These figures correspond fairly with those previously published except that the minimum one for length is the least thus far recorded. A part of the differences can doubtless be attributed

TABLE 1
Showing the length and width in millimetres of thirty-nine individuals of C. marginatum

FROM BASS		FROM HERON		FROM HERON	
FIXED IN COLD SATURATED AQUEOUS CORROSIVE		FIXED IN CORROSIVE		FIXED IN CHROMIC ACID (0.3 PER CENT)	
Length	Width	Length	Width	Length	Width
3.1	1.2	3.0	0.9	4.3	1.1
3.5	1.3	3.0	1.0	5.5	1.5
3.8	1.5	3.5	1.0	6.0	1.5
4.0	1.6	3.7	1.0	6.5	1.5
4.0	1.6	4.0	0.7	6.5	2.0
4.0	1.6	4.0	0.9	7.0	1.7
4.0	1.6	4.0	1.0	7.0	1.7
4.1	1.6	4.1	1.0	7.2	1.7
4.5	1.5	4.5	1.0	7.5	1.8
5.0	1.3	5.0	1.2	7.7	1.7
5.5	1.5	5.0	1.2	7.9	1.8
		5.2	1.2	8.2	1.9
		5.5	1.7		
		6.0	2.2		
		6.5	1.5		
		6.5	1.7		

to the great mobility of the animal, but the series is too regular to be wholly due to mere differences of degree of extension of animals of a constant length and indicates also the existence here of a length variation such as is common in all animal groups. They furnish further an indication that the encysted worms, which are slightly younger, are smaller than the heron worms. The average length of the eleven specimens from the bass is only 4.1 mm. while that of the sixteen worms from the heron, fixed with the same reagent, is 4.59 mm. It is interesting to note the larger figures for the chromic acid material. The average length of these individuals is 6.77 mm.

The form of the anterior end of the body is remarkable. In many distomes the walls of the body converge anteriorly and meet at the mouth, here they run parallel until they intersect the margin of a peculiar area, the oral field, which closes the anterior end of the body. In an animal in which the oral field is in the resting position, as in fig. 4, it is oblique to the axis of the body, with the dorsal side projecting somewhat beyond the ventral. It is this obliquity to which Leidy's generic name alludes. Fig. 1 shows the margin of the oral field where it meets the side wall. Often there is a slight depression in the margin of the field at this point. In fig. 5 the field is retracted, a very frequent act of the living animal; this section is from a specimen which was caught by the fixing reagent in this act. A fuller account of the oral field structure will be given later.

The ventral sucker (figs. 1 *w*, 3 *C*, and 4) is a very conspicuous organ, both in the whole animal and in sections and is much larger than the oral sucker. In all living and preserved animals which I have seen it is entirely enclosed within the contour of the body. Its opening is always very distinctly visible and is usually triangular, with one of the equal sides anterior and the apex posterior. In some cases, however, as in fig. 1, the three angles are rounded, or the opening (as in Linton, '98, pl., 44, fig. 6) may be circular or even almost square (Wright, '79). The sucker has a length of 0.7 mm. and the same width and measures 0.4 mm. dorsoventrally. It is about half as thick as the body and reduces its space very much, as shown in fig. 4 *C*. The reason for the

great size of the ventral sucker has not been indicated by the behavior of the animal. I have not seen it used at any time. Its histological structure is such that it would seem to be fully functional and its great size indicates a function of considerable importance but no activities have been observed in connection with it.

POSITION OF THE GENITAL PORE

MacCallum ('99, p. 699) states that "at about the middle portion of the body behind the ventral sucker the genital *openings* [italics mine] are seen, close together, that of the female apparatus being directly in front of the male." Also on page 703 he says that the female genital opening is located "directly in front of the male genital pore." These statements certainly imply that there are *two genital pores*, a condition not found elsewhere in trematodes. The statements are however contrary to fact and are not consistent with MacCallum's figs. 3 and 7 where a single genital pore is clearly shown, so that it is difficult to see how they crept into his paper. The exact position of the genital pore was determined for twenty-two individuals, the data for which are shown in table 2.

The total length and width measurements are given and the distance from the anterior end to the genital pore. In order to make direct comparisons possible the position of the pore in percentage of total length is given in the column on the right. The opening is thus shown to lie posterior to the center of the body in every instance and to vary between 53.7 per cent and 68.3 per cent as extreme limits. A part of this difference may perhaps be attributable to individual differences of contraction or reagent action but in addition to these we must attribute it in part to variation in the actual position of the pore. If we take the average of these figures we should have 56 per cent as the point of location. The fact that some of the worms of this table show a greater length than any given in table 1 is because they are specimens killed under compression and are consequently unnaturally elongate. I have however admitted them to this table, as the

TABLE 2

Measurements used in determining the position of the genital opening

CATALOGUE NUMBER OF SPECIMEN	TOTAL LENGTH OF SPECIMEN	GREATEST WIDTH OF SPECIMEN	DISTANCE FROM ANTERIOR END TO GENITAL OPENING	PERCENTAGE OF TOTAL LENGTH TO GENITAL PORE
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	
b.....	3.8	1.2	2.5	65.7
x.....	4.4	1.3	2.8	63.6
l.....	6.0	1.5	3.4	56.6
750 b.....	6.0	2.3	3.4	56.6
n.....	6.2	1.6	3.8	61.2
750 c.....	6.7	2.3	3.6	53.7
734.....	6.8	1.75	4.1	68.3
g.....	6.9	1.7	3.8	55.0
e.....	6.9	1.75	3.8	55.0
d.....	6.9	2.0	3.8	55.0
h.....	7.0	1.8	3.8	55.0
a.....	7.2	1.8	4.2	58.3
762.....	7.5	1.8	4.2	56.0
750.1.2.....	7.5	2.0	4.25	56.6
f.....	7.5	2.3	4.3	57.3
762.....	7.75	1.75	4.5	58.0
k.....	7.8	1.9	4.5	57.7
i.....	7.9	1.75	4.6	58.2
m.....	8.3	2.1	4.9	59.0
c.....	8.8	2.1	5.3	60.2
750.1.....	9.2	2.3	5.1	55.4
750.a.....	10.8	3.0	6.1	56.4

compression may be supposed to have acted equally in all directions and so has not influenced these results.

A comparison of the figures of the different species of this genus as given in Braun's paper ('00) shows that the position of the genital pore differs very much in them, it being very near the posterior end of the body in *C. heluans* (fig. 10 *a*) 80.3 per cent, and very posterior also in *C. dimorphum*. It is nearly 79 per cent in the maximum case of *C. marginatum* of the table just given.

The genital pore opens into a chamber, the atrium (fig. 3) in which the male and female genital systems end. The opening of the terminal part of the uterus, the 'metraterm,' lies in this atrium anterior to the position of the cirrus of the male system.

This fact corresponds with the statements of MacCallum except in so far as he gives the impression that these openings are located on the outer surface of the body. The excretory pore opens (as shown in fig. 4) dorsally very near the posterior end of the body.

THE STRUCTURE OF THE BODY WALL

In general the trematode body is encased in a wall made up of a non-cellular cuticula, which may or may not be spinous, resting upon an outer zone of the parenchyma in which muscles run in various directions. For convenience we may consider the oblique muscles as marking the inner boundary of the wall though there is no break in histological structure at that point. The fibers of the oblique muscles lie in groups considerably spaced from each other so that the central parenchyma passes up between the muscles to the cuticle. This well-known structure is shown by Braun in *Fasciola hepatica* ('93, pl. 29, figs. 1, 2 and 3); it is also found in *Cotylaspis* (Osborn, '04, fig. 21) and in many other forms.

In *Clinostomum marginatum* there is a decided departure from the usual type which, since a similar structure has not been reported for any other trematode so far as known, merits a detailed description. Figs. 3, 4 and 5 show the relation of the wall to the body as a whole. The wall seems to be distinctly marked off from the central substance in these figures, due to the prominence of the large oblique muscles.

The cuticle is as usual. It measures from 0.01 to 0.015 mm. in thickness, is entirely structureless, is reinforced by spines which ordinarily do not project beyond the surface. The spines are acute and taper from a broader base seated on the deeper surface of the cuticle. They are set close together. Twin spines of smaller size sometimes occupy the position of one spine of ordinary size, as if the amount of embryonic material apportioned to one spine had been subdivided between two. Spines are found in all parts of the general surface of the body, they are more numerous on the ventral surface and on the posterior parts of the dorsal surface. They are not found generally on the surface of the oral field, with the exception of a small area immediately adjoining

the mouth opening. The spines have a strong affinity for stains and in the iron-haematoxylin preparations are deeply tinged by it, while the cuticle remains unaffected. We know nothing of the process by which spines are formed.

The principal peculiarity of the wall of *Clinostomum* is the existence in its inner layer of an extensive system of cavities, an extension of deeper cavities pervading the parenchyma everywhere, connected ultimately with the excretory collecting vessel. A full description of these cavities will be given in connection with the excretory system of which it forms a part. They are conspicuous in longitudinal sections and can be seen in fig. 4 G and figs. 6 and 7. The subcuticular cavities run in such a direction as to encircle the body, with connections inward to the collecting vessel as seen in transverse sections.

Organs in the cuticle, perhaps sensory. Certain cavities in the cuticle (see fig. 8) are possibly parts of sensory organs. They can be found in the areas in the oral field immediately around the mouth and on the dorsal surface near the anterior end, but not in the surface generally. In fig. 8 two of these are shown. They are spherical cavities excavated in the substance of the cuticle by which they are entirely enclosed except at the base of where they are open to the parenchyma on which the cuticle rests. The cavities thus have no communication whatever with the exterior. A fine deeply stained fiber can be traced into these cavities from the parenchyma. The indication from views like that of the cavity on the right in the figure is that this thread expands into a disk resting against the upper surface of the cavity. The best interpretation of the function of these organs which we can make on the basis of their structure is that they are the terminations of a pressure sense apparatus, the fiber being regarded as a prolongation from a more deeply seated nerve cell.

We have very few references to such organs in the cuticle of other trematodes. They are doubtless not uncommon but not many forms have been examined for them. Nickerson ('95) found a very similar organ in *Stichocotyle*, one of the *Aspidobothridae*. The organ shown in his fig. 15 differs only in size from the one in fig. 8 of this article. Bettendorf also ('97, fig.

30) found organs of much the same kind in the oral sucker of certain distomes. Pratt ('09, fig. 10) copies a figure of a section of the body wall of *Ligula*, one of the cestoda, from Zerneke. The section made by the Golgi method shows nerve cells located some distance below the cuticle from which threads run outward to small spherical 'sense organs' located in the basal level of the cuticle. These organs and those of Nickerson are similar in structure to those of *Clinostomum*. In *Cotyiaspis* (Osborn, '04, fig. 33) the cuticle contains organs apparently of sensation but of a different type from these. They are in the surface of the cuticle and communicate with the exterior. They have a number of stainable fibers which unite and pass as a single thread inward through the cuticle and disappear in the parenchyma. Nickerson, in the article just referred to, in his fig. 14 has shown an organ in the cuticle which communicates with the exterior.

Muscles of body wall. The usual muscles are present as in trematodes at large. Figs. 6 and 7 show them in longitudinal and transverse section respectively. In addition to them there is a layer of longitudinal muscle, which lies immediately below the cuticle. This is an unusual layer of longitudinal muscle, the usual one being located inside the circular muscle, while this is external to it. We may designate it the outer longitudinal muscle (*mo*) the other being then called inner (*mi*) in the figures. The fibers of this outer longitudinal layer were seen by Looss ('85) and are shown in his fig. 23. According to his figure they are very much stronger than I find them in my sections. In my material the fibers are exceedingly small, having a diameter of only 0.0009 mm. In fig. 7 they are shown under a magnification of 1100 diameters. Their size can perhaps be better appreciated by a comparison with those of the inner longitudinal layer as seen in figs. 6 and 7. In the latter the fibers are cut transversely. These fibers lie at equal distances apart, in a single layer, and in direct contact with the cuticle.

Writers who have given attention to the finer structure of trematodes (Braun, '93; Otto, '96; Stafford, '96, to mention three at random) agree that there are three layers which compose the musculature of the body wall, viz: circular, longitudinal and

oblique. I have recently made a re-examination of the sections on which my paper of 1904 was based to test the possibility of the coat being present in *Cotylaspis*; as a result I am entirely convinced that there is no outer longitudinal muscular layer. It thus seems safe to conclude that *Clinostomum* is peculiar in the possession of this layer, though a similar may perhaps be found later in some other forms. My observations of the other coats also confirm those already reported by Looss. The fibers of the circular coat lie in several layers (fig. 7); they are very small, though larger than those of the outer layer, measuring 0.0012 mm. They do not fall into groups or bundles like those of the inner longitudinal layer. These fibers are seen in sections generally at various levels between the sub-cuticular excretory cavities, which thus seem to occupy an area produced by the expansion of that part of the body wall, in correlation with the presence of these cavities.

The inner longitudinal muscles lie much deeper than usual. Instead of lying quite near the cuticle as they do in many cases, and in close contact with the circular muscles, they are located here, as shown in fig. 6, below the vessels of the excretory system at a distance of 0.04 mm. from the outer muscles. The inner muscles are thus seen to be pushed down to a considerable depth below the bottom of the cuticle near which they usually lie. This departure from the ordinary arrangement is clearly a structural adaptation correlated with the presence of the sub-cuticular vessels. We may further perhaps regard the presence of the outer longitudinal muscles as a part of the same adaptation; they may have been developed thus near the surface to offset the disadvantage due to the increased distance of the inner longitudinal layer from the cuticle.

The inner longitudinal muscle fibers are very distinctly grouped into bundles alternating with intermediate areas from which they are absent. Fig. 7, *mi*, shows one of these bundles in cross section; it is made up of a cluster of fibers without other muscles in close proximity. The fibers of the inner longitudinal muscle differ in size, as can be seen in fig. 7; the largest ones are much larger than those of the circular muscle, measuring 0.004 in diam-

eter. The oblique muscles running in the usual two directions are more deeply located.

Certain interesting points were noted in the cytology of the body wall muscles which will receive attention later in connection with those of the parenchyma.

Wall structure in the oral field. The wall of the oral field presents a structure decidedly unlike that of the general surface of the body. Figs. 3 and 5 show the wall under low magnification. It is very much thinner, owing to the great reduction of all its components. The cuticle becomes so thin as to be barely recognizable. The spines, which are so general over the rest of the surface of the animal, are entirely wanting on the oral field with the exception of a small area immediately around the mouth opening where spines of a much smaller size exist. Sub-cuticular cavities so conspicuous elsewhere are scarcely recognizable. They do not in any case take on the regular arrangement so usual elsewhere in the body wall, but are merely irregular cavities underlying the surface and communicating internally with the vessels of the excretory system. The musculature of the oral field does not agree with that of the rest of the body. The various layers are not continued from the wall into the field. Fibers can be found lying parallel with the surface but they cannot be connected with the fibers in the wall beyond. The longitudinal muscles of the parenchyma (*pml* in fig. 3) run on anteriorly until they meet the surface of the field to which they are then vertical. They are shown in fig. 5 at *ml* running directly to the wall, their position enabling them to act as retractors of the field as shown in the figure.

Glands (?) in the body wall. There are certain nucleated cells lying in the body wall, as shown in fig. 7 at *gl*, which seem to be probably of a glandular nature. They are very long and slender, consisting of a globular body, which lies on the level of the oblique wall muscles, and a tapering portion which can be traced outward to a termination on the inner surface of the cuticle. The outer end of the cell may branch so as to present in sections two terminations. No passage through the cuticle has been seen or any indications of secretions passing from these cells to or through it.

The globular body of the cell is entire on its inner side; that is, there are no processes given off from it. The bodies of these cells contain a large clear nucleus. There is no cell wall. The cells stain readily with iron-haematoxylin. Their bodies which lie on nearly the same level constitute a faint zone parallel with the surface of the body.

The position and, to a certain extent the structure of these cells, remind one of the cells found by Blochmann ('96) in trematodes and cestodes in a similar situation. I have not had access to this paper of Blochmann, but several writers have reproduced his figures, among them Pratt ('09) who, in his recent paper on the cuticula, copies a figure from Blochmann of the wall of the cestode *Ligula* and designates 'sub-cuticular cells' certain cells which show great resemblance to those of *Clinostomum* to which I have just referred. There are some differences between the cells in Pratt's figure and those in fig. 7 of this article. In *Ligula* the cell body is sharply angulated on its inner side and produced into fine threads, which are lost in the deeper parenchyma. Externally also the cells soon taper to a very fine thread. In spite of these differences however it seems quite reasonable to regard these cells in their relations to the body structure as a whole as identical with the sub-cuticular cells of *Clinostomum* just described. Benham ('01) gives a diagram of the structure of the body wall of *Ligula* which has cells more like those seen in *Clinostomum*. It is held by some writers that these are epithelial cells which have sunken from a position originally on the surface. The *Clinostomum* sections do not supply any evidence in support of the view that these cells are epithelial in origin.

THE PARENCHYMA

The interspaces among the organs within the body are permeated by the usual network of branching fibers emanating from large nucleated cells. In places where the parenchyma comes in contact with the surface of the walls of various organs such as the oesophagus (fig. 8) and the uterus, but not of all (not of the intestine, for example) its fibers become much more numerous

and denser, so as to form a compact capsule for the support of the parts beneath.

There are certain cells loosely clustered together in a mass which lies in the parenchyma in the region directly anterior to the ventral sucker. They are shown in figs. 1, 3, 4 *B* and 5. These cells are oval, and measure 0.03 by 0.015 mm. The nucleus, which lies near the margin of the cell, is clear and round and contains one or more nucleoli and a few minute grains of chromatin. These cells lie among those of the parenchyma but differ from them in appearance, having no processes and no connection with the fibers of the parenchyma. Each cell is sharply bounded. They also have no connection with the surface, no processes can be traced from them going off toward the surface and their long axes lie in all directions. If they communicated with the surface the cell bodies would point in that direction. There seems to be no doubt that they are purely internal in their physiological action. They are similar in cytological appearance in both bass and heron worms. The cells contain a clear homogeneous material which has a marked affinity for stains.

The physiological significance of this organ is entirely unknown. Looss recognized these cells in the immature worms from the fish cysts. He suggests ('85, p. 46 of separate) "vielleicht sind es die Anlagen von Drüsen, die später . . . erste ihre Funktion antreten werden." But this suggestion cannot be accepted since the cells are identical in structure in the mature worms. MacCallum's suggestion ('99, p. 700) that they are parenchyma cells cannot be accepted, for they are not found outside of certain limits and parenchyma cells pervade all parts of the body. The great number of these cells leads one to believe that they are important. Their entire absence of connection with other organs implies an independent function. It seems therefore most likely that they are concerned in some way in internal secretion.

Parenchymal muscles. The muscles of the parenchyma are very well developed in *Clinostomum*. The usual two sets are found, (fig. 3) namely, the longitudinal muscles and the dorso-ventral ones. There are no horizontal muscles. As the longi-

tudinal muscles pass forward they ultimately meet the oral field almost vertically to its surface and attach there so that they thus become its retractors. Fig. 5 is a camera drawing from a specimen which died with the oral field retracted. In this section the longitudinal parenchyma muscles can be traced forward directly to the in-bent parts of the field. Observations of sagittal sections furnish evidence that, at least in many cases, a single muscle reaches across from the dorsal to the ventral surface, for nucleated myoblasts can be seen in connection with these muscles and these are grouped in the center of the body.

Some cytological features are well shown in the muscles of *Clinostomum*. Both the inner longitudinal wall muscles and the longitudinal parenchyma muscles frequently show transverse subdivisions into stained and unstained zones such as has been noted in other forms by various writers on trematode histology, but in no case with which I am familiar are they shown so distinctly as here. Nickerson ('95) states that in *Stichocotyle* the longitudinal muscles of the body wall appear to be tubular "with nodes of deeply staining substance filling the lumen at intervals," and shows the appearance in fig. 16 of his paper. Stafford, too, in *Aspidogaster* ('96, fig. 26) noted the presence of 'transverse lineations' in the parenchyma muscles which he speaks of as 'contraction centers.' He does not note any in connection with the body wall muscles. Bugge ('02) in his paper on the excretory system in cestodes and trematodes incidentally mentions 'Querstreifung der Muskelfasern' which he observed in the circular and longitudinal muscles of *redias* and *cercarias*, "wie wir sie bei Arthropoden und andern Wirbellosen auffinden," and also quotes Cerfontaine, to whose article (in *Bull. Acad. Sci. Belg.*, 27, no. 6) I have not had access, and Nickerson as having seen the same thing. In 1904 I saw and recorded ('94, figs. 11 and 12) a similar muscular structure in *Cotylaspis*, a form related to *Stichocotyle* and *Aspidogaster*.

Turning now to *Clinostomum*, figs. 9, 10 and 11 are from immersion objective camera drawings of longitudinal wall and parenchyma muscles. Figs. 9 and 11 are from the body wall and parenchyma muscles respectively from the same series. Both

were drawn with the same objective but 11 is made with a higher eyepiece. Such views are found generally in many different series so that we are justified in regarding them as a normal feature of the cytological structure of *Clinostomum* muscle. In fig. 11 it is clearly seen that the muscle is made up of several parallel-sided filaments of considerable length, composed of a substance which is not strongly influenced by haematoxylin and a second deeply staining substance. The swollen globular appearance of the latter leads us to believe that it is a peculiar 'contractile substance.' The fibers do not all present this appearance. One is represented in fig. 10 in which also the myoblast and nucleus are shown. The myoblast is large, measuring 0.014 mm. across, and the nucleus has a diameter of 0.005 mm. Fibers can be traced from such myoblasts. These appear differently from those in figs. 9 and 11, showing a dark contour on the wall and a clearer center. In cross sections the appearance is that of two substances, a clearer central and a darker surface material. These seem to be the 'hollow muscles' of writers. Fig. 10 shows the fibers, probably in an uncontracted state, while 9 and 11 are contracted fibers. A more adequate study of the cytology of this muscle is beyond the scope of this article.

THE ALIMENTARY APPARATUS

Oral sucker. The mouth opening lies in the center of the oral field and leads into the cavity of the oral sucker. The sucker is nearly spherical and is very much smaller than the ventral sucker, measuring 0.28 mm. long and 0.25 mm. across. It has the usual cuticular lining and heavy muscular wall composed of fibers running in the various directions.

Oesophagus. The pharynx, which is generally present in trematodes and usually follows close after the oral sucker, is entirely wanting. There is a short tube immediately behind the oral sucker which, after running ventrally a short distance, makes a dorsal bend to meet a transverse portion of the intestine. This is the oesophagus. The structure of the two bends is somewhat different. The more anterior portion is very thin walled and is lined with a thin cuticle continuous with that of the oral sucker.

The posterior chamber is a globular dense body as seen in a whole-mounted worm. The wall is thick and heavy, due not to the presence of a heavy muscular coat as it would be if the organ were a pharynx, but to the very peculiar structure of the cuticular coat. The cuticle here, which is continuous with the thin layer of the anterior chamber, suddenly changes its character and becomes a mass of tall slender processes springing vertically from the wall and projecting freely into the lumen of the organ. Their appearance is shown in fig. 12. They bear some resemblance to the tall processes of the epithelium of the intestine just above them, with which they are directly continuous. They have every appearance of having arisen from a cuticularized epithelium. In *Cotylaspis* (Osborn, '04) there are similar indications of an epithelial origin of the cuticle which lines the oesophagus.

The posterior chamber of the oesophagus has almost no muscular tissue in its wall. A very few circular and longitudinal fibers can be recognized, evidently strictly comparable with the muscles of the intestine. There is however a coating on the inner surface of the organ which is a condensation of the parenchyma at large. This has a fine but definite boundary next the parenchyma.

Oesophageal glands. Numerous cells lie in close proximity to the oesophagus (*oegl* in fig. 12) which are not ordinary parenchyma cells. Their massing too goes to show that they constitute a definite organ whose position requires us to regard it in its work as in some way a part of the oesophagus. The cells are not angular like those of the parenchyma but have rounded outlines. Each cell has a nucleus poor in chromatin and a distinct nucleolus. The sharp line in the figure passing on the left side of this group of cells marks the boundary of the denser parenchyma which ensheathes the oesophagus. It will be seen that the cells are located outside of this sheath and so are somewhat remote from the lumen of the oesophagus. An organ of this sort is usual in trematodes; it is often called a 'salivary gland.' One writer however, (Otto, '96) questions the salivary function of the cells in the Amphistomes, since he does not find any connection between them and the lumen of the oesophagus. We shall however go on calling these organs 'oesophageal glands' though we have no definite

information as to their physiological significance. It is possible that they are merely mucin-forming organs and that they acquire a temporary connection with the oesophagus.

The intestine. The intestine consists of a part crossing the body transversely (fig. 4 A) which, after bending, continues as the two long lateral caeca. The caeca lie in the center of each half of the body and extend (fig. 2) to the level of the excretory bladder. The walls of the caeca are not entire; blind pouches extend outward from them. These pouches are not as large and distinct in the material after fixation as they are in life. Fig. 13 a is a free-hand drawing of the living organ in a specimen just liberated from a bass cyst. The pouches arise on both sides of the intestine; they are very numerous and close together and are not long and slender. The form of these pouches distinguishes *C. marginatum* from *C. heterostomum*. In the latter (Braun, '00, fig. 1) there are a few very long and slender diverticula which are confined to the outer side of the intestine. In the presence of these intestinal pouches *Clinostomum* resembles *Fasciola hepatica* and the planarians. Fig. 15 is a camera drawing of the wall of the intestine. The pouches are conspicuous in some places and absent in others. This corresponds with the facts seen in life; in bass specimens the pouches are contractile and at moments are drawn back into the wall. The wall itself is contractile; in life its movements are very conspicuous. The lumen is filled with a fine grained material lemon yellow in color. This flows back and forward, streaming, the pouches empty themselves of it or fill with it and the contractions of the wall may obliterate the intestine entirely for a moment.

The structure of the intestine wall from a fully matured heron worm is shown in fig. 13. The epithelium presents two distinct zones, a deeper basal one and, arising from it, a second zone of separate columnar structures. The basal zone is a continuous protoplasmic layer, in which distinct nuclei occur at somewhat regular intervals, but without any walls dividing it into cells. This basal syncytium takes the stain readily. The processes of the outer zone show a relation to the nuclei below though they are not always strictly over them. In a section, (fig. 13 on the

left) this may be due to slight differences in level. It is planned to treat certain cytological points connected with this epithelium in a later paper so that for the present I will only state that these processes are apparently amoeboid and capable of being projected from the deeper body of the cell or retracted. They are clear and barely stained. They are filled with minute black pigment grains which have been traced to the decomposed blood corpuscles of the heron on which the worm had fed. While the intestinal epithelium in some instances shows the appearances just described there are other cases in which its form is quite different as shown in fig. 14. Here it is a low, level surface consisting of a layer of protoplasm with imbedded nuclei. There are no division walls and the layer has the appearance of a syncytium. Cells of the type shown in fig. 15 are found in the intestine of worms from the bass. I have interpreted them as being in a resting or non-digestive state while those in fig. 13 are actively engaged in the work of digestion. In many trematode sections and figures with which I am familiar the cells of the epithelium are entirely distinct and independent to their very base. They do not show any fusion as if syncytial as is the case here. In connection with the structure of *Cotylaspis* ('04, fig. 19) I called attention to the entire independence of the cells of the intestinal epithelium. In *Cryptogonimus*, on the other hand, the cells of the epithelium of the intestinal caeca are fused into a syncytium.

The circular muscular coat is very scanty, its fibers lie close to the epithelium. The longitudinal coat is also very feeble. Its fibers lie at a distance in the parenchyma.

The cavity of the intestine of the worms obtained from bass cysts is filled with thin, flat, four-sided crystalline bodies. As soon as the worm has escaped from its cyst the strong peristaltic contractions already mentioned force this substance backward and forward. At frequent intervals portions of it are expelled from the body through the mouth. In worms obtained from the heron this material is not found in the cavities of the intestine. In such worms on the contrary the intestine has been found to contain a coagulated fluid substance with blood corpuscles scattered through it, which upon careful examination were found to be

identical with blood corpuscles taken from the heron. This substance is evidently food, but the content of the caeca of the bass worm cannot be so considered. Its prompt rejection from the body as soon as it is liberated from the cyst would be evidence sufficient to justify this conclusion. Its crystalline form and the fact that it is discharged as soon as the animal becomes free, point to the hypothesis that the cavities of the intestine are made use of for storage during encystment and that the substance therein is a waste product.

THE EXCRETORY SYSTEM

The excretory system of *C. marginatum* has never been described. The indications of it which are given in Looss ('85, fig. 22) are purely diagrammatic and somewhat misleading. The system, moreover, presents some features which are very unusual, so that the whole subject needs a careful revision.

The location of the excretory pore has already been noted. It opens from a very short duct (fig. 15) which is in communication with the v-shaped bladder. Internally the two branches of the bladder receive the termination of the collecting tube in the center of a flattened area. At the excretory pore there is an invagination of the cuticle which covers the outer surface of the body. As this passes more deeply it gradually changes into a cubical epithelium composed of nucleated cells identical in structure with those which make the wall of the collecting vessel. At intermediate points epithelial cells of the bladder show all stages of degeneration in structure and pass insensibly into cuticle. There is no muscular tissue in the walls of the bladder. Living specimens were observed particularly with reference to contractions in the bladder as I had found this organ in *Cotylaspis* interesting in this respect ('94, p. 216) but the walls were not contractile. In correlation with this is the absence of a sphincter at the surface pore (one is present in this place in *Cotylaspis*) and the presence of a sphincter at the junction of the collecting vessel and the bladder. We may conclude from the position of the sphincter and the non-contractility of the bladder that the latter in *Clinostomum* is

merely a passage and not a place of storage and that the collecting vessel is the functional bladder.

The collecting vessels are very large in the posterior region of the body, but anteriorly their identity is lost. It is usual in trematodes for a collecting vessel to run from the terminal bladders forward to a point near the anterior end of the body and then to bend suddenly on itself and run backward again. The second vessel, called the recurrent vessel, is supplied with a strong vibratile apparatus, while the collecting vessel lacks these. In *Clinostomum* the collecting vessel is readily traced forward as far as the ventral sucker. In fig. 2 it is shown on the right side omitted on the left, in fig. 3 it is shown as far forward as the genital organs and then omitted, in fig. 4 it is shown in sections *B-G*. It disappears a short distance in front of section number 150. The level of the vessel is seen from the cross-sections. It always lies externally to the caeca and generally slightly ventrally. Its diameter is quite variable as in fig. 15, a camera drawing of a section passing in its plane for a long distance. The wall is epithelial and muscular.

I am not able to give a definite account of the relation between the collecting vessel and the recurrent vessel. I have devoted much time to the study of this in different ways without being able to follow the collecting vessel forward to where it meets the recurrent vessel. The body is too thick anteriorly to allow this point to be seen in an entire compressed specimen. I have repeatedly examined the youngest individuals I could find but without success. The network of anastomosing vessels (described in a moment) are so complicated in the anterior of the body that it is impossible to recognize the collecting vessel if, indeed, it has remained distinct from them. It is, of course, possible that the collecting vessel does not remain distinct but is lost in the network of vessels.

Allusion was made above to the system of cavities which lie in the body wall immediately under the cuticle. In living worms just removed from bass cysts these cavities are filled with a cream-colored fluid composed of minute highly refractive droplets which has the effect of an injection, making it very easy

to distinguish the different vessels and their connections. In such a preparation the collecting vessel as well as the smaller vessels which are derived from it are readily seen. The appearance of this system of vessels is shown in a free hand drawing (text fig. 1, for which I am indebted to Mr. Faus Silvermale) made from an unusually young live bass worm under slight compression. The collecting vessel can be followed, its size diminishing as it advances until it is lost in the network of its subdivisions. The network shows a predominance of transverse vessels, though

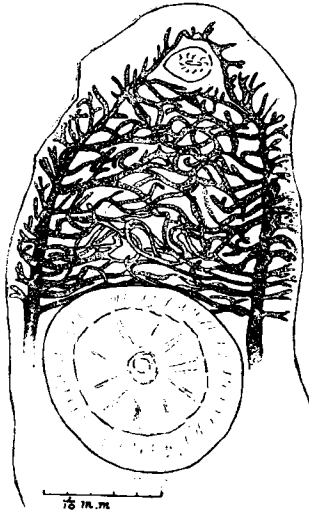


Fig. 1 Free hand drawing from a young, living, slightly compressed bass worm. Zeiss 2 A.

with many communicating vessels connecting them, and some which cross over to the other side of the body and become continuous with those of that side. In this preparation the recurrent vessel could not be seen, but as I shall point out later it seems most likely that it is present and joins the collecting vessel in this part of the animal.

In the posterior part of the body these vessels may be somewhat more definitely subdivided into two sorts according to their destination, a superficial system which runs to the surface and becomes the subcuticular vessels and a deep system which passes

inwardly among the inner organs. The origins of both kinds can be seen in fig. 15; they arise at close intervals from the collecting vessel on both sides. Some of the vessels of the superficial system run directly to the surface (one of these is shown in fig. 4 G) where they become circular vessels immediately under the cuticle. Since these tubes encircle the body they are readily seen in sections passing tangentially in the plane of the surface. The tubes frequently anastomose and all communicate directly with the collecting vessel. They are cut across in longitudinal sections and produce the appearance seen in figs. 3 and 5.

Looss ('85, p. 49) expressed a suspicion that these subcuticular vessels communicate directly with the exterior: "so halte ich es doch für höchst wahrscheinlich, dass—der Excretionsporus nicht die einzige Stelle ist von welcher dieses Maschenwerk von Kanälen mit der Aussenwelt in Verbindung tritt und zwar sind es die subcuticularen Maschen des Gefässnetzes welche diese Kommunikationen vermitteln." And later he says "Ich halte es nun nicht für unmöglich dass sie (i.e., subcuticular vessels) auch nach aussen münden," etc. But he closes his account with an admission to the effect that he has not succeeded in demonstrating the presence of openings from them to the exterior. The observations of the movements of the fluids in these passages described above render it very certain that outlets from them directly to the exterior do not exist; were such outlets present we should undoubtedly have seen stuff from within issuing through them.

Turning now from these superficial vessels to the deep ones we find that they pass inward, permeating the parenchyma everywhere. The vessels of one side tend to remain entirely confined to that side, they anastomose with one another but do not often become continuous with those of the opposite half of the body.

Allusion has been made to the flow of the contents of the excretory vessels in life. Pulsations were seen in bass specimens in the wall of the collecting vessel, forcing a stream out into the dependent vessels. Later these streams reversed their direction and the droplets course back again into the larger vessel. As already noted there is no escape distally; the movement is an ebb and flow.

An observation made recently upon a worm from a frog cyst seemed to be inconsistent with a circulatory movement of the droplets as just described. The cyst attracted my attention because of its small size, it being globular, compact and only 1.3 mm. in diameter, quite unlike frog cysts and much like those found in the bass. When the worm had been liberated and arranged in a compressor for observation it was seen that the vessels of the anterior region were filled with highly refractive droplets. These droplets were not in a state of flux but were stationary. The pulsations mentioned above and the flow of droplets were seen in bass specimens in the posterior region. In the frog worm the vessels of the posterior region were empty. It is possible that this worm was a very recent arrival in the frog and that the movements of the droplets had not yet begun to take place.

A system of branches derived from the collecting vessel and permeating the body in this way is very unusual in trematodes. The usual structure is a network of minor vessels uniting to form larger vessels which finally merge into a single collecting vessel. In three widely separated forms however we find an arrangement somewhat similar to that of *Clinostomum*. In a young stage of *D. echinatum* Looss ('04, fig. 192) figures a collecting vessel much resembling that of *Clinostomum*, especially in the anterior body region where side branches are given off, the main vessel meanwhile continuing until it meets the ciliated recurrent vessel at the extreme anterior end of the body. A comparison of Looss' fig. 192 with that of the younger stage shown in 191 indicates that the branching is a late feature in the life history, a fact of interest since it is uncommon in trematodes at large. In adults of *D. echinatum* (Looss, '94, fig. 114) these vessels are very much branched but the branches do not assume the form of a sub-cutaneous system like the one so well developed in *Clinostomum*. In *Cephalogonimus* also the excretory collecting vessel is branched. This point was first noticed by Poirier ('85, fig. copied by Braun, '93, pl. 20, fig. 9) who says "Ces canaux lateraux comme le canal impair median, emittant sur tout leur parcours des branches ramifiees se dirigeant vers le bords lateraux du corps. Ces ramifications s'entendent en avant, jusque un peu au-dela du point de bifurcation de l'oesoph-

age." In his illustration, as well as in this description, there is no recognition of subcuticular vessels like those of *Clinostomum*. In a study of the parasites infesting the frogs of Minnesota I have happened to find specimens of this genus. The study of living and sectioned material of this form demonstrates that, while there is an extensive system of vessels derived by branching from the collecting vessels and one which bears considerable resemblance to that found in *Clinostomum*, encircling subcuticular vessels are not developed.

A third form with somewhat similar branching excretory vessels was encountered at Chautauqua, New York. In the livers of sun-fishes certain cysts were found which contained immature flukes belonging to the holostomes. These forms are peculiar in having a broad thin anterior body region bearing a resemblance to the foot of a gasteropod mollusc and posteriorly a globular mass carried vertically over it. The excretory pore is located at the summit of the latter. In the thin anterior part there are a median and two lateral longitudinal vessels, extending from which are branching vessels extending everywhere in the foot, anastomosing and forming a complete network. All of the vessels of this system contained minute highly refractive droplets, similar in appearance to those found in the excretory cavities of *Clinostoma* which had been recently liberated from bass cysts. In the living worms masses composed of these droplets were discharged from time to time from a point located at the posterior end of the body, the excretory pore, thus indicating that the passages are members of the excretory system. In life the droplets were in constant motion in the vessels, coursing rapidly in all directions as they had been seen doing in *Clinostomum*.

This observation, taken in connection with the presence of such droplets in the encysted specimens of *Clinostomum* and their absence in the heron specimens of *Clinostomum* and the free living *Cephalogonimus*, constitutes an argument in favor of the supposition that the droplets are composed of chemical wastes. In an encysted organism these must be disposed of in a way that will prevent their damaging the animal, accordingly they cannot be discharged from the body in the ordinary manner but must be

stored during the period of encystment. It is thus reasonable to look upon the extensive equipment of spaces possessed by the excretory collecting vessel as a storage apparatus. In favor of this interpretation is the further fact that in both *Clinostomum* and the holostome just mentioned the contents of these cavities begin to be discharged as soon as the worm has escaped from its cyst. I think that the substances contained in the intestinal caeca may also prove to be waste matters and that these cavities are also being employed for storage.

The recurrent excretory vessel. Reference has already been made to a vessel which parallels the collecting vessel. It is readily seen in the parts of the body behind the ventral sucker; anteriorly it is lost in the maze of vessels which are derived from the collecting vessel. Posteriorly (fig. 16) it bends sharply forward, as the vessel into which the capillaries drain. This, which I have called the recurrent vessel, is spirally coiled in all sections and even shows this state in living animals. It is located externally and somewhat dorsally to the collecting vessel, but is much smaller, having a diameter of only 0.02 mm. The wall is composed of a very thin membrane. The tube is uniform in diameter in all parts; unlike the collecting vessel the wall possesses no contractility, there being no muscular tissue present. The wall of this vessel is supplied at close intervals with peculiar ciliary organs. In life these vibrate at a very rapid rate so that they become visible only after their vitality has become lowered. Then it is seen that the organ is attached posteriorly in the wall of the vessel, the rest being free and pointing anteriorly so that its vibration produces a current running forward in the tube. These organs are located in the recurrent vessel at close intervals. Bugge ('02, fig. 62) finds that in certain cercarias occurring in certain helices the chief canals are supplied with 'Wimperschopfen' which correspond with the organs just mentioned and in addition that there is a lining of ordinary cilia clothing the rest of the inner surface of the wall. There are no similar ordinary cilia in these vessels of *Clinostomum*. The ciliary organs are many times longer than the diameter of the vessel in whose lumen they lie. In life I am unable to recognize individual cilia in them but in sec-

tions after the application of iron-haematoxylin, cilia are clearly seen as sharp black wiry looking lines.

In view of the fact that these ciliary organs produce strong current which flows forward, we are compelled to suppose that the recurrent vessel discharges directly into the collecting vessel, although as already noted it has not been possible to recognize the connection.

Flame-cells and capillaries. The ultimate members of the excretory apparatus of *Clinostomum* are very imperfectly known as yet. Much attention and time have been dedicated to the effort to trace these parts in the living animal with very inadequate reward. Some glimpses of them have been obtained however both in life and in sectioned material. Flame-cells have been seen; they are very tall and slender with a narrow base where the elongate and narrow mass of cilia are attached. A detailed account, with illustration of these flame-cells together with some other finer points, must be reserved for a later article.

It has not been possible to determine the mode of arrangement of the capillaries and connecting vessels. In some places the capillaries have been recognized. They ran in a posterior direction. Vibrating ciliary organs could be seen within them. It was not possible in any case to trace these vessels to a point of connection with the recurrent vessel and I feel very strongly convinced that the recurrent vessel does not receive any branches.

EXCRETORY SYSTEM IN TREMATODES IN GENERAL

There is considerable difference in the plan of anatomical organization of the excretory systems of different trematodes. In all there is a system of flame-cells and their capillaries and one, or occasionally two (e.g., *Aspidogaster*), posteriorly located excretory pores. But there is great difference as to the vessels lying between the external pore and the capillaries. All degrees of distance between the terminal bladder and the capillaries can be found. In *Opisthoglyphe endolobum* (Looss, '94, fig. 157) a large forked chamber, confined to the posterior third of the body, receives directly a vessel formed by the junction of the capil-

laries. In *Allocreadium isoporum* (Looss, '94, fig. 15) a collecting vessel can be recognized which reaches the first body third and there receives the capillary vessels. In *Gorgodera cygnoides* (Looss, '94, fig. 125) a collecting vessel runs the whole length of the body and at its anterior end meets a vessel which runs backward a short distance before the connecting vessel from the capillaries meets it. This might be considered as a short recurrent vessel. In *Distomum echinatum* (Looss, '94, fig. 191) a fully developed collecting vessel meets a still longer recurrent vessel. In *Harmostomum leptostomum* (Looss, '94, fig. 113) the collecting vessel is fully developed and the recurrent vessel runs nearly to the posterior end before the two vessels enter it from the capillaries. Finally in *Cotylaspis* (Osborn, '04, fig. 26) the recurrent vessel as well as the collecting vessel, is fully developed, the capillaries discharging into a canal which is a forward bend of the recurrent vessel. We see from this brief survey of these different forms that, with the gradual development of both collecting and recurrent vessels, an increasing interval is interposed between the capillaries and the exterior. The structure of these two vessels is entirely different, one being entirely destitute of ciliary apparatus and furnished with muscular tissue, the other being ciliated and devoid of muscle. Clinostomum has its place among the forms with complete collecting and recurrent vessels, and in addition possesses the remarkable system of branches derived from the collecting vessel.

It would be possible to find a series of forms showing reciprocal developments of excretory collecting vessel and bladder. Thus in *Stichocotyle* there is no bladder and the collecting vessels are very large; in the closely related *Cotylaspis* the collecting vessels are narrow tubes and there are two well developed excretory bladders which are rhythmically pulsatile.

While noting that the parts of the excretory system thus exhibit a series of degrees in the development of complexity of organization we must not forget that this series is found not in a group of genetically related animals but among forms which are widely separated in the system. On the other hand when we examine forms which are closely related we find great differences. Thus

in the three genera of the Aspidobothridae we find in *Stichocotyle* (Nickerson, '95, fig. 23) no recurrent vessel, a very voluminous collecting vessel and no bladder; in *Aspidogaster* (Stafford, '96, fig. 15) a partly developed recurrent vessel, moderate collecting vessel, no bladder and two excretory pores; in *Cotylaspis* (Osborn, '94, figs. 5 and 26) a fully developed recurrent vessel, a small collecting vessel and two well developed bladders. So that it is impossible to attach any phylogenetic value to differences shown in the organization of the excretory system.

THE REPRODUCTIVE SYSTEM

My observations are in substantial accord with those of Looss and MacCallum with regard to the chief facts in the anatomy of this system. Figs. 1, 2 and 3 show the parts in situ as they appear in various sectional planes. Fig. 17 is an outline of the organs based largely on a study of the total preparation from which fig. 1 was drawn. The two testes lie in the last body third; ovary, shell-gland and ootype, oviduct, Laurer's canal and yolk receptacle are all compactly grouped in the space between the testes. There is a peculiar uterine sack in the course of the uterus, the yolk follicles are small and very diffuse. Since no detailed account of the members of this system has ever been given I will give here a brief description of it.

Genital pore. The genital pore lies in the mid-ventral line (fig. 4 *E*). Its distance from the anterior end has been considered already in this article. There is an atrial cavity below the surface from which a single pore opens to the exterior. The relation of these parts is shown in figs. 3 and 4 *E*.

Cirrus sack. MacCallum's figure shows the cirrus sack and its contents very adequately. The sack occupies a position on the right side of the animal in front of the anterior testis. At its posterior border it receives the two vasa deferentia. The wall of the sack is supplied with muscles whose powerful fibers lie so as to form a circular and longitudinal coat. The sack contains a tube of varied diameter, coiled so as to accommodate its length to that of the sack. Posteriorly the tube expands to a larger, thin-walled, non-muscular seminal vesicle filled with spermatozoa.

Continuing it anteriorly is a smaller portion whose wall is supplied with a very strong coat of circular muscles. It is followed by a less muscular portion at the outer end of the sack. This portion is surrounded by what are apparently to be regarded as prostate cells. This part is not as strongly muscular as the middle region of the tube. Following the usual nomenclature I have designated the middle and outer parts respectively as prostate portion and ductus ejaculatorius. It seems however that the more glandular part and the more muscular parts are out of the usual order. Thus in *D. isoporum* (Looss, '94, fig. 104), the outer part is strongly muscular, and coiled and between it and the seminal vesicle there is a small chamber with which the large prostate cells communicate.

Testes. The testes are somewhat pyramidal in shape, their bases slightly concaved and facing each other. In some cases the remaining surfaces are more or less deeply indented, in many others they are entire. A sharp line bounds the testes. The vasa deferentia have a wall of epithelium with flattened nuclei. This epithelium can be recognized in the wall of the testis where it connects with the vas deferens but no epithelium can be seen in the wall of the organ elsewhere. Apparently trematodes differ on this point. In *Cotylaspis* (Osborn, '94, fig. 88) the wall of the organ is distinctly epithelial. Schwartz ('85) found nuclei in the wall of the testis of certain early stages of trematodes while Ziegler ('83) claimed that the wall is non-cellular in *Bucephalus* and *Gasterostomum*.

The testes are filled with cells which, in some bass worms, are almost completely filled by the very large nucleus, poor in chromatin, and with a very large, readily staining nucleolus which indicate the inactive stage preceding spermatogenesis; in other bass specimens with cells showing various phases of spermatogenesis. In the heron worms the testes contain fully developed spermatozoa scattered among the active cells.

Ovary. The size and form of the ovary as shown in fig. 26 of Looss' paper ('85) is unlike anything which I have found in my material, in which it is oval, entire and measures 0.4 mm. by 0.2 mm. In MacCallum's figure it is also small and entire. It is

bounded by a thin non-cellular membrane, which encloses cells, some of which, near the opening to the oviduct, are much larger than the rest and are about to descend to the ootype.

Oviduct and uterus. The oviduct passes inwardly from the ovary and crosses to the opposite side of the body. Its wall is composed of cubical epithelium and circular muscle fibers. At a point near the ovary there is a small sack, the spermatic receptacle, opening from the oviduct, this narrows dorsally to a tube—Laurer's canal—runs to the dorsal surface of the body (fig. 4 G), and opens to the exterior. The epithelium of the oviducal wall is replaced by cuticle in Laurer's canal, which becomes continuous with that of the general surface of the body. The oviduct, at a point a little farther to the left, meets the duct coming from the yolk receptacle. There is no marked change in the diameter of the oviduct at this point but it is surrounded by glandular cells and doubtless serves as the ootype. The duct from this point continues as the uterus, at first without change in diameter or direction, next with several loops it recrosses toward the ovary, then abruptly bends again and runs a straight course, passes externally to the anterior testis on its left side and runs forward to enter a large sack which we may call the uterine sack. The relation of the uterus to this sack is shown in fig. 3; it passes on its dorsal wall for a distance and opens into it at about the center of its dorsal surface.

Uterine sack. The uterine sack is a large cavity capable of considerable distension; in the case of mature worms it is filled with eggs, as in fig. 1; in bass worms (fig. 2) the cavity is merely a narrow slit. The form of the cavity in transverse section is shown in fig. 4 D. It extends posteriorly to a point near the anterior border of the anterior testis; anteriorly it does not reach the ventral sucker. The outlet from the sack is located at its posterior end. The histological structure of the wall of the sack is quite unlike that of the uterus. In the latter there is a nucleated epithelium and a coat of muscle fibers. In the sack the cavity is lined with cuticle and there is a muscular coat consisting of circular and longitudinal fibers. In addition to these there is a condensation of the parenchyma immediately surrounding the uterus. The nuclei of these parenchyma cells lie in definite

lines parallel with the surface from which the fibers of the parenchyma radiate. A sharp line bounds this mass of specialized parenchyma which thus constitutes a capsule enclosing the uterine sack. The fact that the uterus enters the sack in the center of its dorsal surface and not at the anterior end prevents us from regarding the sack as merely a dilatation of the uterus. We must however think of it as having arisen as a differentiation which has taken place in a loop of the uterus. In most of the species of this genus (Braun, '00) there is a similar blind sack into which the uterus enters and which extends blindly in front of the end of the uterus. In one species however, (*C. heterostomum*, Braun, '00, fig. 1), the uterus passes forward to the posterior border of the ventral sucker where it bends and runs straight back again to end at the female genital opening. This is doubtless the more primitive anatomical arrangement, and the one from which the sack form has been developed. We note also in passing that this species is more primitive, too, in possessing well developed diverticula of the intestinal caeca.

The form of the uterine sack in the *D. reticulatum* of Looss, as described and figured in his paper ('85), is decidedly different from that which I have just described. The sack in that species is elongated posteriorly to reach a point posterior to the posterior testis (fig. 22). In fig. 26 we learn that the part of the sack which leads to the exterior is a lateral offset from the main sack. This posterior portion of the sack of Looss is wholly wanting in my material. Cross sections (e.g., fig. 4 *F*) show that the sack does not extend into the testis region of the body. This is an interesting point. It does not seem possible to doubt the fact as related by Looss for his form. In every other respect the form *D. reticulatum* bears the closest resemblance in organization to *C. marginatum*, and writers from Leuckart down have considered them identical. Thus Leuckart ('89, p. 401) says *D. reticulatum* "Mit Leidy's *Clinostomum gracile* zusammenfällt." Stiles and Hassall ('98) say "Looss described as *Distomum reticulatum* a form which is evidently identical with Leidy's *Clinostomum* as Leuckart has already surmised," etc. And MacCallum ('99, p. 705). The description given by Looss of *D. reticulatum* applies so exactly in every particular to the forms we

have just considered [*C. marginatum*] that I have not the least hesitation in concluding that they are the same." The form of the uterine sack however is very different in *D. reticulum* from that of *C. marginatum* or of any other member of the genus. According to fig. 26 of Looss' article ('85) the sack is extended posteriorly dorsally to the testes until it reaches a point posterior to the posterior testis. This posterior development of the sack is a feature not found in any of the species of this genus so far as I am aware. In the several species included by Braun ('00) in his article on the group, the sack ends in advance of the genital pore. If Looss was not in error in regard to the form of the sack (and this seems very improbable) then we must recognize that *D. reticulatum* differs decidedly in this respect from the rest of the genus. However in any case we should not attach very much importance to differences in the shape of an organ like the uterus or its parts.

Metraterm. There is a short slender tube running from the sack to the atrium (figs. 3 and 4 *E*) which, following the nomenclature suggested by Ward ('94), may be called the metraterm.

Vitellaria. The vitellaria are shown in fig. 1. They are diffusely scattered in all parts of the region behind the ventral sucker. As shown by transverse sections they lie in a thin zone, concentric with the surface and next to the outer wall of the body. They are entirely absent from the anterior part of the body. The vitellaria are made up of ultimate follicles, all of them very small and numerous measuring 0.07 mm. These are bounded by a distinct membrane which encloses a few yolk cells which measure 0.0125 mm. in diameter. Usually the vitellaria cannot be seen in total preparations made from bass worms, but sections of similar immature individuals show the follicles with their thin wall enclosed cells whose structure is then identical in appearance with that of the immature germinal cells of the testes and ovary. In sections from mature worms the follicles contain similar immature cells and also fully formed yolk cells with large nucleus and nucleolus and a cytoplasm containing granules, some of them measuring 0.001 mm. in diameter. These are food granules; in producing them the follicle cells differ from ovarian cells with which they are very likely homologous.

The yolk receptacle lies near the ovary and its duct reaches the oviduct as already noted. The shell gland surrounds the ootype at this point. Its cells radiate from the ootype and their long tapering portions seem to communicate with its cavity, though it is not possible to recognize absolutely the manner of connection.

The egg. The eggs measure 0.099 mm. by 0.66 mm. There is a distinct operculum very near the end of the shell. The shell in some cases is deeply stained by the haematoxylin and looks as if composed of the same substance as the spines. In other cases the shell is not influenced by the stain. The shells contain as usual a fertilized cell derived from the ovary and several cells derived from the vitellaria. The eggs, both those of the uterus and the older ones of the uterine sack are practically undeveloped. In some cases the true egg cells may undergo one or more of the early stages of cleavage but in the vast majority of eggs no development takes place during the time that they are lodged within the body of the parent. In view of this we must recognize the sack as merely a place for the storage of eggs. The reason for this storage remains for the present unknown.

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ABBREVIATIONS

<i>crs</i> , cirrus sack	<i>mt</i> , metraterm
<i>cu</i> , cuticle	<i>nv</i> , nerve collar
<i>dej</i> , ejaculatory duct	<i>nvs</i> , sensory nerve endings
<i>epo</i> , outer part of epithelium of intestine	<i>oegl</i> , oesophageal glands
<i>epi</i> , inner portion of the same	<i>oes</i> , oesophagus
<i>exbl</i> , excretory bladder	<i>os</i> , oral sucker
<i>excv</i> , collecting vessel of excretory system	<i>otp</i> , ootype
<i>expo</i> , excretory pore	<i>ov</i> , ovary
<i>exrv</i> , recurrent vessel of excretory system	<i>pgl</i> , parenchyma glands
<i>gl</i> , glands communicating with the surface	<i>pi</i> , parenchyma sheath of wall of intestine
<i>gpo</i> , genital opening	<i>pn</i> , parenchyma cell nucleus
<i>int</i> , intestine	<i>prs</i> , prostate part of cirrus organ
<i>lc</i> , canal of Laurer	<i>ps</i> , parenchyma sheath of oesophagus
<i>mc</i> , circular muscle of body wall	<i>spn</i> , spines of body wall
<i>mi</i> , inner longitudinal muscle of body-wall	<i>ta</i> , anterior testis.
<i>mo</i> , outer longitudinal of the same	<i>tp</i> , posterior testis
<i>mob</i> , oblique muscles of body wall	<i>ut</i> , uterus
<i>mpl</i> , longitudinal muscles of the parenchyma	<i>utsk</i> , uterine sack
<i>mpt</i> , transverse muscle of the parenchyma	<i>vs</i> , ventral sucker
	<i>vsm</i> , seminal vesicle
	<i>vd</i> , vas deferens
	<i>vt</i> , vitellaria
	<i>vtd</i> , duct from vitellaria
	<i>utr</i> , yolk receptacle

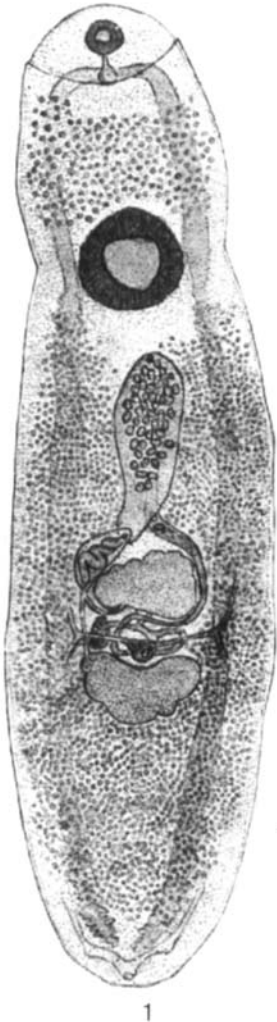
PLATE 1

EXPLANATION OF FIGURES

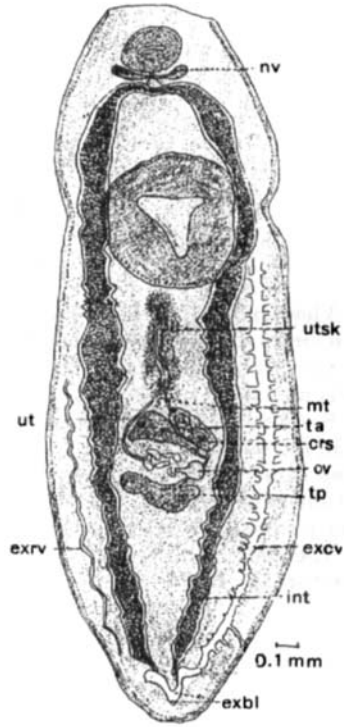
All the figures (except 10, 13 a, and 16) were drawn with the Abbe camera lucida. Most of them have been reduced one-third in reproduction; the magnifications are after this reduction.

1 View from the ventral surface *C. marginatum*, from a specimen from the throat of *Ardea herodias*, fixed under compression in aqueous corrosive sublimate, borax-carmine. $\times 12$.

2 A partly schematic view from the dorsal side, combining facts from several sections from a frontal series. From a bass worm. The vitellaria are not yet developed, the uterine sack is not dilated, the excretory collecting vessel is omitted from the right side and the recurrent vessel from the left, parts on different levels are shown on the same level. $\times 27$.



1



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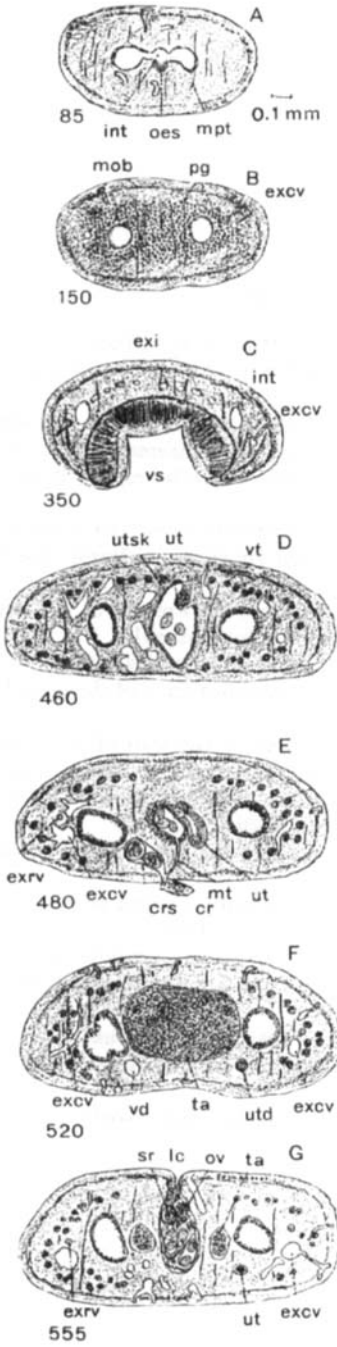
PLATE 2

EXPLANATION OF FIGURES

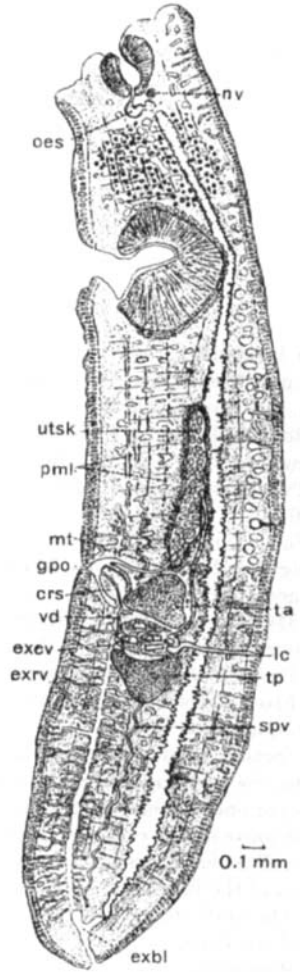
3 View combined from sections of a sagittal series, showing together organs which are on different levels, mouth, ventral sucker, genital organs and excretory pore are median while the intestine and the collecting and recurrent excretory vessels are lateral. $\times 27$.

4 Sections from a transverse series. The numbers show the number of the section in the series; *A* is in the level of the oesophagus; *B*, in front of the ventral sucker; *C*, at the ventral sucker; *D*, at the uterine sack; *E*, at the genital pore; *F*, at the anterior testis; *G*, at the canal of Laurer. From heron, corrosive and acetic, iron-haematoxylin. $\times 27$.

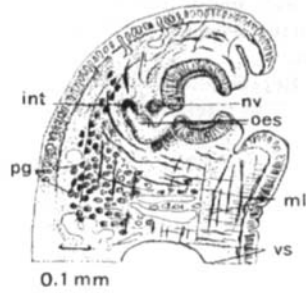
5 The center section of a sagittal series, from a worm which died with the oral field inverted. Heron, after chromic acid fixation and iron-haematoxylin. $\times 40$.



4



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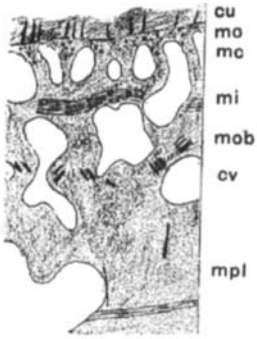


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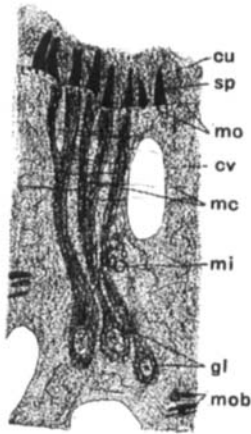
PLATE 3

EXPLANATION OF FIGURES

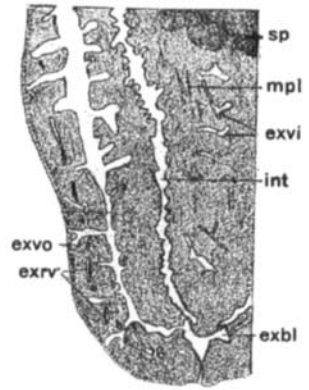
- 6 A longitudinal section of the body wall of the dorsal surface, showing the position of the various excretory vessels with reference to the muscular layers. Heron, corrosive, iron-haematoxylin. $\times 240$.
- 7 Body wall from a transverse section near the center of the ventral surface, showing the uni-cellular glands (?); chromic acid, iron-haematoxylin. $\times 1100$.
- 8 Two sense organs of the cuticle from the dorsal anterior region of the body. $\times 1100$.
- 9 Part of one of the fibers of the inner longitudinal muscle of the body wall, showing the alternation of stained and unstained substance. Heron, corrosive, iron-haematoxylin. $\times 560$.
- 10 Myoblast and its nucleus and adjoining muscle fibers of one of the longitudinal parenchyma muscles. Heron, chromic, iron-haematoxylin. $\times 1100$.
- 11 Part of one of the parenchyma muscles from the same series as fig. 9. $\times 1100$.
- 12 From a section passing vertically to the posterior region of the oesophagus. Heron, chromic. $\times 560$.
- 13 Section from a fully matured worm vertical to the wall of the intestine, showing the pseudopodial inner borders of the epithelium; the darker shading of the deeper ends of the cells indicates the distinction between the stained and little stained parts of the cell, corrosive, iron-haematoxylin. $\times 1100$.
- 13 a Free hand drawing from a living worm from bass, showing the lateral pouches of the intestinal caeca.
- 14 The epithelium of the intestine from an immature worm showing resting stage of the tissue. Corrosive, iron-haematoxylin. $\times 1100$.
- 15 Reconstruction from several adjoining sections of a frontal series, showing the relation of the collecting to the subcuticular cavities and to the bladder, also the recurrent vessel and the intestine. $\times 36$.
- 16 Free hand drawing from the posterior ends of the chief excretory vessels as seen in a living worm from the bass under slight compression. \times Zeiss oc. 2, ob. A.
- 17 Reproductive system as seen from the ventral surface, from total preparations. The vitellaria have been omitted.



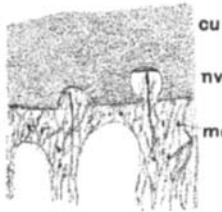
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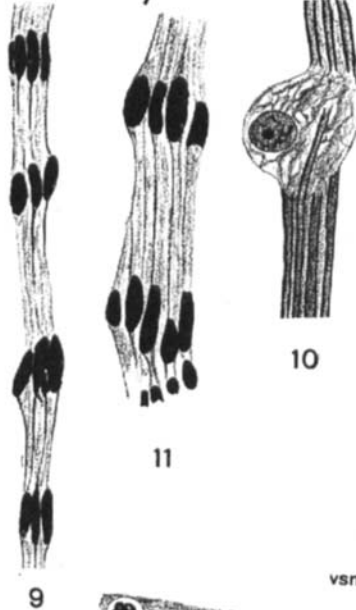
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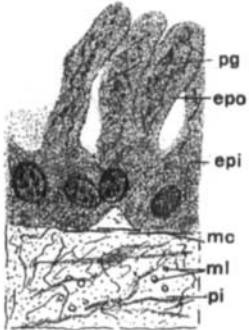
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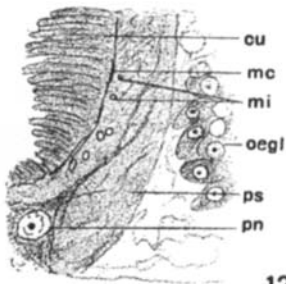
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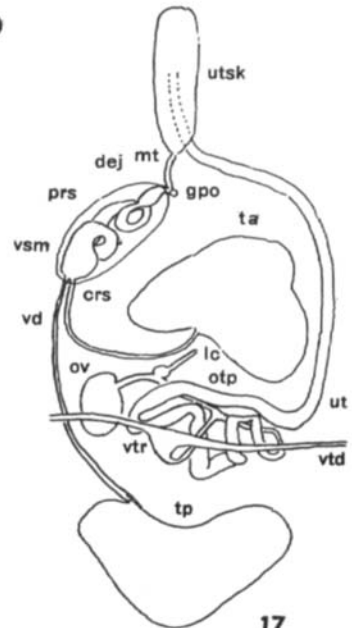
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13a



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