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TRANSACTIONS
OF THE
American Microscopical
Society

VOLUME XXXVIII

1919

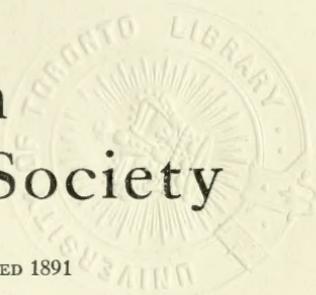
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T. W. GALLOWAY

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TRANSACTIONS OF American Microscopical Society

(Published in Quarterly Instalments)

Vol. XXXVIII

JANUARY, 1919

No. 1

ILLUSTRATING BIOLOGICAL MANUSCRIPTS

By E. A. Smith

Learn drawing—“*that you may set down clearly and usefully, records of such things as cannot be described in words, either to assist your own memory of them, or to convey distinct ideas of them to other people.*” Ruskin.

Although many excellent books have been published concerning drawings and the processes involved in reproducing them, a beginner in science can consume hours in attempting to find within their covers the kind of information he desires. The books on drawing are usually written for artists who desire effect and not scientific accuracy, and those that deal with methods of reproduction serve as text-books for journeymen interested in the commercial phases of the work. In this article, therefore, an attempt will be made to set forth simply and concretely what points should be considered in making a drawing; to tell which media are best for certain classes of work; to give some idea of how the drawings are reproduced, together with the limitations imposed by photo-mechanical methods.

A black picture on a white background gives the best contrast for reproduction. In numerous subjects the form and texture can be brought out equally well in black ink, in crayon, in water-color, or a combination of these. One of these media may show the points which need emphasis better than another. Therefore, before making drawings for a paper, an author should study the illustrations in the journal in which he expects to publish and select the style best suited to his subject. Skill may well be considered also, as a simple outline in ink is preferable to an elaborate picture poorly executed. Manuscripts which contain drawings that can be reproduced as line or half-tone engravings are more readily accepted by editors as they can be published with less expense. The extra cost of plates requiring lithography, heliotype, or photogravure sometimes must be paid by the author.

I. METHODS OF ILLUSTRATION

This is no place to enter into the history of graphic reproduction, interesting as it is, nor to describe all the processes in commercial use to-day. Processes available for reproduction may be divided into three distinct groups: (1) intaglio; (2) planographic; and (3) relief.

Intaglio

The intaglio processes include all those in which the impression is printed from incised or depressed surfaces. Engraving, the oldest known of these methods, consists of lines cut by hand into a copper or steel plate with a graver. On account of its cost, it is seldom used except by the government where it is employed in the making of plates for paper money and maps, as many copies may be printed without injuring the plate.

Planographic

Planographic methods include Lithography, Photolithography, and Photogelatin or Heliotype, in which printing is done from a flat surface. In all these, the surfaces are so treated that certain parts repel ink while other parts take it.

Lithography. In Lithography advantage is taken of the fact that grease unites with limestone to form a substance insoluble in water. Zinc and aluminum plates may be substituted for the stone, using practically the same procedure. The picture to be reproduced may be drawn in reverse order upon a smooth, flat piece of limestone with a special ink or chalk composed of wax, shellac, tallow, and soap. For a shaded, scientific drawing, the surface of the stone is grained, either a fine or coarse grain, depending upon the effect desired. Instead of drawing directly upon stone, the picture may be drawn in proper position upon a smooth or grained paper with lithographic chalk, as it is then reversed when transferred to the stone. To transfer, the paper is dampened and placed upon the warmed stone, then both are run through a press. Considerable skill is required in making scientific drawings upon grained transfer paper as a smudge results in the transfer if the chalk is too soft or the lines too heavy. On the other hand, if the lines are too light, the transfer is blotchy. Sometimes a thin, gelatine sheet is placed directly over a drawing, every detail of which is outlined on the gelatin with a fine etching point.

After ink is rubbed into the lines, the gelatine is dampened and a transfer made upon stone.

Dilute nitric acid applied to the surface attacks the parts of the stone free from ink, producing a chemical change so that the gum arabic solution next put on renders the surface grease-resisting. For chalk work an equal mixture of gum and nitric acid is added. More than one acid bath and several coats of gum may be given in preparing the surface. Often powdered rosin or asphaltum is dusted over the surface. It adheres to the ink, thus protecting it from the acid.

Before a print is taken from the stone, the gum is washed off, leaving the etched parts wet. These, then, repel the printing ink. The latter adheres to those parts of the stone covered with lithographic ink or chalk. The clearness of the print depends to a large extent upon the skill of the operator.

Photolithography. In Photolithography, the picture is photographed and from the negative thus obtained, a print is made upon paper coated with bichromatized gelatine. The picture to be used for the illustration is placed in front of a camera which contains a piece of glass coated with a film of gelatine or collodion mixed with a salt sensitive to light—a photographic plate. When the camera shutter is opened the white parts of the picture reflect light upon the plate and cause a chemical change. The dark parts of the picture have little or no effect upon the salt. Colors reflect light in varying degrees, thereby modifying the plate. The plate is next treated with the chemicals which cause the parts unaffected by light to wash out while the other parts become opaque. A negative is thus produced in which the light parts of the picture are opaque and the black parts clear. If this negative does not present enough contrast between the opaque and clear parts, it is put into a solution which makes the opaque areas denser.

From the negative, a print is made upon transfer paper first coated with gelatine and then immersed in bi-chromate of potash and dried in the dark. The negative is placed upon the coated side of the paper and both are exposed to light in a printing frame. Gelatines, gums and other organic compounds when sensitized with certain chromic salts are rendered insoluble wherever exposed to the action of light rays. The gelatine protected by the opaque parts of

the negative are still soluble whereas that under the clear spaces is made insoluble by the light. The print when sufficiently exposed is taken out and covered with ink. It is now placed in cold water which causes the gelatin surface to swell in the soluble spaces. When rubbed over with a sponge, the ink comes away from all parts not affected by light, leaving a print of the picture in ink slightly in intaglio. This is then transferred to stone which is prepared as for any lithograph.

Heliotype. A negative and print are made in the same manner as described above, except that the negative is reversed before it is printed and the gelatine coating on paper or glass is thick enough to be detached from its original support and printed from directly instead of transferring it to a stone. The gelatine acted upon by light takes ink, the other parts absorb water and repel the ink.

Relief

In the photo-mechanical processes such as zinc-process and half-tone, the plate is so treated that the picture remains in relief upon a sunken background.

Zinc-process. From the negative, a print is made upon a piece of polished zinc which has been coated with a sensitized substance such as a mixture of egg-white, fish glue and ammonium bichromate. The negative is placed upon the coated side of the zinc and both are exposed to light in a printing frame. Instead of developing as a photographic plate, the zinc is covered with an even coating of special ink put on with a roller—"rolled up," is the printer's phrase. This ink is rather thick and contains wax and tallow to make it greasy. When the inked plate is placed in water, the parts of the coating not affected by light wash away, leaving the image in black lines upon a metal background.

The plate is now covered or powdered up with a red, resinous powder called dragon's-blood. This powder is obtained from any one of several different kinds of tropical trees. Upon warming the plate the powder unites with the ink and makes it more resistant to acid. After painting the back with a varnish, the plate is put into nitric acid which acts upon the uninked and unvarnished zinc surface. Usually the plate is sufficiently etched after four treatments in the etching bath so that the image stands up in relief upon the metal

surface. When finished the thin metal plate is mounted upon a block, type-high. Cuts of this kind can be printed as text-figures if the details are not so fine that a slight spreading of ink in printing will destroy the contrast.

Half-tone. In this process a screen is placed between the camera and picture before the negative is made. This screen usually consists of two glass plates, each engraved with lines an equal distance apart which are filled with an opaque black substance. The plates are cemented together in such fashion that the lines cross at an angle. The number of lines per inch vary from 50 to 400 and the crossing may be at various angles. For microscopic enlargements 200 to 250 lines per inch are used and the plates are fastened together so that the lines cross at an angle of 45° . When a picture is photographed through the screen, the negative is covered with small dots the shape of which depends upon the camera diaphragm. The contrast of the original picture is lessened since the white parts are covered by black dots and the black areas by white dots.

If a coarse screen is used the negative can be printed upon coated zinc and etched as for zinc-process. A coated copper plate is necessary where the dots are fine. After the copper plate has been exposed under the negative it is heated until the sensitized coating turns brown where it has been affected by light. This brown oxidation product is insoluble in water and acids. The plate is now etched in a solution of perchlorid of iron until the picture stands out in relief. If clear white spaces are desired in the print, the dots produced by the screen must be tooled out of the plate in these places. As this is done by hand, it adds to the expense of the plates. Prints may be made directly from the plate which has been backed with base metal or wood, or electrotypes may be made from the original.

In printing there is an increase in the size of the black dots and a decrease in size of the white dots due to the spreading action of the ink. If the block is printed on a coated paper, this spreading action is reduced somewhat and can be overcome through the use of an overlay, which is a pad placed on the roller.

Various modifications of the half-tone process are obtainable through the use of different screens—such as mezograph, but the principles involved are the same.

II. DRAWING FOR PUBLICATION

Outline

In free-hand drawing of objects, care must be taken to get a properly proportioned outline. First, determine the size the drawing should be. A small object can be represented several times natural size whereas a large one needs reduction. Upon two faint lines which cross at right angles in the middle of the field, indicate by dots the length and breadth of the object. Other important points can also be marked with dots which, when connected with light lines, roughly block in the object. For this preliminary mapping, an HB pencil lightly clasped between first finger and thumb, and resting on the second finger, should be held in such a manner that the side of the lead comes in contact with the paper at a small angle. Lines thus made can be easily erased as the surface of the paper is not injured by the lead. This crude picture can now be worked over until the finished outline consists of a continuous line of uniform thickness with no overlapping edges where the pencil has been removed from the paper and put down again. The pencil lines should be removed with an art gum eraser until the outline remains only as a faint impression which must be gone over again with the pencil. If guide lines and dots are drawn in light blue, they do not need to be erased since pale blue photographs as white in the reproduction of the picture. In picturing microscopic sections and objects, a correct outline is easily obtained by tracing around the image projected upon the drawing-paper by a camera lucida.

Perspective

Perspective, a subject which can not be treated in any great detail here, must be considered in drawings in which three dimensions are depicted. Objects should be drawn as they appear to the eye and not as they are known to be. They present their true appearance only on that side parallel to the eye. The apparent change in shape which takes place when an object is situated at an angle to the eye is known as foreshortening.

Since drawings of tubes and cylindrical objects are frequently made in biological papers, an understanding of a few principles of the perspective of a cylinder is necessary. If an ordinary tumbler is raised, the visible top becomes narrower and narrower until it appears as a straight line when directly opposite the eye—or at eye-level, as

it is technically called. When the tumbler is lowered from eye-level, the top, though actually round, first appears as an ellipse. Moreover, this ellipse is narrower than that formed by the round bottom, for the latter is farther below the eye-level (Fig. 1). In drawing a tube, the

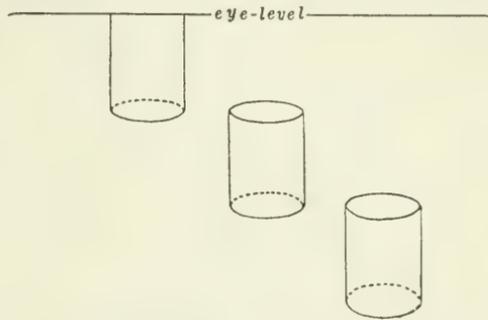


Figure 1. Three cylinders drawn to show the appearance of the top and bottom at different distances below eye-level. Reproduced $\frac{1}{2}$ original size by zinc-process. bottom edge will be more curved than the top as it represents one side of an ellipse broader than the one at the top (Fig. 2).

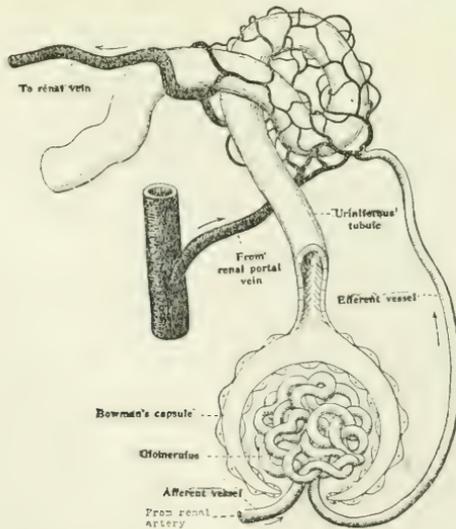


Figure 2. Diagram to show relation of Malpighian body and uriniferous tubules to the blood vessels. Reproduced $\frac{1}{2}$ original size by zinc-process. Taken from Laboratory Directions by M. F. Guyer. Labels typed and pasted on drawing.

It is a familiar fact that from the rear coach of a train the rails of a straight piece of track converge toward a point in the distance directly in front of the eye. If all the outlines of a building seen through a window should be traced on the pane, they would converge so that if continued they would meet at two or more points. Likewise, if a book is placed horizontally in front of the eye, the parallel lines which represent the sides appear nearer together at the back than in the front, and if continued would meet at a point directly in front of the eye. This point is spoken of as the vanishing point.

If the book is moved so that the front edge is no longer parallel to the eye, the vanishing point changes, and instead of one point there are now two, one on either side of the object. These points, however, are on an invisible line which runs at the eye-level. Study the illustration (Fig. 3) to see how the lines vanish. All lines of the object that are parallel vanish to the same point. If two books do not lie at the same angle there will be four vanishing points, two for each book, but these points all lie on the line drawn at the eye-level.

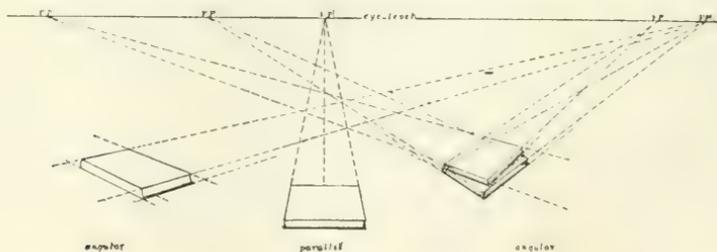


Figure 3. Diagram to explain parallel and angular perspective. The printed letters cut out and pasted on the drawing are too small for the reduction used. Reproduced $\frac{1}{3}$ original size from a zinc plate.

Shading

Often form can be rendered more aptly by the use of light and shade (Plate I). Objects exposed to direct light generally appear lighter on the side near the source of light and darker on the other side. Furthermore, the shadow cast by the object is always darker

Plate I. Crayon drawing illustrating shading on plane, convex and concave surfaces. From a half-tone cut $\frac{1}{2}$ the size of the original. Made with a screen containing 150 meshes per inch.

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PLATE I

SMITH

than the shaded side of the object itself. In the zone between the lightest and darkest part of the object, the half-light or half-zone, details are most prominent. To indicate all the variations in shade would complicate the drawing unduly, hence it is the practice to use only those shadows which are necessary to bring out the form of the object disregarding all others.

Likewise, color, texture, and shape of the surface can be expressed through the proper placing of shadows. Shadows upon different colored surfaces have different values since colors vary in the way in which they reflect light. This is easily illustrated in photographs where a yellow surface takes dark, red shows up as black, and blue comes out white. Also the nature of shadows differ on shiny and on dull surfaces.

A flat surface usually has a continuous shadow of even tone, whereas on a curved surface the shadow must grade off into darkness on one side and into light on the other, with no sharp edges defining it. On a concave surface, the sunken appearance is produced by placing the darkest tone nearest the source of light, shading to a lighter tone away from the light. For here, the higher edge around the concavity prevents the light from striking the side nearest it. Convex surfaces, on the other hand, are illuminated on the side toward the light while the elevated center keeps the light from the opposite side.

Drawing

Ink Drawings. Pen drawings reproduce well by the zinc-process method as the black absorbs all light and the white back-ground reflects all, thereby producing great contrast in the negative.

For ink-drawings a good water-proof India ink such as Higgins' should be applied with pen or brush upon bristol-board (2 or 4 ply), upon Whatman's hot-pressed (smooth) water-color paper, or upon ledger paper. Whatman's paper is the same texture throughout; moreover, it can be used for wash or ink-work. Ledger paper, while little known, is excellent as erasure will not damage the surface. Gillott's pen points are satisfactory but inasmuch as each person handles a pen differently, exerting varying degrees of pressure in drawing, one number may not suit all workers. For fine line work and stippling, lithographic pen No. 290 is good. Fine red sable

brushes can be used although the pen drawing is likely to prove more successful.

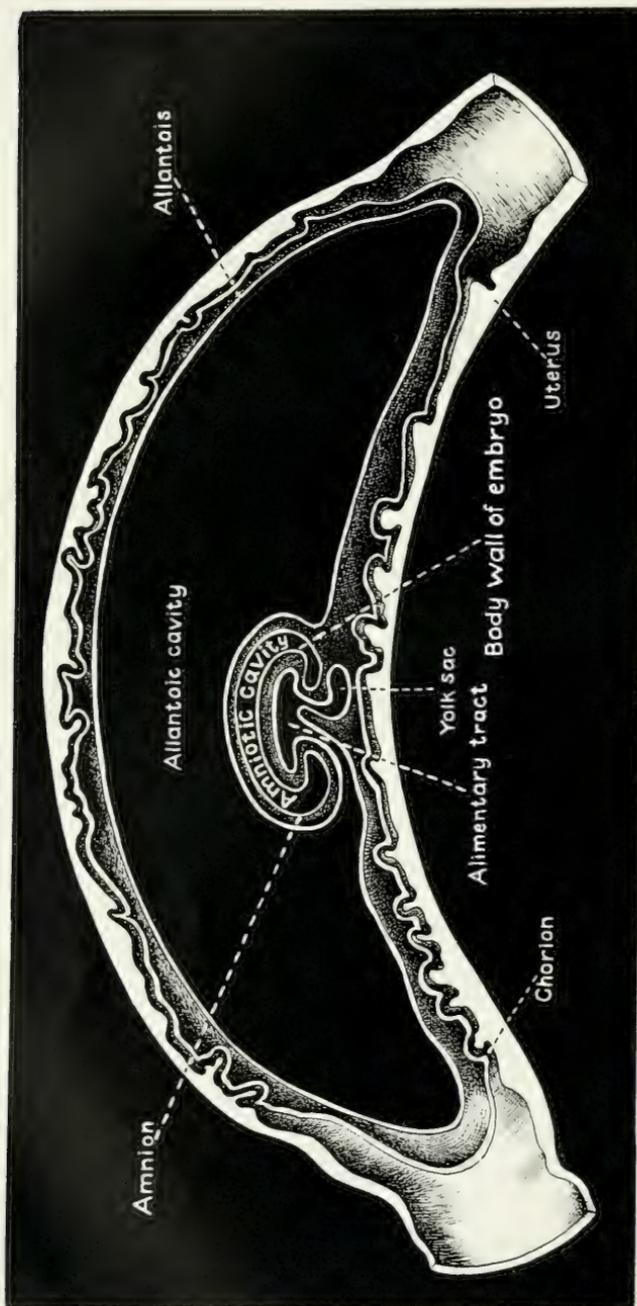
After the pencil outline is correct, the drawing is ready for ink. On a clean pen-point place the ink by means of the quill attached to the cork of the ink bottle. A ragged line results if the pen is held so that one nib bears more heavily upon the paper than does the other, or if it becomes sticky with dried ink. To secure a line of uniform thickness the pen should be held at a wide angle to the paper so that only the very point touches, and a firm steady pressure be applied.



Figure 4. The fur is suggested by the use of uneven lines. Reduced $\frac{1}{2}$ and reproduced from a zinc plate.

While ink thinned to a gray gives a difference in tone in the original drawing, in reproduction gray lines may behave erratically, often appearing as broken black lines. To avoid graying black lines the pen must be wiped frequently and refilled.

Effects may be obtained by the use of lines alone, either by varying the thickness of the lines or the distance between them. How-



ever, extremely fine lines may break, whereas coarse lines when placed close together tend to merge. Lines in the foreground should be heavier and farther apart than those in the background. Lines should follow the shape of the shadows, but they should end unevenly to avoid stiffness. In the darkest areas lines may be placed in an opposite direction across the first set.

Plate II is an ink-drawing in which the background and part of the cavities were inked, leaving the picture in white. This is an excellent method to follow in diagrams as the chief parts are strongly emphasized.

Texture and surfaces can be expressed by the use of lines of different character. Thus broken lines and dots indicate fur (Fig. 4), while fine, smooth lines may suggest feathers. Short, uneven lines in the depressions, suggest rough surfaces, while a few parallel lines on the heavily shaded areas are necessary for a smooth surface.

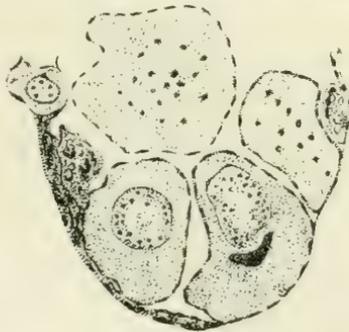


Figure 5. Ovary of frog in stippled ink. This drawing required much more time in its execution than A and B of Plate V. It was reproduced by zinc-process with same reduction as Figure 10.

Although bold line-drawing is admirable for some things, subjects often require the reproduction of the half-lights. These can be suggested by dots in a pen drawing (Fig. 5). All dots must be round and of the same size. Round dots are made by grasping the pen firmly and holding it in such manner that it meets the paper squarely. If it strikes at an acute angle, three-sided instead of round dots will result. Heavy shading is indicated by placing dots close together; light shading, by keeping them farther apart. Dots that are too fine

Plate II. Diagram of pig embryo surrounded by membranes. Lettering done by hand in white ink. Printed from a zinc plate $\frac{1}{2}$ the original size.

can not be reproduced on zinc as the acid undercuts the sides of the dots, thus destroying the stippled effect.

Slight irregularities and defects are easily removed from the drawing by scraping them away or covering them up with Chinese white paint. Before applying white paint clean away all pencil marks and completely cover the part to be obliterated with several coats if necessary. Alternations are then made as desired since the white paint does not interfere with inking. Only a little ink should be placed on the pen when attempting to re-ink a spot erased with a knife as the ink spreads on the damaged surface.

Wash drawings. Wash drawings are ordinarily reproduced by half-tone process. The contrast between the black and white parts of the original picture should be exaggerated, as the screen intersperses white dots in the black areas and black dots in the white spaces thereby lessening the contrast of the picture.

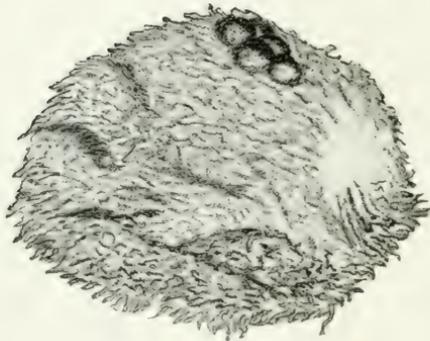
For wash-drawings, Whatman's paper mentioned previously, or any smooth water-color paper, will prove satisfactory. A grained or rough surface usually causes unpleasant effects. Winsor and Newton's Ivory Black or Charcoal Grey makes a good working medium. These colors come in solid cakes from which the wash is prepared as follows: With a wet brush remove some pigment from the cake and put in water in a mixing pan. Repeat this process until the wash is slightly darker than the desired background as the tint lightens in drying. Good sable brushes keep their shape if they are washed after using, brought to a point, and kept inverted in a jar.

Although from the artistic standpoint the use of the brush alone is preferable, the accuracy demanded in a scientific drawing makes a faint pencil outline more practical; and a hard pencil is better than a soft one if the surface of the paper is not injured with pressure, as the graphite is not so likely to smear when wet. After the outline is finished, the paper should be fastened to a board with thumb tacks and the entire surface dampened with a large brush, removing the surplus water with brush or blotter. If the picture is large or complex a dampened blotter placed under the paper will keep the surface moist for a long period. The wash is applied over the entire background and the paper allowed to dry partly before darker tones are added. Where a very dark portion is confined to a small area, the

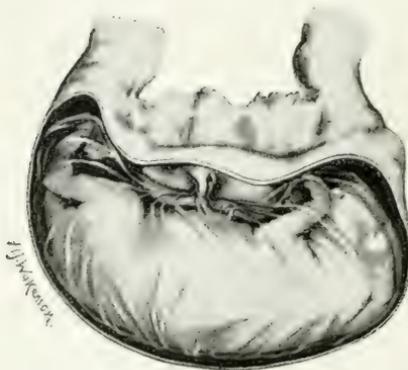
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A



B



C

paper should be quite dry, otherwise the wash will run into the surrounding part. With wash alone, good effects may be obtained (Plate III, C). Details may be stippled either in wash or in ink or drawn in with lines.

Where several wash-drawings require the same tones, it will be found simpler to put in all the backgrounds first. Mix up plenty of wash for this purpose, as it is not easy to duplicate the exact shade. Wash allowed to stand becomes darker upon evaporation of the water; hence if, after the backgrounds are put in, the work must be deferred until later, the same wash will do for darker tints. When the work is continued it is not necessary to redampen the entire surface as the dark tones can be blended into the background with a clean, wet brush.

Some artists prefer a dry paper but this requires more skill in applying the wash. For, unless the work is done rapidly, the pigment dries leaving a dark line or water-mark where each brushful of wash ended.

With an air-brush beautiful wash-drawings can be turned out in a short time. The wash is placed in a receptacle and by air-pressure sprayed over the parts of the drawing in a fine stream. Considerable skill is necessary in regulating the spray. Moreover, the instrument is expensive and requires an air-gage and cylinder. But if the brush is at hand it will repay the time spent in learning its use, especially if one contemplates making many drawings.

Crayon. With crayons, drawings scientifically accurate and artistic as well are obtained with least expenditure of energy. Moreover, mistakes due to lack of skill in execution are more readily remedied.

While Conte crayons in pencil-form come in five numbers ranging from hard to soft, No. 2 is suitable for most work. Varied stipple effects may be produced by using them upon Ross stipple board. This paper has a grained chalk surface to which the crayon adheres in the form of dots when it is rubbed back and forth over the chalk. The grain of the paper differs in the different numbers. No. 8 is good for general

- Plate III, A. Reproduction of a photograph by the half-tone process. No reduction.
B. Crayon drawing of embryonic vesicle of young human embryo reproduced $\frac{1}{2}$ size of the original by half-tone process.
C. A half-tone reproduction of a rabbit embryo in the uterus made from wash original reduced $\frac{1}{2}$.

work. The edges of such a drawing may be cleaned up or straightened by scraping off the chalk surface, thus revealing the white paper below.

Dot or line crayon-drawings may be satisfactorily reproduced by zinc-process if the drawings are coarse enough to stand a reduction to one-half their original size. Here as in ink drawings, brown lines are apt to break.

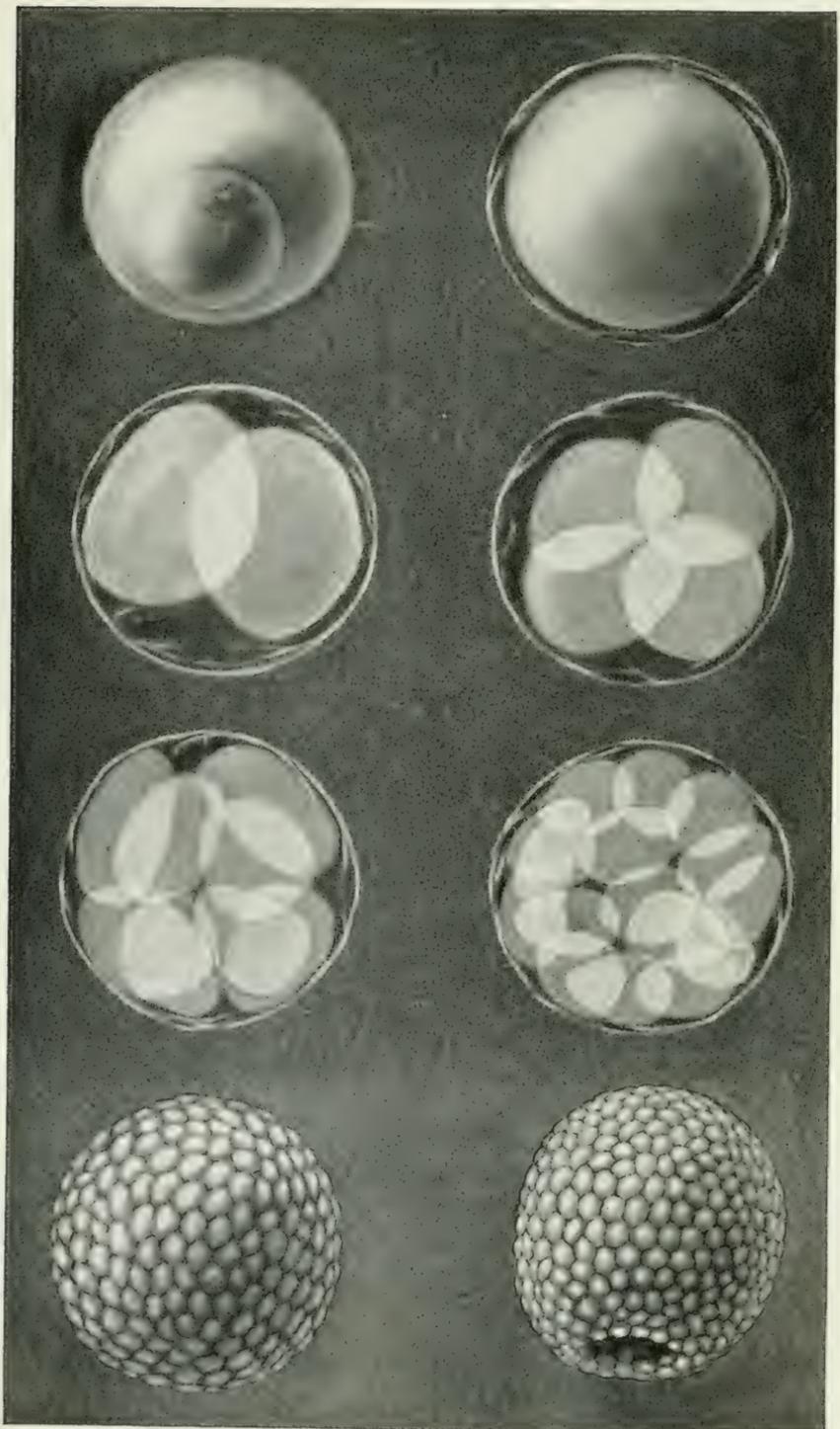
Crayon-drawings closely resembling wash-drawings in appearance may be made. For the ground-coat lightly rub the crayon over the paper and then with a stub blend the crayon marks into an even coating. In the following manner a paper stub is made from a strip of uncoated paper 1 inch wide and 5 inches long: Begin to roll the paper at one end and let each turn overlap the preceding turn slightly, until an elongated coil results. Paper or chamois stubs ordinarily used for charcoal work may be used for crayon. Details are put in with lines or dots of crayon. Where white spaces or high-lights occur, the ground-coat may be removed with an eraser cut to a fine point. If much white occurs, it is better to put in the details first and then use a stub on parts which need shading. Dark parts can be accentuated with ink. (Plate III, B).

White crayon on gray or black board makes an effective illustration especially for transparent objects such as medusae or for membranes (Plate IV).

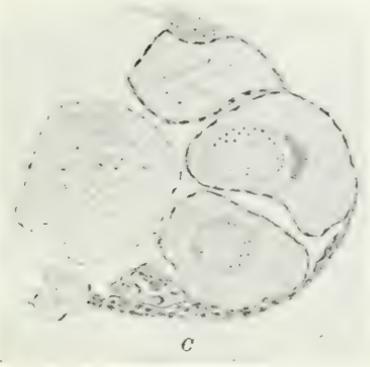
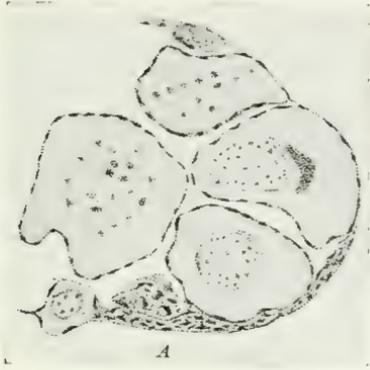
Pencil drawings. While pencil-drawings permit of subtle differentiation in detail, in making they consume an amount of time out of all proportion to the effect obtained. Half-tone reproductions of pencil-work not only are inferior to the originals, but do not begin to compare with half-tones obtained from wash or crayon-drawings. Good illustrations may be secured from pencil-drawings reproduced by lithography. The expense involved from such reproduction makes them undesirable for scientific purposes if any other method will do.

Either dots, lines, blended graphite, or a combination of these may give results desired in pencil-drawings. In stippling with a pencil, the point should be sharp and rounded on all sides. With an HB or 2B pencil graphite is sometimes placed on the part of the drawing

Plate IV. Series of drawings to show developing starfish egg. Original made in black and white crayon on dark gray background. Reproduced by half-tone $\frac{1}{2}$ the original size.



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where the darkest shadows occur and then worked over with a stub in the same manner indicated under *Crayon*. Plate V contrasts a half-tone made in pencil, wash, and crayon. Compare with Fig. 5.

Fixing Pencil and Crayon Drawings. Pencil and crayon drawings which are likely to rub must be fixed. A fixing solution is sprayed on the drawing with a special atomizer which can be bought at any art store. To prepare the fixative, make a saturated solution of white shellac in alcohol; allow this to stand for a day or so; dilute one-half; then filter off the liquid. To prevent evaporation, this should be kept in a tightly stoppered bottle when not in use.

The drawing is placed in an upright position, about two feet from the spray. To avoid a glossy surface, only a light coating of fixative should cover the picture.

Combinations. Any combination of wash, crayon or ink is allowable so long as the picture retains sufficient contrast to reproduce well. Ink and wash are effectively used in Plate V, A. Where ink is combined with wash, lines and solid black areas are broken up by the screen in the half-toning. Only by printing from a zinc-plate over the half-tone print can definite lines or black areas be indicated. The same applies to ink used with crayon if the picture is half-toned.

Methods for Special Subjects. Upon a survey of the leading journals certain methods were more often used for special subjects, the departures from those indicated in the following table were common.

<i>Subject</i>	<i>Method</i>	<i>Process</i>
1. Cell structure	Stippled ink	Zinc-process
2. Protozoa	Stippled ink	Zinc-process
3. Animals of phyla	Stippled ink or line	Zinc-process
4. Histological slides	Wash or Wash and Ink	Half-tone
5. Embryological slides	Wash or Wash and Ink	Half-tone
6. Whole embryos	Crayon or Wash	Half-tone
7. Dissections and anatomical subjects	Crayon or Wash	Half-tone

Plate V. Section of ovary of frog drawn in (A) wash with details in ink, (B) crayon, (C) pencil. Reproduced in half-tone $\frac{2}{3}$ size of original. Note that the pencil drawing does not reproduce as well as A and B. D is printed in color from the same plate as A.

Generally the object of a scientific drawing is to get a good likeness of the subject, using whatever method that gains this end with the minimum effort.

Colored Drawings and Their Reproduction. In order to make a drawing in colors, water-color paints, preferably those that come in solid cakes, must be obtained. If one desires to mix his own colors, a number of reds, blues, yellows, browns, black, and white are necessary, for while a variety of shades can be made from red, blue, and yellow—the primary colors—they are not always the ones desired. A red diluted with water gives pink, whereas another entirely different pink results from red mixed with white. It is easier to purchase the colors wanted and to prepare a plentiful supply of wash from the cake in the same way as the black wash is made. Where several colors are used, they can be prevented from running into each other by mixing the pigment in a gum-arabic solution to which a few drops of glycerine have been added.

Inasmuch as elaborately colored drawings require reproduction by lithography, they are to be avoided wherever possible. Some papers on blood corpuscles contain plates in which ten separate printings are used to obtain the fine distinction between the staining reactions. A separate stone is necessary for each color and the artist who transfers the work to stone must understand what colors to superimpose in order to gain the desired effect. These separate stones are called tint-blocks. In printing, the lightest tones are printed first, followed by the stronger colors. An outline stone to define the picture and one printed in gray to deepen the shadows gives the finishing touches.

Where color gradations are not fine, a three-color process is used. Tint-blocks made for each of the three primary colors are printed one upon the other in such a way that various shades result. This process is more often employed in reproducing colored-drawings by the half-tone method. In making the negatives, photographic plates rendered sensitive to these colors together with properly colored screens are necessary. Ordinarily it takes twelve separate photographic operations to produce three blocks. The screen is turned at a different angle for each plate; otherwise the dots would be directly superimposed in printing.

Half-tone or zinc-process plates from black and white originals may be printed in color instead of black. Where tissues are shown the plate may be printed in a color to resemble the stained microscopic slide (Plate V, D). In case one color is desired upon a black and white background, a zinc-process plate is printed in color over a half-tone in black. Prints of this kind are frequently found illustrating embryological and anatomical papers.

Lately, a few journals have obtained effective plates in one-tone or a combination of two tones by using the heliotype process.

Photographs. Photographs are usually reproduced by half-tones (Plate III, A). Prints on Azo hard X, glossy white Velox paper, and solio paper of a brownish tinge, reproduce best, as the hard finish brings out strong contrast between the blacks and whites. Prints should be squeegeed, that is have the moisture removed from the wet print with a roller so as to leave the gelatin film hard when it dries. While photographs will stand some reduction, on the whole prints should be the same size as the intended cut.

If desired, certain parts of a photographic print may be outlined in water-proof ink, and the print then bleached with potassium iodide or potassium ferricyanide until nothing but an outline ink-drawing remains. This is a simple way to get an accurate outline that will reproduce by the zinc-process method (Fig. 6).

Graphs. Graphs should be made upon coördinate paper in which the lines are blue. As blue does not photograph, the coördinates, which are to appear in the cut, both perpendicular and horizontal, must be inked.

Reduction of Drawings. It is advisable to make drawings larger than they will appear in the finished print as in reduction many irregularities are lessened. But under no circumstances make a crude drawing with the idea that the print from it will be perfect, for while reduction minimizes, it does not obliterate defects. Ordinarily, the original drawing should not be more than twice the size of the intended print, while a reduction of $\frac{1}{4}$ or $\frac{1}{3}$ usually gives better results. Where line-drawings are made to an enlarged scale, the lines should not be thickened.



Figure 6. A photograph in which the outline was inked and then bleached. Reproduced by zinc-process. Contrast with plate III, A.

The Wistar Institute of Anatomy and Biology prefers that authors make their drawings of microscopic objects or sections of such size that when reduced the magnification will accord with one of the numbers suggested by Professor Simon Henry Gage (See Table 1).

Table 1—Standard Magnifications.

(Taken from Style Brief of Wistar Institute)

1, 2½, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 75, 80, 90, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1250, 1500, 2000.

Reduction always refers to linear measurement and not to area. In reducing a drawing to $\frac{1}{2}$ its original size, every line is made $\frac{1}{2}$ as long as it was drawn and the finished print occupies $\frac{1}{4}$ the area of the original. The accompanying diagram (Fig. 7) illustrates this reduction in area.

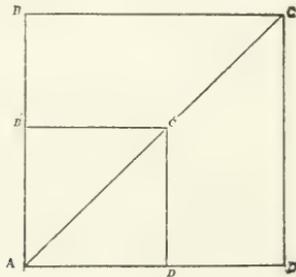


Figure 7. Diagram to show reduction in area. A B C D reduced $\frac{1}{2}$ occupies the space A' B' C' D'.

Arrangement of drawings for reduction. Drawings that require a fine screen in half-tone reproduction can not be printed as text figures unless a superior quality of glazed paper is used. Such draw-

ings are usually so arranged by the author that they may be printed on a special coated paper in the form of plates. While line-drawings generally appear as text-figures, they may be made into a plate, particularly where many small drawings are used. In order that a plate may be the proper size, the amount of reduction it will undergo must be considered when it is planned. If all the drawings on it are to be reduced one-third, the plate must be one-third longer and one-third wider than the printed portion of the journal page. Drawings may be pasted upon a bristol board in their proper sequence or they can be drawn directly upon the board or drawing paper in such order.

The amount of reduction must also be considered in labeling. Too large or too small letters may spoil an otherwise excellent picture. A cut is more legible if the names printed in full are connected to the proper part of the drawing by leaders parallel to the base-line. Since gummed sheets containing printed letters, numerals and words such as "Plate" and "Fig." can be bought in several sizes for such work, and even black typewritten words may be pasted on, an amateur had better not try hand-printing other than a few letters or numerals.

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THE OCCURRENCE OF *TRYPANOPLASMA* AS AN ECTOPARASITE

By Olive Swezy

The question of the adaptation of a protozoan parasite, either in its morphological modifications or in its physiological reactions, to its habitat is one that presents many interesting and at the same time puzzling aspects. In the group of so-called facultative parasites, largely composed of flagellates and amoebas, some forms seem to present such adaptive modifications, particularly among the flagellates. This is shown in the development of certain structures, such as undulating membranes, and more especially of the neuromotor system which reaches its greatest development among the parasitic flagellates.

On the other hand, structural modifications are almost totally lacking in the amoebas, either as obligatory or facultative parasites. Undoubtedly physiological adaptations are present but are such as leave no record in structural organization.

Such modifications or the apparent lack of them may be found in both the true entozoic parasites, as well as those that are merely commensal in the intestinal tract of the host. Thus *Entamoeba histolytica* presents no distinct structural modifications indicative of its dangerous parasitic career. The flagellate *Bodo* is apparently a harmless commensal in the intestinal tract of frogs, etc., and differs in no wise from the free-living representatives of the genus. The mode of adaptive response in both cases is physiological and not structural.

However, when this comparison is extended to protozoans infesting other parts of the body a different state of affairs is found, as most if not all, these parasites are forms which have no representatives among free-living species. This is true of the large group of Sporozoa and of the haemoflagellates as well, with a single exception which will be pointed out below. This fact is probably accounted for by the higher degree of specialization which an organism must attain before it finds itself at home in an environment such as the blood, which differs so greatly from the habitat from which it originally came.

The degree of difference between conditions in the intestinal tract of a frog and of the water in which it lives is not so great as that between the intestinal tract and the highly oxygenized blood-stream.

The single exception to this among the true haemoflagellates is found in the genus *Trypanoplasma*. This genus contains a number of species which may be divided into three groups according to their habitat. The first of these are parasitic in the blood of fresh water fishes, the second in the digestive tract of marine fishes and the third in various invertebrates. Minchin (1912) doubts the validity of placing all the flagellates that have been thus described in a single genus. Habitat alone, however, cannot be used as a generic distinction.

This small flagellate to which had earlier been given the name *Trypanoplasma carassii* (Swezy, 1915), is found as an ectoparasite on gold-fish. No evidences are forthcoming to indicate that it is truly parasitic in its habits, but it is rather a commensal, living in the mucus that is ordinarily present on the surface of the fish. *Costia necatrix* is usually found associated with it. Examinations were frequently made of the water in the aquarium containing the gold-fish but no flagellates were found in it. To secure them the body of the fish must be lightly scraped with a scalpel or other instrument.

The body of the flagellate is roughly triangular in shape with the broader end anterior (pl. M, fig. 3). Its length varies from 5 to 12 μ and its width at the anterior end about 3 to 7 μ . In many cases the body is long and slender, more nearly approaching the trypanosome-like form of the blood-inhabiting species (pl. VI, fig. 2).

The neuromotor system consists of a vesicular nucleus, parabasal body, blepharoplast, with their connecting rhizoplasts, and two flagella (pl. VI, fig. 3). The nucleus is situated near the middle region of the body or slightly anterior, and usually at one side. It is relatively large and vesicular in type, with a large central karyosome bordered by a distinctly light area. In the more slender, attenuate forms the vesicular structure is often obscured, the entire nucleus taking a dark stain. On the opposite side of the body, at the same level or slightly anterior to it, is the parabasal body. This is usually rounded in shape but may be slightly elongated or even somewhat irregular in outline. It stains a deep black with iron haematoxylin and shows no differentiated structure. It is connected with the nucleus by a slender, deeply staining rhizoplast.

In front of this structure and near the antero-lateral margin of the body, is the blepharoplast. This is composed of a single granule from which arise the two flagella (fig. 3). It is connected with the parabasal body and apparently also with the nucleus itself by slender, deeply staining rhizoplasts. In some cases the rhizoplast connecting the nucleus and blepharoplast cannot be distinguished (fig. 1). In most of the flagellates observed, however, the three rhizoplasts are present, i.e. from the nucleus to the parabasal body and also to the blepharoplast and from the blepharoplast to the parabasal body (fig. 3), closely linking together these three parts of the neuromotor system.

One of the two flagella is directed anteriorly, the other posteriorly. The former may have a length two or even three times that of the body. The posteriorly directed flagellum is usually much shorter, sometimes not exceeding the body in length. It is attached to the surface by a thin membrane which may show an undulating line (fig. 3) or may present a nearly smooth contour (fig. 2).

A comparison of *Trypanoplasma carassii* with the blood-inhabiting species of the genus shows no important differences. In *T. borreli* as figured by Keysselitz (1905) the body has the same general form, narrower posteriorly, but, unlike *T. carassii*, shows a strongly marked curvature or sickle-shaped outline. It is also much larger than the latter species, its length varying from 10 to 40 μ . The parabasal body is elongate, extending from near the blepharoplast backward along the lateral margin of the body with a length that may sometimes equal one-third or more of the total length of the body (fig. 4).

Intestinal species do not differ materially from the blood-inhabiting forms. *Trypanoplasma congeri*, from the stomach of the conger eel (Martin, 1910), has a slender, elongate form (fig. 5) with the same type of parabasal body and nucleus found in *T. borreli*. The nucleus shows a tendency to assume an oval shape in some individuals.

It is thus evident that this flagellate with an ectoparasitic habit, presents no specific structural differences distinguishing it from other species of the same genus inhabiting the blood-stream. Its occurrence is noteworthy as being the only example of the haemoflagellates which has thus far been described from a habitat outside the body of a living animal. The genus probably represents a transition stage between the strictly intestinal group of flagellates and the haemo-

flagellates or blood-inhabiting forms, with this species still retaining the ability to live outside of its normal host as do other of the ordinary intestinal flagellates. No trypanoplasmas were found in the intestinal tract or blood of the gold-fish from which these flagellates were taken. This precludes the possibility of an accidental infection of these fish from the faeces.

Trypanoplasma carassii is much smaller in size than either of the other two species and is one of the smallest that has been described for the genus. In the structure and arrangement of the nucleus and parabasal body it presents some striking resemblances to *Prowazekia* to which genus it could properly be assigned were it not for the presence of the undulating membrane. These resemblances suggest an evolutionary development of this genus from *Prowazekia* or a similar form.

The interrelations of the various parts of the neuromotor system in *Trypanoplasma* have not been figured by previous investigators. It is probable that the same connecting rhizoplasts are present in all the species of this genus, linking together the blepharoplast, nucleus and parabasal body.

The facts concerning the life cycle of *Trypanoplasma* are still obscure and await further investigation. Even its methods of division have not been thus far satisfactorily accounted for. Nor can any light be thrown on these questions from present work on this species from gold-fish. Division forms were not observed. Its minute size renders it a difficult object to work with satisfactorily.

University of California

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EXPLANATION OF PLATE

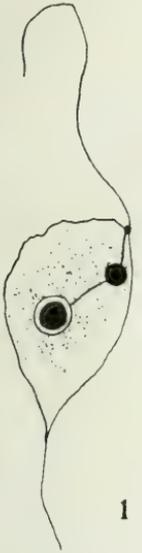
Fig. 1. *Trypanoplasma carassii* Swezy. Ordinary trophozoite showing the typical outline with single blepharoplast, flagella, undulating membrane, parabasal body and nucleus. The rhizoplast connecting the blepharoplast and nucleus could not be detected. $\times 2235$.

Fig. 2. Slender elongate form of the same. $\times 2235$.

Fig. 3. Typical form of trophozoite with the rhizoplast connecting the nucleus and the blepharoplast. $\times 2235$.

Fig. 4. *Trypanoplasma borreli* Laveran and Mesnil. After Keysselitz (1906, fig. 13). Normal trophozoite showing the elongate parabasal body. Rhizoplast are lacking. $\times 2235$.

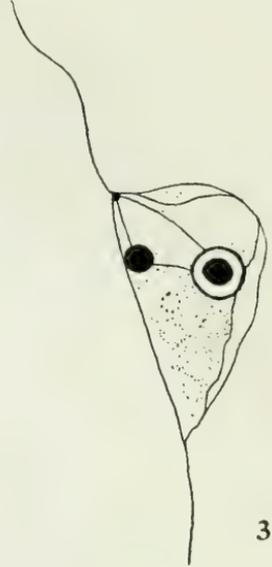
Fig. 5. *Trypanoplasma congeri* Martin. After Martin (1910, fig. 1). Normal active trophozoite. A line of faintly marked cytoplasmic granules follows the base of the undulating membrane. $\times 2235$.



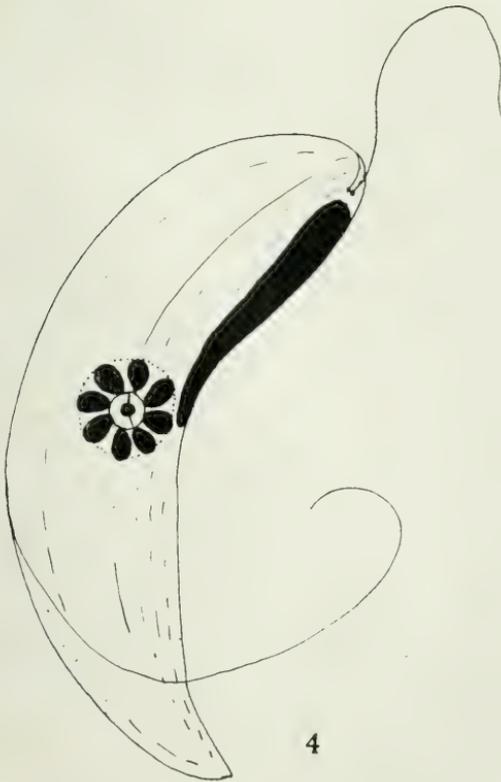
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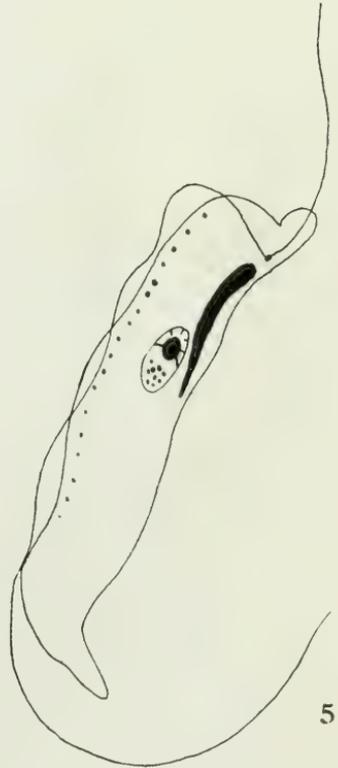
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DEPARTMENT OF NOTES AND REVIEWS

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

THE NEW TREASURER

Owing to press of duties, Dr. H. J. Van Cleave, at the end of his three year term, has felt it necessary to offer his resignation as Treasurer of the American Microscopical Society. In as much as no meeting of the Society was held in 1918, this resignation was acted on by the Executive Committee. In accepting Dr. Van Cleave's resignation the Committee in both formal and personal ways recorded their great regret and their thanks to him for a most capable and constructive administration of the office.

Mr. Wm. F. Henderson, Instructor in The James Millikin University, Decatur, Illinois, was named Treasurer for the ensuing term, subject to ratification at the next constitutional meeting.

THE PROBLEMS OF THE FUTURE

The Secretary wishes to call the attention of the Society to the fact of the great loss, especially among our younger members, during the last four years. This is neither a surprising, unique, nor prostrating experience. It is common among societies of this kind. It does, however, call for heroic and thoroughgoing coöperation among the membership.

I am sure that every member of the American Microscopical Society has confidence that science and scientific research will not suffer permanently after the war. Whatever of our predilections and practises have been shown to be either ineffective or false by the events of the war, science and scientific men showed that the future is helpless without them.

Our own Society, publishing as it does from 300 to 400 pages each year of the results of research in fundamentally important fields of human interest, ought to enter firmly and confidently into its portion

of this future progress. The fields opened up by the microscope—whether in zoology, botany, histology, pathology, medicine, bacteriology and sanitation; or in the hundreds of more specialized industrial aspects of food examination, textiles, agriculture, chemistry, mineralogy, and the like—will be greatly enlarged during the next quarter of a century.

The Secretary feels that our Society has some very definite advantages to offer to the younger generation of students who must use the microscope. Our scope is much more catholic and general than that of most societies, and is yet quite specific and technical enough to serve the specialist. Our publications ought to be peculiarly valuable to those who do not have access to large lists of special journals.

He desires, therefore, to ask very earnestly that all members, beside keeping alive their own membership, aid him in the following ways:—

1) Send to the Secretary the names of any persons likely to be attracted by our program. These may well be of your present advanced students, recent students who have gone out into teaching or other work of a scientific kind, colleagues, acquaintances among progressive high school or college teachers, and isolated workers with the microscope.

2) Send in, suitably illustrated for publication, the best discoveries you are making of methods of work, of technical appliances, or of making truth clear to others. We aspire to become one of the best channels in the country for the presentation of such technical notes, in our Department of "*Notes and Reviews.*"

In a "mutual" association like this, where there is no endowment, there is at once the necessity and the privilege of complete and hearty coöperation. As he enters the tenth year of service to the Society, the Secretary feels more than ever that this may be made the most prosperous period in the whole history of the Society.

CRYSTALS IN AMEBAS

Schaeffer reports (Baltimore meeting Am. Soc. of Zoologists, 1918) that crystals, reasonably distinctive in shape and size, characterize different species of amebas. These crystals are within vacuoles and

are thus not in direct contact with the endoplasm in which they occur. The author believes them excretory, altho their composition has not been determined. Their presence seems to be correlated with the physiological states leading to division. Actively dividing amebas have few crystals. Those that are not dividing become so loaded up with crystals as to produce opaqueness and to impede locomotion. It has not been possible to determine whether the crystals, once formed, are ever resorbed again.

MONTANA TREMATODES

Faust (Ill. Biol. Monog. Vol. IV, No. I, July 1917) reports on the larval trematodes found infesting the snails of Bitter Root Valley, Montana. Fourteen new species are described from this biologically isolated fauna. Two of these are monostomes, two are holostomes, and ten are distomes. This is a large number of species for so limited a territory. The percentage of infection of the snails is very high.

The author has studied only the cercariæ and the parthenogenetic stages whereby these larvae are produced, the mature worms not being known in any of the species. The principal results of the study are summarized as follows:

1. The history of the germ cells in sporocysts and rediæ shows that they are true parthenogenetic ova, with one polar body and no reduction of chromosomes.
2. Consequently the parthenitæ (sporocysts and rediæ) are not "larval" in any real sense; but we have an alternation of parthenogenetic generations with hermaphrodite generations.
3. The manner of forming the egg cells, the origin of the layers, etc. give evidence that the parthenitæ, cercariæ, and miracidia are homologous, tho distinct, life histories.
4. The trematode integument is mesodermal in origin.
5. The fundamental systems (e.g. excretory, genital, and nervous systems) of the adult are manifest in the cercaria and may be used to show relationships.
6. Holostomes are probably of distome origin, and have, in common with the other families, the alternation of hermaphrodite and parthenogenetic generations.

7. The parthenitæ are to be looked upon as physiologically young, and thus able to continue the parthenogenetic cycle for several generations without the hermaphrodite generation.

8. This youth and the attendant simplicity as compared with the miracidium is to be looked upon as secondary results of parasitism.

THE CRYSTALLINE STYLE OF LAMELLIBRANCHS

Nelson (*Jour. Morph.* Vol. 31, June 1918) presents a review of the work done on the crystalline style in Lamellibranchs, and contributes some interesting results of his own about this singular organ.

The lining of the intestine and of the communicating style sac is ciliated. This ciliary mechanism is regarded as having power to separate food from the foreign particles within the tract. Little discrimination is shown as to material taken into the stomach.

The style arises as a thin core of bubbly mucus upon which co-axial layers of a gelatinous protein, containing enzymes, are deposited. The style rotates in the sac, according to the observer. He confirms the conclusion that it contains strong amylolytic ferments and believes that the style serves as a means of restoring to the stomach undigested food particles which might otherwise be lost, at least in those forms in which the style sac is not separated from the intestine. The store of ferment is thought to be of peculiar value because of the long period during which feeding is impossible in many mollusks.

REVIVIFICATION OF EXSICCATED EARTHWORMS

Schmidt (*Jour. Exp. Zool.*, October 1918) has shown that earthworms are capable of being revived after 39 to 48 hours (depending on temperature) of exsiccation in which they have lost one-third to one-half their length and volume, and show no signs of life that can be detected. The worms were of course very gradually dried. The body must be allowed to retain its elasticity and the skin its softness, if revival is to be expected. Life was normally regained after as much as 61.6 per cent of the weight of body (nearly 73 per cent of the weight of water in the body) had been lost.

The earthworms differ from lower animals like rotifers and nematodes in that they cannot be preserved thru such long periods of time. This is probably due to the fact that they are more complicated, cannot be so completely desiccated, and hence decomposition

changes, thru the presence of microorganisms in the gut, are more likely.

The adaptive quality of this power to sustain loss of water without loss of life is manifest, when we remember the fact that earthworms must meet considerable range of variation in the moisture of the soil crust which they inhabit.

LIFE BEHAVIOR OF ASCARIS

Ransom and Foster (Baltimore meeting of the American Society of Zoologists, 1918) report interesting points in the life history of *Ascaris lumbricoides*. It was found by them that partial development may take place in many hosts which are not suitable for the complete life history. Rats and mice are less favorable than lambs and goats. The partial development in the rats and mice led Stewart to believe that these animals were the intermediate hosts of the *Ascaris* found in man and the pig.

The normal life behavior is stated as follows: Eggs after being swallowed hatch in the intestine. Shortly after hatching the larvae occur in the portal vein and the liver. The lungs, reached thru the circulation, are a point of rapid development. The larvae pass back to the intestine by way of trachea and esophagus. If the animal is a suitable host mature development is reached here. If not, the larvae are lost with the feces.

REVERSAL OF ORIENTATION TO LIGHT

Mast (J. Exp. Zool. Jan. 1919) records that *Volvox* and *Pandorina* react similarly to light. He finds dark-adapted colonies which are usually positive in weak illumination and negative in strong. Light-adapted colonies are sometimes positive in strong and negative in weak light.

If dark-adapted colonies are exposed to continuous illumination they suffer a series of reversals of orientation, the time required for which depends on the intensity of light. They are neutral for a short time; then become positive, passing thru a maximum; after this they become neutral again; then they become negative, passing thru a maximum; again they become neutral, and then pass finally into a positive state.

Green and blue rays are most influential both in stimulation and in producing the reversal of orientation. This sense of orientation is

modified by changes in temperature, by the constitution of the culture medium, by the age and physiological state of the colonies.

REACTIONS OF LAND ISOPODS TO LIGHT

Abbot (Jour. Exp. Zool. Nov. 1918) finds that the land isopods, *Oniscus* and *Porcellio*, are negatively phototactic to all intensities from 0.01 C.M. to 100 C.M., whenever not immersed in water. He concludes that the orientation is direct and not by selection of random movements; and that this negative phototaxis is apparently a factor in fitting them for life on land by aiding to keep them in a suitably moist habitat. The negative quality is more pronounced in *Oniscus*, which has the more restricted habitat.

ASSORTIVE MATING IN CHROMODORIS ZEBRA

Crozier (Jour. Exp. Zool. Nov. 1918) finds that there is high degree of assortive mating in the large nudibranch mollusc *Chromodoris zebra*. This assortive mating expresses itself in the correlation in the size of mates—large with large and small with small. Since the species is hermaphrodite and a mutual exchange of sperm is normally to be effected, this selective mating on the basis of similar size and consequently appropriate position of the reciprocal organs is an advantageous adjustment. It is a conservation of sexual elements; and when large individuals mate together the numbers of eggs fertilized is greater than would be true in mismatings at random.

ADAPTIVE COLORATION IN CHROMODORIS ZEBRA

Crozier (Baltimore meeting of Am. Soc. of Zool. 1918) concludes that the coloration in *C. zebra* has no adaptive significance either in its origin or at present. This, in spite of the fact that the organism has brilliant yellow pigment, that there is sufficient variation in coloration to furnish basis for selection, that the species actually suffers extensive injuries from animals capable of seeing the color, and that it possesses "an efficient repugnatorial apparatus" which would conceivably make "warning" coloration useful. The types of injury seem in no way correlated with either the intensity or the distribution of the pigment.

CAMOUFLAGE IN REEF FISHES

Longley (Baltimore Meeting Am. Soc. of Zoology, 1918) reports studies on the coloration and habits of West Indian and Hawaiian

reef fishes. He finds that their fixed colors, excepting red, repeat the dominant color of the surroundings, and that the change of color in moving from place to place is induced by, and on the whole in accordance with, the nature of the places into which they go.

When the following varieties of color are possible to the individuals of a given species, cross-banded markings are likely to be shown when at rest, and longitudinal or self-colored phases when about to move or when actually moving.

To the fact of change of color when moving horizontally, the author adds the observation that there are similar definite phases of color change in some fishes for vertical movement. A vertical change of even a few inches may be followed by definite changes of color. The author feels that some of the changes of color usually charged as being connected with mating are probably so to be considered only because of place changes at the reproductive season, rather than as directly related to reproduction.

GLYCOGEN IN THE NERVOUS SYSTEM

Gage (*J. Comp. Neur.* June 1917) uses the methods of microchemical analysis to determine the presence and quantity of glycogen in the nervous system of Vertebrates. He finds abundant glycogen in the cells of the nervous system, at some stage of development, in all groups of vertebrates from amphioxus to mammals. Amphioxus, the lamprey, *Amblystoma*, the chick, and the pig were carefully studied. Glycogen is also found plentifully in sensory epithelia and in related organs.

The author feels from his results that glycogen is an essential accompanier of the development of nervous (and all other) tissues, especially in their functional stages;—being produced and used by the protoplasm as an essential feature of its metabolism. After the tissues, nervous and other, reach their final form this glycogenic function, as we know it in the higher forms, may be given up largely by the various tissues, and be taken over by the liver and the muscles.

EFFECT OF STRAIN ON DEVELOPMENT OF BONE

Howell (*Anat. Rec.* Vol. 13, Oct. 1917) produces paralysis of the muscles working the bones of the arm and shoulder by cutting the main nerves of the brachial plexus in young puppies. This removed

the stresses usually experienced by these bones. The results show definitely that the strains put upon the bones by the muscles are not necessary to the growth of the bones. Such unstressed bones grew as much as 56% to nearly 100% in four and one-half months. On the other hand bones unstressed by muscles were much smaller in diameter, in the thickness of compacta, in the size of the trabeculae; were reduced in weight and in their resistance to crushing. Growth in length seems little influenced.

EPITHELIAL MOVEMENTS IN VITRO

Shinichi Matsumoto (Jour. Exp. Zool. Vol. 26, Aug. 1918) reports experiments in the culture of corneal epithelium of adult frogs *in vitro*. This is a favorable material because the transparency of the cornea is such as to allow direct observation of the cell movements. Various substrata were used—as flat surfaces of glass, celloidin, and dead cornea; spider web, silk fiber, glass wool, asbestos fiber; and porous bodies, such as thin pieces of pith.

The movements are amoeboid, with the cells tending to cling to their own kind and thus to form sheets. This is a most essential quality in forming and extending epithelial surfaces. The author believes this to be thigmotactic rather than chemotactic in nature, extending as they do over various types of surfaces. Rapid extension of epithelium may thus take place with no mitotic divisions at all.

The same author (Jour. Exp. Zool. Oct. 1918) discusses the technic and results of vital staining of these corneal cells in neutral red. When this was done by immersing the whole animal in a weak solution (1:100,000 to 1,000,000) the excised cells behaved *in vitro* just about as the unstained cells do, and were more readily followed because of the distinctness of the granules in the cytoplasm.

The corneal epithelium, incidentally, showed clear phagocytosis of the pigment of broken iris cells and of finely powdered granules of various stains.

ENTOMOLOGICAL ABSTRACTS

Physiology of Chironomus Larva.—In a study of the biology and physiology of the larva of *Chironomus gregarius*, Pause (1918, Zool. Jahrb., Abt. f. all. Zool. u. Physiol., 36:339-452) finds, among other things, that this larva has four molts. Tracheae, absent in the first instar, appear in the second, and are confined to the head and thorax.

They play no part in the process of respiration and are to be regarded as rudimentary organs. Exchange of gases is accomplished through the circulatory system. A membrane, in the 11th and 12th segments, is so located that the blood stream is directed into the blood gills before it can return to the heart, thus making the latter strictly an "arterielles Herz." The haemoglobin which occurs in diffused form in the blood first appears in the second instar, its formation being coincident with the reversal of the distinct positive phototropic reaction of the young larva into a strong negative phototropic reaction. There is evidence that the amount and time of formation of the haemoglobin are not influenced by differences in nutrition or light reduction. The circulatory system supplies the tissues with oxygen, carries away the carbonic acid, and serves as a means of storing oxygen which can be utilized when the dissolved oxygen in the surrounding water becomes reduced. The larva of *Chironomus gregarius* requires some oxygen, but shows considerable resistance to oxygen reduction, this resistance increasing with the formation of haemoglobin. It succumbs to an oxygen content of 0.10 cc. per liter and it appears that 0.2 cc. per liter is very near the minimum quantity.

Wing Development in Aphids.—Shinji (1918, Biol. Bull., 35:95-116) reports that for a number of the common aphids certain chemicals in the soil in which food plants are grown—salts of the alkalis (Na, Cl K, etc.) and alkaline earths (Ca, Br), constitute "non-wing-developing substances" while others (salts of the heavy metals, of magnesium, sugar, etc.) are "wing-developing substances." The former were only effective when applied within a certain period after birth, the period varying with the temperature and the species (in early summer—2-3 days in *Macrosiphum rosae*; 5-7 days in *Macrosiphum solanifoliae* and *Aphis brassicae*). A M/100 solution of magnesium sulphate operating for 12-24 hours produced nearly 100% winged individuals in *Macrosiphum rosae*. Aphids reared on twigs charged with non-wing-developing substances and subjected to sudden temperature changes of 100° F. to 35° F. failed to produce winged forms.

Sex Determination in Hymenoptera.—Whiting (1918, Biol. Bull., 34:250-256) finds that in *Hadrobracon brevicornis*, a parasitic wasp which deposits its eggs upon caterpillars of the Mediterranean flour

moth, both bisexual and parthenogenetic reproduction occurs. Fertilized eggs produce *females* and unfertilized eggs produce *males*. Comparison with the honey bee leads to the supposition that the males are haplonts and the females diplonts.

Locomotion of Caterpillars.—Turner (1918, Biol. Bull., 34:137-148), in a series of experimental studies on surface feeding caterpillars, finds no evidence that their locomotions are tropisms. Such movements are "identical with those made by animals that learn by the trial and error method." Physiological unrest due to unusual environmental influences induce random movements which continue until stopped by fatigue or by attainment of more favorable environmental conditions.

Zoraptera.—Caudell (1918, Can. Ent., 50:375-381) describes a new species of Zoraptera (*Zorotypus hubbardi*) from ten specimens taken from termite galleries in Florida. The order Zoraptera was established by Silvestri in 1913 for three species of a single genus (*Zorotypus*): *guineensis* from Africa, *ceylonicus* from Ceylon, and *javanicus* from Java. He also described (1916) a new species (*Z. neotropicus*) from Costa Rica. The presence of this very primitive insect on the continent of North America is thus made known for the first time.

Immunity Principles in Insects.—Glaser (1918, Psyche, 25:39-46) finds that the value of the insect blood cells in ridding the body of foreign substances has been greatly exaggerated and that in reality they are usually rather passive. In grasshoppers and caterpillars, the blood cells do not seem to "phagocytose bacteria in an amaeboid fashion" and when bacteria occur in blood cells they may have entered of their own accord or have been included through some physical factor. However, blood seems able to overcome bacterial invasion to some extent due to elaborated substances which constitute extracellular antagonistic substances. Immunized grasshopper blood exhibits a marked degree of antagonism towards the bacteria used in producing immunity. The existence of an agglutinin in immune grasshopper blood was demonstrated.

Polyembryony in Insects.—Gatenby (1918, Quart. Journ. Micr. Sci., 63:175-196) presents a review of polyembryony as it occurs in the parasitic Hymenoptera, giving particular attention to the work of Marchal, Silvestri, Martin, and Patterson. Polyembryony—

the production of numerous embryos by a single egg—in insects is known at present in only two families of the parasitic Hymenoptera (Chalcididae and Proctotrypidae). Eggs of these parasites, fertilized or unfertilized, are deposited in the eggs of various insect hosts and develop in the subsequent larval stages of the latter. Polar bodies, given off by the parasite's egg, develop into a growing mass of nuclei, and the polar cytoplasm forms an investing sheath about the embryonic ooplasm, the latter ultimately producing the embryos. By repeated division, the primary embryonic cell gives rise to many germinal masses (polygerms) which continue to divide, ultimately resulting in numerous masses each containing an embryonic mass surrounded by two membranes. These final masses produce individual embryos, which later, as larvae, break away from their membranes and are free-living in the haemocoel of the host for a time, consuming the body tissues of the latter. Resulting broods may be exclusively male, exclusively female, or mixed, the latter probably resulting from two or more eggs, fertilized or unfertilized. As known at present, fertilized eggs produce females and unfertilized eggs, males. Gatenby claims that the "germ-cell determinant" is possibly a "nutrient cytoplasmic-mass" and does not later form the germ cells of each embryo; that there is no evidence of a germ-track and that "mere position in the morula is all that seems to determine whether this or that cell will be endoderm or ectoderm cell, etc." He also predicts the discovery of species which are polyembryonic or monoembryonic according to the season of the year, or according to some condition of the host egg or caterpillar.

Light and Muscle Tonus of Insects.—Garrey (1918, Journ. General Physiology, 1:101-125) has investigated the relationship between the tonus or tension of the skeletal muscles and the illumination of the eyes of heliotropic insects. Experiments were conducted mainly on the robber flies *Proctacanthus*, *Promachus*, and *Deromyia*. A number of butterflies (*Circionis alope*, *Vanessa huntera*, *Argynnis aphrodite*, et al), dragon flies, certain Vespidae, and representatives of several genera of Diptera were also used. The desired difference in illumination was obtained by coating the eyes wholly or in part with asphalt black varnish. When both eyes were blackened, inactivity, muscular weakness, and incoördination resulted. One eye blackened led, in most of the animals tried, to circus movements towards the

side of the uncovered eye. Difference in illumination produced an asymmetry when the insect was at rest, and if it moved, it was forced toward the side of greater illumination. Tonus changes in the muscles were approximately proportional to the area of the eye blackened. Blackening the lower halves of the eyes caused the anterior end of body to be lifted far up from the horizontal support, head tilted back against thorax, and caudal end of abdomen pressed against the support. The exact opposite occurred on blackening the upper halves of the eyes. Blackening the upper half of one eye and the lower half of the other produced a combination of the effects just described. Symmetrical blackening of outer or inner halves of the eyes resulted in weakening of some groups of muscles but no asymmetry was produced. Insects which normally walk directly up a vertical surface or rest with the body axis in a vertical line, veered off at an angle towards the unblackened eye.

All heliotropic insects with one eye blackened ascended vertical cylinders obliquely. It thus appears that the muscle tonus of heliotropic insects is due chiefly to light. Each eye controls the tonus of a different group of muscles on both sides of the body and unequal photo-chemical reactions in the two eyes lead to asymmetrical conditions of muscle tension. The reactions vary directly with the intensity of the illumination. These experiments yield results which are in accord with Loeb's muscle tension theory of heliotropism.

Gynandry in Arachnida.—Hull (1918, Journ. Genetics, 7:171-181) divides arachnid gynandromorphs into three types: "1. One side male, the other female—sexual structures perfect except for a distortion resulting from the union of dissimilar halves on the median line." "2. As 1, but one side imperfectly developed before, the other behind," and "3. One side perfectly female before and male behind, the other perfectly male in front and female behind." So far as known at present gynandromorphs in Arachnida are confined to one order, Araneae.

Somatic Chromosomes in Coleoptera.—Hoy (1918, Biol. Bull., 35:166-169) finds that developing eggs and embryos of *Epilachna borealis* show eighteen chromosomes and have two kinds of chromatin content in somatic cells, an XY combination and an XX combination. Embryos of *Diabrotica vittata* contain two chromosome groups, one

with twenty-one and the other with twenty-two, each type being constant in number and form in the various tissue cells studied.

Stoneflies and Plants.—Newcomer (1918, Journ. Agr. Research, 13:37-41), has studied the life history and habits of certain western stoneflies belonging to the genus *Taeniopteryx*. While adult Plecoptera are usually referred to as possessing the biting type of mouthparts, several American species have mouthparts which are more or less rudimentary. However, four species were found to possess well developed biting mouthparts and feed actively upon certain plants. *Taeniopteryx pacifica* was found to have the mandibles, maxillae, and labium completely developed and functional. While the foliage and buds of apricots, peaches, and plums are eaten, it is probable that such food habits are acquired. Of the native vegetation along streams, the foliage of the wild rose, wild cherry, alder, and elm and the leaves and catkins of willows are used as food. Two other species (*Taeniopteryx nigripennis* and *T. pallida*) feed actively upon thimbleberry, alder, willows, wildrose, serviceberry, and maple. Another species (*Taeniopteryx, sp.*) was observed feeding upon young cherry leaves.

Gynandromorphism.—Petty (1918, South African Journ. Sci. 14:425-426) describes a case of right-left gynandromorphism in the moth *Metanastria pithyocampa* Cram. Sexual dimorphism in this species is exhibited by such characters as coloration, antennae, size, and pubescence, thus making a gynandromorph rather easily distinguishable. A male was found in connection with the gynandromorphic individual and in the copulatory position.

Oviposition of Notonectidae.—Hungerford (1918, Ent. News, 29:241-245), in a study of the oviposition habits of certain Notonectidae (*N. undulata*; *N. variabilis*; *N. insulata*; *N. irrorata*), finds that the first three mentioned have ovipositors poorly adapted for making incisions in plants of sufficient size to receive eggs and that while they are capable of abrading the surface of stems, only *N. irrorata* possesses an ovipositor capable of inserting the eggs in plant tissues. *N. irrorata* was observed to deposit eggs in the stems of moneywort, *Juncus*, and dead *Typha*, while the other species attach their eggs to the surface of stems. It is suggested that the structure of the ovipositors should be taken into account in determining the relationship of various species of Notonectidae.

Development of Flesh flies.—Kunkel (1918, Journ. Exp. Zool., 26:255-264) has tested the effects of mammalian thymus and thyroid on the development of flesh flies (*Lucilia caesar* and *Lucilia sericata*). When fed exclusively upon thyroid, growth of the larvae is slightly retarded; the resulting pupae are reduced in size, pupation is initiated earlier than normal and the period of pupation is shortened. Thymus tends to increase the size of the larvae. The results resemble those of similar experiments with vertebrates but are not so striking.

Terminology of Metamorphosis.—Comstock (1918, Ann. Ent. Soc. Am., 11:222-224) points out that in insects usually designated as having incomplete metamorphosis two distinct types of metamorphosis occur, one represented by such orders as Hemiptera and Orthoptera in which the development is direct, and the other represented by Plecoptera, Odonata, and Ephemera in which there is cenogenetic development. The recognition of the distinct differences existing between the two groups of insects heretofore associated together gives support to a proposed revision of the following form: (1) *Gradual Metamorphosis* or paurometabolous development, characteristic of Orthoptera, Hemiptera, et al. (2) *Incomplete Metamorphosis* or hemimetabolous development, characteristic of Plecoptera, Odonata, and Ephemera. (3) *Complete Metamorphosis* or homometabolous development, characteristic of Diptera, Lepidoptera, et al. Comstock proposes the restriction of the term *nymph* to the immature stages of *gradual metamorphosis*; the term *naiad* for the immature stages of Plecoptera, Odonata, and Ephemera; and the term *larva* for the immature stages of all insects having complete metamorphosis.

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NOTES ON TECHNIQUE

(Abstracted by Dr. V. A. Latham)

A Mounting Medium.—The best mounting medium is liquid petrolatum. It has the proper consistency for mounts, is less sticky, does not become acid as is so common with the usual Canada balsam

of these times, does not require thinning with its solvent which changes the refractive index and causes blood stains, such as that of Romanowsky, to fade. It has superior optical qualities. It is easily used for small insects and for sporangia of fungi, especially moulds. For permanent mounts the cover must be sealed with gold size or other cement. For the introduction of this liquid medium I believe we are indebted to Dr. Alfred C. Coles of England, who gave the method of using. ("Paraffin as an Oil Immersion Fluid," in *English Mechanic*, February 14, 1914). His work on *Spirochæta pallida* in the same journal for Dec. 1909, p. 267-8 and on the flagellæ of Bacteria, *idem*. Dec. 1909, page 308, will interest many.

Further Note on Mounting in Liquid Petroleum "Sea-cure" (*English Mechanic*, Jan. 17, 1919) suggests the following: Make the smear of bacteria or prepared Diatoms on thin glass. After drying, fix in 1/20 solution of carbolic acid before staining, if bacteria. Then stain, put on one drop of liquid petroleum, and use a cover glass and cedar oil for immersion. When the examination is finished wash off the petroleum by slipping the slide into a stoppered bottle of petrol. Keep the slide in a vertical-rack slide box. Number the slide and keep your notes in the box with it. This method is much easier for use with malarial slides in the tropics, and with preparations where the aniline stains have been used as these are not affected by petroleum in the way they are by the cedar oil. If microscopists would study Bacteria as they do Diatoms with dark ground illumination and a 1/12 oil immersion and with oblique light, some new results might be achieved for science.

Simplified Technic for Determination of Pale Spirochetæ.—Quioc. (*Paris Medical*, p. 73, July 27, 1918, 8, No. 30; Abstract, J. A. M. A. p. 1616, Nov. 9, 1918) describes the superior and unfailing advantages of the Fontana-Tribondeau technic in the early or late differential diagnosis of syphilis. The organism debris and red cells are partially dissolved, while the pale spirochete is shown up clearly from other spirochetes. (T) Dissolve cold 1 gm. AgNO₃ crystals in 20 cc. of distilled water. Reserve part of this solution, and add to the rest ammonia, a little at a time, stirring constantly, until a sepia precipitate is thrown down, and then disappears anew. Now add the reserved solution, fractionated, until there is a slight turbidity, persisting during agitation. This reagent sheltered from light, keeps well.

Dry the specimen carefully, and cover it for 30 seconds, 2 or 3 times, according to its thickness, with Rugés solution (1 cc. of crystallized acetic acid in 100 cc. of a 2% solution of formaldehyd). This dissolves the haemoglobin. Rinse in alcohol; then pass thru flame to burn off all traces of the alcohol. Cover specimen with a solution of tannin (5 gm. of tannin and 1 gm. of glacial phenol in 100 gm. water). Heat till it steams. Let it steam 1 minute, then rinse till all trace of tannin solution is gone. Dry. Cover with the nitrate solution. Rinse and dry. All the spirochetes take an even deposit of the silver, and look uniformly thicker and extremely distinct. The pale spirochete retains all its special characteristics, showing up dark purple against a transparent background or against the light yellow background of the decolored red cells.

Enlarged photographs in Forensic Medicine.—Martin of Lyons University, France, says the most valuable information is derived from enlarging a photograph of a fire-arm wound in criminal cases. The stereoscopic view, enlarged several diameters brings out details which otherwise entirely escape notice.

Improved Staining Technic. P. del Rio-Hortega (Revista Española de Medicina y Cirugia, Barcelona. Sept. 1918, L. No. 3: and J.A.M.A., p. 1620, Nov. 9, '18) gives details of a method for histologic specimens with an ammoniacal solution of silver carbonate, prepared with lithium carbonate from silver nitrate. Histological details are said to be shown up much clearer than with the classic technics. Especially useful in amylosis and for nerve fibers and tumors.

The Amoebas infesting man. H. Aragao (Annaes Paulistas de Medicina Cirugia, S. Paulo, page 25, February 1918, 9, No. 2; and J.A.M.A., 29 December 14, 1918) mentions the increase, in cases reported for S. Pau'o within the last 6 years, from 4 to 543. Drugs do not act directly on the encysted forms, but they check the multiplication of the parasite into the encysted states. None of the drugs that act on the *E. histolytica* seem to have the slightest action on the *E. coli*. Differentiation is difficult if they are dead, especially when any epithelial cells and dead leucocytes are present. He therefore incubtes at 37° C., for from ½ to 1 hour to restore if possible, their mobility if lost. To stain, the faeces are diluted—0.5 cc. in 2 or 3 cc. of a 0.1% solution of gentian violet in physiological solution to

which has been added 0.3% of acetic acid. This keeps the elements for several days.

A Flagellate parasite occurring in a species of Euphorbia is mentioned by J. Iturbe (Gaceta Medica de Caracas. Venezuela. August 31, 1918, 25, No. 16, page 173).

Spirochetosis, filariasis, bilharziasis, pellagra, lepra occur in Porto Rico. See page 247, 14, No. 120 Boletin de la Assoc. Medica de Puerto Rico, S. Juan, September 1918.

Preparing and Mounting Slides of Crystals. Maurice E. Parker (English Mechanic, Jan. 10, 1919) discusses methods for making permanent mounts of crystals. These mounts are suitable for low powers. Take a test tube $\frac{1}{4}$ in. bore and 2 in. long, pour in a teaspoonful of distilled water, then add enough of the chemical to be used to make a saturated solution, i.e. so that just a small amount remains undissolved. Now take a thoroughly clean slide, place a drop of the solution on the center of it, spread the drop so it covers about $\frac{3}{8}$ in. diameter, allow to dry, *taking great care* no dust settles on it, as dust shines up brilliantly in polarized light. To obtain large and well formed crystals *dry slowly*; to obtain very fine, feathery crystals dry by *gentle* heat. When thoroughly dry mount in Canada balsam, being very careful not to displace the delicate crystals when pressing down the cover. (This usually draws down to place if only just enough medium is used.) Remember Canada balsam dissolves some chemicals; therefore mount the same object in castor oil and label the mountant on *all* slides. This should always be done, otherwise in remounting valuable slides in Colleges and Societies, no one can tell what treatment they should receive. Excess of the mounting material must be well cleaned off in order to ring the slides, if castor oil is used. Shellac is preferred for this by the technician, but Canada balsam is a useful one as it mixes in well.

Some workers advise the use of alcoholic or ethereal solutions, instead of aqueous, so that smaller crystals are formed, thus allowing higher powers to be used. Some of the best chemicals to try out are found among the materials used in the photographic room—hydroquinone, potassium bichromate, pyrogallol, sodium carbonate, sulphite of sodium, sodium borate, salol, picric acid, potassium cyanide (the last needs care as it is dangerous under certain conditions). Others are menthol, potassium chlorate from the head

of a safety match, kinnate quinia, salicin, sugar, etc. Try sodium benzene sulphonate, hippuric acid, and anthracene with polarized light. Hippuric acid can be made to vary its crystal forms. If dissolved in alcohol and warmed, on drying they resemble the leaves of flowers. If breathed on during cooling they take the form of rosettes. Ortho-nitro-phenol is a complex compound of the "Ring" series and if very thin on a slide its color effects are very beautiful. Coumarin shows another type.

V. A. L.

MEASURING CARBON DIOXIDE PRODUCED BY PROTOZOA

Lund (Baltimore Meeting Am. Soc. Zool. 1918) has devised a simple procedure to determine the production of CO_2 by small organisms. A wide mouth glass-stoppered bottle is used, from the stopper of which is suspended a small flat stender dish containing the organisms. A small quantity of weak $\text{Ba}(\text{OH})_2$ is placed on the bottom of the bottle. This absorbs the CO_2 which gets into the bottle. By proper controls the amount due to the animals can be determined.

It was found by using small quantities of some substance, as Na_2CO_3 , which would set free known quantities of CO_2 , that only about 5% of error existed in measuring the quantity of CO_2 set free by an acid from even one milligram of Na_2CO_3 . Similar accuracy is insured for the production of the organisms.

METHOD FOR DEMONSTRATING GLYCOGEN IN TISSUES

Gage (J. Comp. Neur. June, 1917) summarizes the methods used by him in his studies of the distribution of glycogen in Vertebrates.

1. Fix in alcohol (67-100%). A medium is necessary which does not dissolve the very changeable glycogen. While other agents may be used, none is so generally satisfactory.

2. Imbed either in paraffin or collodion, or the combined method may be used.

3. For staining iodine is the only reliable and satisfactory agent. An alcoholic iodine stain was found most satisfactory (95% alcohol, 150 cc.; water, 150 cc.; 10% alcoholic solution of iodine, 15 cc.; iodid

of potassium, 3 grams; sodium chloride, 1.5 grams). Spread the sections with the iodine stain instead of water. The glycogen stains a mahogany red, which is permanent in the paraffin.

4. If permanent mounts for high power work are needed, the sections may be immersed in the iodine stain for a few minutes, dried thoroughly and then deparaffined by xylene. They may be mounted in melted yellow vaseline.

SPENCER-TOLLES MEMORIAL NUMBER

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TRANSACTIONS
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CAMALLANUS AMERICANUS, nov. spec.,*

A MONOGRAPH ON A NEMATODE SPECIES.

By Thomas Byrd Magath

I. INTRODUCTION

It is as lamentable as interesting that a group of animals presenting so many unusual and important objects of investigation as do the nematodes, should have received so little attention from a general morphological point of view, despite the fact that they have been studied from so many other angles. It is for this reason that systematic studies upon the Nematoda have been so unsatisfactory in the past. With the hope that a very detailed study of the morphology of this group will throw some light on the present chaotic situation, the author has undertaken the study of a single species to find out its precise structure. This work has been done with the hope that it will not only contribute definite information on certain features of anatomy heretofore altogether unknown or at best little described, but will furnish a stimulus to others for investigation along morphological lines in the Nematoda. If as many forms are accurately described among this group as have been in other parasitic classes, there can be no doubt that this difficult branch will be as easily handled systematically as any other.

This work has been done under the direction and with the criticism of Professor Henry Baldwin Ward. To him I express my earnest

* Contributions from the Zoological Laboratory of the University of Illinois under the direction of Henry B. Ward, No. 129.

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thanks and appreciation. I further wish to thank Dr. Howard B. Lewis and Dr. D. Wright Wilson for suggestions concerning the work on the chemistry of the cuticula, but they assume no responsibility for the results. For material the author is indebted to Dr. Morris M. Wells, the United States Bureau of Fisheries and certain members of the Laboratory of Parasitology at the University of Illinois.

The material used for this study was a parasitic nematode, found usually in the upper two inches of the intestine of certain turtles. This species cannot be identified from any description previously given and is named *Camallanus americanus*, n. sp.

In the table given below it will be seen that this parasite is found in five species belonging to three genera of turtles from different localities as indicated. In addition to these, the same species has been identified from the following hosts in the collection of Professor Ward: *Chrysemys trossti*, *Chrysemys elegans* and *Aromochelys odoratus* from unknown American localities. Seven individuals of *Trionyx spinifer* and two of *Trionyx muticus* examined at Fairport failed to reveal these forms. From these and a few other records it is evident that this parasite occurs from Texas to South Carolina, northward to the great lakes and along the Mississippi river. It seems likely that the parasite is found fairly generally east of the Mississippi river. No information is at hand as regards its distribution west of that stream.

I. TABLE SHOWING EXTENT OF INFECTION

Host species	No. examined	No. infected	No. un-infected	Per Cent infected	Locality
<i>Chelydra serpentina</i> ..	4	3	1	75	Fairport, Ia. Urbana, Ill. Raleigh, N. C.
<i>Chrysemys marginata</i>	17	11	6	64	Fairport, Ia. Chicago, Ill.
<i>Chrysemys picta</i>	6	5	1	83	Raleigh, N. C.
<i>Chrysemys scripta</i>	8	7	1	87	Raleigh, N. C.
<i>Malacoclemmys lesueuri</i>	12	11	1	91	Fairport, Ia.
Total.....	47	37	10	

Thus, in the forty-seven turtles examined the percentage of infection averages seventy-eight. In Table I, among the five host species examined, the percentage of infection is lowest in the western painted terrapin. It is interesting to note that in the soft-shelled turtles caught in the same net with the hard-shelled species which were infected, none of these worms were found.

The number of parasites of this species per host ranges from one to several hundred, altho most infected turtles yield about fifteen to twenty. These are found in the intestine just below the stomach with occasionally one or two in the pyloric end of the stomach, or more rarely in the lower region of the gut.

The methods of technique used have been thoroughly discussed in an earlier paper (Magath 1916) and little has been added.

II. GENERAL DESCRIPTION OF PARASITE

These worms are of medium size, rather slender and, when first collected, slightly reddish-brown in color. Perhaps the most noticeable feature is the presence of the golden-brown mouth apparatus, readily seen with the naked eye, and characteristic of the genus. The worms are as a rule moderately active, coiling themselves up into loose knots, but never rolling themselves up spirally, as is the custom of some nematodes, in particular those which are characterized by possessing soft lips.

If a bit of the intestine is placed in a dish with the worms, those coming in contact with it sometimes seize it, and indeed they may be seen to grasp each other in various parts of the body. There is in the anterior region a short clear space, which is occupied by the esophagus, and back of this region is seen the gut, usually dark in outline or even black in some cases, as it appears thru the transparent cuticula. The latter shows fine striations under magnification, and the two sets of three prongs of the oral apparatus can be made out, embedded in the cuticula at the anterior end. Two minute papillae are at the level of the thickest portion of the anterior region of the esophagus; the excretory canal opens about 20 μ anterior of these papillae.

The posterior region of the males (Fig. 6) is inturned so as to look like the letter "J," the bottom of which presents two medium sized, narrow alae, which are lateral in position; sometimes the longer spiculum is protruded thru the ano-genital aperture.

Nearly all authors have noted the presence of caudal alae on the males within this genus, but the description of the structure is very incomplete. At the anterior margin of the alae, just behind a slight indentation in the body wall on the ventral side, one sees in sections, that the cuticula is split, so that the alae are covered with the outer layer, the body with the inner and a space intervenes. The alae at first arise as a single ventral swelling from the cuticula, but on passing posteriorly they divide and separate more and more from each other, until they come to lie one on either side of the mid-ventral line, and with their ventral margins removed quite a distance from each other. These right and left ala extend to the tip of the tail, remaining about the same size until within a short distance from their terminations, they taper sharply to the tip.

The alae are not very high nor broad (Fig. 87), projecting along their course about $20\ \mu$ ventrally and $30\ \mu$ laterally beyond the body wall. The outer contour is fairly regular, but here and there is indented or swollen. If the body of the worm be divided in frontal section along the mid-lateral line, it will cut at the level of the dorsal margin of the alae, their ventral margins run parallel to each other, but some distance apart.

Between the two layers of split cuticula there is sometimes present a deeply staining fibrous or granular substance, varying in amount in different individuals. I am unable to state what this substance is, but two possibilities present themselves. The first being that as the cuticula is laid down by the subcuticula underneath this region, it is sloughed off of the lower layer into the alar cavity; the fact that the mass increases with the age of the worms somewhat bears this out. A second theory, suggested to me by Professor Ward, is that this material represents precipitated fluid expressed from the body cavity.

The alae are not without support. Anterior to the anus there are seven pairs of ribs, which are slender and extend at definite intervals between the two split layers of cuticula. Since these supports are discussed elsewhere in the paper, only a few points need be mentioned here. The number and arrangement is like that in *C. seurati*. A rib is like a little slender rod, and anterior to the anus the seven pairs are about equally spaced from each other. In addition to these there are two pairs which are para-anals, very near the anus and on either side; finally there are five pairs situated posteriorly, the anterior

three of either side being grouped together and the last two (Fig. 77). Superficially these last five pairs seem to be pedunculated, with a slight distal enlargement. All of these ribs, unless the postanals are exceptions, are capable of being bent and returning to their original position. They are covered with an exceedingly thin layer of cuticula which is continuous with that of the body wall on the one hand and of the alar wall on the other.

It is interesting to note in passing that Leidy (1851) refers to these structures as "respiratory canals"; he found only six pairs anterior to the anus and did not mention the presence of others posteriorly; whether they were present in *C. trispinosus* is a question. At present one is unable to say whether the number of papillae, ribs or rays is constant for a genus or not; the reports of the number in the different species of the genus *Camallanus* cannot be looked upon as being reliable.

The females are usually larger than the males, but of about the same ratio so far as the length and thickness of the body are concerned. They are easily recognized from the males by the presence of the large projecting vulva, situated about the middle of the body. The tail is drawn out into a long conical point, without alae, and under a medium magnification shows the presence of three minute spines (Figs. 8,113). Under the same magnification the uterus can be

II. TABLE OF MEASUREMENTS OF ADULT *C. AMERICANUS*

Structure measured	Male	Female
Length.....	4.9-11.3	7.4-19.4
Greatest width.....	0.15-0.27	0.16-0.46
Ratio width to length.....	1:26-1:49	1:33-1:64
Length of postvulva.....		3.2-10.4
Length prevulva.....		4.0-9.2
Ratio postvulva to prevulva.....		1:0.8-1:1.3
Length of tail.....		0.14-0.31
Length of anterior portion of esophagus.....	0.36-0.44	0.39-0.49
Length of posterior portion of esophagus.....	0.41-0.70	0.45-0.70
Thickest diameter of esophagus (anterior).....	0.086-0.120	0.095-0.131
Length of right spiculum.....	0.840-0.920	
Length of left spiculum.....	0.310	
Length of caudal alae.....	0.439-0.793	

seen to contain motile embryos and some of the coils of the ovary and oviduct can be made out in the anterior end of the body.

Worms kept in water for twenty-four hours or more, are almost transparent as the red color of the body fluid disappears, whereupon the intestine stands out even more clearly.

III. SPECIAL ANATOMY

CUTICULA

The cuticular covering of these worms is not unlike that of other nematodes in general appearance. Altho the worms are slightly colored in life, this fact is not due to the presence of coloring matter in the cuticula itself, but rather to a color present in the body fluid, for if worms are cut in two this fluid runs out, leaving the worms without the red color; if kept in water for several days, they get almost colorless, if kept in blood they do not lose their color. The material of which the cuticula is composed is, physically, a highly refractive substance, which may be slightly straw colored. It is elastic and and when stripped off and moist, reminds one of softened gelatin. Reports of pigment in the cuticula of nematodes, especially the parasitic ones, are rare and probably due to misinterpreted facts.

The thickness of the cuticula in these forms varies with the age of the worms to some extent and is thinner in males than females, tho perhaps no thinner in proportion to the length of the worms. The table below gives the average of several measurements made from average adult males and females.

Region measured	Male	Female
Region of jaws	1 μ	3 μ
Anterior third of esophagus	3 μ	4 μ
Middle of body	4 μ	10 μ
Posterior region of body	4 μ	9 μ
Vulva swelling		16 μ
Region of alae	4 μ	
Postanal region	1 μ	1 μ

From this it will be seen that the thickest part of the cuticula is found in the middle of the body and that at either end the cuticula is thinned out. There are slight thickenings of the cuticula extending for a short distance in the anterior region of the body underneath the lateral cephalic ganglia and just outside of the lateral bands.

As in the case of most other nematodes the cuticula of this form is striated (Fig. 29), but the striations are not very pronounced. Striations are seen in embryos which are only 0.542 mm. long. These markings seem to be more conspicuous in the males than in the females and in the latter they are deepest in the posterior region. In the largest individuals these striations are 6μ apart and half so far apart in females that are no longer than 4.6 mm. It seems probable that as the worms grow the distance of the striations from each other grows so that a worm about 9.0 mm. long will have the same number of striations as the female of half the length; the growth of the cuticula pulls them apart. So far as I could determine these striations do not appear below the outer layer, altho Looss (1905) states that they make an impression upon the subcuticula in *Ancylostoma duodenale*.

In *C. americanus* the cuticula is not made up of a number of complex layers as has been described in the case of the larger *Ascaris* species. Four layers are found in the largest specimens and each one of these is homogenous. The layers have been named as indicated by the reaction to stains. The thickness of each layer is given as observed in an adult female.

1. Outer dark layer.....	0.3 μ
2. Outer light layer.....	4.0 μ
3. Inner dark layer.....	3.0 μ
4. Inner light layer.....	2.0 μ

No fibrous layer (Fibrillenschicht) nor supporting fibers could be detected within the cuticula, neither could hyaline bodies as described in *Ancylostoma* be seen. Each layer is homogeneous within itself and the only difference that could be detected between the several layers is their difference in taking stains. The third layer is divided in the middle by a dark line, indicating that it was laid down at two different times, or in two layers, this being also true of the fourth layer (Figs. 26, 27).

In the embryos within the uterus the cuticula is a single homogenous layer and is a little over one micron thick. In a very young female about 5 mm. long the cuticula is only 4μ thick and shows in its structure only the outer two and a half layers. In a young female, after fecundation but with the embryos mostly in cleavage stages, the cuticula is 6μ thick in the mid-region of the body,

the outer light layer is formed and is of the same thickness as in the most mature females, the inner dark layer is in the process of being formed and the subcuticula is rather thicker than in the older forms (Fig. 32).

The cuticula is easily stained with any one of the following stains among others: all hematoxylins, orcein, eosin, acid fuschin, gold chloride, thionin (weakly) and orange G. It does not stain with methylene blue or polychromatic methylene blue.

Most authors have used the word "chitin" for this cuticula and the name is also used for the covering and other hard parts of animals in widely separated groups; hence the question has arisen as to the justification of its usage. There seem to be two distinct meanings of the term, one of which is morphological and the other chemical. In morphology the word has been used to cover a great variety of structures for it is applied to coverings of animals, mouth parts, spicula, linings of organs, setae, etc. In chemistry the word has been used to designate a definite chemical compound. Following is given a brief survey of the usage of the word, especially as regards the group of worms, and from this I am inclined to reserve the name for those parts or organs which are chemically chitin, and to use other words for substances which are not of this chemical constitution.

Odier (1832) first used and applied the word chitin to the material composing the covering of certain insects upon which he was working. This early worker not only appreciated its physical but many of its chemical properties, and regarded it as being closely related to the cell coverings found in some plants. He sums up his results in the statement that chitin is a substance which is not dissolved in potash; is soluble in cold sulfuric acid; does not become yellow in nitric acid (negative xanthroproteic test); does not melt on heating and does not contain nitrogen.

More recent investigation has supported this first summary of the properties of the substance, with the exception of the very last statement, there being in the neighborhood of 6% nitrogen in chitin. It is evident that if the term be considered as a morphological one, then only the structures which envelop an organism can be called chitin, while if it is taken in a chemical sense, only those structures which agree with this substance in the coverings of the insects can be called chitin, in short, they must be composed of glucosamine and

acetic acid plus an as yet unidentified nitrogen fraction (Morgulis 1916).

Grube (1850:253) who worked on the cuticular coverings in various forms, stated that among others, *Ascaris* was covered with a chitinous cuticula; just what he considered to be his evidence is unknown.

It is perhaps due to the authority of Leuckart (1852) that the misunderstanding concerning the name chitin has arisen. This eminent parasitologist maintained that the word "chitin" was a "Collectivbegriff" and stated that the cuticula of *Ascaris* (Nematoda) and the annelids was composed of chitin. He further included in this list many other forms which do not concern this discussion. Altho he called the substance chitin he knew that it was soluble in alkalis and that chitin of Odier was not, because he gives the properties of the two substances in his paper. Other authors have followed him in his usage of the terms.

Goodrich (1897) recognized a difference in the substance which composed the covering of certain worms and that of the Arthropoda. He states that "so far as the solubilities show, the cuticula appears to be formed of a substance closely allied neither to chitin nor mucin." In addition to this he stated that he obtained a positive xanthroproteic and a modified Millon's test with the cuticula and certain cuticula appendages of these worms.

Sukatschoff (1899) worked on the cuticula of *Lumbricus* and *Ascaris*. In the former, in which he was particularly interested he corrected the erroneous statement of Grube, and said that it was not chitin, but conjectured that it belonged within a group of proteins known as albuminoids.

Finally Reichart (1902) proposed the name *cornein*, a name first used by Valenciennes (1855), for the substance which covers the bodies of annelids, or most of them and *Ascaris*, basing his claim on the quantitative chemical analysis in the case of annelids and corresponding qualitative tests of both forms. With this last investigator I can agree and present here the results of my investigations. The form used was *Ascaris suum* from the hog, since the same qualitative tests hold good for *C. americanus* and other nematodes, the chemical composition of the cuticula is essentially the same in the entire group of nematodes.

The material was obtained fresh, and prepared by scraping the cuticula free from the underlying tissue and then washing it thoroly in distilled water, after which it was dried to a constant weight in an oven at 70° C. Cuticula prepared in this way gives the following results in chemical analysis:

It is insoluble in cold water, but goes partly into solution in boiling water, swelling to some extent in either. It is insoluble in alcohol, ether, chloroform, or acetic acid, but swells in the last reagent. It is further insoluble in dilute mineral acids, but will dissolve upon standing in either concentrated sulfuric or nitric acid. It is soluble in hot concentrated acids and in cold caustic alkalis, even when only 1% concentrated upon standing and readily when heated to 70° C. It is soluble in ammonium hydroxid.

According to Burge and Burge (1915) and Reichard (1902) the cuticula of *Ascaris* is digested by the action of enzymes; I have not repeated these experiments but can see no reason for doubting them.

Tests for the presence of uric acid, creatin and urea have been negative, as have also been the repeated attempts to obtain a reduction with Fehling's solution, either before or after hydrolysis.

With Millon's reagent the test does not result in a very strong red color and sometimes seen to be totally negative. Xanthroproteic and Hopkin-Cole tests are positive. With the biuret test a deep purple color develops like that resulting in the presence of peptones and gelatin. The test for unoxidized sulfur was positive. On hydrolysis no tyrosin could be detected.

The total amount of sulfur was determined in two samples with the following results:

I. 0.2318 gm.....	1.25% sulfur
II. 0.2384 gm.....	1.16% sulfur
Average.....	1.20% sulfur

Total nitrogen was determined by the Kjeldahl method and the two samples yielded:

I. 0.2020 gm.....	16.90% nitrogen
II. 0.2113 gm.....	17.04% nitrogen
Average.....	16.97% nitrogen

A small amount of cuticula was boiled in water for several hours and the solution filtered. After the filtrate was precipitated with

alcohol and filtered, the dried precipitate was tested for free and combined tryptophane. No free acid was found but the Hopkins-Cole test was still positive. A test for cystine was also positive in the filtrate.

From the foregoing observations it is at once evident that the substance of which the cuticula is composed is not chitin but an albuminoid. On closer observation it becomes obvious that chemically it more nearly resembles the group of albuminoids represented by collagen, elastin and gelatin than any others in the group. In all these the total nitrogen is high, ranging around 17% and the sulfur is usually above 0.5% (of course is pure gelatin there is no cystine and hence no sulfur) altho in my particular samples the sulfur is a little high. If the total nitrogen analysis is compared with those offered for cornein in other animals, it will be seen that they all are very near alike, thus the list below demonstrates.

16.8 %.....	Fremy
17.06%.....	Krukenberg
16.76%.....	Krukenberg
16.60%.....	Krukenberg
16.97%.....	Magath

In addition to this the qualitative properties are the same in all these cases. If one looks a little more closely into the relation of cornein with gelatin, collagen, etc., he will at once be struck by the fact that with formol all are hardened and rendered insoluble. This is the basis for the statement (Magath 1916) that formol is useless as a killing and fixing agent for nematodes, except in one special technique. The result with the biuret reaction is again significant, the absence of tyrosin, the swelling phenomenon with acetic acid and water, and the general physical appearance relate it very definitely to this series of proteins.

The fact that the cuticula is digested by the action of enzymes, is relatively low in sulfur and high in nitrogen excludes it from the keratin series, which is characterized by the opposite properties; the fact that it has no sugar in combination with it excludes a close relationship with the mucoids. To compare this substance with chitin one should recall, the low percentage of nitrogen present in chitin, its insolubility in caustic alkalis, its resistance to the action of

enzymes and its glucose molecule; these facts make it impossible to call the cuticula of nematodes chitin.

Attention should be called to a paper by Flury (1912) who presents some work on the chemistry of the cuticula of *Ascaris*. The results agree very closely with these presented here with the exception of the determination of sulfur. Flury found 4.3% in his samples which is much higher than I have found. He concludes that the substance is keratin, but objections to this conclusion have already been pointed out.

In conclusion then, the cuticula of nematodes, and as previous authors have pointed out the cuticula of most of the worms, is composed of cornein, an albuminoid closely related to the albuminoids of connective and supportive tissue and is a differentiation product and not a solidified secretion (Leydig, 1888, and Rauther, 1905).

SUBCUTICULA AND LONGITUDINAL LINES

A. Female

In general, the subcuticula of this species is not unlike in arrangement that of *Ancylostoma duodenale* as described by Looss.

No nuclei can be found in the very thin layer between the muscle cells and the cuticula. In the oldest worms no subcuticula layer can be demonstrated at all except in the thickened areas known as the longitudinal lines. In some cases the muscles seem to be applied directly to the inner margin of the cuticula. In the younger individuals there can be occasionally seen a few strands of tissue, continuous with the longitudinal lines, and because it lies underneath the cuticula it has been interpreted as being the subcuticula, but at best this layer is very poorly developed in *C. americanus*. Because no nuclei appear in this tissue it should be considered as being a syncytium which embraces the longitudinal lines and certain other parts to be mentioned in other sections of the paper.

The anterior origin of the four longitudinal lines, there are no subdorsals or subventrals in this species, is very interesting and at the same time extremely difficult to work out. Unexplained structures have been noted by previous authors in the "head" region of nematodes and even in *Mermis*, Rauther (1906) has described them briefly, but as will be shown, correctly attributes them to special modifications on the longitudinal lines.

Looss (1901, 1905) referred to structures, which from his text and figures, I consider to be homologous structures in the Sclerostomidae and *A. duodenale*. He named these structures the "ligamentum cephalo-oesophageale" stating that this was a structure "sui generis" and while he admits (1905:53) that the lateral lines rise slightly in height in this region and offer support for the cells of the "ligament," he mentions no dorsal and ventral connections with the subcuticula, nor does he make it clear that he considers this structure of subcuticula origin. His idea of the function of the structure is set forth in the following statement: (1905:77) "it is intended to attach and to secure the chitinous mouth capsule to the muscular oesophagus." Looss (1901) suggested for this structure a function in the motion of these parts, thus indicating a muscular connection.

The description which Looss gives of these structures in *A. duodenale* is not very complete, since he himself admits the difficulty of working out the region on account of the sections breaking out, due to the hard parts of the mouth capsule, but I am inclined to believe from the description given, that in the following parts of *C. americanus* I am dealing with homologous structures and not greatly unlike those in *A. duodenale*.

Figure 14 shows four pairs of loose granular masses lying in the angles formed by the lateral plates of the jaws and the tridents. Passing posteriorly the two members of each pair unite; each pair is therefore formed by the division of a single structure. This union takes place just below the anterior margin of the oesophagus. The anterior ends of these pairs of protoplasmic masses are seen just posterior to the anterior margins of the jaws. In a series of sections passing anteroposteriorly, the lateral lines appear just behind the anterior insertions of the four giant jaw muscles as narrow bands dividing each right and left pairs. They soon send out tissue which applies itself around the outer margins of the lateral plates and soon joins with the median member of each pair of granular areas. A single large nucleus appears in each lateral line anterior to the beginning of the oesophagus. The lateral lines increase in size as one passes posteriorly until at the level of the beginning of the oesophagus they branch out, tissue from them serving to fill in the space between the esophagus, body walls and the muscle cells. Here three more nuclei

appear in each lateral line. The tissue of the lateral lines is continuous with the end of the esophagus in the young forms, but in older animals this tissue has formed the esophageal cap and still retains its connection with that structure. The cap is not smooth but looks as though it had a scalloped border.

Fifteen micra below this level the dorsal and ventral lines make their appearances and then in the region from here to the nerve ring, the esophagus is surrounded by subcuticula tissue which originates from the longitudinal lines and forms a commissure around the oesophagus. The mass of tissue is syncytial, but presents very definite structures or thickenings around very large spherical nuclei; the rest of the tissue is chiefly fibrous. Of these thickenings there are five dorsal and an equal number in the ventral field, the two most lateral of each represents the united granular masses which extend anteriorly, the middle one of each five being the inner end of the dorsal and ventral lines. Each thickening has two large nuclei, all twenty nuclei being contained within a distance of 50μ . Here and there, scattered thruout this region, are nerve cells which will be considered in another section. In the middle of this region appear two more nuclei in each lateral line, making six in each before they divide into dorsal and ventral halves.

Near the end of this region under discussion appear two ovoid nuclei in the dorsal line, there are two more spherical ones in the ventral but further separated from each other. In the region of the nerve ring the lines present broadened surfaces towards it. In addition to these nuclei in the dorsal and ventral lines are two others in each, small and just at the anterior margins of these lines.

This tissue seems to me to represent nothing more nor less than the anterior origin of the subcuticula. It acts in this region as support, primarily perhaps, for the nervous tissue, but undoubtedly forms in some manner the esophageal cap and in all probability it contributes to the mouth parts, which are cuticula products and should be formed from the same type of tissue as the body covering; here is where the two structures make intimate contact with each other. It is impossible to consider this as a ligament for mouth apparatus and esophageal connection in *C. americanus* for here the chief parts of the structure lie too far posteriorly and from Looss' description this must be true in *Ancylostoma* as well. Furthermore

there are similar structures in species without hard oral parts and these interesting thickenings of subcuticula certainly occur in *Mermis*.

Roughly speaking the subcuticula fills up the region between the esophagus and the body wall, the nerve ring and base of the oral apparatus; this is the tissue seen within this region as so complicated a structure.

Interesting in this connection is the fact that the position of the nuclei and number in the various parts of this region have been found to be constant in all the specimens examined, perhaps as many as ten in all, but the fewness and apparent individuality of these nuclei offer no surprising revelations in the field of "cell constancy" (Figs. 34, 35, 46-49).

The thickening of the subcuticula forming the dorsal line is by far the smallest and most inconspicuous of all the longitudinal lines, but extends nearly the entire length of the body. It begins anterior to the nerve ring and extends to the posterior tip of the tail. Thruout the middle region of the body it becomes so insignificant as to be entirely overlooked, but posteriorly it increases rather suddenly and remains rather thick until within a few micra of the posterior tip. There are in this structure a few nuclei, and below the anus there are three, equally spaced and rather large. The muscles in the dorsal half of the body send more projections to this band than those in the anterior half.

The ventral line is much more conspicuous than the dorsal one, but like it diminishes greatly in size thruout the mid region of the body. Anteriorly it begins about at the level of the beginning of the dorsal line and has quite the same fate in the posterior part, with the exception of becoming involved with other structures which will be taken up separately. About the level of the posterior fifth of the body the ventral line enlarges greatly and is like a flat cushion extending along the mid-ventral line. In the region of the anus it becomes very wide and is even more conspicuous post-anally. There are three nuclei in this region, equally spaced to correspond to the three in the dorsal line (Fig. 116). Preanally there are nuclei in the ventral line, but they are very small and far apart. The regions of the lines posterior to the anus have been called the "pulvillus postanalís" in *A. duodenale* by Looss, but little justification can be found for the

continued use of the term, for this region is merely the posterior part of the dorsal line and deserves no particular name. Special modifications of this structure will be discussed elsewhere as for example in the sections on the vulva, rectum, etc.

As in *A. duodenale*, the lateral lines (Figs. 46, 50, 57, 117) of this species arise in the anterior region of the body as a thickened region of the subcuticula which is undivided at first. Looss says that these lines arise shortly behind the anterior margin of the oral apparatus in *A. duodenale* which is also true of *C. americanus*. As a matter of fact they seem to begin just posterior to the region of the anterior insertion of the four giant muscle cells of the jaws; below their posterior insertions they are narrow but project well out into the body cavity. Shortly behind their origin the divisional septum is seen and from thence posteriorly they are divided into dorsal and ventral halves. Two regions are fairly well marked out in the lateral lines. Around the outer membrane which covers them on the interior side, the protoplasm is very dense and granular, in this region and lying towards the mesiad, there appears as Looss suggested, a tissue of "softer" material and "watery." Often in older specimens this area is totally devoid of stainable material and when there is material present it is not unlike that which precipitates in the body cavity. Around the "partition wall" the protoplasm is thickened; no nuclei appear either in this region or in the inner area. In the part of the lateral lines applied to the inner margins of the cuticula one recognizes the second region. In here are found nuclei, rather large and frequent in distribution, especially in the posterior region of the body. The tissue is decidedly of the nature of a syncytium and very fibrous in character, the Stütz fibrillen originate here, and in larger species have been traced out into the subcuticula and into the muscle cells; in this form the structures are too minute to be demonstrated if they exist. Occasionally a nucleus is seen at the outer base of the "partition wall" and these nuclei are believed to belong to the structure K. C. Schneider (1902) speaks of as a row of cells, "mediale Zellreihe," in *Ascaris*, being homologous in these species.

During the course thruout the length of the body, no special details of the lateral lines need mention; in older females they become compressed as the uterus fills with embryos, but here and there extend out into the body cavity when not interferred with by other organs.

Near the posterior tip of the tail the lines occupy a large percentage of the entire circumference of the body and have less "watery" material within them. They disappear in the subcuticula of the tip of the tail. In young forms the lateral lines occupy a large percentage of the body cavity (Fig. 124).

B. Males

The conditions described for the females as regards the subcuticula and longitudinal lines are almost duplicated in the males, but with some slight modifications, chief of which are due to the apparent displacement of the lateral lines in the posterior region of the body. These are located so that they appear to lie on the dorsal wall, but in reality this is not the case. The enormous development of the muscles of the male tail takes place entirely below the lateral lines, i.e., in the ventral half of the body, which causes them to appear far dorsal, so that they no longer divide the body into approximately equal halves. The arrangement of the parts of the subcuticula in the posterior region of the body below the anus is like that in the females.

THE EXCRETORY SYSTEM

Altho Bojanus and Cloquet (1824) noted the presence of canals in the lateral lines of nematodes, it remained for von Siebold (1838) to attach a significance to these organs, and it was he who first noted that they were connected with a duct which opened on the surface of the body. He however admitted the puzzling nature of their function: "Zu welchen Zwecke das in diesen Organen abgesonderte homogene und farblose Sekret dienen soll, wurde noch nicht ermittelt." Subsequent authors up to the time of Schneider (1866) added nothing of value to the observations of von Siebold and even Schneider was able to make only a few guesses as to the true nature of these organs. About this time the work of Bastian (1866) appeared in which he stated that the whole structure came from a single cell and compared the organs to the so-called "water-vascular system" in trematodes. Leuckart added nothing of value to the statements of Schneider, but upon his authority nearly all workers since have looked for the excretory function of the nematodes in the lateral lines and the canals anatomically connected with them.

Stimulated by the observations of Kovalesky followed by Metalnikow (1897), Nassanow (1897 to 1900a) investigated this interesting system by means of injecting certain substances into the body cavity and observing the results. The second of these authors mentioned obtained chiefly negative results; in only two instances did he observe the massing of the stains; he used suspensions of carmin, etc., within the lateral lines. However, he noted the appearance of certain stains in the cells of the middle intestine and was forced to the conclusion that while the lateral lines may play some part in the excretory function, the gut itself must be quite a factor in the elimination of certain materials from the body. Nassanow repeated the experiments and also noted the action of the midgut, but was able to detect the presence of frog's blood, when injected into the body cavity, within the canals themselves, and so attached some importance to these structures. He, it was, who investigated the phagocytic organs, and came to the conclusion that they are like lymph glands, giving rise to ameboid cells which pass through the body cavity, collecting foreign materials and destroying it. He is not very clear as to the final elimination of the destroyed materials, but one may surmise that this also passes through the lateral canals or gut wall.

Golwin (1902) carried on a very extensive investigation of the problem and his results, so far as the excretory system proper is concerned, may be summarized as follows:

1. Most of the stains used by the former investigators are precipitated in the body cavity, and hence their negative results are explained. They must be in solution before they can get into the lateral canals.

2. When colored solutions are injected they may be watched as they pass into the lateral lines, canals, and finally out thru the excretory pore, and the amount of excretion can be determined quantitatively by means of the colorimeter.

3. Staining of the lateral lines in the few cases in which it was noted in the use of suspensions, is explained by the fact that the animals died first, this is true as well in the case of the staining of the phagocytic organs, midgut, etc.

4. The lateral lines are engaged in the excretory process as well as the canals.

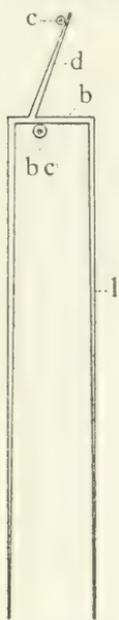
Looss (1905) after investigating the cervical glands in *Ancylostoma* came to the conclusion that they represent "integral component parts of the excretory apparatus," and not glands, but he wisely reserved his final decision of the whole matter until further investigation of the system has been made.

One year later Goldschmidt, (1906) in his characteristic dogmatic way, gave his conception of the system. It arises from a single cell, a radiating nucleus lies in the left leg posterior to the arcadelike portion. The canal is lost in a circle of nuclei in a smaller canal, which itself then narrows before a nucleus. The two lateral canals, lying in the lateral lines, connect in the anterior part of the body thru an arcade, called a "bridge." There is in *Ascaris* some complications of the canals in the anterior region, of no importance here, but finally an exit is made by means of a single ventral pore. Posteriorly the lateral canals end blindly. The lining of the canals is difficult to describe, but looks something like glass. There is around this inner lining a plasmatic substance, but neither of these structures suggests in the least the excreting cells of other forms. The tissue on either side of the canals in each line is glandular in nature, and Goldschmidt thought that he had recognized an excretion from them into the canals themselves, thru small pores. The lateral lines in part then, are excretory in function and the syncytium around them is a passage way for materials going into the canals.

The research of Rauther (1907) is very interesting in a comparative way. He concerned himself with the free-living nematodes and after working with indigo carmin, found that whether it was taken in by the mouth, e.g., with food, or thru the skin, that the excretion was indirect. It was finally excreted thru the gut, which he compared to a urinary duct, and the esophagus, which he compared to the Malpighian capsules. He eliminated the glands of the esophagus as functioning in the process and found that the process was carried on by the muscles of the organ. The chemicals were absorbed and eliminated by the gut. He suggested that the pigment masses in the intestines of certain parasitic forms were stages in the excretion process.

If Goldschmidt and Rauther are both correct it is interesting to note that there is a marked difference in the method of elimination of excretory products between the free-living and parasitic nematodes

and such forms in the parasitic groups as do have no lateral lines and canals, or only poorly developed ones, present interesting cases. It occurred to the author that there must be forms present in which the function is divided between the alimentary canal and the lateral lines, but may function or there may remain some indication of function in either case. The species used in this work at once suggested a possibility, from the anatomical relations of its parts. In the first place there is a divided esophagus, the most posterior portion of which it histologically quite different from the anterior portion.



Textfigure A.
Diagram showing the general arrangement of the excretory system. *b*, excretory bridge; *b. c.*, excretory bridge cell; *c.*, carrying cell of excretory duct; *d.*, excretory duct; *l.*, lateral excretory canal.

In the next place the bridge is in the region of the lower part. Finally this portion is covered with an out-growth from the tissue of the lateral lines.

In *C. americanus* the canals themselves lie in the lateral lines, in a V-shaped area formed by the union of the dorsal and ventral halves, which is turned towards the body cavity. Here there is a thickened portion and very granular, but devoid of nuclei (Fig. 57). These canals are in the lateral lines since their inner boundary is always seen between the canals and the body cavity, except where they leave to form the bridge, to be spoken of later. The canals begin in the posterior third of the body as blind tubes and pass forward to about the level of the anterior fifth of the posterior part of the esophagus, where they each bend towards the ventral line, anastomosing with each other; then there passes, anteriorly and slightly to the left of the median line, a small duct, which after making a sharp turn outwards and medially, opens about 0.35 mm. from the anterior tip of the body and between the later cervical papillae and the nerve ring (Fig. 50, also Textfig. A).

The histological details of this system in nematodes have been discussed by the previous workers of whom K. C. Schneider gives a correct account of the condition in *Ascaris*: that in *C. americanus* is not greatly different except in certain features. It seems well to consider the canals being composed of two

layers, the inner of which is highly refractive and of a substance recalling the cuticula from which it is believed by most authors to have been derived; it must be stated that it stains differently and does not seem to be continuous with the cuticula in the adults. To this statement must be added that the mechanics of getting this long bifid tube lined by the invasion of cuticula thru a very minute pore at one end is not at all easy to explain, and the suggestion that it is lined with a transformation product from the outer layer of its own wall is not an unreasonable one; in the absence of absolute embryological evidence for either position the latter seems as plausible as the first suggestion. The outer layer (*sarc*) is almost as monotonous as the inner. This layer is granular in nature, stains with the cytoplasmic stains much more intensely than the lateral lines, and often has in it rather deeply staining granules, which stand out sharply. These are nearer the periphery of the wall than the lumen, which is about one to two micra in diameter.

Perhaps the granules have been mistaken by some authors for nuclei and would account for the statement made by Shipley (1910) that there are nuclei in this layer. One is unable to find the best authors considering this as a nucleated layer and it has been shown that the whole structure proceeds from a single anterior cell.

As the ducts pass anteriorly they enlarge, especially does the wall get thicker, while the lumen enlarges but slightly. At a level with the anterior margin of the intestine the entire duct is, in the females, about 8μ in diameter, while just posterior to the bridge it is 10μ .

At a position which varies within a distance of the level of the anterior fifth of the posterior region of the esophagus, the right and left canals bend towards the mid-ventral line and here lie in a thickened portion of the wall of the ducts, in a substance known as the "bridge" (Figs. 3, 51, 56). Lying to the left of the mid-line is a single large nucleus, oval or nearly round in shape, and 15μ in diameter, containing a nucleolus 5μ in diameter. The tissue of the bridge is continuous with the outer layer of the canals and of the same histological properties, within it can be seen the minute lumen of the duct and the inner layer of refractive material. On the ventral side of the esophagus where the bridge lies, the two are in close contact

with each other. The only wall existing between them is that of the tunica propria of the esophagus, and in some specimens even this cannot be detected. In either event the bridge partially encloses the esophagus on the ventral side. Furthermore, there is enveloping the whole of the posterior region of the esophagus a tissue (Figs. 31, 33) not unlike the outer layer of the canals and which seems to be made of a tissue from them and partly from the inner margins of the lateral lines. A small nucleus can be seen in this tissue between the left side of the esophagus and the inner portion of the lateral lines below the bridge.

Where the duct turns to open to the exterior, there is a nucleus (Fig. 50) which is located just on top of the duct and medial to it. This is considered the nucleus of the carrying cell of the excretory vesicle and this cell envelops the canal in this region. It probably functions as a supporting cell as well, since no other cell is present.

The rather suggestive histology of the posterior portion of the esophagus and the lack of excreting tissue with nuclei in the lateral lines as in the case of *Ascaris*, together with the fact that the bridge and the accessory tissue are so closely associated with the esophagus has led to a conclusion, which if true, has some bearing on conceptions of the excretory function of these forms.

The bridge is so closely associated with the esophagus that the latter stands in the same relation to the lumen of the canals as do the lateral lines, and I believe from the nature of the structure of this portion of the esophagus, that it has to do with the excretory function, that the excretory products instead of passing thru the lumen of the gut as Rauther found in the case of the free-living nematodes, passes thru the tissue of the esophagus and then into the lateral ducts in whatever fashion this could take place in the lateral bands, whether thru minute pores or by absorption, in which event hydrophylic proteins or their derivatives should be looked for as agents. Under the last condition the thin lining will have to be permeable, which would increase the possibility of this system being excretory.

If one allow that the posterior portion of the esophagus can and does act as the excretory apparatus in part or in the whole, these forms will be intermediate between the free-living and the more highly developed parasitic nematodes, so far as this function is concerned.

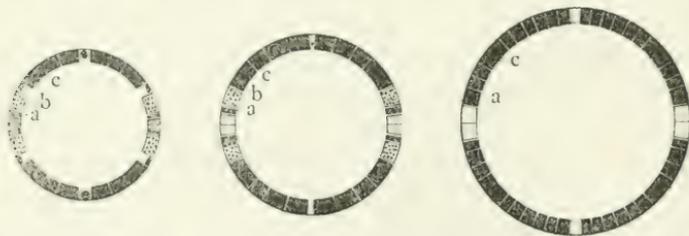
It is rather interesting to note in this connection that most of the nematode parasites so far recorded from water hosts are characterized by the possession of two or more regions of the esophagus, ceca, etc.; forms which are found in both water and land hosts have, to a great extent, an esophageal bulb, with this group are numerous nematode parasites of insects which live in moist decaying material, and some hosts which spend part of their lives in the water; the strictly land parasites have usually a simple esophagus. It is not unreasonable to suppose that the first forms to become parasitized would be the water hosts, since the free living nematodes are mostly found in water or mud. If this be true one would expect the nematodes of these hosts to be closer related to the free-living forms than those of the land hosts. It is an interesting speculation and one with some foundation, to suppose that these forms found in the water hosts represent forms that stand below the bulbed esophageal species, e.g., the Heterakidae, which in turn are below the forms with the type of the esophagus found in *Ascaris*, so far as parasitism is concerned. These more primitive nematodes have retained in part, if not entirely, the function of excretion within the esophagus and are in this respect but slightly more advanced than the free-living species. Interesting are the various members of the Superfamily Spiruroidea, in which one can find varying degrees of esophageal division, some of which have been pointed out by Ward and Magath (1917).

Finally in this connection should be mentioned the rather peculiar group of Trichosyringata, in which the esophagus is not muscular but composed of a capillary tube, passing thru a row of cells. These forms have very small lateral lines and Rauther (1906) has maintained that in *Mermis*, a form he has studied, that the "spindelförmigen Zellen des hintern Oesophagus" are the "Excretionzellen" of this form and are homologous to the ventral bridge cell in *Ascaris*. *Camallanus americanus* then would lie between the two great groups proposed by Ward. Stephens (1916) suggests an excretory function for certain "skin glands" found in some nematodes in the group of Trichosyringata, but Rauther's suggestion seems better founded both on fact and theory.

SOMATIC MUSCULATURE

The somatic muscles begin anteriorly on a level with the ring of the mouth apparatus. Here they first appear on the dorsal and

ventral sides of the body, closely applied to the cuticula and between the fields marked out by the tridents. The lateral fields are still occupied by the four giant muscle cells which open the jaws. Three cells between each median and lateral branch of the tridents come into existence at once, so there are three cells in each quadrant, the one nearest the lateral margin being nearly twice as large as either of the other two. A few micra posteriorly three other muscles enter each field, the large cell in each case being pushed laterally, so that the last cells enter between them and the mid-dorsal or ventral line, as the case may be. Other cells enter shortly and the giant cells in the lateral lines are quickly undermined by the fibrillar portions of the general body muscle cells, which are large cells of the same size as those previously mentioned. At the level of the nerve ring there are, if one quadrant is considered, six cells between the first large cell and the mid-dorsal or ventral line; this cell makes the seventh, and between it and the mid-lateral line there are two more, of which the most lateral is a large one. Finally there enters another small one between the last and the lateral margin, thus making ten cells in each quadrant. This last muscle cell can be traced to the level of the anterior margin of the esophagus, where it appears in each quadrant and as a single fibrillar element. It remains thus until at the level of the nerve ring it increases in size, takes on the characteristic shape of the general body muscle cells, and possesses a sarcoplasmic portion when the similar portion of the giant cells disappears below the level of the nerve ring. The series of diagrams shows how these muscle cells originate (Textfig. B).



Textfigure B. Schematic representation of the anterior origin of the somatic muscle cells *a*, is the same muscle cell in each figure, *b*, is the giant muscle cells of the valves; *c*, is the same cell in each figure. The lateral lines are indicated as are also the dorsal and ventral ones, by fine stipling.

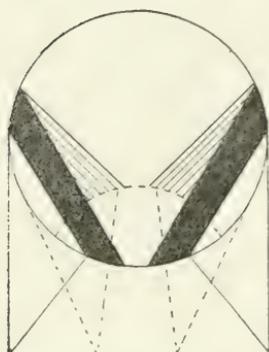
On passing posteriorly the differences in the sizes of the various cells is lost, so that below the level of the posterior margin of the esophagus they seem to be of uniform size. Processes bend over to the dorsal and ventral nerves, none were seen going to the lateral lines, so that a cross section gives the impression that they lean from the sides towards the mid-lines (Figs. 55, 123). In the posterior region this tendency is even more marked, where the processes can be seen very clearly and to come into contact with the nerve, especially the ventral nerve. As the cells are anteriorly of about the same height, the appearance is that of an even circle when seen in cross section, formed by the tops of the cells (Fig. 51).

Posteriorly the number of cells diminishes, first in the ventral fields, so that there are but two in the ventral quadrants and four in the dorsal ones, at the level of the anus. Posterior to the anus they are diminished further, until in the tip there are finally but four muscle cells, one in each quadrant.

In the males the somatic muscles in the caudal end are evidently modified to be useful in the act of copulation (Figs. 87, 92).

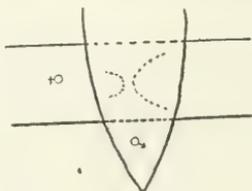
Beginning about the level of the lateral alae the fibrillar portions of two regions of the somatic muscles elongate towards the body cavity. The two regions are found just ventral to the lateral lines on the one hand, and on the other hand just lateral on either side of the ventral line. At first only one or two muscle cells are involved and so a corresponding cell from each region on the same side comes to have its fibrillar portion connected and thus there extends a muscle from a ventral-lateral to a lateral-ventral position, a cross section of the body presenting the appearance of oblique muscles on either side of the ventral field. More cells become involved as one passes posteriorly so that there are about as many bridging the gap as are left between the two places of insertion. This means that there are more than a fourth of all the muscles of the caudal end of the body involved, since the lateral lines have already been pushed far dorsally. The nuclei of these cells are surrounded by a small amount of protoplasm, in the center of the cell. This condition continues even a short distance below the anus, then the regular somatic muscles continue and end in the same manner as in the case of the female.

Action of the muscles in the caudal end of the males. The diagram (Textfig. C) shows the mode of action of these muscles. Their con-



Textfigure C. Diagram illustrating the action of the caudal muscles of the male. The dotted lines indicate the position of the alae when the muscles have contracted pulling up the ventrum of the body. By this method the male grasps the vulva of the female.

traction raises the ventral side of the body. Normally the lateral alae stand out from the body of the male leaving quite a little space between the right and left alae, but when these caudal muscles contract they pull the ventral side of the body up thus serving to bring together the two lateral alae. These muscles are antagonized by the elasticity of the body cuticula, and when they relax the lateral alae are again allowed to swing outward. The usefulness of this arrangement is clearly seen in the act of copulation. Here the male comes to lie at right angles to the female and with the alae straddling the vulva (Textfig. D). Then the caudal muscles contract and this pulls



Textfigure D. Diagram showing the position of the male and female during copulation.

in and down the alae, thus forming a firm hold over the two lips of the vulva, which are suited by their structure to just this sort of action. The connection is made not by the use of suction nor by the

use of cement, but rather by the mere mechanical grip of the two wings. Of course the insertion of the spicula—either one or two, for I cannot say whether the smaller one functions—helps to hold the two worms together.

Here the methods of copulation as known in the Nematoda may be briefly reviewed.

(1) The action of cement and a bursa. An example is found in *Ancylostoma*, where the bursa opens and closes by the action of special muscles and also furnishes a broad surface for the application of cement, which is the chief means of holding the male against the female.

(2) The use of a sucker. An example is *Heterakis* in which a large sucker exists in the male and is used to attach it to the female. The action of this sucker, according to Schneider, is effected by a series of muscles radiating from the bottom of the organ to the edges of the lateral lines. Their contraction creates a small vacuum which is released after copulation by the action of the fluid of the body.

(3) The case given in this paper, where a mechanical grip serves to make the male fast to the female.

There are undoubtedly other means of copulation in the nematodes, but no others are sufficiently well-known to be given here. These three methods are dependent upon certain distinct morphological differences in the anatomy of the forms in question and presents an interesting field for research which may lead to a good means of classification. The vulva of the females will also need to be studied for it may furnish a clue, because it is modified according to the *modus operandi* of the males.

Histology of the somatic muscle cells. These cells are, like the muscle cells of other nematodes, composed of two portions, the fibrillar and sarcoplasmic. In the cells of the anterior region of the body, anterior to the posterior margin of the esophagus, the latter portion is larger than the former, while in the rest of the body they are of about equal size. The fibrilla part is in the shape of a V or U, with the notch varying in depth from a barely perceptible one, until in some cells, it appears to cut in half the depth of the outer layer. In the fibrillar layer the muscle bands (Muskelleisten) are placed very closely together so that it is not possible to count the number accurately, altho they are estimated as being nearly one hundred in each

cell (Figs. 25, 28). Supporting fibers (Stütz fibrille) cannot be seen in this layer. The muscle bands are arranged along three faces of the cell, on the face nearest the cuticula and on the two vertical sides, so that the bands come together in a sharp angle in two diagonal lines. A little thickening of the protoplasm occurs around the margins of the cells. The sarcoplasmic parts of the cells stick out into the general body cavity, somewhat beyond the inner margins of the fibrillar layer. In this portion one can see a very large nucleus, oval in shape and $6\ \mu$ long, containing but one nucleolus, which is rather large and stains deeply with hematoxylin stains. Around the nucleus is a dark staining area, the "Gitterkörbchen" of Bilek (1909), which he has shown to be made up of the supporting fibers of the cells, and from this area, in well preserved specimens can be seen very minute fibrills, which can be traced out thru the sarcoplasmic part of the cell. They probably pass into the contractile layer and thence into the subcuticula as Bilek (1910) has shown in the case of *A. lumbricoides* and *megalocephala*. The sarcoplasm consists of fine granular material, which stains a light red with van Gieson's stain and blue with Mallory's and Delafield's hematoxylin.

The fibrillar portion stains with picric acid and the anilin stains, or with chrom-hematoxylin. The bands stain but slightly darker than the ground substance of the fibrillar layer.

The anterior cells are perhaps no longer than 0.2 mm. while posteriorly the cells are as long as 1 mm. in some cases. A typical anterior cell is compared with a typical posterior one in the following table:

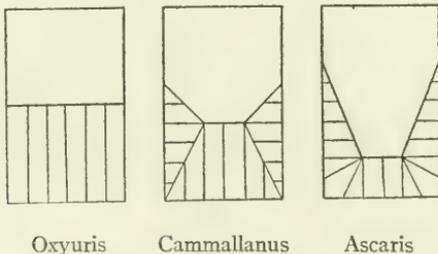
Place of measurement	Anterior cell	Posterior cell
Width	0.013 mm.	0.013 mm.
Thickness	0.020 mm.	0.015 mm.
Thickest part of sarcoplasma	0.016 mm.	0.008 mm.

The muscle cells of this form are very much like those of *Ancylostoma duodenale* as described by Looss (1905) both as regards size and structure. It is interesting to note that while this is true, there are in the hookworm only eight cells around the body as seen in cross section, while there are about forty in the new species. As in the case of *A. duodenale* there may be several sarcoplasmic processes from

each cell, and these also anastomose with each other, and seem in some cases to come into contact with the internal organs.

As compared with the muscle cells of *Oxyuris curvula* (Ehlers 1899) they are quite different. Here the cells are 8.69 mm. long and 0.51 mm. wide. There is next to the subcuticula a flat layer of contractile material, then placed on this is a granular unstainable portion, and finally an intensively staining rind layer. Each muscle cell has a nucleus.

The type of muscle cell in the larger members of the genus *Ascaris* is so well known that it needs little attention here. The contractile portion is in the shape of a very deep U with the sarcoplasmic portion extending out from it and containing a large nucleus with its accumulation of supporting fibers around it. These fibers pass into the ground substance of the contractile part and finally into the subcuticula. The sarcoplasmic layer is in contact with the fibrillar layer nearly all the way down to the bottom of the U, which is very near the subcuticula layer. The diagram illustrates the three conditions mentioned above (Textfig. E).



Textfigure E. Diagram illustrating the relative amount of Sarcoplasmic and Fibrillar portions of muscle cells in different nematode genera.

Schneider (1860) proposed two names to be applied to divisions of the Class Nematoda, based on the structure of the muscle cells. The name "Platymyariet" was applied to all nematodes in which the fibrillar portion of the muscle was flat towards the body cavity, typically in the case of *Oxyuris*. The "Coelomyariet" include the nematodes in which the fibrillar portion was notched so that the sarcoplasmic part dipped down into and between the contractile layer, which was present on either side of the cell and on the outer margin.

Further Schneider recognized a difference in the muscular system of *Gordius* and *Mermis* and he (1886) proposed the name Holo-

myarii to include these forms along with certain other Nematoda. However, Bütschli (1873) showed that this division was unwarranted, and so the term must be rejected.

Cognizant of the fact that there were forms in which the muscle cells because of their great variation could not be accurately placed in either the group of Platymyriarians or Coelomyriarians, and further that some worms were found in which the cells were different in different parts of the body, Schneider (1863) proposed to abandon these original names and use instead Polymyrii and Meromyrii, basing his classification on the number of cells that appear in a cross section. In the former, as the name signifies, many cells occur, in the latter there are only eight, or two in each quadrant.

It is evident that *C. americanus* is a Polymyrian on this basis and on the dividing line as regards the original groups. *Ancylostoma duodenale* stands about on the level with the new species as regards the type of cell, but is a Meromyrian according to the division of 1866.

A discussion of the advisability of continuing this classification will be found in the section on systematic position of the form, but it is interesting to note here that Martini (1909) states that all Polymyriarians studied by him have many cells in a cross section of the gut, but, while there are many Meromyriarians with an intestine composed of only two rows of cells, there are those in which the intestine has as many cells in cross section as in any Polymyrian, e.g., *Oxyuris*.

SPECIAL MUSCLES

The intestinal muscles. The intestinal muscles (dilators) in the female arise about 0.4 mm. from the anterior end of the rectum and extend to that level. They arise as four separate bundles of fibers, each about 7μ in diameter. After a rather long insertion on the inner side of the cuticula, pass down diagonally thru the body cavity to run posteriorly along the side of the intestine, one at each "corner," so that they lie, two in the dorso-lateral and two in the ventro-lateral fields (Fig. 118). Their anterior insertions are in each quadrant just between the first muscle cell to the dorsum or ventrum of the lateral lines and the lines themselves.

About half way in their course each dorsal bundle unites with a ventral bundle on the same side (Fig. 129), so that there is a widened

region on each side of the intestine, curving around it in the shape of a horse-shoe. It is in this widened region, on either side, that a single nucleus is found, lying towards the dorsal side of the place of fusion. This demonstrates that each lateral pair of fiber bundles belongs to one cell, which branches anteriorly into two parts. Below this anastomosis of the two bundles, each cell branches out (Fig. 120) and these fine divisions become attached to the outer wall of the intestine. When the branches become as numerous as ten, there is seen covering all of the branches and enclosing the intestine, a thin fibrous tube (Fig. 123), which binds these smaller bundles of the intestinal muscle cells to the intestine. This tube of fibrous material extends to and is continuous with the fibrous element of the sphincter muscle cells, becoming along its course much thicker than at its beginning. Near this region there appear two nuclei, one of which is always more posterior and lies a little to the left of the mid-ventral line, (Fig. 125). The outer varies in position but is usually dorsal in location (Fig. 126). In the section on the rectum a more complete account of these structure will be given, but here a few words are necessary. Looss has not mentioned the existence of such a fibrous tube as here described, but has stated that there is a sphincter, muscle composed of a small number of fibers and a single nucleus in *Ancylostoma*. It seems probable that this tube here described in reality is the sphincter, greatly developed and serving as a means of effecting a good insertion for the intestinal muscles, as well as for the constriction of the lower portion of the gut. The second nucleus may have been overlooked by Looss or may be present in *C. americanus* on account of the greater development of the sphincter.

Branches from this tube pass over to the ventral line and some few to the lateral lines, and undoubtedly carry in them the nerves to supply this structure. Just in front of the rectum one can count as many as thirty-five branches spreading out all around the intestine, and these are held in place by the fibrous tube, and partly, by the fusion to the gut wall in the very posterior region.

As to the function of these fibers which go to the intestine a word should be added based on their position and insertion. I believe that they oppose the anal muscles in part and the sphincter muscle as well. Their contraction would raise the intestine and at the same time expand its lumen, the anal muscles by contraction would pull

down the gut into position and the sphincter would close it. The sudden elevation of the gut and expansion of the lumen would of course act to expel the intestinal contents. There is no evidence to show that peristaltic movements occur in the guts of nematodes.

A continued contraction of these muscles would tend to inroll the tail, a fact taken into account in the discussion of the *musculus ani*. Looss states that the branches of these muscles which partly encircle the gut in *A. duodenale*, by contraction would not only open but also close the gut, an action which would be hard to conceive in any case, much less in *C. americanus*. In *Ascaris* these muscles are so placed that it would be impossible for them to close the gut, for here branches radiate all the way around the intestine and are inserted on the cuticula (Voltzenlogel, 1902).

In the males, the intestinal muscles are not so well developed as they are in the females, and are much shorter. They are anteriorly inserted a little above the level of the beginning of the caudal muscles and have their posterior insertions effected by branching out over the intestine just above the rectum. However, the branches are by no means so numerous as in the case of the females.

Musculus ani. Altho Looss described this muscle correctly he called it "musculi anales" for which I can see no justification, since it is clearly one muscle and furthermore composed of one cell, which fact Looss points out; no one would speak of a biceps as being "muscles" simply because it has two places of origin.

This muscle is present in the females only and is in the shape of a fan, spreading out antero-posteriorly as well as laterally. One insertion of this muscle is along the dorsal side of the rectum in its posterior sixth, immediately above the anus. From here it spreads out in all directions, (Figs. 8, 113, 119), but with the main divisions running on the right and left sides of the body, these in turn break up into branches which are inserted on the inner side of the cuticula between the regular somatic muscles. Previous authors have called attention to the peculiar shape of this muscle, which is roughly that of the letter H, for between the two places of insertion there occurs a narrow strip of sarcoplasm in which is present a large spherical nucleus (Figs. 113, 119), demonstrating that the structure is really composed of a single median cell.

The action of this muscle has been indicated in the section on the intestinal muscles, and only a word need be added here. When the gut is elevated and opened by the latter muscles, the body is inrolled to some extent, this being true when the feces are expelled, when the *musculus ani* contracts, its broad outer insertion allows it not only to pull the gut down into place but to straighten out the tail as well. Thus these two sets of muscles are even more closely related to the general somatic muscle cells in function than has been suggested by previous authors. The function of this cell is taken over in the male by the modified caudal somatic muscle cells.

THE DIGESTIVE TRACT

Altho the *oral apparatus* of the genus *Camallanus* is so very characteristic and prominent, none of the previous writers have given good descriptions of the parts or have interpreted their functions aright.

The earliest description which was available to the author was that of Rudolphi (1809) who wrote of the structure in the following manner: The mouth (the principal food passageway) is globular and longitudinally densely striated, with a posterior apophysis, short and extending transversely, which seems to end in two short internal hooks, obtuse and incurved. There are two other external, longer and decurrent ones; or if the total apparatus (*vasa*) is short, the hooks seem to be set into the intestine. As for the shell (*cucullum*) itself, it does not entirely fill up the body, a part is empty and appears clear, which is called the clear spot (*macula pellucida*), a peculiar organ but not considered.

The next author to describe the structure was Dujardin (1845) and his meager account of the apparatus of manducation is that it is formed of a shell, with a short transverse bar at its base, and two intermediate pieces forming a longitudinal body with two or four divergent, oblique and posteriorly directed branches.

In Schneider's monograph (1866) appears the following description: The mouth is slit right and left, occupying the entire region of the head; it is built into a thick capsule, somewhat elliptical, more circular posteriorly and opening into the esophagus in a cross-shaped opening. On the internal surface of the capsule occur a number of ridges or teeth, parallel and forming small teeth on the margin of the

buccal orifice. The sides of the capsule are not equally thick; in the anterior part of each side, they are reduced to a thin membrane. The dorsal and ventral portions, which are brown and thick, give to the eye the appearance of two opposed shells. Behind the capsule, on each side, one sees an apparatus of three branches, made of the same substance and continuous with the shells. These prolongations are morphologically, and without doubt physiologically, of the same nature as the apparatus with the three branches in *Filaria pungens*, which they resemble. The trifurcated apparatus is situated, not in the esophagus, but outside of it.

Perrier's description (1872) is more lengthy and while superficial and incorrect in some respects, gives the best idea of the structure. His figures are copied in Plate XVI. He writes: In the first place the two buccal valves are very evident and are not simple in appearance, due to the thickening of certain parts of the capsule, analogous to the cephalic capsule of the strongyles and related Nematoda. These valves are perhaps joined to each other by a ligament as different bones are joined; no one would consider two adjoining bones as having been formed at one and the same time, so in the case of the capsular articulation. I believe that the formulation of the opinion of our adversary of the present moment, Schneider, allows the entertainment of strange, preconceived, morphological ideas held in regards this genus and the strongyles.

Each valve is composed of a part, more or less semi-elliptical, concave towards the interior and situated anterior to the esophagus: it is the active part of the mouth; inferiorly this part is prolonged into a sort of median point, rectangular, short, somewhat transparent, and engaged in the esophagus where one can easily distinguish it. On each side, the two valves are separated, the one from the other, by a chitinous nodule on which they are simply supported by their inferior angles, and is not made in so sharp a fashion as the body of the valves; on the inferior side this nodule rests on the superior margin of the esophagus. It gives origin to two kinds of chitinous structures:

- (1) Three lateral branches which are spoken of by other authors.
- (2) Two transverse chitinous bands, a superior one, and a ventral inferior one.

These chitinous bands unite the two nodules, making an absolutely firm contour. Each band is formed of three parts, of which

the two laterals are convex towards the exterior, while the middle scallop, weaker and less colored than the other two, is convex towards the interior and supported at its summit on the middle of the inferior border of the corresponding valve.

These are the analogues of the two chitinous bands, which Rudolphi wished to call the apophysis, as he designated this transverse bar. Unfortunately, the peculiarities presented by this bar in the species under discussion are not recognized, and its physiological rôle has completely escaped helminthologists in illustrations, as it has escaped those who have occupied themselves with the form from the perch only.

The lateral branches are three in number on each side; with a length of 60 μ . Of these branches one is median and unpaired on each side; the other two are symmetrical and formed in consequence of a sort of angle which the median branch bisects. This last branch is straight, pointed at its summit, oblique from before and behind, and from within outwards, in respect to the axis of the body; it is found immediately in contact with the sides of the body, which it serves to support. The other two branches are strongly curved and divergent, one is high and interior, the other low and largely exterior. It is a little underneath their junction with the chitinous nodule from which the apophysis arises. Each of these nodules terminates in a large swelling of chitin, in which is inserted a large muscular cord, which passes from before backwards and from without inwards towards the axis of the body. One can distinguish very clearly four muscular cords among the longitudinal muscles of the body and among the cords which unite with the esophagus, with the sides of the body and the chitinous branches themselves.

Perrier thought that if the muscles attached to the posterior tips of the prongs should contract, they would tend to pull together the posterior tips and because the middle scallop was weak it would give in, allowing the anterior margins of the valves to be sprung open. When these muscles were relaxed the simple elasticity of the material, it being under compression, would cause it to resume its normal shape. Outside of this explanation, which will be shown to be totally incorrect, there is in literature no explanation of the action of these parts, so far as my information goes. Before going into that it is necessary to give a full account of the exact morphology of the parts of the

oral region. Judging from either the text or the figures of the earlier authors, there is some confusion as to the position of the two lateral valves, as I shall designate the most prominent structures of the mouth region. Altho it is difficult to tell from the poor figures given by Rudolphi, and there is no statement in the text, it seems probably that he considered the valves as being dorso-ventral in position. Schneider and Perrier certainly considered them as being in this position as well as does von Linstow (1909), if one is to judge from his figures. Railliet and Henry (1915a) give as a generic character the position of the valves, which they state is dorso-ventral. Dujardin, Seurat (1915a) and Ward and Magath (1917) have correctly stated that they are lateral, and with this view I agree: reference to the figures and descriptions will prove this contention.

Authors have applied various terms to the description of these valves; some are: kappenförmigen Mundkapsel (Goeze 1782), cucullo striato (Rudolphi), coquille (chaperon cucullus Rud.) (Dujardin), valves buccales (Perrier), camail d'apicalteur (Railliet and Henry) and Seurat refers to them as being "buccal valves shaped like the valves of pecten." This last phrase very nearly describes them, for as viewed from the side they present a very close resemblance to such shells, except they are a little more convex (Figs. 1, 2, 3, 4). These two valves, of which one is a right and one left, are united in their posterior half; a cross section in this region shows a complete, rather oval-shaped structure of valve substance, (Figs. 13, 16). A section taken more anteriorly shows they are free of each other, and appear as two jaws (Figs. 12, 15). The whole apparatus is a golden brown color. These valves appear longitudinally striated which is due to the presence of ridges projecting a few micra towards the interior. The number of ridges varies a little in different specimens and in the regions of the valves, so that a section taken near the posterior region will show six ridges and as the sections are examined anteriorly more ridges make their appearance until there are ten or twelve in all, divided into two fields, so that there is a little distance in the middle in which there are no ridges. Near the anterior margin of the valves these ridges suddenly increase in depth so that the end shows little hooks formed which project out into the buccal cavity as rather sharp teeth (Fig. 12). The anterior median margin of the valves is notched in the shape of a U. From either side of this

notch the anterior margins pass off in a slight curve, which proceed posteriorly and dorso-ventrally; at the point where the valves are united to each other is the widest part of the jaws, it being in each female 0.16 mm. and 0.12 mm. in each of the males. Another curve which forms the posterior and lateral margin of the valves passes posteriorly and to the mid-lateral lines; it is along this line that the two valves are united. Posteriorly (Fig. 14) there is a round hole which opens into the esophagus. The valves are made up of two layers, varying in thickness in different regions but around $7\ \mu$, except along the line of union where they are half so thick. Length: in the males, 0.089 mm., in females, 0.105 mm.

Around this posterior hole is placed a ring (Figs. 1, 4, 24, 34) which is made of the same substance as the valves, as all other parts seem to be. This ring is thin and curved so as to fit down over the anterior margin of the esophagus. It is about 0.1 mm. in diameter, and is rather tightly joined to the valves but can be parted with a needle and then breaks off smoothly. In the fourth stage the ring is formed, but appears more curved over the esophagus, perhaps when growth of the esophagus takes place it pushes the ring up and straightens it out, at least it gives one that impression.

Both of these parts described lie well within the body of the worm and nowhere do they touch the cuticula save at the most anterior margin, where the valves are in contact with the inner margin of the thinned cuticula which runs up just over the edge of the valves to end on a level with their inner surfaces, and again at the widest place in the valves (Figs. 17, 19).

The third important structures of the oral region are two sets of three posteriorly directed spikes (Figs. 1, 2, 18) known as the tridents. These lie one dorsal and the other ventral in position with the posterior ends radiating out, so that the total amount of circumference included between the points of each set is over one-fourth of the total circumference of the animal's body in that region. Each of the tridents is constructed as follows: The anterior margins of the three spikes are brought together into a solid nodule which is hollowed out on the interior and fits over the region of the valves where they are united, this articulation looks very much like that made by the humerus with the scapula and may be spoken of as a ball and socket joint, however the juncture must be well made because it is not an

easy task to separate them from the valves, except by special treatment. From this socket the three spikes radiate and about half way of their length push into the cuticula itself, so that they lie for the rest of their way in the cuticula, between the two chief divisions, the points firmly embedded within it (Figs. 46-49). The middle spike runs exactly mid-dorsal or ventral, as the case may be, and is nearly square in cross section, coming to a point at its posterior tip, and being in the males 0.08 mm. long and in the females 0.05 mm. The two lateral ones are almost oval in cross section and have a slight swelling on their posterior ends; they are about the same length as the middle ones.

Two other kinds of structures are present in this complicated apparatus. The first of these to be mentioned are the so-called papillae of the earlier authors, and here called the anterior wings (Figs. 1, 2, 4, 15, 22, 23). These are a right and left pair firmly attached to either valve just posterior to the anterior margin. Each wing is attached by its inner margin to the valve and the rest is free, extending laterally for a distance of four to six micra. Their shape is roughly that of the wings of a beetle and are drawn from a top view and slightly stretched out in the figures. They are about one micron wide.

The last structure to be described is the pair of valve covers (Fig. 20) never before referred to in literature. There is one to cover each valve and is triangular in shape, with the apex rounded, medianally notched and placed anteriorly. The whole structure is very thin, but apparently made of the same material as the rest of the apparatus. The sides as well as the base are curved inwards. The two basal corners are pointed and are weakly attached to the inner margin of the socket of each trident. The anterior margin is strongly attached to the anterior outer region of the valves, just below the insertion of the wings. The covers are curved to fit, and lie closely applied to the outer surface of the valves.

If worms are treated with concentrated caustic alkalies the entire worm, including the cuticula is dissolved on standing, or immediately on boiling, except the mouth apparatus. This remains intact so long as strong currents do not break it up into its several parts. The easiest parts to come off and those which do so first are the tridents, then the covers, then the ring. The wings and the two valves always

remain intact and none of the parts dissolve so far as can be detected with the aid of the microscope. It should be said that while the head parts have been kept for days in very hot alkalis they have never been boiled in experimental tests, since they are so small that it is not practical to recover them after such radical treatment. It seems evident enough then that the oral parts are not of the same substance as the cuticula. The fact that they are not soluble in hot concentrated alkalis is quite suggestive, as this is one of the chief characteristics of chitin. Every known test for chitin has been tried on these structures and each gave negative results, but since these structures are so deeply colored, I am under the impression that should the purple tint be formed it could not be recognized. Until enough material can be obtained to make a chemical analysis I cannot say what the substance really is. The only absolute way that could be used to show this apparatus was made of chitin would be to isolate glucoseaminehydrochlorid from it and this is impossible with the present method known. The fact that it is soluble in acids makes it certain that it is not keratin, and considering its color and the fact that it is insoluble in concentrated alkalis, it seems rather probable that it is made of chitin.

The last point of interest is how does the apparatus operate and what is the physiological significance of the whole as well as the parts? In beginning this answer it is necessary to look at the pathological picture presented in the intestine of the host. If the intestine is sectioned with worms still attached it will be seen that the ability to hold onto the wall is developed to a great extent, which fact is observed in nature when they are pulled off of the gut for study or preservation; the amount of traction necessary to dislodge them is remarkable. Within the mouth and filling up nearly the whole cavity will be found a plug of intestinal mucosa Figs. 132, 133 the shape of the oral cavity. Where the plug is in contact with the rest of the wall, the cells show a great degree of distal suction-result, so that they are compressed and the nuclei drawn towards the plug. No marked edema seems to occur at the foci and comparatively little accumulation of leucocytes. At the proximal end of the plug, the end hooks of the internal valvular ridges are seen firmly embedded in the tissue.

If a worm is taken from the wall of the intestine and placed with a piece of the gut under a binocular and watched, it will be seen to go thru very characteristic motions which finally result in its attachment to the wall. Sometimes this will take place within a few seconds and the jaws can be seen to open and close; a motion can be observed whereby the jaws are made to rock upon the tridents. The power of motion and the ability of the jaws to attach themselves to the intestinal wall is due to the presence of a right and left pair of muscles (see figures of mouth parts), each composed of two cells, so that there are four cells in all. In addition to this there is the fact that the jaws are kept from being pushed posteriorly by the firm attachment of the tridents and the fact that there is a powerful suction exerted upon the tissue by the esophagus.

These four muscle cells have the following positions and take their names from this fact: right dorsal and ventral and left dorsal and ventral. The anterior insertions of these is at the anterior margin of the valves, where there is yet no sarcoplasmic process and where they are closely applied to the wings, which help in supplying insertion surface. Here the fibrillar portion is in contact with the wings and the valves on the one hand, and with the cuticula on the other, the muscles being between the cuticula and the valves. Just below the attachment of fibrillar portion of the muscles, they break away and thus the most anterior part of the valves is the only place of insertion, this gives more leverage as will be seen later. The sarcoplasmic portion appears between the fibrillar portion and the jaws, and the nuclei of each cell is seen on about the level of the buccal ring. These muscles extend down the lateral margins on either side of the very narrow lateral lines, the sarcoplasmic portion extending a little below the nerve ring and to the level of the lateral cervical ganglia. The fibrillar portion continues only to the nerve ring. These cells are enormous as compared with the other muscles of the body, they being 0.08 mm. wide and the sarcoplasmic process being 0.03 mm. in thickness.

Being longitudinal muscles, on contraction they pull the anterior margin of the valves open, because the tridents prevent any backward traction, being firmly held in the cuticula. The thinning of the valves at the line of juncture, and the fact that they are convex provides a lever which is made use of by these muscles. If only one pair of the

muscles is contracted the valves will rock upon the tridents and this motion orients the mouth with respect to the gut wall. Thus attachment is made by the rocking of the valves to get the mouth in position, correlated contraction of the two pairs of muscles to open the jaws, suction by the esophagus, and relaxation of the muscles when a portion of the gut is in the mouth. Of course the normal motion of the worm's body helps to keep the mouth in close contact with the intestinal wall. Once the mouth surround a plug of tissue, the hold is made more effective by the anterior hooks, for these work somewhat like the barbs on fish-hooks. The only function of the ring seems to be that of support, for it furnishes a large surface to fit over the end of the esophagus.

It would seem then that the explanation of Perrier is in error, chiefly from the fact that he was ignorant of the exact structure of the parts of the oral apparatus. The prongs are not connected to a "middle bar," but the "ring" really belongs to the valves, is well joined to them and is developed before the tridents. The tridents when they are developed come from a region at the extreme lateral margins of the valves and not from this ring. In the next place there are no muscles connected to the ends of the spikes of the tridents, an error carried by Railliet and Henry even in 1915. These are so firmly embedded in the cuticula that they are not capable of motion and by this very fact make it possible for other parts to move. The restoration of the apparatus after opening is in truth due to the elasticity of the structure, but not to "the middle of three scallops" which do not exist, but to the elasticity along the line of union of the valves.

Some other points regarding this interesting mouth apparatus in particular as regards its condition in young specimens, will be discussed in other sections of the paper, to which the reader is referred.

The esophagus. The esophagus of nematodes has been suggested by previous authors to be of importance both from a phylogenetic and an ontogenetic standpoint. This type of esophagus has been said by many to be characteristic of the group of Nematoda, and so far only one kind of exception has been found, and that in the division made by Ward (1917) as the Trichosyringata. In this group the esophagus is a capillary tube, enclosed within a row of cells, a condition which is not very well understood. The fact that in certain species at least, the esophagus reaches a maximum length early in

the growth of the individual, makes this organ of some value in identification and its constancy within a given genus shows that it is subject to little variation thruout different species within a genus. Taking it all in all it seems that the esophagus should be considered as a very important organ in the nematodes, and its shape, size and structure should be given in descriptions of these animals.

In *C. americanus* there exist two portions of this interesting organ (Fig. 3), which divisions are called the anterior and the posterior parts or regions. As evidence for considering these two regions as belonging to one structure, I offer the following considerations:

(1) The outside covering, the tunica propria, covers both regions as a continuous structure.

(2) The inside lining is of the same structure and substance and continuous; it is tripartite in both.

(3) The muscles and nuclei are arranged alike and have similar histological elements in both regions.

(4) No valve exists between the anterior and posterior regions.

(5) There is a typical valve between the posterior margin of the posterior portion and the intestine, just as found in all other nematodes studied for this particular structure.

(6) The dorsal gland is continuous in both regions.

The use of the expression "first and second esophagus" is misleading and is on the whole unfortunate.

The anterior portion, to follow Cobb's (1898) nomenclature, is conoid in shape but expanding very gradually towards the posterior portion, until it is about as thick as anteriorly; rounding off, it presents a nearly straight margin where the posterior portion begins (Fig. 3). There is no evidence that esophageal torsion exists in this species as in the case of *A. duodenale*.

This region is lined thruout with a substance similar in appearance and staining reactions with the cuticula and it, like the latter, dissolves in alkalis; in these facts it offers some evidence for considering it as being formed by the invagination of the external cuticula as most authors have held. Owing to its size and position I have been unable to obtain enough of it for an analysis. Its solubility in alkalis and its failure to respond to a chitin test removes it from consideration there, altho nearly all authors have called it a "chitinous lining." Anteriorly the lining is in the shape of a circular layer, and narrows

down like a funnel, until a few micra posterior to its anterior opening, which is within the field of the mouth apparatus ring, it changes into a triradiate shape and encloses a very small cavity. This "funnel" (Looss 1905) is composed of a substance which is continuous with the rest of the lining (Fig. 34), altho it appears set off from it, staining very deeply and being much thicker. Anteriorly it abuts on the lower margin of the jaws, as tho it was at one time continuous with them also. This region of the lining seems to act as a cap for the anterior portion of the esophagus as well as a lining. From here on the lining is triradiate and six thickenings appear, two on each third, and at the center of each field. They are oblong in cross section, and are like ridges extending almost the entire length of the anterior portion of the esophagus. The lining is further thickened at the juncture of each side, this acting as a hinge for opening and closing the lumen. These thickenings get larger posteriorly and just before the beginning of the posterior region they become smaller, disappearing entirely 15μ anterior to this level; the triradiate lumen continues then to the posterior margin of the esophagus (Figs. 41 to 45 give a series of views of the lining, drawn on the same scale).

The marginal thickenings are for the insertions of the marginal muscles (called so by Looss, 1905) as well as serving for a hinge, and these muscles appear as a small group stretching out from this inner insertion to the tunica propria. The nuclei of these cells lie in groups of three at the same level and there are two such groups, so that six nuclei are found in these muscles. The protoplasmic strands with them are sarcoplasmic, and the nuclei lie half way between their two insertions.

The other set of thickenings are for the insertion of the ordinary muscles of the esophagus, which are more numerous than the marginal fibers and usually stain a little darker. Their nuclei appear in groups of six at one level, two nuclei in each field; being three groups, there are in all eighteen nuclei for these muscles. The marginal and the ordinary muscle cell nuclei alternate with each other thruout the entire esophagus. The latter nuclei lie a bit more peripherally than the former, in most cases. The sarcoplasm of these cells is more abundant than in the marginal muscle cells (see Figs. 30, 47, 49 for the structure of this region of the esophagus).

A gland lies in the dorsal third of the esophagus, which will be discussed later.

The action of the esophageal muscle cells is obvious. Their contraction opens the lumen and in living specimens this can be seen, and that the entire lumen opens at once. The elasticity of the lining closes the opening. The marginal muscle cells keep the lumen in position and so have a kind of supportive function as well as helping in the opening process.

The posterior region of the esophagus is made up of a different looking tissue than that found in the anterior portion. The general shape of this region, which is cylindrical, is shown in Figure 18 where it can be seen to be of nearly equal diameter thruout, being slightly swollen in the anterior region and expanding up to its widest parts from a narrowed portion just behind the anterior enlargement.

The tunica propria of the anterior portion of the esophagus extends over the posterior portion, somewhat invading the tissue between the two regions, serving to set them off from each other (Fig. 40). The lumen of the two regions, as has been remarked before, is continuous, the characteristic lining of the anterior portion giving way to a simple triparted lining with no special places for the insertions of muscles in the posterior region. This lining extends to the end of the valve.

Four different kinds of tissue can be recognized in this region of the esophagus.

(1) The sarcoplasm of the dorsal esophageal gland, with its single nucleus, will be treated under a separate division of the paper.

(2) The second tissue is of a nature not greatly unlike the muscle cells of the anterior portion, and I consider it to be weakly developed muscles for the opening of the posterior lumen of the esophagus. In this tissue can be seen small nuclei (Fig. 31), with a single nucleus in each, near the lumen and therefore near the insertion of the muscle fibers of the ordinary muscle cells; there are also marginal nuclei. The arrangement of these nuclei follow the general rule of the anterior portion. Just posterior to the anterior tissue cone (see below) there is a set of three marginal muscle nuclei, further down there is a group of six nuclei, two in each field; posterior to these appear another group of three marginal nuclei and then the number and arrangement is not, so far as I could find, constant in the individuals examined. There are usually several (2 to 6) scattered nuclei, sometimes apparently marginal and sometimes ordinary muscle nuclei; perhaps

typically there is another group of six, following the general rule, but have been overlooked for some reason.

The ordinary muscle cells are inserted in the middle of each side of esophageal lining and have their outer insertions along the tunica propria, spreading out more like a fan than do the cells of the anterior region. The marginal muscle masses are, as in the anterior portion, inserted at the angles of the lumen. While these two different kinds of fibers are made out in the best preparations and by following thru series of sections, specimens show some variation in the distinctness of these two kinds of fibers, and more than that, there are often insertions of muscles all along the lining of the lumen, with fibers radiating out to the inner side of the tunica. The muscle fibers stain deeply and show a fibrous structure with little or no granular material. There are perhaps no more than twenty nuclei in this tissue.

(3) A third type of tissue is confined to a region in the anterior region of this general part of the esophagus, and is in the shape of an inverted cone (Figs. 40, 39). This tissue, a syncytium, is very granular and stains deeply, lies in the center of the esophagus around the lumen and just posterior to the division between the two regions. There are twelve nuclei within it, three in each of the three fields. These nuclei are a little larger than the muscle nuclei, and each has its nucleolus. There are numerous vacuoles in the esophagus surrounding this plug of tissue and muscle fibers invade some of this region. The function of this region is unknown and no suggestion so far can be advanced until a further study is made of it.

(4) Concerning the last type of tissue I feel more certain. This also is granular in nature and not very closely packed together, is made up of larger granules than in the preceding case, but they stain very lightly. This tissue fills up all the space not occupied by the others so that there is considerably more of this type than all the rest put together. One very interesting fact is its composition of but two cells, their nuclei lying one in each of the two sub-dorsal fields of the esophagus in their posterior tenths (Figs. 3, 33, 38). These nuclei (Fig. 37) are spherical in shape with a spherical nucleolus in each. The nucleoplasm is highly granular and the nuclei stand out sharply as black staining bodies. This tissue, or rather these two cells, I consider to be involved in the excretory function of this animal. There is in the third division (dorsal) anterior to these two nuclei

which are on a level with each other, another large nucleus belonging to the dorsal gland and by comparison with the other two seems to be quite different not only in being smaller but also in being ellipsoidal. In the table below the measurements of these nuclei are given.

	Size	Volume
Gland nucleus	0.0083 x 0.0140 mm.	0.000001182 cmm.
Excretory nucleus	0.0161	0.000002245
Gland nucleolus	0.0050	0.000000065
Excretory nucleolus	0.0061	0.000000108

The histology of this part of the esophagus and the small number of nuclei, prevents it from being considered a gland, but rather favor its being concerned with excretory process, especially when its relation to the rest of the excretory system is considered. Just how the esophagus functions in this process is as yet unknown to me, but from a theoretical standpoint it seems possible to imagine the excretory products of the organs to be passed into the body fluid, and then taken out from here by specific action of the two giant cells of the esophagus, to be given up into the bridge and accessory tissue and finally into the excretory ducts themselves. This would presuppose a discriminating action on the part of the esophagus against the food materials which must be present in the body fluid, but this assumption is not at all unreasonable, since the same thing has been observed in the cells of every plant and animal known.

A rather interesting error is committed by Stephens (1916). He writes: "In others (*Cucullanus* [now *Camallanus*], *Ascaris*, etc.), a tube, the so-called glandular stomach, lined only by epithelial cells, follows behind the muscular esophagus. This glandular stomach is, from its structure, easily distinguished from the midgut, or chyle intestine, which is like-wise cellular." From the foregoing study it is evident that for *Camallanus*, at least, this statement does not hold true.

Like most, if not all nematodes, this species has a dorsal esophageal gland, but glandular tissue is in no other field of the esophagus. This gland extends from the very posterior margin of the esophagus to within a few micra of the anterior margin of the anterior region.

Anteriorly there is at its beginning an expansion of the glandular tissue which includes most of the entire dorsal field and here a minute

duct from this gland empties into the esophagus in the mid-dorsal region (Fig. 30) between the two dorsal thickenings of the lumen; this duct continues thruout the entire gland, lying in its central region. Posteriorly the gland narrows, occupying an elongated ovoid area in the mid-dorsal line. At the juncture of the two regions of the esophagus the gland narrows greatly but expands in the posterior region. Thruout this region the gland is larger than in the anterior region and here and there can be seen little knob-like projections on either side, but these never extend beyond the area of the dorsal third of the esophagus.

Very near the posterior end of the gland there is a large ellipsoidal nucleus (Figs. 3, 37) with a single spherical nucleolus, their sizes being respectively 0.0083×0.014 and 0.005 in diameter. In some cases the gland seems to extend down into the dorsal member of the esophageal valve.

The sarcoplasm of the gland is rather hard to analyze (Figs. 30, 31, 33, 38, 51). In sections stained with Mallory's connective tissue stain, the gland is colored red, due to the fuschin, while the rest of the tissue in the esophagus is colored from a purple to a deep blue. The gland stains with thionin, as also a little tissue within its immediate neighborhood. In any good preparation of the gland can be seen a structureless outer membrane and an inner granular portion. The granules are large and relatively few, so that there are open spaces within the gland, hence there must be a rather large amount of hyaloplasm present. Around the nucleus the granules are more numerous.

As to the function of the gland, if indeed it be a gland, I can offer no suggestions other than those made by previous authors. These particular worms being blood suckers, suggests an hemolytic or anticoagulative function for this gland, yet the opening of the gland duct is perhaps to far posterior to the mouth to favor this view. It seems more logical to agree with those authors who think this structure is a digestive gland, altho the evidence is by no means plentiful and referring to it as a "salivary gland" is certainly open to criticism.

In most nematodes studies there has been described some kind of a valve between the esophagus and the intestine, and most authors have called it the "esophageal valve." On the contrary Looss (1905, 1911) has claimed that this is really an "intestinal valve" and

is here formed by a telescoping of parts in this region. Lane (1916) states that this is true in the genus *Ancylostoma*. Looss has even gone so far as to locate four cells in the anterior portion of the intestine of the developing hookworm larvae which he claims give rise to these valves, altho I can find no complete history of these cells given. He (1905:91) bases his claim on the following statement: "The fact that, in the valvular apparatus, as in the intestine which follows it, only two cells appear in cross section, while, in the esophagus, three cells are always found composing such a section, indicates that the whole apparatus is to be regarded as a product of differentiation of the intestine," yet he admits a three-cell arrangement in the rectum. However, he states else where that the inner lining of the valves is continuous with the esophagus and thus "we see," writes the author (page 90), "that the outer tunica propria of the esophagus does *not unite* with its inner lining but that there is a *direct connection* between the tissue of the esophagus and the valves, the connection taking place on their *inner edges*." Even his nuclear counts he admits were made under difficulties and noted considerable variation in their number and position.

In *C. americanus* there exists at the posterior margin of the posterior part of the esophagus a structure which I wish to call the "esophageal valve." This structure is remarkably like that described by Looss for *A. duodenale*. It is composed of three valvular projections (Figs. 38, 36) which are well in the lumen of the intestine. Each projection is composed of the muscular tissue of the esophagus plus its own cell which is granular and stains lightly. Each third possesses a nucleus of medium size which can be seen in good preparations. Around the three members of the valve there is a sphincter muscle, containing one or two nuclei (Fig. 36). The cells of the intestine come up around the valves covering the grooves round their bases but *do not cover their free margins*; these project in the gut lumen. The lining of these members is continuous with the esophageal lining and they are somewhat set off by its invasion between the esophagus and the valve, which is not so complete as to cut it off. Each projection represents the most posterior part of its respective third of the esophagus.

I can see no justification for assuming, in *C. americanus* at least, that this valve is intestinal, and on the other hand very good ground for

believing that it is an esophageal structure. In this I agree with Cobb (1898), Quack (1913), Railliet and Henry (1915), Lane (1916a) and others. The fact that the cells are continuous with the esophageal syncytium, that they are lined with the continuous layer of the esophagus, and covered with the same tunica propria, are three in number and with a nucleus in each, that they are set off from the intestine and of entirely different histological structure, and finally that they function to prevent the backflow of food into the esophagus points to their belonging to this organ rather than to the intestine.

Leuckart (1876) in his work on the life history of *Camallanus lacustris*, figures a group of cells at the posterior region of the esophagus which he found to develop into this valve like structure in that species. This, of course is additional proof for considering this valve of esophageal origin, for one would not expect the conditions to differ in the same genus.

It is difficult to believe that this valve is not homologous with that in *Ancylostoma* and the other nematodes, and yet if Looss be correct, this could hardly be true. I have been unable to convince myself either by a study of the text or his figures, that in the form described by Looss, this valve is intestinal, and his failure to find the third nucleus in the third valve member (he does not say in which projection this was lacking, nor is he clear that it is always lacking in a particular one) I consider due to his technique, and the condition of the tissue at the time of killing and fixing. He admits his difficulties in counting the nuclei in this region, which has been noted above for the posterior region of the esophagus and these are often very near the valve itself in contracted specimens, so that the chance for confusion is greatly increased. After all, in *C. americanus* there may be more than three nuclei concerned with this valve, but at least there is one in each division. If Looss be correct in his statement that the "two-cell" condition is carried in both the valve and the intestine, on this reasoning one would expect many more nuclei than it would be possible to have in the valves, because in *C. americanus* there are many nuclei in cross section of the gut and in some forms, there would be even more.

I am forced to the conclusion, therefore, that the valves of various nematodes are homologous and are specializations of the posterior portion of the esophagus; that the cells of the intestine have crept

up around their bases and a sphincter muscle has been developed out of the intermediate edges of the tissue, perhaps in part from both, or entirely from either one. This conclusion seems warranted from a study of the present literature, although subsequent study may show that there is here a fundamental distinction between certain nematodes.

The intestine. The portion of the alimentary tract which lie between the esophagus and the rectum is known as the intestine, or chyle intestine, an unfortunate name, since there are no villi in nematodes.

In *Camallanus americanus* this portion of the tract is made up of tall, hexagonal, columnar epithelial cells. The inside of the gut is not smooth in cross section, but is thrown into irregular low folds. In some nematodes the inside of the gut is very smooth, in others there are definite and very deep folds. In adults the cell walls usually break down to some extent, so that often none can be seen, and with this degeneration comes an apparent degeneration of the nuclei (Ehrlich 1909). As in most nematodes, there are definite regions in the cells. On the outside is a thick basal membrane and just inside of this a slightly thickened portion or layer of sarcoplasm.

Towards the center of the cells are numerous reddish-brown concretions or granules (Figs. 60, 115), about one micron in diameter, more numerous in the older individuals than in the young, and chiefly in the anterior part of the gut. Such concretions have attracted the attention of many students of other species and by nearly all are thought to be excretory products of some nature (Exkretkörnen). Looss (1905) describes them for *A. duodenale*, but here they are far less numerous than in *C. americanus*, where they more often than not almost fill the cells. Looss considered them as some product of blood digestion, which seems very logical and would therefore represent what are sometimes termed "coffee-grounds" in human pathology. These bodies are probably a part, at least, of the pigment group of the turtle's blood corpuscles.

In *Ascaris* this same kind of material has been noted and studied to some extent by Flury (1912) and Fauré-Fremiet (1913). The latter found them to be insoluble in the solvents of fats and resistant to digestion with pepsin and trypsin. He expresses his belief in the following words: "Il est donc tout a fait probable que ces grains

sont l'expression de la transformation d'une partie appréciable de l'hémoglobine ingérée par l'*Ascaris*." I am inclined to think that they are not excreted in the form in which they appear in the gut wall, because they have never been found free in the lumen, and they accumulate with the increasing age of the worms, so that very often they are nearly or totally lacking from the intestines of young animals. However, this fact must be kept in mind; the body fluid is colored red, and this color most likely comes from the blood of the host. It may be that these pigment bodies represent stored up material in the walls of the gut, and that they are intermediate products in metabolism; they would then be changed into a fluid, giving the red color to it. Thus some of the material might be used as food, or it may be that parts are excreted, even before being used for food, passing out through the excretory duct. (See the section on the body fluid for a further discussion of this problem).

Among others who have noted the presence of non-cellular materials in the intestines of nematodes Cobb (1914) should be mentioned. He called attention to "rhabditin" in the intestinal cells of *Rhabditis monhystera*, and came to the conclusion, after his very brief study of the material, that it was a carbohydrate, basing his conclusion on the following results: slowly soluble in water, rapidly so in alkalis and acids; insoluble in most organic solvents; the aqueous solution gives no precipitate with barium salts; the substance does not stain with iodine-potassium iodide solution, and the crushed bodies of the worms gave a Fehling's reduction; no trace of the substance remained when the bodies were burned, a faint flicker over the sodium line of the spectrum indicated to him the absence of the earthly constituents that might be expected in certain excretory salts, such as calcium. It is unfortunate that he saw fit to name this substance without more knowledge of its nature. Of course his Fehling's test was absurd, since the bodies of nearly every animal will give a reduction with this agent, especially nematodes, and he indicated no way whatsoever of telling that these granules played a part in the reduction. The failure to stain with iodine solution certainly excludes a great many carbohydrates. It is impossible to tell just what he really had from the very meager information given in his paper, and since he does not refer to any special work on the subject one is inclined to think that he did not consult these particular articles. One of the most recent

and there is that of Marie Quack (1913), who concludes that certain granules, which agree in description very closely with "rhabditiin" and found in free-living and parasitic nematodes, are calcium salts, and her evidence is very conclusive on that point. However, these "Sphaerokristallen" are insoluble in water, alkalis and dilute acids. Until Cobb publishes a more complete account of his work one is forced to discount his conclusions, at least one cannot accept his name without better justification from a chemical standpoint.

The nutritional zone (nutritive Zone) can be recognized as the layer of thickened protoplasm on the inner border of the cells and inside of this another membrane. The "Stäbschensaume" is very tall in this species, being nearly equal to the height of the cells themselves. In young specimens, this layer shows very clearly its arrangement. Each cell bears a definite clump of little bristles, which proceed from each cell towards the lumen of the gut. In the older individuals these bristles become matted together so that the layer seems almost like a continuous one. Many authors have called this a "chitinous layer," but this is not the nature of the material of which it is composed for Quack has shown that it is digested by the action of pepsin and is soluble in caustic alkalis.

The nuclei of the intestinal cells are ellipsoidal in shape, with a single nucleolus in each, and lie either within the nutritional zone or immediately below it. This position is unusual, for the intestinal cell nuclei usually are said to be well in the middle of the cells, or more typically near the outer margins. Supporting fibers can be seen in the best preparations, stretching out from a slightly thickened area around the nuclei.

Just anterior to the rectum the intestine shows a very poorly developed valve (Figs. 116, 125, 126). The appearance is that of certain cells being pushed up and in posteriorly, so that a small pocket is formed and the lumen is made smaller. The nuclei are very numerous in this region.

The rectum and cloaca. Between the posterior end of the intestine and the anus there is a small region known, in nematode anatomy, as the rectum. The region has excited the interest of workers in the field, chiefly because of certain bodies which always seem to be present, even in the most diverse forms. Various functions have been ascribed to these organs, which are considered to be a group

of three or four cells. Leuckart, Cobb and others have called them "anal glands," but none have been able to propose a logical function for glands in this region of the alimentary tract, since a secretion poured out by them would pass into a cuticula-lined canal and right at the opening of the tract to the exterior. In addition to this, the demonstration of ducts from these cells is by no means certain. Others have spoken of them as "giant cells" and "ganglion cells." Looss could not convince himself that these explanations would explain the function of such cells and finally came to the conclusion that they were cells which belonged to a syncytium of connective tissue in the forms in which he studied the structure, and that "the structure to which they are attached is a ligament for fastening the chyle intestine to the rectum." Before giving my interpretation of the structure, it will be necessary to give in detail the condition found in *C. americanus*. The modification in the males, due to its entrance into the cloaca, makes it necessary to describe the condition in the two sexes separately.

The female. In the females the rectum is a short tube 85 μ long and greatly compressed dorso-ventrally. In the middle region it assumes a slightly triparted shape on the inside and towards the end it shows several prominent excavations or indentations, the largest of which occurs on the ventrum (Fig. 113). Other indentations are shown in Figure 128. Posteriorly the anus terminates the rectum, and is in the shape of a slit (Figs. 113, 114). Where the anus is located the ventral band is divided, its posterior two halves uniting.

The lining is rather thick and as a rule stains more deeply than the cuticula, from which it is supposed to be derived and with which, even in adults, it seems to be continuous, altho the division between the two is usually well marked off. Anteriorly the lining ends abruptly, a few micra posterior to the regular cells of the intestine, and between these two structures is a little space surrounded by three cells, which lie immediately posterior to the lower margin of the intestinal muscle cells.

These three cells (Figs. 8, 114, 127, 128) lie one dorsal and the other two sub-dorsal; they are large and spherical in shape being about 17 μ in diameter. Each spreading out at its base, they form a solid syncytium around the central cavity continuous with the lumen of the gut. Anteriorly these cells pass into the fibrous tube which has

been mentioned previously, and thus they are connected with the sphincter muscle; this fibrous material even extends back over the cells, involving some of their inner and anterior margins. A large spherical nucleus with a single nucleolus is found in each cell. Posterior to these three cells are three others, nearly as large and whose anterior parts push up under the anterior three larger cells, thus replacing them. These cells are not globose but are flattened (Fig. 131), contain large nuclei and each is in one of the three fields as in the case of the other cells. The last three extend almost down to the anus. over the whole of the outer surface of the rectal lining, being rounded out in their peripheral surfaces, they give the rectum the appearance of a regular cylinder (Figs. 130, 131).

The male. In the males this region (Figs. 99, 100) of the gut is essentially like that of the females but of course differs somewhat on account of the modifications caused by the rectum opening into the cloaca rather than at the anus, also on account of the presence in this region of the termination of the male reproductive organs, which become involved with the alimentary tract.

Here as in the case of the female there is a sphincter muscle around the narrowed portion of the posterior end of the intestine and extending just below it, the three giant cells. These cells are not quite so large as in the case of the female and the two sub-dorsal ones are pressed to an almost lateral position on account of the presence of the genital duct lying along the ventrum of the gut. The cells covering the rectum are also present, and these seem to be a part of a syncytium covering the cloaca. The rectal lining is continuous with that of the cloaca. The rectum itself is much shorter than in the females, being only about half so long; it opens on the dorsal side of the cloaca above the opening of the spicular canal.

The cloaca. In the male nematode the common cavity just before the anus which receives the end of the gut and the reproductive organs, is known as the cloaca. This very short passageway is a cuticular lined cavity with an outer cellular covering, containing a few nuclei and part of the syncytium covering the rectum. On the dorsal side, as has been noted above, enters the rectum, and along side of it (Fig. 92) on the ventrum, is found the terminal of the genital duct. Just posterior to the opening of the alimentary canal is seen the opening of the spicular canal. After a short course the cloaca

opens to the exterior in the midventral line as the ano-genital aperture (Fig. 79), $10\ \mu$ in diameter and in a slightly elevated portion of the cuticula, on either side of which are the two pairs of para-anal papillae.

The interpretation of these structures in this case is not an easy task and the final word cannot be said until the embryology is known. I consider the posterior three cells described above to be the "posterior ring" cells of Looss, and think they are nothing more than the rectal cells. They may even form the lining of the rectum, their position and granulation would suggest this: at any rate they support it and of course must help to connect up the posterior structures, but I am unable to consider them as being separate parts of a special ligament, as Looss would have one believe the corresponding structure in the hookworm serves. The three anterior and larger cells, are evidently the cells Looss thot made up the "anterior ring" and these are near the region which is supposed to be contracted by the sphincter muscle. Looss admits that there is a very close relationship between the rectal sphincter muscle and the "anterior ring of the rectal ligament," and in reality the two structures seem to be one and the same. The nuclei of the large cells are then in part nuclei of the sphincter, and the globose portions of the cells are the sarco-plasmic parts of the entire structure. The outer parts of the cells are granular in character but the line around which the three cells are united with each other is towards the anterior side, fibrous, and continuous with and exactly like, the circular tube enclosing the posterior portion of the intestine, and which is in reality the rectal sphincter. A contraction of the basal ring of the three cell syncytium serves to close the posterior end of the gut, helped by the action of the tube of fibrous tissue. It is opened by the intestinal muscles, which section see for details. In *C. americanus* the interesting bodies are nothing more nor less than parts of the rectal sphincter. Under this interpretation the two nuclei in the sphincter proper are not well explained, unless they be considered as accessory in nature.

There is some doubt in considering these as purely connective tissue cells as Looss thinks; they serve as such in a way, but the two sets of three cells are better interpreted as separate structures and as given above, at least in this species. From the text and figures of Looss one cannot see that he has, in his own case, demonstrated his

contentions, for he clearly shows that the fibers of the sphincter muscle are "intercalated" among the protoplasm of the three giant cells.

Food. All of the evidence at hand points to the fact that the normal food of this species is the blood of the host. This is indicated by the red color of the body fluid and the pigment in the intestinal walls.

Looss devoted some time to a study of the condition in *A. duodenale* and came to the conclusion that in this species the normal food was not human blood but rather the intestinal mucosal cells. This he demonstrated by the fact that the amounts of pigment varied in different worms and not with their ages and in addition to that, the pathological condition of the intestine of the host and the presence of the intestinal cells in the lumen of the gut of the worms bore out this conclusion.

In the case of *C. americanus* neither of these conditions exists. I have never found host intestinal cells in the guts of these worms nor does the pathological picture in the intestine of the turtle indicate that the intestinal lining was being eaten. The great amount of pigment and the fact that it is found in greatest quantities in the older individuals indicates further, that blood is the normal food of this animal.

From an anatomical standpoint there is further evidence to this effect. The presence of the small sharp hooks at the anterior end of the jaws affords instruments for the laceration of the tissue and the movements of the mouth apparatus are conducive of the same effect. The strong suction made by the action of the muscles of the esophagus serves to draw in blood. The blood corpuscles must be rapidly digested for they are seldomly found within the parasite. All evidence goes to demonstrate that these animals are blood suckers.

An interesting experiment was carried on to see if these worms could be kept alive in a cultural medium and if they would use the medium for food. After several trials a mixture of Witte's peptone and gelatin was obtained so that the warmth of the hand would melt it and at room temperature (about 21 degrees C.) it remained solid. Before the worms were introduced the medium was tested and gave the following properties after sterilization:

- (1) Tryptophane present in combination only.

- (2) Biuret reaction was positive.
- (3) Clear solution, liquid at hand temperature.
- (4) Micro Kjeldahl determinations gave the following results:
 - I. Total nitrogen per cc. 0.0039 gm.
 - II. Total nitrogen per cc. 0.0042 gm.
 - I. Non-protein nitrogen per cc. 0.0015 gm.
 - II. Non-protein nitrogen per cc. 0.0017 gm.
(The trichloroacetic acid method was used)Average total nitrogen per cc. 0.004 gm.
Average non-protein nitrogen per cc. 0.0016 gm.

Six worms were introduced into a flask of this fluid and incubated at 25 degrees C. The worms were first washed thru a liter of steril water in fifty different wash waters, using steril Stendor dishes, and a sterile platinum needle to handle the worms. Every precaution to avoid contamination was exercised. After allowing the worms to remain for six days an examination of the medium was made and a portion of it plated out in agar after twenty-four hours incubation at the same temperature at which the worms were kept. In addition to this a small portion was introduced into another flask of the same medium and kept in the incubator for six days. The medium after the action of the worms showed the following properties:

- (1) Tryptophane as before.
- (2) Biuret as before.
- (3) Clear supernant liquid with a heavy precipitate, but the liquid at temperatures below that of the room did not congeal.
- (4) Nitrogen analyses:
 - I. Total nitrogen per cc. 0.0036 gm.
 - II. Total nitrogen per cc. 0.0042 gm.
 - I. Non-protein nitrogen per cc. 0.0033 gm.
 - II. Non-protein nitrogen per cc. 0.0030 gm.Average total nitrogen per cc. 0.0039 gm.
Average non-protein nitrogen per cc. 0.0032 gm.

The worms remained alive in this medium for over two months.

The control inoculation was steril at the end of three days and the inoculated medium gave the same results for total and non-protein nitrogen as it did in the original case.

This demonstrated conclusively that these worms can live on other materials perhaps only substances which are like those they get from the blood. Just what they did to the medium was not learned,

but it is evident that they reduced the gelatin to non-protein nitrogen how much further it went could not be investigated on account of insufficient apparatus. This work will form the nucleus of a subsequent investigation to be undertaken by the author.

Worms were kept for two months on turtle blood agar, they being transferred to fresh tubes when the contamination became great. The maximum length of time they can be thus kept was not ascertained in my experiments.

BODY CAVITY.

For a long time it has been shown that the body cavity of nematodes was not empty but filled with "a fluid probably containing albumen, which curdles under the influence of acid and, when poured into water becomes milky" (Looss 1905:67, translated from Schneider). In the light of modern physiology it seems that the statement of the early investigator of nematodes, Anton Schneider, is by no means incorrect but has an element of suggestion which is probably not far from the truth.

In the absence of a circulatory system and the presence of a large, fluid-filled body cavity, which surrounds the organs, it seems logical to suppose that this acts for the nematode much as does the blood system for other animals; the circulation of the fluid can be seen in living specimens, due to the contortions of the body. If this be true all intermediate metabolic processes and compounds will be found here, and so the digested materials going from the intestine into the organs, and the waste materials from the organs to the esophagus and the excretory ducts will be found, as in other animals, within the same limits and the selection out of the fluid will rest in the power of discrimination of the individual cells.

If one cuts or injures the body wall of an individual of *C. americanus* there runs out of the lesion a quantity of reddish fluid, rather thick and somewhat opaque, which seems to "congeal" within a few minutes. Alcohol and the usual killing and fixing fluids precipitate it and thus it is preserved in sections of worms. Here the fluid appears as a fine granular substance, staining with almost any stain and partially or entirely filling up the space not occupied by the internal organs.

Attempts to obtain hemin crystals from the fresh fluid have been unsuccessful, but the fluid must contain some part at least of the

pigment group of the blood of the host. There is something very suggestive in the presence of the brownish-red granules in the intestinal walls (see that section) and it seems that these granules are in part responsible for the color in the body fluid. The fact that these pigment masses accumulate with the age of the individual indicates that their breaking down process is slow, hence they may be storage products, and the fact that the worms lose their reddish color after remaining for some time in water indicates to some extent that the color of the body fluid comes directly thru the intestine into the body cavity from the digested blood corpuscles, in this event part of the color group would be retained by the gut in the form of the pigment masses in its walls. The whole problem is an important and interesting one and offers possibilities of solution which are more favorable than many others within the same group of worms.

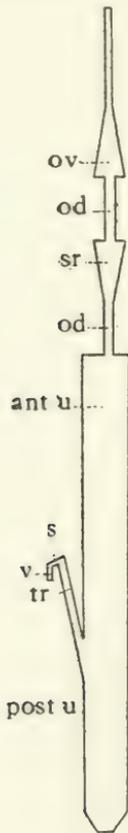
Flury and Fauré-Fremiet (1912) have studied the body fluid of *Ascaris* and they have found the following substances within it: water, sodium chloride, albumin, globulin, some pure bases, free fatty acids, phosphorous compounds, cholesterol, some reducible sugars and finally hemoglobin and often oxyhemoglobin.

Here and there appear in the body cavity, connected to various organs and invading interstitial spaces, small strand-like materials, which give one the impression of a loose connective tissue. This material was called by K. C. Schneider (1902) "Bindegewebe" and by Looss "strand-like organs." The former did not locate nuclei within the mass, while the latter has reported finding them in two places, and suggests an homology between these organs in *A. duodenale* and the phagocytic organs or "büschelförmigen" organs of other species. Goldschmidt (1906) has called this tissue "Isolationsgewebe" and in the large ascarids has located a few cells in this structure situated just posterior to the nerve ring. I have been unable to find nuclei in this tissue in *C. americanus* but this is not surprising, since one would expect them to be very minute and might easily be overlooked. No function nor homology can be proposed at the present time for this structure in our species, and the literature on the subject, tho large, is very much confused; that the whole problem will have to be worked over carefully before a conclusion can be reached is evident; the larger forms will have to be examined first before the smaller ones are studied. I am under the impression

that authors have been dealing with the same structures here and that it will prove to be nothing more nor less than a structure similar to the mesenteries and supporting ligaments of other animals, for holding in place the various internal organs. If this be true the name proposed by Schneider is much better than that given by Goldschmidt. Looss' name is at best a make-shift as would be any that I could propose.

THE REPRODUCTIVE ORGANS

Female. The female reproductive organs (Textfig. G) are of espe-



Textfigure G. Diagram showing the general arrangement of the reproductive system of the female. *ant.u.*, anterior uterine branch; *o.d.*, oviduct; *o.v.* ovary; *post.u.*, posterior uterine branch; *s.*, sphincter; *tr.*, trompe; *v.*, vestibule.

cial interest on account of the absence of the posterior ovary, altho its uterine branch is found. This is all the more interesting because in the Trichosyringata, an order not greatly removed from the Camallanidae, there is only one ovary found.

On superficial examination one might be lead to think that the posterior horn of the uterus was in reality a result of the mere mechanical lengthening of a single uterus, due to the enormous number of embryos developing within; but this is not the case, since in the very young females in which the ovary has not yet begun to function, this posterior branch is seen long before the female has been fertilized. At this stage it is merely a posterior thread of cells leading from the ovijector, corresponding to a similar anterior fundement. The details will be found elsewhere in the paper.

In the anterior division of the reproductive organs can be found, in general, the regions which have been found in most nematode species. There is distinguished an ovary, oviduct, with a "receptaculum seminis," and anterior branch of the uterus.

The ovary is pyriform in shape, attenuated anteriorly and ending in a rather long, slender cylindrical tube. Figure 66 shows the shape and the fact that the tube is about half the total length of the ovary. At the widest place the ovary is about 0.15 mm. The cylindrical tube increases gradually from $10\ \mu$ to $20\ \mu$ in diameter. Within this tube are recognized two areas as in the case of *Ascaris* and referred to by K. C. Schneider as the "Keimzone" and "Wachstumszone," each composing about half the length of the tube. In the former zone occurs an unorganized mass of primitive germ cells which begin in the posterior part of this zone to arrange themselves around the inner margin of the wall (Fig. 74). These cells are spherical in shape, $5\ \mu$ in diameter and with relatively large nuclei. Division of the cells takes place within this zone for here mitotic figures can be seen.

By growth, these cells come to fill the cavity of the tube so that only one layer around is seen and thus in the center they fuse (?) with each other, their peripheral ends being free. So is formed the characteristic structure found in most, if not all, nematodes (Fig. 73). The ovogonia appear like the spokes of a wheel, fastened by their fused ends, called a "rachis." In this species there is but a single rachis and about five or six ovogonia in each cross section. Under the force of this mechanical pressure they are compelled to assume

a cone shape but still have relatively large nuclei. This zone extends well down into the enlargement of the posterior region of the ovary where the last zone begins, the so-called "Reifungszone," where the cells begin to break away from the rachis and gradually assume a spherical shape again. In this region the first polar body is begun to be formed altho the process may be continued thruout the passage of the eggs into the oviduct. On leaving the ovary the eggs are about 25μ in diameter with very large neuclei and prominent nucleoli, one in each nucleus.

There is nothing especially interesting about the histological structure of the ovary. A rather thick deeply staining basal membrane exists, and the wall is composed of a single layer of cells, very much flattened out and like typical pavement epithelial cells, with medium sized nuclei. As many as eight or ten cells can be seen in a cross section. As the ovary lies in the body, it is more or less coiled, but never more than twice, and these are very loose coils.

Posteriorly the ovary is rather sharply delimited by the appearance of a second layer of cells on the outside of the epithelial layer. These are cells with their inner sides muscular and a sarcoplasmic portion towards the periphery, giving the oviduct an irregular outline in cross section (Fig. 76). The nuclei of the muscle cells are in the sarcoplasmic portion. This layer of cells furnishes the oviduct with a circular layer of muscle and by peristaltic motion the eggs are shoved along. Coincident with the appearance of the muscular layer, the epithelial one becomes much higher so that the cells stick out more all around the wall into the lumen and here and there very large projections are seen, especially in one region, which I have designated as the receptaculum seminis.

In fairly mature worms, three regions of the oviduct are distinguished (Fig. 66); the first, about one-third the total length of the organ in 30μ in diameter, then follows a second third, which suddenly expands to a diameter of 130μ and contains shortly after copulation a mass of spermatozoa; few are to be found elsewhere in the system. Here the projections into the lumen (Figs. 75, 78) are very much larger than elsewhere, and groups of spermatozoa can be seen clinging around such out-jutting pieces. Fertilization (Fig. 68) takes place here or perhaps in the very anterior region of the uterus. Posteriorly this receptaculum seminis reduces in diameter to about 17μ and the

musculature becomes very much thinner, until it disappears at the anterior margin of the uterus (Fig. 66). The epithelial cells remain high thruout its entire course and no valves are encountered in this region.

It is interesting to note in passing, that in cross section of the receptaculum seminis the spermatozoa are cut transversely as well, showing that their orientation is with the long axis of the uterus and oviduct; they apparently progress up the entire length of the uterus and posterior third of the oviduct, head first, maturing as they travel, to waylay the egg cells as they pass thru the oviduct. The fluid of the uterus and oviduct offer a medium for their progress.

Five worms were used for the compilation of the following table. The reproductive organs were carefully dissected out and preserved, stained and measured in xylol before they were sectioned. Unfortunately the total length of the worms could not be gotten since the dissections had to be made while the animals were still alive and they were so active that it was impossible to ascertain their lengths at that time.

A study of the sections showed that the shortest ovary and oviduct belonged to the youngest worm, and that they then arranged themselves according to the table, with the fifth as the oldest female. It becomes obvious that both the ovary and the oviduct grow with the worm, but that there is a tendency for the ovary to outgrow the oviduct, an altogether logical condition. Altho there are few cases given here it is perhaps a fair sample since they were picked at random from among a great many individuals.

III. OVARY AND OVIDUCT MEASUREMENTS

Organ	a	b	c	d	e
Ovary.....	1.9	2.0	2.4	3.2	3.5
Oviduct.....	2.0	2.1	2.5	2.6	2.7
Total.....	3.9	4.1	4.9	5.8	6.2
Ovary: oviduct.....	1:1.0	1:1.0	1:1.0	1:0.8	1:0.8

This uterus is simply a huge sac in which are contained the developing eggs and embryos, for this species is viviparous. Its walls consist of a single layer of pavement epithelial cells with fairly large nuclei (Figs. 64, 71) and cell walls more or less indistinct, the

basal membrane which is present stains deeply. There are no muscular fibers in the walls of this uterus, altho Cobb as well as other authors have described such in some nematodes. Looss failed to find them in *A. duodenale*. Within the uterus is a fluid which has been mentioned and this is precipitated by the killing and fixing fluids, stains well and is undoubtedly nourishing for the growing embryos (Fauré-Fremiet).

The anterior uterine branch is considered as beginning posteriorly at the inner end of the ovijector and continuing to the beginning of the oviduct (Fig. 65). The posterior branch ends blindly in a cul-de-sac (Fig. 67), and takes its origin at the common juncture of the two uterine branches and the vagina. Needless to say the uterus grows, probably by stretching as it becomes filled with embryos.

In living animals the uterus passes to and fro (antero-posteriorly) in the body cavity, undoubtedly due to the hydrostatic pressure within the body and the uterus itself, for the uterus lies free in the body, with no muscular fibers attached to it, being held somewhat in place by the before mentioned strands of tissue.

Adopting the recent terms of Looss and Seurat (1912, 1914) for the specific regions of the tube which leads out from the uterus to the genital aperture, I shall refer to this as the ovijector, for the sake of simplicity (Fig. 62). Taking up its structure from within outwards, it begins at the uterus as a tube 18 μ in diameter, posterior to the level of the vulva, being then directed antieriad at a sharp angle and lying at its origin on the ventral side of the body. Its course is by no means straight and its length varies from 1.3 mm. to 2.1 mm. It usually bends from side to side and in one female made a loose loop. Its angle is rather sharp where it starts towards the vulva.

The ovijector is lined thruout its course, save for a short distance at either end, with four rows of epithelial cells (Figs. 61, 72). These cells are rather unique in shape, being more or less round as seen in cross section and spindle-shape longitudinally (Figs. 21, 59). Their nuclei are in about the same level, so that in a given transverse section one sees four nuclei. These cells do not fill the entire cavity and a space extends between the four rows. The cells are 42 μ long. At the inner end of the ovijector the rows become a little irregular and not well marked out because of the appearance of more cells, so that it passes into the uterus without a valve and presenting no radical

histological difference. The covering of the tube is composed of a very heavy layer of circular muscles, which is anteriorly $10\ \mu$ thick and gradually diminishes in thickness until none can be seen at the exact juncture with the uterus. Many nuclei occur within this layer and the muscles obviously act as do those of the oviduct; by peristaltic motion they help to pass the embryos to the genital opening.

From a region just where the ovijector begins to turn towards the ventral side to its openings, this tube has a cuticular lining, which seems to be continuous with the external cuticula (Fig. 63). During the short distance in which the sphincter lies in the body cavity, there is around the cuticular lining a very heavy layer of circular fibers (Figs. 58, 62), with many nuclei, and which clearly function as a sphincter, their sarcoplasmic portions being towards the periphery. Sometimes the cuticular lining extends posteriorly over the four rows of cells, but then only for a short distance, and these cells do not appear very far forward of the end of the cuticular lining.

Thus the ovijector extends anteriorly past the level of the genital opening and suddenly makes a very sharp turn within the anterior vulvar lip, loses its musculature and passes posteriorly to open near the mid-ventral line of the body, but in the side of the vulvar lip towards the tail. This final tube (Fig. 63) is the "vestibule" of Seurat and is only a few micra in diameter and will allow of the passage of only one embryo at a time.

Just at the highest point of the turn a very interesting cuticular structure is present, which from its position and structure is considered as a valve, opening by its own elasticity and closed by the action of the sphincter (Figs. 58, 60). This valve is nothing more nor less than two, somewhat spherical, masses of material, apparently derived from and attached to the inner lining as tho they were mere thickening in its wall, and occupying a right and left position. Compression by the sphincter would push these two bodies together and thus close the passage way. There is the further possibility that they serve in some way during the act of copulation, e.g., in holding the spiculum within the vulva.

The vulva is very conspicuous in this species and is provided on both sides with a very prominent lip, of which the anterior one is the greater developed (Figs. 5, 58, 63). The posterior lip increases as the worms grow older so that in females just before giving birth

to embryos this lip is rather large and becomes pushed out to one side or the other by the overhanging anterior projection.

The anterior lip is about 0.3 mm. long and juts out about 0.12 mm. ventrally from the body; the shape is difficult to describe but can be easily seen in the figures as a swelled out portion of the ventral wall, overhanging its own posterior limit on the body wall.

In sections of the vulva one can see that the ventral band spreads out over the inner margin of the cuticula, and is very much thickened in the whole general region (Fig. 62); it becomes divided around the actual opening of the vestibule, which seems to pierce it, then unites below the opening to continue posteriorly as a single line or band. The cuticula is transversely ridged on the inner side so that it presents eleven definite places for muscle insertion (Figs. 5, 63).

Special muscles exist in this region, there being twelve large cells, each with a nucleus and having its greater insertion on the cuticula, then converging, they find their second insertion around the end of the sphincter and vestibule. Thus one sees in longitudinal sections a radiating structure of muscle cells in this region.

Contraction of these muscles will pull up the lower overlapping portion of the vulvar projection and at the same time turn the opening away from the body wall, while the elasticity of the wall itself will tend to restore it to its original position, aided by the contraction of the muscles in the lower portion of the vulva. These muscles are evidently modified somatic muscle cells. Their function, while possibly related to the expulsion of the embryos, is most likely involved in the act of copulation as well.

Larvae. It does not lie within the scope of this paper to discuss in detail the larval stage of this parasite, however, a few words will not be amiss. The larvae appear within the uterus of the female in two conditions, but in the first stage only. The two conditions referred to are (a) a phase in which the first cuticula is closely applied to the body and is the youngest phase, and (b) a phase in which the first skin is loose and the larvae are contained within it. When the larvae are freed from the female they are very active and the first skin is shed within a few hours, they then being in the second stage.

In the uterus these larvae are at times very active and can be stained *in vitro* with methylene blue. Those in the first phase stain

readily but those with the two skins do not stain until after several hours. They range in length from 200 μ to about 360 μ , and occasionally they are found as long as 540 μ , but this is unusual. Several regions can be located, in particular an anterior region, which has a great many nuclei and is about one-third the total length of the body. This is evidently the start of the esophagus. A light staining area passes around this regions and is interpreted as being the beginning of the nerve ring.

In cross section the larvae are seen to be made up of a tube of cells, about nine appearing in such a section (Figs. 121, 122). The anterior end is bluntly rounded off, while the posterior tip is sharply pointed (Fig. 10). Even in young individuals there are four nuclei in the anterior tip (Fig. 11) which stain deeply and are more distinct than any others in that region. Perhaps they are the foundation of the complicated subcuticular head structure and will form the oral apparatus. On the whole the nuclei of the body are very numerous, and occasionally one or two can be seen inside the tube of cells of which the worms are composed.

Some attempts have been made to find the life history of this species, but so far they have led to negative results. The larvae will live in water for several weeks, but they grow little and do not moult after the first skin has been cast, so far as was learned. These young stages are not as a rule in the intestine of the host. Every thing points to the necessity of an intermediate host in this parasite as in the case of *Camallanus lacustris*.

Male. The reproductive organs in the male nematodes are characterized by their simplicity and in this respect *C. americanus* does not differ from the general type, altho some peculiarities exist. There are three regions in the reproductive organs proper (Textfig. H), all within a single tube on the ventral side of the body, which may be displaced to the right or left, or parts of the tube may occur on the dorsal side in old animals where the tube has become long and sinuous.

The anterior region is known as the testis. This varies in length in different individuals according to their ages. In the young it is almost straight, in older forms it may have several bends, a loop, or may turn back on itself in the esophageal region and grow posteriorly almost to the anus. This tube is at its extremity 10 μ in diameter

and, after about $130\ \mu$, has usually a small enlargement about $20\ \mu \times 30\ \mu$ (Fig. 101). Then the tube very gradually enlarges up to a diameter of $35\ \mu$, passing without much differentiation into the seminal vesicle, which, at its anterior end is about $65\ \mu$ enlarging up to $130\ \mu$ at its widest place in the posterior region. This part of the



Textfigure H. Diagram showing the general arrangement of the reproductive system of the male. *de.*, ductus ejaculatorius; *s.v.*, seminal vesicle; *t.*, testis.

system is about 3.3 mm. long. It passes into a small short tube and then into a region known as the ductus ejaculatorius continuing to the cloaca into which it empties on the ventral side (Fig. 92). This latter organ is about 2.2 mm. long and has an anterior diameter of $66\ \mu$, narrowing to $20\ \mu$ just before the cloaca and to about $10\ \mu$ at its entrance into the common posterior ending of the genital and alimentary ducts.

In toto mounts these regions are well marked by their differences in staining. Of the three, the last region stains the deepest, the testis next and the seminal vesicle very lightly.

The spicula should be mentioned here as being accessory genital organs.

There is nothing especially interesting about the testis which has not been previously considered by zoologists. It consists of a tube, made up of a single row of cells with small nuclei, rather hard to find and comparatively few in number. The cells are very much flattened out and have a rather heavy outer basal membrane (Fig. 83). In the very anterior end of the testis, which ends blindly, are found the primordial germ cells and these can be seen, as in the case of the ovary, as very small cells which divide rapidly, as seen by the frequent mitotic figures. Further down in the testis the rachis formation is noted.

The seminal vesicle is but an enlarged portion of the system and contains in mature forms a great mass of germinal cells, before copulation has taken place. The cells here are free from the rachis. The walls of this region (Figs. 80, 104) are in direct continuation with those of the testis and but for an external constriction there is not a sharp delimiting area. The cells are more spread out here so that they become very narrow and with small nuclei. The size of this region is determined chiefly by the amount of germinal products present.

These products are considered by most authors as still being immature until after they have been for sometime in the uterus of the female. Looss, however, speaks of "mature spermatozoa" in this region of the system in *A. duodenale* and refers to a "mantle" surrounding nematode spermatozoa (1905:111). Concepts differ from other evidence and I am at a loss to explain his observations. The passage of the seminal vesicle into the last region of the system is indicated by the appearance of high epithelial cells and an outer muscular layer. The epithelial cells crowd in at the anterior end and thus form a kind of valve (Fig. 112).

The ductus ejaculatorius of the male reproductive system (Figs. 81, 97) is composed of two layers, the outer of which, consists of a circular musculature containing a very few nuclei, which appear in the sarcoplasmic part, and project out a little on either side of

the tube. This muscle layer is thin and must act as a muscle to produce peristaltic motion, functioning in the removal of the spermatic fluid from the male generative organs.

The inner layer of this tube is cellular and composed of rather tall columnar epithelial cells, about twelve being seen in a transverse section. These cells are extremely granular and have moderately sized nuclei. Figure 81 shows a cross section of this organ. Reproductive cells are sometimes seen in this region but when crowded down by the formation of the products in the tube above, and hence only in the older specimens.

For the function of the epithelial cells I can offer but one suggestion, they may secrete, at the time of copulation, a fluid, perhaps gelatinous, which accompanies the sperm into the uterus of the female. This fluid may be nutritive or may be merely a mechanical carrier for the cells. I have sought in vain for information which would lead me to believe that this organ secretes a cement to stick the male to the female during copulation. The fact that the male cells are not generally retained in the ductus ejaculatorius for a very long time, presents some difficulty for considering it as secreting a nutritive fluid, yet after they have gotten into the uterus of the female there is yet necessity for some nutrition of the developing spermatids. It would seem as tho some fluid medium was necessary for conveying the male cells into the female and it seems most logical to believe that this organ secretes such a fluid.

The statements of Looss concerning this organ in the hookworm are not easily followed for he has included the description of the cement glands in the same chapter, but one is led to believe that he did not see a muscular layer around the organ; such a layer certainly exists in our species and has been noted in many other forms as well.

A further word concerning the spermatic cells. They reach maturity in the seminal receptacle of the female and there as has already been noted they may be seen oriented with their long axis parallel with that of the uterus and oviduct. They apparently have, their "heads" pointing anteriorly. Their shape is of interest; they are elongated and about 16μ ; have a short pointed tail and three more or less deeply staining areas, about equal in size and bulging out somewhat (Fig. 82). They are 3μ wide in the widest place.

The spicula present a rather characteristic appearance in this species. The right one (Fig. 94) is by far larger than the left and differs in some other respects. The smaller (Fig. 95) of the two is but slightly curved and has no embellishments of any nature, being slender, acuminate in form and tapering from a diameter of $8\ \mu$ to a very fine point. Its total length is about $310\ \mu$ in all specimens, and ends anteriorly in an acute angle.

The right spicula is $18\ \mu$ in diameter anteriorly and it also has an acute angular anterior ending, so that a cross section near its anterior end shows only a crescent-shaped structure (Fig. 90). This speculum is about $870\ \mu$ long and is also acuminate in form. It is slightly curved and $75\ \mu$ from its tapering point has on its dorsal side a small point (Fig. 93) projecting dorsad and $5\ \mu$ long, curving slightly anteriorly. Posterior to this point the spiculum dips sharply ventrad and then rapidly tapers to a fine point.

The structure of the spicula is very interesting and has been given in detail by Looss with whom I agree in the fundamental points. They consist of tubes (Fig. 88) of cuticular material, which dissolve in concentrated alkalis and stain deeply with almost all stains. The shell of the right spiculum is $4\ \mu$ thick and about eight times as thick as the smaller one (Fig. 89). Near their posterior tips they become solid. The cavity is filled with a very granular "pulp" which contains nuclei at the anterior end only. Sections show that this pulp is continuous with the outer covering of the spiculum (Figs. 90, 103), which is called the "spicular sheath" or the *musculi exsertores spiculorum*. In this mass of tissue which appears just at the head of each spiculum are from three to five small nuclei, evidently the nuclei of this tissue, although others will be mentioned later.

The extensor muscles are essentially alike those described for other species. In *C. americanus* this muscle (Fig. 96) is about $5\ \mu$ thick in the case of the large spiculum and $2\ \mu$ in the smaller, the thickness varying a little with the degree of contraction of the muscle. It seems to present two distinct layers of which the outer is a little wider and more granular, corresponding to a sarcoplasmic layer, and in this layer from time to time are noted large spherical nuclei (Fig. 103), especially near the posterior end of the organ. The inner layer is differentiated into fibers and is evidently the contractile layer of the muscles. Because of its intimate connection with the heads of the

spicula and its lack of attachment along their courses, its acts like a spring to shoot the spicula out when they are to be protruded. For this purpose they have been anchored firmly at their posterior ends and this is provided for by the places of insertion (Fig. 103) on the dorsal wall of the cloaca in a manner to be described a little later.

Other muscles have yet to be mentioned in connection with the spicular apparatus; these are the muscoli retractores. They are seen in single masses of fibrous tissue attached to the heads of each spiculum (Fig. 103) and extending for a short distance underneath the spicular sheath. On the oblique side of the anterior end of the spiculum this mass sends out several small branches which become attached to the spiculum and somewhat embedded in its pulp (Fig. 86). In each case the single mass divides a short distance anteriorly into two long slender muscles, which proceed, two on either side of the body, free in the body cavity, (Fig. 97) for a distance of about one-fourth the total length of the body to their respective anterior insertions. Each muscle has about half way in its course a small amount of sarcoplasm surrounding a nucleus (Fig. 105). The muscles of the left spiculum are correspondingly smaller than those of the right and are not quite so long. After the muscles have divided, they pass anteriorly, each member of a pair running close together and become inserted at each of the four sides of the lateral lines.

The spicular canal (Fig. 100) has been discussed at length by Looss and to his account I have little to add. The structure has been called by some authors the "spicular sheath" but as Looss clearly points out, it is a separate structure, and should therefore be given another name.

The canal appears on the dorsal side of the cloaca where two very small cuticular wings arise on either side of a small groove, which has a cuticular lining. The lining is covered with a granular layer of protoplasm which contains a few nuclei, three according to Looss. This single canal continues anteriorly at a sharp angle from the cloaca and shortly divides into two grooves into which the spicula project, their sheaths being firmly inserted on this structure; the cuticular lined canals continue but a short distance anteriorly.

THE NERVOUS SYSTEM.

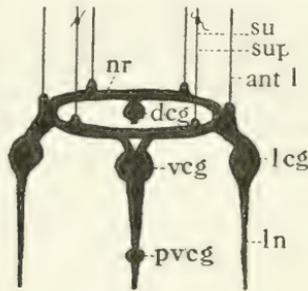
Comparatively few points are known and understood concerning the morphology of the nervous system of the nematodes as a

whole. The enormous mass of literature that has accumulated on the subject has concerned itself with the nervous system of *Ascaris megalocéphala* and *A. lumbricoides* and while it has contributed to the morphology of these systems, it has chiefly been concerned with the various problems in the neurone theory, cell constancy, etc., offering considerable information on these subjects. In addition to this, these workers (Apáthy 1908; Goldschmidt 1907, 1908, 1908a, 1909, 1910; Deineka 1908, 1912) have investigated the members of the genus *Ascaris* admittedly the highest specialized of the group, and many conditions are found in this genus and in particular these two species which appear in other nematodes in a much simpler and more elementary stage or are totally lacking.

It does not lie within the field of the present paper to consider these problems and no attempt has been made to present a review of the literature which has been so well done by de Rouville (1910, 1911). The work of Looss (1905), because of its strictly morphological character, will demand consideration and since it is really the only piece of work that is at all complete, reference will constantly be made to it.

One is struck in looking over this work with the apparent ease with which Looss was able to trace out the minute fibers and locate the most delicate connections and for all of this he gives no special technique; one must conclude that he did the work on material preserved in glycerol-alcohol and handled according to his method which is at best poor for working out the finer details. One cannot help but marvel when he reads of nerves which "are small and may consist at most of two or three fibers," or "the first fiber which leaves the ganglion," etc. Every method known has been tried to demonstrate the nervous system in *C. americanus* and it is only after many trials and piecing out gaps in each method that the conclusions about to be set forth have been obtained, which I regret to say are all too fragmentary and liable to error. If the finer connections, comparable to those described by Looss in *A. duodenale* really exist in my species, I have failed to locate them and have difficulty in believing that they are present in one species and not in the other, altho one is not surprised at even greater differences between *C. americanus* and the genus *Ascaris*. In one or two of the major connections are to be noted differences in my species and Looss' description.

Like most nematodes, there exists in this species a nerve ring, better referred to as the *cephalic commissure*. There are associated with this commissure twenty cells in each lateral half, in all forty cells, of which some may be adventitious, and the greater number are just anterior to the nerve ring. The rest are just below it, altho some are scattered in the subcuticula surrounding the esophagus. Some of these cells anterior to the nerve ring are found in groups in definite places. From these groups six nerves pass anteriorad, while two are laterals, two sub-dorsals and two sub-laterals. (See Textfig. I for diagram of the nervous system). A further word will dispose of



Textfigure I. Diagram of the cephalic part of the nervous system. *ant.l.*, *d.c.g.*, dorsal cervical ganglion; *l.c.g.*, lateral cephalic ganglion; *sub.l.*, anterior sub-lateral nerve; *sup.l.*, anterior supra-lateral nerve; *v.c.g.*, ventral cervical ganglion, *nr.*, nerve ring; *p.v.c.g.*, postventral cephalic ganglion.

these nerves; they supply the region anterior to the nerve ring, and each runs forward very close to the esophagus and supported by surrounding subcuticula. The two sub-ventrals have within their course near the anterior tip one nerve cell in each (Fig. 14), and these give out branches to supply the adjacent tissue. Nerves in *Ascaris* and *Ancylostoma* are found passing anteriorly also, but these go to papillae, none of which exist in *C. americanus* so that I refrain from homologizing these structures until more is known about them. Under the present condition I believe that the nomenclature for these nerves should be based upon their position alone.

Below the nerve ring one can recognize five ganglia which are called the dorsal cephalic ganglion, the ventral cephalic ganglion, the post-ventral ganglion and the two lateral ganglia. Commissures

connect the latter two with the nerve ring on the one hand and the ventral cephalic ganglion on the other. Longitudinal nerves arise from each ganglion.

As has been clearly pointed out by previous authors, the cephalic commissure, or nerve ring, is composed essentially of fibers which originate from the ganglia, hence this structure is not regarded as the fundamental part of the nervous system. In *C. americanus* the nerve ring (Figs. 3, 34, 52) is in direct contact with the esophagus all the way round and is contributed to by tissue from all four longitudinal lines, altho Looss maintains that the dorsal one plays no part in this formation in the hookworm. The fibers themselves are chiefly, if not entirely, from the ventral and lateral cephalic ganglia. The nerve ring is slightly oval in cross section and about $10\ \mu$ in diameter. The fibers are supported by loose tissue network of subcuticula origin.

The dorsal cephalic ganglion is the smallest of the anterior ganglia (Fig. 54) and is situated on the inner side of the dorsal longitudinal band just beneath the nerve ring. It consists of but three small cells which give rise to the dorsal nerve, which in turn continues posteriorly in the dorsal line.

The ventral cephalic ganglion is horse-shoe-shaped (Figs. 52, 54) and lies at a little more posterior level than the dorsal cephalic ganglion. From it pass two large masses of fibers to the nerve ring (Fig. 53), and posteriorly the collected fibers from the ganglion pass towards the tail in the ventral line. This ganglion consists of about twenty-five cells of varying sizes, each having a relatively large nucleus with a single nucleolus. Further, from this ganglion pass a right and a left commissure in the subcuticula to reach the two lateral cephalic ganglia.

The post-ventral cephalic ganglion appears a short distance posterior to the ventral cephalic ganglion and in the course of the ventral nerve (Fig. 55). As a matter of fact it appears at the opening of the excretory duct, lying immediately above it, and is composed of three or four small cells. Goldschmidt has described a similar ganglion within the course of the ventral nerve in *Ascaris* and these two are probably homologous.

The lateral cephalic ganglia (Figs. 35, 54) are by far the largest of the ganglia and are directly connected to the nerve ring, altho Looss failed to find such connections in *A. duodenale*. These ganglia begin

on about the level of the ring and gradually increase in size, occupying the inner margins of the lateral lines, even dipping in between their two halves. Posteriorly, they diminish in size until a few cells string out nearly to the level of the cervical papillae. The commissure to the ventral cephalic ganglion has already been mentioned. A nerve goes from each ganglion to the cephalic papillae on the same side and posteriorly the lateral nerves arise. I can be certain of only one on each side, and these continue caudad in the lateral lines, in the anterior region of the body, near the cuticula and in the mid-lateral line. There are about thirty cells in each ganglion.

At the hands of both Apáthy and Goldschmidt, the minute structure of the ganglionic cells in *Ascaris* have received special attention, and these authors have offered many interesting considerations, most of which involve physiological inferences and explanations which lie outside the present work. In brief, Goldschmidt has found that typically three zones exist in the ganglionic cells, an outer, middle and inner. The outer zone is made up of a rather coarse alveolar structure and borders the cell. The middle layer has smaller alveoli and is continuous with the nerve process. The inner zone, also alveolar in structure, lies immediately around the nucleus. Various modifications occur in the various cells of the ganglia so that in most of the bipolar cells one fails to recognize separate zones. Furthermore, one often notes that the borders of the alveoli are so arranged as to give the cell the appearance of being made up of radiating structures, and again this latter type of cell may be further modified to present a "central body" around the nucleus, which is continuous with the neurofibrills. Fibroid substance is present in varying amounts in all the cells.

While so complete a study of the histology of the nerve cells in *C. americanus* has not been made as Goldschmidt has done in the case of *Ascaris*, I have nevertheless satisfied myself that the conditions here, while essentially like those in this genus are not so complicated. These cells are small, the largest having a short axis of about 30μ and the structure is therefore hard to make out. In only a few instances could neurofibrills be recognized, but in good preparations one can see and follow for some distance the nerve processes of the cells. Figures 106 to 109 show several cells drawn from one of the lateral ganglia. In none of these cells have I been able to recognize

three distinct zones altho there is usually a thickened area around the nucleus. Figure 108 shows a central body and distinctly radiating protoplasm and the nerve fiber continuous with the central body, exactly as Goldschmidt has figured it for *Ascaris*. The other cells show beautiful alveolar structure but zones are not marked off. In the bipolar cells the alveoli are very small.

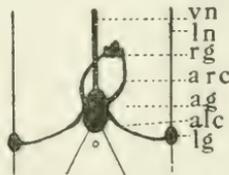
Within the cephalic ganglia has been noted only two types of cells, agreeing with Goldschmidt's unipolar and bipolar types. Perhaps multipolar cells exist also, but these have not been seen.

The longitudinal nerves are extremely difficult to trace thruout their courses and I cannot give a positive statement of their behavior except in certain regions. The dorsal nerve continues to the tail in the dorsal longitudinal line, supplies the somatic muscles with nerves and diminishes to an uneventful ending near the posterior end of the body.

The ventral nerve is the largest of all and can be found in the ventral line (Figs. 53, 119). This nerve also supplies the muscles of the body and in the posterior region enlarges greatly. Its ultimate fate will be considered in a special section.

I am not at all sure of the lateral longitudinal nerves, they are small and arise at the base of the lateral cephalic ganglia. Each gives a branch to its corresponding cervical papilla (Fig. 111) and continues posteriorly in the lateral lines. One cannot say whether these nerves are doubled or single thruout their courses. They become involved in the complicated structure of the caudal end of the body, which is so different in the two sexes as to demand separate consideration.

In the female as the ventral and lateral nerves approach the tail they become much thicker and here there is only one lateral nerve in each lateral line, carried near the excretory canal and taking its place in position when the excretory canal ends. (See textfigure J).

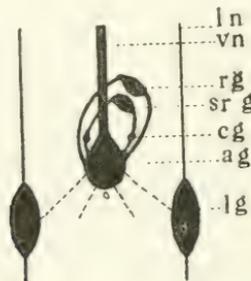


Textfigure J. Diagram of the caudal part of the nervous system of the female *a.g.*, anal ganglion; *a.l.c.*, anolumbar commissure; *a.r.c.*, anorectal commissure; *l.g.*, lumbar ganglion; *l.n.*, lateral nerve; *r.g.*, rectal ganglion; *v.n.*, ventral nerve.

Just anterior to the anus a ganglion appears in the ventral nerve and contains from four to five cells (Fig. 130). A relatively large commissure passes on either side from this, the anal ganglion, around the rectum to join with the rectal ganglion which lies on the dorsal side of the rectum a little anterior to the anal ganglion. This rectal ganglion has only three cells in it, a large median one and two smaller laterals.

There are right and left ganglia in each lateral nerve called the lumbar ganglia, each consisting of five or six cells and these lie a little posteriorly to the level of the anus on the inside margins of the lateral lines. A small commissure connects the lumbar with the anal ganglion. Nerves continue into the tail from each lumbar ganglion and two small nerves pass posteriorly from the anal ganglion, one on either side of the anus.

It will be noted that the caudal nervous system of the male very closely corresponds to the condition in *A. duodenale*, the most important difference, which is of no consequence, being that in the hook-worm there is a paired anal ganglion; in this respect it differs from the condition found in *Ascaris* and *C. americanus* (Textfig. K). The



Textfigure K. Diagram of the caudal part of the nervous system of the male. *a.g.*, anal ganglion; *c.g.*, cloacal ganglion; *l.g.*, lumbar ganglion; *l.n.*, lateral nerve; *r.g.*, rectal ganglion; *sr.g.*, sub-rectal gland; *vn.*, ventral nerve.

same statement might hold true in the case of the female as well. One other difference will be pointed out later on as regards the males.

While differences occur in the two sexes in respect to the nervous system in the caudal region of the body, they can still be homologized in most instances, if not entirely.

The ventral nerve becomes very much enlarged at the beginning of the region of differentiation in the caudal end of the body of the somatic muscles (Fig. 87) and here and there, branches from these caudal muscles bend over to the ventral nerve for their innervation. Just anterior to the ano-genital aperture there is found the anal ganglion (Fig. 92). In the males as in the females this ganglion is not paired, but in the former it is very much larger, for it contains about twice as many nuclei. From this ganglion I have been unable to trace nerves but two commissures were found. These arise very close together and pass dorsally (Fig. 99) and at the same time anteriorly, one of them assuming a more anterior position than the other. This one goes to the rectal ganglion (Fig. 98) which lies on the dorsal side of the rectum in the same relative position as the rectal ganglion in the female.

As in *A. duodenale* this ganglion seems to have been divided for another small one is found posterior to it and between the spicular canal and the rectum (Fig. 98). To this ganglion, the sub-rectal, the second commissure from the anal ganglion passes. In its course are found ganglia lying on either side of the cloaca, for this commissure passes obliquely around the cloaca, and since corresponding cells in *A. duodenale* have been called by Looss, the cloacal ganglia, the name for the structure here described will be retained.

In the lateral lines, beginning a little above the anus are found ganglionic cells in the course of the lateral nerves. At first these are but a few cells, but on passing backwards, the number of cells in each lateral line becomes greatly increased (Fig. 84), until below the anus there is a considerable mass of ganglionic cells, which continue almost to the tip of the tail. Nerves from these ganglia supply the ribs of the caudal region of the male. I have tried to find one or more commissures to the anal ganglion, for they surely must exist, but so far the attempt has been unsuccessful, for the region is very hard to study on account of the condition of the tail in preserved specimens, it usually rolls in such a manner as to preclude the possibility of getting good sections.

Looss has noted in *A. duodenale* three pairs of lateral ganglia in this region and he has termed them beginning anteriorly, the lumbar, postlumbar and costal ganglia, but since in *C. americanus* there is no definite division of the ganglia, I propose to call this pair in this

species, the lumbar ganglia, believing them to be homologous with those in the female and possibly with all three pairs in the case of *A. duodenlae*.

Nothing of especial interest happens in the case of the dorsal nerve in the caudal region of the males.

While sensory endings are more varied and of greater frequency in the free-living nematodes, they are by no means lacking in the parasitic species. Unfortunately no detailed study has been made on the free-living forms and only in the genus *Ascaris* has a careful and more or less complete description been given of the sensory endings in parasitic nematodes.

By position there are three groups of sensory endings in most nematodes, altho one or more, or perhaps all may be lacking in special cases. Typically then, are found papillae with sensory endings in the head region, a pair in the "neck" region in a lateral position and finally papillae in the caudal region of the male. So far as known there are no nerve endings in the caudal papillae of the females when such structures occur, but no statement is found in literature which points to a careful study of this point.

It is out of the place to enter into the discussion between Goldschmidt and Deineka and I have little to offer in support or against either of the two authors. In *C. americanus* the structures are all very much simpler and almost totally unlike those in *Ascaris*; in addition to this, there is the unfortunate fact that the structures are so small as to preclude the kind of work done by these two authors mentioned above. Since there are no "head" papillae in *C. americanus* one can omit a discussion of their work on the papillae of the lips of *Ascaris*; a few words will indicate some of the points brought out by Goldschmidt, since his work is very complete and differs from that of Deineka only in interpretation and a few minute points of anatomy which being so different in *C. americanus* do not concern this paper.

The cervical papillae are found laterally in *Ascaris* and do not project out into the exterior. They are rounded masses of subcuticula which have pushed up into the cuticula. In the lateral line, connected with these papillae, are found two cells, the Stützzellen and the Geleitzelle; the former contains the nerve, which in this case, before its end, has an enlargement, then it has a deeply staining swelling

and finally a very dark staining "Trichterplatte" and an end body for the very terminal portion of the nerve. The neurofibrillae do not pierce the covering of the body, the entire structure of the papillae being below the surface and covered by the cuticula.

In the anal papillae one sees a different structure in the genus *Ascaris*. Here the cuticula is projected out into a more or less pointed papilla and at its periphery is a minute hole leading into a canula which is a part of the Stützzelle and contains the nerve. The sensory apparatus consists of from one to three nervefibers which are very simple. There is a chromatic portion of the nerve near its periphery which stains deeply. The Geleitzelle is lacking.

Looss mentions the innervation of the cervical papillae in *A. duodenale* but has studied the entire structure very little. He said that the pulp of the organ was of subcuticula and that its nerve supply was from the post-lateral cervical ganglia. No details of the nerve endings were given. The innervation of the few papillae in the male tail he stated to be from the posterior ganglia. No details of their structure were given either.

Strictly speaking there are no true papillae in the tail of the male but the structures usually referred to under that name are really ribs, if one considers a papilla as a formation from the entire cuticula, while a rib is covered, except at its tip, by only the lower layer or layers of the cuticula.

In *C. americanus* the cuticula divides into two layers at the beginning of the lateral alae (Fig. 87), and as has been mentioned previously, the tubes are short, cylindrical ribs (Figs. 79, 87) which occupy certain positions extending between the two layers. These are tubes covered over by the lower layer of cuticula from which they can be seen to arise in Figure 87. Into these tubes the subcuticula extends.

At the periphery of such a tube is a minute canula which extends down into the tube one micron and is evidently a product of the subcuticula. Over the peripheral end of the tube of the lower layer of the cuticula, there extends the outer layer, so that this end of the rib becomes enclosed in a cap of outer cuticula.

The nerve endings in the papillae are interesting. They consist in each case of two minute fibrillae which run in the subcuticula very close to the inner sides of the tube and terminate in two chromatic

portions (Fig. 91), spherical in shape and lying at the base of the little canal. If, as in the case of the anal papillae of *Ascaris*, minute fibers run into the canula from the chromatic portion, I have been unable to trace them. Special supporting and accompanying cells for these papillae could not be found but their innervation is from the posterior ganglia. In short they seem to be combined ribs and papillae. All of these ribs have the same kind of structure except that the postanals and paraanals are shorter and thicker (Fig. 79). Usually the peripheral end of the tube is slightly dilated, this condition being more marked in the post- than in the pre- anal papillae.

The cervical papillae are but very slightly raised above the general contour of the body and are not exactly lateral, they being slightly dorsal to the middle of each lateral line.

The *pulp* of the papillae lies well embedded in the cuticula and is in the shape of a little knob, not perfectly smooth in outline, but slightly bulging, so that in sections one sees little swollen sides of the knob (Figs. 102, 110, 111). This knob is composed of subcuticula. Here again I have been unable to locate the two cells found by Goldschmidt in connection with this organ in *Ascaris*.

The nerve supply is from the lateral nerve (Fig. 111), a branch coming off anterior to the papilla and then running into it as a moderate sized bundle of fibers. These fibers end peripherally in a deeply staining mass at the termination of the knob. Set into this structure thru a hole in the cuticula is a minute spine which has its inner end directly above the nerve ending.

Here it would seem that the whole structure is remarkably simple and its mechanism is easier conceived than in the case of *Ascaris*, for in *C. americanus* there is a spine to communicate the stimuli to the nerve endings, while in the case of *Ascaris* it is necessary to assume that the stimuli are transmitted to the nerve endings thru the direct effect on the cuticula itself.

In most free-living nematodes the so-called amphids are found in a somewhat similar position to the lateral cervical papillae in the parasitic species and it would be decidedly worth while to study these two structures in a comparative way to see if the two are homologous.

IV. YOUNG FEMALES

In the examination of turtles for *C. americanus* only twelve young individuals have been found. These are all females and are near the same age if the lengths of the bodies, thickness and conditions of the genital organs are indications of their ages.

They are about of the same proportions as the adults so far as the body size is concerned and each has already the three spines (Fig. 7) on the tip of the tail. The esophagus is divided into two regions and the "bridge cell" is present (Fig. 56). The intestine shows some pigment and the tail is about 0.1 mm. long. The ovijector can be seen near the middle of the body and from it stretch out, as two arms, the beginnings of the genital organs. The oral apparatus is present, light yellowish-brown in color and somewhat different from the adult, the details of which will be taken up later.

Seven individuals have been studied in regards to certain measurements which appear in Table IV.

It will be seen that these specimens range in length from 3.3 mm. to 4.7 mm. As they increase in length the diameter of the body also increases so that the smallest one has its greatest diameter 0.1 mm. which was taken a little anterior to the fundement of the ovijector, the longest worm is 0.14 mm. thick. It will be seen at once that the ratio between the length and the thickness is not exactly constant but very nearly so, which is not the case in the adults. In these young worms the ratio is about 1:33. It would seem therefore as if the mature worms grow longer faster than they grow wider, while in the young forms both dimensions grow alike. Undoubtedly the development of the embryos within the uterus accounts for the difference to a great extent, so in the males the development of the testis explains this, since elongation is probably more easily accomplished than enlargement in diameter.

The average of the lengths of the anterior region of the esophagus is 0.36 mm. which is not greatly below that of the adult females, while in two individuals this region of the esophagus is as long as in some adults. In other words this region is almost grown in individuals in which the oral apparatus is not yet completely formed and the reproductive organs are extremely immature.

The average length of the second region of the esophagus is also but little shorter than in the case of adults, but here again one finds

in certain individuals that this region is already as large as in some adults. The average length of these younger forms is 0.46 mm. That the esophagus develops early and to its maximum length is clearly shown in the case of this species.

While the lengths of the two regions of the esophagus compare very favorably with those of the adults, the thickness of this organ is less in the younger individuals as would be expected, since the body width is so much less. The greatest thickness of the anterior region of the esophagus in the younger forms is about 0.055 mm. while in the average of the adults it is about twice as great. The growth then comes in the diameter of the organ rather than its length. It would be interesting to know if there is a correlation between the two but the material at hand is not abundant enough to study this point.

Another very interesting ratio is that between the post- and prevulvar regions. It will be recalled that in the smallest females which were mature, the prevulvar region was longer than the postvulvar region. This is also true in the case of every one of the immature individuals, so that the ratio between the two regions is, in the worm 3.3 mm. long, 1:1.2 and this ratio diminishes with a fair degree of regularity, considering the few specimens, to a ratio of 1:1.13. The ratio between the two regions in the youngest mature female is 1:1.1 which is in series with the ratios of the immature specimens. This demonstrates that at first, even in the very young specimens the prevulvar region is greater in length and that as the female grows the postvulvar region outgrows the anterior portion of the worm. This seems to be due not merely to the presence of the growing embryos, but rather to the growth of other tissue as well, since the same thing is noted for the individuals without embryos.

Since the structure of most parts of these young forms is essentially like that of the adults and so much detail has been given for the latter, only those organs which differ greatly will be discussed here. Under the section on the cuticula of the adult, that of the young form has been treated so that there remains but to take up the oral apparatus and the genital organs; of these the latter will be considered first.

IV. MEASUREMENTS OF YOUNG FEMALES

No.	Body length	Body thickness	Length anterior portion esophagus	Length posterior portion esophagus	Length prevulvar region	Length postvulvar region	Ratio prevulvar: postvulvar lengths
1*	3.3	0.10	0.36	0.41	1.8	1.5	1:1.20
2*	3.5	0.10	0.34	0.46	1.9	1.6	1:1.18
3*	3.7	0.11	0.33	0.44	2.0	1.7	1:1.17
4	4.0	0.12	0.39	0.48	2.1	1.9	1:1.11
5	4.2	0.13	0.36	0.49	2.3	1.9	1:1.12
6	4.7	0.14	0.36	0.45	2.5	2.2	1:1.13
7*	4.7	0.14	0.40	0.50	2.5	2.2	1:1.13

*Without dorsal and ventral spikes (tridents?) on oral apparatus

Female reproductive organs. The reproductive organs are in the shape of the letter T with one arm bent over after a short distance and running back parallel to the cross bar. The T slants posteriorly (Fig. 9). The upright of the T is represented by a small cavity surrounded by cells, lined near its base with an extremely thin layer of cuticula. This portion (Figs. 69, 124) is the ovijector and in the longest forms at hand is about 70 μ and 25 μ wide. In the shorter specimens it is about as long as wide. There are as yet no projection of the cuticula in this region, so that the typical vulva does not exist, and there is no opening of the organ to the exterior.

In sections this ovijector shows that it is composed of two layers of cells (Fig. 69) just as the corresponding region of the adults, but the inner layer here has at first more than four nuclei in cross section, differing therein from the condition found in adults. Since by position this region is the vestibule and is later heavily lined with cuticula, the suggestion is here made that these cells in the younger specimens give rise to this lining. Mediad (Fig. 70) this condition changes so that there are but four cells seen in cross section as in the case of the adult and the circular musculature is quite well represented. This is the region of the vagina and "trompe," which passes into a very small tube of cells running off in two strings, one passing anteriad and the other posteriad.

These tubes do not have a muscular layer and the tubular condition is not very evident after a short distance (Fig. 85) for the tube is so small and the cells so large that they fill in the cavity. The nuclei are especially large as compared with the total size of the cells.

Posteriorly this string of cells continues to a little over half way between the vulva and the posterior tip of the body and must be the foundation of the posterior uterine branch. The branch which passes anteriorly represents the future anterior uterine branch, the oviduct and the ovary. About 0.6 mm. from the vulva it makes a sudden turn and then runs posteriorly to within a short distance of its origin. This condition was noted in every individual and leads me to believe that the region from the ovijector to the point of the turn is the foundation of the anterior uterus, for in the adult there always occurs a turn at the end of the uterus and the oviduct passes posteriorly. The rest of the string of cells is a little thicker but with no differentiation in it. A muscular layer of the oviduct could not be found in this material.

The mouth apparatus. Altho some features of the oral apparatus as it exists in the young individuals have been considered earlier in the paper, there are other points which call for description here.

In general the same plan of construction is present in the young worms (Fig. 4) as in the older ones, but in the former the structures are much smaller and more delicate. Table V gives comparative figures for both.

V. MEASUREMENTS OF ORAL APPARATUS

PARTS MEASURED	YOUNG FEMALES	OLD FEMALES
Length.....	0.070 mm.	0.105 mm.
Width.....	0.080	0.160
Thickness outer layer.....	0.007	0.010
Thickness inner layer.....	0.004	0.005
Height of teeth.....	0.004	0.005
Diameter of the ring.....	0.050	0.100
Extension of ring below valves.....	0.030	0.017

The shape and structure of the lateral valves is almost identical with that of the adults. They are of a slightly lighter color but have as many longitudinal ridges as in the adults (Fig. 16). The two layers which have been mentioned before are demonstrable by staining reactions and these are slightly thinner than in the case of adults and the ridges are not quite so high. The two valves are united along their dorsal and ventral margins (Fig. 16, 19) and as in the case of the adults they are in contact with the cuticula.

The four giant cells are developed at this stage and apparently can function, for the worms are as tightly attached in proportion to their size to the mucosa of the host's intestine as are the adults.

The anterior wings (Fig. 15) are well developed but are smaller than in the adults and essentially like them. Whether the valves covers exist or not could not be ascertained from the material at hand.

Two striking differences exist in this young oral apparatus, the first of which is the condition of the ring. It will be noted by reference to the table that while the ring is only half so large in diameter, it is twice as broad as in the adults, that is, it extends further posteriorly from the margin of the valves. It must be remembered that the esophagus is narrower in these young forms and the appearance in the adults is as tho it had pushed out the margin of the ring when it expanded.

The second characteristic of this mouth apparatus is the absence of the tridents. In some of the individuals the dorsal and ventral margins of the valves are perfectly smooth while in others there projects out a small process from a point just below the union of their anterior margins. These projections vary in length in different specimens, the longest being 5μ (Fig. 4). No indication of a cleft condition has been found and in all individuals they appear as single spikes. The significance of this will be discussed in the next section of the paper.

Discussion. From a study of the material at hand it is not wise to state positively the stage of the larvae which have just been described. However, the majority of facts indicate that they are, as yet, in the fourth stage, following the nomenclature of Maupas (1899). That is to say, they will moult again in the intestine of the host, just as do the hookworms.

There are some minor objections to this hypothesis; no indication has been seen in any of the individuals which suggests the preparation for the final moult. No deposits appear beneath the cuticula, and the coverings of these forms corresponds to the outer layer of the cuticula of the adults, as has been previously stated. Further, no indication of the formation of a second or final oral apparatus has been noted and the series of dorsal and ventral projections suggests the start of the tridents, which by further growth would develop into

the adult structure. Finally in no individuals found has anything been seen which would suggest a moult within the intestine of the host.

At the same time there is no positive proof that another moult will not take place. The failure to find intermediate stages is of course not sufficient ground for assuming that there will be no further development, and since the individuals are all so near the same condition of development, one would hardly expect to find different phases among them.

Up to the present time the most conclusive evidence for considering these forms as being in the fourth stage, is in the first place, the growth of the oral apparatus; in particular its growth in outside dimensions, is very difficult to explain. Before the form reaches maturity that organ will have to grow to twice the size it is in the young specimens. The second point in the evidence is found in the condition of the genital organs. The fact that no opening to the exterior has been found is very strong proof that this form is not yet in the fifth stage, especially since the vulva is absent and the ovijector is so extremely undeveloped. Until more material is found a conclusion on this point cannot be reached.

Considering all the information given in this paper on the morphology of the adults and the young, it becomes evident that this is a very important nematode species. In the first place this form is from a water host, and members of the genus *Camallanus* are found parasitic in host of three phyla. The hosts are from among the fishes batrachians and reptiles, all animals inhabiting water for the greater part of their lives, and species of this genus have been reported from both fresh- and salt-water hosts, in Europe, Africa and America.

In the next place this particular species is not only a member of the genus *Camallanus* but is in the great superfamily *Spiruroidea*, one of the most interesting and fundamental groups which exists. Upon the correct interpretation and description of its members will depend in a great measure the future knowledge of nematodes, especially from a systematic standpoint for this family clearly consists of a group of intermediate species. The divided condition of the esophagus and the two lateral lipped condition certainly constitutes one of the fundamental and important divisions of the *Nematoda*.

The nematode parasites of these water hosts as a rule show characters which more closely resemble the free-living species, than the parasites of the strictly land hosts. In this connection, a full discussion of the significance of the divided esophagus and the condition of the excretory system has been given in the paper and no further word is necessary here.

Again, the simplicity of the special endings of the nervous system indicates primitive conditions. Not only are these endings in themselves very simple, but they are connected to the exterior while in *Ascaris* this is not the case. The rest of the nervous system, while complete in the essentials, is much simpler than in the case of the higher species of nematodes, as for example in *Ascaris*.

Not only does the condition of the excretory system and the function of the large cells in the esophagus suggest a relationship with the family Trichotrachelidae, which certainly are not very advanced above the free-living state, but the reproductive organs of the female are very much like those of this family. Even in the viviparousness of the family the genus *Camallanus* resembles it.

V. THE GENUS CAMALLANUS RAILLIET AND HENRY 1915

The name *Camallanus* was introduced by Railliet and Henry in 1915 as a designation for the genus *Cucullanus* of some recent previous authors which was not the original genus *Cucullanus* of Müller 1777.

Cucullanus was created by Müller to include two species, parasitic in the intestine of the cod, which he named *C. cirratus* and *C. muticus*, and later united in one species which he called *C. marinus*. Railliet and Henry have not considered these two names as referring to one species, thus they have come to select the species *cirratus*, because it was first named, as the type of the original genus. Dujardin (1845) gave the name of *Dacnitis* to certain members of the genus *Cucullanus*, but by the law of priority his name is no longer in good standing. Since members of this genus are distinct from those in the *Cucullanus* of Dujardin and subsequent authors, Railliet and Henry have given them a new name, that of *Camallanus* (*camallus*, a hood). The characters of this genus are stated by these authors as follows:

Camallanus Railliet and Henry 1915 (*Cucullanus* Auct., non Müller 1777). Polymyarians (Schneider), secernantes (Linstow). Cuticula finely transversely striated. Body usually red, obtuse anteriorly, more attenuated posteriorly. Head with two dorsoventral valves, limited by a mouth which is a transverse slit and an elliptical buccal capsule at its entrance; rounded behind, where the internal walls present longitudinal parallel ridges, usually terminating at the margin of the mouth in the form of small teeth. Behind this buccal capsule is a chitinous apparatus in the shape of two transverse bands united into a sort of band (apophysis Rud.); this part, on each side has a trident, diverging posteriorly, of which the lateral branches serve for the insertion of muscles to move the buccal valves. The valves are terminated by a circular "bourrelet" (pharynx Duj.) at its entrance into the esophagus. In general the esophagus is formed in two portions; the anterior muscular and clear, and the posterior glandular, more opaque and swollen.

Males with recurved and inrolled tails, carrying caudal alae which project a little and have a variable number of riblike papillae. A single spiculum, sometimes accompanied by a very small accessory piece.

Females larger, tails straight and conical, sometimes with two subterminal lateral papillae. Vulva projecting from the middle of the body. Viviparous.

In the development, an intermediate host functions.

Habitat: The adults live in the intestine or stomach of fishes, batrachians and reptiles. The larvae have been found in the body of crustaceans (Copepoda) or the larvae of aquatic insects, sometimes in the eyes of fishes.

Type *Cucullanus elegans* Zeder 1800 = *Echinorhynchus lacustris* Zoega 1776.

From the very careful study made of *Camallanus americanus*, another species of this genus sent from Africa by Seurat and placed at my disposal, and the two species, *Camallanus ancyloDIRUS* and *Camallanus oxycephalus* described jointly with Professor Ward, the author is able to correct somewhat at length the generic description given by Railliet and Henry. The errors in their account are the following:

- i. The valves are lateral and not dorso-ventral.
- ii. The mouth apparatus is not a buccal capsule, but is composed of a pair of jaws, which indicate an origin from a lipped-condition.
- iii. Two transverse bars, the so-called apophysis, do not exist.
- iv. No muscles are inserted on the lateral branches of the trident.
- v. The *bourrelet* is the esophageal cap.
- vi. The second region of the esophagus is not glandular in the true sense of the word.
- vii. There are two spicula in the males and no accessory piece.
- viii. Some females have terminal papillae.
- ix. The vulva is not always in the middle of the body, but may be a little anterior or posterior to it.

As a result the generic description should read:

Cuticula finely transversely striated. Worms usually appear reddish; obtuse anteriorly, more attenuated posteriorly. Mouth an elliptical dorso-ventral slit; oral cavity bounded by two lateral, pecten-shell-shaped valves united posteriorly along dorsal and ventral margins; internal walls present longitudinal posteriorly converging ridges, usually terminating at oral margin in small tooth-like spines. Valves united posteriorly at bases; a circular band-like ring is joined to valve bases covering the esophageal cap. Valves supported by two sets of dorsal and ventral prongs, usually three in each set, extending into the cuticula from valve joints. Two pairs of jaw muscles extending from the anterior valve margins to the cuticula posterior to the oral apparatus. Esophagus divided into two regions, anterior muscular, transparent; posterior opaque, probably excretory in function.

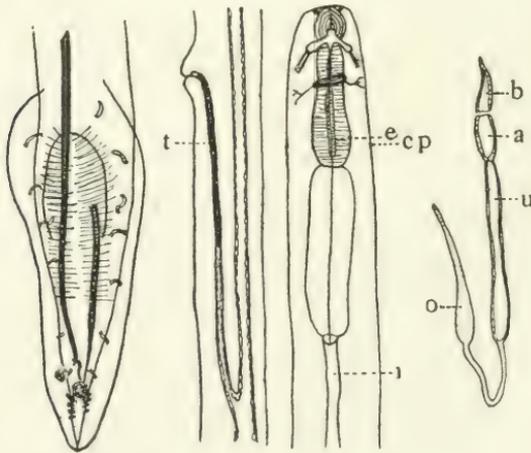
Males with recurved tails, inrolled and carrying lateral caudal alae supported by ribs. Two spicula, acuminous.

Females larger than males; tails, straight, conical, sometimes bearing minute papillae. Vulva projecting, on ventral side near middle of body; one ovary. Viviparous.

In the genus *Camallanus* Railliet and Henry have placed eleven species. Two others are usually considered as belonging to this group of worms but these authors do not include them in their revision of the genus. Two species have since been described by Ward and Magath (1917) and the form under consideration in the present paper adds still another.

The French authors already referred to in this paper have placed the genus in the superfamily Spiruroidea Railliet and Henry 1915 and in the family Camallanidae Railliet and Henry 1915. At present this position seems to be satisfactory.

In the past the descriptions of the members of this genus have been so brief that little information can be found in them save a few measurements; many forms will therefore have to be placed ultimately among species inquirendae. The species discussed in this paper suggests very strongly a close relationship with three others which have been previously described. One of these is a form recently discussed by Seurat (1915a) under the name *Cucullanus microcephalus* Duj. On comparing this description with that of Dujardin one is forced to say that the two forms are not the same if indeed one can identify any form from the latter's description. I therefore propose to call Seurat's material *Camallanus seurati* (Textfig. L) in honor of



Textfigure L (See page 168)

its discoverer. By a close analysis of the facts certain points of difference come out of a study of the species *C. americanus*, *C. seurati*, *C. trispinosus* and *C. microcephalus*.

II. TABLE OF COMPARISONS

ORGAN	<i>C. americanus</i> Magath		<i>C. seurati</i> Magath		<i>C. trispinosus</i> Leidy	
	Male	Female	Male	Female	Male	Female
Mouth apparatus						
Length.....	0.089	0.105	0.110	0.140		
Width.....	0.120	0.160	0.160	0.180		
Prong length.....	0.080	0.105	0.110	0.140		
Number ridges.....	10 or 12				16	
Female tail	Three spines		Bifid		Three spines	
Female genital organs- lengths	1.3-2.1 mm.		3.1 mm.			
Ovijector.....	2.0-2.7 mm.		1.5 mm.			
Ovary.....	1.9...3.5 mm.		1.3 mm.			
Ration, ovary: ovi- duct.....	1:1.0-0.8		1:1.1			
Ova.....	24x25 μ		77x80 μ			
Spicula						
Right.....	870 μ		840 μ		450 μ	
Left.....	310 μ		420 μ		120 μ	
Embellishment.....	Single curved prong, 75 μ from from tip		Shaped like "ar- dillon d'hame- con," 60 μ to tip			

By reference to the descriptions one can make the following comparisons between *C. americanus* and *C. seurati*:

i. The size of the mouth apparatus is not the same in the two species. A great deal of importance should be attributed to this fact, since the structure is so constant in individuals of the same species. In *C. seurati* this structure is on the whole rather larger than in *C. americanus*.

ii. The female tail is bifid in the former species and terminates in three spines in the latter.

iii. The ovijector is longer in *C. seurati*, and the ovary and oviduct are shorter.

iv. The ova are much greater in size in *C. seurati*.

v. While the right spiculum is perhaps about the same length in the two species, the left one is not, that in *C. seurati* being a third longer. On the right spiculum there is an embellishment, which, in

the American species is, 75μ from the tip and a simple spine in shape; in *C. seurati* the embellishment is 60μ from the tip and in the shape of a battle axe.

There is but one item which can be compared with the new species and *C. microcephalus* as described by Dujardin. This is the size of the head, which is given by him as being 0.1 mm. in the female and 0.09 mm. in the males. While this agrees with the length of the mouth apparatus in the new species it is by no means certain that Dujardin meant to give this measurement for the length of the valves in his species. Since there are no other data which are characteristic, I regard his form as a species inquirenda.

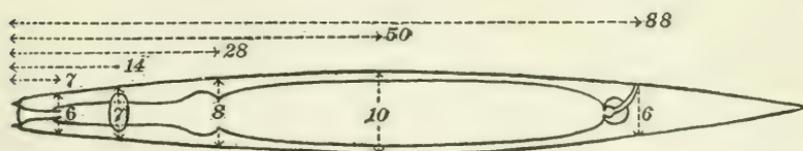
Mention should be made here that Diesing (1851) adds to the description of Dujardin's species the fact that there are three spines on the end of the female tail, but if the spines are as large as those in *C. americanus* it is hard to see how the earlier author failed to see them. It is by no means certain that Diesing had the same species that Dujardin described.

The third species is *C. trispinosus* Leidy (1851). Here again the description is so meager that one is forced to consider this as a species inquirenda for no one can identify with certainty this form from the data at hand. Leidy gave the length of the spicula as being 450μ and 120μ , which would be far too small for *C. americanus*, but the three spines on the end of the tail of the female is a little suggestive. He states definitely that there are eight ridges on either side of a medium on each valve, while there are only five or six in the case of *C. americanus*.

VI. NEMATODE MEASUREMENTS

Perhaps no one factor has contributed more to the chaotic condition which now exists in systematic nematode literature than the fact that authors have been content in the past to describe species by giving a few measurements and a comment or two concerning the general size and shape of some of the more prominent organs. The utter folly of describing these complex forms with a running stereotyped characterization was pointed out by Looss (1911), who stated that the trouble lay chiefly in the "way in which the new species are described." He further urged that more stress be laid upon the descriptions of parts and comparative features in nematodes.

It is not surprising that under the existing conditions some one undertook to work out a scheme for classification based upon measurements. Cobb (1890) proposed the first and only definite scheme of this kind. He devised (Textfig. M) a formula to show two kinds of



Textfigure M (See page 168)

measurements, absolute and relative, first the length of the worm and its thickness at certain points in millimeters, and secondly the percentage of that length which is represented by the distance from the anterior tip to definite points in the body. It is clear that this formula is acceptable for a species only if during the process of growth all parts increase so as to retain the original proportions, for if this is not the case then at a given age the worms will yield a different formula than at other ages. While Cobb has used the formula for over a quarter of a century he has never attempted to defend it against the occasional criticisms of other authors, further he has never recognized that there might be some chance for unequal growth in different regions.

Fracker (1914), who confined his discussion to the parasitic nematodes, published a criticism of the formula which cast considerable reflection upon it. It is not out of place here to call attention to the fact that the table given by this author is not arranged with the animals according to size, so that, since no specific summary of the table is made, a little difficulty arises in interpreting the figures. However, he concludes that in *Oxyuris vermicularis* that "the locality of the cephalic parts of the alimentary canal tend to vary from 1 to 4 per cent., about one-third of the maximum." He also found a variation of 15% in the location of the vulva, 7% in the anus and variation in the total length and width of the body.

Looss in the work already referred to, shows quite plainly by a short table of measurements how futile it would be to try to identify *Uncinaria criniformis* or *Uncinaria polaris* on measurements alone,

and suggests in these cases that while the relative position of the genital aperture in the female is in some degree constant during individual growth, this is true in other species in the same genus, and is therefore a generic and not a specific character. All the other comparative figures vary during growth and would be of no earthly value either in the description of the species or of the genus; he has given the proportions of the length of the body to that of the esophagus, the length of the tail to the body, and the prevulvar to the postvulvar section. On the other hand he points out that the absolute length of the esophagus is fairly constant, as is also the length of the spicula and the length of the female tail, as these parts do not grow much, if at all after maturity is reached or even before. Thus he shows that in general, proportional figures are of little value, especially when they are involved in the length or thickness of the body, and that only a few organs will yield absolute measurements which can be relied upon. The points to be decided then are, which organs will yield such useful facts, how far they can be applied, and whether they are generic or specific in their compass. Such a decision will be of value because it will furnish some reliable measure or will eliminate unreliable ones, which can never do anything more than confuse literature.

In order to test the formula system further and to see just how much weight should be placed on measurements, the author undertook a somewhat extended set of observations on individuals of *C. americanus*. For this purpose twenty females and seventeen males, picked at random from among several hundreds, have been measured and the ratios and curves worked out with a view of seeing just how much variation of parts occurs and which measurements could be relied upon. All of these worms were handled in the same manner and mounted in damar, so that the errors due to technique should be about the same in each case. Of course the number used is not very great but there can be little doubt but that, while the actual figures would be changed somewhat if more individuals had been used, the same general conclusions would be reached.*

* The tables of these measurements and the plots of curves are not published in this paper on account of the limited space. They are, however, on file in the Library of the University of Illinois, Urbana, Ill., and the Department of Zoology, University of Illinois.

(1) That an enormous variation occurs in the individuals of *Camallanus americanus* becomes evident from a study of the tables and curves. While the table includes the longest male found, the longest females are not included; the longest one ever obtained measured 30.9 mm., which if included, would give a much higher percentage of variation than is indicated in the table. Each one of the females has many embryos in the uteri, so that they must all be considered as mature females and to give a single measure for the length of the species would be extremely incorrect. The same holds true for the males altho they reach a maximum length earlier, for no embryos complicate the length factor here. Thus it appears that if the length of this species is to be given in its description, it should be accompanied with a full statement of how it was obtained, the stage to which it applies, etc., since the length variations are over 100%.

(2) While it also appears that as the worms increase in length they increase in thickness, yet one ratio will not express the relation in all individuals, since the increase in width does not parallel the increase in length; a variation of 69.4% in the females and 53.8% in the males was recorded.

(3) The size of the oral apparatus has been discussed at length elsewhere in the paper and does not appear in the table, for that region is so constant in size in both males and females that practically no difference can be detected between individuals of one sex. A ratio between the length of this structure and the length of the body or the width of each would be of no significance, since it would vary so greatly.

(4) The importance of the esophagus from a systematic and functional standpoint has been pointed out in the paper, but here it is fitting to note that this organ presents a remarkable constancy in absolute length and thickness in each sex. There seems to be no tendency for the anterior region of this organ to increase at all in length after perhaps the time of the assumption of the definitive stage, for extremely young males and females have an esophagus as long as in the older individuals, but there is some slight tendency for an increase in diameter, especially up to the time of maturity from the fourth stage. With the growth of the animals the second region of the esophagus elongates a little, not greatly and not very consistently. The data in the table shows the absolute length of the

esophagus given for any individual may vary but 11% on either side of the average, but that a ratio between it and the length of the body varies 110.5% in the females and 69.9% in males. Accordingly, in this species at least, the esophagus obtains its final length early in life and this is especially true of the anterior region. The ratio of the first or anterior portion of the esophagus to the posterior portion varies a little more in the males than females.

(5) As the females get older and more embryos accumulate and grow in the uterus, that organ is enormously stretched and tends to fill the body cavity. As it grows the posterior horn is pushed down into the tail region; by comparison it is noted that the distance between the posterior tip of the uterus and the tip of the tail lessens as more embryos develop. This distance is, in turn, somewhat inversely proportional to the total length of the body, so that, the length of this space is, roughly speaking, an indication of the age of the worm.

(6) The length of the female tail, that is, the distance of the anus from the extreme posterior tip, is often given as a diagnostic point. In this species there is a great variation in the item. The tail grows as well as the rest of the body but not in the same proportion. Its absolute length varies 77.2% while in ratio with the length of the body a variation of 48.6% is found.

(7) As females get older the ratio between the pre- and postvulvar region varies 50.0%, the prevulvar portion being more constant in length than the postvulvar region, this latter tending to overgrow in the large individuals. It will be noted that in the young females the postvulvar region is shorter, while in the older ones this region surpasses the length of the anterior part. Without doubt the factor governing this to a great extent is the development of the embryos.

(8) It is seen that the length of the caudal alae increases with the length of the body, but that this increase is not so rapid in the caudal alae, so that a ratio between the two in no way expresses the relationship for all males, nor does the average indicate the true state of affairs.

(9) It is unfortunate that so few measurements of the spicula could be obtained, and that it is impossible to measure the length of the left spiculum in toto mounts. However, it will be seen that

very little difference in length was found in the right one, with no tendency for increase with the length of the body, the variation in length, therefore, is individual. The author has dissected out many spicula of both sides in worms varying greatly in size, and they always give a length of very nearly 870μ and 310μ . It therefore appears that these structures reach a full growth early in the development of these worms and do not grow later in life. A ratio between them and the body length would naturally vary considerably.

From these considerations it becomes evident that there are few if any ratios commonly given that will distinguish individuals of this species, for the range of individual variation is so great as to overlap many other species, and then too, no one set of ratios is even approximately correct or accurate for this species. The absolute length of the anterior region of the esophagus is reasonably constant, but as will be pointed out later, does not distinguish other worms of this genus. The absolute lengths, thicknesses, etc., of the mouth apparatus and the spicula are very constant and will be shown to be specific. Lengths, widths and ratios of other parts of the body are misleading and inaccurate within this species.

In connection with this work it is interesting to compare the two tables of measurements given by Breinl (1913) for a group of individuals of *Onchocerca gibsoni*. One can see from his tables that there is even more individual variation than in *C. americanus* since the esophagus and spicula vary considerably. The ratios between the parts like those taken in *C. americanus* show in the same way no close relation between the growth of the different parts, such as the length of the body and body thickness, length of the esophagus, spicular lengths, etc.

From a detailed study of the tables of measurements made from the records of previous authors and the tables already referred to of *C. americanus* the following conclusions seem justifiable:

(1) The measurements which exist are nearly as incomplete as the descriptions of the species themselves. If the facts in the case are as in *C. americanus* these measurements will not differentiate the species from one another. Even when several measurements are at hand, some species could not be differentiated from others. In the cases of *A. duodenale* and *A. conepti* there is proof for this statement. Only when the accurate descriptions of these two species are known

can they be separated, for no major differences are noted in the measurements of several of the most important organs. It is plain that two spicula might have the same length, yet one may be heavy and the other filiform, while one may be straight and the other spirally bent, conditions which would hardly justify classifying them within the same species.

(2) Tho the tables are incomplete, there seems to be a tendency for the esophagus to be fairly constant within a genus, but here and there are found exceptions. In a few cases the amount of variation found in individuals within a species has been noted, and here it can be seen that this variation will include, to a great extent, that within the genus.

(3) As in the case of the single genus *Camallanus*, so also with other genera, the lengths of the spicula furnish the best single characteristic. If a gubernaculum is present, its size adds additional specific information.

(4) The size of the eggs is of little or no value in the general separation of either species or genera, as the variation within a species overlaps that within a genus or even a family. Diagnosis of human parasites by observation of the eggs, e.g., in the feces, is entirely practical, for here the number of species involved is relatively small and the eggs are recognized not by the size alone, but also by their peculiar characteristics, such as the rough shell of *Ascaris*, the plugs at the poles in *Trichuris*, etc.

(5) It has been shown in the case of the species discussed in the paper, that ratios of parts and most of the absolute measurements are quite misleading in a great many respects. Cobb has used in his formula ratios and absolute lengths and thicknesses, maintaining that the value of these lay in having a number of measurements and not, as some authors give, a single item. Since it has now been shown that organs vary so greatly, a formula for one individual in a species will be as totally different from that of another as it will be from a formula of an individual in another species (Fracker 1914). In practically all the cases given in the table where this variation has been indicated there is as much individual variation in a single species as there is in different species within a genus. Altho organs may increase in thickness as they do in length, the ratio is by no means

constant, and hence these ratios could not be used to designate species.

(6) It must be admitted, therefore, that the lengths and thicknesses of organs are of little value in systematic descriptions of nematodes, and if such measurements are excluded there remains only *the accurate morphological description of every organ and part of the form in question*. If this kind of information is collected there can be no doubt that it will yield results, as it has in the organization of other parasitic groups. Because of the uniformity in structure the nematodes constitute a difficult division of the animal kingdom for study, and much has yet to be done before their structures are well known as those of other parasitic divisions. Characters which are most constant in individuals should receive the greatest attention and these are usually found in the anterior and posterior regions of the body. The internal organs should of course not be overlooked, tho interest in them does not lie in measurements but in their chemical characters and morphological structure.

VII. THE CLASSIFICATION OF PARASITIC NEMATODES

In the past only one broad classification of parasitic nematodes has been offered to the attention of zoologists generally, and it was proposed by A. Schneider in 1866. He grouped the nematodes into three main divisions including certain genera under each, and omitting all other subdivisions. In free translation his classification is as follows:

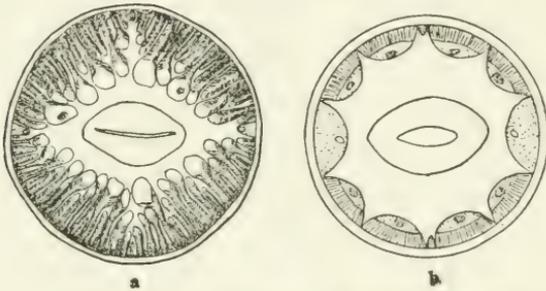
A. *Polomyarii*. Many somatic muscles are seen around the body wall in cross section. In this group are included the following genera: *Ascaris*, *Eustrongylus*, *Enoplus*, *Physaloptera*, *Heterakis*, *Filaria*, *Ancyracanthus*, *Hedruris*, *Ceratospira* and *Cucullanus* (*Camallanus*).

B. *Meromyarii*. Muscles of the body built up of eight longitudinal rows. Under this division are placed *Nematoxys*, *Oxysoma*, *Oxyuris*, *Labiduris*, *Dermatoxys*, *Atractis*, *Spiroxis*, *Strongylus*, *Pelodera*, and *Leptodera*.

C. *Holomyarii*. The muscles of the body not divided, or divided only by lateral bands. *Anguilla*, *Trichina* (*Trichinella*), *Trichosoma*, *Pseudalius*, *Ichyonema*, *Mermis*, *Gordius* and *Sphaerularia*.

Bütschli (1873) showed in the forms grouped as *Holomyarii* there is a separation of the ventral musculature due to the presence of the ventral nerve cord, while he and others have shown that in the other

members of the group one can find the typical longitudinal bands. As all of these members have many muscle cells in each quadrant they were placed in the first division and thus only two groups have resulted from Schneider's three (Textfig. N). However, this is not



Textfigure N. Diagrammatic representation to illustrate the muscle cell arrangement described by Schneider. *a*, Polymyarii, *b*, Meromyarii. The lateral, dorsal and ventral bands are indicated.

the only objection to the system. *Gordius* has no place in such a classification since it is not a member of the Class Nematoda. A very heterogenous mixture resulted from the combination of the first and last groups and even in the original scheme, worms which usually have been regarded as members of the same family or superfamily are further removed from each other than from those of different superfamilies or even tribes. Thus the genera *Heterakis*, *Ascaris* and *Oxyuris* are separated from each other altho *Mermis* and *Filaria* are associated with *Ascaris*. There is no regard for any general external feature in this classification and it is conceded by most systematists that external features are very important in the separation of groups in most cases. Further it is evident that the structure of important organs of the nematodes is not considered by Schneider. In a recent work Hall (1916) names three families under the superfamily Strongyloidea, two of which, Strongylidae and Trichostrongylidae, include Meromyarian forms while the third, Metastrongylidae, represents a Polymyarian group. He further described the superfamily Ascaroidea in which the Ascaridae and Heterakidae represent Polymyarian species while the Oxyuridae are Meromyarians. On the basis of Schneider's divisions the Oxyuridae, Strongylidae and Trichostrongylidae would be grouped together, while the Ascaridae, Heterakidae

and Metastrongylidae would belong to the group of Polymyarii. It would be absurd to think of such a grouping on the basis of present knowledge of these families. Many other objections can be pointed out and it is evident that this method of classification is not only not practical but is entirely artificial.

The following scheme for classifying nematodes was suggested by von Linstow (1897):

I. Sererentes. Along each side a lateral field with slender basis which broadens centrad and spreads out over the muscles; in one or both fields a longitudinal vessel that empties forward in an excretory pore located in the ventral line. The species live mostly in the alimentary system when sexually mature, or are free-living. The lateral fields function as kidneys. Species in the following genera are included: *Ascaris*, *Physaloptera*, *Cheiracanthus*, *Lecaocephalus*, *Heterakis*, *Cucullanus* (*Camallanus*), *Sclerostomum*, *Peritrachelius*, *Ancryacanthus*, *Dacnitis* (*Cucullanus*), *Spiroptera*, *Spiroptenina*, *Leptosomatum*, *Oxyuris*, *Oxysoma*, *Nematoxys*, *Strongylus*, *Anchylostomum* (*Ancylostoma*) and *Trichina* (*Trichinella*).

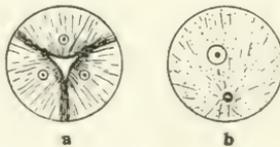
II. Resorbentes. The lateral lines are broad fields, at times one-sixth the entire circumference of the body; they have the same thickness as the muscles and carry no vessels; the excretory pore is lacking; the lateral fields appear to have an absorptive function. The species when mature do not live in the alimentary canal of their hosts. Here are included *Filaria*, *Filaroides*, *Dispharagus*, *Dracunculus*, *Eustrongylus*, *Ichthyonema*, *Pseudalius* and *Angiostomum*.

III. Pleuromyarii. In the lateral lines stand muscles; esophageal lumen often a narrow chitinous tube, in some genera the intestine is entirely lacking. *Trichosoma*, *Trichocephalus*, (*Trichuris*), *Gordius*, *Nectonema*, *Mermis*, and *Echinorhynchus*.

There are several objections to this grouping. In the first place the basis of classification is purely artificial and not practical, on account of the difficulty of preparing sections to demonstrate the facts in question. The separation of *Trichnella* from *Trichuris* is of course unwarranted. The last group is a grand mixture. *Gordius* and the *Acanthocephala* are not *Nematoda* and have no place in such a division. *Nectonema* should not be considered here either. The whole system and grouping is of very little value and should not be used in the future.

In recent times certain French zoologists have been studying this group of parasites, creating many superfamilies which they divide into families, subfamilies, genera and subgenera. So far very little description of these divisions has been offered and while these investigators have brought to light many interesting and important facts, it is yet too early to accept their conclusions as final.

The last general division of the Nematoda has been made by Ward (1917), who divided them into two great divisions, the Trichosyringata and the Myosyringata. He defines the former as follows: With the esophagus of the capillary type, consisting of "a row of cells pierced thruout the entire length by a delicate tube of minute caliber." Functionally it is evident that this type of esophagus is adapted for the passage of fluids, which must flow into the esophagus without its aid, for no musculature has been demonstrated which could assist in the process. He has termed the second group, the Myosyringata, having a pronounced muscular esophagus, with the fibers contracting transversely to the long axis of the body. "The esophagus is tripartite in cross section" and functions for the taking in of food by opening up a lumen lined with cuticula and triangular in cross section when open. This causes a powerful suction and draws in fluids or solid food. This grouping included in the Myosyringata all the families of the nematodes save the Trichotrachelidae and the Mermithidae, which are placed in the Trichosyringata (Textfig. O).



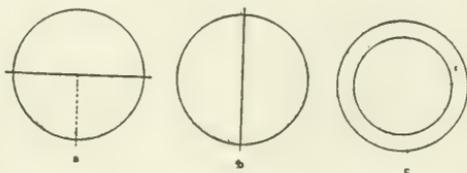
Textfigure O. Diagrammatic representation illustrating the basis of Ward's nematode divisions. *a*, Myosyringata type of esophagus in cross section. *b*, Trichosyringata type of esophagus in cross section.

Many authors have suggested in the morphological descriptions of nematodes the importance of the esophagus and it has been stated that as a class, the Nematoda have a tripartite muscular esophagus shaped like a club. This statement is not entirely true for there are several forms known in which the esophagus is not of this character

but as has been mentioned before, is of the capillary tube type. As yet there is no very good explanation as to how these two types have arisen, but some very suggestive facts have been learned which at a later date may help to clear up the matter. However this be, it is certainly true that for purposes of classification these two types are easily distinguished and furnish a natural and logical division of the class. The author has pointed out some of the important features of the esophagus and it is very fitting that this structure should be selected as the primary basis for the separation of the nematode orders. For the subsequent division of these groups a great deal of investigation will be necessary, yet at the present time some suggestions are not out of place.

Since the smaller group contains forms of such varied nature there will be no difficulty in finding characters which will differentiate groups among them. For example, there are forms in which the males have but one spicule, while others have two, some in which the males have but one testis. Again there are forms which have bacillary bands, etc. These are more or less radical departures from the usual types and careful study will, without doubt, separate out groups very easily. Since the *Myosyringata* contain the majority of nematodes and these are more nearly alike each other than the *Trichosyringata*, the subdivisions here will be harder to make, all the more so when the morphological data are so scanty.

Several possibilities have presented themselves, one of which seems to be particularly fundamental, and uses the oral apparatus for the separation of groups. It has been⁸⁵ shown that at least three distinct conditions exist which are as follows (Textfig. P.):



Textfigure P. Diagrammatic representation of the three fundamental types of oral parts which have been demonstrated for certain nematodes belonging to the *Myosyringata*. *a*, the dorsalventral type, *b*, the lateral type, and *c*, the circular or true buccal capsule type.

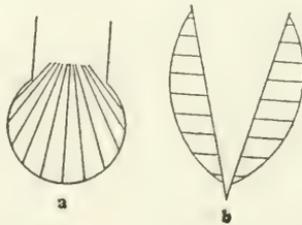
1. A mouth built up, on the dorso-ventral plan. An example of this is found in *Ascaris*, where there are three lips, but so placed that one is dorsal and two ventral and that the division between the lips for the mouth opening extends from right to left.

2. Lateral mouth parts, of which the genus *Camallanus* furnishes an interesting case. Here the whole structure is devised on the lateral plan, there being two lateral lips or jaws, with the mouth as a dorso-ventral slit. It is obvious that the two types are radically different and can be readily distinguished.

3. In the last type the mouth is arranged on the circular plan. Here there is a true buccal capsule which is perhaps best understood in the hookworms. The mouth parts of *Camallanus* are therefore fundamentally different from those of the group to which the hookworms belong and from a functional standpoint the mouth parts of the former are really jaws.

Physiologically there seems to be some difference between these three types. The first are munchers, the second grasping forms and the last suckers. Other groups based on different conditions of the oral parts will be found when more species are carefully studied.

A great deal of confusion has resulted from the loose usage of the terms "bursa," "alae" and "wings." The caudal end of the male is often modified so that there exists an expansion of cuticula enclosing the whole posterior end. This is supported by cuticular tubes filled with muscles, which radiate outwards like the spread out fingers of the hand (Textfig. Q). For this structure the term bursa



Textfigure Q. Diagrammatic representation to show the difference between a "bursa" and "wings" at the posterior end of certain male nematodes. Note that in *a*, the bursa is supported by *rays* which arise from a common locus, while in *b*, the wings are supported by *ribs* which have separate origins.

should be used. In other forms the cuticula is split laterally forming narrow wings, which are supported by small ribs of cuticula, or the wings may meet medially on the ventral side, the median wall of each wing breaking down and leaving the space open all the way across. However in either case the supporting structures arise from separate places along the body wall. These forms should be stated to possess wings or alae. Still other male nematodes have no modification of the posterior cuticula save the presence of papillae, while some may not even have these. Thus at least four subdivisions are possible on the basis of differences in the male tail. It is also important to note that corresponding differences are to be noted in the morphology of the vulva of the females which must be accommodated to the characteristics of the male tail of that species.

Not only does the esophagus furnish a good organ upon which to base the first separation of groups, but it also furnishes possibilities for further subdivision. Ward and Magath (1917) have pointed out some of the possibilities and at present at least four distinct types are known. The first of these consists of the simple muscular esophagus without any modifications; in the second type the esophagus has a bulbous enlargement on its posterior end; in the third type one finds ceca attached to the posterior end and these may be associated with ceca from the intestine. Several different varieties may be distinguished according to whether the ceca point anteriorly, posteriorly etc. The fourth type of esophagus modification occurs in those cases in which one finds regional differentiations in the esophagus, which may take the form of granular portions, septal divisions, etc. Other types probably exist but lack of information of a morphological character necessitates this point being left open.

Generic and specific classification will not be such a great task once the larger divisions are made, because one deals in the nematodes with so many different organs and variations of these organs. It is not the author's intention to point out these items at the present time, but the careful study of the exact morphological details of many species of nematodes will result in as firm a basis of classification of this group of animals as exists in any other group.

VIII. SUMMARY AND CONCLUSIONS

1. The material used for this study was *Camallanus americanus* nov. spec., found in the small intestine of turtles of the following species and in different parts of the United States of America: *Chlydra serpentina*, *Chrysemys marginata*, *picta*, *scripta*, *troosti* and *elegans*, *Malacoclemmys lesuerri* and *Aromochelys odoratus*. The percentage of infection is nearly eighty and most turtles yield about fifteen to twenty parasites. They have not been found in the few soft-shelled turtles examined.

2. The description of the genus *Camallanus* Railliet and Henry 1915 is corrected and amended according to new facts learned.

3. *Camallanus americanus* is distinguished from the most closely related species by the size of the hard parts and their shape, by conditions in the female reproductive system and by the female tail. These are the only points that can be compared since these are all that are given by previous authors. A nematode called by Seurat "*C. microcephalus*" has been shown to be a new species and is named *Camallanus seurati*.

4. Evidence is given to demonstrate the inadequacy of nematode ratios as distinguishing features. Some absolute lengths seem to be specific, others tend to embrace the whole genus. Descriptions of nematodes based on a few measurements and ratios of organs are valueless. Measurements are secondary to the careful description of the parts of the worms and only in the case of the hard parts can one place any confidence in absolute lengths or thicknesses. Cobb's nematode formula is fallacious, at least as regards the parasitic species. The esophagus obtains a maximum length early in life, but later grows somewhat in thickness. The members of the genus *Camallanus* are poorly described and many cannot be identified from the descriptions given since they contain only a few measurements and little of fundamental morphological description.

5. The morphology of every system of *Camallanus americanus* has been studied and given in detail.

(a) The cuticula is uninteresting morphologically but from a chemical standpoint presents a number of important problems. It has been shown that the cuticula is not chitin but cornein, an albuminoid probably related to the supportive tissue proteins of other animals.

(b) The structure of the subcuticula and the longitudinal lines has been given in detail; the "filling in" tissue of the anterior end is not a ligament as Looss supposed, but represents the anterior mass of the subcuticula, which supports the nervous structure of the anterior end and perhaps forms the oral apparatus. Here there is cell constancy.

(c) The function of excretion is undoubtedly divided between the lateral lines, canals and the posterior portion of the esophagus. There is a single bridge cell which is in contact with the accessory tissue around the esophagus.

(d) The somatic musculature is of the type designated by Schneider as Polymyarii, but is on the dividing line between the types called Platymyaria and Coelomyaria. Part of the somatic muscles of the ventral half of the caudal end of the male are modified for helping in the act of copulation. They pull up the ventrum of the body thus drawing together the two caudal alae, which in turn grip the projecting vulva of the female.

(e) The intestinal muscle cells are described for the male and female, and the musculus ani for the female. Their mechanisms are given.

(f) The mouth apparatus is built on the lateral plan. It is opened by two pairs of muscles, large and modified from the somatic muscle cells.

(g) The esophagus is divided into two portions; the anterior muscular and the posterior granular and probably excretory. Cell-constancy perhaps exists. The dorsal esophageal gland has a single nucleus and the excretory tissue of the esophagus has two very large nuclei. There is an esophageal gland.

(h) The intestine is typical and has in it a great deal of pigment. There is little doubt but that this pigment is the result of some stage in the metabolism of blood of the host.

(i) Details of the rectum and the cloaca are given. Looss' position taken as regards the rectal cells and his so-called "rectal ligament" is not accepted.

6. The chief food of this species is the blood of the host.

7. The red color of the worms is due to the color of the body fluid; it is without doubt some product from the host's blood. Tissue acting like mesenteries to hold the organs in place is present.

8. The reproductive system of the female is interesting. Only the anterior ovary and oviduct are developed and the latter contains a seminal receptacle. The species is viviparous and the uteri of the adults are filled with embryos. Some of these are already contained within their first skin which is shed a short time after birth.

9. Two unequal, acuminous spicula exist in the males and the typical nematode male reproductive organs are present. The spermatozoa in the seminal receptacle are oriented with their long axes parallel to the long axis of the oviduct. Circular muscles are around the ductus ejaculatorius and this region probably secretes a carrying fluid for the spermatozoa.

10. The nervous system is simple and like most nematodes, consists of a nerve ring, anterior ganglia, longitudinal nerves and posterior ganglia. The anal ganglion is simple and in the male there are large posterior lateral ganglia which supply the anal papillae or ribs. These ganglia are most likely homologous with the three pairs of posterior lateral ganglia in *Ancylostoma*.

11. The innervation of the two lateral cervical papillae and the alar ribs of the male have been studied and the details of the nervous endings given. Both are connected to the exterior by special structures.

12. A few young females have been found in the several examinations made for these parasites. They are probably in the fourth stage, and differ from the adults in three essential respects, viz., the condition of the cuticula, the oral apparatus and the genital organs. In them the vulva is not indicated and the opening to the exterior is not yet effected.

13. The importance of the species is pointed out and the members of the superfamily Spiruroidea are given a place between the free-living forms and the Trichosyringata, on the one hand, and the higher forms such as the ascarids on the other. They are therefore very important and are mostly parasites of water hosts.

14. Possibilities for the future classification of the Nematoda are shown and Ward's fundamental divisions, the Trichosyringata and Myosyringata, are accepted. His secondary division, based on the condition of the oral parts is deemed logical and natural.

15. Nematode classification cannot hope to make a great advance, however, until more species are accurately and minutely described.

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LIST OF ABBREVIATIONS

<i>ac t</i>	accessory excretory around the esophagus	<i>l e c</i>	lateral excretory canal
<i>a g</i>	anal ganglion	<i>l g</i>	lumbar ganglion
<i>a-g o</i>	ano-genital opening	<i>l l</i>	lateral line
<i>al</i>	caudal alae	<i>l n</i>	lateral nerve
<i>an</i>	anus	<i>l sp</i>	left spiculum
<i>ant e</i>	anterior portion of the esophagus	<i>l v</i>	lateral valve of the oral apparatus
<i>ant u</i>	anterior uterine branch	<i>m</i>	ordinary muscle cells of the esophagus
<i>a sv c</i>	anterior subventral nerve cell	<i>m a</i>	musculus ani
<i>b c</i>	excretory bridge cell	<i>m a n</i>	musculus ani, nucleus
<i>c c</i>	carrying cell of the excretory duct	<i>m c</i>	somatic muscle cell
<i>c g</i>	cloacal ganglion	<i>m l</i>	muscular layer of the ovijector
<i>cl</i>	cloaca	<i>m m</i>	marginal muscle of the esophagus
<i>c p</i>	cervical papilla	<i>m n</i>	nucleus from the spermatozoon
<i>c t</i>	cone tissue, posterior portion of the esophagus	<i>n r</i>	nerve ring
<i>d e</i>	ductus ejaculatorius	<i>n r c</i>	nerve ring cells
<i>d l</i>	dorsal line	<i>n z</i>	nutritional zone
<i>d n o</i>	origin of the dorsal nerve	<i>o</i>	ovum
<i>e d</i>	excretory duct	<i>ob m</i>	oblique muscles of the tail of the male
<i>e g</i>	dorsal esophageal gland	<i>od</i>	oviduct
<i>e g n</i>	dorsal esophageal gland nucleus	<i>o dk</i>	outer dark layer
<i>e m</i>	extensor muscle of the spiculum	<i>o l</i>	outer light layer
<i>e n</i>	posterior portion of the esophageal excretory tissue nucleus	<i>ov</i>	ovary
<i>e v</i>	esophageal valve	<i>ov j</i>	ovijector
<i>f</i>	nucleus of the ovum	<i>p-a p</i>	para-anal papilla
<i>fb</i>	fibrillar portion of muscle cell	<i>p b</i>	polar body
<i>f i m</i>	fibrous tube enclosing the intestinal muscles	<i>p j</i>	projections from the lining of the seminal receptacle
<i>g</i>	genital fundement	<i>po-a p</i>	post-anal papilla
<i>g m</i>	giant muscle cell of the oral valve	<i>post e</i>	posterior portion of the esophagus
<i>i</i>	intestine	<i>post u</i>	posterior uterine branch
<i>i dk</i>	inner dark layer	<i>pr-a p</i>	pre-anal papilla
<i>i l</i>	inner light layer	<i>p v c g</i>	post ventral cervical ganglion
<i>i m</i>	intestinal muscle	<i>p v l</i>	projections from the somatic muscle cells to the ventral line
<i>i m n</i>	intestinal muscle nucleus	<i>rach</i>	rachis
<i>i sph</i>	intestinal sphincter	<i>rec</i>	rectum
<i>l c</i>	lateral commissure	<i>rec c</i>	rectal cells
<i>l c g</i>	lateral cephalic ganglion		

<i>rec l</i>	rectal lining	<i>s v</i>	seminal vesicle
<i>r es</i>	esophageal cap or the ring of the oral apparatus	<i>td</i>	trident
<i>r g</i>	rectal ganglion	<i>tr</i>	trompe
<i>r m</i>	retractor muscles	<i>u</i>	uterus
<i>r sp</i>	right spiculum	<i>v</i>	vestibule
<i>sb t</i>	subcuticular tissue, "filling-in tissue"	<i>vc</i>	valve cover of the oral apparatus
<i>sc</i>	sarcoplasmic portion of a somatic muscle cell	<i>vc g</i>	ventral cervical ganglion
<i>se</i>	esophageal sphincter	<i>vc g c</i>	ventral cervical ganglion cell
<i>s il</i>	spindle cells lining the ovijector	<i>vl</i>	ventral line
<i>sp</i>	spiculum	<i>vul</i>	vulva
<i>sp c</i>	spicular canal	<i>vv</i>	vulva valve
<i>sperm</i>	spermatozoa	<i>w</i>	anterior wing of the oral apparatus
<i>sph</i>	sphincter		tus
<i>sr</i>	seminal receptacle	<i>x</i>	shedding skin of the first stage
<i>sr g</i>	sub-rectal gland	<i>z</i>	juncture of the anterior and posterior portions of the esophagus

EXPLANATION OF FIGURES

PLATE VII*

- Fig. 1. Lateral view of the oral apparatus of a male.
 Fig. 2. Dorsal view of the oral apparatus of a female.
 Fig. 3. Anterior end of a male, lateral view.
 Fig. 4. Anterior end of a young female, lateral view.
 Fig. 5. Vulva of an adult female, lateral view. The uterus is filled with living embryos.
 Fig. 6. Posterior end of an adult male. Only the right spiculum is shown.
 Fig. 7. Posterior end of a young female, immature. Lateral view.
 Fig. 8. Posterior end of an adult female. Lateral view.
 Fig. 9. Vulval region of an immature female.
 Fig. 10. Larva of the first stage in the uterus of the female. Note the four anterior nuclei.
 Fig. 11. Head of a larva beginning the fourth stage. Drawn from life, note the four anterior nuclei. The reference line is $10\ \mu$ long.

PLATE VIII†

- Fig. 12. Transverse section thru the anterior portion of the oral apparatus.
 Fig. 13. Transverse section thru the middle of the oral apparatus, section in series with figure 12.
 Fig. 14. Transverse section thru the ring of the oral apparatus and in series with figures 12 and 13.
 Fig. 15. Transverse section thru the anterior tip of the oral apparatus of an immature female.
 Fig. 16. Transverse section thru the middle of the oral apparatus of an immature female and in series with figure 15.
 Fig. 17. Detail of the angle of the oral valves of an adult.
 Fig. 18. The trident, drawn from a dissection, medial view.
 Fig. 19. Detail of the angle of the oral valves of an immature female.
 Fig. 20. The valve cover, drawn from a dissection.
 Fig. 21. A spindle cell from the lining of the ovijector.
 Fig. 22. The anterior wings of the oral apparatus, drawn from a dissection, slightly displaced.
 Fig. 23. The anterior wings of the oral apparatus, drawn from a dissection.
 Fig. 24. The ring of the oral apparatus, drawn from a dissection.
 Fig. 25. Structure of a somatic muscle cell from the anterior half of the body, transverse section.
 Fig. 26. Longitudinal section of the cuticula of an adult.
 Fig. 27. Transverse section of the cuticula of an adult.

* Each line on this plate represents a length of $40\ \mu$, except in Figure 11

† Each line on this plate represents a length of $10\ \mu$.

Fig. 28. Structure of a somatic muscle cell from the posterior half of the body, transverse section.

Fig. 29. Outline of a longitudinal section of the cuticula, to show the striations.

Fig. 30. Transverse section of the anterior portion of the esophagus.

Fig. 31. Transverse section of the posterior portion of the esophagus.

Fig. 32. Longitudinal section thru the cuticula of an immature female.

Fig. 33. Transverse section of the posterior portion of the esophagus, thru the excretory tissue nuclei.

PLATE IX*

Fig. 34. Sagittal section thru the anterior portion of the body.

Fig. 35. Frontal section thru the anterior portion of the body.

Fig. 36. Transverse section thru the esophageal valve.

Fig. 37. The two excretory nuclei and gland cell nucleus of the esophagus.

Fig. 38. Longitudinal section thru the lower end of the esophagus and upper end of the intestine.

Fig. 39. Transverse section thru the anterior cone of tissue in the posterior portion of the esophagus.

Fig. 40. Longitudinal section thru the juncture of the anterior and posterior portions of the esophagus.

Figs. 41-45. Transverse sections of the lining of the anterior portion of the esophagus, in order passing posteriadly. Figure 41 is at the extreme anterior end and figure 45 at the extreme posterior end.

PLATE X†

Figs. 46-55. Series of transverse sections from the same worm, beginning with the first section just at the anterior level of the esophagus. The last figure in the series is figure 51, which is thru the bridge cell. The figures are arranged in sequence on the plate but not in regard to the numbering.

Fig. 56. Transverse section thru the bridge cell of an immature female.

Fig. 57. Transverse section thru lateral line and lateral excretory canal.

PLATE XI‡

Fig. 58. Transverse section thru the vulval valve.

Fig. 59. Longitudinal section thru the ovijector.

Fig. 60. Transverse section thru the vulva.

Fig. 61. Transverse section thru the ovijector near the uterus.

Fig. 62. Transverse section thru the ovijector sphincter at the point where it turns outward.

Fig. 63. Sagittal section thru the vulva.

Fig. 64. Longitudinal section thru the juncture of the oviduct with the uterus

* Each line on this plate represents a length of $10\ \mu$, except in Figure 38 where it is $40\ \mu$ long.

† Each line on this plate represents a length of $20\ \mu$.

‡ Each line on this plate represents a length of $10\ \mu$, except in Figures 65, 66, 67 where it is $100\ \mu$ long.

Fig. 65. Outline of the beginning of the ovijector and the uterus.

Fig. 66. Outline of the anterior portion of the reproductive organs of a female.

Fig. 67. Outline of the posterior ending of the female reproductive organs.

Fig. 68. Fertilization of the ovum.

Fig. 69. Transverse section of the anterior region of the ovijector of an immature female; the beginning of the sphincter.

Fig. 70. Middle region of the ovijector of an immature female, in transverse section.

Fig. 71. An uterine wall cell.

Fig. 72. Transverse section of the ovijector; middle region.

Fig. 73. Transverse section of the zone of "growth."

Fig. 74. Transverse section of the germ zone.

PLATE XII*

Fig. 75. Longitudinal section thru the seminal receptacle.

Fig. 76. Oblique section thru the oviduct.

Fig. 77. Oblique section below the anus of a male.

Fig. 78. Transverse section thru the seminal receptacle.

Fig. 79. Transverse section thru the ano-genital opening of a male.

Fig. 80. Transverse section thru the middle of the body of a male.

Fig. 81. Transverse section thru the ductus ejaculatorius.

Fig. 82. Spermatozoa.

Fig. 83. Transverse section of the anterior region of the testis.

Fig. 84. Sagittal section thru the posterior region of the body of a male.

Fig. 85. Transverse section of the reproductive organ in an immature female.

Fig. 86. Transverse section at the insertion of the retractor muscle on the head of the right spiculum.

Fig. 87. Transverse section thru the posterior portion of the body of a male.

Fig. 88. Transverse section thru the right spiculum, below the head.

Fig. 89. Transverse section thru the left spiculum below the head.

Fig. 90. Transverse section thru the head of the right spiculum.

Fig. 91. Detail of the external end of a preanal papilla.

Fig. 92. Sagittal section thru the posterior portion of the body of a male and thru the juncture of the digestive and reproductive tracts.

PLATE XIII†

Fig. 93. Distal end of the right spiculum, from a dissection.

Fig. 94. The right spiculum, from a dissection.

Fig. 95. The left spiculum, from a dissection.

Fig. 96. The right spiculum within its sheath, from a dissection.

* Each line on this plate represents a length of 10μ , except in Figures 84, 87 and 92 where it is 20μ long.

† The lines for Figures 93-101 represent a length of 20μ , those for Figs. 102-112, a length of 10μ .

- Fig. 97. Transverse section thru the posterior region of a male, above the region of the caudal alae.
- Fig. 98. Sagittal section thru the posterior region of a male.
- Fig. 99. Oblique frontal section thru the posterior region of a male.
- Fig. 100. Oblique frontal section thru the posterior region of a male and passing thru the spicular canal.
- Fig. 101. Outline of the anterior ending of the testis.
- Fig. 102. Detail of the cephalic papilla to show the entrance of the nerve. The section is longitudinal.
- Fig. 103. Longitudinal section thru the right spiculum, proximal end.
- Fig. 104. Transverse section of the seminal vesicle.
- Fig. 105. Longitudinal section thru the retractor muscle of the spiculum to show the type of nucleus.
- Figs. 106-109. Details of the nerve cells from the lateral cephalic ganglion.
- Fig. 110. Detail of the lateral cephalic papilla, showing the chromatin ending of the nerve and the "trigger."
- Fig. 111. Longitudinal section thru the posterior portion of the lateral cephalic ganglion and the cervical papilla.
- Fig. 112. The juncture of the seminal vesicle and the ductus ejaculatorius; longitudinal section.

PLATE XIV*

- Fig. 113. Sagittal section thru the posterior portion of the body of a female, passing thru the anus.
- Fig. 114. Sagittal section of the posterior region of the body of a female and passing thru the middle of the rectum.
- Fig. 115. Transverse section thru the intestine of a mature female.
- Fig. 116. Sagittal section thru the posterior region of the body of a female, and passing thru the anus. Almost a median section.
- Fig. 117. Transverse section in the post-anal region of a female.
- Fig. 118. Transverse section thru the posterior third of the body of a female.
- Fig. 119. Transverse section thru the musculus ani of a female.
- Fig. 120. Transverse section of the same worm as figured in figure 118 and somewhat posterior to the latter figure.
- Figs. 121-122. Transverse sections thru larvae of the last part of the first phase, in the uterus of the female.
- Fig. 123. Transverse section posterior to figure 120, and from the same worm.
- Fig. 124. Transverse section thru the ovijector of an immature female.
- Figs. 125-128. Transverse sections in series thru the rectal sphincter region.
- Fig. 129. Transverse section thru the nucleus of the intestinal muscles of a female.
- Figs. 130-131. Two sections in series passing thru the rectum of the same female as figure 118.

* Each line on this plate represents a length of 10μ , except in Figures 113, 114, 116, where it is 40μ long.

PLATE XV

Fig. 132. Longitudinal section thru the oral apparatus of *C. americanus*, showing the mode of attachment in the intestine of the turtle. Photomicrograph.

Fig. 133. Transverse section thru the oral apparatus of *C. americanus*, showing the mode of attachment to the intestine of the turtle. Photomicrograph.

Textfigure F. Illustrating Perrier's principle of the action of the lateral valves of *Camallanus*. After Perrier (1872).

PLATE XVI

Figure 134. Redrawn from Seurat. *C. seurati*; *a*, distal region of the anterior uterine branch; *b*, terminal region of the posterior uterine branch; *c*, excretory pore; *i*, intestine; *o*, ovary; *p*, postcervical papilla; *t*, "trompe"; *u*, region of the uterus which is occupied by the eggs.

Textfigure O. Cobb's nematode formula (Redrawn from Cobb), 6, 7, 8, 10, 6 are the transverse measurements, while 7, 14, 28, 50, 88 are the corresponding longitudinal measurements. The formula in this case is:—

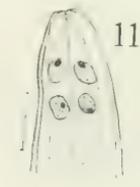
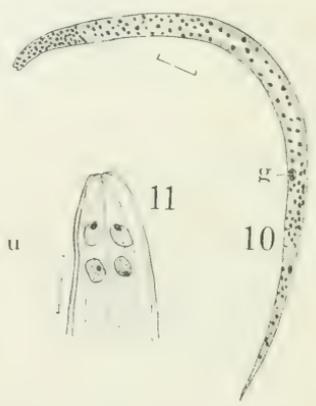
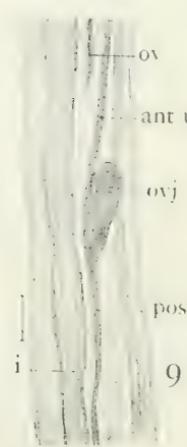
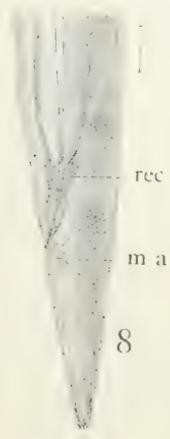
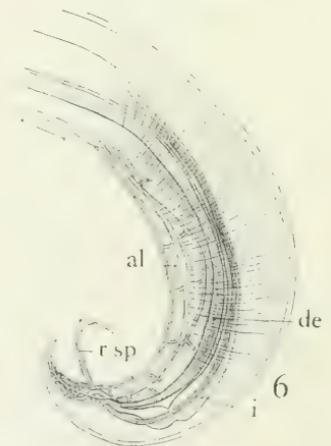
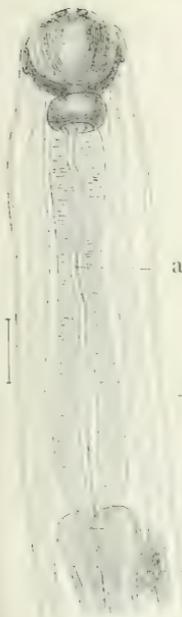
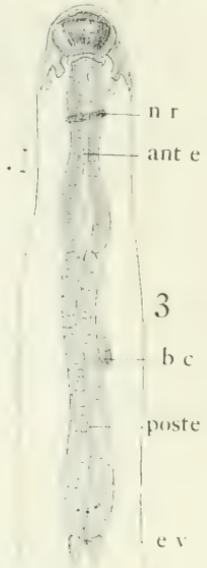
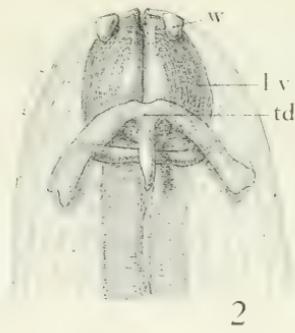
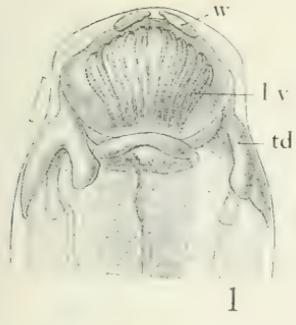
7. 14. 28. 50. 88.

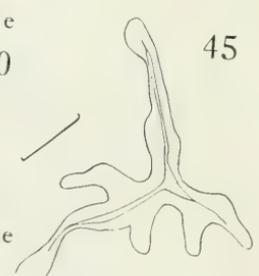
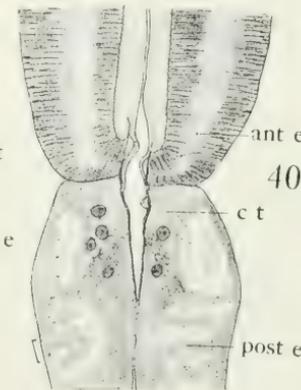
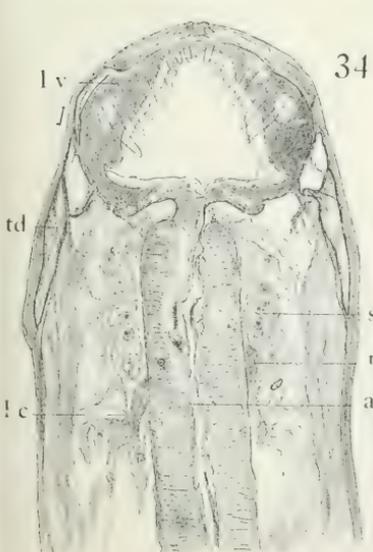
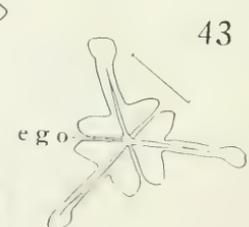
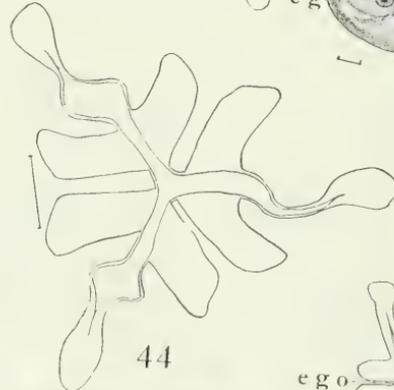
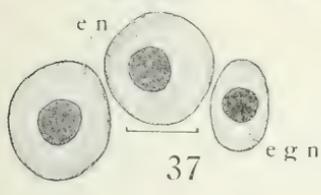
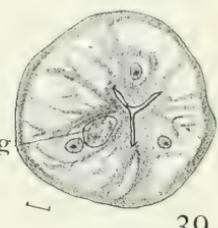
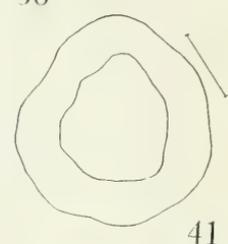
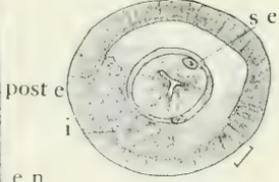
6. 7. 8. 10. 6.

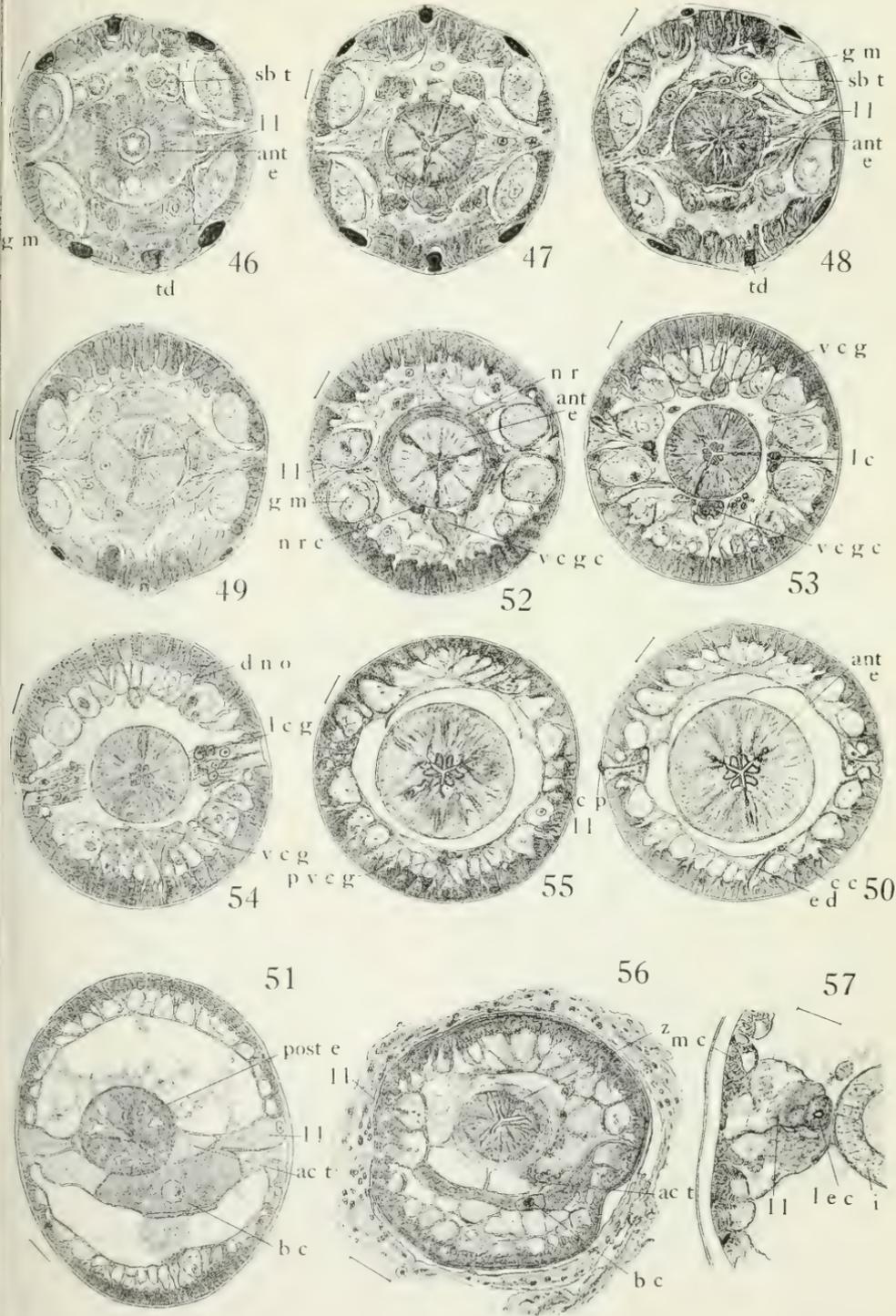
The unite of measurement is the hundredth part of the length of the worm, whatever that may be. The measurements become, therefore, percentages of the length. The measurements are taken with the animal viewed in profile; the first is taken at the base of the pharynx, the second at the nerve-ring, the third at the cardiac constriction, the fourth at the vulva in females and at the middle (*M*) in males, the fifth at the anus.

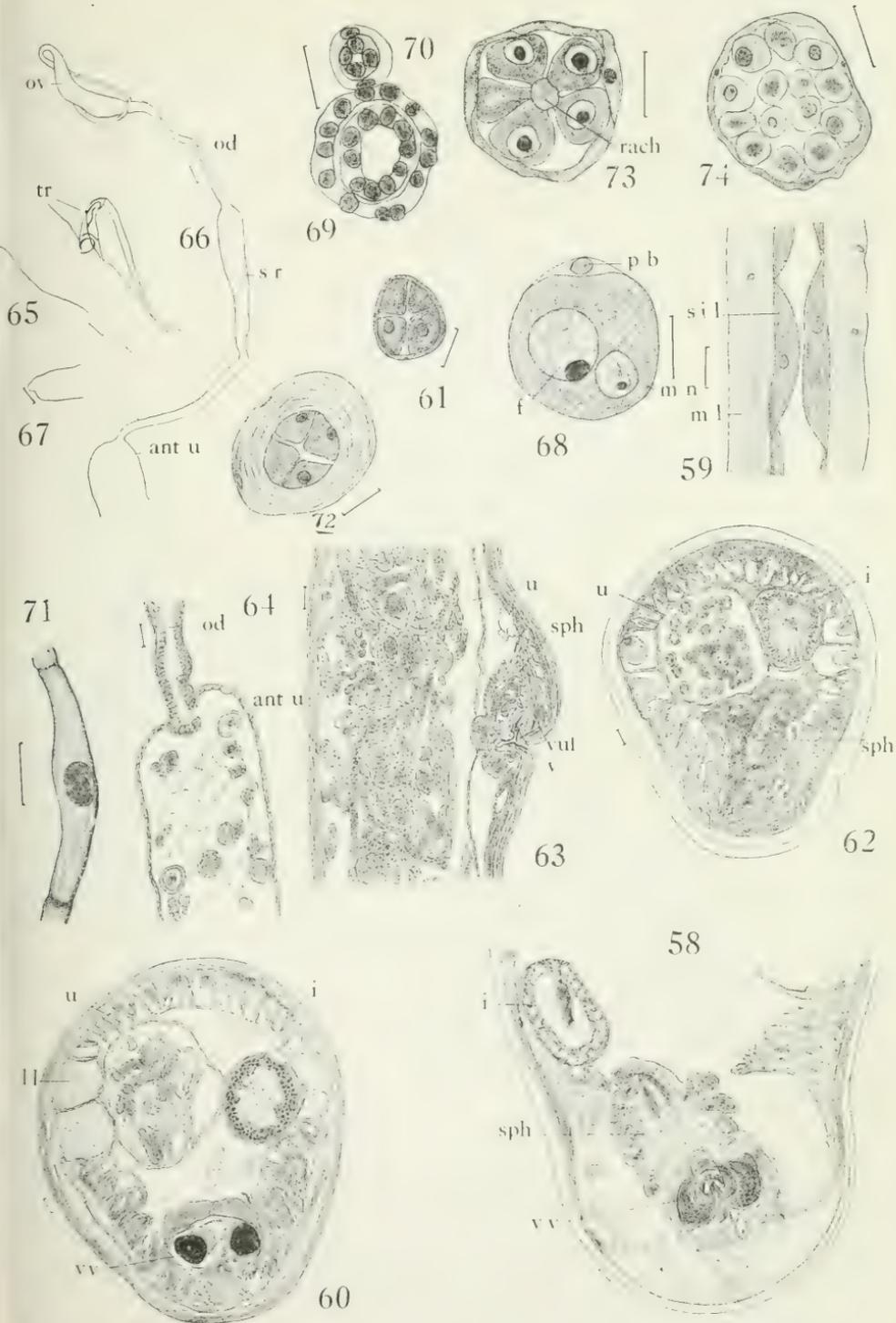
Most of the drawings were made by Mr. C. W. Shepard of the Department of Anatomy, College of Medicine, University of Illinois.

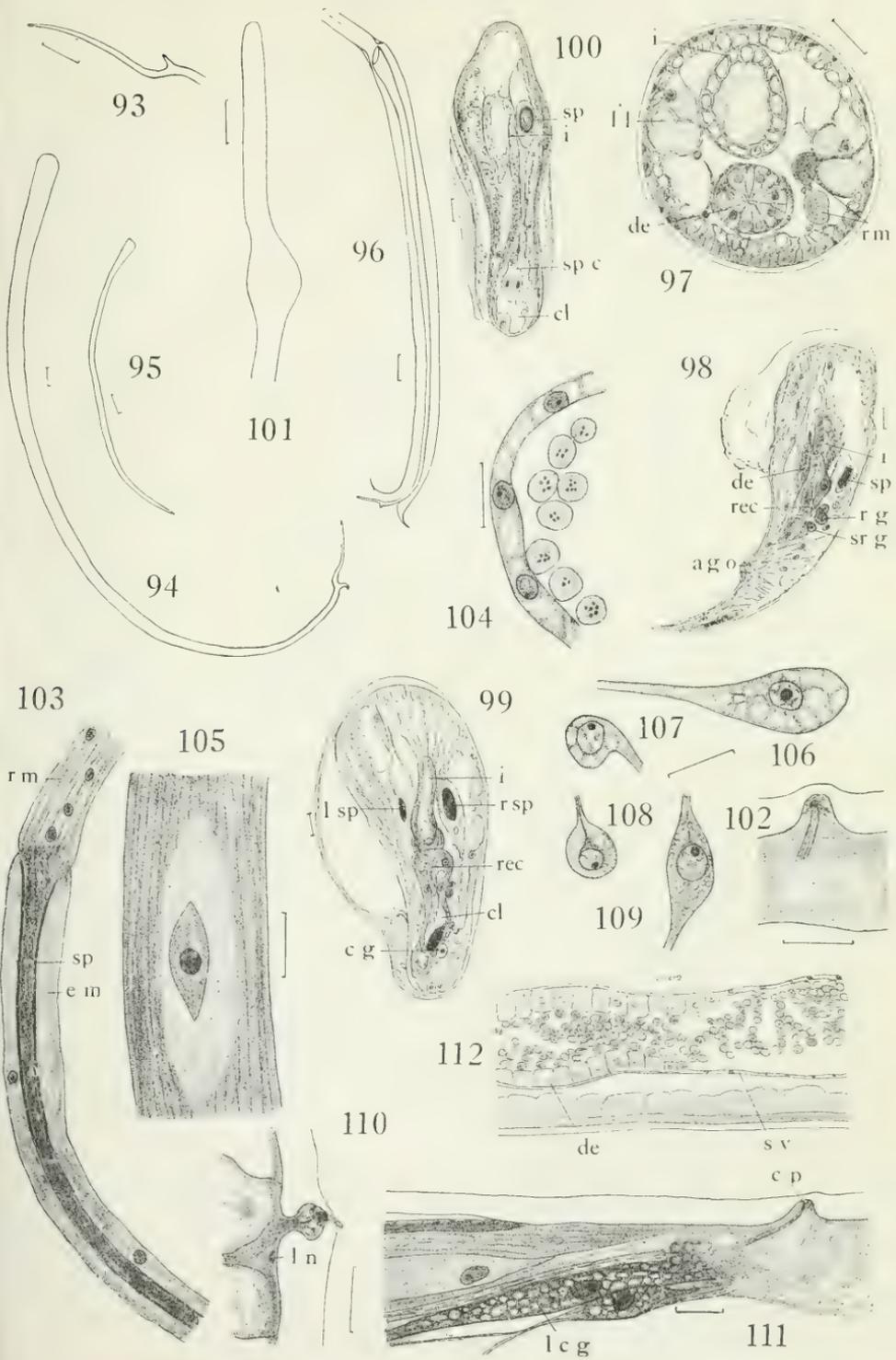
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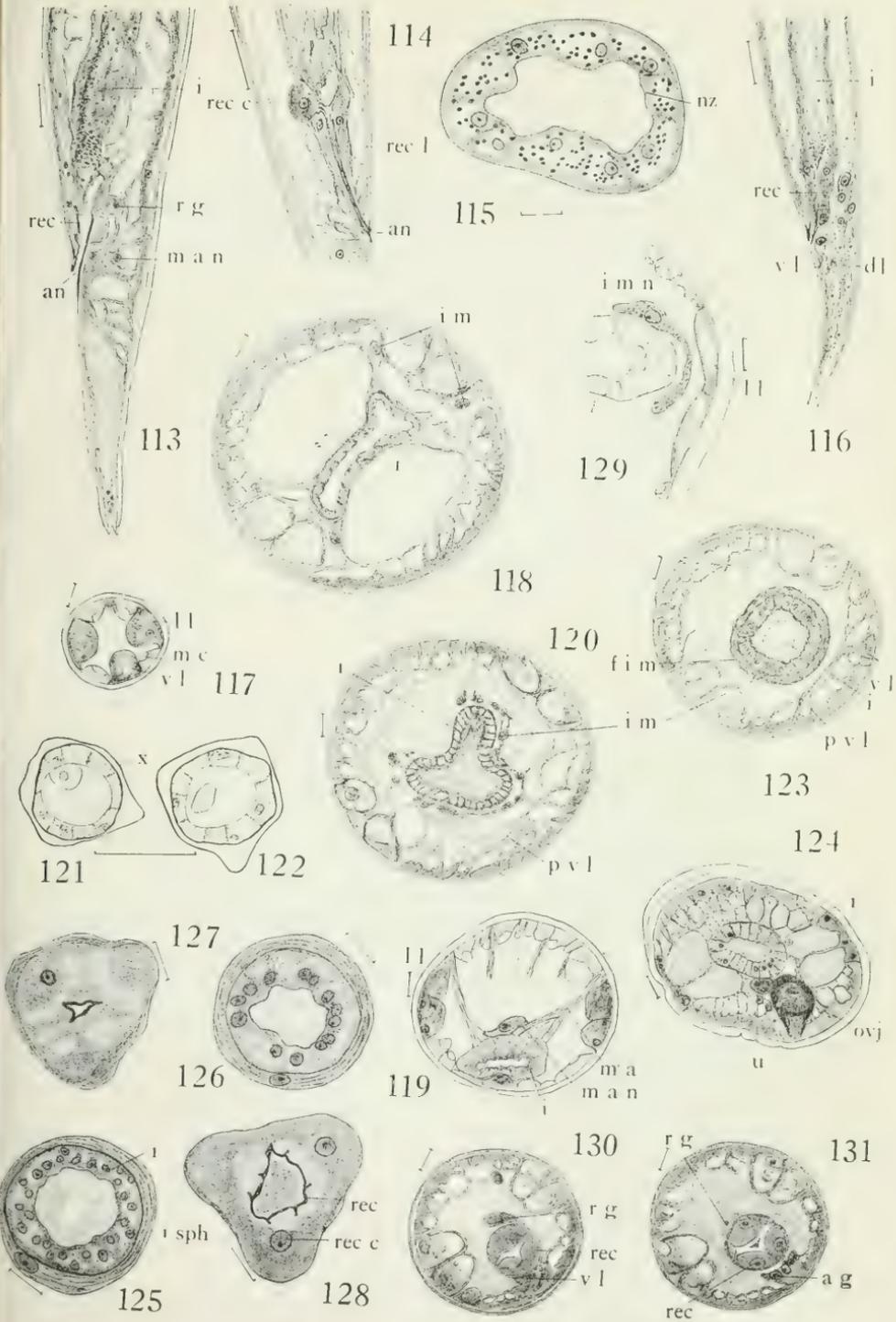


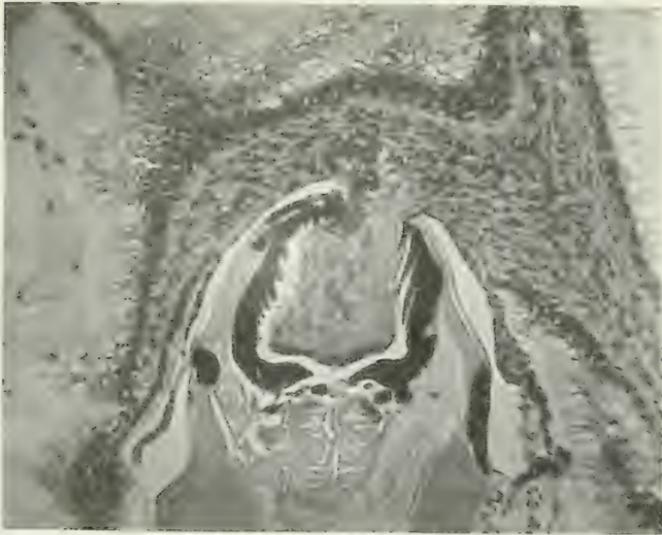


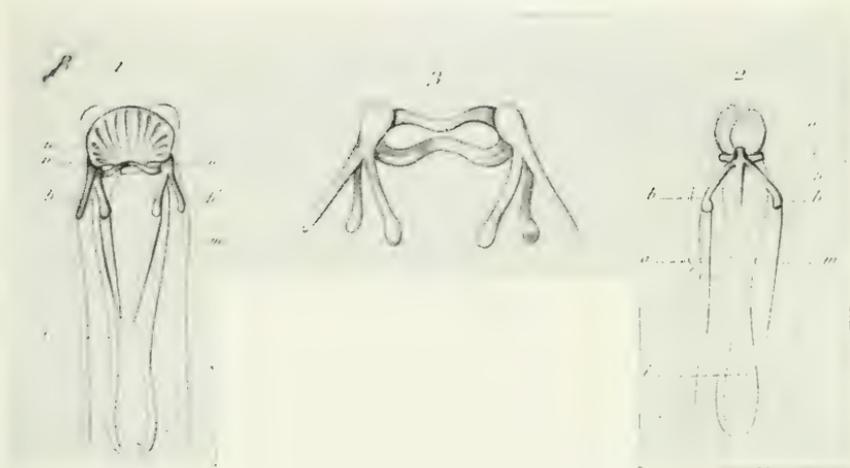












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FURTHER STUDIES ON NORTH AMERICAN MESEN-
CHYTRAEIDS (OLIGOCHAETA)*

BY PAUL S. WELCH

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PART I. *MESENCHYTRAEUS HYDRIUS* N. SP.

Habitat

The following description is based upon a collection of enchytraeids made by Mr. J. B. Flett in the Mt. Rainier National Park, June 15, 1917, at an altitude of 3400 feet. This collection contained 91 specimens of which 24 were sexually mature and all apparently belonged to the same species, definite identification of the immature specimens being, of course, out of the question. They were found in slowly moving water in close proximity to melting snow, thus existing under conditions of low temperature. Mr. Flett reports that some of the specimens were taken from the sand in the bottom of the stream and that they offered considerable resistance when pulled from their burrows. They exhibit the usual crawling movements but dispersal is facilitated by their ability to loosen, or become loosened from their hold on the bottom and drift down stream. He also states that they

* Contribution from the Zoological Laboratory of the University of Michigan.

may possibly occur in the snow but since the color of the living specimens is so nearly that of the snow they would be overlooked easily. No information is available concerning the abundance of this form but judging from the number in this single collection, they evidently occur in large numbers in the Mt. Rainier habitat.

Identity

A study of this annelid based upon dissections and serial sections showed it to be a new representative of the genus *Mesenchytraeus* and another form belonging in the list of enchytraeids having the striking character of much enlarged and elongated spermathecae—a character which, as the writer (1916b, pp. 99–100; 1917a, p. 74) has already pointed out, seems to be restricted to North American species. The excellent state of preservation and the complete sexual maturity of 24 specimens made it possible to work out thoroughly all of the structural characters bearing on the identification of the species. In many respects, it resembles *Mes. seichelli* Eisen closely, the important differences being as follows: (1) larger number of setae per bundle; (2) distinctly larger number of somites; (3) much longer spermiducal funnel; (4) absence of sperm sacs; and (5) marked difference in the internal structure of the penial bulb; (6) crossed spermathecae.

External Morphology

The body of *Mes. hydrius* is cylindrical, elongate, smooth and approximately of the same diameter throughout except at the extremities and at the clitellar somites. The length (alcoholic specimens) varies from 17 to 24 mm. inclusive, and the maximum diameter (clitellar region) from 0.76 to 0.91 mm. inclusive. Externally, the segmentation is distinct only on the anteclitellar and the posterior portions of the body. Between inter-segmental grooves, the body surface is smooth, and free from secondary annulations of any sort. In the specimens examined, the somites vary in number from 82 to 97 inclusive. The well developed clitellum, occupying $\frac{1}{3}$ 11–13, is continuous around the body except for a small area between and anterior to the penial bulbs where the clitellar thickening is lacking. The setae conform to the usual condition in the genus *Mesenchytraeus*, being distinctly sigmoid and arranged in four rows of fan-shaped bundles. In the lateral rows, the number varies from 4 to 7 inclusive:

in the ventral rows from 5 to 9 inclusive. Specialized setae are absent. The small, smooth, rounded prostomium carries the head pore near its tip. Color light yellow; pigmentation entirely absent, internally and externally.

Internal Morphology

Chloragog Cells. In the somites occupied by the elongated spermathecae, the chloragog cells are greatly reduced in number, being represented only by a few scattering cells or small groups of cells. These cells are, however, frequently much elongated. In the somites occupied by the long ovisac, more of the chloragog cells occur and while their ental extremities cover most of the surface of the alimentary tract, they are diverted from their usual radial arrangement, the free extremities being pushed around to the dorsal side. Somites caudad of the ovisac contain elongated chloragog cells arranged radially about the intestine.

Brain. The brain (Pl. XVII, Fig. 6) lies almost entirely in 1. It is about as long as wide, has parallel lateral margins, a truncated posterior margin, and a concave anterior margin. Two supporting strands extend to the body-wall.

Nephridia. A nephridium (Pl. XVII, Fig. 4, 5) consists of a small anteseptal region, little more than a mere nephrostome supported on a short, narrow pedicel, and a large, loosely constructed postseptal part of the usual mesenchytraeid type. The efferent duct is as long or a little longer than the entire nephridium and arises from the ventral surface of the postseptal part about midway of its length. There is no evidence of a reservoir at its ectal opening. There is considerable variation in the shape of the nephridia in the different parts of the body, although all conform to the general type described above.

Dorsal Blood-vessel. The dorsal blood-vessel arises in or very near 18. Distinct swellings occur in several somites just anterior to its origin.

Spermiducal Funnel. Two prominent spermiducal funnels (Pl. XVII, Fig. 7) lie in the clitellar region and, owing to their size and length, more than one somite is involved, one and sometimes both extending into 12. Each funnel is about seven times longer than the maximum diameter and fills most of the coelomic space in the somites occupied. Since the space within each somite is insufficient for both funnels, a

common arrangement is that in which one funnel, bent in a sigmoid figure, lies more or less transversely in one somite while the other extends longitudinally into the following somite, septum 11/12 being pushed caudad to septum 12/13. Usually the extremity of the funnel is reduced somewhat in diameter and terminates in a thin, flaring rim. The sperm duct is greatly coiled and massed in 13-14, the posterior coils lying in the ovisac.

Sperm Sacs and Ovisac. Definitely developed sperm sacs are not present. In some specimens, 11/12 is reflected caudad in such a way as to suggest the formation of incipient sperm sacs but such septal reflections do not extend beyond the confines of the clitellar somites. A large part of the coelom posterior to the clitellum is occupied by an extensive, unbranched ovisac which ends in 31-33.

Penial Bulb. The essential features of the "mesenchytraeid bulb" (Eisen, 1905, p. 7) are presented in the penial apparatus (Pl. XVII, Fig. 2, 3) of this species. In the retracted condition, each bulb surrounds a deep invagination which, in transverse section of the worm, appears as a bifurcating slit, thus forming a mesal compartment and an ectal compartment, both of similar shape and extent. This double chamber is lined throughout by a continuation of the external cuticula. The body of the bulb is firm, compact, and composed mainly of muscle tissue closely built together. A single layer of inner bulb cells surround the mesal side of the penial lumen but it composes only a small part of the mass of the organ. Peripheral gland cells are entirely lacking.

The ectal end of the sperm duct expands into a fusiform atrium which penetrates the bulb at a point on the mesal surface about mid-way between the ventral and the dorsal extremities. This atrium is composed of two portions, the larger fusiform portion lying in the coelom between the mesal surface of the penial bulb and the ectal end of the sperm duct proper, and the smaller, shorter portion enclosed within the body of the bulb. Structurally, the two parts are similar except that in the latter there is a much greater development of the longitudinal muscle-layer. At or very near the junction of these two parts the ducts from about five large, irregular, multicellular atrial glands enter the wall of the atrium but in the material at hand it has not been possible to follow these ducts further. The ducts of these atrial glands are difficult to follow, even outside the

atrium, and since the free ends of the glands lie about the base of the bulb, often in contact with it, they have, at first sight, the appearance of accessory glands. However, no true accessory glands were found.

Spermathecae. In form, size, and structure, the spermathecae (Pl. XVII, Fig. 1) resemble very closely those of *Mes. gelidus* Welch. In the specimens studied in this connection, each spermatheca is composed of three distinctly differentiated parts: the duct, the diverticula, and the ampulla. The duct is straight, slender, and uniform in diameter, extending caudo-mesad into the anterior part of 6. The ectal opening occurs laterad in 4/5 and is devoid of glands. At the junction of the duct and the ampulla, two, opposite, elongated, finger-like diverticula arise. These diverticula are, in the specimens examined, somewhat shorter than the duct. The ampulla composes the bulk of the spermatheca, extending caudad through the succeeding somites to the end of 10 and filling the greater part of the coelom in the somites involved. In fact, the ampulla is, in some cases, longer than the combined length of the somites through which it extends so that it may be doubled in varying degrees about the digestive tract. Constrictions at the septa are well marked but elsewhere the ampulla is, in the sexually mature specimens, distended with great masses of spermatozoa, the whole presenting something of a moniliform appearance when isolated from the body of the worm. The two ampullae of the animal are not symmetrical and may be slightly dissimilar in length but diversity, such as occurs in *Mes. gelidus* (Welch, 1916b, pp. 97-98), was not observed, both extending practically throughout the anteclellar somites. In 10, the extremities of the ampullae may lie in very close contact with the wall of the digestive tract producing an apparent union with the latter which is very deceiving. Critical examination of both transverse and longitudinal sections through these regions has failed to reveal any true connection between the ampullae and the alimentary canal, the former ending blindly. Structurally, an ampulla is composed of two regions, a short ectal portion which adjoins the ental end of the duct and a very long ental part which constitutes the storage region of the organ. The ectal portion is rather thick-walled and the lining epithelium is thrown into a series of transverse folds while in the ental part the wall is reduced in thickness to the appearance of a mere membrane.

A remarkable peculiarity of these spermathecae appears in the fact that the right organ crosses to the left side of the body and the left organ to the right, the intersection occurring in the posterior part of 5 or the anterior part of 6, at about the level of the origin of the diverticula. This phenomenon is a constant feature in all of the specimens studied and may be regarded as a character of the species. In an earlier paper (1917a, p. 74), the writer pointed out a similar crossing of spermathecae in *Mes. altus* Welch, although in the latter case these organs pass each other about midway of the length of the greatly elongated ampullae. Such a crossing of spermathecae must have been coincident with the development of the organs.

PART II. KEY TO THE SPECIES OF MESENCHYTRAEUS
KNOWN TO OCCUR IN NORTH AMERICA

There are two outstanding difficulties which hinder investigation of the Enchytraeidae, (1) the extremely scattered condition of the literature involved, and (2) the incomplete, fragmentary data on so many of the foreign species described in years past. It is indeed fortunate that practically all of the North American forms have been adequately treated and, so far as they are directly concerned, form a fairly satisfactory basis for work in this country. The world-wide roster of mesenchytraeid species presents at the present time about sixty names, although the standing of a few of them is a matter of uncertainty. Investigations indicate that the genus *Mesenchytraeus* is rather generously represented on our continent and with the hope of facilitating studies involving this genus, the writer has made several attempts to construct a usable key to all the known species but has found it a hopeless task, due to the fact that many of the Old World forms lack both uniformity and completeness in the description of essential details. In lieu of this more desirable but at present seemingly impossible treatment of the whole genus, the writer presents the following key to the identification of North American mesenchytraeids which may serve at least as a partial basis for work in this country.

- | | | |
|--------|---|---|
| 1 (26) | Spermathecae short, confined to 5 | 2 |
| 2 (3) | Spermathecae without diverticula; no atrium; no accessory glands; 1 set of penial glands; 2 sperm sacs; | |

- 1 ovisac; spermathecae twisted at ectal openings;
dorsal blood-vessel arising in 18
. *unalaskae* Eisen (See p. 185)
- 3 (2) Spermathecae with diverticula 4
- 4 (5) Spermathecae with 3 diverticula (occasionally 2); ac-
cessory glands present; 8 atrial glands; 1 sperm sac
rudimentary, 1 fully developed sperm sac extending
to 20, not enclosed in ovisac; sperm ducts extending
to 18, only one enclosed within ovisac; ovisac ex-
tending to 22; dorsal blood-vessel arising in 12;
enlarged setae in ventral bundles of 11
. *solifugus* (Emery)
- 5 (4) Spermathecae with two diverticula 6
- 6 (7) Sperm ducts absent; spermiducal funnels club-shaped
and opening directly into penial pores without ducts;
penial bulbs absent; no glands of any sort at ectal
openings of spermiducal funnels; diverticula of
spermathecae greatly reduced; 1 ovisac and 1 pair of
sperm sacs, both extending posteriorly for several
somites *nanus* Eisen
- 7 (6) Sperm ducts present; penial bulbs present; diverticula
of spermathecae distinct 8
- 8 (9) Penial glands absent; penial bulbs composed of muscle
and connective tissues only; atria absent; no acces-
sory glands *kincaidi* Eisen
- 9 (8) Penial glands present within penial bulb; atria present . 10
- 10 (15) Atrial glands absent 11
- 11 (12) Accessory glands absent; atria within penial bulbs .
. *beringensis* Eisen
- 12 (11) Accessory glands present 13
- 13 (14) Dorsal blood-vessel arising in 19; sperm sacs extending
to 16; ovisac extending to 18 *fontinalis* Eisen (See p. 185)
- 14 (13) Dorsal blood-vessel arising in 14-15; accessory glands
very large; copulatory papillae exceptionally prom-
inent *pedatus* Eisen (See p. 185)
- 15 (10) Atrial glands present 16
- 16 (17) Accessory glands present; 5 atrial glands; both sperm
sacs equally developed, extending to 15-16 and en-

- closed in ovisac; dorsal blood-vessel arising in 13-14
 *solifugus* var. *rainierensis* Welch
- 17 (16) Accessory glands absent 18
- 18 (19) Two atrial glands; spermiducal funnels small, almost
 globular, wider than long, bases twisted.
 *eastwoodi* Eisen
- 19 (18) More than 2 atrial glands; spermiducal funnels cylin-
 drical, longer than wide 20
- 20 (25) Two sperm sacs; no glands at ectal openings of sperm-
 athecae 21
- 21 (22) Sperm ducts very short, about as long as spermiducal
 funnels; 3-4 atrial glands; sperm sacs extending
 through 15 or more somites . . . *penicillus* Eisen
- 22 (21) Sperm ducts distinctly longer than spermiducal fun-
 nels; 6-8 atrial glands 23
- 23 (24) Spermiducal funnel very long, extending posteriorly
 for 6 somites; sperm sacs beginning in 7; length
 170 mm.; 105 somites . . . *grandis* Eisen (See p. 185)
- 24 (23) Spermiducal funnels very large, length but little great-
 er than diameter; sperm sacs arising in usual posi-
 tion in clitellar somites and extending to 27 or be-
 yond; sperm ducts extending to 21; dorsal blood-
 vessel arising in 20 *fuscus* Eisen
- 25 (20) One sperm sac, extending to 16, bifurcating at poster-
 ior end; a few glands at ectal openings of sperm-
 athecae; spermiducal funnels short, only about 1½
 times longer than diameter; 1 ovisac present,
 extending to 16, bifurcating at posterior end, con-
 taining sperm sac; dorsal blood-vessel arising in
 22-23 *johanseni* Welch
- 26 (1) Spermathecae long, extending through more than 1
 somite 27
- 27 (28) Spermathecae without diverticula; spermiducal fun-
 nels large, about 5 times longer than diameter;
 sperm sacs within ovisacs, extending to 15-17; 8
 atrial glands; penial glands present; spermathecae
 extending to 7-8, no connection with digestive tract;

- sperm ducts extending to 19 within ovisacs; 2 ovisacs extending to 22-26; dorsal blood-vessel arising in 18 *altus* Welch
- 28 (27) Spermathecae with diverticula 29
- 29 (30) One small diverticulum on spermatheca; 5 atrial glands; no accessory glands; penial glands present; spermathecae not connected with digestive tract, extending to 6; 2 long sperm sacs; 1 ovisac
 *asiaticus* Eisen
- 30 (29) Two diverticula on spermatheca 31
- 31 (32) Spermathecae connected with digestive tract by narrow ducts in 7-8; 12-14 atrial glands; penial glands of 1 kind only; no accessory glands . . . *vegae* Eisen
- 32 (31) Spermathecae not connected with digestive tract . . . 33
- 33 (34) Penial glands absent, body of penial bulbs composed of muscle fibers and connective tissue only; diverticula of spermathecae minute, globular; spermathecae extending to 10; more than 5 atrial glands; no accessory glands; dorsal blood-vessel arising in 15
 *orcae* Eisen
- 34 (33) Penial glands present; diverticula of spermathecae well developed, elongate 35
- 35 (36) One prominent accessory gland present at each penial bulb; spermathecae extending to 10-12; 8-10 small, globular atrial glands; penial glands present; dorsal blood-vessel arising in 16; sperm ducts short, but little longer than spermiducal funnels; spermiducal funnels large, length about 4 times greater than diameter, extending through 2 somites
 *franciscanus* Eisen
- 36 (35) Accessory glands absent 37
- 37 (40) Not more than 10 atrial glands; body pigmentation absent 38
- 38 (39) Spermathecae extending into 7-8; spermiducal funnels with length about twice diameter, constricted in middle; 5 atrial glands; 2 sperm sacs extending to 18 or beyond; 1 ovisac; penial invaginations simple, undivided *setchelli* Eisen

- 39 (38) Spermathecae extending to 11, crossing each other near 5/6; spermiducal funnels approximately cylindrical, length about 7 times the diameter; about 5 atrial glands; no accessory glands; no sperm sacs; 1 ovisac extending to 31-33; each penial invagination divided into an ental chamber and a similar ectal compartment, the former receiving the sperm duct; 1 set of penial glands *hydrius* Welch
- 40 (37) More than 10 atrial glands; body pigmentation present 41
- 41 (42) Sperm ducts long, extending to 17; 16-20 small, sessile, globular atrial glands; spermathecae extending to 9-10; spermiducal funnels trumpet-shaped, length about twice the diameter, rim long and recurved; sperm sacs extending beyond 18 . . . *obscurus* Eisen
- 42 (41) Sperm ducts short, confined to clitellar somites; atrial glands elongate 43
- 43 (44) Spermiducal funnels long, extending cephalad through 3 somites, length about 9 times diameter; sperm ducts about 3 times longer than spermiducal funnels; spermathecae extending to 10-11; about 16 atrial glands; sperm sacs extending caudad about 30 somites; 1 set of penial glands . . . *harrimani* Eisen
- 44 (43) Spermiducal funnels of the usual extent, confined to 11 45
- 45 (46) Penial glands exclusively unicellular; spermathecal diverticula united with ampulla at its ectal end; spermathecal duct short, diverticula longer than duct; 2 small groups of glands at ectal openings of spermathecae; spermathecae extending to 9-11; spermiducal funnel 3-4 times longer than diameter; sperm sacs extending to 31-35; 1 ovisac, bifurcating in 16, extending to 35, enclosing sperm sacs *gelidus* Welch
- 46 (45) Penial glands in part multicellular; spermathecal diverticula attached to middle of long spermathecal duct, diverticula shorter than duct; spermathecae extending to 7-8; no glands at ectal opening of spermathecae; 2 long sperm sacs; 1 ovisac *maculatus* Eisen

Discussion

Mes. unalaskae. The only record of this species is the original description by Eisen (1905, pp. 20-21) based upon specimens collected at Unalaska, Alaska, Aug. 10, 1899, which are described as "not fully developed." The clitellum was absent and there appears to be reason for questioning the maturity of the sexual organs. In fact, the structure of the spermathecae and the penial bulb, as presented in Eisen's figures (text fig. 1c, c), suggests a certain degree of immaturity. On the other hand, the connection of the spermathecae with the digestive tract and the presence of well developed sperm sacs and an ovisac argues nearness to sexual maturity. However, there is, at present, no alternative other than to give the species this tentative position in the key.

Mes. fontinalis var. *gracilis*. Eisen (1905, p. 54) describes a new variety of this species under the name *gracilis* but the differences as described are so slight that the writer questions its validity.

Mes. pedatus. A discrepancy occurs in Eisen's original description (1905) of this species which demands notice here. In his key to the species of *Mesenchytraeus* (pp. 18-20), *pedatus* is placed under "d. No atrial and no penial glands, but many accessory glands at the lower apex of sperm-ducts." In the formal description (pp. 55-57), no statement appears concerning the presence or absence of penial glands but, on plate IX, fig. 5, a figure appears representing penial glands within the small penial bulb and indicated by an abbreviation "*pb*." No such abbreviation appears in the explanation of this figure but an abbreviation "*p. blb*," not on the figure at all, is explained as "penial bulb containing unicellular glands." There is every reason for believing that "*pb*" on the plate is a typographical error and should have been "*p. blb*." The writer regards the figure as indicating more correctly the structure of the penial bulb, hence the place of treatment in the key.

Mes. fuscus var. *inermis*. A variety of this species, described by Eisen (1905, pp. 49-50) under the name *inermis*, differs from the original species in minor respects only.

Mes. grandis. Eisen, the original describer of this species, points out (1905, pp. 46-47) the close relationship which seems to exist between it and *Mes. harrimani*, and suggests the possibility that

grandis may be identical with *harrimani*, "the spermathecae having become accidentally reduced." The original description was based upon a single specimen "which was carefully narcotized and fixed in sublimate," implying that it was in good condition. It is not clear just how a spermatheca might become "accidentally reduced" except through some unfavorable dissection or sectioning. No statement is made as to the method of preparation of the specimen for study but there is a hint that it was dissected. The sperm sacs are described as beginning "as far forward as somite VII, where they appear to spring from the septum VI/VII. They gradually increase in size posteriorly, except in the somites of the clitellum, where they are thin, even and tubular. The walls of the sperm-sacs are thick, a cross-section resembling a cross-section of a spermatheca." The anterior position, as described, of the sperm sacs is a very unusual one and, while Eisen was experienced in recognizing elongated spermathecae, the writer is inclined to raise the question as to whether the above-mentioned "sperm-sacs" in the antecitellar somites might not have been portions of the spermathecal ampullae. If such was the case then *grandis* would fall into the group of species having elongated spermathecae and possibly might have to be regarded as the same as *harrimani*. However, definite settlement of this matter must await study of additional material.

Mes. beumeri. In the above key no account is taken of a doubtful record of *Mes. beumeri* (Mchlsn.) given by Moore (1899, p. 141) as occurring in the vicinity of Philadelphia.

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EXPLANATION OF PLATE

ABBREVIATIONS

<i>atr</i>	atrium
<i>atr gl</i>	atrial gland
<i>dis</i>	spermathecal diverticulum
<i>in b c'l</i>	inner bulb cells
<i>m</i>	muscle tissues within penial bulb
<i>pen b i</i>	penial bulb invagination
<i>pen po</i>	penial pore
<i>sp'r</i>	spermatheca

PLATE XVII

Mesenchytraeus hydrius n. sp.

Fig. 1. Diagram of 4-10, showing position, extent, gross structural features, and crossing of the elongated spermathecae.

Fig. 2. Penial bulb and associated structures. Somewhat diagrammatic.

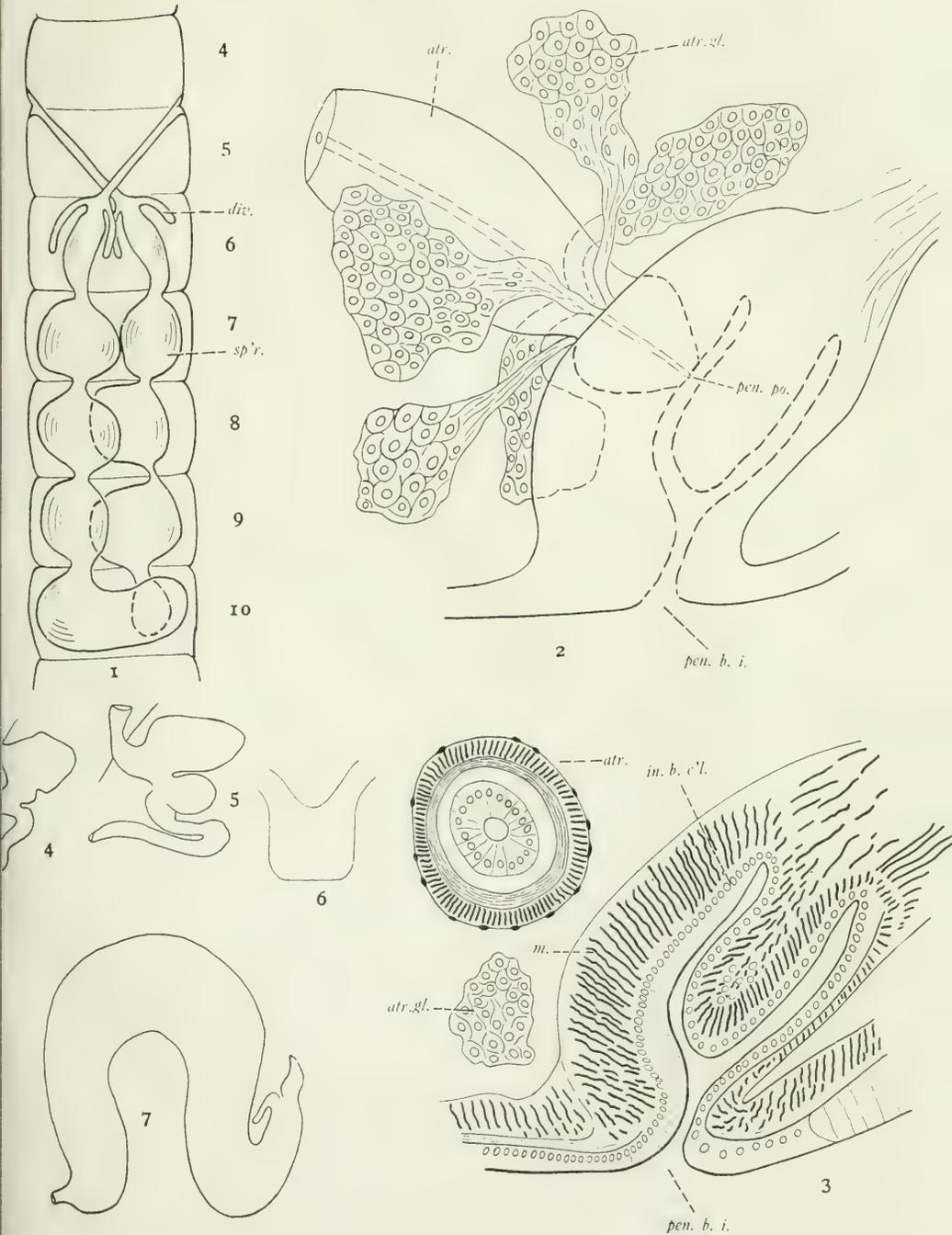
Fig. 3. Penial bulb as it appears in transverse section of worm. Bulb in retracted condition.

Fig. 4. Nephridium from a postclitellar somite.

Fig. 5. Nephridium from an anteclitellar somite.

Fig. 6. Brain.

Fig. 7. Spermiducal funnel.



THE LATERAL LINE OF POLYODON SPATHULA*

BY HOMER B. LATIMER

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I. HISTORICAL

The lateral line system of fishes has long been known as a system of dermal canals lying upon the head and along the sides of the body. It was described in 1664 by N. Stenois and until 1850 it was regarded as an organ for the secretion of mucus.

Leydig ('50) describes the general appearance and location of the canals, the nerve supply and histology and concludes that it is sensory in function. Vogt ('56) advanced the theory that it was connected with the lymph system, altho he agrees with Leydig that it is not a mucus producing organ. Franz Schulze ('61) in working on the lateral line organs of *Perca fluviatilis* agrees with Leydig and Vogt in calling it a sensory structure, but the following year M'Donnell ('62) states that it "secretes some fluid which is poured forth from the skin as an excretion." Following these earlier workers we have the papers of R. R. Wright, Allis, Collinge, and Cole. R. R. Wright ('84) in describing the lateral canal of *Amiurus*, gives the number of pores and nerve hillocks (sensory ridges) as equal in number and forty on each side of the fish. In the specimens of *Polyodon* examined I find that the number of sensory ridges is much greater than the number of the branchlets and not equal as he found them in *Amiurus*.

*Studies from the Zoölogical Laboratory, of the University of Nebraska. No. 122.

The condition of the canal as found in *Amia calva* by Allis ('89) resembles in many ways that of *Polyodon*. He describes the canal as turning downward at the caudal end and passing between two tail fin rays and not in a ray as in *Polyodon*. In fig. 49, Plate XLII he shows the ridges as intermediate between the branchlets, not at the bases of the branchlets as was found in *Polyodon*.

In the description of the lateral line of *Polyodon folium* by Collinge ('94) he states that "during its course from the caudal to the cranial region there is a gradual but distinctly appreciable enlargement in its diameter." He gives the number of branchlets as varying from 32-35, part on the ventral side and part on the dorsal side. The smallest number found in *Polyodon spathula* was 41. He found the branchlets from 12-17 mm. in length, while in the specimens of *Polyodon spathula* which I have examined none of the branchlets exceeded 9 mm. in length, but this may be due to the comparatively small size of the specimens examined.

Mention should be made of the work of G. H. Parker ('02 and '04) upon the function of the lateral line organs. In the summary of his paper he says, "The lateral line organs are stimulated by water vibrations of low frequency—6 per second." Some other work has been done on the physiology of these organs and a good deal in attempting to determine the embryology.

Brohmer ('08) in working with embryos of *Spinax niger* found that in 36 mm. embryos the lateral line, seen in toto, appeared as a white line. In the 45 mm. stage he found that the white dots along the sides of the lateral line which he had supposed, in the general examination to be Lorenzian ampullae, were in reality the openings of the branchlets. He says (free translation) "On either side of the lateral line, were noticed rows of white spots, the openings of the sense bodies. The openings arranged themselves with somewhat of regularity on the right and left sides. The adjoining bodies united to form the lateral line, and the communicating cavities formed the lateral canal." If this represents the embryonic condition of the canal of *Polyodon* as well as that of *Spinax*, it would account for the position of the branchlets always on the dorsal or ventral sides of the canal, never on the lateral side, which would naturally be expected if the branchlets were simple evaginations from the canal, or if they were the tubes connecting the depressed organs with the surface.

This would also explain the enlargement of the canal at each sensory ridge, as well as the tendency of the sensory ridge to pass up onto the opposite side of the canal to that from which the branchlet is given off. The closing of the openings of some of the bodies would account for the fact that some of the ridges are without a branchlet. In speaking of this alternating opening of the bodies upon the right and the left sides he says that the two sided opening of the canal perhaps aids in the determination of the direction of the sensation, since the stimulus will have a different effect upon some of the bodies or nerve ridges than upon others whose branchlets are turned away from the source of the stimulus. In this way the fish is enabled to orient itself with respect to the stimulus. An interesting fact in connection with this theory is that in the *Polyodon*, a bottom swimmer, the majority of the branchlets come from the ventral side of the canal, which is not what would be expected if this suggestion is correct.

Johnson (1917) in his description of the lateral line system of *Selachians* finds that the sensory epithelium is practically uniformly distributed along the canal except for the decrease in size toward the caudal end, and that it is located on the superior medial part of the canal. He finds the branchlets (tubules he calls them) all opening from the ventral side of the canal.

II. MATERIAL AND METHODS

The material used in this investigation consisted of the right lateral lines of two *Polyodon spathula*. Both lateral lines had been removed from the fishes, so there was no way of knowing definitely the total lengths of the specimens. In order to gain some idea of the size of the fish from which the lateral lines had been taken thirteen *Polyodon* of various sizes were examined. The total length of the fish from the tip of the "bill" to the tip of the caudal fin, as well as the length of the lateral line measured from the first branchlet posterior to the gills to the termination of the canal upon the dorsal part of the caudal fin, was carefully determined for each specimen. The length of the lateral line expressed as a percentage of the total length of the fish was found to be approximately 47%, the average for the thirteen specimens examined. Thus the length of the larger fish whose lateral line was 17 inches long must have been about 36 inches in length, and the smaller fish with a 7 inch lateral line must have been about

14.5 inches long. Of course, this gives only an estimate of the lengths of the fish. All the measurements were made from preserved material.

The two lateral lines had been removed by cutting a strip about three quarters of an inch wide, in the case of the larger fish, and one half inch wide from the smaller fish, and deep enough in both cases to ensure getting all of the dermis. The lateral line from the larger fish had been fixed in Formol-Corrosive-Acetic and had been kept in 80% alcohol for about a year. The shorter lateral line had been preserved but a short time in alcohol, after fixation in Trichlor-Acetic.

Charts showing the number of the branchlets and tube pores as well as the lengths of these branchlets and the intervening lengths of the canal were drawn to scale before the strips were cut (Figs. 1 and 2). Both strips were placed in acid alcohol (.5% HCl. in 70% Alcohol) for from two to four hours and then stained in toto in Haemacalcium for from 24 to 36 hours. The canal from the larger fish was then cut into convenient lengths run thru the alcohols and xylol, imbedded in paraffin and cut longitudinally. The other canal, stained and imbedded in the same way, was cut transversely. The longitudinal series was cut ten microns in thickness while the transverse series was cut twenty microns. The larger canal was the better preserved altho neither was in first class histological condition. The epidermis of the strip containing the shorter canal was nearly all destroyed in attempting to remove the hardened opaque mass of mucus so that the branchlets could be distinguished, with the aid of a dissecting lens, in making the surface drawing. The epidermis of the other strip was in a little better condition. The lengths of the sensory ridges in the lateral canal, which was cut longitudinally, were determined by means of an ocular micrometer; the lengths of the ridges in the transverse series were determined by counting the number of sections containing each ridge.¹

¹This work was done some time ago in the Animal Biology Laboratory of the University of Minnesota, at the suggestion of Professor Henry F. Nachtrieb, to whom I wish to express my most hearty thanks for his stimulating and very helpful advice. I had hoped to get more material and then make a more complete histological study but there seems to be no immediate prospect of doing this.

III. GENERAL STRUCTURE OF THE LATERAL LINE

The lateral line system as described by various investigators consists of several cranial branches, a main branch, and the lateral branch, or lateral line. The lateral line was the only part of the lateral line system studied. In *Polyodon spathula* the lateral line unites with the main canal by passing dorsad to the gills, and extends caudad from the gills along the sides of the fish. In the anterior portion of the body it is situated upon the dorsal half of the fish, but as it approaches the tail it comes to lie about half way between the dorsal and ventral portions. It is deflected downwards slightly as it reaches the tail, but upon entering the dorsal part of the caudal fin it runs upward, parallel to the fin rays and terminates a short distance from the tip of the tail. The lateral line, in preserved specimens, when viewed from the surface, appears as a whitish line extending along the sides of the fish, and the branchlets appear as shorter white lines extending dorsad and ventrad from this. These branchlets divide, as a rule terminating in the tube pores. (Figs. 1 and 2).

The number of branchlets varies in different specimens and even on the two sides of the same specimen. The number of branchlets found on four specimens of *Polyodon* is given below:

Right side.	Left side.
41	41
56	50
64	61
52	46

For convenience the lateral line from the larger fish, which was cut longitudinally, will be spoken of as the "Longitudinal series," and the lateral line from the smaller fish which was cut transversely will be spoken of as the "Transverse series."

a. The Longitudinal Series

The lateral line from the first branchlet posterior to the gills to the last branchlet on the caudal fin was 42.85 cm. in length. Thruout this entire length there were in all, 54 branchlets, ten of these were given off from the dorsal side of the canal, and 44, from the ventral side of the canal. In no instance did a branchlet start from the lateral or medial sides of the canal. Four of the ventral branchlets passed between the canal and the epithelium and terminated in the

epithelium, either entirely, or partly on the opposite side of the canal. All four of these branchlets were in the anterior region. On account of the amount of cartilage in the tail it was impossible to obtain a series of the posterior part of the canal; so the length of the canal which was sectioned, was 37.9 cm. in length and contained 44 branchlets. These branchlets terminated in from two to seven tube-pores. The more anterior branchlets, as a rule, had the larger number of pores, and hence were more branched. No opening of these branchlets was more than 9 mm. distant from the canal. Thus but a relatively narrow band, not over 18 mm. wide, contained the entire lateral canal and its branchlets. The cartilaginous nodules and "pit organs" could also be seen in the surface view, situated either over or near the canal. The nodules seemed more numerous in the posterior part, where they were scattered irregularly, while in the anterior part, they were nearly always found lying over the lateral canal. These nodules and "pit organs" will be described a little more in detail later on.

The features most clearly shown in the longitudinal sections were, the relation of the cartilaginous rings surrounding the canal and the unevenness of the sensory ridges, (fig. 4). The determination of the lengths of the ridges was a little more difficult than in the transverse series, due to the fact that it was hard to get the sections perfectly parallel to the canal. The lateral canal itself was not perfectly straight. Where a branchlet was given off there was often a slight divergence toward the side from which the branchlet originated. These irregularities in the plane parallel to the surface were, however far less pronounced than the wave like course in the plane perpendicular to the surface.

The cartilaginous rings surrounding the lateral canal thruout its entire length were of two kinds, (a) ring-like cartilages which enclose the canal in the troughs, or parts of the canal most distant from the surface, and (b) cylindrical or "drainpipe-like" cartilages, found always at the crests or parts of the canal nearest the surface. The ring-shaped cartilages enclosed those portions of the canal between the ridges, while the sensory ridges were always enclosed in the cylindrical cartilages, (fig. 4). The distance from one crest to another in the anterior part of the canal varied from 3.2 mm. to 4.5 mm. The crests of the canal very often came up to the base of the epithelium while the troughs were from .24 mm. to .48 mm. distant from the

base of the epithelium. Between the cylindrical cartilages, or "drainpipe-like bones," as they were called by Collinge ('96), there were from 12 to 14 of the ring-shaped cartilages in the anterior part of the canal. In the body region, at the base of the caudal fin, there were but one or two and sometimes none of the ring-shaped cartilages between the long cartilages. This change in the number of the ring cartilages was not abrupt, for from the middle of the body region there was a gradual diminution till the extreme condition, the absence of the ring cartilages was found at the base of the tail.

The cylindrical cartilages terminated either at the crest or a little caudad to it at the place where the canal began to descend. From this point they extended cephalad and mediad often reaching to nearly the lowest part of the curve. Thus the sensory ridges which lay primarily within these cartilages were situated in that part of the canal facing the anterior. The sensory ridges as well as the cylindrical cartilages in some instances extended a short distance caudad to the crest.

The branchlets were always given off from either the dorsal or the ventral side of the cylindrical cartilages at a point near the surface. A continuation of the connective tissue extended out surrounding the branchlet for some distance. The sensory ridge extended but a short distance caudad to the origin of the branchlet. Neither the sensory ridge nor any part of it extended out into the branchlet. This relation of the sensory ridge to the branchlet was followed out more carefully in the transverse series and will be discussed later. The sensory ridges which were situated in the cylindrical cartilages from which branchlets were given off showed apparently no difference in structure from those ridges which were not near a branchlet. Every branchlet, however, originated from one of the cylindrical cartilages containing a sensory ridge. In some instances branchlets arose from adjoining crests, while as many as five, in one instance, and often two, three and four ridges intervened between two ridges which were located at the base of a branchlet.

From one crest in the anterior part of the canal a small tube was given off from the lateral side of the canal. This was much smaller than the branchlets, both in length and in diameter. The sensory ridge and canal showed no special modifications at this point. It resembled a similar structure found in the transverse series, which

will be described more fully in that part of the paper. This opening was not counted as a branchlet.

That part of the canal which was cut was 37.9 cm. in length, and contained 126 sensory ridges and 44 branchlets, or an average of 2.86 ridges for every branchlet. The branchlets were not distributed uniformly, for there was a tendency for the branchlets to group themselves.

The measurements of the sensory ridges will be given more concisely and perhaps just as clearly in the following table. The eleven groups are the eleven pieces into which the lateral canal was cut for convenience in sectioning. The first column shows the number of branchlets in that section, the second column gives the number of sensory ridges, the third, the average length of the sensory ridges found in that division, and the fourth and fifth columns give, respectively, the longest and shortest ridges found in each division.

Branchlets	Ridges	Av. Length	Longest	Shortest
5	15	1.194 mm.	1.666 mm.	.830 mm.
6	9	1.473 mm.	2.075 mm.	.797 mm.
3	11	1.045 mm.	1.328 mm.	.581 mm.
4	11	1.004 mm.	1.411 mm.	.498 mm.
4	12	.837 mm.	1.666 mm.	.415 mm.
4	14	.652 mm.	1.328 mm.	.249 mm.
4	15	.655 mm.	.896 mm.	.332 mm.
4	14	.792 mm.	1.411 mm.	.581 mm.
5	13	.745 mm.	1.162 mm.	.249 mm.
2	6	.766 mm.	1.162 mm.	.581 mm.
3	6	.830 mm.	1.079 mm.	.415 mm.
—	—	—	—	—
44	126	.908 mm.		

This table and the chart of the lateral line (fig. 2) do not show the same number of branchlets but it must be remembered that only a part of the lateral canal was sectioned, or that part between the second branchlet from the anterior end and the eighth from the caudal end. There were 54 branchlets in the entire canal which measured 42.85 cm., and but 44 branchlets in the part sectioned which measured 37.9 cm.

b. The Transverse Series

The material used for this series was the right lateral line canal of the smaller fish, as stated above, and on account of the smaller size of the fish as well as the hardened opaque mucus covering the skin, it was much more difficult to chart the line and branches. It was possible, however, to make a series thru the entire lateral canal, from the gills to its termination upon the caudal fin.

The canal measured 18.1 cm. from the first branchlet posterior to the gills to the last branchlet on the dorsal part of the caudal fin. Thruout its entire length there were 61 branchlets, 53 of which were given off from the ventral side, and 8 from the dorsal side, (fig. 1). There were thirteen branchlets in the tail, all of which were on the ventral side of the canal. In studying the sections an additional branchlet was discovered originating from the lateral side of the canal. This branchlet resembled the others in everything but its position, which was between the canal and the epidermis, and parallel to the canal, so that it was not seen while making the surface drawing. This would raise the total to 62 branchlets. All of the branchlets were less branched than in the larger specimen, terminating in but from one to three tube pores. The majority of the branchlets did not branch at all, or else had a short dichotomous branching. Many of the branchlets would remain unbranched thruout nearly their entire course, then very near the end they would divide terminating in from one to three tube pores. The two charts show a marked difference in the branching of the branchlets and the number of tube pores, tho the branchlets are distributed along the canal in much the same way. Immediately posterior to the gills, the first two or three branchlets were not so close together as the next ten or twelve. Then followed a longer region extending nearly to the base of the caudal fin, with the branchlets more scattered. On the body, at the base of the caudal fin and on the fin itself, the branchlets tho shorter and less branched, were closer together. No branchlet terminated more than two and one-half or three millimeters from the canal: in other words, the entire canal and its branches were contained in a strip six millimeters wide on the body, and gradually diminishing posteriorly, till not over three millimeters wide on the caudal fin. As was found in the other series, the more anterior branchlets had a tendency to turn caudad after leaving the canal. Only one of the branchlets in this series

passed laterad to the canal in reaching the surface on the opposite side of the canal. In one case two branchlets were given off from the same ridge, one from the dorsal side, and the other from the ventral side of the canal.

The number and arrangement of the canal cartilages was not so carefully noted in this series. As in the longitudinal series, the sensory ridges were always found in the cylindrical cartilages, which were separated from each other by a greater number of ring-shaped cartilages in the anterior part of the canal than in the posterior part. The canal had the same wavy course tho perhaps not quite so marked as in the longitudinal series. In this series the crests scarcely ever came in contact with the dermal epithelium. The few exceptional cases where the cartilages of the canal came up to the epithelium were just about as numerous in this series as were the exceptions in the longitudinal series, or those cases where the cartilages did not come to the surface. In a short portion of the canal about one-third of the distance caudad, the top of the canal varied from .08 mm. to .22 mm. distant from the base of the epithelium. These figures do not mean that the above is the variation in one curve, but the longest and the shortest distances from the base of the dermal epithelium in the two slides in which the distances were measured. The difference between a crest and an adjoining trough would probably be quite a little less.

In this series as in the other, the sensory ridges were found in the anterior slope of the crest, terminating at, or just posterior to, the base of the branchlet, or at the crest if no branchlet is given off, and enclosed in the cylindrical cartilages. As before, there was no apparent difference in the structure of the sensory ridge whether a branchlet was given off or not. In the case of the double branchlet, or the one where a branchlet was given off from each side of the canal, the sensory ridge was a normal one, being .380 mm. in length. The average for this region was .336 mm. and the longest ridge was .600 mm. in length and had no branchlet. The number of sensory ridges intervening, between ridges in the cartilages from which branchlets were given off, varied from none to as many as six in one case (two-thirds of the distance back on the body). In seventeen instances there was no intervening ridge; in fourteen instances there was one ridge; in eleven instances there were two ridges; nine instances.

three ridges; five instances, four ridges; two instances, five ridges, and in one instance there were six intervening sensory ridges. As many as three consecutive ridges were found with branchlets, again showing the grouping of the branchlets.

The method of arrangement found in the body region seemed to be very much altered in the tail region. There were nine branchlets on the caudal fin with no sensory ridge at their bases. In two places two adjoining branchlets were found with no ridge at their bases nor an intervening ridge. The arrangement of the cartilages seemed less typical in the caudal fin. The canal toward the later part of its course in the caudal fin seemed to enter what might be called a jointed cartilaginous, fin ray. The differences in the epithelial lining of the canal will be described later.

The location of the sensory ridge in the canal was shown very well in this series (figs. 6 and 7). A narrow circular space filled with an indifferent mesenchymatous tissue, separated the cartilaginous canal from the inner or epithelial canal, which consisted of a single layer of flat epithelial cells thruout about one-half to three-fourths of the circumference of the canal, the remaining portion being occupied by the columnar epithelium or sensory ridge. The sensory ridge was uniformly found on the medial side of the canal, tho in some places there was a slight tendency toward the movement of the ridge up onto the side of the canal opposite to that from which the branchlet was given off. In one case, the anterior end of the ridge started a little way up on one side of the canal and in going caudad it passed to the normal position on the medial side of the canal and then passed up onto the opposite side from that on which it started. The longitudinal band of columnar epithelium, or sensory ridge, was always raised up upon a thicker mass of mesenchymatous tissue, which was well supplied with blood vessels and nerves, both of which entered thru openings in the cylindrical canal cartilages. The blood vessels often entered on the dorsal or ventral side, while the nerves entered thru an opening in the medial side. The central position of the sensory ridge, the top of the sensory epithelium reaching often to the center of the canal, was due, not only to the higher cells composing it, but also to the infolding of the ridge. This infolding was supported upon the mass of tissue underneath. The ridges terminated quite abruptly at both ends.

Just caudad to each ridge the canal lost its circular outline and assumed an oval shape, with the long axis perpendicular to the surface. There was in addition a rapid decrease in the size of the lumen, with a more gradual increase toward the cephalic end of the following ridge. This was especially noticeable in the tail region, where the size of the lumen diminished appreciably caudad to each ridge; in some places the lumen was almost entirely closed. The cylindrical portion containing the ridge was quite uniform in diameter in the transverse series but in the longitudinal series it was of considerably larger diameter in the median portion than at either end. In some cases the lumen of the "drainpipe-cartilages" assumed an elongated ellipsoidal form. This may have been due to a distortion during the preparation of the material.

In addition to the differences in the arrangement of the canal cartilages in the tail region, which has been described above, there was also a change in the epithelium of the canal itself. In general the epithelium of the body canal was of the flat pavement type, but in this region it was cuboidal. The entire lateral canal as well as the lumen became much smaller and the ridges were not so prominently elevated into the lumen of the canal. As nearly as could be determined with the available material there was a difference in the character of the ridges. In the caudal fin the sensory ridges were composed of deeply staining, long, narrow cylindrical cells, while in the body region, in addition to these cells, there was a type of cell less intensely staining and which often seemed to have its upper end dilated. A group of these cells would apparently make a little elevation above the rest of the ridge. The upper end of these cells was often much clearer and with proper stains it might have been shown to have contained mucus. These may be the surface mucus cells carried down with the nervous elements in the formation of the canals as suggested by Cole (98). Better material and a more careful study of these conditions would be necessary before a positive statement could be made.

The height of the epithelium in the various ridges, and the average height for the three regions of the canal was quite different. The average height of the sensory epithelium of the ridges of the first region, or the first thirteen ridges posterior to the gills was .032 mm.; that of the second region, or from a region about two-thirds of the way

back on the side of the body, was .027 mm. and the average height of several ridges on the dorsal caudal fin was .025 mm. The highest epithelium, .048 mm. in height, was found in the anterior region, while the lowest, .020 mm. in height, was found in the posterior region.

A short distance caudad to the branchlet which originated from the lateral side of the canal one of the sensory ridges was observed to commence upon the lateral side of the epithelial canal. Passing caudad in the series of sections the ridge was observed to pass onto the dorsal side of the epithelial canal and then almost over to the medial side. At the point where the ridge left the lateral side and came to lie more upon the dorsal side of the canal, an exceedingly fine tube was cut off from the lateral side of the canal, and passing thru a longitudinal distance of seven sections, or .14 mm., it opened to the surface. The average diameter of the tube was .02 mm. It broadened out at its termination on the surface to about .06 mm. in diameter, and posteriorly it was followed by a slight groove which gradually became shallower and finally disappeared. In many respects this tube resembled a branchlet, and very likely may have been either a developing branchlet or one that was closing, but I am unable to explain its position. Anterior to this opening the lumen of the canal was unusually large for the region, being .244 mm. in diameter. The lumen narrowed down to .112 mm. in diameter in the cylindrical cartilage immediately following which enclosed the posterior part of the ridge. The later part of the ridge was normal both in structure and position. At the caudal end of the cylindrical cartilage the lumen narrowed as usual, but to a much greater extent, being only .064 mm. in diameter.

The lateral canal used in this series was divided into seven pieces for convenience in handling and cutting. The following table will give the data grouped into seven divisions corresponding to the seven pieces into which the canal was divided for sectioning. There were 62 branchlets and 151 sensory ridges, or an average of 2.45 ridges for every branchlet. The first column shows the number of branchlets in each of the seven divisions, the second, the number of sensory ridges, the third column, the average length of the ridges in each division, the fourth and fifth columns show respectively, the longest and the shortest ridge in each division.

Branchlets	Ridges	Av. Length	Longest	Shortest
7	14	.536 mm.	.92 mm.	.14 mm.
8	16	.470 mm.	.62 mm.	.34 mm.
7	20	.336 mm.	.60 mm.	.14 mm.
12	35	.293 mm.	.78 mm.	.12 mm.
13	35	.250 mm.	.46 mm.	.10 mm.
3	7	.211 mm.	.34 mm.	.12 mm.
12	24	.290 mm.	.52 mm.	.08 mm.
—	—	—	—	—
62	151	.3408 mm.		

One of the most striking things shown in the table is the decrease in the average length of the ridges toward the caudal end of the canal. The table giving the averages for the longitudinal series shows the same decrease in length. It must be remembered, that the eleven divisions of the longitudinal series correspond to the first six divisions of this series, the seventh being the part on the caudal fin which it was impossible to cut in the longitudinal series. In the above table there is a gradual decrease in the length of the ridges in the first six divisions, or that part of the canal lying on the body of the fish, as the canal approaches the tail, and then a marked increase in the seventh section or that part of the canal lying on the caudal fin. In the smaller fish the ridges do not begin to increase in length until the canal reaches the tail, while in the larger specimen the increase begins on the posterior part of the body.

The fact that in nearly every instance the ridges terminated so near the base of the branchlet and extended some distance cephalad suggested that the sensory ridge or some portion of it might be continued out into the branchlet. Nothing of the kind had been observed in either series, but over one-half of the branchlets in the transverse series were carefully reexamined for the presence of sensory epithelium in the branchlets. As has been stated before, the ridge occupied the medial part of the canal extending up slightly, at times, upon the side of the canal opposite to that from which the branchlet was given off. The branchlet emerged from the canal from the lateral part of either the dorsal or ventral sides of the canal and ran nearly parallel to the surface for some distance. Thus the sensory ridges occupied the medial half of the canal and the branchlets opened into the lateral

half of the canal. The sensory ridge was never seen to divide or branch. A careful examination of the epithelial lining of the branchlets failed to reveal any sensory epithelium. The flat pavement epithelium of the canal, very soon after entering the branchlet became more cuboidal, resembling that found in the caudal portion of the canal. Toward the peripheral ends of the branchlets the simple cuboidal epithelium gradually became stratified, and passed over at the tube-pore without any abrupt change into the general epithelium of the body surface. Only the proximal parts of the branchlets were enclosed in the cartilaginous rings.

No careful investigation into the nature of the pit organs was attempted, on account of the conditions of the material and the thickness of the sections. All of these organs observed in the neighborhood of the canal were encased in a cup-shaped cartilage with a layer of columnar epithelium across the top of the "beaker" or cup-shaped cavity. Eleven of these pits were observed in the transverse series.

A rather unusual structure was observed in both series, namely, the apparent combination of one of the pit organs and a crest of the lateral canal (fig. 6). Three of these were found in the longitudinal series and one in the transverse series. The caudal end of the drain-pipe cartilage where it approached the surface was elongated laterally forming a cup or "beaker-shaped" cavity. The rim of this cavity was on a level with the lower columnar layer of the epidermis and the bottom was open into the lumen of the cylindrical cartilage. There was apparently no open communication between the pit organ and the sensory canal for the opening was covered by quite a thick membrane. As far as was observed there was no difference in the structure of these organs and the pit organs which were in no way connected with the canal. This relation may have no special significance but be due to the accidental fusion of the cartilage forming the cup-shaped cavity with the canal cartilage. The investigation has not been extensive enough to warrant any conclusions with regard to these structures.

The location of the nodules, or placoid scale-like structures, pits and these openings will be shown in the following table as they were found in the longitudinal series. The grouping into eleven divisions corresponds to the eleven pieces into which the lateral canal was cut

for convenience. The first column gives the number of pit organs found in the immediate vicinity of the canal, the second column, the number of "openings" or combination of pit organ and canal cartilage, while the third column gives the number of nodules in each division. Where two or more scales had a common base they were counted as one. The last column gives the number of crests in each division which had no surface marking; neither pits, "openings," nor nodules. The table shows very clearly the anterior position of the pits and the "free crests," and the posterior location of the nodules.

Pits	Openings	Nodules	"Free crests"
2	2	2	10
1	0	6	3
2	0	6	4
1	0	5	4
2	0	8	3
1	0	11	4
1	1	13	1
0	0	12	1
0	0	11	2
0	0	7	0
0	0	7	0
—	—	—	—
10	3	88	32

IV. SUMMARY

1. As the lateral canal passes caudad from the gill region its diameter gradually becomes smaller.

2. The sensory ridges are located upon crests or parts of the canal approaching the surface. The lumen of the canal here is always larger than just anterior or posterior to the ridge.

3. The longest ridges are in the anterior region. There is a gradual diminution in length going caudad upon the body until just before the tail is reached, or upon the tail itself, where there is a slight increase in length.

4. No branchlet, except on the caudal fin where there seems to be a great irregularity, is given off without a sensory ridge at its proximal end. Ridges may or may not occur between the branchlets.

5. The branchlets tho grouped to a slight extent are given off thruout the entire length of the lateral canal.

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FIG. 1. Line of a Larger Fish.

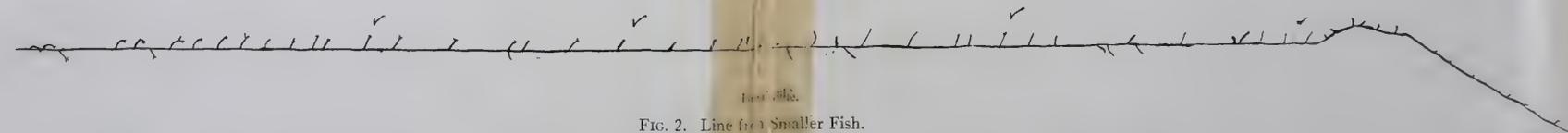


FIG. 2. Line of a Smaller Fish.

V indicates places where line has cut.
X posterior to this point tubercles were not indicated



EXPLANATION OF PLATES XVIII and XIX

- Fig. 1. Diagram of the right lateral line from the smaller fish.
 Fig. 2. Diagram of the right lateral line from the larger fish.
 Fig. 3. A crest from the longitudinal series showing an "opening." Outlined with camera lucida. Zeiss objective A, ocular no. 4.
 Fig. 4. A crest from the longitudinal series showing the cylindrical cartilage projecting above the surface as a nodule (c.n.) Magnification as for fig. 3.
 Fig. 5. A section from the longitudinal series showing a crest in sagittal section and a free nodule (n). Same magnification as fig. 3.
 Fig. 6. A section from the transverse series showing an "opening." Outlined with camera lucida Zeiss objective D, ocular no. 2.
 Fig. 7. Section from the transverse series showing a pit organ (f.p.) and passing thru the anterior part of a branchlet (br.), showing the continuation of the canal cartilage out around the branchlet. Drawn with the same magnification as fig. 6.

X=posterior to this point the nodules were not indicated.

V indicates places where line was cut.

- | | |
|---|---|
| <i>b</i> ring-shaped canal cartilages. | <i>p t</i> pit organ attached to the cylindrical cartilage. |
| <i>br</i> base of branchlet | <i>n</i> free nodule |
| <i>c</i> cylindrical canal cartilage | <i>o</i> opening for the entrance of nerve |
| <i>c n</i> nodule attached to the cylindrical cartilage | <i>p</i> pigment cells |
| <i>e</i> surface epithelium | <i>r</i> sensory ridge |
| <i>f p</i> free pit organ | |

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SOCIETY, VOL. XXXVIII

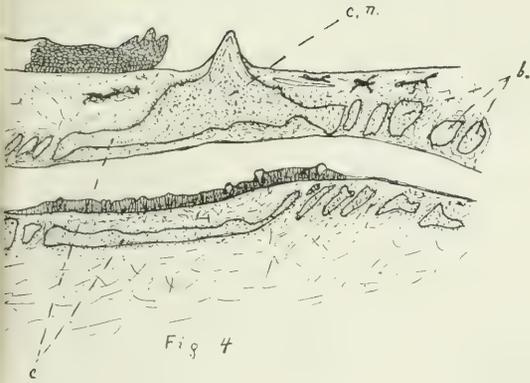


Fig. 4

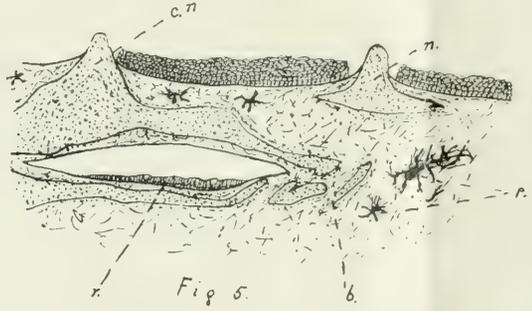


Fig. 5

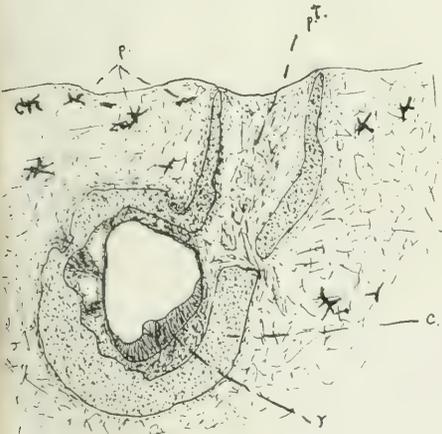


Fig. 6

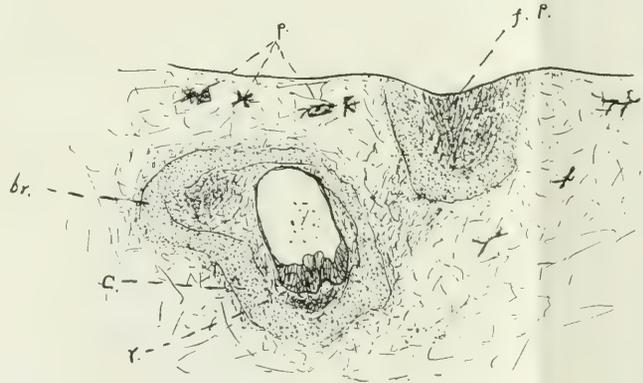


Fig. 7

A NEW TREMATODE, *ACANTHATRIUM NYCTERIDIS*,
NOV. GEN., NOV. SPEC., FROM THE LITTLE
BROWN BAT.¹

BY ERNEST CARROLL FAUST

The material described in this paper was taken from the small intestine of a female *Nycteris borealis-borealis* (Müller). The bat was found in the vicinity of Urbana, Illinois and brought to the writer during the summer of 1918. With the female were two suckling young which were uninfected. Of the ten individuals of the new parasite found, two were studied alive and the remainder preserved and studied as totos and sectioned material. In the living specimens the details of the excretory system were worked out and the process of fertilization observed.

This species conforms to the previous diagnostic rules prescribed for the genus *Lecithodendrium* in the shortness of the digestive ceca, the position of the uterine loops, the general type and position of the testes and vitellaria and in the absence of a muscular cirrus. However since the structure of the organs immediately surrounding the genital pore are important criteria on which generic diagnosis is made, it seems necessary to create a new genus to include this species and *Lecithodendrium sphaerula* Looss 1896, which possess in common a genital atrium lined with numerous lanceolate spines. I propose the name *Acanthatrium* for this new genus.

Diagnosis of Acanthatrium nov. gen. Small-sized Brachycoeliinae, spherical to pyriform, with a genital atrium lined with numerous integumentary spines; prostate glands numerous; testes preacetabular, in a plane with the genital pore; vitellaria anterior to the digestive ceca; excretory system with four groups of flame cells of three each for each half of the body; in intestine of bats. Type species: *A. nycteridis*.

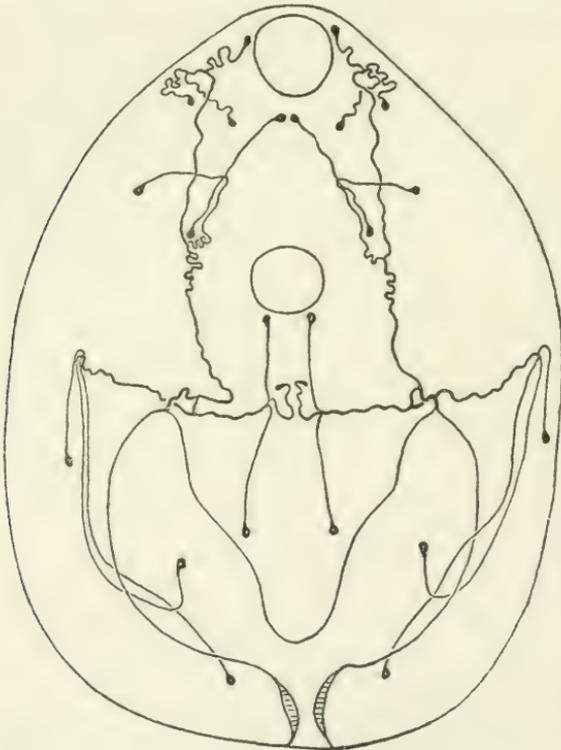
¹ Contributions from the Zoological Laboratory of the University of Illinois, No. 138, and from the Department of Pathology, Peking Union Medical College.

Acanthatrium nycteridis nov. gen., nov. spec.Host: *Nycteris borealis-borealis* (Müller), small intestine

Locality: Urbana, Illinois

Date: July, 1918

Acanthatrium nycteridis is a broadly oval to pyriform fluke which normally measures 0.185 to 0.2 mm. in length by 0.15 to 0.16 mm. in width, but on contraction may become much broader or longer. All specimens examined have been found to be spineless on the external integument. The oral sucker measures up to 25 μ in diameter and is

Text fig. 1. The excretory system of *Acanthatrium nycteridis*.

consequently much larger than the acetabulum which always measures less than 16 μ . The latter organ is situated some two-fifths distance from the anterior end of the fluke. The oral opening leads directly into a very muscular pharynx, 6 μ in diameter by 4-5 μ in

length. The ceca compose a short broad furculum lying between the vitelline glands on the anterior face and the prostate glands on the posterior face. They extend laterad as far as the testes. The walls of the ceca are heavy, and the cells of which they are composed distinctly glandular.

The excretory system of this species has been studied very carefully in the living animal subjected to slight pressure of the cover glass. Not only have the main canals of the system been made out but the exact number and relationship of the capillaries and flame cells have been determined. These latter comprise four triplet groups for each side of the body, making a total of twenty-four cells for the worm. The excretory pore is posterior. It leads into a single muscular shank of short length (see text fig.) which soon expands into two long pouch-like cornua. These cornua extend anteriorly to a plane somewhat back of the middle of the body. There each cornu receives a single very short main collecting tubule. Into this main tubule flow two secondary tubules, one of which is derived from a double set of three capillaries each lateral and posterior to the main tubule and a second which is derived from a double set of three capillaries each arising anteriorly and somewhat mesad. At the end of each capillary is a small flame cell. Designated from the anterior end backward (Faust 1919) group α' lies ventrad to the median frontal plane of the fluke, laterad and slightly posteriorly to the oral sucker. Group β' also lies ventrad, but laterad to the prostate glands. Group α'' lies dorsad, in the region just posterior to the acetabulum. Group β'' lies ventrad to the cornu on each side. A view of the individual flame cell under the highest powers of the microscope shows the "flame" to consist of a relatively small number of flagella which are noticeably thick at their distal ends. No excretory granules appear in the system.

Previous studies on the excretory system of the Brachycoeliinae (Looss 1896: Fig. 50) are fragmentary, but are consistent with the data I have secured from the study of the species *Acanthatrium nycteridis*, namely, that there is a common flame-cell plan for the Brachycoeliinae. On the basis of this scheme it is expected that there will be found in each species of the sub-family twenty-four flame cells, consisting of four triplet groups of flame cells on each side of the body. Furthermore, the work of Wright (1912:167-169) on the

related family Microphallinae gives weight to the view that there is a fundamental plan of flame-cell grouping in the family. For, in *Microphallus opacus* altho there are only sixteen flame cells in the adult worm, they consist of four couplet groups for each side of the body, so that the four groups are to be regarded as elemental, hence fundamental. Moreover, the rather inadequate study of Jäger-skiöld (1900) on the excretory system of *Spelotrema pygmaeum* demonstrates at least the fourfold structure in this species. Thus as I have remarked (1919) "the mathematical exactness of flame-cell formation of this family makes it possible to calculate the flame-cell formula of the cercaria from the structure of this system in the adult."

The genital system in *Acanthatrium nycteridis* is characteristically lecithodendrine. The ootype is located to the right and somewhat posterior to the acetabulum. The vitelline follicles, ten to twelve in number, lie lateral to the pharynx and esophagus and anterior to the ceca. They are somewhat lobed. Two vitelline ducts, dark brown in color, arise from the two groups of follicles. Extending over the ceca and prostate glands, they bend toward the median line and converge behind the acetabulum. From this junction a common vitelline duct proceeds to the ootype. The ovary is an irregular pyriform body, somewhat smaller than the testes and located anterior to the ootype, in the plane of the acetabulum. It is connected with the ootype by a short duct. A minute seminal receptacle opens into the ootype from the right side. From the side of this receptacle a delicate Laurer's canal extends toward the integument of the dorsal side of the worm. The uterus arises from the posterior side of the ootype. Its convolutions first fill the posterior half of the right side of the fluke, then turn to the left side, crowding the entire region up to and often encroaching upon the left testis. The uterus enters the genital atrium to the left of the ejaculatory duct.

The testes are ovoid to pyriform glands occupying the same transverse plane as the genital pore and the surrounding prostate glands. They are definitely antacetabular. Vasa efferentia carry the spermatozoa mesad to the base of the seminal vesicle on the anterior border of the acetabulum. The latter organ coils to the left and then forward. It passes almost imperceptibly into the short ejaculatory duct. This duct opens into the genital atrium thru a small pore. The prostate glands surrounding the metraterm and opening

into the ejaculatory duct consist of a large spherical mass of unicellular glands.

The genital atrium lies mostly anterior to the genital pore. It is elongate and coiled on itself several times, and is lined with a large number of sharp lanceolate spines. The genital pore is large with a prominent sphincter muscle.

The mature uterus is filled with fertilized eggs. They have a distinct operculum at one end, are oval, and are light brown in color. They measure 33 by 19 μ at the inner end of the uterus and 44 by 23 μ at the opening of the uterus into the genital atrium. In spite of the crowding of the uterus with eggs spermatozoa were observed to pass in large numbers from the genital atrium down the uterus to the ootype. The activity of these sperm frequently caused the eggs to back up into the ootype and even into the distal end of Laurer's canal. At times the sperm masses occupy half of the uterus.

Fertilization occurs in the ootype. The naked protoplast comes down the oviduct at irregular intervals. Material from the common vitelline duct is then laid around the ovum, after which the shell is added.

DISCUSSION

The Brachycoeliinae as originally constituted by Looss (1899:608) have come to include Brachycoelium, Pycnopus, Phaneropsolus, and Lecithodendrium. To this group must now be added the new genus Acanthatrium, previously embodied in part in Lecithodendrium. European investigators have divided this group into two parts, one consisting of those genera in which a muscular cirrus pouch is present and one containing those genera where a cirrus bulb is wanting, or, at most, parenchymatous in structure. Lühe (1901:173) and Looss (1902:815) have even suggested that those genera including forms without muscular cirrus should be withdrawn from the Brachycoeliinae and placed in a new subfamily, the Lecithodendriinae. The genus Acanthatrium readily falls into the latter group, because species of this genus lack any evidence of muscular structure in the region of the ejaculatory duct.

An examination of the various species comprehended up to this time under the head Lecithodendrium shows that exact data on the seminal receptacle are lacking in many species. Moreover the out-

line of the ovary is decidedly varied, being entire in certain species and decidedly lobed or sinuate in closely related ones. But the position and type of the vitellaria and of the testes, and the presence or absence of spines in the genital atrium give a basis for a natural division of these species into three groups. The first group contains those in which the genital atrium is lined with conspicuous spines and in which the testes are antacetabular in a plane with the genital pore. These species, *nycteridis* nov. spec., and *sphaerula* Looss belong to the genus *Acanthatrium*. The second group consists of those species in which the genital atrium is aspinose, in which the vitellaria are lateral to the pharynx, but in which the testes are in the plane of the acetabulum. To this group belong the species *ascidia* van Beneden, *chefrenianum* Looss, *chilostomum* Mehlis, *cordiforme* Braun, *glandulosum* Looss, *obtusum* Looss, *posticum* Stafford, and *pyramidum* Looss. These species belong to the genus *Lecithodendrium sensu stricto*. The third group contains species, which, like those just mentioned, have an aspinose genital atrium and testes in the plane of the acetabulum, but in which vitellaria are conspicuously posterior to the ceca and near to the acetabulum. To this group belong the species *granulosum* Looss, *hirsutum* Looss, and *urna* Looss, and for them I propose on the basis of this distinction a new genus, *Mesodendrium*. Other species at one time or another referred to *Lecithodendrium* have either been removed from this genus or are so inadequately described that their exact position can not be fixed with certainty.

SUMMARY

1. *Acanthatrium nycteridis* nov. gen., nov. spec., from *Nycteris borealis-borealis* is described.
2. The excretory system of this species is based on a fundamental four-fold grouping of triplet flame cells, which, by comparison, suggest that the four-fold grouping is a common denominator of the several sub-families of the *Brachycoeliidae*.
3. Analysis of the species of *Lecithodendrium sensu lato* makes it necessary to recognize three genera from this group, *Acanthatrium* nov. gen., *Lecithodendrium sensu stricto*, and *Mesodendrium* nov. gen.

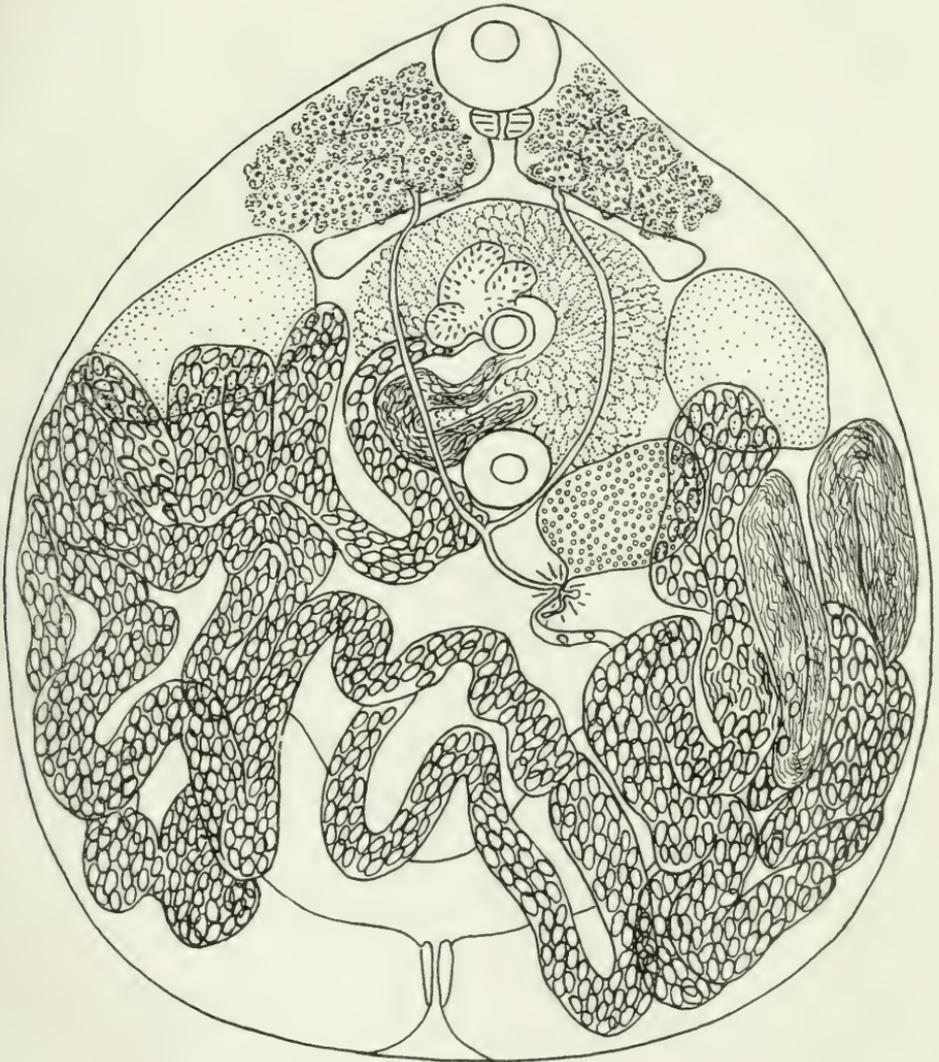
EXPLANATION OF PLATE

Dorsal view of *Acanthatrium nycteridis*, showing reproductive organs. X 340.

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THE PRESERVATION OF FRESH-WATER BRYOZOA

BY F. SLATER JACKSON, M.D.

The following method is the outcome of a series of experiments made by the writer during the summer of 1916, while engaged in a preliminary study of the fauna of certain of the Laurentian lakes in Argenteuil County.

Attempts were made to preserve several species of Bryozoa in an expanded condition by treating specimens (previously narcotized with chloretone, cocain, chloral hydrate, etc.) with alcohols of gradually increasing concentration, formalin and other reagents, but without success. Nor did the sudden killing and fixation of small colonies by means of well-known reagents, such as hot corrosive-acetic, chrom-acetic, osmic acid mixtures or Bouin's fluid, give better results.

For reasons afterwards to be discussed, the following method suggested itself to the writer, and has afforded good results for several species.

(1) *Narcotization.* Elongated colonies attached to twigs or to the stems of aquatic plants, are placed in water in a tube of convenient length, and of a diameter sufficient to permit of the colony lying obliquely, so that it may not come in contact with the tube except at the extremities. In the case of flattened forms, e.g., those occurring on leaves, etc., the operations are best conducted in Petri dishes. A saturated aqueous solution of cocain* is slowly added by means of a pipette. At first a small quantity of the solution (say 1 c.c.) is dropped upon the surface of the water and allowed to diffuse. A similar amount may be added at intervals of five minutes, a small quantity of the water being removed from time to time if necessary.

The retraction of the zooids caused by the first addition of the cocain is only temporary, and uniform expansion of the whole colony will be attained in about half an hour.

* A saturated aqueous solution of chloretone may be substituted, of which, however, a larger quantity will be required.

When this stage is reached, about 5% of the entire fluid may be withdrawn, and replaced by the cocain solution, which is to be forcibly injected into the remaining fluid by means of the pipette, in order to ensure its uniform admixture.

After this time the colony should be observed at intervals with a lens, and if ciliary motion has ceased (as indicated by the cessation of vortices containing fæcal and other particles) no further addition of cocain is necessary.

Periodical stimulation (either by touching the individual extended lophophores with a needle, or, preferably, by setting up currents with the pipette) will indicate the degree of narcotization. This will probably be complete in from one to one and a half hours, but will vary with the species.

In every case it will be advisable to wait for ten minutes after there is no further response to stimuli, before adding the preserving fluid, otherwise immediate and permanent retraction may follow its addition.

(2) *Preservation.* The tube or dish containing the narcotized colony is placed in a convenient vessel (to permit of overflow) and the narcotizing fluid gradually replaced by a fluid having the following composition:—

Cane sugar	10 parts
Formalin	2 parts
Distilled water to	100 parts

(The sugar is to be dissolved in the water and the formalin subsequently added.)

The replacement is made by injecting the preserving fluid (a pipetteful at a time) with sufficient force to ensure its admixture with the narcotizing fluid, and its uniform contact with the colony. When it is estimated that the fluid consists of approximately 50% of the sugar solution, the preparation may be allowed to remain in this for about half an hour. At the expiration of that time the preserving fluid may be added more rapidly until it almost completely replaces the former mixture. The colony is then left undisturbed for half an hour or more, and may then be transferred to a fresh vessel containing the undiluted preserving fluid.

The time required for the entire process, from the commencement of narcotization, is about 2½ hours, but will be found to vary slightly for different species.

Numerous preparations of *Plumatella*, *Fredericella* and *Cristatella*, made according to this method in August, 1916 are still in an excellent state of preservation.

It is suggested that the fluid be changed after the lapse of a week or ten days.

While not primarily designed as a microscopic or histological method, the above treatment has been found to afford an adequate means of preliminary preservation for material to be subsequently employed in the preparation of slides, or even of sections.

For this purpose individual zooids or small portions of the colony are to be dropped into Bouin's fluid,* allowed to remain in this for a few hours, and then transferred to 70% alcohol.

When free from picric acid they may be stained *in toto* and mounted, or embedded in paraffin in the usual way.

It has been found that the tissues, and even the cilia, are well preserved.

The formalin-sugar solution has, with modifications, been successfully employed for the preservation of insect larvæ and pupæ,¹ and may also be used for various small invertebrates.

A consideration of some aspects of the question of the permeability of cell-membranes, and of the adsorption phenomena of cane sugar, suggested the possibility of the application of a solution such as the above in the preservation of Bryozoa. Since the surface tension at the interface between water and other phases is but slightly reduced by sugar, and, as has been pointed out by Michaelis and Roona,² and by Parkin,³ adsorption of sugar does take place at such surfaces, the employment of a sugar solution offers possibilities for the preservation of delicate tissues. The observations of Küster⁴ on the cells of the onion, plasmolyzed by hypertonic sugar solution, seem to indicate that there is a certain degree of fixation of the surface membrane.

Furthermore in view of the fact that, as established by Freundlich,⁵ gelatin will adsorb sugar only after preliminary treatment with formalin, and since the experiments of Lloyd⁶ have indicated the essential similarity of protoplasm and gelatin as regards their behavior in imbibition and similar phenomena, it was thought that the

*Picric acid, saturated aqueous solution.....	75
Formalin.....	20
Glacial acetic acid.....	5

employment of sugar and formalin in combination might provide an efficient preservative for these organisms.

It is suggested that the efficacy of the method is due to the fixation of the protoplasm by the formalin, and to the protective action of the adsorbed sugar.

The employment of formalin alone as a preservative, although recommended by Green⁷ and by Davenport,⁸ has, in the writer's hands, invariably proved fatal.

As is well known, formalin combines chemically with proteins, and in the case of fresh-water Bryozoa, it results in the formation of a flocculent precipitate, and the ultimate separation and disintegration of the lophophores. Similarly, while one of the best preservatives for marine Cœlenterates, it gives poor results with Hydra. On the other hand, it proves excellent for the free-swimming larvæ of Bryozoa, and for statoblasts.

*Zoological Laboratory, McGill University,
June 10th, 1919.*

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For the accompanying photographs, I am indebted to Mr. W. B. Stokes, Secretary of the Montreal Natural History Society. Plate XXI is *Cristatella mucedo* and Plate XXII is *Fredericella* sp.

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PLATE XXII

JACKSON

SOME EXPERIMENTS CONDUCTED WITH PURE CULTURES OF BREAD YEAST

BY WILLIAM F. HENDERSON

Study of the conditions under which yeast colonies most rapidly develop, also of the conditions under which yeast produces carbon dioxide, have always been very interesting as well as practical. The failure to secure satisfactory yeast growths and gas productions when ordinary bacteriological media were used, led the author to pursue the problem more in detail, with the discovery of some very interesting facts concerning yeast development.

ISOLATION OF THE YEAST COLONIES

Some pieces of "Yeast Foam" were mixed with distilled water. Agar and gelatin plates were made from diluted portions of this stock solution and the agar plates were incubated at 38° C. The gelatin plates were allowed to develop at room temperature, 20° C. The yeast colonies developed on the agar plate along with bacterial colonies, but none of the yeast colonies grew very large. The largest was not quite one mm. in diameter, circular, soft, and dull gray. The colonies on gelatin developed much more slowly and were no larger than those on agar.

Sub-cultures were made in sloped agar tubes. The maximum development gave a very thin streak of circular colonies, closely packed together. The development was scanty and far from satisfactory. Microscopical mounts were made from one of these streak cultures and no organisms were found to be present save the yeast. This pure culture was saved and was used as the source of material directly or indirectly, for all later experiments.

EXPERIMENTS ON SOLID MEDIA

The familiar relationship which exists between yeasts and sugar fermentation at once suggested the addition of sugar to the agar medium. This was tried as follows:

Author's Note: During the course of these experiments many helpful suggestions were made by Dr. A. A. Tyler to whom the author wishes to express his appreciation.

Small amounts of cane sugar, lactose, and glucose were added, respectively, to three agar tubes. These tubes were sterilized and cooled in a sloping position, and when cold, were inoculated from pure yeast cultures. The material was incubated for 24 hours at 38° C. and the results noted. The cultures on cane sugar agar and on lactose agar had scarcely developed at all; only thin, transparent streaks were visible. The yeast on glucose agar, however, had grown so rapidly that the entire surface of the medium was covered with the thick creamy-white mass of colonies. Upon microscopical examination, this material on glucose agar proved to be pure yeast culture. It would seem then, that the monosaccharides, of which glucose is an example, furnish much more favorable conditions for yeast development than do the disaccharides.

Further experiments were performed, using a larger variety of sugars. Seven agar tubes were prepared, six of which contained the following sugars, respectively:

Monosaccharides:	Disaccharides:
Glucose	Saccharose (cane sugar)
Galactose	Maltose
Levulose	Lactose

The seventh tube was used without sugar and served as a check.

One cc. of sterile water was poured into each of seven Petri dishes which had previously been sterilized. This water was inoculated with a small amount of yeast material and the plates poured in the usual manner, using the seven tubes of agar described above. These plates were incubated at 38° C for 28 hours. Development on two of the monosaccharides (glucose and levulose) was very rapid, while on the galactose and on the disaccharides, development was slight. Of the disaccharides, cane sugar seemed to furnish the most favorable conditions. The largest colonies on the glucose and levulose agar plates were surface colonies 6 to 7 mm. in diameter. The largest colonies on any of the other plates were 2 to 3 mm. in diameter. These occurred on the cane sugar plate and were surface colonies. The accompanying illustration shows the results obtained on these plate cultures. The plate containing galactose agar was not included in the photograph as it was identical in appearance with No. 6 (agar without sugar). It seems, therefore, that galactose, maltose, and lactose contribute very little, if any, to the conditions favorable for

yeast development on solid media; that cane sugar improves the conditions slightly, and that glucose and levulose produce conditions exceedingly favorable for yeast growth.

VARIATIONS IN MORPHOLOGY

When yeast grows in liquid media, the cells produced are of the familiar form viz., round, or more often, oval. As the budding occurs, small round or oval cells are produced, and these break off very readily, especially if the medium is agitated by shaking or stirring. In the case of the yeast used in these experiments, the maximum diameter attained by the cells in liquid media was from 7 to 9 microns. It is evident at the outset, that the conditions encountered by the yeast in or on a solid medium were entirely different from those encountered in a liquid medium. A careful examination of the plate cultures described previously showed that while the colonies were still minute, those on the surface, as well as those below the surface of the agar, were composed of cells identical in appearance with yeast cells grown in liquid media. As the colonies became older and larger, those on the surface retained their generally circular form, composed of ordinary oval yeast cells closely packed together. At the edge, the layer was one cell thick, but in the center budding had occurred vertically, thus rendering the colonies more or less opaque. Cells in all stages of budding could be seen, as the solidity of the medium held all the cells and buds in their respective positions.

As the deep, embedded colonies grew older, they attained a distinctly stellate appearance. These colonies possessed a small circular "nucleus" of ordinary oval yeast cells, but at the edge, some of the cells had become greatly elongated, growing in a direction away from the mass of the colony. Repeated budding and elongation of cells, together with the solidity of the medium resulted in the formation of long branched filaments, extending radially from the central colony. When cells from these stellate colonies were transferred to a slide and examined, it was found that the cells had all broken apart in the transfer. The cells observed were almost all single, some being oval in shape and others very long and slender. It is evident then, that the solidity of the medium (causing the cells to be immovable in reference to each other) was the cause of the filamentous formation. Furthermore, as the initial cells of the colony grew and multiplied,

a paucity of food would soon arise in the immediate vicinity. In liquid media, diffusion would maintain the supply of food for a considerable length of time, but in a solid medium diffusion would be slow.

As the young colony grew and spread in all directions, the food supply in the medium within the colony soon became almost exhausted. Very soon, those cells at the edge received the food stimulus only from the medium outside the colony. They responded to this more localized stimulus by growing and budding in a direction away from the mass of the colony. The location of the cell contents proved also to be interesting. The older, elongated cells were highly vacuolated. The younger, elongated cells (farther away from the mass of the colony) were less vacuolated. The vacuoles appeared first in the older part of the cell (nearer the mass of the colony) and the cell protoplasm tended to collect in the end of the cell away from the mass of the colony. Finally, the terminal cell contained only a few very small vacuoles and in many cases was found to be budding at the free end. The occurrence of more than one bud resulted in the formation of a "branch" in the filament. The accompanying figures show some of the types of filamentous growths and elongated cells.

EXPERIMENTS ON GAS FORMATION

A Smith gas tube was filled with 1% lactose bouillon and inoculated with pure yeast. Development occurred in the open arm only and no gas was registered in the tube. This suggested the thought perhaps the yeast was aerobic in character and would not develop to any extent under anaerobic conditions.

Gas tubes were prepared with two-hole cork stoppers; through one hole was placed a glass tube drawn out to a fine capillary and reaching to the bottom of the gas tube. Through the other hole was placed a short glass tube to allow exchange of gases as changes of pressure required. Both tubes were bent down outside and plugged with cotton. The gas tubes were filled with sugar bouillon, corked, and sterilized. The tubes were carefully inoculated with pure yeast and then from 30% to 40% of sterile oxygen forced into the closed arm through the capillary. A check tube was prepared in which no gas was admitted. These tubes were incubated 120 hours after which time no further changes seemed to occur. The residual gas in

the tubes was tested for carbon dioxide by absorption in 5% potassium hydroxide solution. The results were as follows:

Media	Initial Gas	Final Gas	% Absorbed by KOH	Residual Gas
Bouillon+ 1% Cane Sugar	Oxygen 38%	8%	5%	3%
Bouillon+ 1% Lactose	Oxygen 34%	6%	3%	3%
Bouillon+ 1% Glucose	Oxygen 35%	8%	0%	8%
Bouillon+ 1% Lactose	Air 32%	27%	3%	24%
Bouillon+ 1% Lactose	No Gas	No Gas	—	—

Where oxygen or air was admitted, development occurred in both arms of the tubes. In the last tube where no gas was admitted, development occurred in the open arm only. These tests strongly suggest that the yeast used (ordinary bread yeast), is aerobic. This conclusion is supported by the fact that in the agar plates described earlier, the deep colonies never attained any great size while the surface colonies grew (when on suitable media) to a large size.

According to chemical laws of gases, one volume of oxygen will produce the same volume of carbon dioxide. However, when we consider that carbon dioxide is 25 or 30 times as soluble in water as is oxygen, the shrinkage of gas volumes in the tubes is easily explained. As the slightly soluble oxygen was replaced by carbon dioxide, this latter gas would remain dissolved in the water and the liquid would rise to replace the oxygen used. This could proceed until the liquid became practically saturated with carbon dioxide. The proof of this explanation lay in the following simple test: A gas tube was filled with distilled water and 33% of carbon dioxide passed into the closed arm. This tube was placed in the incubator overnight. In twelve hours all but 3% of the gas had been absorbed. Even though the liquid might become saturated and an excess of gas

be produced by some organism, the slow diffusion and escape through the open arm of the tube would result in a gradual shrinkage of gas volume. This was shown by placing 35% of carbon dioxide over water previously saturated with the gas. In thirteen days the gas volume read 15%.

The last experiment performed consisted of the inoculation of gas tubes which contained different percentages of several kinds of sugars. The sugars used were glucose, lactose, cane sugar, and maltose. The percentages used were 1%, 5%, and 10% in each case except maltose, where 1% and 5% were used. No air nor oxygen was passed into the closed arms. All the tubes were incubated at 38° C. Those containing lactose and maltose developed a very slight scum over the surface of the liquid in the open arm. The liquid in the closed arm remained perfectly clear and no gas was registered. The tubes containing 1% and 5% cane sugar developed fairly well in the open arm, but not at all in the closed arm. Where 10% cane sugar was used, 1.5% of gas appeared on the sixth day. The maximum percentage in this tube was 1%, acquired on the seventh day. There was an excellent development in the open arm and a fair development (cloudiness) in the closed arm.

The tubes containing glucose developed without delay. Scum formed on the surface of the liquids in a few hours. A record of the gas formation follows:

Medium Used	42 hrs.	45 hrs.	50 hrs.	53 hrs.	56 hrs.	66 hrs.	70 hrs.	74 hrs.	117 hrs.	130 hrs.
1% Glucose	3%	5%	14%	30%	48%	77%	88%	90%	30%	20%
5% Glucose	2%	4%	20%	42%	100%	100%	100%	63%	63%
10% Glucose	9%	100%	100%	100%	100%	100%

At 100% the gas overflowed and bubbled out through the open arm. In the tube containing 5% glucose, a shrinkage from 100% (full) to 63% occurred, at which time evaporation of the liquid allowed air to bubble in and stop the shrinkage. The tube containing 10% glucose remained full of gas as long as observations were taken.

Food became nearly exhausted first in the tube containing 1% glucose. Diffusion and consequent loss of carbon dioxide caused shrinkage to begin first in this tube. The food in the 5% glucose tube became scarce a few hours later and shrinkage began at once. Food in the 10% glucose tube evidently was still available at the time of the last observation.

When these results are compared with those obtained from the plate cultures, we find admirable agreement. The glucose (as would probably levulose) gave very favorable conditions for quick growth and rapid gas formation. Of the disaccharides, cane sugar was the most suitable, but did not compare favorably with glucose.

CONCLUSIONS

The determination of the exact or total amounts of carbon dioxide produced by yeast is beyond the scope of this article, as are also such problems as those concerning the ease of hydrolysis of the various disaccharides by the yeast enzymes. However, from the simple experiments cited above, several concluding statements might be made:

1. That glucose and levulose cause yeast to grow much more rapidly than any of the other common sugars.
2. That glucose, (and probably levulose) causes the most rapid production of carbon dioxide.
3. That yeast grows better under aerobic conditions, but will develop in the proper medium under at least limited anaerobic conditions.
4. That in order to register gas in a gas tube, the gas must be produced in sufficient amount to more than saturate the liquid, and at a sufficient rate to overcome loss by diffusion through the open arm.
5. That a solid medium may materially alter the morphological characters of the individual yeast cells by a tendency to localize the food supply.

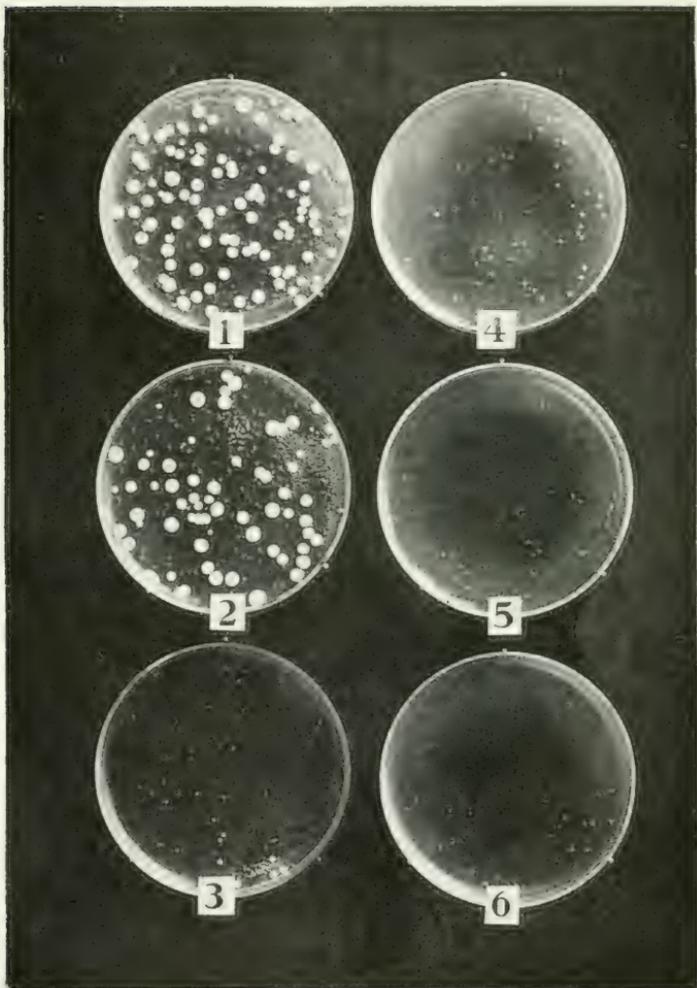
James Millikin University.

EXPLANATION OF PLATES

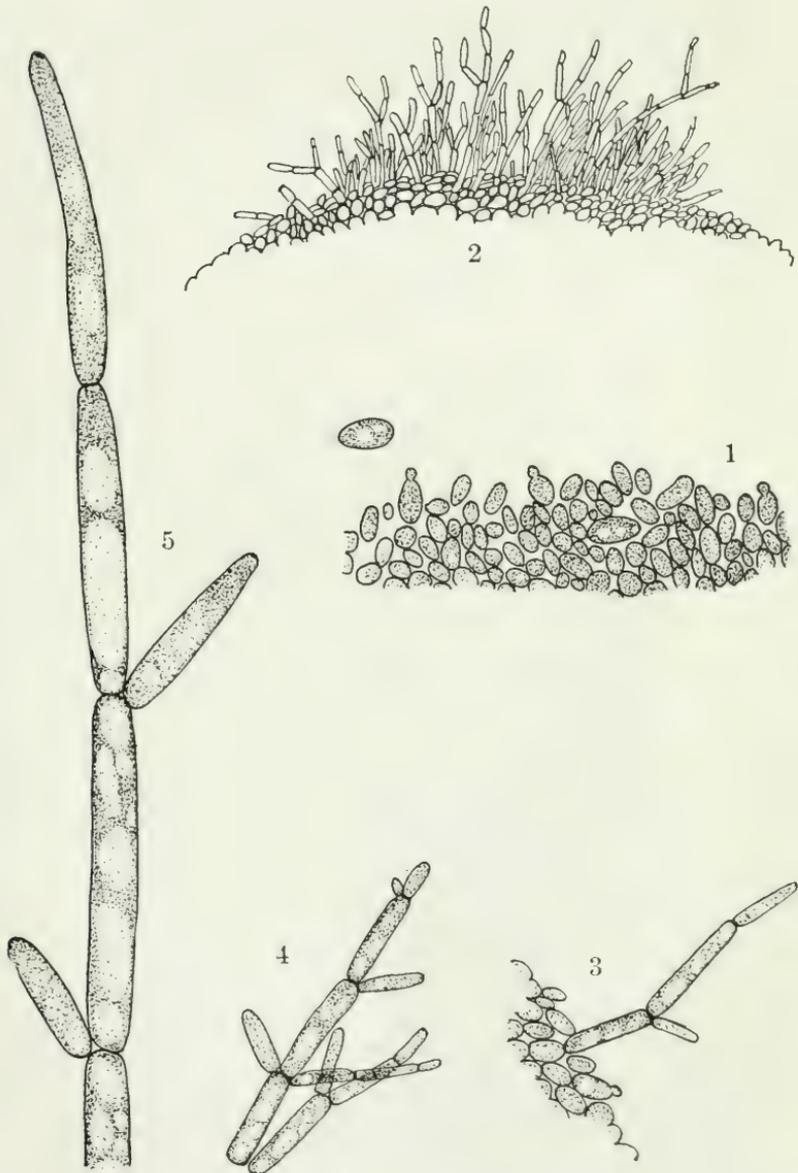
Plate XXIII. Plate cultures on sugar agar. (1) Glucose; (2) Levulose; (3) Cane sugar; (4) Maltose; (5) Lactose; (6) Agar without sugar.

Plate XXIV. Fig. 1, Margin of surface colony on sugar agar plate. Fig. 2, Edge of deep agar colony showing filaments. Figs. 3, 4, Elongated yeast cells, showing origin, also method of branching. Fig. 5, Elongated cells, showing large vacuoles especially in older cells.

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SECONDARY PROTHALLIA OF *NEPHRODIUM*
HIRTIPES HK

By W. N. STEIL

Although the prothallia of a large number of species of ferns were grown under different cultural conditions to determine, so far as possible, what factors are concerned in the formation of secondary prothallia, the results herein described are based chiefly on experiments with the prothallia of *Nephrodium hirtipes* for a description of which the reader is referred to an earlier paper (Steil, 1919).

In the first culture of *Nephrodium hirtipes*, made December 14, 1913, most of the prothallia were destroyed by a parasitic fungus. The healthy prothallia were carefully transferred to cultures made by placing sphagnum into small Stender dishes. The sphagnum was saturated with Knop's solution, and the medium was then thoroughly sterilized before the prothallia were transplanted. The cultures were then placed in subdued light for a period of two weeks. In consequence of the different light conditions, a large number of short filaments, each consisting of a single row of cells, were produced from the margins and occasionally from both surfaces of the prothallia. The cultures were then placed for a like period of time under favorable conditions of illumination in a Wardian case. It was now observed that the filamentous prothallia broadened out and became heart-shaped. The experiment was repeated several times with the same culture and as a result of the changes in illumination numerous secondary prothallia were obtained which upon separation from one another became independent prothallia (Fig. 5, Plate XXVI). Fig. 1, Plate XXV represents a large secondary prothallium obtained from one of the cultures. From a margin of the prothallium a lobe (a) has been produced. A smaller heart-shaped prothallium (c) has been formed as an outgrowth of the lobe. The posterior portion of the large prothallium consists almost wholly of dead cells. The prothallium bears an apogamously produced embryo (e) of considerable size. An embryo of like origin had just begun its development on the

smaller prothallium. The secondary prothallia which were produced after the primary prothallia were transferred from subdued light to favorable illumination resembled those represented in Fig. 6, Plate XXVI. These, however, as will be described later, were produced under different cultural conditions.

A culture of *Polypodium crassifolium* L. was placed under the same conditions of illumination as those under which the culture of *Nephrodium hirtipes* produced numerous secondary prothallia and it was found that the former species exhibited a similar tendency to form such prothallia. It thus appears that, although the prothallia of *Nephrodium hirtipes* produce embryos apogamously, they possess no greater tendency to vegetative growth than do those of *Polypodium crassifolium* which produce embryos only as a result of fertilization.

In many cultures of *Nephrodium hirtipes* and several other species of ferns, the method just described for producing secondary prothallia was invariably successful.

Large portions of prothallia were removed from cultures in the Wardian case and were placed on media like that of the cultures just described. Some pieces were also floated on the surface of sterilized tap water, and sterilized nutrient solutions. Even when the illumination was favorable for the formation of heart-shaped prothallia in cultures made by sowing the spores, numerous secondary prothallia were produced from the margins and surfaces of the larger pieces (Fig. 2, Plate XXV).

When prothallia, growing under favorable light conditions, were cut off near the substratum with a sharp razor, the remaining portions of the prothallia likewise produced many secondary prothallia (Steil, 1918).

In some of the cultures the prothallia were attacked by parasitic fungi to such an extent that only small portions of the older prothallia remained. From the apparently normal cells of such prothallia, filaments were produced which developed into heart-shaped prothallia when the cultural conditions became more favorable.

Occasionally the prothallia of *Nephrodium hirtipes*, especially in the older cultures, became discolored or brownish, perhaps on account of certain "physiological" conditions. The large majority of the cells in such cases died. From the living cells secondary prothallia were usually formed when more nutrient solution was supplied

to the culture. The writer has observed in the vicinity of Madison similar instances of regeneration of the prothallia of *Onoclea sensibilis* L. which had lived over winter. In some cases only small portions of the original prothallia had survived the winter conditions. From these portions secondary prothallia were observed to form in profusion.

When the illumination was somewhat subdued, but not sufficiently to produce only filamentous prothallia, one or more lobes of the primary prothallium formed secondary prothallia (Fig. 3, Plate XXV, and Fig. 6, Plate XXVI).

Prothallia of *Nephrodium hirtipes* on which apogamous embryos had begun their development were placed under conditions of weak illumination and these also formed secondary prothallia (Fig. 2 Plate XXV). The two regions, a and b, shown in the photograph, are composed of cells containing few chloroplasts. Embryos of apogamous origin have already begun their development in the paler regions.

Under certain conditions of light to be described at a later time, many branched cells were produced (Fig. 7, Plate XXIV). When the cultures were placed in the Wardian case, the branched cells formed prothallia precisely like those originating from the germination of a spore (Fig. 8, Plate XXIV). Branched cells have been described by Atkinson (1894), Miss Black (1915) and Miss Wuist (1916). Atkinson (1894) reported the formation of prothallia from branched cells of *Adiantum cuneatum*.

In one of the cultures of *Nephrodium hirtipes*, a peculiar secondary prothallium was observed (Fig. 4, Plate XXV). The "light" region present in the portion just back of the apical notch indicates that an embryo of apogamous origin was about to make its appearance. A number of clearly defined regions are shown at *a*, Fig. 4. At *b* is a larger and more distinct area. All of those at *a* in a few days formed small prothallia. The prothallium was composed of only a single layer of cells in thickness where the peculiar regions were present. The writer is unable to give a satisfactory explanation the prothallium described above.

Secondary prothallia were readily induced by any of the methods which have been described. In every respect, such prothallia

resembled apparently the primary ones, producing also embryos of apogamous origin.

The formation of secondary prothallia from primary prothallia have been described by a large number of investigators including, Wiegand (1849), Hofmeister (1851), Kny (1870), Goebel (1877), de Bary (1878), Bauke (1878), Beck (1880), Dodel-Port (1880), Campbell (1892), Heim (1896), Britton and Taylor (1902), Lagerberg (1906), Woronin (1908), Pace (1913), Heilbron (1910), Fischer (1911), Schlumberger (1911), Wuist (1913), Nagai (1914), Pickett (1914), Black (1914), Wuist (1916).

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DESCRIPTION OF PLATES

PLATE XXV

The prothallia from which the photo-micrographs 1, 2, and 3 were made, were magnified about 25 times. Photomicrograph 4 represents a magnification of about 30 times.

- Fig. 1. A prothallium of *Nephrodium hirtipes*. A large lobe, *a*, has been produced from the prothallium. From the lobe a smaller heart-shaped prothallium, *c*, has been formed. Apogamous embryo, *e*.
- Fig. 2. A prothallium of *Nephrodium hirtipes* from which many secondary prothallia have been formed. Regions in which apogamous embryos are beginning their development, *a* and *b*.
- Fig. 3. A prothallium of *Pteris cretica albo-lineata* from one lobe of which regeneration has taken place. An embryo of apogamous origin has also begun its development at *a*.
- Fig. 4. A peculiar secondary prothallium of *Nephrodium hirtipes*. Distinct prothallial regions at *a* and *b*.

PLATE XXVI

- Fig. 5. A culture of *Nephrodium hirtipes* containing numerous secondary prothallia. X $1\frac{1}{4}$.
- Fig. 6. Prothallia of *Nephrodium hirtipes* from the lobes of which secondary prothallia have been produced. X $2\frac{1}{2}$.
- Fig. 7. Branched cells of *Nephrodium hirtipes*. X About 42.
- Fig. 8. A young prothallium of *Nephrodium hirtipes* produced from a branched cell. X 42.



Fig. 1



Fig. 2



Fig. 4

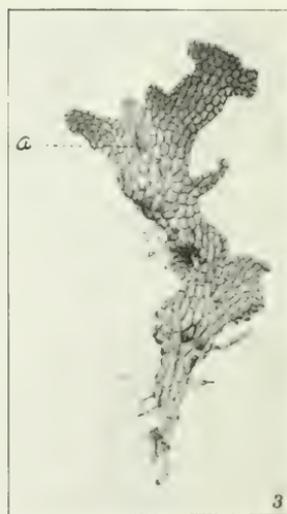


Fig. 3



Fig. 5

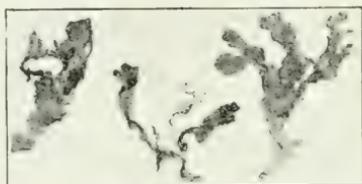


Fig. 6

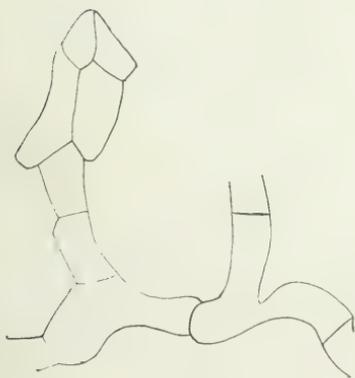


Fig. 7

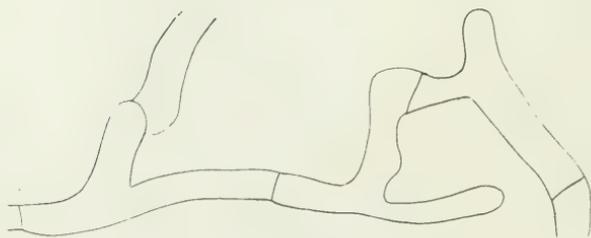


Fig. 8

DEPARTMENT OF NOTES AND REVIEWS

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

RECENT CHANGES IN ILLINOIS RIVER BIOLOGY

Forbes and Richardson (Bull. Ill. Nat. Hist. Survey, XIII; 6, April 1919) present an abstract of changes in the biology of the Illinois River in the last twenty years. The chief causes of change are: The increase in the volume of the water due to the opening of the Chicago drainage canal in January, 1900; the great increase in the sewage content of the water due to the same cause; and reclamation of the river bottoms for agricultural purposes.

To illustrate the first of these items it is stated that just one-half the average flow at Peoria for 1913 was thus derived from Lake Michigan. This tends to give constancy to the height of the river, but increases the overflow in high times and extends the period. Depth of river and rate of flow are both increased. The bottom-land lakes which stand at approximately the river level are also correspondingly heightened. Numerous summer shallow-water weedy belts are eliminated. The increased rate of flow insures that the decomposition and assimilation processes formerly occurring in a given length of stream take place further down stream. The results are as tho the stream had been shortened. Carried organisms have less time to multiply and less chance of being devoured in given length of river. The point where the decomposing sewage becomes available for green plants and other plankton is further down stream than before. All the dependent vital phenomena take place further down the river. In spite of the increase of sewage in the river, the rate of flow and volume of water make it true that the river water contains a smaller percentage of sewage in 1914 than before 1900.

The total result of this is that optimum conditions for green plankton which apparently occurred at or above La Salle before 1900 now occur not much above Peoria. From this point to the mouth of

the river the food supply in smaller and larger plankton organisms is greatly increased.

The results of levees preventing overflow of the bottoms, together with the draining of the tributary lakes operate in the opposite direction. These stagnant and semi-stagnant waters are perpetually productive of plankton and feed the river with it. With the progressive elimination of the sources a further reduction in the river plankton may be expected.

The total effects of the changing conditions, expressed in terms of the fisheries, are interesting. The increase of yield of fish for the five years preceding the opening of the drainage canal was about 9 per cent per annum; that for the eight years following averaged an annual increase of about 3.5 per cent; while for the next four years there was an average *decrease* of 15 per cent per annum, based on statistics from Havana.

Three factors during this time tended to increase the yield; the introduction of the sewage with its increase of organisms; the rapid increase of European carp which in 1908 furnished 64 per cent of the total product; and increased interest in fishing due to this increase. The rapid progress of reclamation would operate to diminish the yield. The factor of increased fishing doubtless operated in the same way for the later years. The catch was greater than the increase.

It is the purpose of the Survey to find by investigation the treatment, both of the river itself and its adjacent and tributary regions, which may so far as possible allow the maintenance of the fishery properties of the state.

FROGS AND TOADS IN BERMUDA

Pope (Bul. Mus. Comp. Zool. Harvard Coll. May 1917) presents a brief account of the three species of Anura found in the Bermudas. These are *Bufo aqua*, the Great Surinam Toad; *Eleutherodactylus johnstonei*, the "whistling" tree frog; and *E. luteolus*. No amphibian is native to Bermuda. The *Bufo* was imported from British Guiana to capture garden insects, about 1885. The "whistling frog" is thought to have been brought from the Barbadoes, and is known to the Islands as far back as 1880. The *E. luteolus* was discovered in 1916, and nothing is known as to its origin.

There are two points of unusual interest in connection with the situation. The first is that *Bufo aqua* is the largest of living toads.

The second is the extremely unfavorable conditions in the Bermudas for amphibians. The limestone of the Islands is so porous that the rain water which falls on the land quickly seeps back to the sea. There are no streams and no permanent pools. The tadpoles of the Great toad have become able to develop in brackish water. In *E. johnstoni* the entire larval development takes place in the egg, and the frog hatches out in the adult form. All that is necessary therefore is that the eggs be laid in moist places—as under leaves and stones.

The first two species grew in numbers rapidly within a few years after introduction, but later gradually established a balance at somewhat lower numbers.

GONADS AS CONTROLLERS OF SOMATIC AND PSYCHICAL QUALITIES

Moore (J. Exp. Zool., May 1919) reports experiments on rats confirming in part Steinach's conclusions respecting the effect of grafting ovarian tissues in completely castrated males. Steinach found that such "feminized males" behaved more like females than males both physiologically and psychically. Growth of mammary glands and the secretion of milk were noted in such males. Similarly females in which testicular tissue was substituted for the ovaries resembled males both in body and temperament.

In these grafting experiments two distinct changes are wrought:—the native sex bodies are removed, and bodies of the opposite sex are inserted. It is necessary therefore, to observe as controls both uncastrated animals, and castrated ones into which no exogenous elements have been introduced.

The distinguishing characteristics of the sexes in the white rats are not sharply marked in features other than the sexual organs themselves. The growth curves of weight and body length differ somewhat in males and females, the male being somewhat higher. Castration of male rats seems not to modify the growth curve, while spaying the female increases the curve over that of the normal female. Hair, mammary glands, changes in skeleton, and fat deposit have all been suggested as presenting differences. The author, however, feels that there is too much variation in all these things for them to have any exact value as criteria. He holds that the characteristic *behavior* of the sexes gives much better means of

measurement than the features mentioned. This would include mating reactions, rivalry and fighting, parental behavior toward young, and the like.

Moore finds definite evidence that masculinized females show exact male copulatory sex reactions and that feminized males show a tendency toward maternal behavior with the young. The interchanged sex hormones appear therefore to modify the psychic nature of one sex in the direction of the other.

CONTINUOUS VARIATION, AND ITS INHERITANCE IN *PEROMYSCUS*

Sumner (Amer. Nat. 1918, p. 177; 290; 439) finds evidence of continuous variation, subject to selection and to blending in inheritance, as well as evidence of other variations which are discontinuous and behave in breeding in accordance with Mendelian expectations in four local races of the wild deer-mouse *Peromyscus maniculatus*. These continuous variations relate both to pigment and to measurable structural features. These observations furnish cogent materials for further denial of the all-sufficiency of the extreme "Mendelian-mutation-pure-line" interpretation of evolution.

MOULT AND REGENERATION OF PELAGE IN DEER-MICE

Collins (Jour. Exp. Zool., Oct 1918) records observations on the normal moult of several varieties of deer-mice and on regeneration of the pelage after artificial removal.

The general body is destitute of hair and pigment at birth. The upper parts of the body begin, on the second day, to assume a bluish-black tinge and the hair begins to come thru the skin. The ventral white hair begins to show a day or two later. The characteristic juvenal pelage is attained in four or five weeks. This is made up of a thin coat of long and coarse overhair, filled between with a fine soft underfur. The hairs of the underfur are agouti, slate colored at base, a narrow intermediate band of pale mouse gray near the tip, and a black tip. The overhairs lack the intermediate band, not being agouti. The ventral surface is similar except that the tips of the hairs are white. The line between the deep gray of the back and the white of the belly is very sharp.

The transition to the post juvenal pelage begins at age of six weeks and requires about eight weeks for completion. It begins to appear at the throat and proceeds dorsally and anteriorly, then

posteriorly by a quite definite route and rate of extension. The ventral moult is completed before the dorsal. This pelage is somewhat longer and coarser, with a distinct color effect dorsally varying from umber to sepia, and due to increased yellow pigment in the intermediate band of the hair.

The young mice were etherized and the hair plucked out over certain areas, without injuring the skin. Where the juvenal hair was removed it was replaced directly by the post-juvenal. The artificial removal of hair modified the normal sequence of appearance of post-juvenal pelage over the body quite definitely. Usually this modification was confined to the regions actually depilated; but not always. It sometimes influenced the succession at a little distance.

A precocious appearance of the post-juvenal pelage may be induced by removing the juvenile hair.

Restoration takes place in removed adult pelage, and occurs irrespective of seasons. It is restored somewhat more rapidly when hair is plucked out than when it is merely cut. Light appears to have no influence in developing the differences of color in dorsal and ventral surfaces.

EXPERIMENTS ON PROTECTIVE COLORATION

Young (Jour. Exp. Zool. May 1916) reports experiments in which crows, hawks, owls, chickens, prairie chickens, grackles, kingbirds and martins are used as preys, and amphibians, small mammals and insects as prey. The work was done in cages, and varying backgrounds, which contrasted with and concealed the prey were used. He concludes; (1) that protective resemblance is effective in protecting motionless animals from attacks by caged birds; and (2) stillness is probably a more important factor than color in protecting animals from their foes.

COLOR DISCRIMINATION AND ASSOCIATION IN FISHES

White (Jour. Exp. Zool., Feb. 20, 1919) concludes from experiments on mudminnows and sticklebacks that they were able to discriminate, in differing degrees, such colors as red and green, and that the discrimination is based on wave length and not upon intensity. Their power of discrimination is less than that of man. Effective associations between certain colors and behavior were shown by their learning to leap out of the water for food announced by various colors. In a similar way associated actions were shown

for movement of objects in the water, for movements of the operator, for jarring the vessel in which they were. There was no evidence of ability to discriminate patterns, altho they discriminated the shape of objects, such as a dobson-larva.

Their behavior is stereotyped. The associations are few and simple—such as relate directly to their life struggle. They were able to learn nothing as complex as passing thru a definite opening to secure food. The associations are fairly permanent, lasting as long as 42 days. They are more difficult to modify than to establish at the outset.

AGE AND FERTILITY IN FOWLS

Pearl (Proc. Nat. Acad. Sci., 1917, 3, p. 354) compares the cycle of progress in the fertility of mammals and poultry. In mammals fertility seems to begin below the maximum, increase, and then decline until sterility is reached. In fowls the maximum seems to occur at the first breeding season—when the combined age of the parents at mating is only two years. There is a strong drop from this to the point where the sum of the ages is three years. From three to four there is little change. In passing from this period to a combined parental age of five years there is another large drop.

The same author (Genetics, 1917, 2, p. 417) formulates a fertility index which represents a practical measure of the reproductive value of mated pairs of domestic fowls. This is that percentage of the maximum total number of chicks physiologically possible, which any given mating shows. It includes the total number of chicks produced which are capable of living three weeks after hatching. In the rapid decline in fertility expressed above, the rate of decline is more rapid in the male than in the female.

SEX RATIO IN CHICKENS

Pearl (Science 1917, 46, 220) states that the normal per cent of cockerels is 48.57. It seems that this ratio is correlated with the laying ability of the hens. In hens which have been bred and selected to a high egg productivity, a still larger proportion of pullets is produced.

NEW PROCESS OF KEEPING FISH BY BRINE FREEZING

Gardiner and Nuttall (Proc. Cambr. Phil. Soc. 1918; 19:185) give an account of a brine freezing process at high temperature which pro-

duces no breaking down of muscle, nor loss of aroma or flavor. The brine is of 18% salt. A temperature of 5°-20° F. will serve to freeze a large fish in three hours; a herring in twenty minutes. The better preservative results are due to the fact that in ordinary freezing large ice crystals are formed in and among the muscle fibres. This breaks up the texture of the flesh. In the brine freezing the tissues are unchanged because only small crystals are produced.

FOOD OF YOUNG FISHES

Lebour (*Jour. Marine Biol. Assoc.* 1918, p. 433) states that some very young fish eat diatoms and other single celled organisms before they begin to eat animal food in the plankton. By a study of some fifty species she concludes, however, that all except a few vegetarian fishes, depend upon the small animals of the plankton rather than upon the algae. These food animals are Cladocera, Copepods, cirriped larvæ, and eggs. These crustacea feed freely on the microscopic plants.

STIMULI AND REACTIONS OF SAND CRAB

Mead (*Univ. Cal. Zool. Publ.* 1917, 16) reports experimental studies upon the sand crab so abundant on the tidal beaches of California. He found that the range of stimuli to which they are adapted is quite limited. Their eyes are effective, and guide them to their feeding beds and in the avoidance of enemies. Their feathery antennae aid them in capturing small organisms for food.

Their most striking reactions are in burrowing when uncovered, and in making their way back to the water when out of it. Two tendencies aid in the latter reaction; (1) they tend to run down slopes; (2) when not further than 200 feet from the ocean they tend to go toward it, even when they cannot see it. Even tho near the ocean they will, however, follow a 7 per cent slope away from it.

REACTIONS UNDERLYING THE DIURNAL MIGRATIONS OF VARIOUS PLANKTON ANIMALS

Esterly (*Univ. Cal. Zool. Pub.*, April 4, 1919) reports experimental studies of the behavior of various plankton animals in the laboratory, conducted with the purpose of determining the factors that account for their diurnal migrational habits in nature. The author calls

attention to several definite reversals of response in laboratory conditions as compared with what is shown immediately after being taken from the sea. This greatly complicates the practical problems.

In general the plankton animals of a given species are more numerous at higher levels and less abundant at lower. One form may range from the surface to 100 fathoms, and another from 100 to 200 fathoms. What are the factors which operate to produce this result? The rhythmic quality suggests that the action of light in day and night is responsible. Clearly, also gravity, increase of pressure, salinity, temperature, and the like enter along with light into the problem. Equally also there may be internal or physiological rhythm apart from anything external.

The following general conclusions are suggested:

- (1) That no general explanation will hold for all the forms, in as much as there are quick specific reversals in behavior under stimulus.
- (2) While there are some experimental reversals of geotropism, change in geotropism because of change in light intensity cannot account wholly for vertical migration as a general phenomenon. Geotropism is, however, usually positive when the light is vertically above the water.
- (3) The same limitations must be made with respect to changes in temperature as explanatory of general migrations.
- (4) Physiological rhythm is shown in some species, under certain conditions, and may enter into the explanation of diurnal migrations.

ARCELLA EXCAVATA NOV. SP.

Habitat: Found in small swamp along with other varieties near Durham, N. C. First noted occurrence Dec. 9, 1918.

Shape: Somewhat like a quarter-section of cantaloup, the mouth being situated in the cup.

Size: Length 55μ , width 50μ , total depth 45μ , depth of depression 25μ , mouth $15 \times 20\mu$.

Color: Brown to almost black.

Differs from *A. discoides* Leidy¹ in contour. Other difference can not be determined since Leidy does not describe the individuals. It differs from *A. curvata* of Wailes² in size and contour. *A. curvata* is about three times as large, and has a thickness about $\frac{1}{3}$ its diameter while *A. excavata* has a thickness nearly equal to its diameter. *A. curvata* is rather saucer shaped while *A. excavata* is U-shaped.

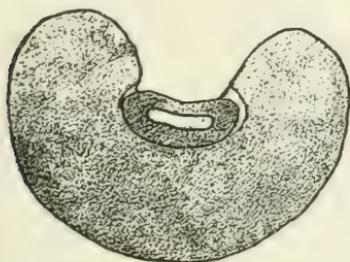


Fig. 1. Side View.

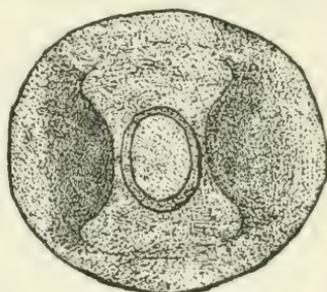


Fig. 2. Oral View.

The author wishes to acknowledge the assistance rendered by Dr. C. H. Edmondson, who agrees with the author that this is a probable new species and has suggested the name given.

¹ Fresh Water Rhizopods of North America. Joseph Leidy. U. S. Geological Survey of the Terr. 1879.

² Fresh Water Rhizopods from N. and S. America. Wailes. Journal. Linn. Soc. Zoology 32:203.

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CARPENTER ANT DESTROYING SOUND WOOD

Graham (Report State Ent. Minn., 17, 1918) refutes the standard statement that the carpenter ant, *Camponotus pennsylvanicus* Degeer works merely in decaying wood, but does not attack sound material. The author finds the ants attacking the solid heart wood of living cedar trees in Minnesota.

It seems true that they always attack a tree by way of some wound or decayed spot. In as much as few trees of pole size are without some such diseased areas, much of the cedar harbors colonies. After a colony is established in a tree the ants work upward from the rotten

area into the sound heart wood, excavating it with longitudinal galleries until only a thin shell of sound wood is left. From the main body of occupied nest they cut lateral openings to the outside, called "windows" by the woodsmen. The topmost window marks approximately the upper limit of damage. The writer makes practical suggestions leading to the conservation of the wood in cutting the poles with respect to this condition and at the same time sufficiently to protect the buyers.

DROSOPHILA IN BOTTLED CERTIFIED MILK

Riley (Report State Entomol. Minn. 17, 1918) reports frequent occurrence of the puparium of a species of *Drosophila* on the inside of bottles of certified milk. The occurrence of these objects has been recognized by many of the distributing companies, whose employees referred to them as "hay-seeds." It was concluded that the eggs are laid in unwashed bottles to which the flies are attracted by the souring milk. The larvae are so nearly transparent as to escape notice, and adhere so tightly that they are not removed by washing. They are of course killed and rendered innocuous by the cleansing treatment in any properly managed dairy, where the bottles are treated by a hot, almost boiling, caustic solution. The inability of the author to rear any flies is reasonable evidence of the soundness of this conclusion. Such episodes certainly make apparent the necessity of enforcement of regulation for cleansing of milk bottles, as soon as emptied, by consumers.

TROPHYLLAXES: A NUTRITIVE EXCHANGE AMONG ANTS

Wheeler (Proc. Amer. Phil. Soc. 1918, p. 293) in a most suggestive paper offers a suggestion as to one of the possible elements underlying the social life of ants. In certain Ponerine ants the workers turn the larvae on their backs while they are being fed. Fragments of insects are placed on the concave ventral surface. This stimulates the larvae to secrete and discharge a fluid, comprizing blood serum, other nutrient matters and a proteolytic enzyme. The secretion may exude thru the pores of the skin or from special glands. There may be special tubercles or other outgrowths. These materials are licked off by the nurses. This exchange of foods between larvae and workers

suggests the relationship known to exist between some species of ants and plant lice. Such a symbiotic relation might well modify community life.

NUTRITION OF INSECTS IN RELATION TO MICROÖRGANISMS

Many insects live on plant food which is rich in carbohydrates but relatively low in protein. Because of the one fact insects may show great activity while, owing to the lack of protein, their growth may be both limited and slow. Some forms have a long life cycle altho ingesting great quantities of the substratum. This argues low metabolic rate in spite of large ingestion. On the other hand, many insects using fermenting vegetable matter with very low protein content have a very short and rapid growth period. This suggests an unapparent protein constituent. Baumberger (Jour. Exp. Zool., April, 1919) reports a study designed to discover the source of the protein supply of such insects. *Drosophila* in fermenting fruit, sarcophagous flies, coprophagous flies, fungus gnats, and other insects were used. The experiments on the *Drosophila* were especially thoro. By crucial experiments the author shows that the insect, while able to live on sterilized fruit alone, only has its normal rapid growth rate when this fruit diet is accompanied by microörganisms, particularly yeast. The yeast is a more adequate food than the fruit because of its higher protein content. Similarly the other insects, mites, and the like studied were shown to feed on microörganisms, thus generally supplementing their diet by the power of the fungi to extract, absorb, and synthesize the many non-protein compounds.

The author gives grounds for believing that this dependence of insects upon fungi is wide-spread. This is another measure of the great adaptability of insects in respect to available foods. Three modes of availing themselves of these high-class fungous foods are mentioned: (1) Ingestion of the microörganisms with the substratum, as in larvae of *Drosophila*, *Musca*, etc.; (2) Feeding directly on the microörganisms, as mites, crickets, many adult Diptera; (3) Preparation of an organic medium for the growth of microörganisms, as leaf-cutting ants, termites. Attention is called to the fact that animals other than insects go to the same source for food—as *Protozoa*, probably nematodes, and possibly earth worms.

In addition microorganisms are internal symbionts in insects and other animals. In the intestine of higher animals, these may elaborate protein from non-proteins, or serve other ends, as preserving a constant digestive flora. In still other locations, as fat bodies, coeca and the like, they may destroy waste products of metabolism, produce digestive enzymes, etc. The exact value of these internal relations, however, is by no means securely established.

PROBLEMS OF FERTILIZATION

In a book with this title, Lillie has placed in brief and semi-popular form a discussion of the problems of fertilization to which he himself has made notable contributions. Fertilization has long been recognized as a critical and decisive process in all plants and animals in which sex appears, and has appealed keenly to human interest from the earliest times. Every modern step in the study of genetics, variation, inheritance, and breeding from any angle whatsoever has paid tribute to and received support from investigations in the chemical and architectural composition and the behaviour of the egg and sperm as these cells unite in fertilization.

In the first chapter the author traces the history of human speculation and discovery in respect to eggs and sperm and the manner and meaning of their coming together. Few items of biological progress illustrate better the gradual passage from the metaphysical philosophical subtleties of *a priori* reasoning than this field shows. The discovery of the living sperm, by Leeuwenhoek and others, soon after the invention of the microscope, at first only gave a more riotous zest to these speculations. But gradually increased knowledge of the facts, coupled with definite limiting experiments, chastened these theories and led to the recognition of the coördinate value and function of the male and female elements.

The intimate cytological investigations of the last quarter of the nineteenth century finally brought into clear view the full significance of the cell—and nuclear—theory and made possible its final application to fertilization. There has been no more brilliant biological work in any field than that which relates to the behavior of the nuclear elements in maturation and fertilization.

There are two sets of problems at this point “where all the strands of the webs of two lives are gathered in one knot, from which they

diverge again and are re-woven in a new individual life history." One group relates to the specific morphological and physiological problems of fertilization. The other includes questions of inheritance. It is to the first of these that the book is directed. Progress in recent years has been in perfecting our knowledge of the actual structures and processes involved in the bringing of these two nuclei together in one cytoplasmic mass; and in the much more difficult and less known realm of the physiology of fertilization, including as it does all the actions and reactions of the external and internal elements of these cells as they unite, as well as the factors external and internal that inhibit or limit these reactions.

Chapter II gives striking expression to the place of fertilization in the life history of organisms. While recognizing the generally accepted view that mature egg and sperm cells are senile cells in the sense of possessing such differentiation that neither alone, under ordinary circumstances, can go on with development, the author feels that the inner meaning of sex and fertilization is not fully expressed by the rejuvenescence and de-differentiation that accompanies union. He recognizes an underlying and "inevitable dimorphism of living matter" which sets them apart from all other specialized and senescent cells. Furthermore fertilization is the vehicle for inheritance both in preserving old qualities and in introducing variations. Thus fertilization furnishes material for natural selection to work on. There is evidence also that the result of the union of contrasting germplasms gives greater vigor in offspring—a further accent upon this underlying dimorphism.

Chapter III deals with the morphology of fertilization. In this chapter the author restates the outstanding steps in maturation, the structure of the elements, the method of bringing the cells into mutual influence, the mechanics of the entrance of the sperm into the egg and the resulting changes in the surface apparatus of the egg itself, the fate of the parts of the spermatozoon and their relation to the ovarian structures.

The author summarizes interestingly the relation of the maturing of the germ cells to the act of fertilization. In the case of the sperm cells the maturation divisions always precede the special differentiation of the locomotor elements. In the case of the ovum, however,

there is a great variety in the relation of maturation and fertilization. In echinoderms and some other animals the maturation divisions of the ovum are completed before fertilization. This is the typical condition—of our text books. In many animals, on the contrary, the ovum is unable to complete or sometimes even to begin the divisions leading to maturation until stimulated by the entrance of the spermatozoon. For example, in many vertebrates the first polar body is formed and the nucleus enters upon the prophase of the second but is unable to complete the process without fertilization. In other instances the ovum is able to effect the prophases of the first division, but is checked permanently in the metaphase unless the egg is fertilized. In *Nereis* and some other Annelids the egg will pass thru none of the maturation steps without precedent fertilization.

These variations impress most strongly the loss of power, almost degenerative in character, seen in the maturation of the germ cells, in contrast with the stimulating character of their union. In these cases where entrance of the sperm is necessary to insure complete maturation, the sperm elements rest quietly in the egg until the maturation steps are finished. The preliminary or external phase of fertilization includes such events as the approach and attack of the sperm cell, penetration of sperm, the response of the cortical egg cytoplasm which aids the sperm nucleus in its progress, the special development of the perivitelline structure, which probably tends to prevent polyspermy and otherwise modifies permeability, respiration, and other metabolic and developmental processes.

The actual or internal fertilization involves the study of the fate of the portions of the sperm cell which enter the egg cytoplasm. These consist universally of sperm nucleus which represents the nuclear chromatin in its most concentrated form; and certain small sperm cytoplasmic elements which differ greatly in different groups of animals—from nothing to a very considerable portion. The author conceives that the course of the male nucleus in the cytoplasm is due neither to initial direction at penetration nor to attractions between the nuclei; but rather that they move together because of independent movements forced upon them by the stresses of a common cytoplasm, somewhat as two chips might come together in an eddy.

A most profoundly important morphological problem is that of the equivalent and representative character of the chromatin (chromosomes) of the male and female nuclei. About this item has arisen our most adequate interpretations of inheritance from the embryological side.

The other constituents of the sperm to enter eggs are the beak and tail of which there is no demonstrable history; the sperm centrosome, and mitochondria. There is great variation in respect to sperm centrosomes. The author believes that the sperm aster which arises shortly after the sperm has penetrated the egg cannot be attributed uniformly, if at all, to a sperm centrosome entering with the sperm. The middle piece of the sperm does not enter at all in *Nereis*; and by mechanical means he has been able to remove the middle piece from sperm nuclei—which nevertheless formed the aster at the customary place and in the usual way. Aster formation then he ascribes to the influence of the sperm nucleus itself rather than to an inherited centrosome.

While not denying that sperm mitochondria, which have been traced for several cell generations in cleavage, may function in the egg, the author holds that they show no evidence of being dimorphic in composition or behavior when compared with those of the egg itself.

One remaining substance, the egg cytoplasm is still to be mentioned. In quantity it is quite the most impressive material to be considered. It must determine in high degree if not exclusively the early embryonic steps. These processes and characters may fairly be said, therefore, to be exclusively maternal in their origin. And the cytoplasm, from the point of view of inheritance, might well be the determiner and conserver of the basal racial trends. The maternal cytoplasm would, however, be gradually used up—and all restoration and increase would be the joint product of the cytoplasm and the biparental nucleus. Thus we might expect the later stages of individual development to be increasingly the product of this nucleus, unless there may be some permanent elements in the cytoplasm which grow and reproduce independently of the nucleus.

Under the caption "Physiology of the Spermatozoon," Chapter IV discusses such questions as these: What sensitiveness and modes of behavior have spermatozoa? To what extent are their actions

determined by the conditions of the media? What are the optimum conditions for them? The principal facts about them may be summarized as follows. Spermatozoa are shown to be exceedingly sensitive to some external conditions. While usually without motion in the testis and the ducts leading from it, they readily become mobile under the operation of various natural and artificial media. They are little influenced by light or gravity. They are sensitive to changes in osmotic pressure, and more so to increase than to decrease of it. Temperature affects the rate of their movement as measured by the time required to aggregate.

The most important factors in determining the behavior of spermatozoa are contacts and the chemical character of the medium. The chief ways in which these factors operate are (a) by increase or decrease of activity, (b) by producing aggregations of spermatozoa thru chemotaxis; (c) by reversible agglutinations; and (d) by thigmotactic adherence to surfaces of various kinds.

Various media, differing in different types of sperm, may activate quiet sperm. In mammals the secretions of the prostate and other glands normally activate sperm. In other instances sperm may be made active by physiological salt solution, by sea water, with or without an excess of OH ions. In general the medium in which insemination normally takes place will render them active. For the most part they are extremely sensitive to changes in these media. Addition of acids to sea water decreases activity and causes paralysis. Saturation of sea water with CO₂ completely paralyzes. Alkalies in general increase activity up to the lethal strength. Other things being equal the degree of activity of spermatozoa is a function of the H ion concentration of the medium.

Fresh active sperm in suspension in their inseminating medium rapidly aggregate in masses. This action is due to rapid CO₂ production by the moving spermatozoa themselves. They collect in any region of increased CO₂ tension. This proceeds as tho the spermatozoa assume a positive orientation in a CO₂ gradient. The author believes this is the actual explanation of the reaction.

Now it is known that the eggs of marine animals normally give off into the sea-water, before fertilization, CO₂ and other complex substances. These egg secretions have striking effects upon the sperm. In some types of sperm these secretions make immobile sperm highly

motile. They also produce aggregation of the sperm, such as is shown about CO₂-producing centers; and, third, in some species cause a coherence or sticking together of the heads of the spermatozoa,—called “agglutination,” which is quite distinct from the chemotactic aggregation. This agglutinating reaction is reversible; that is, the masses may later break up without the sperm suffering any toxic or other effect. The specific substance that causes agglutination is a peculiar product of the egg. It is not contained in the blood or other extracts of the female body. It is produced only by mature eggs, and ceases when they are fertilized. Its production is coincident with the period during which the egg may be fertilized.

The agglutinating substance is colorless; will not pass thru a Burkefeld filter; will pass thru specially hardened filter paper; is non-dialyzable; is only slowly destroyed at the boiling point; preserves its power in sea-water for months, tho it does slowly disintegrate. Chemical and efficiency tests suggest that it has analogies to the ferments.

The union of egg and sperm depend upon motion of sperm to the egg, adhesion to it and penetration of it by the sperm. It is clear, therefore, that the egg secretion by stimulating motion in the sperm, by guiding its orientation, by developing an adhesive surface thru the agglutinating substance—all coupled with the well established thigmotropic reaction of the sperm, furnishes a highly adjusted situation leading toward this event.

In Chapter V the general relation of the physiological events involved in fertilization is treated. There are certain essential the elementary features about the intimate process of fertilization which the author does well to stress. For example, fertilization must not be thought of as synonymous with the penetration of the egg by the sperm. Fertilization is as a matter of fact a complex and progressive reaction, of which penetration is an initial portion. It is a very definite series of events complete only when the inseminated egg is fully capable of development and of transmitting the inherited characteristics. Furthermore, fertilization is an “irreversible” reaction when considered as a series. That is to say, after responding normally in the cycle of reactions neither of these cells can go back to the physiological state that existed before that response. Most cells, after being stimulated and functioning, are able sooner or later to

return to the beginning phase and repeat the cycle. The egg and sperm are in a critical phase which prohibits reversibility.

Fertilization is possible only for a very limited and very definite time in the life of the spermatozoon and egg. Unripe sperm will not fertilize and unripe eggs cannot be fertilized. Both become aged and cease to be functional. The time required for this differs. Ordinarily it is very short, especially after sperm or eggs reach an external medium; altho sperm may remain functional in the sperm ducts of the male for weeks or months. Similarly, where internal insemination occurs, sperm may remain potent for considerable periods; as in fowls, for two or three weeks, or in bats for as much as six months.

The author adduces some most interesting experiments to show the rate of decrease in fertilizing power in sperm, and that motility is not the sole measure of fertilizing power. In respect to both sperm and ova the writer believes that the power of fertilization depends upon the presence of a specific substance produced by the cells. In the case of the sperm he regards it as probable that this is identical with the agglutinable substance of the spermatozoa. In the egg the onset and waning of the capacity to be fertilized is due to the formation and loss of a (hypothetical) substance which he calls *fertilizin*. Fertilization itself, in all kinds of eggs, causes loss of power to be fertilized.

Fertilized eggs differ from unfertilized eggs in that the permeability of the membrane is increased, as is shown by increased use of oxygen after fertilization in some eggs, by increased escape of CO_2 and other substances, by more rapid transfer of water by osmosis, by taking up of intra-vitam stains, etc. Fertilized egg cytoplasm is less fluid than before fertilization—which is interpreted as a gelation phenomenon. Chemically the fertilized egg loses the power to produce the substance that causes agglutination of the spermatozoa.

The activating effect of the sperm upon the egg consists physiologically of two different and progressive parts; (1) the cortical changes which usually closely follow upon penetration, but which in some types of eggs may be shown not to depend on penetration; and (2) the internal progressive series of processes which seem to be completed about the time of the union of the nuclei. In this internal work does the sperm activate the egg by means of a substance, which

itself gradually releases? Or does it progressively activate some substance already in the egg?

In Chapter VII this special problem of activation of the egg is taken up in detail, and may be considered in three phases; the production of the changes at the surface of the egg in the plasma membrane and the cortex; second, the activation of the internal protoplasm; and finally the operations of the combined nuclei, leading up to karyokinesis and cleavage.

The evidence seems conclusive that the egg produces and possesses all the activating substances. The spermatozoon merely releases these or causes their development, and does not furnish the substance itself. This is evidenced by the fact that polyspermy does not in any way accelerate the process. The author's view may be summarized as follows:

First interaction—egg on sperm: The egg secretes in the process of maturing substance whose first manifestation is its agglutinizing or binding effect on the sperm. This, supplemented by the thigmotaxis of the sperm, secures contact—and penetration of the egg by the sperm.

Second interaction—sperm on cortical portion of egg: Upon contact and penetration by the sperm there is a cortical change in the egg, which prevents further reaction of the egg to other spermatozoa. In some eggs this shows as an elevation of a "fertilization membrane." This starts at the point of impact and proceeds at a rapid, but measurable and varied, rate around the egg. Beneath this membrane, however, in the cortex there is a "wave of negativity" that is more rapid than the membrane formation and makes the whole cortex immune to further sperm action sometime before the membrane is elevated. This seems to be the really significant reaction.

The author believes that the essential activating substance which produces this cortical fertilization change in the egg is the same as that which produced the agglutinating of the sperm. He calls this substance "fertilizin." The sperm introduces a substance which releases the activities of the fertilizin. This produces the instantaneous change in the cortex. The identity of the fertilizin with the agglutinating substance is based upon the following considerations. Eggs before beginning the secretion of the agglutinizing substance

cannot be fertilized; eggs after fertilization are incapable of reaction to entering sperm, and in such eggs all the agglutinating substance also disappears; eggs artificially activated likewise cease to produce agglutinating substance; washings which cause a decline in the presence of this substance cause the decline also of power of being fertilized. Thus the sperm agglutination and the fertilization activation are completely parallel and coincident, and hence are either produced by one substance with a dual action or by two substances most remarkably yoked. The former is the simpler hypothesis.

Eggs which have been producing abundant fertilizin, sufficient to charge many hundred times their own bulk of sea-water, immediately cease after fertilization, and the eggs no longer agglutinate spermatozoa nor react to them. The author believes this does not mean that the power of secretion is merely exhausted, but that the entrance of the sperm causes this agglutinating side chain of the fertilizin to combine with some substance, and thus the changed cortex becomes completely incapable of further sperm reaction. This substance he calls anti-fertilizin.

The existence of such substance in the interior of the egg which unites with and neutralizes fertilizin is shown by this experiment. The fertilizin of maturing eggs may be collected in sea-water. Then the eggs themselves may be repeatedly washed until they almost cease to produce fertilizin. If these washed eggs be shaken to pieces in the sea water containing their own fertilizin the latter is neutralized. Normally it would have kept for considerable periods. Fertilizin then is necessary for fertilization. As soon as fertilization is effected it disappears from the cortex and further fertilization is impossible. It has been shown that a concentration of egg fertilizin may produce parthenogenesis in eggs of the same species. On the other hand sperm extracts will not activate eggs.

Third interaction—the cortex and sperm with the interior: The activation of the deeper cytoplasm might be effected by the advancing sperm, or progressively from the changed cortex, or both. Spermatozoa that succeed in penetrating into immature eggs or into eggs already reacting have no effect on the deeper protoplasm. Portions of eggs in a state to be fertilized can themselves be fertilized. The author, quoting from work of Chambers yet unpublished, cites experiments showing that dissections of fertilizable eggs *containing*

the internal protoplasm alone can not be fertilized. Whereas if this inner protoplasm remains connected with cortical material the mass is fertilizable, and the perfection of the latter events is a function of the amount of cortical material present. This determination seems crucial in respect to the essential soundness of distinguishing the superficial from the deeper phenomena, and of considering the processes in the cortex rather than the spermatozoon itself as the activating agency in the deeper protoplasm.

It is suggested that this fertilization product in the cortex may exert a ferment-like action, or bodies formerly solid may become liquified, and thus penetrate into the depths of the egg. These problems are yet obscure. Evidently the cortical combinations release substances that produce metabolic changes, including increased consumption of oxygen, and the initiation of development.

Fourth interaction—of the nuclei and the cytoplasm: Little can be said at present relative to this. The action that brings the two nuclei together the author holds to be merely the parallel effect of the cytoplasmic stresses working upon both, rather than to any attraction between the nuclei themselves. The supreme problem in this connection is the coördination of the various factors and processes that lead up to nuclear division. It is a matter of timing these two nuclei with quite diverse history—and both of these to the cytoplasm. In this process the leading role is taken by the sperm nucleus. The intricate character of this timing process is realized by recalling the various periods in which the sperm enters in various eggs, in relation to the maturation divisions of the egg itself: it may enter during the resting stage following completed maturation; or in the midst of the maturation mitosis; or before the process is well under way. This tuning or timing process may well be considered a mutual one. It is not unreasonable to suppose, with the author, that the sperm nucleus has been "fertilized," and thus rendered physiologically potent as well as synchronized by its experiences in the cortex and thus made relatively dominant in the later stages of internal reaction. The sperm aster would be an instance of this increased rôle.

Chapter VI deals with problems of *specificity*. This means the investigation of the question as to whether ova and sperm, with this well analyzed group of fertilization interactions we have been study-

ing, are thus attuned to each other by such specifically exact adjustments that they are limited to each other. There are two possible aspects of this: (1) Can sperm penetrate other kinds of cell beside the ovum? or (2) Can sperm and eggs of different species of organisms unite and effect fertilization?

In respect to the first problem some claims have been made of sperm penetrating epithelial cells of the uterus, and even cleavage cells of the developing blastula. These findings are not confirmed and are in great doubt. Numerous negative findings are recorded where the sperm have abundant opportunity to penetrate the epithelia of oviducts. Furthermore, the agglutinating product of eggs is specific. No other body fluid, secretion, nor tissue-extract is capable of agglutinating the sperm. We may say then that eggs and sperm seem to be utterly specific from the point of view of their tissue differentiation.

The specificity of the fertilization can be advantageously studied in those animals in which fertilization is external—as echinoderms, teleosts, and amphibia. The studies involve the crossing of different species, genera, classes, and even phyla. Attempted self-fertilization in those forms which usually cross fertilize is also a fruitful field for experiment. Incompatibility—an inverse measure of specificity—may show itself in very varied degrees. In certain combinations, as some species of frogs, the eggs do not react at all to the foreign sperm. In other animals, foreign sperm may start the cortical changes but be unable to penetrate. This is often the case in crossing different genera, classes, etc. In some cases they may penetrate and fail to activate the deeper protoplasm or unite the nuclei. In some cases where the nuclei unite, the sperm chromatin may be eliminated during cleavage, as between some species of *Echinus* and in crosses of *Arbacia* with *Echinus*. In these cases the resulting embryos have purely maternal characteristics. Even in cases where the male chromosomes are retained the development may be partial or otherwise abnormal. In some instances there is complete and normal development, but the hybrid itself is sterile. The hybrids from certain crosses seem perfectly normal and fertile. This makes a most instructive series showing all degrees of compatibility from perfect to zero. It expresses itself in the possibility or ease of fertilization; in the completeness of the reaction; in the tolerance of the chromosomes;

in the initiation of development; in the stage to which it may go; in the viability, the normality, and the fertility of the offspring.

From a survey of the numerous experiments with eggs and sperms of echinoderms, fishes and amphibians—the author concludes that there are both specific and non-specific factors in fertilization. These may be outlined as follows:—

1. There is some specificity in the agglutinating effect of egg secretions. Some sperm have been shown to be uninfluenced by heterologous egg secretions. More investigation is needed on this point.

2. There is unquestionably specific resistance in the normal cortical reaction of eggs to heterologous sperm. Here is the most common block on fertilization. It very generally exists in crosses outside the species and increases with the remoteness. It is found, however, in self-incompatible hermaphrodites. It may be overcome by staling and by the use of chemicals. While there may be physical elements in this cortical adaptation, whereby homologous sperm is favored and heterologous discouraged, the chief factor is almost surely a chemical one.

3. The next stages of activation seem little or not at all specific. Any spermatozoon, apparently, after having produced the cortical reaction may pass on and call forth the same general internal events as the homologous sperm. If chemical means have been used to secure the entrance of the sperm, these can still pass into the cytoplasm and accomplish union. This has been done by means of Mollusk sperm in Echinoderm eggs.

4. Another block, which is of a specific nature, appears in the later stages of activation after the union of the nuclei. This is shown by the elimination of the male chromatin. Clearly similar incompatibility might exist, quite equal to the prevention of normal development, which might still fall short of this elimination. The author believes that the whole problem of specificity in fertilization is closely related to that of sperm agglutination by the egg secretions, which has been shown to be completely parallel to the cortical reaction of eggs in fertilization.

The reviewer has undertaken thus to give a somewhat extended account of the argument in this very suggestive book because the book itself does so well what the *Transactions* have been trying to do

for the members of the Society by the "Summaries of Progress" that have been given from time to time. The necessary brevity of such an exposition is a shortcoming; but possibly even this may guide the reader to the book. The whole discussion illustrates most brilliantly the method of approximation seen in physiological analysis at its best.

The Problem of Fertilization, by F. R. Lillie. 278 pages. University of Chicago Press, 1919. Price \$1.75 net.

APPLIED EUGENICS

Popenoe and Johnson have undertaken in this book to magnify the application of the principles of genetics to human society rather than to discuss at length the biological foundations of inheritance.

In the study of human biology we are quite disposed to ignore the fact that we cannot always judge the germ plasm of an individual by his own personal accomplishments. The authors impress well the fundamental fact that we must get a germinal rather than a mere cultural basis for human improvement, if we would encourage the production of superior and discourage that of inferior persons. A eugenically superior person is defined as one "who has, to a greater degree than the average, the germinal basis of the following characteristics:—to live past maturity, to reproduce adequately, to live happily and to make contributions to the productivity, happiness, and progress of society."

The authors leave to others the statement of the problems of genetics underlying eugenics, and concern themselves much more largely in consequence with the applied aspects of the question. The chapter headings sufficiently indicate the thoroughness with which the subject is presented: Nature or Nurture? Modification of the Germ Plasm; Differences among Men; Inheritance of Mental Traits; Laws of Heredity; Natural Selection; Origin and Growth of the Eugenic Movement; Desirability of Restrictive Eugenics; The Dysgenic Classes; Methods of Restriction; Improvement of Sexual Selection; Increasing the Marriage Rate of the Superior; The Color Line; Immigration; War; Genealogy and Eugenics; The Eugenic Aspect of such Specific Reforms as Taxation, Rural Movement, Democracy, Socialism, Child Labor, Compulsory Education, Vocational Guidance and Training, Minimum Wage, Mother's Pensions,

Housing, Feminism, Sex Hygiene Movement, Trades Unionism, Prohibition, and the like; Religion and Eugenics; Eugenics and Euthenics.

This book is a thoroly well organized and well-reasoned contribution to a subject of immense importance and timeliness. It is sure to be much used.

Applied Eugenics, by Paul Popenoe and R. H. Johnson, 459 pages. The Macmillan Company, 1918.

THE CAUSES AND COURSE OF ORGANIC EVOLUTION

The last few years have seen a revival of interest in the task of furnishing a statement of the problems and progress of general evolution. While perhaps none of these except that of Osborne makes a notably original contribution to this synthesis, no such attempt is ever without great interest to the student of life.

The author of the book under review calls it a "Study in Bio-energies." He says that *energy*, *continuity*, and *evolution* may be considered the triune key-note of the volume. His early chapters have to do with "Ether and Energy in Evolution of Matter," "Relation of Inorganic to Organic Bodies," "Relations and Transformations of Energy," and "Energies of the Organic World."

In the spirit of this idea that evolution is to be conceived primarily as transformations of energy in relation to ether, the author undertakes to arrange an ascending series of energies under the two heads of inorganic (crystalloid) energies and organic (or colloid). Under the former he grades heat, light, chemical activity, and electricity as progressive manifestations, and he deems the physical states of matter—gaseous, liquid, viscous, and solid—as parallel with, and the result of, these progressive transformations in energy. Between the inorganic and organic he conceives a "transition" energy which he calls "duplo-electric." The distinctive organic energies he names biotic, cognitic, cogitic, and spiritic. Parallel with the evolution of these types of energy we have the specialized manifestations of matter in the ascending scale of living objects, as protoplasmic, nuclear-chromatin, and nerve cell substance ("neuratin"). Later in the volume the author gives specific chapters to the discussion of what he conceives to be the essential nature of each of these, their relation to one another and to the "inorganic energies" of heat, light,

chemical affinities and electricity. The author continually uses in this connection a conception like this: the progress in the realization of these energies is marked by increased and "more condensed energy activities." Great ingenuity is shown in correlating these energies, the materials they are associated with, and their various manifestations. On the one hand a tremendous encyclopedic amount of chemical, physical, and biological data are thus correlated; on the other the definiteness of these correlations seem at times naïvely forced and fanciful—and often ponderously worded.

The active causes of organic evolution are mentioned as (1) heredity, (2) Environment; (3) Proenvironment; (4) Selection, and (5) Reproduction. Of these the author regards his "law of proenvironment" as a distinctive contribution. He defines this as the "capacity of organized beings of being stimulated by and then positively growing or moving, in part or in whole, toward an environment that represents the *satisfying resultant* or mean between all of the environal stimuli by which they are surrounded"; "the correlated resultant response by any body to the summated correlation of stimulatory action, that leads to a temporarily satisfied state." So far as the writer can see this "law" is only a roundabout statement of the idea of ontogenetic recapitulation (a form of inheritance), plus a measure of the idea of orthogenesis, and possibly a dash of determinism. It seems to say little more than that responses are really adaptive and that they seem to *satisfy* the organism before the response can actually bring about the adaptation to which the completed response ultimately leads.

Next follow a chapter on the origin of sexuality, three on the evolution of plants, and five on the evolution of animals. The last third of the book is given to a discussion of human evolution, the later chapters being given to a consideration of "Morals as a Factor in Organic Evolution," "Religion as a Factor in Human Evolution," "History of Religious Evolution," "Probable Future Advances in Evolution."

As protoplasm is the material formed by biotic energy and is its avenue of expression; and chromatin the product and vehicle of cognitic energy; and neuratin the foundation of the cogitic energy of the nerve cells—so hypothetical "spiritin" is conceived to be the

material concentrate which is at once the product and the mechanism of "spiritic energy" which inspires those specialized phases of personality which are more social, sacrificial, moral, religious—counsels of perfection—the partial qualities of present men, the prime qualities of the superman.

Idealized and even fantastic as some of the author's restatements and interpretation of the scientific data may seem, the reader is certainly made to feel the writer's sense of *continuity* and inclusiveness of evolution, as well as the remarkable function which imagination must play in any such enterprize of synthesizing and actually including all the higher human states as truly as those more elemental forms of energy of which we usually make so much.

The Causes and Course of Evolution, by J. M. Macfarlane. Pages 875. Illustrated. The Macmillan Company, New York, 1918. Price \$4.00.

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A PECULIAR ENTOMOPHTHOUS FUNGUS

BY
E. M. GILBERT

The writer has for some time been interested in the collection and study of fungi which are found under conditions of a more or less aquatic nature. In this connection a study has been made of the fungi found on fern prothallia grown in water cultures or on moist sphagnum. Both the sphagnum and liquid cultures were carefully sterilized before the sowing of the spores took place.

Among the fungi which from time to time made their appearance in these cultures was one which seemed to all appearances to be a vigorous parasite, and an effort was at once made to isolate it for further study and identification. Thaxter's Potato-hard agar, to which had been added a small quantity of Lofflund's Malt Extract, was poured into sterile Petri dishes and dilution transfers were then made, giving in two days a vigorous growth of the fungus. Pure cultures were easily obtained as it was found that the spores of the fungus were thrown to a considerable distance and by means of the binocular microscope it was possible to pick out individual spores which could then be transferred to test tubes containing media and in a few days a number of pure cultures were available.

The preliminary study seemed to indicate that the fungus was of an Entomophthorous nature and an effort was then made to find the insect upon which it may have been growing. No infections were secured upon any of the insects found in the fern cultures nor upon any of the various insects found in the greenhouse. No successful infections were secured when vigorous fern cultures were inoculated, but it was found that the fungus would grow on dying fern prothallia and upon prothallia which were infected by other fungi. The evidence so far collected seems to indicate that the fungus, altho seem-

ingly very much like *Empusa* in morphological characteristics, is of a decided saprophytic nature as it has been possible to grow it on many of the common fungal media such as potato-hard agar, oatmeal agar, rice agar, pumpkin agar, but best results have been obtained on agar containing the Malt-soup or a beef extract.

Other investigators have observed a saprophytic condition in members of the Entomophthorales. Mr. Torrey of the Storrs Experiment Station, Storrs, Connecticut, has made a study of such a fungus which he has described in a paper, I believe now in print. Molliard (13) describes *E. henrici* originally obtained as a parasite on *Culex pipiens*, which he has found capable of growing on the sterilized grub of *Euchelia jacobaeae*, on sterilized ox-liver and even on vegetable material such as sterilized carrot. It was found, however, that the growth on the vegetable substratum was not as vigorous as that obtained on animal tissue. These observations indicate that it may well be possible that other members of the group may be able to live at least in part as saprophytes.

THE FUNGUS

The mycelium grows very rapidly and at the end of from 48 to 72 hours forms a thin, compact growth on the surface of the medium. No haustoria or rhizoidal growth has been found and there is very little penetration of the substance upon which the fungus may be growing. The hyphae branch profusely and soon become septate with cells of varying length. The individual cells compare favorably with those described by Thaxter (19) and Olive (16) for *Empusa*, but contain a greater number of vacuoles and are not as a rule as irregular in shape (Fig. 1). The cytoplasm is of a very heavy granular nature and does not as a rule contain the conspicuous fat bodies described by these authors. This of course may in part be due to the fact that the media used differ greatly in food content from the bodies of insects upon which the *Empusas* grow. Fat or oil bodies are more often found in the older hyphal cells and in the mature conidia.

The shape and size of individual cells vary greatly, dependent upon the nature of the medium upon which the fungus is growing. On a medium poor in food material the cells are more elongate and

narrow in diameter, while on a rich substratum they are thicker, shorter, and less vacuolate.

REPRODUCTION

At the end of from 36 to 48 hours the conidiophores begin to make their appearance in considerable numbers. These may arise from any portion of the hyphae, but usually arise from the terminal cells. Search has been made for the hyphal bodies described by Thaxter (19) and Olive (16) but no cells quite comparable to these have been found, although it is evident at times that the cells which give rise to the conidiophores are filled with a denser cytoplasm and are often more irregular in shape than neighboring cells. Nothing comparable to the sclerotia described by Sorokin (17, 18) have been found in any of these cultures.

The conidiophores arise as branches from the cell and are usually simple, each producing a single conidium (Figs. 2, 3), but many cases of compound conidiophores are found, in some instances quite comparable to those described by Thaxter for *Empusa occidentalis* and *Empusa Aphidis* (Figs. 6-9).

The conidiophore becomes club-shaped and filled with a very heavy granular cytoplasm which at once distinguishes it from any of the cells of the vegetative hyphae. The apical portion soon expands into a "basidium" which finally reaches about the diameter of the mature conidium. By this time the basal portion of the conidiophore has become greatly vacuolated and there seems to be a decided movement of the cytoplasm into the rounded upper end. It has not been possible to definitely decide just what takes place during the next stage but a columella like structure soon makes its appearance, cutting off the enlarged portion of the basidium, and within this cell a membrane is laid down to form the wall of the single spore. Figs. 5, 10, 11.

A portion of the content of the basidium continues to pass into the maturing conidium which finally becomes filled with a very dense granular cytoplasm, in which appear at times a few fat bodies. During this later stage the portion of the conidium which is in contact with the "columella" becomes decidedly conical with the apex of the cone usually pressed against the columella. The cytoplasm within the basidium at this stage is decidedly hyaline and at times appears to contain little or no cytoplasm.

The process by which the basidium ruptures and projects the conidium is not fully understood. Many instances have been found where it appears as if the pointed portion of the conidium tends to weaken or pierce the columella of the basidium which is continually becoming more turgid, due to the intake of water, and finally the pressure becomes so great that the entire end of the basidium is torn asunder, thus discharging the conidium with such force that it is often thrown to a distance of 65 mm.

A portion of the content of the basidium is probably thrown with the conidium and it seemingly is due to this material that the conidium will readily adhere to any substance with which it may come in contact. Although this seems to be the usual procedure, other cases have been observed where it appears as if the basidial wall ruptures without any damage to the columella.

THE CONIDIA

The mature conidium is perfectly spherical except for a conspicuous "appiculus" found at the point of contact with the columella. The cytoplasm is usually quite dense, finely granular, with an occasional fat body. The primary conidia have a diameter varying from 48μ – 60μ , the secondary conidia average 35μ – 40μ , while the tertiary conidia at times have a diameter of not more than 20μ . The secondary and tertiary conidia are often characterized by the presence of a very large vacuole and conspicuous fat bodies are at times also apparent.

GERMINATION OF THE CONIDIA

When a conidium falls upon a substratum containing some moisture, it germinates in from 6–12 hours, putting out from one to four germ tubes which in a very short time develop into the typical septate mycelium previously described. Fig. 16. If, however, a conidium falls upon a surface free from moisture, it at once develops a very short tube, often less than one-tenth as great as the diameter of the spore, at the end of which is produced a secondary conidium averaging about two-thirds the diameter of the primary spore. Figs. 18, 22. This secondary conidium is formed like the primary spore and is discharged in the same manner, and may now produce either a mycelium or a "tertiary" spore, dependent upon the nature of the substratum.

In some cultures it has been found that the primary conidia do not germinate if discharged upon an unfavorable substance, but instead there appears a slight thickening of the spore wall, while at the same time the contents become decidedly yellowish in color. These were at first thought to be resting spores but it has so far been impossible to obtain any evidence of germination.

In a few rare cases the germ tubes continue to elongate for a time and then produce a basidium and a secondary conidium. In such cases the secondary conidium is of a smaller size than the normal secondary spore, probably due to the fact that much of the content of the primary spore was used in formation of the hyphae. Figs. 19, 20.

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EXPLANATION OF PLATES (XXVII—XXVIII)

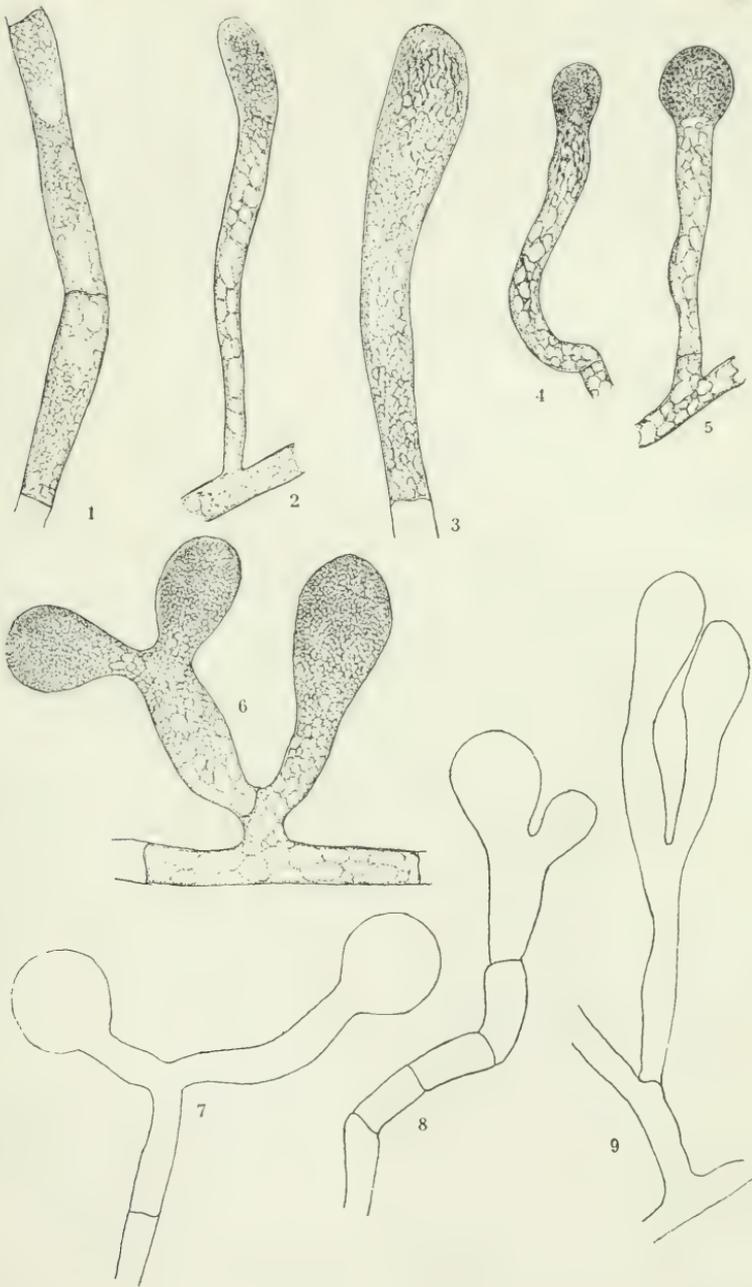
All figures were drawn with the aid of the camera lucida, with a Leitz No. 3 ocular and Leitz No. 6 objective.

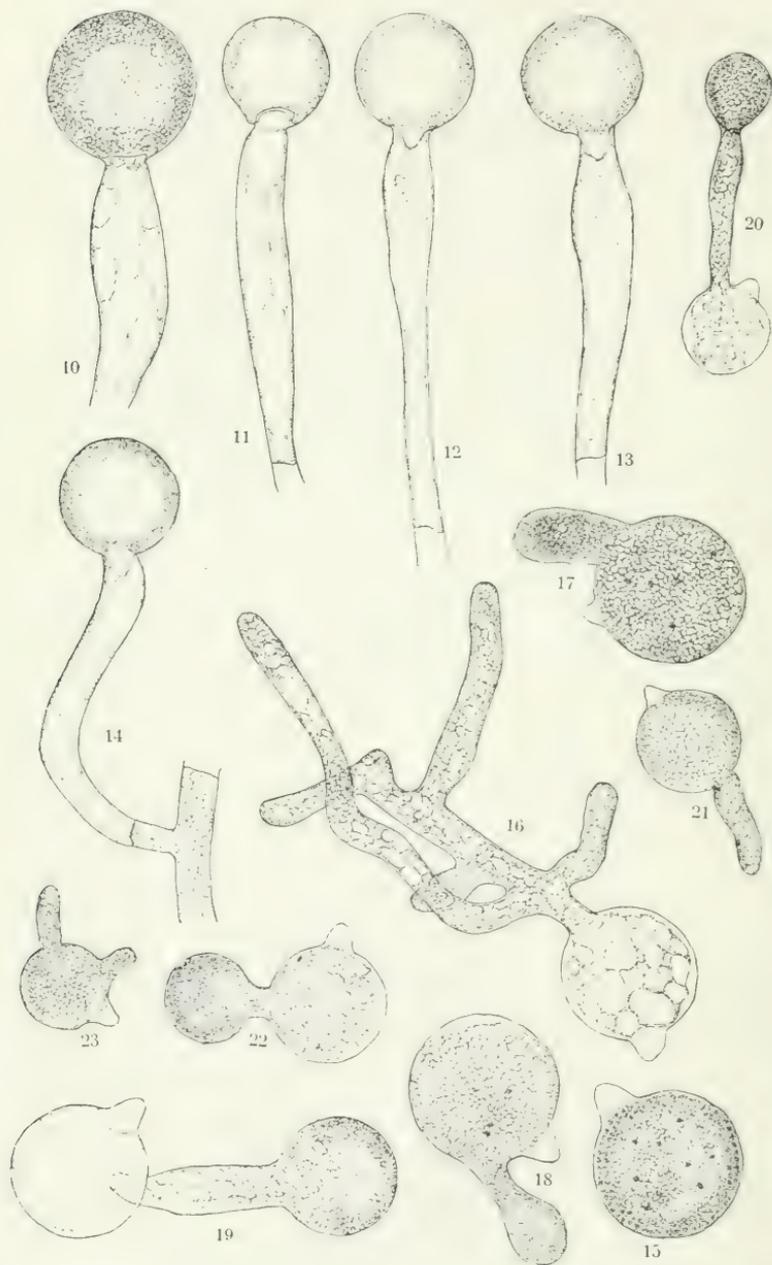
Plate XXVII

- Fig. 1. Typical cells from mycelium grown on agar containing beef extract.
Fig. 2. Early stage in formation of conidiophore.
Fig. 3. Conidiophore separated from balance of mycelium by cell wall.
Fig. 4. Conidiophore with dense cytoplasm gathered at apex which is beginning to increase in size.
Fig. 5. Terminal portion of conidiophore fully enlarged and "columella" partly completed.
Figs. 6, 7, 8, and 9. Abnormal types of conidiophores which however produce typical conidia.

PLATE XXVIII

- Fig. 10. Upper portion of conidiophore, showing "columella" and also indications of spore wall.
Fig. 11. Conidial wall completely laid down. At this stage the columella usually forms an indentation in the maturing spore.
Fig. 12. Slightly later stage. The "appiculus" is in process of development and has forced the columella backward into the basidium.
Figs. 13 and 14. Fully matured conidia still attached to basidium which at this stage contains a very small amount of cytoplasm.
Fig. 15. Primary conidium showing heavy granular appearance.
Fig. 16. Germination of primary spore sown on agar containing Malt-soup extract.
Fig. 17. Germination of primary spore sown on potato-hard agar (sown at same time as spore in Fig. 16.)
Fig. 18. Early stage in formation of secondary spore.
Figs. 19 and 20. Abnormal formation of secondary spore. In each case a conidiophore has been formed.
Fig. 21. Germination of secondary spore.
Fig. 22. Formation of tertiary spore by secondary spore.
Fig. 23. Germination of tertiary spore.





THE DISTRIBUTION OF THE ARCHEGONIA AND THE
ANTHERIDIA ON THE PROTHALLIA OF SOME
HOMOSPOROUS LEPTOSPORANGIATE FERNS

BY
W. N. STELL

It is a well-known fact that in the ordinary *Polypodiaceae*, the archegonia are formed exclusively on the so-called cushion and directly back of the apical notch. Usually the antheridia are produced on the posterior portion of the prothallium, especially among the rhizoids. In some species antheridia are produced also on the lobes and the margins of the prothallium. The small "male" prothallia are frequently covered with antheridia, but under favorable conditions of nutrition, as Miss Wuist (1910) has shown, become monoecious. From some cultures of *Pteris aquilina* L. in which the prothallia were crowded the majority were removed by the writer, and it was found that the smaller remaining prothallia bearing only antheridia assumed the typical heart shape and formed also archegonia as shown in Fig. 1. In this case the original antheridial bearing portion (a) can still be readily recognized.

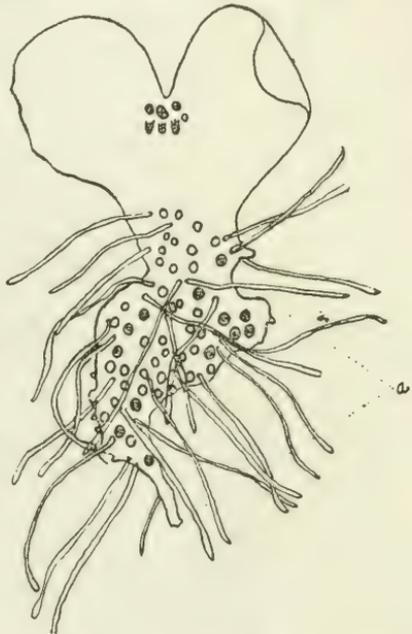


Fig. 1. A prothallium of *Pteris aquilina*. a, the original antheridial bearing portion. The archegonia back of the apical notch and the antheridia are diagrammatically represented. x25.

In the *Osmundaceae* the archegonia are produced on the sides of the "mid-rib" which in *Osmunda regalis* is very conspicuous. On

the younger prothallia a single row of archegonia is usually present on each side of the mid-rib and extending from the apical notch to the posterior part of the prothallium where many antheridia are borne, although a large number are frequently produced on the lobes. On the older prothallia numerous archegonia are formed along the sides of the mid-rib. The distribution and the development of the sex organs of *Osmunda regalis* were first described by Kny (1872).

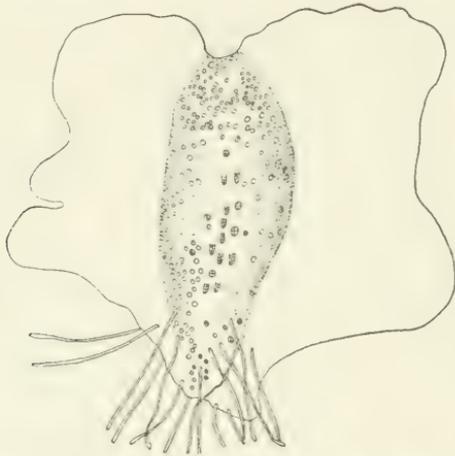


Fig. 2. A prothallium of *Pteris ensiformis* var. *Victoria*, showing archegonia entirely surrounded by antheridia. The sex organs are diagrammatically represented. $\times 12.5$.

The writer found a peculiar arrangement of the sex organs on the prothallia of *Pteris ensiformis* Burn. var. *victoria*. The prothallium in this species produces a prominent and elongated cushion. The archegonia, however, occupy only the highest portion of the cushion. In some instances a few archegonia are developed directly back of the apical notch among numerous antheridia which are always produced on this portion of the prothallium of the *Pteris* species. Many antheridia are also formed on the lower portion of the cushion and on the posterior end of the prothallium. The archegonia are thus wholly surrounded by antheridia (Figure 2). A large number of the prothallia in some of the cultures produced antheridia but in no case archegonia, on both surfaces. Some of the prothallia assumed a nearly vertical position. The two sides of the

prothallia were, therefore, almost equally illuminated, and in consequence dorsi-ventrality was not completely established. Rhizoids and antheridia were, hence, formed on both surfaces.

In some cases the small Stender dishes, used for the cultural work, were only about half filled with sphagnum saturated with a nutrient solution and prothallia of several species were grown on the substratum. Antheridia and archegonia were produced, in consequence of the illumination thus secured, on both surfaces of the prothallia. The same results were obtained by Pierce (1906) who grew the prothallia on a clinostat.

It has been frequently demonstrated that a sufficient reduction in the amount of light inhibits the formation of heart-shaped prothallia and in consequence archegonia are never produced. If favorable cultural conditions are maintained, prothallia may be grown in weak light, and for an indefinite period of time only antheridia will be developed. If the illumination is sufficiently strong for the formation of archegonia, such sex organs will develop with the continued growth of the prothallium provided fertilization is prevented. The prothallia of *Osmunda regalis* were grown under these conditions for about a year and a half. On one of the prothallia, thus produced, approximately a thousand archegonia were counted (Steil, 1918).

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NEW SPECIES OF WATER MITES OF THE GENUS ARRHENURUS

BY
RUTH MARSHALL

About ninety genera of Hydrachnidae are now recognized, with some eight hundred species. About one-fourth of these species belong to the single genus *Arrhenurus*. Fifty-five have been described for North America, chiefly from the upper Mississippi valley. This paper adds one new species.

The hydrachnid fauna of South America has received little attention. Only eleven species have been described, of which five are for the females only. Dr. F. Koenike, in two papers (1894, 1905), published descriptions of two species (*A. corniger*, *A. ludificator*) found in material sent to him from Brazil. Dr. C. Ribago (1902) described a single species from Colombia (*A. oxyurus*). Dr. E. von Daday (1905) described seven new species from material collected in Paraguay; these were designated *A. anisitsi*, *A. apertus*, *A. meridionalis*, *A. multangulus*, *A. propinquus*, *A. uncatius*, *A. trichoporus*. Dr. C. Walter, in a paper published in 1912, described one new species (*A. fuhrmanai*), from Colombia. The present paper adds six new species.

Material from Asia has been very scanty. Twenty-two species of *Arrhenuri* have been recorded; of these eight are from Asia Minor, mostly species found also in Europe. The remaining species have been found in the islands of Ceylon, Java and Sumatra. This paper adds two new species from China.

The author has been very fortunate in securing the material for the descriptions of the nine new species of *Arrhenuri* included in this paper, and thanks are extended to the collectors who generously contributed it. The greater part of the material was found in collections made in northern South America by the late Harriet B. Merrill, in 1908 and 1909, and now in the possession of Dr. E. A. Birge, of the University of Wisconsin, who kindly permitted the author to sort out the water mites. Professor A. S. Pearse, of the

University of Wisconsin, contributed material from Venezuela. Mr. C. Juday, of the Wisconsin Natural History Survey, gave some material in his possession which had been collected by Professor N. Gist Gee, of Soochaw, China. Dr. R. A. Muttkowski sent material found by him in the lakes at Madison, Wisconsin, and which contained one new *Arrhenurus*.

Arrhenurus serratus nov. spec.

Pl. XXIX, Figs. 1-7

In form this mite resembles *A. brachyurus* Viets (1914), found in Germany. The latter, however, is shown without a petiole, a conspicuous feature of the new species. The enclosed dorsal area is large and runs over on to the small appendix. The petiole is a long, slim transparent structure with an upward curve in its posterior half where it has a saw-toothed appearance. At its base, on the ventral side of the appendix, there are two saber-like bristles. There is a delicate hyaline appendage and numerous stout hairs on the appendix, with several smaller ones on the rest of the body. The fourth joint of the fourth leg shows a well-developed spur with a few short curved hairs at its end. The body measures 1.0 mm. without the projecting petiole which measures 0.2 mm. The female has a length of 1.3 mm., and is ovate in form. The genital area is large and the wing-shaped areas are broad and extend nearly straight outward from the cleft. The color is olive green in preserved material.

Three males and four females were found in Lake Mendota, at Madison, Wisconsin, June 18, 1915, by Dr. R. A. Muttkowski.

Arrhenurus asiaticus nov. spec.

Pl. XXIX, Figs. 8-10

In dorsal aspect this species bears a general resemblance to that of the North American species *A. montifer* Marshall, and shows the characteristics of the subgenus *Micrurus* in the stout form, small enclosed dorsal area and short scalloped appendix with a medial incision. The petiole is stout and turned abruptly upward and has no hyaline appendage. The fourth leg lacks the spur on the fourth joint, but this and the preceding segment have sharp points on the distal ends. The palpi are stout. The color of the preserved specimen is pale blue green with brown mottles. The body length is 0.9 mm.

The single male on which this description is based was found by Mr. N. Gist Gee, of the University of Soochow, China, in material taken from canals and small lakes in the region.

Arrhenurus distinctus nov. spec.

Pl. XXX, Figs. 14-16

This form resembles *A. orientalis* Daday (1898), found in Ceylon. The large and well-developed petiole has a similar form in the two species, but in the related form it is more flaring at the end and relatively larger. The new species has a longer body, the entire length, including the petiole being 1.10 mm., the petiole alone being 0.75 mm. long. No trace of a hyaline appendage was found. There is a well-developed spur on the fourth leg. The palpi are stout. The stout body with the well-developed appendix having conspicuous lateral projections directed outward, and the pair of sickle-shaped projections within the dorsal enclosed area place the new species in a well-defined group of the subgenus *Arrhenurus* noted by the author in a former paper (1908). The color is a pale brown green.

A single male of this species was found with the preceding species in the material from Soochow, China.

Arrhenurus valenciis nov. spec.

Pl. XXIX, Figs. 11-13

A single female from Lake Valencia, Venezuela (July 18, 1918), sent to the author by Professor A. S. Pearse for identification proved to be a new form and its description is now given. It resembles *A. multangulus* Daday fem. from Paraguay, but is larger (1.53 mm.). The large conical elevations on the dorsal side of the body which are so conspicuous in both species do not have the same arrangement. In the new species there are four large humps at the posterior end of the body, while the anterior ones and those within the enclosed dorsal area are small. The genital region is small and the wing-shaped areas bend abruptly outward. The color of the specimen is blue green.

Arrhenurus merrilli nov. spec.

Pl. XXX, Figs. 17-18

This species and the four which follow belong to the Merrill collection. It is noteworthy that these five new species as well as most of the other described South American *Arrhenuri* for which the

males are known belong to the "long tailed" group, the subgenus *Megalurus*.

A. merrilli is a small water mite, its total length being only 0.80 mm. It resembles the North American species *A. manubriator* Marshall. The elevations on the body and appendix are only moderately developed. The epimera end in blunt points. The line enclosing the dorsal area runs over to the ventral side where it closes at the narrow genital wings. There is a small spur on the fourth segment of the fourth leg and segments two and three end in sharp points. The color of the specimen is brownish green.

One male was found by Miss Merrill in a small clay puddle near Marajo, Brazil, May 5, 1908.

Arrhenurus triconicus nov. spec.

Pl. XXX, Figs. 19-24

Abundant material was found for the study of this species. It resembles *A. ludificator* Koenike and *A. uncatu*s Daday; these three species, together with the following species and its related form, are all characterized by the possession of a large conical elevation in the middle of a long slim appendix, a feature which so far has been found in only two others, the North American species *A. petiolatus* Piersig and *A. cornicularis* Marshall. *A. triconicus* has also two small but well-developed outstanding humps near the end of the appendix. The line enclosing the dorsal area runs ventrally below the small genital wings. The epimeral groups are close together; the first and second develop horn-like projections as in the next species. The entire length of the animal is 0.70 mm. The palpi are characterized by an unusual development of the fourth segment.

The female of this species was identified. The body is obovate and measures 0.50 mm. The epimera show the same tendency to develop horns as in the male. The color is blue green.

Seventeen males and twenty females were found in Miss Merrill's collections. These were taken in May, 1908, at Calama, Rio Madeira, and Marajo, Brazil; and in March, 1909, from canals near Christ's Church, Georgetown, British Guiana.

Arrhenurus epimerosus nov. spec.

Pl. XXXI, Figs. 25-28

As noted in the preceding description, this species belongs to a small and sharply defined group of the subgenus characterized by

the development of a large conical hump near the base of the appendix; like *A. triconicus* it also shows an unusual development of horn-like extensions on the first two pairs of epimera, very striking in this species in the case of the second pair. The latter feature, together with the general form of the appendix, relates this species closely to *A. corniger* Koenike. Fortunately the author is in possession of a specimen which Dr. Koenike had kindly sent of his species and a careful comparison of the two forms was possible. The new species is smaller, measuring only 0.65 mm.; the appendix is slimmer and its conical hump is larger and more anteriorly placed than in the related form. The color is blue green.

Two males were found in Miss Merrill's collections from Brazil (Calama and Marajo, Lake Aray) in 1908.

Arrhenurus maderius nov. spec.

Pl. XXXI, Figs. 29-32

The formation of humps on the body and appendix is characteristic of several of the described species of South American Arrhenuri. In this and the following species it is the body rather than the appendix which has them. Herfe our large lateral elevations, two dorsal and two posterior, besides two smaller ones by the eyes, give the body an angular form. The dorsal enclosed area is without them; its enclosing line runs ventrally over to the small genital wings. The appendix is broad, scarcely as long as the body proper, and ends in a scolloped border. (In one individual the end is more truncated and the rounded corners more pronounced than is shown in Fig. 29.) Near the end is a small but distinct peg-like petiole (P, Figs. 29, 30), similar to one sometimes found in other males of the subgenus. The palpi have a scanty patch of short fine hairs on the second segment. There is a small spur on the fourth joint of the fourth leg. The length of the entire body is 1.15 mm. The color is brown green in preservation.

Two males of this species occur in collections made by Miss Merrill in 1908, one from Rio Maderia, Brazil, the other from British Guiana.

Arrhenurus quadricornicus nov. spec.

Pl. XXXI, Figs. 33-37

This is a very striking form, characterized by the enormous development of horns on the body in both sexes. In the male there

are four large curved ones, two anterior ones on the protuberances over the eyes, and two lateral ones on elongated elevations. The appendix is small at the base, increasing in width and thickness toward the end, and free from conspicuous elevations. The fourth segment of the fourth leg has a moderately long spur with a bunch of long curved hairs. The entire length of the body, including the anterior horns, is 1.30 mm.

In the female, besides the four horns, smaller but similarly placed, there are two larger ones on the posterior corners of the body and four small rounded humps, two within the enclosed dorsal area and two at the extreme end. The palpi are somewhat unusual in form; the second is very broad and bears a small patch of fine hairs on the inner surface. The entire length of the body, including the horns, is 0.90 mm. The color is dark brownish green in preservation.

One male and one female were found in Miss Merrill's collections from Georgetown, British Guiana (canals near Christ's Church), March, 1909.

Lane Technical School, Chicago.

September 1, 1919.

EXPLANATION OF THE PLATES

PLATE XXIX

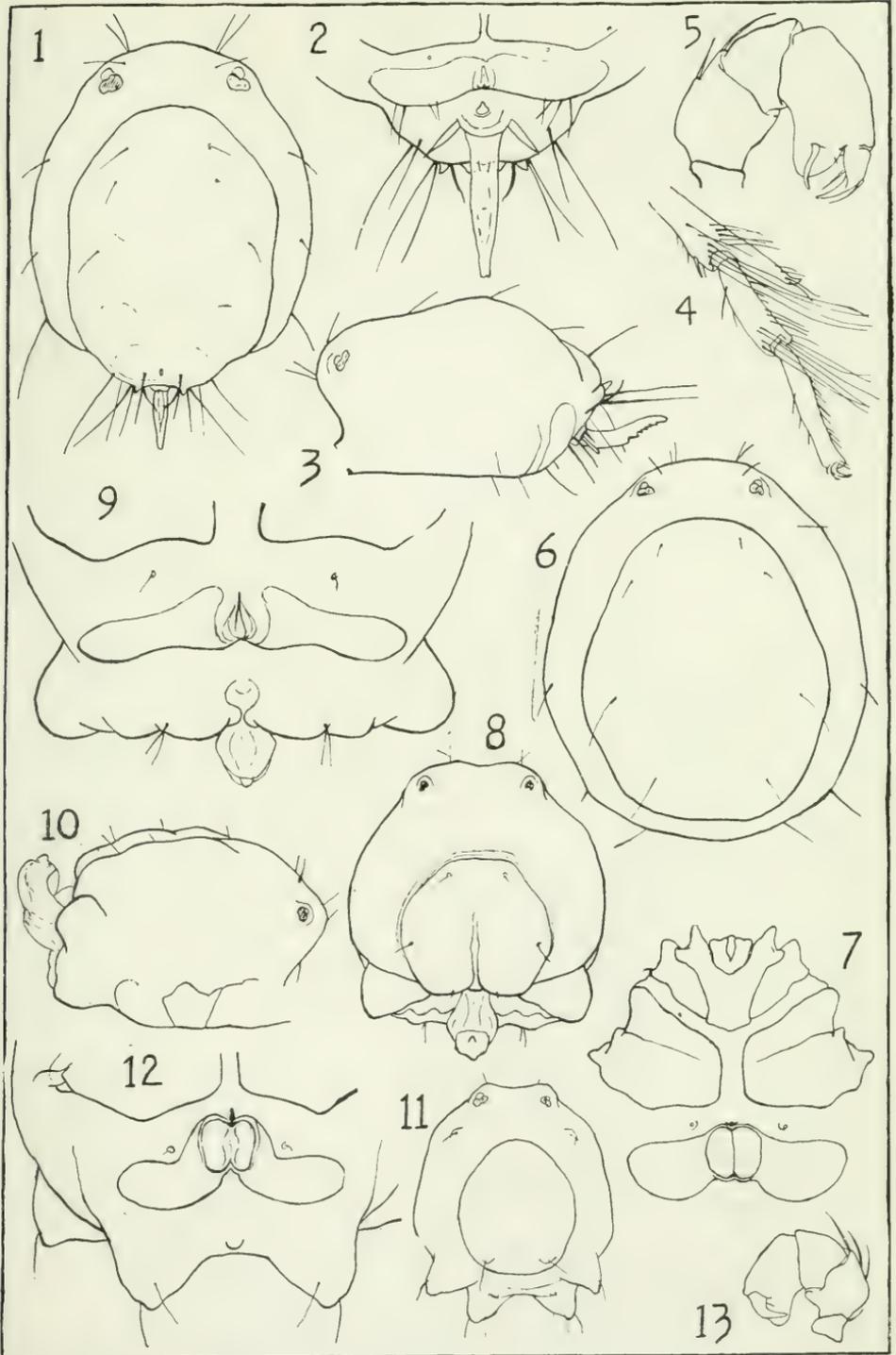
1. *Arrhenurus serratus*, dorsal view.
2. *Arrhenurus serratus*, ventral view of the appendix.
3. *Arrhenurus serratus*, lateral view.
4. *Arrhenurus serratus*, left fourth leg, last three segments.
5. *Arrhenurus serratus*, palpus.
6. *Arrhenurus serratus* female, dorsal view.
7. *Arrhenurus serratus* female, epimera.
8. *Arrhenurus asiaticus*, dorsal view.
9. *Arrhenurus asiaticus*, ventral view of the end of the appendix.
10. *Arrhenurus asiaticus*, lateral view.
11. *Arrhenurus valenciensis* female, dorsal view.
12. *Arrhenurus valenciensis* female, ventral view of the posterior end.
13. *Arrhenurus valenciensis* female, palpus.

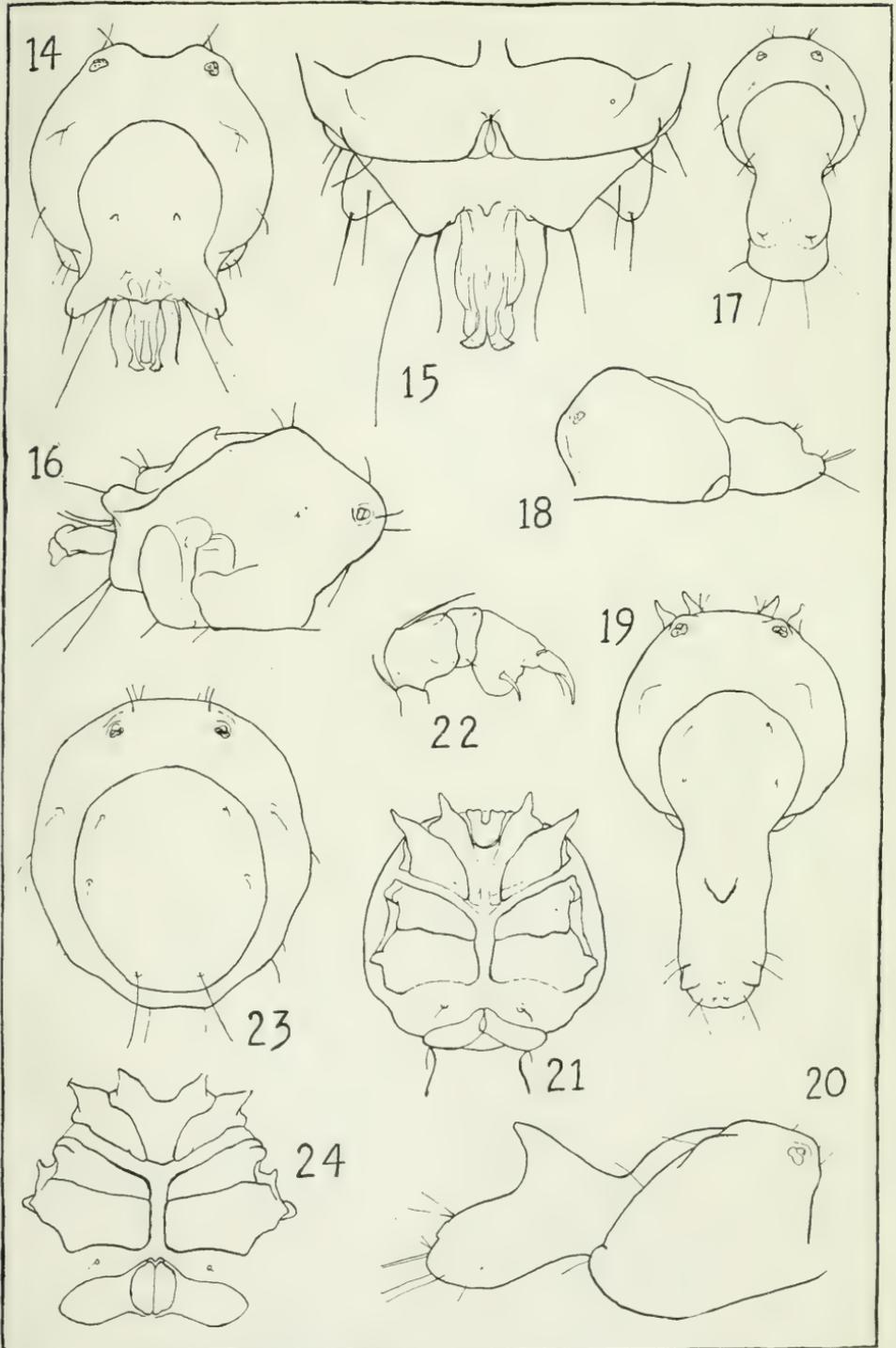
PLATE XXX

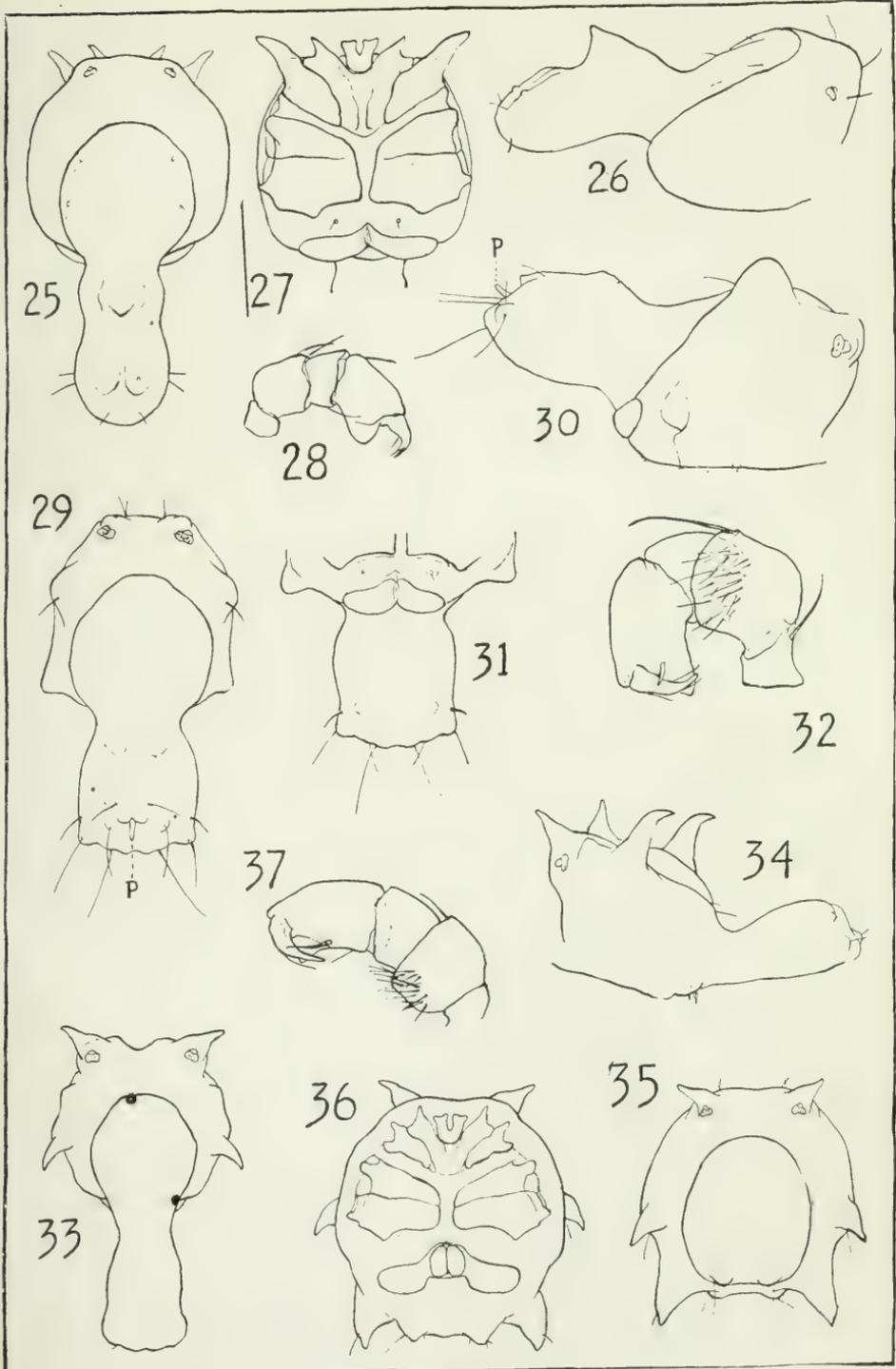
14. *Arrhenurus distinctus*, dorsal view.
15. *Arrhenurus distinctus*, ventral view of the appendix.
16. *Arrhenurus distinctus*, lateral view.
17. *Arrhenurus merrilli*, dorsal view.
18. *Arrhenurus merrilli*, lateral view.
19. *Arrhenurus triconicus*, dorsal view.
20. *Arrhenurus triconicus*, lateral view.
21. *Arrhenurus triconicus*, ventral view of the body.
22. *Arrhenurus triconicus*, left palpus.
23. *Arrhenurus triconicus* female, dorsal view.
24. *Arrhenurus triconicus* female, epimera.

PLATE XXXI

25. *Arrhenurus epimerosus*, dorsal view.
26. *Arrhenurus epimerosus*, lateral view.
27. *Arrhenurus epimerosus*, ventral view of the body.
28. *Arrhenurus epimerosus*, left palpus.
29. *Arrhenurus maderius*, dorsal view; P, petiole.
30. *Arrhenurus maderius*, lateral view; P, petiole.
31. *Arrhenurus maderius*, ventral view of the genital area and appendix.
32. *Arrhenurus maderius*, right palpus.
33. *Arrhenurus quadricornicus*, dorsal view.
34. *Arrhenurus quadricornicus*, lateral view.
35. *Arrhenurus quadricornicus* female, dorsal view.
36. *Arrhenurus quadricornicus* female, ventral view.
37. *Arrhenurus quadricornicus* female, right palpus.







DIATOMS—NEW GENERA AND SPECIES

BY FRED B. TAYLOR

The only catalogue of diatoms which can be regarded as exhaustive, is De Toni's volume on the Bacillarieae in his *Sylloge Algarum*. This was published in 1891–1894, and contains a list of all diatoms known at that date. The price is 115 francs.

In this book diatoms are classified into Rhabdidae, Pseudorhabdidae, and Cryptorhabdidae. This practically agrees with Van Heurck's arrangement, and is followed by Schuett and later writers. The book gives a latin description of the genera and species with a list of references and illustrations. Five thousand, seven hundred and forty-one species are numbered and the index, which includes synonyms, has about double that number of entries.

Several books and monographs have appeared since then, and I have myself collected references to some 1,450 new species and genera and varieties since published; so we may reckon about 7,000 species at the present date.

Various suggestions for new genera have been made during this period. Cleve in his *Naviculoid Diatoms* has revived and separated the old genera *Diploneis*, Ehr., *Pinnularia*, and *Neidium*, and has added new genera *Caloneis*, *Cymatoneis*, *Cistula*, *Pseudo-Amphiprora*, *Stenoneis*, and *Trachyneis* for various forms of *Navicula*. Of these Schuett recognizes *Cistula* and *Cymatoneis*, but regards the others as synonyms or subdivisions.

Cleve has also carved out other new genera, *Disconeis* and *Pleuroconeis* out of *Cocconeis*; *Heteroneis*, *Actinoneis*, and *Microneis* out of *Achanthes*; and *Gomphoneis* out of *Gomphonema*. He would also make a new genus *Mastoneis* for *Stauroneis biformis*; *Scoliotropis* for *Scolioptera late-striata*; and *Tropidoneis* for certain forms of *Amphiprora* and *Plagiotropis*; in this last instance he is followed by Schuett. Pantocsek's *Pseudo-Dictyoneis hungarica* appears to be a *Dictyoneis*.

Cleve further proposes to divide *Pleurosigma* by separating under *Gyrosigma* those forms in which the striae are at right angles to one another.

Mereschkowsky has proposed *Placoneis* and *Sellaphora* for certain forms of *Navicula*, basing his distinction on the form of the endochrome plates; and similarly he has made new genera *Stauronella* and *Staurophora* for *Stauroneis constricta* and *Stauroneis salina* respectively.

Other new genera are:—

ANNELLUS, Tempère. *A. Californicus*, Fossil, Sta. Monica and Sta. Maria Cal. The valve is folded on itself in the form of a tubular ring, covered with large separated granules arranged in quincunx. "Diat. du Monde Entier, p. 60."

AZPEITIA, Paragallo. *A. Temperei*, Fossil, Spain. Valve triangular, sides almost straight, angles rounded; cellular structure not reaching the edge of the valve. Cellules hexagonal, in short and decussate radiating lines, smaller at the centre and border. Margin hyaline, with a line of fine puncta divided by larger dots. *Triceratium antiquum* Pant. I. 13.115 belongs to this genus. *D.M.E.*, p. 326, and *Diatomologia Española*, Azpeitia, p. 177, xii.2.

BACTERIOSIRA, Oestrup.

CAPSULA, Brun. *Le Diatomiste*, II., p. 235; cf. Brun and Tempère, *Diat.* Japan, 1889, p. 62; Van Heurck, *Treatise on Diat.*, p. 469. Under the name of *Capsula* Brun has separated from *Triceratium* certain exotic forms having an internal plate with a triangular space fashioned so as to recall the structure of *Entogonia*. *Triceratium acceptum*, *Hardmanianum*, *radiatum*, *trilineatum*, *exornatum*, *neglectum*, *balaniferum*, *scopus*, *Normanianum*, *Trinacria coronata*, *princeps*, *ventricosa*, *rugosa*, and *Capsula Barboi* and *Capsula biformis*, *Diat.* II, plate xx, are included in this genus.

CATENULA, Mereschkowsky. Fossil, San Pedro, Cal. Outline of valve plano-convex, girdle view as *Eunotia* or *Fragilaria*, frustules united in bands. Extremities of valve acute, terminal nodules near the margin.

CENTRONELLA, Voigt. *C. Reicheltii*, Fresh water, recent, Holstein. The valve is in the form of a three-legged star, outer extremities of the limbs capitate, inner slightly bent at the point of junction, the intermediate portion striate. In girdle view the ends are not inflated. It belongs to the Centricae. Schmidt's Atlas, 306, 32-34: Von Schönfeldt, Diat. Germaniae, p. 240, ix., 398; Süss. Flora, f. 378.

CLEVEIA, Pantocsek. Diatomiste II, p. 162; proposed for *Alloioneis Castracanei*.

CLEVIA, Mereschkowsky = *Pseudo-Navicula* Karsten. Mereschkowsky and Karsten unite Van Heurck's *Lyratae* and *Granulatae* of *Navicula* in this genus because of the disposition of their endochrome.

COSCINOSIRA, Gran. Arctic, marine, recent = *Coscinodiscus polychordus* Gran. Nansen's North Polar Expedition, p. 80, ii. 33. Nord Plankton, fasc., xix., p. 20, f. 17. A series of *Coscinodiscus lineatus* united by plasmic filaments. Peragallo, Diat. Marines de France, p. 427 does not consider the form or genus warranted.

CYCLOSIRA, Peragallo. Marine, recent = *Thalassiosira subtilis* Ost. A small circular diatom living in globular colonies in a gelatinous mass, attached to one another by plasmic filaments like *Cyclotella socialis*, which inhabits fresh water. Diat. Mar. France, cxx. 10. Peragallo in his note on page 427 admits that this is not a valid genus.

DETONULA, Schuett. Marine, recent. Sub-genus of *Lauderia*, = *Lauderia pumila*. Valves plane, with a border of spines at the edge of the disc. It is included in *Dactyliosolen* by Mann.

DIDYMOSPHENIA, Martin Schmidt. A sub-genus of *Gomphonema*, having the rhaps arcuate, and the valve bent accordingly, as in *Cymbella*. Atl. Schmidt, 214. 1-12. *D. sibirica*, *D. curvirostrum*, and *D. geminata* v. *stricta*, are the forms included.

DIMEROSIRA, Peragallo. Marine, recent. Sub-genus of *Dimerogramma*, with convex instead of flat valves, growing as *Dimerogramma* in banded filaments. Diat. Mar. de France, p. 333., lxxxii., 13, 14, 15, 19, 20.

DOSSETIA, Azpeitia. Fossil, Spain. Valves unequally convex, more or less hyaline, with a considerable laminar expansion or frill irregularly undulate. Also a hyaline frill with irregularly serrate edge surrounds the less convex valve. *Diatomologia Española*, p. 202, ix 3, 7.

FRICKEA, Heiden. Brackist, recent = *Frustulia Lewisiana*, Batavia, India, Brazil, United States, West Africa; Fossil, Japan. *Atl. Schmidt* 264.1.

GOMPHOCYMBELLA, Otto Müller. Marine, recent, California. Fossil, Fresh water, Ethiopia; Fresh water, recent, Austria. Valve as in *Cymbella*, but with one extremity smaller than the other. *Atl. Schmidt* 294. 29-32.

GOMPHOPLEURA, Reichelt = *Reicheltia Van Heurck*. *G. nobilis* *Atl. Schmidt* 215: 15, 16. Valve with one extremity smaller than the other, but cuneate also in girdle view. Fossil, Japan, Hungary, Bohemia.

GONIOCEROS, Peragallo. *G. armatum* = *Chaetoceros armatum*, West. *Diat. Mar. France*, p. 471, cxxxv. 6. *Frustule quadrangular* with the corners cut off, whence proceed long curved spines with blunt ends; there is also a smaller spine at each end of the straight sides.

HANDMANNIANA, Peragallo. *H. Austriaca*. *Mitt. Mik. Vereins Linz*, 1913, I, p. 36, taf. und fig. *Bot. Cent.*, cxxv, 1914, p. 622, no figure. Cocconeis in form with border: "die mitte von einen stark aufgetriebenen buckel (15-17 streifen) durchzogen; auch der rand zeigt feine linie mit perlen. Die unterschale zeigt mittel streifungen, welche von der schwach sichtbarn rhapshe aus alternieren."

I have been unable to find the Proceedings of the Linz Microscopical Society, which contains the figure of this species, and am unable to reconstruct it mentally from the description. Fresh water, recent. Alm See, Austria.

HERIBAUDIA, Peragallo. Fossil, Auvergne. *H. ternata*, *Diat. d'Auvergne*, p. 196, v. 25. Van Heurck, *Treatise on Diat.*, p. 542, fig. 291. Valve circular, disciform, hyaline or finely punctate, bearing

on its edge three small expansions or conical wings, between which extend three larger wings, rounded or plicate.

LICMOSPHENIA, Mereschkowsky. Marine, recent, Adriatic Villefranche, Sumatra. Frustules as in Licmophora, but with two openings in the septa instead of one opening. The superior opening is small, the inferior is larger.

OESTRUPIA, Heiden. Marine, recent = *Navicula* (*Caloneis*) *Powellii* with its varieties and *Navicula quadriseriata* Atl. Schmidt., 264. 4, 5, 8, 9. Adriatic, United States, Egypt, and Balearic Islands.

PERAGALLIA or PERAGALLOA, Schuett. Marine, recent, Baltic. It has the body of a *Dactyliosolen* with the horns of a *Chaetoceros*. *P. tropica*. The horns at the two extremities are turned in the same direction. Schuett, *Bacillariales*, p. 86, fig. 142: Van Heurck's *Treatise on Diat.*, p. 419, fig. 137; *Diat. Mar. France*, p. 475, cxxvi., 9.

PETITIA, Peragallo. Marine, recent. Nassau, Bahamas. Valve bacillar, arcuate, covered with transverse striae interrupted by two longitudinal lines. *Diat. du Monde Entier*, p. 146.

PHAEODACTYLON, Bohlin. Fresh water, Finland. *P. tricornutum*. Valve a three pointed star, the arms in one plane; the outer half of the arms generally hyaline. Von Schonfeldt's *Süsswasser Flora Deutschlands*, etc., p. 173, fig. 379. Something like the *Manx* arms cut off at the knees.

PLANKTONIELLA, Schuett. Marine, recent = *Coscinodiscus sol*. The valve is surrounded by a broad membranous frill; in girdle view it is linear. *Trans. Mic. Soc.* 1860, p. 38, ii., 1, 2: Atl. Schmidt 58, 41, 42, 45: Van Heurck *Syn.* 129. 6: V. H. *Treatise*, p. 534, fig. 279: *Diat. Tar. France*, p. 426, cxvi. 5. Bay of Bengal, Indian Ocean, Fossil, Barbadoes.

PSEUDO-AMPHIPRORA, Cleve, *Syn. Naviculoid Diat.* I., p. 70 = *Navicula lepidoptera*, = *Nav. arctica* Cl. Van Heurck includes it in *Orthotropis*, Peragallo considers the genus good, as the structure of the frustule and the endochrome are typical. *Greg. Diat. Clyde*, p. 34, iv. 60.

PSEUDO-NITZSCHIA, Peragallo. For forms between *Nitzschia* and *Synedra*, includes *N.* (?) *seriata*, *Rhaphoneis cuneata*, and *Synedra sicula*. The power of movement indicates the presence of a rhaphe or of longitudinal openings on the keel. *Diat. Mar. France*, p. 298, lxxii, 25-29., Italy, Scotland, Arctic.

PSEUDO-PHXILLA, Forti. *Nuova Notarisia*, XX., 1909, pp. 25, 29: plate ii. Fossil, Italy; Richmond, Va., etc. Valves unequal, cylindrical, with or without spines at the end of the longer valve, smaller valve more or less convex, often included in the longer valve.

PSEUDO-STICTODISCUS, Grunow = *Stictodiscus Eulensteinii* Castr. *Challenger Diat.*, i. 7. Van Heurck and Schuett include in *Triceratium*; it resembles *Stictodiscus*, but wants the radiating folds or plicae.

RHOPALODIA, O. Müller. = *Epithemia partim*. Frustules cuneiform or sub-globular, valves keeled, without lines of junction, rhaphe and central nodule distinct, terminal nodules indistinct. Zone striate or plicate. Transverse section of valve is like a wide V with unequal arms, making the section of the frustule trapezoidal; the rhaphe occupying the acute angle is seldom visible, as the valve falls flat. *Jl. R. Mic. Soc.* 1900, p. 228: *Atlas Schmidt*, plates 253-256, 265, 294: *Diat. Mar. France*, lxxvii. *Rhopalodia* includes *Epithemia gibba* and certain forms from Nyassaland.

SCHMIDTIELLA, Ostfeldt. *S. pelagica*. Frustules in chains, valve broadly elliptical, surface undulate, minute processes at extremities, hyaline: akin to *Grayia*. *Kohchang Flora*, 1902, p. 24, fig. 20.

SCHROEDERELLA, Peragallo. Marine, recent. = *Lauderia delicatula* Schroeder = *Detonula delicatula* Gran = *Lauderia Schroederi* Bergon = *Detonula Schroederi* Gran. Not = *Lauderia delicatula* Peragallo. *Nuova Notarisia* April, 1914, p. 131, Naples, Arcachon.

SECALLIA, Azpeitia. *Diat. Esp.*, pp. 217, 176., vi. 6, 7. Moron. *S. Caballeroi*. Valve elongato-rhomboidal with rounded angles, and with a deep depression across the shorter diameter. Granules scattered over the valve without fixed direction, but disposed in transverse lines on connecting zone, no rhaphe or nodules.

SEMSEYIA, Pantocsek. Fossil, Kertsch and Hungary. Valve arcuate, capitate, with transverse striae, marginal or complete; resembles in shape *Eunotia gracilis*. Frustule in zonal view with transverse striae interrupted by a longitudinal hyaline line inflated at the extremities. Klebschiefer von Kertsch von Dr. Jos. Pantocsek. Verhand. der Russ. Kais. Mineral Gesell. Serie 2, Band 39. 1901, 2., p. 644, taf. xii. 30, 31.

SMITHIELLA, Peragallo. *S. marina* = *Himantidium marinum* W.S. Ann. Mag. Nat. Hist. 1857, ii. 14. = *Eunotogramma debile* Grun. Van Heurck Syn. 126, 18, 19; Diat. Mar. France, p. 343. lxxxii. 36. A cymbiform *Odontidium*. Marine, recent, Biarritz.

STREPTOTHECA, Shrubsole. Leisure Hour, Nov. 1890, p. 34. J. Quekett Mic. Club, 1890, p. 259., xiii. 4-6. Peragallo, Diat. Mar. France, p. 458, cxxi., 10. Estuary of the Thames. Peragallo places it with *Rhizosolenia*, Schuett considers it "incertae sedis," Ostenfeldt and Van Heurck place it with *Eucampia*. According to Cleve *S. Tamesis* = *S. maxima* from the Indian Ocean and Malay Archipelago. It is also found in the Red Sea and North Atlantic.

SYNEDROSPHENIA, Paragallo. Diat. Mar. France, p. 312, a subgenus of *Synedra*, to include *Sceptroneis cuneata*, *Synedra clavata*, and *Synedra dubia*. Bahamas, Cayenne, Barbadoes, Samoa, Banyuls. Valve cuneate, Marine, recent and Fossil.

SZECHENYIA, Pantocsek. Hungary. Diat. Szliacs, pub. Friedlander, p. 16, ii, 58-61. Valves cylindrical, domed, hyaline; with longitudinal septa and hyaline rays; zone with transverse lines. Grows in a chain. It is like *Cladogramma* in girdle view, and is placed among the *Melosireae*.

TEMPEREA, Peragallo. *T. Mephistopheles*. Marine, recent. Tamatave, Madagascar. Diat. du Monde Entier, Nos. 98-100. Valve circular, convex, with border of radiate oval granules. Cf. *Skeletonema mediterraneum* Grun.

TEMPERELLA, Forti. *T. miocenica*. Fossil, Bergonzano, Italy. = *Aulacodiscus miocenicus* Forti, Nuova Notarisa, Jan. 1909, p. 39: April 1914, p. 109 note, vi. 1, 3, 4, 5. Valve circular, divided

into 18 or more sectors each with rows of puncta parallel to the middle row. There is a minute process near the outer margin of the valve in each alternate sector. Puncta minute, arranged in quincunx.

VALDIVIELLA, Schimper.

VANHEURCKIELLA, Pantocsek. Diat. Foss. Hung. III, 1, 4. Van Heurck Treatise on Diat., p. 540, fig. 288. Fossil, Oamaru. *V. Admirabilis* Grun. Van Heurck considers it a spongiolithum.

WEISSFLOGIA, Janisch. Gazelle Exped. i., 12-17. *W. Macdonaldii*, King George's Sound, Australia, resembles a twisted *Surirella* with short transverse markings across the spaces between the costae.

It will be noticed that many of these genera are formed for single species already described, or are very limited in extent. I have failed to find any description of *Valdiviella* or *Bacteriosira*, and doubtless this list is in other respects incomplete, but it represents a considerable amount of work in literature not easily accessible.

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DEPARTMENT OF NOTES AND REVIEWS

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

THE NEW SECRETARY AND EDITOR

Owing to the pressure of other duties the undersigned has been compelled to retire as Secretary of the American Microscopical Society and Editor of the *Transactions*, at the end of the current year and volume. This concludes ten years of service which is probably as much as one member should be called upon to render, and doubtless as much as the Society can afford to endure.

The Executive Committee has voted to appoint, subject to confirmation at the coming business meeting of the Society in St. Louis in affiliation with the American Association for the Advancement of Science, Professor Paul S. Welch of the University of Michigan, Ann Arbor, Michigan, as Secretary-Editor for the next term of three years. Indeed, Dr. Welch has kindly undertaken to begin his work with the October number of Volume 38. All correspondence relative to membership, *Transactions*, and exchanges should therefore be addressed to him.

The former Secretary had hoped to present a statement to the Society of the work of the decade, but this does not seem possible at the present time. He must content himself with making a final request of the membership to give the new Secretary the utmost support in his effort to make the *Transactions* still more effective in stimulating research and in publishing its results.

T. W. GALLOWAY

ENTOMOLOGICAL ABSTRACTS

Sex Determination in Trialeurodes.—Stoll and Shull (1919, *Genetics*, 4:251-260) have investigated the previously reported statement that in *Trialeurodes (Aleurodes) vaporariorum* the parthenogenetic eggs produce females in the English representatives of the species and males in those occurring in the United States, and that fertilized eggs produce both sexes in equal numbers. A series of carefully planned breeding experiments with American stock indicated that the unfertilized eggs do produce males, but there was no evidence for the belief that the fertilized ones result in both males and females in equal numbers. Instead, the evidence supports the conclusion that all fertilized eggs produce females, thus corresponding with the case of the honey bee.

Pentatomoidea.—Hart (1919, *Bull. Nat. Hist. Surv., Illinois*, 13:157-223) has summarized the *Pentatomoidea* of Illinois and constructed keys to the Nearctic genera, thus presenting a very useful and important work on this group of Hemiptera. This paper is exclusively systematic in nature and has much to commend itself to workers in entomology.

Canadian Bark-beetles.—Swaine (1918, *Dep't Agr., Dominion of Canada, Entomological Branch, Bull.* 14) has published a treatise on "Canadian Bark-beetles" which makes readily available much important data on Canadian *Scolytidae*. An extensive account is given of the structure and general biological features of these insects, followed by descriptions of the various species concerned accompanied by keys for the identification of the same. Thirty-one plates and four text figures add much to the value of the paper. Owing to the nature of the paper it is not readily summarizable but it is a work worthy of commendation and is indispensable to students of Coleoptera.

Drosophila and Disease.—Sturtevant (1918, *Journ. Parasitology*, 5:84-85) reviews the circumstantial evidence that flies of the genus *Drosophila* may be carriers of disease. In the tropics *D. repleta* and *D. caribbea* have habits which place them under strong suspicion, but contrary to the meager literature on this subject, there seems to be little reason for regarding *D. melanogaster (ampelophila)* as particularly dangerous.

Nematode Parasite of Sciara—Hungerford (1919, *Journ. Parasitology*, 5:186-192) reports certain biological features of a nematode,

Tetradonema plicans, which parasitizes the mycetophilid fly, *Sciara coprophila*, in the larval, pupal, and adult stages. From two or twenty nematodes representing both sexes were found in a single host. A striking sexual dimorphism is manifested, the females reaching a length of 5 mm. while the males are less than 1 mm. long. Likewise the sexually mature female is characteristically swollen about midway between the anterior and posterior end, due to accumulation of great numbers of eggs "beneath the cuticula, which serves as a retaining capsule." It seems probable that the parasite gains entrance into the host by the latter swallowing the eggs of the nematode, the eggs hatching and the young parasites boring through the walls of the alimentary canal into the body cavity of the larva. There is no evidence of an alternate host in the life cycle. In connection with the copulation activities as many as four males may become attached to one female and so remain until the female completes her egg capsule and dies. A maximum of 5,520 eggs per female is recorded.

Propleura of Orthoptera.—Du Porte (1919, Can. Ent., 51:147-153) has made morphological studies of representatives of the principal orthopteran families in investigating the problem of the presence or absence of the propleura and the significance of the pronotal sulci. Evidence secured indicates that the propleurum has not been eliminated by the downgrowth of the pronotum but persists on the inner side of the latter which has descended over it. The typical pleural sclerites are present and possess a musculature similar to the homologous sclerites on the mesopleurum and metapleurum. The sulci are mere folds resulting from mechanical stresses and do not mark off areas homologous to the prescutum, scutum, scutellum and postscutellum in the other thoracic segments. It is therefore claimed that these terms should not be applied to the pronotal areas.

Grylloblatta campodeiformis.—Walker (1919, Can. Ent., 51:131-139) reports on seven additional specimens of the remarkable orthopteroid insect, *Grylloblatta campodeiformis*, one of which is a mature male and four are nymphs representing both sexes. Males and nymphs are described for the first time and important morphological data are presented. The genitalia have been given critical examination since the phylogeny of this insect is of particular interest. The opinion is expressed that the "Panisoptera" (which includes the

Blattoidea, Mantoidea, and Isoptera) and the Orthoptera represent two branches from the same stem and that *Grylloblatta* is the sole survivor of a branch "which separated from this stem before the two main branches had become differentiated."

Staining Coccidae.—Gage (1919, Ent. News, 30:142-144) has found a method for permanently staining the exoskeleton of scale insects. After trials with several stains, it was found that säuerfuchsin gave best results but had the serious disadvantage of fading after a time, due to the alkali remaining in the tissues from the KOH cleaning fluid. This difficulty was eliminated by introducing hydrochloric acid to form an excess and mounting in acid balsam. The following formula represents the most satisfactory proportions of the ingredients:

Säuerfuchsin.....	0.5 gram
10 per cent hydrochloric acid.....	25.0 cc.
Distilled water.....	300.0 cc.

Remove specimens to be stained from the KOH and wash thoroughly in three or four changes of distilled water; place in Syracuse watch-glass containing a few cubic centimeters of the staining fluid and leave for from 20 to 40 minutes; remove and use subsequent treatment ordinarily employed in preparing scale insects. Care should be used that the specimens remain in such liquids as carbol-xylene, clove oil, or alcohol long enough to insure the complete clearing or dehydration.

Olfactory Sense in Larvae.—McIndoo (1919, Ann. Ent. Soc. Am., 12:65-84) presents morphological and experimental data on the presence of an olfactory sense in the larvae of Lepidoptera. The larvae of five species, four moths and one butterfly, were tested with a variety of substances giving odors and, in general, found to respond to such chemical stimuli although differences in the average reaction times seemed to depend upon the degree of sluggishness of the animal rather than specific sensitiveness to the odors. Pores, widely distributed on the head and its appendages, legs, dorsal surfaces of prothorax and last abdominal segment, and on the anal prolegs are structurally well adapted to receive chemical stimuli and may constitute the morphological basis for the olfactory response of the animal although experiments were not performed to determine their exact function.

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PAUL S. WELCH.

I. FORCED MOVEMENTS, TROPISMS, AND ANIMAL CONDUCT

Dr. Loeb is chairman of the board of editors which is issuing a series of monographs on experimental biology and general physiology. These monographs which collectively cover a wide field are designed to encourage quantitative experimental work as against descriptive and speculative; and to this end each one presents a summary of the exact work that has already been done in its particular field.

Dr. Loeb introduces the series with a volume treating forced movements and tropisms in relation to animal conduct. His analysis is designed to illustrate the application of the quantitative method to the study of animal behavior. He holds that such study supports his own well-known theory of tropisms or forced movements. This hypothesis, first propounded by him some thirty years ago, is in antithesis to the more anthropomorphic idea that animal behavior is the result of trial and error, of pleasure and pain, of curiosity or other internal physiological states.

In Chapter II the author, in a most suggestive and lucid way, shows how the fundamental symmetry of animals is the starting point and foundation for an exact analysis of behavior. The importance of animal symmetry lies in the fact that the morphological plane of symmetry is also the dynamical plane of symmetry. This morphological symmetry is the gross expression of equivalency of chemical constitution and of reacting stuffs, and this gives a basis for quantitative and comparative experiments involving the similar elements both of reception and response.

To illustrate by a quotation: "When symmetrical elements of the eyes are struck by light of the same wave length and intensity, the velocity of photochemical reactions will be the same in both eyes. Symmetrical spots of the retina are connected with symmetrical elements in the brain and these in turn with symmetrical muscles. As a consequence of the equal photochemical reactions in the symmetrical spots of the retina, equal changes are produced in the symmetrical brain cells with which they are connected, and equal changes in tension will be produced in the symmetrical muscles on both sides of the body with which the active brain elements are connected. On account of the symmetrical character of all the changes no deviation from the original direction of motion will

occur. If, however, one eye is illuminated more than the other eye, the influence upon the tension of symmetrical muscles will no longer be the same and the animal will be forced to deviate from the original direction of motion." The bilaterally symmetrical organism serves as a kind of pair of physiological scales or balances, by which one may appraise the forces causing movement. Couple this with the asymmetrical polarity of head end and tail end (or free end and base) and one can anticipate the general method of the book. But no one can anticipate the brilliant and vivid illustrative experiments and the special interpretations of these in the support of the general hypothesis.

In Chapter three on "Forced Movements," the author illustrates three kinds of forced movements: circus motions (involving some destruction of symmetry of tension), the tendency to go backward, and the tendency to move forward. These latter movements are related to the antero-posterior polarity.

In the remaining chapters the author gives experiments and their interpretation under the following heads: Galvanotropism, Heliotropism, Geotropism, Rheotropism, Anemotropism, Stereotropism, Chemotropism, and Thermotropism. Except in the case of the first of these a large part of the account is of new experiments.

A relatively large part of the book is given to the discussion of heliotropism. There are special chapters dealing with particular problems involved in heliotropism, as: light of different intensity from double sources; the Bunsen-Roscoe law for heliotropic reactions; effect of rapid changes in intensity of light; relative effectiveness of different wave lengths; charges or reversals of heliotropism.

The concluding chapters XVIII and XIX deal with instinct and memory images in relation to the theory of forced movements. This the author rightly states is the real test of the theory. While suggestive, these are the least convincing and satisfactory chapters of the book.

Instincts are tropistic reactions modified by hormones,—or otherwise. A quoted example will give the author's method. "The fact that eggs are laid by insects on material which serves as a nutrient medium for the offspring is a typical instinct. An experimental analysis shows that the underlying mechanism of the instinct is a positive chemotropism of the mother insect for the type of substance

servicing her as food; and when the intensity of these volatile substances is very high, i.e. when the insect is on the material, the egg-laying mechanism of the fly is automatically set in motion. Thus the common house-fly will deposit its eggs on decaying meat but not on fat; but it will deposit it on objects smeared over with assafoetida, on which the larvae cannot live. It seems that the female insect lays her eggs on material for which she is positively chemotropic, and this is generally material which she also eats. The fact that such material serves as food for the coming generation is an accident. Considered in this way, the mystic aspect of the care of insects for the future generation is replaced by the simple mechanistic conception of a tropistic reaction."

The author's treatment of memory images and the general phenomena of association seem to indicate a negative psychotropism on his part which results in "forced" conclusions.

It is not often, in spite of his mechanistic determinism, that the author's logic actually nods. But surely the fact that "Passenger pigeons when reared by ring doves refuse to mate with their own species but mate with the species of their foster parents" does *not* "show incidentally that racial antagonism is not inherited but acquired." The most that it can "show" is that if inherited in any degree such aversion can be lost thru experience.

The book is wonderfully suggestive and is a strong exposition of the purely mechanist thesis.

Monographs on Experimental Biology: Volume I, Forced Movements, Tropisms, and Anima Conduct, by Jacques Loeb. 210 pages, illustrated. J. B. Lippincott and Company, Philadelphia. 1918. Price \$2.50.

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II. THE ELEMENTARY NERVOUS SYSTEM

This is the second in the series of volumes summarizing the results in various special fields of experimental physiology. It is written with the directness and clearness characteristic of Professor Parker's writings.

"Elementary" in this title is used in a strict sense, as the author confines his discussion to the conditions found in the three simpler phyla of multicellular animals,—the sponges, coelenterates, and ctenophores.

In an introductory chapter the author calls our attention to the fact that we take an anthropomorphic view of the behavior and capacities of the lower animals because our first studies of nervous structure and phenomena were done upon man and the higher animals. In this connection he emphasizes that the unitary neuro-muscular mechanism of the higher animals, consisting as it does of a group of receptors connected with a well-organized adjustor or internuncial group of neurones which in turn control a specialized effector apparatus, is found only in the differentiated animals. While elementary in relation to the whole complex system, this reflex arc is not elementary in a primitive sense.

For the most part in the groups studied the neuro-muscular system consists of many peripheral sensory cells, often with well specialized receptive portions, with deep branching ends which connect more or less directly with the muscular elements without any complex adjusting or central organ. This latter may be fairly looked upon as a later, indeed the latest, and higher step in the evolution of the apparatus. And yet, as we might well expect, conditions are found which suggest gradations toward this higher type.

This direct connection of receptors and effectors is itself not the primitive condition. The author conceives that the antecedent of this apparatus is a still simpler condition in which is only the effector or muscular element. With this foundation of independent effectors, themselves directly but slowly sensitive and responsive to the essential tensions and stresses, the gradual differentiation of the other parts as accessory to them is entirely plausible.

Dr. Parker organizes his presentation of the subject on this interpretation of his findings by discussing in Section I the "Effector Systems," as in the sponges and in certain independent effector systems of higher animals; in Section II, the "Receptor-Effector Systems" as illustrated in the Sea-anemones, Jelly fishes and Hydroids; and in Section III, with certain anticipations in later chapters of Section II, he outlines a scheme correlating these more primitive conditions with one another and with those animals in which the adjustors or central organs also appear.

By a series of experiments upon suitable sponges it was found that the common flesh is contractile, the oscula open and close, the incurrent pores may close either by the modified ameboid motion

of the pre-membrane or by the contraction of a sphincter-like band of cells in the wall of the canal itself. These operations may be studied directly or by the modification of the currents of water maintained thru the sponge by the flagellate cells in the specialized canals of the sponge. Various, tho somewhat limited stimuli may operate in producing response in these effectors,—as presence or absence of sea water, existence of currents in the water, injuries, and changes in the chemical condition of the water. Critical experiments, detailed at length, warrant the conclusion that the stimuli operate directly upon the simple independent contractile elements, and not thru specialized receptors.

In Chapter IV it is shown that such independent muscular effectors are not confined to sponges but are present also in certain organs of higher animals. The sphincter of the pupil of the eye, the amnion of the chick, the embryonic heart of vertebrates (and probably in some degree the adult heart) show this immediate responsiveness of muscle without the intervention of special sensory elements. These conditions may also be accompanied by ordinary nervous control of the muscles.

The transmission of impulses from one part of the sponge to another takes place by a sluggish neuroid rather than a nervous process. This is protoplasmic and primordial, and apparently back of the more specialized nervous transmission. This too is a condition retained in certain effectors in the higher animals. Evidences of such neuroid transmission in the absence of actual nervous structures are seen in the coördinations of ciliary action in many higher animals including vertebrates, and in the swimming plates of Ctenophores.

Section II with eight chapters deals with the more prompt and effective receptor-effector system as illustrated in the Coelenterates. There is added here a more specialized sensory surface fitted to receive more exactly the various stimuli and to transmit their influence thus indirectly to the responding apparatus. In the sea-anemones there are four types of effectors:—the mucous glands, the cilia, the nettle cells, and the rather numerous special sets (13 in *Metridium*) of muscle fibres. Among these only the muscles give any experimental evidence of nervous control. The others are independent effectors.

Various observations show that there is wide spread transmissive connection of a nervous kind between the epithelium and the deep

muscular layer which makes it possible to bring the whole muscular effector apparatus into response by a surface stimulus at any point of the body. The author holds that this nerve net is not in the supposedly nervous layers of ectoderm and entoderm, but rather in the supporting lamella between them.

In Jelly fishes there is both an increased specialization of the receptor system (e.g., the marginal sensory bodies) and of the muscular effectors, as well as of the nerve net which connects them. There is in them a corresponding promptness of response, with a remarkable coördination of the total bodily reaction thru a very definite wave of contraction evidently made possible by the nerve net.

In Chapters IX and X the author traces the contrasts and correspondences between this nerve net as the main connecting apparatus in Coelenterates and its existence in special organs in the higher forms—as in the heart and intestine of vertebrates (often side by side with the neurone synaptic system). This nerve net is particularly suitable to autonomous structures and organs and to situations in which the transmission needs to be diffuse and general rather than to produce specific and local reflexes. Yet in even such highly autonomous organs as tentacles in the Coelenterates show themselves to be, there is some degree of physiological polarity present, in that transmission occurs more freely in one direction than in others; and they may fairly be said to show evidences locally of the beginnings of nervous organization of a grade higher than the diffuse nerve net.

Functionally the transition to the condition which we know as reflexes, in which a local and particularized muscular response follows regularly from equally local and specific stimulus might well come in this gradual way thru increased polarization of originally diffuse apparatus. A localized esophageal response in *Metridium* by a specific stimulation of the tentacles by fish meat has all the earmarks of such a reflex—superimposed upon the more diffused net reactions.

Chapters XI and XII analyze some of the more complex effective operations of Actinians (in which field the author and his students have done leading and conclusive work) to determine whether these indicate any evidences of the higher internal coördinative and unifying functions which characterize those animals having a central apparatus. Among the most promising of these are the operations

of feeding, rhythmic or other contractions and expansions, the creeping activity of the pedal disc, and the general modifiability of behavior thru the experiences of the animals. No such associative results are revealed as would imply a nervous integration higher than the net would supply. The best that can be said seems to be that there is a small group of feeding activities which localize as reflexes.

Chapter XIII is devoted to Hydroids, particularly illustrated by *Corymorpha*, in an effort to discover whether these animals, regarded as more primitive than the Actinians, have a receptor-effector apparatus more simple than they, and thus furnish a clue to the possible evolution of the higher coelenterate condition from that in the sponges. In these forms the muscular effector system reduces essentially to an ectodermal longitudinal system and a circular entodermic one. These are further differentiated into the stalk muscles, those of the hypostomial ("proboscis") extension of the stalk, and those of the two groups of tentacles. Of these muscles the circular entodermic ones seem to be directly stimulated (i.e., without nervous intermediation) and therefore are more like the slow acting, primitive type found in sponges. They operate in connection with the vacuolated cells to bring the hydroid back to its elongated form. The ectodermic muscles on the other hand are relatively prompt in action and are controlled by a nervous system of ectodermic receptors and a nerve net—as in actinians. *Corymorpha* also shows specialized reflexes analogous to those in *Metridium*. The author holds that *Corymorpha* evidences behavior and nervous organization of *reduced actinian type* rather than intermediate between the sponges and the actinians.

Section III, which comprises one final chapter of "Conclusions" is devoted to giving an outline of the differentiating steps in passing from the elementary independent effector to the complex central nervous system of the higher animals. The whole chapter is a concise, condensed outline, and no brief abstract of it can do justice to its lucid presentation. It can serve only as a Table of Contents to the chapter.

1. The starting point in the evolution of the nervous system in metazoa is the simple independent effector of smooth muscle cells. Such an apparatus is functionally limited to the reception of the grosser, more physical types of stimulus, is slow and sluggish in its responses, and the diffuse transmission is of a protoplasmic neuroid

rather than specialized nervous type. This condition is realized in sponges.

2. Around this starting point of response the receptor system next develops, making for promptness and elaboration of reception of stimuli. In this way comes distinctions in the categories of possible stimulation, and extension of its range to milder and more refined types of stimulus. Hypothetically, there are several possible steps in the elaboration of such a receptor-effector system. The first of these would be the immediate connection of nearby epithelial cells with the Effectors. This simple condition is as yet hypothetical. The simplest form actually found is where the inner branching ends of the receptors connect not alone with the muscles but with one another in a more or less complex network. This is illustrated in the tentacles of Actinians. Functionally this increases the dispersal of the effective results of stimulation in a diffuse way to many muscles, and gives a nervous instead of a neuroid character to transmission. A further complication of this is seen where special branched cells are connected with this nerve net. These have been called "ganglia." The author thinks that the term "protoneurone" may rightly express their nature. This is illustrated in the Coelenterates at many points. There are experimental evidences that a still further specialization of this nerve net is in limiting or specializing the routes and directions of transmission. Functionally the result in such as we usually describe under the term reflexes. It involves a localized response as characteristic of a localized stimulus. This involves some sort of polarity in the nerve net. This is found in certain actinian reactions. All of this is without any organization or any responses which simulate the central nervous stations of the higher animals.

3. Broadly anticipating the transitions to anatomical and functional conditions in the perfected receptor-adjustor-effector scheme the author suggests the main changes to be: the inward migration and concentration of the primary receptors and of the diffuse net elements into sheets, bands, and masses; more elaborate and perfect polarization and the increase of the special reflexes; the appropriation in many cases of secondary sensory cells by which the receiving function is again differentiated; and perhaps most significant of all the introduction of the neurone-synaptic apparatus whereby the route of the passage of impulses is more definitely determined. This carries

the polarization phenomenon a step further. There follows too in higher forms, apparently, the mechanism of the control of synaptic or other resistances by which inhibition or augmentation of impulses takes place.

The book will aid the general teacher and student of biology at a most interesting point.

Monographs on Experimental Biology, Volume II: The Elementary Nervous System, by G. H. Parker. 229 pages, illustrated. J. B. Lippincott, Philadelphia, Price \$2.50.

Neerology

DR. WILLIAM GILSON FARLOW

Died at his home in Quincy Street, Cambridge, on the third day of June, after an illness of three weeks. He was born in Boston, December 17th, 1844, and graduated from Harvard College in the Class of 1866, obtaining the degree of A.M. in 1869 and of M.D. in 1870. After receiving the medical degree he studied Botany in Europe for several years, for the most part at Strassburg in the laboratories of the distinguished botanist, Professor A. de Bary. After his return to America he was for a time assistant to Professor Asa Gray, and was also connected with the Bussey Institution. In 1874 he was appointed Assistant Professor of Botany in Harvard, receiving in 1879 the title of Professor of Cryptogamic Botany which he held for a period of forty years. On June 10th, 1900 he was married to Miss Lilian Horsford, daughter of Eben N. Horsford.

The honorary degree of LL.D. was conferred on him by Harvard in 1896, by the University of Glasgow in 1901, and by the University of Wisconsin in 1904. In 1907 he also received the degree of Ph.D. from the University of Upsala. He was a member of the National Academy of Sciences, the American Philosophical Society, the American Academy of Arts and Sciences, the Philadelphia Academy of Natural Science, the American Association for the Advancement of Science, of which he was President in 1906, the Linnaean Society of London, the Paris Academy of Science as well as of numerous other scientific bodies in this country and abroad.

In America, Professor Farlow was a pioneer in Cryptogamic Botany, and for many years has been justly regarded as preëminent in his profession, both in this country and abroad. Through his published writings, the inspiration of his teaching, his high ideals, versatility and extraordinarily wide learning he has long occupied a unique position among his scientific friends and associates, and has exercised an influence on the study and development of this chosen field the importance of which can hardly be overestimated.

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