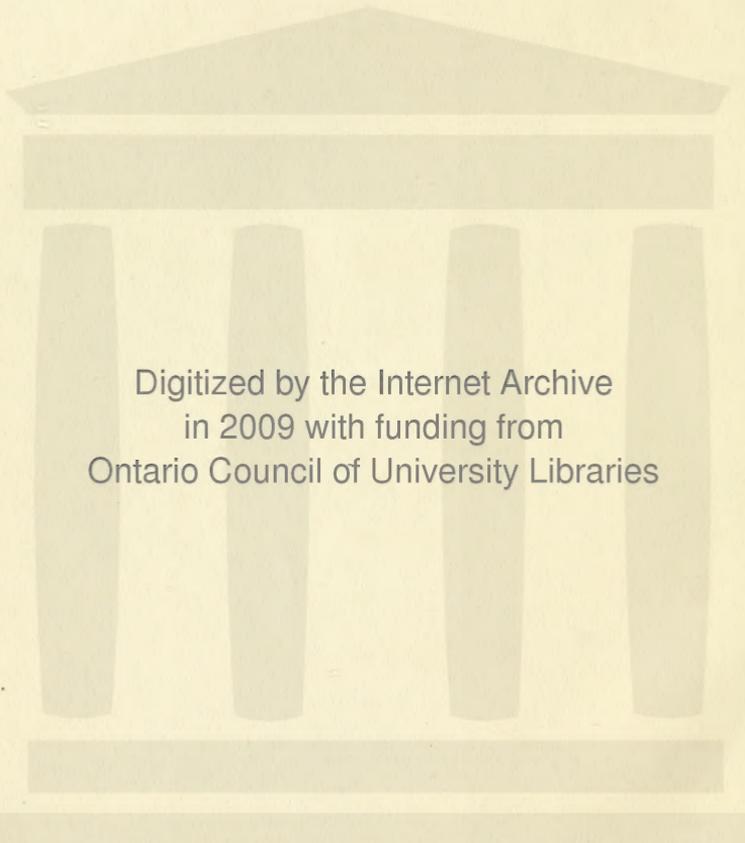


UNIV. OF  
TORONTO  
LIBRARY







Digitized by the Internet Archive  
in 2009 with funding from  
Ontario Council of University Libraries

13101  
A

4727

UNIVERSITY OF TORONTO LIBRARY

# TRANSACTIONS

OF THE

## American Microscopical Society

ORGANIZED 1878

INCORPORATED 1891

---

PUBLISHED QUARTERLY

BY THE SOCIETY

---

EDITED BY THE SECRETARY

PAUL S. WELCH

ANN ARBOR, MICHIGAN

---

VOLUME XXXIX

NUMBER FOUR

---

Entered as Second-class Matter August 13, 1918, at the Post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in Section 1103, of the Act of October 3, 1917, authorized Oct. 21, 1918

---

The Collegiate Press  
GEORGE BANTA PUBLISHING COMPANY  
MENASHA, WISCONSIN  
1920

174599  
14/10/22



111

TABLE OF CONTENTS

FOR VOLUME XXXIX, Number 4, October 1920

---

Micro-Technique. Suggestions for Methods and Apparatus, with five figures, by N. A. Cobb.....	231
List of Members.....	243
Index to Volume XXXIX.....	254



TRANSACTIONS  
OF THE  
American  
Microscopical Society

ORGANIZED 1878

INCORPORATED 1891

---

PUBLISHED QUARTERLY

BY THE SOCIETY

---

EDITED BY THE SECRETARY

PAUL S. WELCH

ANN ARBOR, MICHIGAN

---

VOLUME XXXIX

NUMBER ONE

---

Entered as Second-class Matter August 13, 1918, at the Post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in Section 1103, of the Act of October 3, 1917, authorized Oct. 21, 1918

---

The Collegiate Press  
GEORGE BANTA PUBLISHING COMPANY  
MENASHA, WISCONSIN  
1920

## OFFICERS

<i>President:</i> T. W. GALLOWAY.....	New York City, N.Y.
<i>First Vice-President:</i> CHANCEY JUDAY.....	Madison, Wis.
<i>Second Vice-President:</i> A. D. MACGILLIVRAY.....	Urbana, Ill.
<i>Secretary:</i> PAUL S. WELCH.....	Ann Arbor, Mich.
<i>Treasurer:</i> WILLIAM F. HENDERSON.....	Decatur, Ill.
<i>Custodian:</i> MAGNUS PFLAUM.....	Philadelphia, Pa.

---

## ELECTIVE MEMBERS OF THE EXECUTIVE COMMITTEE

FRANK SMITH.....	Urbana, Ill.
J. E. ACKERT.....	Manhattan, Kansas.
B. H. RANSOM.....	Washington, D.C.

---

## EX-OFFICIO MEMBERS OF THE EXECUTIVE COMMITTEE

Past Presidents Still Retaining Membership in the Society

SIMON HENRY GAGE, B.S., of Ithaca, N.Y.,	at Ithaca, N.Y., 1895 and 1906
A. CLIFFORD MERCER, M.D., F.R.M.S., of Syracuse, N. Y.,	at Pittsburgh, Pa, 1896
A. M. BLEILE, M.D., of Columbus, Ohio,	at New York City, 1900
C. H. EIGENMANN, Ph.D., of Bloomington, Ind.,	at Denver, Colo., 1901
E. A. BIRGE, L.L.D., of Madison, Wis.,	at Winona Lake, Ind., 1903
HENRY B. WARD, A.M., Ph.D., of Urbana, Ill.,	at Sandusky, Ohio, 1905
HERBERT OSBORN, M.S., of Columbus, Ohio,	at Minneapolis, Minn., 1910
A. E. HERTZLER, M.D., of Kansas City, Mo.,	at Washington, D. C., 1911
F. D. HEALD, Ph.D., of Pullman, Wash.,	at Cleveland, Ohio, 1912
CHARLES BROOKOVER, Ph.D., of Louisville, Ky.,	at Philadelphia, Pa., 1914
CHARLES A. KOFOID, Ph.D., of Berkeley, Calif.,	at Columbus, Ohio, 1915
M. F. GUYER, Ph.D., of Madison, Wis.,	at Pittsburg, Pa., 1917
L. E. GRIFFIN, of Pittsburg, Pa.,	at Baltimore, Md., 1918

The Society does not hold itself responsible for the opinions expressed by members in its published *Transactions* unless endorsed by special vote.

## TABLE OF CONTENTS

FOR VOLUME XXXIX, NUMBER 1, January 1920

Glaridacris catostomi gen. nov., sp. nov.: A Cestodarian Parasite, with Plates I and II, by A. R. Cooper . . . . .	5
The Genera of the Enchytraeidae (Oligochaeta), by Paul S. Welch . . . . .	25
An Ecological Study of the Algae of Some Sandhill Lakes, by Emma N. Andersen and Elda R. Walker . . . . .	51
Notes and Reviews: Leeches considered as Oligochaeta Modified for a Predatory Life, reviewed by F. Smith . . . . .	86
Minutes of the St. Louis Meeting . . . . .	89
Report of the Custodian . . . . .	89
Report of the Treasurer . . . . .	90



TRANSACTIONS  
OF  
American Microscopical Society

(Published in Quarterly Instalments)

Vol. XXXIX

JANUARY, 1920

No. 1

*GLARIDACRIS CATOSTOMI* GEN. NOV., SP. NOV.:  
A CESTODARIAN PARASITE

BY A. R. COOPER

INTRODUCTION

In a preliminary paper Ward (1911) stated that he had found in fish from the Illinois River a cestodarian tapeworm which showed certain features common to the well known European genera, *Caryophyllaeus* and *Archigetes*. "It resembles the former in the absence of a caudal appendage and in the location chosen by the adult parasite, viz., the intestine of a fish, whereas, so far as known in Europe, *Archigetes* always possesses a tail and has been found only in the body cavity of tubificid worms. In general appearance and structure the American form resembles the European *Archigetes* very strongly. It has a scolex of fixed form with prominent suckers or phyllidea and also the musculature of *Archigetes*. The general arrangement of the reproductive organs, especially the two rows of testes in the central field, and the genital pores, correspond also closely to conditions in *Archigetes*." Much later the same writer (Ward and Whipple, 1918) merely stated in his key to the Cestodaria that, as regards *Archigetes*, "a form which undoubtedly belongs here has been described to me as found in native earthworms." Neither under *Archigetes* nor under *Caryophyllaeus* does he make any further mention of the above form, and concerning *Amphilina* says only: "Not yet reported from North America but present." Nor have I been able to locate any other reference to members of the Cestodaria, sensu latu, having been found on this continent up to date.

Before proceeding with the detailed account it should be mentioned as a matter of introduction that, apart from being evidently

the first member of the group to be described from America, the species to be dealt with here is of special interest in that it seems to stand intermediate in the family, Caryophyllaeidae Lühe 1910, between *Archigetes* and *Caryophyllaeus*. Excepting for the scolex, however, which is quite similar at least in outward appearance to that of *Archigetes brachyurus* Mrázek, it closely resembles the species of *Caryophyllaeus*, of which three, namely, *C. laticeps* (Pallas), *C. tuba* (Wagener) and *C. fennicus* Schneider, have been found in Europe, and one, *C. syrdarjensis* Skrjabin, in Asia (Turkestan).

### MATERIAL

The material for the present study was obtained at the Douglas Lake Biological Station of the University of Michigan during the summer of 1917 while the writer was paying particular attention to the bothriocephalid cestodes of fishes. In all thirty-six specimens of *Catostomus commersonii* (Lacépède), the host species, were examined. These fell into two lots as regards size: ten younger ones ranging in length from 90 to 115mm. and twenty-six adults from 250 to 325mm. The latter were caught in the trammel and fyke nets used in the lake proper, while the former were seined out of Maple River which drains the lake. No parasites belonging to the species described here were met with in the younger hosts, but from two to at least sixty-three were found in the stomachs and intestines of eleven of the adults. The table shown on page 7 gives their number, distribution and kind in nine of the hosts, the exact numbers not having been recorded for the other two fish.

From this it is seen that the degree of infestation of the host is comparatively small. Whereas the number of adults met with was quite limited, larvae were very plentiful when present at all. In situ all of the adults and most of the larvae were found free in the stomach or intestine, but many larvae—forty-one in the case of the third fish in the table—were attached to the bottoms of deep pits in the mucosa of the pyloric region of the stomach. These pits were not mere depressions of the wall of the stomach but actual cavities, as shown in figure 7, bordered by a pronounced annular thickening of the mucous membrane and as much as 2mm. in diameter. Larvae ranging in size from almost the smallest met with to those near the adult stage in development were tightly crowded into these pits and at the same time strongly contracted longitudinally.

Length of host	STOMACH		INTESTINE	
	Number	Kind	Number	Kind
285 mm.....	9	Adults		
250 mm.....	4	Adults		
	14	Larvae		
275 mm.....	63	Larvae		
313 mm.....			2	Larvae
282 mm.....	9	Larvae	52	Larvae
295 mm.....			2	Adult
			1	Larva
295 mm.....	7	Larvae	21	Larvae
280 mm.....			20+	Larvae
265 mm.....			12+	Larvae

## EXTERNAL FEATURES

On account of its possessing a well developed musculature for its size this species exhibits considerable differences in degree of contraction and elongation on fixation. If no care is taken in applying the fixing reagent nor in slightly manipulating the specimen, it usually contracts to such an extent that it becomes almost useless, at least for the making of toto preparations. However, the adults, which are here considered to be those whose uteri may be seen in toto preparations to contain a few or many eggs, may be said to range in length from about 5 to 25mm. and from 0.4 to 1.0mm. in maximum breadth.

In immature individuals the scolex, when not strongly contracted, has somewhat the form of a truncated rectangular pyramid with the longer diameter in the transverse direction. As shown in figures 1 and 2, the edges of the base and the apex protrude markedly, in the latter case forming a terminal disc comparable to that of many of the bothriocephalid cestodes. The dorsal and ventral faces of the organ are each divided by two ridges converging towards the apex into three sucking grooves or loculi, of which the middle is best developed and most efficacious during life. It is also the last to become smoothed out with strong contraction of the whole scolex. The lateral loculi are, furthermore, not in the same plane with the medial one but inclined towards the corresponding ones of the opposite surface so that the edges of the scolex, especially just behind the

terminal disc, are often not much thicker than the ridges between the loculi. As regards these features the organ consequently resembles that of *Archigetes brachyurus* Mrázek 1908, which is here reproduced (Fig. 5) for the sake of comparison. In adults, on the other hand, the edges of the terminal disc are usually found in preserved material to be contracted to the point of obliteration, so that the whole organ is shaped more like a wedge or chisel with oftentimes rather thick margins (Figs. 3, 4 and 9). As a matter of fact the scolex of this form assumes a greater variety of shapes than that of any other tapeworm I have yet examined, in which respect it is comparable to the leaf-like anterior end of *Caryophyllaeus*. The dimensions of the organ are as follows: Length, 0.30 to 0.45mm.; width (posteriorly), 0.45 to 1.10mm.; depth (posteriorly), 0.50 to 0.75mm.

Behind the scolex the strobila narrows down for a short distance and then much more gradually enlarges again to the region of maximum diameter, which is usually behind the genital openings. Yet in many specimens, especially the more relaxed ones, the whole strobila is all but uniform in width thruout its length. The region between the scolex and formost vitelline follicles, which includes the narrowest portion of the strobila and is consequently called the neck, varies from 1.5 to 2.5mm. in length. Finally the posterior end, as shown in figure 6, is somewhat triangular in outline with a slightly indented tip where the excretory vessels open to the exterior, but bears nothing in the nature of an appendix such as it present in *Archigetes*.

#### CUTICULA, SUBCUTICULA AND PARENCHYMA

The cuticula, which varies in thickness from 7 to 11 $\mu$ , is bounded on the inside by a comparatively heavy basement membrane, about one-sixth of the thickness of the whole layer, and on the outside by a smooth membrane about one-half as thick as the basement membrane. The remainder of the tissue has the appearance of a reticulum enclosing numerous distinct granules. This reticulum is in reality a meshwork of fine canaliculi which freely pierce both limiting membranes, thus giving them the appearance in tangential sections of fine sieves. Nowhere is the cuticula modified to form spinelets nor distinct cirri, altho over the scolex it is considerably folded and

irregular, the outer membrane being all but absent, especially within the suckers. For *Caryophyllaeus laticeps* Will (1893) described a cuticula 5 to 6 $\mu$  in thickness and composed of only two layers, an outer showing radial striping, as if formed by fine bristle-like hairs, and an inner, more deeply staining stratum, comparable to the basement membrane of this form. He saw no distinct pores in the cuticula, and thought that perhaps the striations might represent prolongations of the subcuticula.

The subcuticula is made up of large flask-shaped cells, closely crowded together and provided with comparatively large nuclei. Whereas the individual cells are not distinctly separated from one another, the whole layer, from 90 to 100 $\mu$  in thickness, is clearly marked off from the underlying parenchyma owing to the very granular nature of its components. The nuclei, which are spherical to oval in shape and provided with distinct spherical nucleoli, vary from 16 to 18 $\mu$  in greatest diameter. They are located at different levels, so that the whole layer has a pseudostratified appearance. The enlarged central ends of the cells are usually rounded off towards the parenchyma, which feature is clearly indicated by their characteristically large granules. On the whole the subcuticula is not very different from that of *C. laticeps* as described by Will.

The parenchymatous cells form an open reticulum showing only a very few nuclei. They are in strong contrast with the subcuticular cells on account of their clear, non-granular cytoplasm. Posteriorly the whole tissue is much limited in amount by the large reproductive organs which are imbedded in it. No such "fibrous strands" of modified parenchymatous cells, as described by Will for *C. laticeps* and by Skrjabin for *C. syrdarjensis*, were seen in this form. In the base of the scolex and in the neck region, however, the medulla is occupied by a more or less X-shaped mass of cells (Fig. 10) containing large nuclei with numerous large granules which have a great affinity for the counterstain. They are probably glandular in their nature since they send long processes, especially in the diagonal direction, to the cuticula covering the scolex, between the cells of the subcuticular layer. Furthermore, no evidence of the presence of calcareous bodies in the parenchyma was met with in an examination of both fresh and preserved material.

## MUSCULATURE

The musculature is comparable to that of the cestodes proper in that it is composed of two sets of fibres, the parenchymatous and the cuticular. The former consists of sagittal (dorsoventral), frontal (transverse) and two sets of longitudinal fibres, of which the latter are much the strongest. Whereas both sagittal and frontal fibres are few in number, they are not equally so, for the sagittal are somewhat larger and more numerous. Both kinds tend to course slightly obliquely where they are greatly interfered with by the reproductive organs. The main or inner longitudinal fibres are, on the other hand, comparatively large and arranged in thick bundles (Figs. 11, 12 and 13). They are situated among the central ends of the subcuticular or just within them, the cortical parenchyma being thus considerably restricted in amount. Posteriorly the fasciculi are very unequal in size and quite numerous. As they are followed forward, however, their numbers diminish while their size increases, until at the base of the scolex there are only eight large bundles arranged as in figure 10. This is brought about by the fusion of the smaller bundles and the passage of the fibres from one fasciculus to another. In longitudinal sections the bundles are irregularly striated owing to there being a considerable amount of myoplasm in the middle of each fibre around the remains of the original myoblastic nucleus. Nevertheless, no distinct nuclei such as described and figured by Will for *C. laticeps* were seen. In the posterior end of the worm many of these longitudinal muscles terminate in the walls of the excretory invagination or run alongside of it to the extremity of the strobila. The outer longitudinal group (Fig. 10) consists of a large number of bundles, smaller but more uniform in size than those of the inner group, situated among the peripheral ends of the subcuticular cells just outside of their nuclei or from 15 to 30 $\mu$  from the cuticula. Posteriorly only a few of them pass beyond the anterior end of the excretory invagination, but anteriorly they are very pronounced and continue into the scolex. Similar fibres in *C. laticeps* were considered by Will to belong to the cuticular instead of to the parenchymatous series.

The cuticular muscles consists of an outer stratum of circular fibres lying close to the inside of the cuticula and an inner of longitudinal fibres situated close within that. The longitudinal fibres, which in some places intermingle slightly with some of the outermost

members of the outer longitudinal parenchymatous group, are arranged in small bundles, each containing at most only about ten or a dozen fibres. In the posterior end of the worm they proceed farther back than the latter, after being closely associated with them opposite the excretory invagination. The same may be said of the circular cuticular muscles, excepting that they are not distinctly arranged in bundles.

In the scolex the cuticular muscles are much less pronounced over the sucking-grooves than on the lateral faces. As shown in figure 9, the eight large bundles of inner longitudinal muscles, mentioned above, are arranged so that four form two sagittal pairs situated towards the lateral faces, while the other four, somewhat larger ones form two other sagittal pairs, each about half way between the nerve trunk and the median line. These are distributed in a radiating manner to the corresponding portions of the tip of the scolex, the median pairs going to the ridges between the loculi and the neighboring parts of the latter. On the whole their attachment is similar to that of the main longitudinal group in *C. tuba* and *C. laticeps*, as described respectively by Monticelli (1892) and Will. The outer longitudinal muscles are more numerous on the lateral surfaces of the scolex than opposite the suckers, to the cuticula of which they are easily traced. The loculi are also provided with a few scattered radiating fibres, lying in both the longitudinal and the transverse directions, and comparable to those used in the Pseudophyllidea for the enlargement of the bothria. They are, however, of much less functional importance in that connection than the sagittal and transverse fibres, which are somewhat larger and more numerous than in the middle of the worm. In fine, the musculature of the scolex is poorly developed as compared with that of *Bothriocephalus*, s. str., for example, which fact is shown in the great diversity of shapes of the organ in preserved material. In fact it might be considered to represent an intermediate stage between that of the anterior end of *Caryophyllaeus* and that of the typical bothriocephalid scolex. But the comparative inefficiency of the individual sucking-grooves is compensated for by their number and by their manner of attachment to the host's alimentary tract, namely at the bottom of the spacious pits described above.

## NERVOUS SYSTEM

The nervous system consists of a pair of ill-defined longitudinal trunks and two equally indistinct and diffuse terminal ganglia situated in the scolex, into which they pass. The main strands can be followed more or less easily in material not especially treated to demonstrate them only in the neck region. There, as shown in figure 10, they are situated symmetrically in the median frontal plane within the trapezium formed by the two pairs of main longitudinal muscle bundles, much closer, however, to the lateral pair than to the more median pair. They supply these muscles with large branches. Whereas in the neck they are fairly uniform in diameter—which varies from 18 to 30 $\mu$ —behind the most anterior vitelline follicles they become quite irregular in transection, all but disappearing in places. In the middle of the worm and posteriorly they seem to break up into a diffuse plexus lying just within the subcuticular cells, that is, among the numerous bundles of the inner longitudinal muscles. No collateral strands such as the eight described by Will for *C. laticeps* were seen in this form.

In the base of the scolex these chief nerve strands expand considerably in the dorsoventral direction and become united by a few transverse fibrils. Farther towards the tip, however, each of these enlargements divides into two parts sagittally, and each of the latter unites with its fellow of the opposite side by a loose strand of transverse fibrils, so that two anteriorly directed loops are thus formed. On the whole the nervous system is comparatively poorly developed, since not only the chief strands but also their connections in the scolex are composed of very fine, indistinct and loosely arranged fibrils.

## EXCRETORY SYSTEM

Thruout most of the length of the worm the excretory system consists of a single layer of comparatively large and much coiled longitudinal vessels situated just outside of the inner longitudinal muscles among the central ends of the subcuticular cells. Whereas the number of these vessels cannot be stated definitely, owing to many transverse connecting channels, there is a tendency, especially in the anterior regions, for eight of them to take the courses indicated in figure 11. Three are located on each surface and one in the median frontal plane at each side. In the anterior part of the neck region

the number increases, and the courses of these vessels become irregular, that is, the plexus becomes more diffuse. There they invade all parts of the subcuticula and the periphery of the cortical parenchyma (Fig. 10). From 1 to 1.5mm. behind the tip of the scolex two branches leave the plexus above and below the nerve cord on each side (Fig. 10) and unite on the medial side of the latter to form one vessel. In these positions the two vessels thus formed pursue spiral courses forward and apparently unite close behind the nerve commissures mentioned above. For *C. laticeps* Fraipont (1880) and Will described an excretory system consisting in brief of four "ascending canals" and ten "descending canals," connected in the mobile anterior end of the worm with each other and posteriorly with the so-called excretory vesicle. Thus it is seen that as regards the main channels of the excretory system at least this species is somewhat less complicated in structure than the European species in question. In the posterior end of the former the plexus just described converges towards the centre of the medulla, as the vessels diminish in size, and unites by several openings with the terminal receptacle. The latter, as pointed out for *C. laticeps* by Steudener (1877), is merely an invagination of the hinder end of the worm, about 0.25mm. in length by about 0.05 in diameter. Its wall is composed of only a lining of cuticula continuous with that covering the posterior end of the worm and also traceable for some distance into the larger branches leading from the plexus into the invagination. In the sections made it was also seen to be quite vacuolated and granular and poorly provided with cuticular muscles, thus indicating that the whole structure is not a true pulsating vesicle.

Nowhere in any of the sections studied was I able to find the typical terminal organs of the excretory system, namely, the flame-cells, which according to Fraipont are present in *C. laticeps* with the same structure as those in trematodes. But in their place there appeared much less specialized cells which are, nevertheless, comparable in some respects to the ciliated funnels of other cestodes. As shown in figure 8, each consists of a large cell provided with a large nucleus with a distinct spherical nucleolus but much vacuolated cytoplasm. The cytoplasm is aggregated close around the nucleus, and from this mass numerous strands pass to the wall of the cell. The latter is directly continuous with one or more canaliculi which lead off from the structure and connect up with the larger vessels

to form the plexus. The whole has the appearance of an enlargement of the terminal vessel, enclosing an amoeboid cell which is suspended in the centre of the vesicle by its pseudopodia. Thus the vacuolated space which surrounds the cytoplasmic mass and is continuous with the cavity of the canaliculi is comparable in part at least to the funnel which accommodates the "flame" in the typical flame-cell. These terminal organs are situated close around the canals in the periphery of the cortex or even farther out among the inner ends of the subcuticular cells. Furthermore, they are much more numerous in the neck region than elsewhere. The only reference I have been able to find to structures at all comparable to these peculiar cells is that by Wright and Macallum (1887) on *Sphyranura osleri*. For this form, a monogenetic trematode, they described as the terminal renal organs peculiar elongated, club-shaped cells which are situated in close proximity to the vitelline follicles and the principal groups of muscles. The cytoplasm of the cell is divided into a number of coarse, granular trabeculae radiating from the nucleus to the wall, thus leaving a system of communicating spaces, "empty in the fixed, but often unobserved in the fresh, condition. . . . Each cell has a process at one pole, with an axial wavy channel connected with one of the neighbouring excretory capillaries . . . , the wall of which passes insensibly into the membrane of the cell." Perhaps also certain large amoeboid cells with nuclei filling up almost the whole of the cell and large nucleoli surrounded by clear areas, found by Will in specimens of *C. laticeps* fixed in Flemming's solution and crude acetic acid and described under the nervous system, may rightly belong to this category of peculiar excretory cells.

### REPRODUCTIVE ORGANS

On the whole the reproductive organs of this species (Fig. 6) closely resemble those of the species of *Caryophyllaeus*. In the longitudinal direction they extend from 1.5 to 2.5mm. behind the scolex, where the foremost vitelline follicles are situated, to the posterior end of the worm. The openings and the central connections of the ducts are located, however, near the posterior end, the former, in fact, only from 1.5 to 2.8mm. from the tip, depending on the degree of contraction of the specimen. Excepting Skrjabin, the European writers emphasize in their descriptions of the species of *Caryophyllaeus* the fraction of the whole length of the worm occupied by the

organs behind the opening of the cirrus. For *C. tuba* the latter opens at the beginning of the last quarter of the body, for *C. laticeps* at the beginning of the last fifth, and for *C. fennicus* in the last fifth. Skrjabin says only that in *C. syrdarjensis* the ovary is situated in the posterior third of the body. Owing to very considerable differences in degree of contraction and elongation it seems to me that, at least so far as the present species is concerned, these proportions are not of specific value. On account of the greater development of the musculature anteriorly that portion of the body ahead of the genital openings is much more variable in length than that behind the apertures—hence the above measurements for the latter only.

The genital openings are situated in the midline on the ventral surface from 0.5 to 1.0mm. apart. The cirrus-opening is somewhat transversely elongated and about 0.15mm. in diameter. The opening of the female atrium has the form of a shallow, transverse, crescentic groove, about 0.35mm. in width, with its concave side directed anteriorly. Both apertures are so close together in most of the specimens at hand that they are located at the bottom of a common depression; or, the slight depression accommodating the male opening runs insensibly into the crescentic female atrium.

*Male genitalia.*—The testes (Fig. 11) are not entirely surrounded by the vitelline follicles as in *C. laticeps* and *C. syrdarjensis*. Anteriorly they begin at the same level as do the latter, and posteriorly they extend to the cirrus-sac or in some cases slightly beyond its anterior border. They are irregularly ellipsoidal in shape, and have lengths, widths and depths of from 0.135 to 0.227, 0.100 to 0.145 and 0.127 to 0.181mm., respectively. Their number as determined by direct count and by calculation from the average number in longitudinal and transverse sections varies from 150 to 160. They are especially noteworthy on account of their showing the various stages of spermatogenesis with almost diagrammatic clearness, a fact which was also noted by Monticelli in the case of *C. tuba* and by Skrjabin in his description of *C. syrdarjensis*. Nevertheless in none of the series of sections cut were any spermatozoa seen in any part of the vas deferens, altho the uteri were in the same preparations well filled with eggs. This would seem to indicate that contrary to the usual procedure among cestodes the female genital organs develop before the male organs and that self-fertilization does not take place.

The vas deferens forms a loose and somewhat triangular mass of coils about 0.32, 0.28 and 0.36mm. in length, width and depth, respectively and situated immediately ahead of the cirrus-sac. Just before entering the latter it expands into a muscular vesicula seminalis having a diameter of from 65 to 90 $\mu$  and a length of about 0.30mm.; but at its beginning it has no seminal reservoir like that attributed to *C. laticeps* by Will. The wall of the duct consists of a lacerated or pseudociliated, syncytial epithelium, provided with widely separated nuclei—excepting in the seminal vesicle where they are fairly numerous—and resting on a basement membrane. The musculature of the vesicle consists of numerous circular fibres with a few oblique fibres distributed among them.

Entering the cirrus-sac anterodorsally with a diameter of 30 $\mu$ , the vas deferens expands in the dorsal third of the latter to form a sort of secondary, but doubtless only temporary, seminal vesicle averaging 60 $\mu$  in diameter. After taking several turns it gradually diminishes to about 35 $\mu$  in the mid-region of the sac and passes insensibly into the cirrus proper. The structure of the wall of the duct within the sac up to this point is the same as that of the seminal vesicle just outside of the sac. The cirrus, which occupies the lower half of the cirrus-pouch, is a comparatively large closely coiled tube with a diameter of 60 to 65 $\mu$ . Its wall, which is much cleft and folded on account of the length of the organ, is similar in structure to that of the vas deferens, excepting that the number of circular muscular fibres is much greater and that the imperfect epithelium of the latter is replaced (in the transitional region) by smooth cuticula, continuous with that of the ventral surface of the worm as in the cestodes proper. Altho in the material at hand there were no cases of extruded cirrus, its structure and disposition within the sac is such as to lead one to believe that when it is evaginated it is a comparatively long and stout organ.

The cirrus-sac (Fig. 12) is ellipsoidal in shape and occupies the whole of the medulla of the region dorsoventrally and almost all of it laterally. Its length, width and depth are, respectively, 0.40 to 0.50, 0.50 and 0.50 to 0.60mm. Its wall is composed of muscular fibres running in all directions and not sharply separated from the retractor muscles within the organ. A few dorsoventral fibres pass from the top of the sac to the dorsal body-wall and a few from

the equatorial region to the ventral body-wall. The contents of the sac are composed of numerous and very compactly arranged retractor muscles, their myoblastic nuclei and a small amount of parenchymatous tissues.

*Female genitalia.*—Into the dorsal portion of the female genital atrium, which is about 0.25mm. in depth and lined with a much lacerated continuation of the cuticula from the ventral surface of the worm, the vagina empties slightly to one side of the median line, the other side accomodating the opening of the uterus. From the atrium it passes backward in the median line (Fig. 6) beneath, or at some levels almost surrounded by, the coils of the uterus. Its diameter near the opening varies from 50 to 55 $\mu$ , but half way along its course this is reduced to 30 $\mu$ . Thruout its length its wall is composed of a lining of cuticula 5 $\mu$  in thickness and surrounded by numerous circular muscles only, the myoblastic nuclei of which form a rather distinct stratum about 10 $\mu$  distant from the fibres. At the level of the posterior end of the ovary it opens into the oviduct with a diameter of 8 $\mu$  and a much reduced cuticular lining and layer of circular muscles. Unlike that of *C. laticeps*, as described by Will, it is nowhere enlarged to form a receptaculum seminis.

The ovary is situated usually half way between the genital openings and the posterior end of the animal (Fig. 6). It is from 0.8 to 0.9mm. in length and consists of a stout almost spherical isthmus, about 0.4mm. in diameter, from which numerous, irregular and thick lobules pass upward and slightly forward to enclose a capacious generative space. In the latter respect this form resembles not only the species of *Caryophyllaeus* but also *Cyathocephalus* and *Bothrimonus* as described elsewhere by the writer (Cooper, 1919). As shown in figure 13, the lobules lie in the periphery of the medulla, close to the main longitudinal muscles. Ova near the beginning of the oviduct average 15 $\mu$  in diameter in sections, and are composed almost entirely of the nucleus, there being very little cytoplasm. A distinct and almost spherical nucleolus taking the counterstain very readily is to be seen in each nucleus.

The oviduct begins at the posterior end of the isthmus and somewhat ventrolaterally in an oocapt, 25 $\mu$  in diameter by 20 $\mu$  in length and provided with only a few circular muscles. About 125 $\mu$  from the oocapt it is joined by the vagina. This first portion

of the oviduct is 25 to 30 $\mu$  in diameter, and takes a dorsal course. Its walls are composed of a thin but uniform layer of circular muscular fibres on the outside, and on the inside of a comparatively thick layer of epithelium, the cells of which are not clearly separated from each other but contain relatively large and deeply staining nuclei. After passing backward and upward about 40 $\mu$  beyond the point of union with the vagina the oviduct receives the common vitelline duct.

As in the species of *Caryophyllaeus* the vitelline follicles are located in the medulla in two distinct and separate regions: a large one extending from 1.5 to 2.5mm. behind the tip of the scolex to the cirrus sac, and a much smaller one in the more or less conical posterior end of the worm behind the coils of the uterus (Fig. 6). In the former situation they form an irregular layer in the periphery of the medulla (Fig. 11), for not only do some dip down among the testes, as mentioned above, but others extend outward to the main longitudinal muscles; in the latter, however, they occupy almost the whole of the medulla, as in *C. laticeps*. In the immature worm there is, furthermore, some tendency for them to be arranged in two lateral fields anteriorly, leaving a free strip in the median line dorsally and ventrally. In the anterior region in particular they are very numerous, irregularly ellipsoidal in shape, and vary greatly in size. From 8 to 14 appear in transections, while their maximum diameter is 0.20mm. Posteriorly they are slightly larger.

The process of the formation of the peculiarly clear yolk-cells which are to be seen in the vitelline ducts (Fig. 14c) can be followed with a considerable degree of satisfaction in the follicles. The cytoplasm of the small peripheral primordial cells from which they develop is very compact, and consequently stains deeply as does the nucleus (Fig. 14a). Numerous vacuoles appear in it and quickly enlarge, so that in the intermediate stages the nucleus appears to be suspended in the centre of the cells by protoplasmic strands radiating from it to the cell-membrane, as shown in figure 14b. These strands become modified into numerous, spherical deutoplasmic granules, migrate outward and eventually come to lie just inside the cell-membrane (Fig. 14c). In the proximal part of the uterus, where from four to six vitelline cells are seen to be associated with each fertilized ovum in the formation of the egg, the nucleus enlarges still

more and becomes more transparent, while the cell-wall gradually breaks down, thus liberating the vitelline granules. The enlarged nuclei remain intact, however, during the passage of the egg thru almost the whole length of the uterus.

The common vitelline duct varies in diameter from 30 to 75 $\mu$ , and is lined by an epithelium similar to that of the oviduct. It is largest immediately dorsal to the posterior end of the ovarian isthmus where it forms a vitelline reservoir, as in *C. laticeps*, as much as 220 $\mu$  in width by 45 $\mu$  in depth when filled with yolk. A little farther forward it receives two main tributaries, varying considerably in calibre according to the amount of vitelline material they contain. Whereas these two ducts collect chiefly from the follicles ahead of the uterus, at least one small tributary on each side drains the follicles situated in the posterior end of the worm, and unites with the main ducts near their point of union with each other.

Shortly after being joined by the common vitelline duct and as it courses a little farther back on one side or the other, the oviduct becomes surrounded by a poorly developed shell-gland. The ootype is consequently inconspicuous. Beyond the ootype the epithelium is syncytial in its nature since no distinct cell-boundaries appear. More than its inner half is deeply cleft to form pseudocilia, yet its nuclei are comparatively large. As the oviduct—now, more properly called the beginning of the uterus—continues backward in a dorsal position in the medulla, it gradually enlarges, according as it becomes filled with eggs, its wall becomes thinner and thinner, and the nuclei diminish in number, flatten out and eventually disappear. The latter takes place particularly after the organ turns in its course—just ahead of the posterior group of vitelline follicles—and starts forward towards the female genital atrium.

From a point just behind the level of the posterior border of the ovarian isthmus to its opening the uterus is surrounded by a voluminous mass of club-shaped, unicellular glands (Fig. 13), similar to those described for the species of *Caryophyllaeus* and closely resembling those described by the writer (1919) for *Cyathocephalus americanus* and *Bothrimonus intermedius*. As to the function of these cells no definite statements can be made as yet. Monticelli likened the similar cells in *C. tuba* to those to be seen along the uteri of many trematodes as well as of *Gyrocotyle urna* (Wagener), and called them

glutin-producing glands. Will described them in *C. laticeps*, and said that they were "fully identical" with those in *Diphyllobothrium latum*. He also incidentally mentioned that Saint-Remy (1890) looked upon them as a shell-gland. Schneider (1902) called them glandular cells in *C. fennicus*, while Skrjabin considered them to be shell-glands in *C. syrdarjensis*. In view of the fact that, as in the species of the subfamily Cyathocephalinae just mentioned, the shell-gland surrounding the ootype is poorly developed—alho it was clearly seen in this species to initiate the formation of the egg-shell—they may act as an accessory shell-gland. Even tho this whole region of the uterus is lined with a deeply cleft cuticula, numerous droplets of material were seen in the sections studied adhering to or lying among the pseudocilia as if they were secreted from the cells in question; and it is only in this portion of the uterus, not in the thin-walled proximal region, that the shells of the eggs are thickest. At any rate, since the uterus is provided with only a very few scattered circular muscles, excepting just before its opening, they cannot be myoblastic in their nature. Distally they diminish considerably in number, yet they are directly continuous with the myoblastic nuclei of the more numerous muscular fibres surrounding the terminal portion of the duct and the female atrium, which in turn are continuous with the subcuticular cells around the atrial opening. As stated above, the uterus opens into the female genital atrium ahead of and slightly to one side of the vagina. The atrium itself is from 0.20 to 0.30mm. in length by about 0.10mm. in diameter and lined with a very irregular and deeply cleft cuticula.

The mature fresh eggs, when examined in normal saline solution, were found to be ovoid in shape and from 54 to 66 $\mu$  in length by 38 to 48 $\mu$  in width. The shell is from 2 to 3 $\mu$  in thickness, and is provided at its larger end with a small button-like boss and at its smaller end with an operculum from 12 to 16 $\mu$  in diameter.

#### LIFE HISTORY

As regards the development and life-history of this species only a few statements can be made at present. Larvae as small as that shown in figure 15 were found in the stomach of the host, but, alho a thoro dissection of the food-contents, which consisted of larvae of *Chironomus* and *Simulium*, Ostracoda, Cladocera, "caddice-worms,"

dragon-fly nymphs and Mollusca, was made, their mode of entrance was not discovered. Possibly further search will show that some member of these groups of animals, if not a tubificid worm as in Europe, is the intermediate host. Finally, from the standpoint of the systematic position of the species it should be emphasized that the smallest larvae found had nothing whatsoever in the nature of appendages.

### SYSTEMATIC POSITION

From the above description it is clear that this species, altho a member of the family Caryophyllaeidae Lühe 1910, does not belong either to *Archigetes* or to *Caryophyllaeus*. As pointed out above, the scolex resembles that of at least one species of *Archigetes*, namely, *A. brachyurus* Mrázek, but is quite different from the simple, leaf-like anterior ends of the species of *Caryophyllaeus*. The reproductive organs, it is true, are much more comparable to those of the latter, but certain features of the muscular, excretory and nervous systems do not permit of its being placed in either genus. Consequently a new genus is erected to accommodate this form, and is given the following characters:

#### *Glaridacris* gen. nov.

With the characters of the family. Medium sized caryophyllaeids with the anterior end modified to form a scolex, provided on each surface with three suckers, of which the median one is the deepest and most efficacious. Main longitudinal parenchymatous muscles in eight large fasciculi in the anterior part of the neck and the base of the scolex. Only two main nerve strands in the medulla, connected in the scolex by two more or less diffuse commissural loops. Excretory vessels form a single cortical plexus with eight principal longitudinal channels; no true flame-cells present, terminal renal organs, peculiar, highly vacuolated, simple cells. Expansion of the vas deferens before entering the cirrus-sac to form a vesicula seminalis.  $\delta\lambda\alpha\rho\iota\varsigma$ , chisel;  $\acute{\alpha}\kappa\rho\iota\varsigma$ , summit.

Type, and as yet only, species: *G. catostomi* sp. nov.

The principal specific characters may be set down as follows:

#### *Glaridacris catostomi* sp. nov.

With the characters of the genus. Small cestodarians, up to 25mm. in length by 1.0mm. in breadth. Scolex, short and broad, chisel-shaped in older specimens, hexagonally pyramidal with prominent terminal disc in younger, base large in both; length, 0.30 to 0.45mm., width (posteriorly), 0.45 to 1.10mm., depth (posteriorly), 0.50 to

0.75mm. Neck only slightly narrower than body, 1.5 to 2.5mm. in length; whole worm, apart from scolex, cylindrical, with somewhat conical posterior end.

Cuticula, 7 to 11 $\mu$  in thickness; subcuticula, 90 to 100 $\mu$ . No "fibrous strands" nor calcareous bodies in parenchyma.

Female genital atrium, 0.5 to 1.0mm. behind opening of cirrus, 0.20 to 0.30mm. in depth by 0.10mm. in diameter, opening crescentic, in same depression with male opening.

Testes not completely surrounded by vitelline follicles; extend to cirrus-sac posteriorly; irregularly ellipsoidal in shape, from 0.10 to 0.18mm. in different diameters; 150 to 160 in number. Vas deferens, a loose somewhat triangular mass ahead of cirrus-sac, 0.28 to 0.36mm. in diameter. Vesicula seminalis, 0.30 by 0.06 to 0.09mm. Cirrus-sac large, almost spherical, occupying almost whole of medulla of region, 0.40 to 0.60mm. in diameter. Cirrus, 60 to 65 $\mu$  in diameter.

Vagina median, ventral, 30 to 55 $\mu$  in diameter. Ovary irregularly lobular, 0.8 to 0.9mm. in length, with nearly spherical isthmus, 0.4mm. in diameter. Oocapt, 20 by 25 $\mu$ . Vitelline follicles not completely surrounding the testes, 8 to 14 in transections, 0.20mm. in maximum diameter. Vitelline reservoir, the expanded common vitelline duct, 220 by 45 $\mu$ . Ootype inconspicuous. Uterus in two portions, a proximal, thin-walled, and a distal, extending from the posterior vitelline follicles to the opening and surrounded by a large number of unicellular glands; empties into female atrium slightly ahead of and to one side of vagina.

Eggs, ovoid, with small boss at larger end, 54 to 66 $\mu$  in length by 38 to 48 $\mu$  in width.

Habitat: In stomach and intestine of *Catostomus commersonii* (Lacépède).

Finally, Lühe's (1910) characterization of the family will have to be slightly emended to include this new species:

#### CARYOPHYLLAEIDAE Lühe 1910, e.p.

Monozootic pseudophyllidea with scolex unarmed; may or may not bear more or less well expressed sucking organs which are set off from the rest of the body by a neck-like constriction or are fused with the same without such. A caudal appendage bearing on its hinder end the hooks of the oncosphere may also be present in the sexually mature animal. Genital organs present only singly. Reproductive openings surficial, ventral, medial and near the posterior end. Testes, numerous, exclusively anterior to the ovary and the female genital ducts. Cirrus unarmed, ahead of the female sexual apertures; vagina and uterus open at the bottom of a common vestibule which resembles in its histological structure the shallow genital atrium and opens into it close behind the cirrus. Ovary two-winged, directly behind the genital opening. Vitelline follicles in the medulla, but peripheral to the testes and more or less completely surrounding them like a mantle; mostly ahead of the ovary, but a group also in the hinder end of the body, separated from the main mass by the ovary and the female genital ducts. Uterus a winding canal, without sack-like expansions. Eggs, operculate.

*College of Medicine,  
University of Illinois.*

## WORKS CITED

- COOPER, A. R.  
1919. North American Pseudophyllidean Cestodes From Fishes. Ill. Biol. Monogr., 4:289-541, 13 pls.
- FRAIPONT, J.  
1880. Recherches sur l'appareil excréteur des trematodes et des cestodes. Arch. Biol., 1:415-36, 2 pls.
- LÜHE, M.  
1910. Parasitische Plattwürmer. II Cestodes. Die Süßwasserfauna Deutschlands, Dr. Brauer, Berlin, Heft 18:1-153.
- MONTICELLI, F. S.  
1892. Appunti sui Cestodaria. Atti d. r. accad. sc. fis. mat. di Napoli, 5, ser. 2(6), 11 pp., 4 figs.
- MRÁZEK, A.  
1908. Ueber eine neue Art der Gattung *Archigeles*. Vorläufige Mittheilung. Centrbl. Bakt., Orig., 46:719-23.
- SAINT-REMY, G.  
1890. Recherches sur la structure des organes genitaux du *Caryophyllaeus mutabilis* Rud. Rev. biol. du nord de la France, Lille, 2:249-60, 1 fig.
- SCHNEIDER, G.  
1902. *Caryophyllaeus fennicus* n. sp. Arch. Naturgesch., 68J, 1:65-71, 82-98, 3 figs.
- SKRJABIN, K.  
1913. Fischparasiten aus Turkestan. I. Hirudinea et Cestodaria. Arch. Naturgesch., 79J, Abt. A, 2:2-10, 2 pls.
- STEUDENER, F.  
1877. Untersuchungen über den feineren Bau der Cestoden. Abhandl. naturf. Gesellsch., Halle, 13:277-316, pls. 28-31.
- WARD, H. B.  
1911. The Discovery of *Archigeles* in America, with a Discussion of its Structure and Affinities. Science, N.S., 33:272.
- WARD, H. B. and G. C. WHIPPLE,  
1918. Fresh-Water Biology. New York.
- WILL, H.  
1893. Anatomie von *Caryophyllaeus mutabilis* Rud. Ein Beitrag zur Kenntnis der Cestoden. Zeitschr. wiss. Zool., 56:1-39, 2 figs., 2 pls.
- WRIGHT, R. R. and A. B. MACALLUM,  
1887. *Sphyranura osleri*: a contribution to American helminthology. Journ. Morph., 1:1-48, 1 pl.

## EXPLANATIONS OF FIGURES

<i>co</i>	cirrus opening	<i>g</i>	glands
<i>cs</i>	cirrus-sac	<i>i</i>	isthmus of ovary
<i>ev</i>	excretory vessel	<i>lm</i>	longitudinal muscles
<i>fa</i>	female atrium	<i>n</i>	nerve(s)
		<i>ns</i>	nerve strand

<i>o</i>	ovary	<i>l</i>	testis
<i>olm</i>	outer longitudinal muscles	<i>u</i>	uterus
<i>rc</i>	renal cell	<i>v</i>	vagina
<i>sg</i>	shell-gland	<i>vf</i>	vitelline follicles
		<i>vs</i>	vesicula seminalis

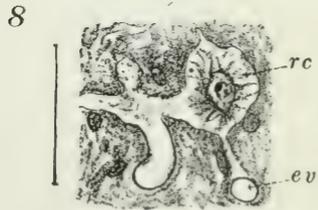
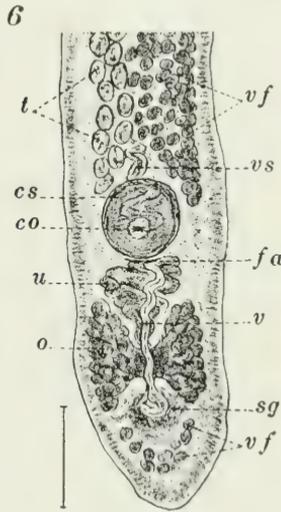
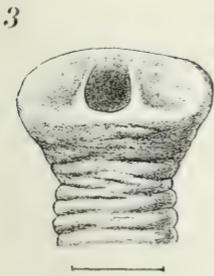
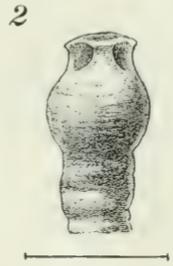
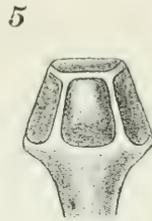
Unless otherwise stated, the lines indicating the magnifications of the figures are 0.5mm. in length.

## PLATE I

- Fig. 1. Surficial view of scolex of specimen 3.5mm. in length.  
 Fig. 2. Lateral view of same.  
 Fig. 3. Surficial view of scolex of specimen 21mm. in length.  
 Fig. 4. Lateral view of same.  
 Fig. 5. Scolex of *Archigetes brachyurus*, surficial view. After Mrázek.  
 Fig. 6. Genital organs in posterior end of worm, toto preparation, surficial view.  
 Fig. 7. Pits in the mucosa of the host's intestine, each showing only two of the several larvae found in them.  
 Fig. 8. A terminal renal cell and its connections, from a frontal section. The line at the side represents 0.05mm.

## PLATE II

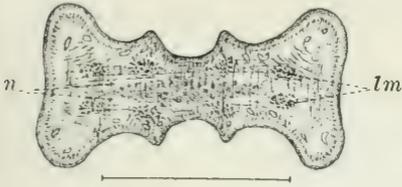
- Fig. 9. Transection thru the middle of the scolex.  
 Fig. 10. Transection thru the anterior part of the neck.  
 Fig. 11. Transection thru about the middle of the whole worm.  
 Fig. 12. Transection thru the cirrus-sac.  
 Fig. 13. Transection thru the ovarian isthmus.  
 Fig. 14. Three stages in the development of the vitelline cells: *a*, the primordial cell from the periphery of the follicle; *b*, an intermediate stage from the centre of the follicle; *c*, the mature cell from the vitelline reservoir. The line represents 0.02mm.  
 Fig. 15. The smallest larvae procured.



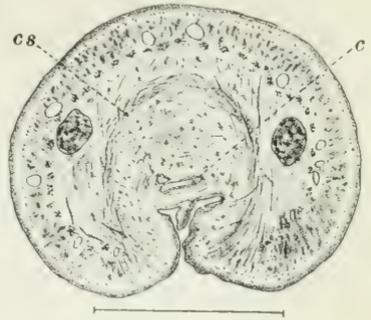


TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY  
VOL. XXXIX

9



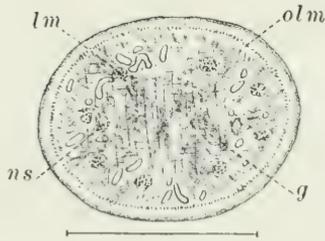
12



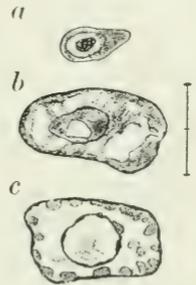
15



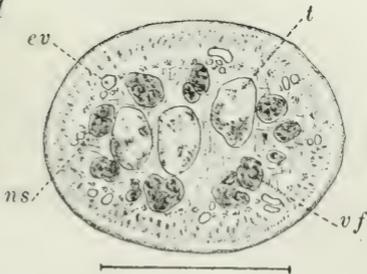
10



14



11



13

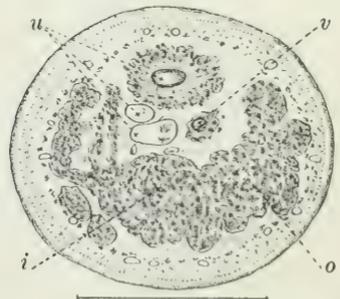


PLATE II

COOPER



# THE GENERA OF THE ENCHYTRAEIDAE (OLIGOCHAETA)<sup>1</sup>

BY PAUL S. WELCH

## INTRODUCTION

Michaelsen's monograph (1900) on the Oligochaeta contains the last general revision of the genera of the Enchytraeidae for the whole world. Eisen (1905) modified, to some extent, the genera then known to occur in North America. Since the publication of the above-mentioned works numerous contributions to the knowledge of these annelids have been made, so that the family has grown from a relatively small group containing 13 genera and less than 100 species to the present status of 16 genera and approximately 325 species. With this marked increase has come the necessity for certain changes and modifications in the limits of most of the groups.

The revision herein presented is the direct result of the discovery of certain enchytraeids which failed to agree exactly with any of the older generic descriptions and in order to properly assign them a careful survey of several genera was necessitated. It was then decided to extend the study to include all of the known genera of Enchytraeidae and thus not only make available a considerable amount of inaccessible material but also present something which will serve as a basis for further revision as soon as more data are secured. The writer wishes to acknowledge indebtedness to one of his graduate students, Miss Helen M. Scott, who gave considerable assistance in testing and rechecking the revised generic descriptions.

This revision can at best be regarded only as an attempt to indicate progress to date. Certain unsurmountable difficulties make it impossible at the present time to do more than work over critically the published records as they now stand and to determine the present status of each group as nearly as possible. The descriptions of all of the species now assigned to the Enchytraeidae (approximately 325) have been re-examined in this connection and the work of the writer on this group of annelids, covering a period of ten years, has been brought to bear upon the task wherever possible. The

<sup>1</sup> Contribution from the Zoological Laboratory of the University of Michigan.

principal difficulties are indicated below in order to point out some of the features which should receive attention in future investigations and revisions:

1. Descriptions based upon sexually immature specimens or upon material not so stated but apparently immature.

2. Species placed tentatively in certain genera but data insufficient to make final disposal of them.

3. Lack of information on morphological features now known to have important systematic value, especially in the older descriptions

4. Deviations which strongly have the appearance of being errors of observation or of printing.

5. Structures recorded as "not seen" or "not found" sometimes the result of faulty methods of study, such as external examination only or dissection only, or the use of poorly preserved specimens.

6. Difficulty in correct interpretation of certain descriptive terms, as for example, does "lobulated testes" mean *divided* testes as represented in *Lumbricillus* or something much less significant. In the absence of illustrations which supplement the descriptions, such expressions are very puzzling.

7. Interpretation of indefinite terms indicating differences of degree, as for example, "setae slightly curved," an expression which when unaccompanied by further explanation or by figures is practically unusable.

8. Difficulty arising from descriptions which fail to mention important organs. Does lack of mention always or ever mean positive absence of the structure? Apparently, many investigators have not realized the importance of stating *positively* that certain characters are absent. To leave these matters without mention is a distinct detriment.

In this revision, the generic descriptions have been so modified as to include what seems to be well founded changes demanded by increased data as well as new features now regarded as having generic value. In making these modifications the procedure has been as follows:

1. The writer concurs with those who hold that the multiplication of genera should be avoided except where the case is perfectly clear.

2. Genera and species founded upon sexually immature material have been disregarded. It is well established that complete, depend-

able data cannot be secured from immature specimens and it is to be hoped that future investigation will frown upon any disposition to write descriptions from such imperfect material. It can only lead to confusion and hinderance.

3. Data easily derivable from illustrations not supplemented by description were regarded as valid.

4. Lymphocytes and the brain have been omitted purposely from generic consideration since the writer doubts their value in generic distinction.

5. In certain cases, statements known to be true for some of the species of a genus have been incorporated in the generic description since they represent all that is known at present about the features mentioned. Re-examination of the other species will decide whether such features will remain valid.

6. When the usual generic characters are not mentioned in the descriptions, such omission is taken to mean that no information is available, rather than that they are absent.

7. There have been incorporated into the genus descriptions certain features which may prove later to be specific characters, as for example, when structures are indicated as "present and absent." This is done largely for the sake of record and to indicate divergences from the former descriptions. It remains for future investigation to make the final disposal.

8. The term *chylus cell* is used to indicate the large, intestinal cells each of which is characterized by a longitudinal, intracellular canal. In the region involved, chylus cells usually alternate with the ordinary epithelial cells which line the lumen of the intestine.

9. Eisen's system of classifying the various forms of penial bulb has been followed to considerable extent. There is increasing evidence that the features of the penial bulb have distinct generic value.

10. No attempt has been made to list more than the most important literature involved in making this review. A complete set of references and a statement of the synonymy up to 1900 is given in Michaelsen's monograph.

#### SUBFAMILIES

Some attempts have been made in the past to establish subfamilies in the Enchytraeidae. Eisen (1905, pp. 11-13) proposed

four subfamilies, *Mesenchytracinae*, *Enchytracinae*, *Achaetinae*, and *Lumbricillinae*, the major basis of distinction being the character of the penial bulb. However, since the structure of the penial bulb was not known for certain genera, the distribution of the genera among these subfamilies was to some extent inferential. Čejka (1910, p. 25) made use of three subfamilies under the names *Fridericiinae*, *Mesenchytracinae* and *Henleinae*.

There seem to be some good grounds for considering the structure of the penial bulb as a basis for the erection of subfamilies, but since its structure is unknown in such genera as *Achaeta*, *Distichopus*, *Chirodrilus*, and *Stercutus*, it does not seem profitable just now to attempt to discuss this problem.

#### THE PENIAL BULB

The first attempt to use the characters of the penial bulb in the classification of the Enchytraeidae was made by Eisen (1905, pp. 6-10) who, after an extensive study of a large number of North American species, thought it possible to recognize three distinct "types" which were definitely related to certain taxonomic groups. The writer (Welch, 1914, pp. 173-180) presented a critical discussion of this matter, pointing out that it seemed necessary to make some modifications in Eisen's original system. Since that time many more of the North American forms have been studied and while it is still probable that certain changes may ultimately be necessary, all of the evidence at hand indicates that the characters of the penial bulb are valuable in generic and possibly in specific diagnoses. For this reason, statements as to the penial bulb have been incorporated into revised definitions of the various genera, retaining Eisen's terms for the different types. If many of the species from the Old World can be re-examined and the structure of the penial bulb described and figured, the taxonomic status of this organ will be made more certain.

For sake of ready reference, Eisen's summary of the three types of penial bulbs will be quoted here.

"The Mesenchytræid bulb is a single muscular structure, containing circular muscles as well as fan-shaped muscular bands connecting the body wall with the periphery of the bulb. Between the muscular bands are generally found numerous penial glands which

open on the surface of the bulb around the penial pore. The sperm-duct penetrates the bulb, opening on the center of its outer surface.

The Enchytraeid bulb is multiple, consisting of several separate cushions grouped around the penial pore. In these cushions we find several sets or fascicles of glands, each fascicle opening by itself on the surface of the body. There are no muscular bands connecting the base of the cushions with its periphery. The sperm-duct never penetrates the bulbs or cushions but opens close to and independently of them. Exterior to the cushions there are numerous muscles connecting the body wall immediately surrounding the pore with other parts of the same somite.

The Lumbricillid bulb is always single and covered with a strong muscular layer, which however never penetrates down between the cells of the bulb. There are generally two or three distinct sets of glandular cells in the bulb. Some of these open in the lower part of the sperm-duct, or rather in a narrow groove in the elongation of the sperm duct. Others open on the free surface of the bulb, either irregularly or in narrow circular fields, bunched into fascicles. The sperm-duct penetrates one side of the bulb. In *Bryodrilus* the gland which opens in the extension of the sperm-duct is covered with a thin cushion of muscular strands, forming a bulb within a bulb."

#### RELATIONSHIPS

It is not intended that any particular significance be attached to the order in which the different genera are treated in this paper. Certain genera are too poorly known at present to justify any attempt to establish relationships, while others are little enough known to make it a difficult and an uncertain task. It thus seems best in this paper to omit efforts to determine phylogeny.

#### PROPAPPUS MICHAELSEN

Setae sigmoid; distal extremity cleft; those of a bundle equal; four bundles per somite, two lateral and two ventral. Dorsal pores absent. Oesophagus passing abruptly into intestine in 8; intestinal diverticula absent; chylus cells absent; peptonephridia absent. Origin of dorsal blood-vessel anteclitellar or intraclitellar. Nephridia with small, slender, funnel-shaped anteseptal part and with loose, scantily lobed, irregularly folded postseptal body, the folds being but

little more than in close contact. Testes undivided; moderately compact. Spermiducal funnel extremely short; shallow bowl-shaped. Sperm duct very short; confined to 12. Penial bulb absent; small atrial chamber at ectal end of sperm duct; atrial glands absent. Spermathecae simple; no diverticula; no connection with digestive tract; long, extending into 6-12.

#### DISCUSSION

Formerly, the genus *Henlea* (Michaelsen, 1903, p. 51) was regarded as the most primitive group of the Enchytraeidae because of the diverse character of the setae manifested by the various species although it presented no distinct transition features leading into the near-standing, more primitive families of Oligochaeta (Phreodrilidae, Tubificidae, Naididae). In 1905, however, Michaelsen (pp. 24-28) described under the name *Propappus* a genus based upon specimens found abundant in Lake Baikal, Southern Siberia, at depths of 2-8 meters. These specimens presented a complex of characters of particular interest. Most of the fundamental features are enchytraeid leaving little doubt as to its membership in that group. However, certain affinities with other families are manifested in the presence of the following structures:

1. Cleft setae are recorded for the first time among the Enchytraeidae, all other known species having the simple-pointed type. Cleft setae are common in Naididae, Tubificidae, and Lumbriculidae.

2. The spermiducal funnel is a very short, shallow, bowl-shaped organ, resembling the funnel in certain other oligochaetes (*Tubifex*, et al) and showing little resemblance to the elongate, cylindrical, glandular, thick-walled funnel found in practically all enchytraeids. Apparently, only two other enchytraeids have spermiducal funnels which at all resemble those of *Propappus*, namely, *Mesenchytraeus bungei* Mchlsn. and *Mesenchytraeus grebnizkyi* Mchlsn. (Michaelsen, 1901, pp. 193, 199), in which they are very short, and "pantoffel-förmig."

3. Each nephridium consists of a small, slender, funnel-shaped nephrostome which constitutes the entire anteseptal part. The postseptal part, however, departs strikingly in form and structure from the typical enchytraeid condition in being very loosely constructed, having the appearance of an irregular knot of adherent loops

or folds, the free end of which composes the efferent duct. This type of nephridium recalls the postseptal coils in the same organ in Tubificidae, et al. Of all the other enchytraeids, *Mesenchytraeus* alone shows any approach to such nephridial structure, although its irregular, lobed, postseptal part in which the wide ducts are close together is definitely coalesced into one mass.

Only two species are known at present to belong to this annectent genus, namely, *glandulosus* and *volki*. The former, found in Lake Baikal, in the one on which the genus was established. Recently, Michaelsen (in a paper dated 1915 but which must have been published in 1916 since papers dated 1916 are referred to in it) described a second species, *volki*. It appears that this same writer first reported it in the "Hamburger Nachrichten, Jahrg. 1916, Nr. 53, vom 30, Januar, 3. Beilage, p. 1," as *Palpenchytraeus volki*, n. gen., n. sp. but later placed it in *Propappus*—a decision which certainly seems more nearly correct. It is worthy of mention that in this species the elongated spermathecae recall the condition in many of the North American mesenchytraeids.

#### HENLEA MICHAELSEN

Setae straight and unequal in size, or straight and equal in size, or slightly sigmoid and approximately equal in size; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus (with possible rare exceptions) expanding abruptly into intestine. Peptonephridia present or absent. Intestinal diverticula usually present. Origin of dorsal blood-vessel anteclytellar; rarely intraclitellar; cardiac body absent. Blood colorless. Nephridia with either large or small anteseptal part; nephridial canal loosely wound and surrounded by considerable amount of cell mass. Ventral glands absent. Testes compact; not divided. Spermiducal funnel cylindrical; sperm duct short, confined to 12, rarely longer. Sperm sacs and ovisacs absent. Penial bulb of lumbricillid type. Spermathecae connecting with digestive tract; diverticula present or absent.

#### DISCUSSION

Perhaps no genus of Enchytraeidae needs a thorough going revision as badly as does *Henlea*. Its heterogeneous nature has been

recognized by investigators for some time but certain conditions surrounding the problem have thus far made such a revision almost impossible. It therefore presents many difficulties in connection with the present attempt to redefine the genus. Friend (1914b, pp. 150-153; 1915, pp. 197-198) has pointed out the existence of certain "groups" within this genus. The writer [Welch] suspects strongly that *Hepatogaster* Čejka should be regarded as a part of the genus *Henlea*—possibly as a subgenus. It seems likely that these "groups" will form the basis for the establishment of several subgenera when the genus is thoroughly worked over, particularly when many of the foreign species have been re-examined and more thoroughly described.

Certain deviations, apparent or otherwise, from the newly modified genus definition require some notice. Some ill-defined species (*lefroyi* Beddard; *scharfi* Southern; et al) seem to offer exceptional features, but the imperfect descriptions leave considerable doubt as to whether they belong in this genus at all. Hence no significance can be attached to them at present.

Eisen (1905, p. 98), in connection with his discussion of *Henlea*, presents the following statement: "Chylus cells in the intestine in the vicinity of clitellum." However, in his subsequent descriptions, no mention of them appears except in the case of *H. guatemalae* (pp. 102-103) which is described as having no chylus cells at all. In none of the American species of *Henlea* examined by the writer have chylus cells been observed.

A number of species have been described in which the origin of the dorsal blood-vessel is specified as intraclitellar and the definition has been modified so as to include these forms. However, Friend (1913b, pp. 460-461) has described a species under the name *insulac* which has the dorsal blood-vessel arising in "17/18 or 19/20." This form is assigned to *Henlea* but taking the original description as it stands, the writer is unable to place it with any more certainty in *Henlea* than in one or two other genera, as for example, *Enchytraeus*. For this reason, the apparent exception has not been given any particular consideration. *H. alba* (Friend, 1913c, p. 83) and *H. hillmani* (Friend, 1914b, p. 135) are reported as having the origin of the dorsal blood-vessel in the region of 13-14.

Of the sixty or more species now assigned to this genus, there are several which future investigations will certainly prove invalid.

### HEPATOASTER ČEJKA

Setae straight and equal; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present, dorsal and ventral. Intestinal diverticulum present, surrounding digestive tract. Chylus cells absent. Origin of dorsal blood-vessel antecleitar; cardiac body absent. Nephridia with small anteseptal part; nephridial duct loosely coiled and with distinct cell mass. Testes not divided. Spermiducal funnel cylindrical. Penial bulb of lumbricillid type. Spermathecae connecting with digestive tract; diverticula absent. Characteristic, longitudinal canals in epithelium of digestive tract in posterior part of body just entad of perivisceral blood-sinus.

### DISCUSSION

The genus *Hepatogaster* was established by Čejka (1910) for the reception of two species which he considered as presenting characters representing a new group. A careful examination of descriptions reveals at least a close affinity with *Henlea*. In fact, it could be included in *Henlea* with practically no change in the limitations of the latter. Only one feature seems to offer any difference, namely, that the oesophagus passes gradually into the intestine, but it seems doubtful if a new genus could be established upon that character alone. The presence of certain peculiar longitudinal canals in the epithelium of the posterior part of the alimentary canal is stressed in the original description and while these characters seem to be unique, their value as a generic character remains to be demonstrated.

The structure of the penial bulb requires some notice. Čejka thought that it resembled the enchytraeid type, interpreting certain peculiar glands which open out through the body-wall in 12 and 13 in the vicinity of the sperm duct termination as parts of the penial bulb proper. Unfortunately, the penial apparatus is recorded in only one of the two species. However, a careful study of the description and Čejka's plates leads the present writer to hold that the bulb is of the lumbricillid type for the following reasons: 1. The sperm duct opens

to the exterior through a compact, glandular bulb which is typically lumbricillid. This duct actually opens out through it into a penial invagination—a thing which does not occur in the typical enchytraeid bulb. (2) Of the nearby groups of problematical glands, the one in 12 is single and median, thus apparently belonging to neither bulb. (3) The other glands are in 13—another somite—a thing which has not been observed in connection with the various parts of a typical enchytraeid bulb. (4) In general appearance these peculiar glands resemble the “ventral glands” found in certain enchytraeids although they are unusual in being free from direct connections with the ventral nerve cord.

Owing to the incompleteness of some of the data on this proposed genus, it is allowed to stand for the present although there seem to be good reasons for believing that it should be reduced at least to the rank of a subgenus of *Henlea*.

#### BRYODRILUS UDE

Setae slightly or distinctly sigmoid; distal extremities simple-pointed; those of a bundle equal in size; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present. Four intestinal diverticula present. Origin of dorsal blood-vessel intracitellar; cardiac body present or absent. Nephridia with small anteseptal part; nephridial canal loosely wound; cell mass large. No ventral glands. Testes compact; not divided. Spermiducal funnel cylindrical; sperm duct confined to 12. Sperm sacs and ovisacs absent. Penial bulb of lumbricillid type. Spermathecae connecting with digestive tract; diverticula absent.

#### DISCUSSION

While a few slight modifications have been introduced into the description of this genus, no important comments are demanded here. No mention of intestinal diverticula appears in the description of *B. sulphureus* (Bretscher, 1904, p. 262) but since the material on which the description was based was immature, this omission may have no significance. The head pore in this same species is recorded as appearing on the tip of the prostomium.

Four species are assigned to this genus.

## BUCHHOLZIA MICHAELSEN

Setae sigmoid; distal extremities simple-pointed; those of a bundle approximately equal in size; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus expanding abruptly into intestine. Peptonephridia present. Chylus cells absent. Origin of dorsal blood-vessel anteclitellar or intraclitellar; arising from summit of dorsal intestinal diverticulum; cardiac body absent. Blood colorless. Nephridia with anteseptal part large or small. Spermiducal funnel cylindrical; sperm duct confined to 12. Structure of penial bulb unknown. Spermathecae connecting with digestive tract; diverticula absent.

## DISCUSSION

In *Buchholzia focale* (Friend, 1914a, pp. 118-119) no mention is made of a dorsal intestinal diverticulum and the origin of the dorsal blood-vessel is given as "Henlean."

But little is known concerning the penial bulb in representatives of this genus. Eisen (1905, p. 12) places the genus under his subfamily Lumbricillinae but explains (p. 6) that he does so on account of its "undoubted relationship to the genus *Henlea*."

*Buchholzia parva* (Bretscher, 1900a, p. 24) is described as showing no connection of the spermathecae with the digestive tract. However, the sexual maturity of the material might be questioned since it is stated that no trace of a clitellum was found.

Six species are now assigned to this genus.

## MARIONINA MICHAELSEN

Setae sigmoid; distal extremities simple-pointed; those of a bundle approximately equal in size; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel postclitellar; cardiac body absent. Blood red, yellow, or colorless. Nephridia with anteseptal part large or small; nephridial canal loosely wound; cell mass large. Ventral glands present or absent. Testes undivided. Spermiducal funnel cylindrical; sperm duct confined to 12. Sperm sacs present or absent. Penial bulb of the lumbric-

cillid type. Spermathecae with or without connection with digestive tract; never greatly elongate; diverticula present or absent.

#### DISCUSSION

In a few species, assuming that they are correctly referred to this genus, there seems to be some deviation as to the origin of the dorsal blood-vessel. Bretscher (1900b, p. 449; 1901, pp. 209-10) described *rivularis* and *guttulata* as having this origin anteclytellar, and Eisen (1905, p. 91) reported it in 12 in the single specimen of *alaskae* which he described although he retained the general generic character of a postclytellar origin (p. 90).

Friend (1912a, p. 224) has described a species, *sialona*, which he assigns to *Marionina*, pointing out at the same time that it is strikingly like an *Enchytraeus*. This species possesses peptonephridia—a feature not represented in *Marionina* and *sialona* is unique in that respect if it actually belongs in *Marionina*. However, the writer has been unable, on the basis of the original description, to see why that species should not be assigned to *Enchytraeus*, rather than to *Marionina*. If this be the proper disposal of *sialona*, then the absence of peptonephridia still stands as an invariable character of the genus.

Eisen (1905, p. 90) held that a generic character appears in the presence of a small sperm sac in connection with each testis. Whether this is true, remains to be determined by future investigations.

In *antipodum* (Benham 1904b, p. 294) the body of the penial bulb appears to be of the lumbricillid type, but it is unique in possessing a single, large accessory gland. Bretscher (1901, p. 210) recorded *guttulata* as "ohne Prostata," but the whole description is so brief that it is impossible to judge accurately as to the sexual maturity of the material studied, or as to the exact meaning of the above quoted statement.

*M. werthi* Mchlsn. (1908, p. 15) has a penial bulb which is described as "einen winzigen, zwiebelförmigen, ganz in der Leibeswand verborgenen Bulbus aus. An diesen Bulbus, der manchmal als winzige äussere Papille etwas heraustritt, sitzt eine schwach gelappte, in die Leibeshöhle hineinragende Prostata."

About twenty-eight species are referred to this genus.

## LUMBRICILLUS ÖRSTED

Setae sigmoid; distal extremities simple-pointed; those of a bundle approximately equal in size; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel postclitellar, rarely intraclitellar; cardiac body absent. Color of blood yellow, red, or colorless. Nephridia with anteseptal part either large or small; nephridial canal loosely wound, and considerable cell mass between the folds. Ventral glands present or absent. Testes divided deeply, forming a number of distinct lobes. Spermiducal funnel cylindrical; sperm duct long but confined chiefly to 12. Sperm sacs and ovisac absent. Penial bulb of lumbricillid type. Spermathecae connecting with digestive tract; diverticula absent.

## DISCUSSION

While the limits of this genus have been but little changed, certain variations may well be mentioned here. *L. viridis* (Stephenson, 1911, p. 48) has the dorsal blood-vessel arising in 13 and in *tuba* (p. 43) it arises in 13, 14, 15. A variety (?) of *minutus* (Müll.) described by Michaelsen (1911, pp. 1-4) has this vessel arising in 12. *Lineatus* (Müll.) (= *agilis* Moore) has also been described by some authors as having the dorsal vessel arising in 13.

Ventral glands do not appear in all representatives of *Lumbricillus*. Furthermore, they are said to occur in a few species of certain other genera (Welch, 1914, p. 141).

Distinct sperm sacs and ovisacs appear to be absent in this genus. A few references to very diminutive ovisacs restricted to the clitellar region occur in the literature (Eisen, 1905, p. 77; Moore, 1905, p. 397) but these can scarcely be regarded as having any special significance. Eisen (1905, pp. 75-76) stated that each division of the testes is capped by a small sperm sac and evidently regarded this as a generic character.

Stephenson (1911) has pointed out the close relation of *Lumbricillus* to *Enchytraeus* on the basis of the discovery of certain species which though assigned to the former, possess some characters strongly suggestive of the latter.

About thirty species are assigned to this genus at present, although there is reason for doubting the validity of some of them.

#### FRIDERICIA MICHAELSEN

Setae straight or nearly so; unequal, those in bundle developed in pairs, outer pair being largest, and enclosing smaller pairs; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores present. Oesophagus merging gradually into intestine. Peptonphridia present. Intestinal diverticula absent. Chylus cells present. Origin of dorsal blood-vessel postclitellar or intraclitellar, usually the former; cardiac body absent. Blood colorless. Nephridia usually with large anteseptal part, always consisting of more than nephrostome; cell mass well developed. Ventral glands usually absent. Testes not divided. Spermiducal funnel cylindrical; sperm duct short, usually confined to 12. Sperm sacs and ovisacs absent. Penial bulb of lumbricillid type. Spermathecae usually connecting with digestive tract; diverticula present or absent.

#### DISCUSSION

While it seems advisable to make but little modification in the description of *Fridericia*, a few variations as recorded in the literature demand notice here.

*F. tusca* and *F. valdarnensis* are described by Dequal (1914, pp. 15, 17) as having setae which are sigmoid but those of a bundle are of unequal length, the inner ones being shorter. The description is very meager but it appears that even though they be sigmoid, they still resemble the typical *Fridericia* arrangement and development. Stephenson (1915, p. 47) describes the setae of *F. carmichaëli* as being of the "*Enchytraeus type*" but it may be that since in this species there are usually only two setae per bundle and since the outer setae of a *Fridericia* bundle are straight and approximately the same size, this statement could be true, although if more were present per bundle the inner ones might be shorter, smaller, and arranged in pairs. Friend (1912b, p. 24) states that the head pore in *F. anglica* occurs on the tip of the prostomium. In *Fridericia peruviana*, Friend (1911, pp. 734-736) described the oesophagus as passing abruptly into the intestine, but since the specimens on which the description was based

were immature, it seems best to attach no particular significance to this case. According to Southern (1909, p. 165) *F. magna* Friend has bright red blood. Friend (1899, p. 263) stated that in *F. magna* an ovisac is present, extending caudad to 16.

At present about 90 species are assigned to *Fridericia* and while it is very possible that some of them are not valid, it appears that this is the largest of all the enchytraeid genera.

#### DISTICHOPUS LEIDY

Setae in two bundles per somite, representing the ventral rows only; nearly straight; simple-pointed but blunt; very stout and swollen in middle; hooked at proximal end. Head pore at 0/1. Oesophagus merging gradually into intestine. Peptonephridia present. Origin of dorsal blood-vessel postclitellar; small cardiac body present. Blood colorless. Nephridia with small anteseptal part. Spermiducal funnel cylindrical; sperm duct short, confined to 12. Penial bulb of lumbricillid type. Spermathecae not described.

#### DISCUSSION

The genus *Distichopus* is known only from a single set of specimens collected in Delaware and Pennsylvania by Leidy (1882, pp. 146-147). Some of these specimens were later studied by Moore (1895, pp. 754-756) who extended the account, although even yet too little is known concerning this unusual form. Certain important structural features, such as the spermathecae, are yet undescribed and thus the relationships of this genus are difficult to determine. Moore holds that it is a close ally of *Fridericia*. The single known species bears the name *silvestris*.

#### ACHAETA VEJDOVSKY

Setae entirely absent; dorsal and ventral rows preserved in some species only as pear-shaped gland cells in body-wall, gland cells also absent in other species. Head pore large; on tip of prostomium. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present or absent. Origin of dorsal blood-vessel antecitellar. Blood colorless. Nephridia with moderate or large anteseptal part. Spermiducal funnel cylindrical; sperm duct short or long but confined to region of clitellum. Penial bulb present; struc-

ture practically unknown; probably of lumbricillid type. Spermathecae with or without connection with digestive tract; diverticula absent.

#### DISCUSSION

*Achaeta* is a small genus to which is assigned, at the present time, eight species, none of which occur in the Western Hemisphere. Its most striking characteristic is the total absence of setae. In most of the species, specialized, pear-shaped seta-glands occur in the positions where setae would be expected, although three species, *vejdovskyi* Bretscher (1902, p. 27), *maorica* Benham (1904a, pp. 221-223) and *camerani* (Cognetti 1899, pp. 1-4) are described as being completely devoid of seta-glands. Peptonephridia have not been found in *minima* Southern (1907, p. 77) and *incisa* Friend (1914b, pp. 133-134) but occur in the other known species. The penial bulb is practically unknown for this group since in no case is it described in adequate detail. It was figured by Vejdovsky (1879, pl. I, fig. 11) for *eisenii* but even there it is difficult to determine its exact composition. It suggests the lumbricillid type of bulb. In *maorica* Benham (1904a, p. 222) the spermathecae are greatly elongated, extending to 9 or 10, thus recalling the greatly elongated spermathecae of some of American species of *Mesenchytraeus*.

#### ENCHYTRAEUS HENLE

Setae straight; those of a bundle equal; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Head pore at 0/1. Dorsal pores absent; Oesophagus merging gradually into the intestine. Peptonephridia present or absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel postclitellar or intraclitellar; cardiac body absent. Blood usually colorless. Nephridia with small anteseptal part; cell mass well developed. Ventral glands sometimes present. Testes not divided. Spermiducal funnel cylindrical; Sperm duct confined to 12, or quite long, extending caudad through several somites. Sperm sacs present or absent. Penial bulb of enchytraeid type. Spermathecae connecting with digestive tract; diverticula present or absent.

## DISCUSSION

While it has been necessary to modify the older definition of the genus, only a few points require mention here. *E. dubius* (Stephenson, 1911, p. 56) is unique in possessing testes which are divided very much as is the case in representatives of the genus *Lumbricillus*. However, Stephenson himself (1915, p. 43) indicates that there is some doubt as to the generic position of this species. The same writer (1915, pp. 43-44) gives a critical discussion of sperm sacs in the genus *Enchytraeus* but makes no attempt to draw a general conclusion. Since the matter of sperm sacs is still in doubt, it seems best, in this paper, to use the data directly from the original descriptions and consider the statements of absence of sperm sacs as valid until they are definitely shown to be in error. The writer (Welch, 1914, pp. 177-178) previously discussed the penial bulb as a generic character and pointed out that not all of the species included in *Enchytraeus* conform to the enchytraeid type of bulb. However, it appears at this time that in the cases in which the penial bulb has been adequately described the large majority have bulbs of the enchytraeid type as proposed by Eisen and may so be incorporated into a revised statement of the limits of the genus, at least until subsequent investigation yields more complete data.

Eisen (1905, p. 61), in his generic description, states that the intestine generally possesses chylus cells. However, no mention of these cells is made later in his descriptions of species.

A genus, *Litorea*, described by Čejka (1913) for the reception of a species which he called *krumbachi*, is certainly the same as *Enchytraeus* and is so treated in this paper.

About thirty-five species are considered as belonging to this genus at the present time.

## MICHAELSENA UDE

Setae straight; distal extremity simple-pointed; one to two setae per bundle, often but one; four bundles per somite, two lateral and two ventral; present only on some of the somites (except in *M. mangeri* Mchln.). Head pore at 0/1. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present or absent. Intestinal diverticula absent. Origin of dorsal blood-vessel post-clitellar; cardiac body absent. Blood colorless. Nephridia with

small anteseptal part, consisting of nephrostome only. Ventral glands absent. Testes not divided. Spermiducal funnel cylindrical; sperm duct confined to 12, or long and extending caudad to 14. Penial bulb of the enchytraeid type. Spermathecae connecting with digestive tract; diverticula absent.

#### DISCUSSION

Formerly the absence of setae from some or the majority of the somites was regarded as one of the chief distinguishing features of this genus but Michaelsen (1914, pp. 177-181) described a new species under the name *mangeri* in which setae are present in four bundles on all of the somites. This species may be regarded as a connecting form between *Michaelsena* and *Enchytraeus* and at the same time, according to Michaelsen, emphasizes the relation of *Michaelsena* to *Fridericia*.

Southern (1913, pp. 8-12) described under the name *Grania* what he regarded as a new genus, pointing out similarities with a form then known under the name of *Enchytraeus monochaetus* Mchlsn. The latter is now known to belong to *Michaelsena* and Michaelsen (1914, p. 181) seems to be right in placing *Grania* there also.

Eisen (1905, p. 73) incorporated in his definition of this genus the statement that there are "No penial bulbs," but this seemed to be based upon the condition which he found in a single specimen to which he gave the name *paucispina* and which did not permit a full description. However, the descriptions of species now assigned to *Michaelsena* indicate that it may be of the enchytraeid type. Eisen (1905, p. 11) placed the genus in his subfamily *Enchytraeinae*.

It seems to be becoming increasingly difficult to separate *Michaelsena* from *Enchytraeus* and it is possible that some future revision based upon more intensive study of all of the species involved may suggest the fusion of the two groups.

Eight species are assigned to this genus at present.

#### MESENCHYTRAEUS EISEN

Setae sigmoid; distal extremities simple-pointed; approximately equal in size in bundle; four bundles per somite, two lateral and two ventral. Head pore distinct; usually at or very near tip of prostomium. Dorsal pores absent. Oesophagus merging gradually into

intestine. Peptonephridia absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel postclitellar; cardiac body present. Blood either colorless or red. Nephridia with small anteseptal portion, consisting merely of nephrostome; post-septal part large, irregularly pluri-lobed, and with cell mass between folds of closely wound nephridial canal greatly reduced. Ventral glands absent. Testes compact; undivided. Spermiducal funnel usually cylindrical; sperm duct short and confined to 12, or very long extending caudad for many somites. Sperm sacs and an ovisac often present. Penial bulb of mesenchytraeid type. Spermathecae confined to 5, or elongated and extending caudad for varying distances, sometimes to clitellum; diverticula present or absent; communication with digestive tract present or absent.

#### DISCUSSION

Eisen (1905, p. 14) stated that a "single median ovisac" and "one pair of sperm-sacs generally of large size" are present in *Mesenchytraeus*. However, *Mes. altus* Welch (1917, p. 71), which unquestionably belongs to this genus, has a pair of ovisacs and it seems possible that other cases of that sort will appear.

Several special cases which depart somewhat from the definition as proposed require mention here. In *eastwoodi* Eisen (1905, p. 50) the head pore occurs on the upper side of the prostomium near 0/1. *Mes. mencli* Vejd. (1905, p. 5) is described as having "Herz im 12," apparently referring to an intraclitellar origin of the dorsal blood-vessel. A similar origin seems true of *celticus* Southern (1909, p. 155), although the statement is not made positively. Bretscher (1902, p. 16) claimed that specimens of *selosus* Mchlsn. (= *megachaetus* Bret.) show the dorsal blood-vessel arising in 11, 13, or 16 — which seems an unusual variation. *Mes. grandis* Eisen (1905, p. 44) is described as having nephridia with broad anteseptal parts. In *orcae*, *mirabilis*, and *kincaidi* (Eisen, 1905, pp. 40–41), the testes are recorded as composed of "lobes" but it is not clear whether they are deeply divided as in the case of *Lumbricillus* or are merely lobulate at the free extremity. Bretscher (1901, p. 212) states that in *alpinus* the spermiducal funnel is in 8, but nothing is given concerning the sperm duct. In *nanus* (Eisen, 1905, pp. 51–52), the penial bulb is described

as absent, but there seems to be some possibility that immature specimens were studied.

This genus contains at the present time about 50 species.

#### HYDRENCHYTRAEUS BRETSCHER

Setae sigmoid; distal extremities simple-pointed; four bundles per somite, two lateral and two ventral. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia present. Origin of dorsal blood-vessel postclitellar. Blood yellow or red. Nephridia with large or small anteseptal part. Spermiducal funnel cylindrical. Spermathecae without diverticula.

#### DISCUSSION

This genus was established by Bretscher (1901, pp. 208-209) for two incompletely described species, *stebleri* and *nematoides*, found in Switzerland. Its status is somewhat uncertain owing to the fact that information on the head pore, relation of oesophagus to intestine, chylus cells, cardiac body, ventral glands, testes, sperm sacs, ovisacs, penial bulb, and relation of spermathecae to the digestive tract is entirely lacking. Likewise certain other features are incompletely described. Since the original description is the only record, nothing further can be done with these forms until specimens are again found and studied critically. The fragmentary information which is available seems to indicate that it is a valid genus.

#### STERCUTUS MICHAELSEN

Setae sigmoid; distal extremity simple-pointed; four bundles per somite, two lateral and two ventral. Head pore absent (apparently) or very small. Dorsal pores absent. Oesophagus merging gradually into intestine. Peptonephridia absent. Intestinal diverticula absent. Chylus cells absent. Origin of dorsal blood-vessel anteclitellar; cardiac body present. Blood colorless. Nephridia with small anteseptal part consisting of but little more than a mere nephrostome; postseptal part large; nephridial canal loosely wound and surrounded by considerable cell mass. Testes small; undivided. Spermiducal funnel short and distinctly funnel-shaped. Penial bulb unknown. Spermathecae not connected with digestive tract; diverticula absent.

## DISCUSSION

The genus *Stercutus* was established by Michaelsen (1888) to receive a species, *niveus*, which was found inhabiting fish excrement in Germany. No other representatives of this genus have ever been described. Lack of information as to the character of the penial bulb, the sperm duct, and the presence or absence of ventral glands, ovisacs and sperm sacs gives some difficulty in determining the affinities of this genus.

## CHIRODRILUS VERRILL

Setae in six bundles per somite, two sub-dorsal, two lateral, and two ventral; those in ventral and lateral bundles distinctly sigmoid, those in sub-dorsal less curved; distal extremities simple-pointed. Blood colorless.

## DISCUSSION

The description of this interesting genus is extremely meager and based entirely upon the original record by Smith and Verrill (1871, pp. 450-451) in which but few of the important details are described. Two species are assigned to this genus, *larviformis* and *abyssorum*, both collected in Lake Superior. Both are apparently deep water forms, *larviformis* being dredged from depths of 17 and 59 fathoms, and *abyssorum* from 47 and 159 fathoms. There is some question as to the position of this genus, certain previous writers having regarded it as a tubificid. Beddard (1895, p. 314) and Michaelsen (1900, p. 88) have classed it among the Enchytraeidae, although the latter (1903, p. 50) later placed it among the Tubificidae. Eisen (1905, p. 13) retains it in the Enchytraeidae. It is unlikely that the matter can receive any positive decision until material is again secured and carefully studied. Since some of the characters as described are apparently enchytraeid in nature, it is included in this review, with the realization, however, that future investigation may show it to have other affinities. If it be an enchytraeid, it is unique for the entire family in possessing six sets of setae per somite. Eisen (1905, p. 13) places it under the Lumbricillinae, but states (p. 6) that it is "appended for convenience sake" and points out correctly that nothing is known concerning the penial bulb and other internal structures.

### GENERA DUBIA

Under the name *Chamaedrillus*, Friend (1913a, pp. 260-263) described a new genus from material collected in England. Considering the generic characters as recognized at present for the family Enchytraeidae, a careful comparison has made it impossible for the writer to distinguish *Chamaedrillus* from *Marionina* and in this paper they are regarded as the same.

Bretscher (1905) described what he regarded as a new genus of Enchytraeidae under the name of *Euenchytraeus* but unfortunately the record was made on sexually immature material and as a consequence nothing could be determined as to the nature of the reproductive organs. Since no further record of this postulated genus occurs in the literature and since the original description is unusable as it stands, it is omitted from consideration in this paper.

#### KEY FOR THE IDENTIFICATION OF THE GENERA OF ENCHYTRAEIDAE

- 1 ( 2) Setae entirely absent; represented in most species only by four longitudinal rows of pear-shaped glands in body-wall  
.....*Achaeta*
- 2 ( 1) Setae present.....3
- 3 ( 4) Setae arranged in two bundles per somite.....*Distichopus*
- 4 ( 3) Setae arranged in more than two bundles per somite.....5
- 5 ( 6) Setae arranged in six bundles per somite, two subdorsal, two lateral, and two ventral.....*Chirodrillus*
- 6 ( 5) Setae arranged in four bundles per somite, two lateral and two ventral.....7
- 7 ( 8) Setae cleft at distal extremities; spermiducal funnel wide, open, shallow, and extremely short; postseptal part of nephridia composed of a few coherent folds not intimately fused, forming very loose organ.....*Propappus*
- 8 ( 7) Setae simple-pointed at distal extremities; spermiducal funnel cylindrical or trumpet-shaped; postseptal part of nephridia compact.....9
- 9 (10) Setae straight, arranged in pairs, inner pairs of bundle successively smaller than outer; dorsal pores present; chylus cells present in walls of intestine.....*Fridericia*

- 10 (9) Setae straight, sigmoid, or in pairs in bundle with smaller ones within; dorsal pores absent; chylus cells absent from walls of intestine . . . . . 11
- 11 (14) Oesophagus expanding abruptly into intestine . . . . . 12
- 12 (13) Dorsal blood-vessel arising from anterior end of single, dorsal intestinal diverticulum . . . . . *Buchholzia*
- 13 (12) Dorsal blood-vessel arising directly from perivisceral blood-sinus . . . . . *Henlea*\*
- 14 (11) Oesophagus merging gradually into intestine . . . . . 15
- 15 (16) Setae usually absent from several somites (except in *Michael-sena mangeri* Mchlsn.); usually one setae per bundle, never more than two . . . . . *Michael-sena*
- 16 (15) Setae regularly present on all somites except first and last and possibly the clitellar . . . . . 17
- 17 (20) Intestinal diverticula present . . . . . 18
- 18 (19) Setae sigmoid; four distinct intestinal diverticula; origin of dorsal blood-vessel intraclitellar . . . . . *Bryodrilus*
- 19 (18) Setae straight; one intestinal diverticulum completely surrounding digestive tract; origin of dorsal blood-vessel ante-clitellar . . . . . *Hepatogaster*
- 20 (17) Intestinal diverticula absent . . . . . 21
- 21 (22) Setae straight; those of a bundle equal . . . . . *Enchytraeus*
- 22 (21) Setae sigmoid . . . . . 23
- 23 (24) Peptonephridia present . . . . . *Hydrenchytraeus*
- 24 (23) Peptonephridia absent . . . . . 25
- 25 (26) Origin of dorsal blood-vessel anteclitellar; spermiducal funnel short and trumpet-shaped . . . . . *Stercutus*
- 26 (25) Origin of dorsal blood-vessel postclitellar; spermiducal funnel cylindrical . . . . . 27
- 27 (28) Testes divided . . . . . *Lumbricillu*
- 28 (27) Testes solid, not divided . . . . . 29
- 29 (30) Cardiac body absent; penial bulb of lumbricillid type; nephridial duct loosely coiled and cell mass of postseptal part well developed; spermathecae never extending through several somites . . . . . *Marionina*

\* A few species, e.g., *hillmani* Fr., *insulae* Fr., *marina* Fr., *alba* Fr., and three or four uncertain forms, have been assigned to *Henlea*, although they are described as having the oesophagus pass gradually into the intestine.

- 30 (29) Cardiac body present; penial bulb of mesenchytraeid type; nephridial duct closely wound and cell mass reduced to minimum. . . . . *Mesenchytraeus*

## LITERATURE CITED

- BEDDARD, F. E.  
1895. A Monograph of the Order of Oligochaeta. 769 pp., 5 pl. 52 fig. Oxford.
- BENHAM, W. B.  
1904a. Some new species of Aquatic Oligochaeta from New Zealand. Proc. Zool. Soc. London, 1903 (Vol. II), pp. 202-232. 3 pl. 1 fig.  
1904b. On the Oligochaeta from the Southern Islands of the New Zealand Region. Trans. New Zeal. Inst., 37:285-297, 3 pl.
- BRETSCHER, K.  
1900a. Mitteilungen über die Oligochaetenfauna der Schweiz. Rev. Suisse Zool., 8: 1-44. 3 pl.  
1900b. Südschweizerische Oligochæten. Rev. Suisse Zool., 8: 435-458. 1 pl.  
1901. Beobachtungen über Oligochæten der Schweiz. Rev. Suisse Zool., 9:189-223. 1 pl.  
1902. Beobachtungen über die Oligochæten der Schweiz. Rev. Suisse Zool., 10:1-29. 4 fig.  
1904. Beobachtungen über die Oligochæten der Schweiz. Rev. Suisse Zool., 12:259-267.  
1905. Über ein neues Enchytraeidengenus. Zool. Anz., 29:672-674.
- ČEJKA, B.  
1910. Die Oligochaeten der Russischen in den Jahren 1900-1903 unternommenen Nordpolarexpedition. I. Ueber eine neue Gattung der Enchytraeiden, Hepatogaster. Mem. Acad. Imp. Sci. St.-Petersbourg, (8), 29: No. 2, 29 pp. 3 pl.  
1913. *Litorea krumbachi* n. spec. n. gen.—Ein Beitrag zur Systematik der Enchytraeiden. Zool. Anz., 42:145-151. 10 fig.
- COGNETTI, L.  
1899. Descrizione dell' *Anachaeta camerani* nuova specie della famiglia degli Enchitreidi. Boll. Mus. Zool. Anat. Torino, 14, Nr. 354, pp. 1-4.
- DEQUAL, L.  
1914. Gli Enchitreidi della Toscana. Monit. Zool. Ital., 25:13-24. 7 fig.
- EISEN, G.  
1905. Enchytraeidae of the West Coast of North America. Harriman Alaska Expedition, 12:1-166. 20 pl. New York.
- FRIEND, H.  
1899. New British Annelids. Zoologist, (4), 3:262-265.  
1911. New British Enchytraeids. Journ. R. Micr. Soc., pp. 730-736. 1 pl.  
1912a. New British Oligochaets. Zoologist, (4), 16:220-226.  
1912b. British Enchytraeids. III. The Genus *Fridericia*. Journ. R. Micr. Soc., pp. 9-27.

- 1913a. British Enchytraeids. V. Species New to Science. Journ. R. Micr. Soc., pp. 255-271. 35 fig.
- 1913b. Some Jersey Oligochaets. Zoologist, (4), 17:456-464.
- 1913c. A Key to British Henleas. Zoologist, (4), 17:81-91.
- 1914a. Rare and Unique Sussex Oligochaets. Hastings and East Sussex Naturalist, 2:114-123.
- 1914b. British Enchytræids. VI. New Species and Revised List. Journ. R. Micr. Soc., pp. 128-154. 5 fig.
1915. Studies in Enchytraeid Worms. *Henlea fragilis* Friend. Ann. Appl. Biol., 2:195-208. 6 pl.

## LEIDY, J.

1882. On *Enchytraeus*, *Distichopus*, and their Parasites. Proc. Acad. Nat. Sci. Phil., pp. 145-148.

## MICHAELSEN, W.

1888. Beiträge zur Kenntniss der deutschen Enchytraeciden-Fauna. Arch. f. mikr. Anat., 31:483-498. 1 pl.
1900. Oligochaeta. Das Tierreich, 10 Lief. XXXIX+575 pp. 13 fig. Berlin.
1901. Oligochaeten der Zoologischen Museen zu St. Petersburg und Kiew. Bull. L'Acad. Imp. Sci. St. Petersburg, (5), 15:137-215.
1903. Die geographische Verbreitung der Oligochaeten. 186 pp. 11 pl. Berlin.
1905. Die Oligochaeten des Baikal-Sees. Wissenschaftliche Ergebnisse einer Zoologischen Expedition nach dem Baikal-See unter Leitung des Professors Alexis Korotneff in den Jahren 1900-1902. Erste Lief. 68 pp. 9 fig.
1908. Die Oligochaeten der Deutschen Südpolar-expedition 1901-1903. Deutsche Südpolar-expedition 1901-1903, 9:1-58. 1 pl.
1911. Litorale Oligochäten von der Nordküste Russlands. Travaux de la Soc. Imp. Nat. St. Petersburg, 42: 1-6. 2 fig.
1914. Beiträge zur Kenntniss der Land- und Süßwasserfauna Deutsch-Südwestafrikas. Ergeb. Hamb. deutsch-südwestafrikanischen Studienreise 1911. Oligochæta, pp. 139-182. 1 pl.
1915. Ein eigentümlicher neuer Enchytræide der Gattung *Propappus* aus der Niederelbe. Verh. nat. Ver. Hamburg, (3), 23:51-53.

## MOORE, J. P.

1895. The Characters of the Enchytraeid Genus *Distichopus*. Am. Nat., 29:754-756.
1905. Some Marine Oligochaeta of New England. Proc. Acad. Nat. Sci. Phil., pp. 373-399. 2 pl.

## SMITH, S. I. and VERRILL, A. E.

1871. Notice of the Invertebrata dredged in Lake Superior in 1871, by the U. S. Lake Survey, under the direction of Gen. C. B. Comstock, S. I. Smith, naturalist. Am. Journ. Sci. Arts, (3), 2:448-454.

## SOUTHERN, R.

1907. Oligochæta of Lambay. Irish Nat., 16:68-82. 2 pl.
1909. Contributions towards a Monograph of the British and Irish Oligochæta. Proc. R. Irish Acad., 27:119-182. 5 pl.

1913. Oligochaeta. Clare Island Survey, part 48. Proc. R. Irish Acad., Vol. 31, 14 pp. 1 pl. 1 fig.
- STEPHENSON, J.
1911. On some Littoral Oligochaeta of the Clyde. Trans. R. Soc. Edinburgh, 48:31-65. 1 pl. 14 fig.
1915. On some Indian Oligochaeta mainly from Southern India and Ceylon. Mem. Ind. Mus., 6:35-108. 4 pl. 2 fig.
- VEJDOVSKY, F.
1879. Beiträge zur Vergleichenden Morphologie der Anneliden. I. Monographie der Enchytraeiden. 14 pl. Prag.
1905. Ueber die Nephridien von Aeolosoma und Mesenchytraeus. Sitz. Gesell. Wiss., Math.-Naturw. Classe, pp. 1-11. 1 pl.
- WELCH, P. S.
1914. Studies on the Enchytraeidae of North America. Bull. Ill. State Lab. Nat. Hist., 10:123-212. 5 pl.
1917. Enchytraeidae (Oligochaeta) from the Rocky Mountain Region. Trans. Am. Micr. Soc., 36:67-81.

R 21

T  
31  
N

T  
30  
N

T  
29  
N

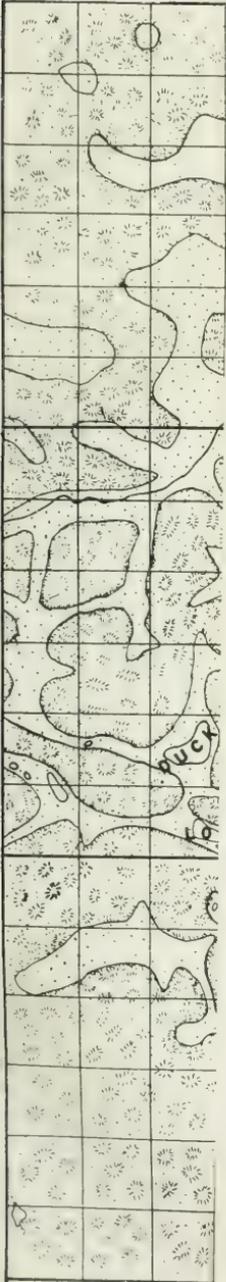
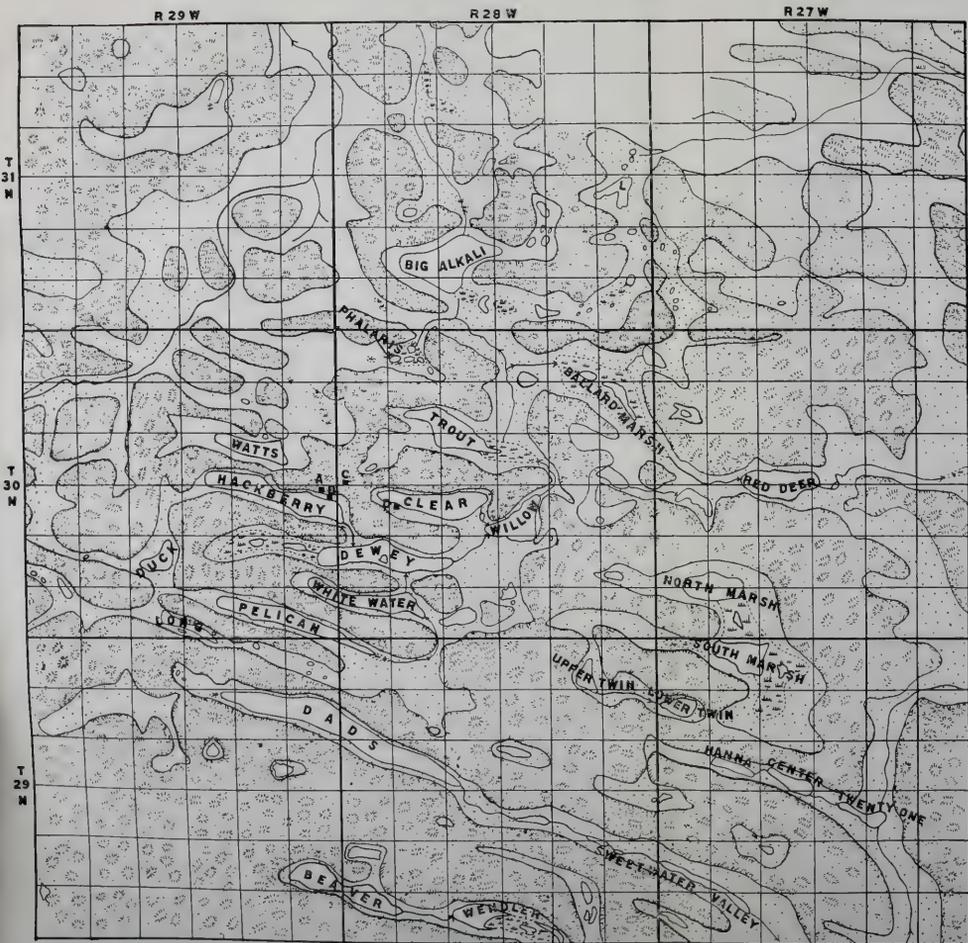


PLATE III



508



PLATE IV

ANDERSEN AND WALKER





PLATE V

ANDERSEN AND WALKER



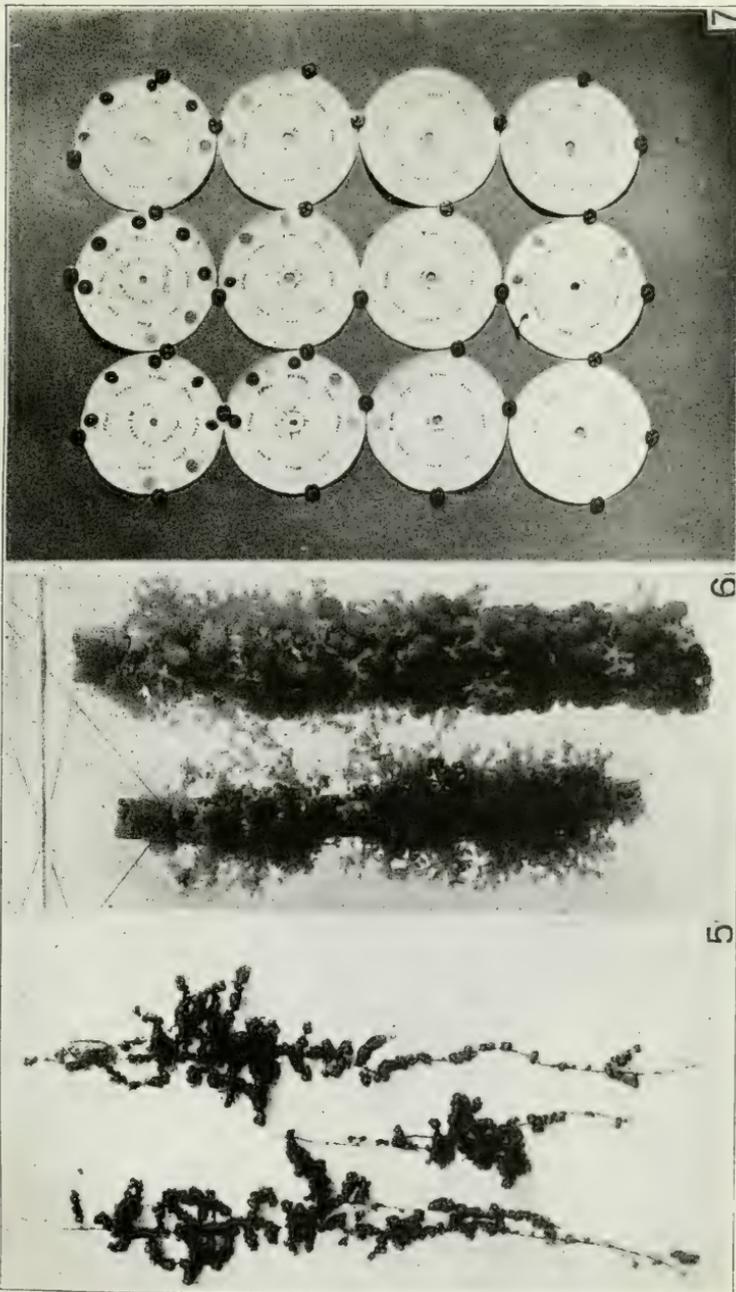
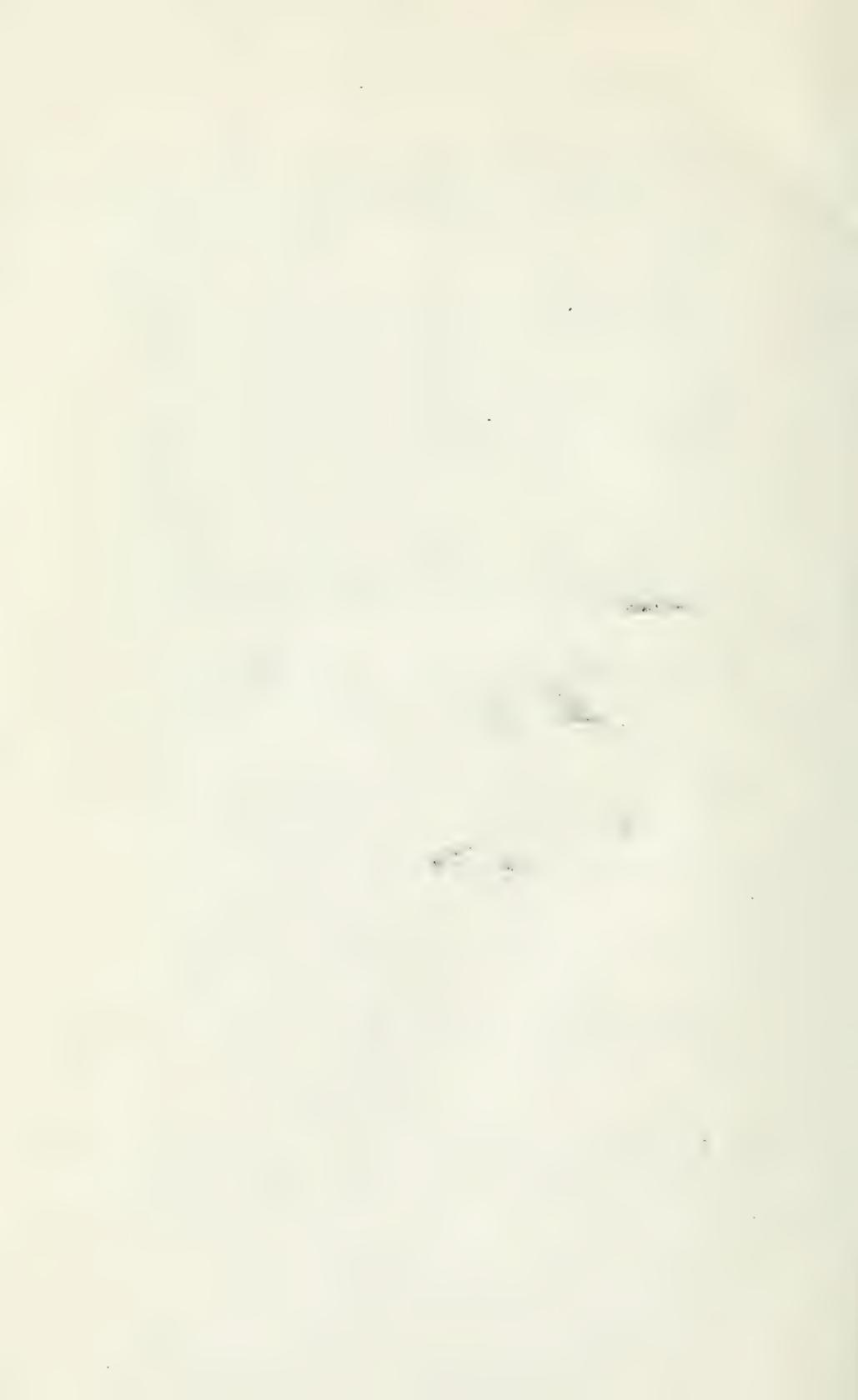


PLATE VI

ANDERSEN AND WALKER



# AN ECOLOGICAL STUDY OF THE ALGAE OF SOME SANDHILL LAKES

BY EMMA N. ANDERSEN AND ELDA R. WALKER

## INTRODUCTION

Much of the western part of Nebraska consists of rolling sandhills covered with bunchgrasses, yuccas, cacti, and other dry-land plants. Cherry county which is situated in the north central part of the sandhills has in its valleys many bodies of water. In one area of 250 square miles there are about 75 lakes. This region is twenty-five miles southwest from the village of Woodlake and about the same distance south of Valentine. These lakes vary greatly in size. Some are ponds a few hundred feet in diameter while the largest of the group is about four and a half miles long and three-fourths of a mile wide. All are comparatively shallow bodies of water, varying in depth from a couple of feet to a maximum of fifteen feet. The accompanying map<sup>1</sup> (Fig. 1) shows the relative size and arrangement of the lakes of this region.

Surrounding the lakes are low meadows covered with grasses and prairie flowers.<sup>2</sup> These meadows extend back from the lakes anywhere from a rod to, in some cases, a mile or more. Surrounding the grasslands rise the "sandhills"—dunes of yellowish sand extending to the next lake with its surrounding grasslands (Fig. 2). The few native trees are stunted and produce no effect on the landscape.

The climate is that typical of the central plains, dry, windy, hot in summer, and cold in winter. In the summer of 1912, the average maximum daily temperature from June 24th to August 3rd was 91.2°, while the average minimum temperature was 59.8°. During the same period the wind velocity reached a maximum of 21.9 miles per hour, the average for a twelve hour period, while the lowest average for a similar period was 1.3 miles per hour.

<sup>1</sup> Furnished by Dr. G. E. Condra of the University of Nebraska.

<sup>2</sup> The vegetation of this region is well discussed by Pool (36).

The high daily temperature warms the water of the shallow lakes and the prevailing high winds stir it, frequently mixing the warm surface layers with those below causing thorough aëration. The climatic conditions together with the abundance of water plants, such as *Chara*, *Myriophyllum*, *Potamogeton*, *Scirpus*, *Nymphaea*, and *Zizania*, which give anchorage for attached forms, make an ideal habitat for algae.

Although complete analyses of the water of the lakes are not available, some idea of their alkalinity may be obtained from analyses made by the department of chemistry of the University of Nebraska of samples of water taken by Dr. R. H. Wolcott. These were made in 1911 and showed the following parts per million of alkali:

Watts Lake, 111

Dewey Lake, 160

Hackberry Lake (no analysis made)

Big Alkali Lake, 622

Clear Lake, 1,129

As the analyses show some of the lakes are adapted to the algae of fresher waters, while others are so alkaline that very few forms can inhabit them. There is evidence that all of the series of lakes belonged formerly to one general system. The larger lakes have well formed shore lines except at their northwest ends where many of them are swampy. This gives farther variations in the habitat for algae.

Surrounded by a large semiarid region, these lakes with their large beds of wild rice and other seed producing plants prove a most tempting resting and feeding place for migratory birds. They flock here in large numbers and, no doubt, bring on their mud-laden feet spores of algae from ponds both north and south—the only explanation for some of the species present.

Earlier students working with the higher plants of the sandhills reported a rich algal flora. This led the writers to spend the summer of 1912 in studying the algae of the region and the conditions under which they live. At first it was hoped to cover the entire group of lakes, but after a few preliminary trips through the region, the work was limited to a few localities so situated that they could be frequently visited. These were chosen to represent so far as possible the different types of habitat found in the region.

From the various localities, specimens were collected by hand either from a boat or by wading. A Birge net was also used to secure free floating forms.

The identifications of species are based upon Tilden's Myxophyceae, Collins' Green Algae of North America, and West's British Desmidiaceae. In groups such as the Oedogoniaceae, Characeae, and Helminthocladiaceae covered by special publications, the identifications were made with the works cited. Constant reference was made to the other systematic works listed at the end of this paper.

The nomenclature of DeToni is followed except for the Desmids where that of West is used. In the case of a few species not given in the above general works, the terminology of the author describing the form is used.

All diatoms were identified by Dr. C. J. Elmore. The one *Volvox* found was identified by Dr. J. H. Powers. Acknowledgments are also due Mr. F. H. Shoemaker for the photographs from which figures 2, 10, 11, and 12 were taken; to the Nebraska Conservation and Soil Survey for help in prosecuting the work; to Prof. B. E. Moore for suggestions as to physical problems; and to Prof. T. J. Fitzpatrick for careful reading of manuscript and proof.

### HACKBERRY LAKE

Hackberry lake (Fig. 1, 3) was chosen for a study of water conditions, because it was representative and conveniently located. It is a lake two and a half miles long and one-half mile wide. In depth it varied during the summer of 1912 from three to seven feet. The maximum depth, however, was found only in a few places. Usually it did not exceed four feet.

The shore is sandy except at the northwest end where it is freshly formed by the filling in of decomposing vegetable matter. This end is swampy and passes gradually into dense beds of *Zizania*, *Scirpus*, *Myriophyllum*, *Potamogeton*, and similar water plants. Here the water is so filled with vegetation that it is almost impenetrable with a boat or otherwise.

The southeast half of the lake was more open. Dense beds of water plants were scattered through it but between them were areas of open water, (Fig. 4). Here the algae were most abundant. It was in this region that records of water conditions were made.

Algae were abundant all over the lake but more so in this part where every rush stem, every lily pad, in fact, every submerged plant was loaded with them (Figs. 5, 6). The number of species was not large, as the list which follows shows, but the number of individuals was very great. For example, a count was made of the thalli of *Chaetophora elegans*. On one old *Scirpus* stem there were 592 thalli. In an area one meter square, there were 103 stems loaded in a similar way, making over sixty thousand thalli of *Chaetophora elegans* in a square meter. *Chaetophora cornu-damae*, *Nostoc glomeratum*, *Gongrosira debaryana*, and *Rivularia natans* were equally abundant, while other species were only slightly less so.

#### CLIMATIC CONDITIONS

The accompanying graphs (Figs. 15, 16, 17) are intended to illustrate weather conditions surrounding the lake. The station, (C on Fig. 1) represented in Fig. 8, was in a blowout about  $\frac{1}{4}$  mile from the lake. In the graph (Fig. 15) line A shows the daily variation in temperature of this station as recorded by a Fries self-registering thermograph. Line D shows the temperature on the north side of a small house, a few rods from the lake (A on Fig. 1) as registered by a maximum and minimum thermometer. Line C shows the temperature of the surface sand as recorded by a Fries self-registering soil thermograph whose bulb was barely covered with sand. B gives the temperature of the sand eight inches below the surface as recorded by a similar instrument. The wind record was made by a standard anemometer (Julien P. Fries), so only the averages of wind velocity for twelve hour periods are available, but it gives some estimate as to wind in the region. The high air temperature and its effect on the surface temperature of the light sand when compared with the temperature of the sand eight inches below the surface shows that comparatively little heat penetrates deeply into the soil—a fact that may have some bearing on the temperature of the water of the lakes in some instances. Fig. 16 is a similar record for a much cooler week taken in the grass of the meadow (Fig. 9) on the lake shore (B on Fig. 1).

## WATER CONDITIONS

*Temperature and Wind*

For a month, July 8 to August 6, the same instruments were stationed in a boat on this lake to determine, so far as possible, the temperature conditions under which the algae were growing. During this time the anemometer was placed on a post about six feet above the surface of the water and a hundred feet from the water's edge to determine the velocity of the wind passing over the water. The thermographs in the boat recorded the temperature (*A*) of the air in the boat, the temperature (*C*) of the surface water, and the temperature (*B*) of the water at the bottom of the lake. Due to drifting of the boat to a slightly different position, the depth at which the temperature was taken varied from three feet the first week to four and one-half the second and fourth weeks and five feet the third week. The temperatures were not taken in deeper parts of the lake because there the algal growth was slight. As can be seen from the accompanying graph (Fig. 17) the temperatures of the surface and bottom water approach each other very closely except on days of low wind, when the temperature of the surface water varies considerably from that at the bottom. This effect of air temperature and wind was especially evident in the fourth week when great variations in air temperature occurred.

It will be noticed that during a large part of this month, the water temperatures were between 70° and 80° F., and that the temperature of the water at all levels was remarkably uniform. It is evident from the divergences of the temperature of surface and bottom water during each period of lower wind that the uniformity of the water temperature is due largely to stirring by wind. Also that a constant stirring would produce good aëration in all parts of the water is an inevitable conclusion.

Hackberry lake is one of the less alkaline of the lakes in this group. It is, however, more alkaline than some of its neighbors as was noted before.

*Light*

A modification of the common solio paper photometer (text Fig. 1) was devised to study the light conditions under which the algae were

growing.<sup>1</sup> This consisted of a water tight circular drum (A) at the end of a metal tube (D). Inside of this drum was a disk (G) which revolved by means of a rod (E) passing through the tube and connected with a lever at the top. This disk (G) carried the paper (F) on its under surface. It was perforated by eight holes (B) of the same size as a clear glass window (C) in the drum. By revolving the inner disk, the areas of solio paper under the perforation of the disk could be exposed to the light through the window (C). The tube and

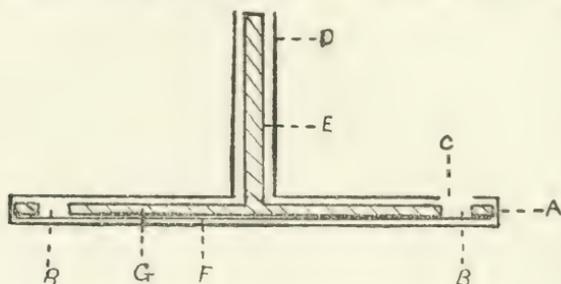


Fig. 1

rod inside of it were made in sections so they could be extended to the necessary length. The tube was marked in decimeters on the outside so the depth could be ascertained at all times. With this instrument, light readings were taken from a boat (Fig. 4) at various depths. Care was taken to have the window in the drum exposed to direct light at all times. In some instances, the period of exposure was uniform and the depth was varied; in other cases both depth and period of exposure were varied and in still others one depth was maintained during the series of readings and the period of exposure was varied.

Records taken by the last method are shown in (Fig. 7). The top row shows exposures made in the air beginning at the following hours, reading from left to right: 10:18 a.m., 10:30 a.m., and 2:10 p.m. In each record the exposures were 60, 45, 40, 30, 20, 10, 5, and 2 seconds.

Below are nine water records taken the same day and at intervals immediately following or preceding the air exposures. These are

<sup>1</sup> This instrument was made by the Spencer Lens Company.

arranged in order by depths, the first being one decimeter below the surface and each succeeding one a decimeter deeper, making the last nine decimeters deep. In all the periods of exposure were the same as the air records, except the last two. Here no records could be secured at the above exposure periods so the time was increased in record 8 to 2, 3, 4, 5, 6, 7, and 8 minutes and in record 9 to 1, 2, 3, 4, 5, 6, 7, and 8 minutes. All water readings were compared with exposures made in the air at the same hour. In no cases were the readings satisfactory but some facts of interest were recorded.

1. Waves on the surface water varied the record of light intensity greatly. On very windy days the intensity of light under the water was so variable that one exposure showed almost no coloration while the next at the same depth and period of exposure was deeply colored. The only explanation evident was that the change in angle of surface layer deflects the light almost entirely at times and not at other times. On account of this variability readings on windy days were early discontinued.

2. Before 9 A.M. or after 3 P.M. it was almost impossible to get a usable tint on the solio paper, although to the eye it was entirely light to the bottom of the lake. Evidently the angle between the sun's rays and the surface of the water was such that most of the rays were deflected and the water light was simply reflected light and not of a kind or quantity to affect solio paper.

3. Exposures made at a given depth but with different periods of exposure gave very different records as to relative light intensity. The shorter the exposure, the greater reduction of light, according to the readings. When the exposure required more than three minutes the error was so great that the reading was useless for comparison with readings of shorter periods. Two series will illustrate the point. The exposures were consecutive in both cases.

Time	Relative intensity All at 4 dm. deep	Time	Relative intensity All at 8 dm. deep
3 min. ....	.111	4 min. ....	.0083
2 min. ....	.125	5 min. ....	.0166
1 min. ....	.083	6 min. ....	.0277
½ min. ....	.05	7 min. ....	.0357
		8 min. ....	.0416

Such a series can only be explained by the well known fact that the reduction of silver in a photographic paper is not a uniform process. Since these results proved a variable period impracticable, exposures were made for a given period at various depths. These readings showed clearly a constant reduction of light as the depth was increased and agreed in general with the results obtained by Needham and Lloyd (33), by Birge (11), and by Oltmanns (34).

4. Exposures made for constant periods at various depths showed the light to be reduced by passing through the water. However, this gave no accurate measure of the light at given depths, because such exposures were compared with air tints of various periods of exposure. These we have just shown to be unreliable because of the inconstant reduction of the photographic film.

5. Solio paper is sensitive only to the blue-violet end of the spectrum. It is generally conceded that it is the red-orange end of the spectrum that is most largely used by plants in photosynthesis (Dangard 22). It is, therefore, evident that tests made with solio paper are of little value in determining the light conditions under which algae grow. Such tests show in a most general way, as noted above, that the direct rays of light are largely deflected except during the middle of the day and that the light is reduced by passing through water. Both facts are well known to physicists. This means that water plants have a shorter day than air plants and that they grow in less intense light at all times. In other words, as Oltmanns (34) states, they are all shade plants.

6. Light readings taken in October show less light penetrating the water than was found in July and August. The following table shows this as well as could be determined from solio paper records. Because of the reasons already given these records can only be regarded as a crude estimate. The figures given are compiled from a large number of readings so that the general diminution of blue light per decimeter of depth and the relative intensity between the light of midsummer and that of October are approximately correct.

Dm.	August	October
1	.5	.2
2	.25	.1
3	.173	.066

Dm.	August	October
4	.083	.05
5	.05	.033
6	.033	.016

No doubt the reduction of light in October is due to the rays of the sun striking the water more obliquely. Hence water plants have not only a shorter day but also a shorter growing season than land plants.

It is evident that the main advantage in the present attempt to measure light with reference to that used by plants under water is to prove the absolute uselessness of the solio paper method.

Later the same photometer was modified slightly so that plates sensitive to all kinds of light could be used in it (Figs. 10, 11, 12). A color screen was added and in that way an attempt was made to measure the red-orange light penetrating the water. Fig. 10 shows the upper side of the drum which holds the photographic plate and the detachable color screen over the exposure window. This is shown again in Fig. 12 (below) with the tube removed. At the top of Fig. 12 is shown the under side of the upper half of the drum. The inner disk shows two clips for holding the plate in place and eight perforations through which areas of the plate may be exposed to the light of the window. This disk is revolved, exposing alternately a perforation and a solid area, by a rod passing through the tube and connected with the lever at the top (Fig. 11). This apparatus for making exposures, while entirely rapid enough for solio paper, proved altogether too slow for the highly sensitive plates. The only results obtained indicated that red light penetrating many decimeters of water was too strong for more than instantaneous exposures on these plates. It is believed that a similar apparatus fitted with a shutter that would make possible exposures of a fraction of a second would come much nearer to giving an estimate of light available for the use of algae. Even here the reduction of the photographic film would not be uniform and some error would remain. Oltmanns (34) states that physicists have shown that the light from each part of the spectrum is deflected and absorbed differently by pure water. The water inhabited by algae is always modified in color and transparency both by its chemical content and by the presence of floating organisms giving turbidity to the water. An instrument such as the above would give an idea as to what effect such water conditions have upon

the quality of light used by algae even tho it did not give accurate information as to the quantity of light available for them.

Murray and Hjort (32) investigating light conditions in the ocean, by means of the Helland-Hansen Photometer and pan-chromatic photographic plates found that red light penetrated the water less than did blue and indigo. This agrees with the results cited by Oltmanns (34) for pure water. This suggests, in some cases at least, that actual light available for submerged plants is far below the amount indicated by the solio paper photometer.

#### DISTRIBUTION

As is readily seen from the physical factors noted above, this lake is characterized by fairly uniform temperature, aëration, and alkalinity. There remain but two factors that can influence the distribution of algae in this lake—light and mechanical support. The distribution may be entirely explained by these factors.

Attached forms as *Rivularia pisum*, *Nostoc glomeratum*, etc., grow at a depth of about 3–4 dm. below the surface of the water. When they are near the margin of the lake they grow on small submerged sedges, chara, etc. In deeper water they grow on Potamogeton, Myriophyllum, and such taller plants, but at remarkably uniform distances below the surface. On the other hand, *Nostoc pruniforme*, lying free at the bottom of the lake, was only found near the margin where the water was two or at most three decimeters deep. At first such forms led to the opinion that there was a zonal arrangement about the shore such as is suggested by Comère (17) but the theory did not stand the test. The distribution was entirely a vertical one caused undoubtedly by the light intensity. There were several conspicuous examples which demonstrate this. *Chaetophora elegans* extended on Scirpus stems, almost its only support, over a distance of two decimeters but the growth was constantly most abundant in the upper region, within  $\frac{1}{2}$ –1 dm. of the surface of the water. *Chaetophora cornu-damae* had almost the same distribution while *Nostoc glomeratum* made its best growth at a depth of 2–4 dm. or even deeper. Evidently it was crowded downward by the Chaetophora with which it grew. Early in the season the Nostoc and Chaetophora grew together. Later the Chaetophora occupied the upper zone while Nostoc grew in equal abundance lower down.

100





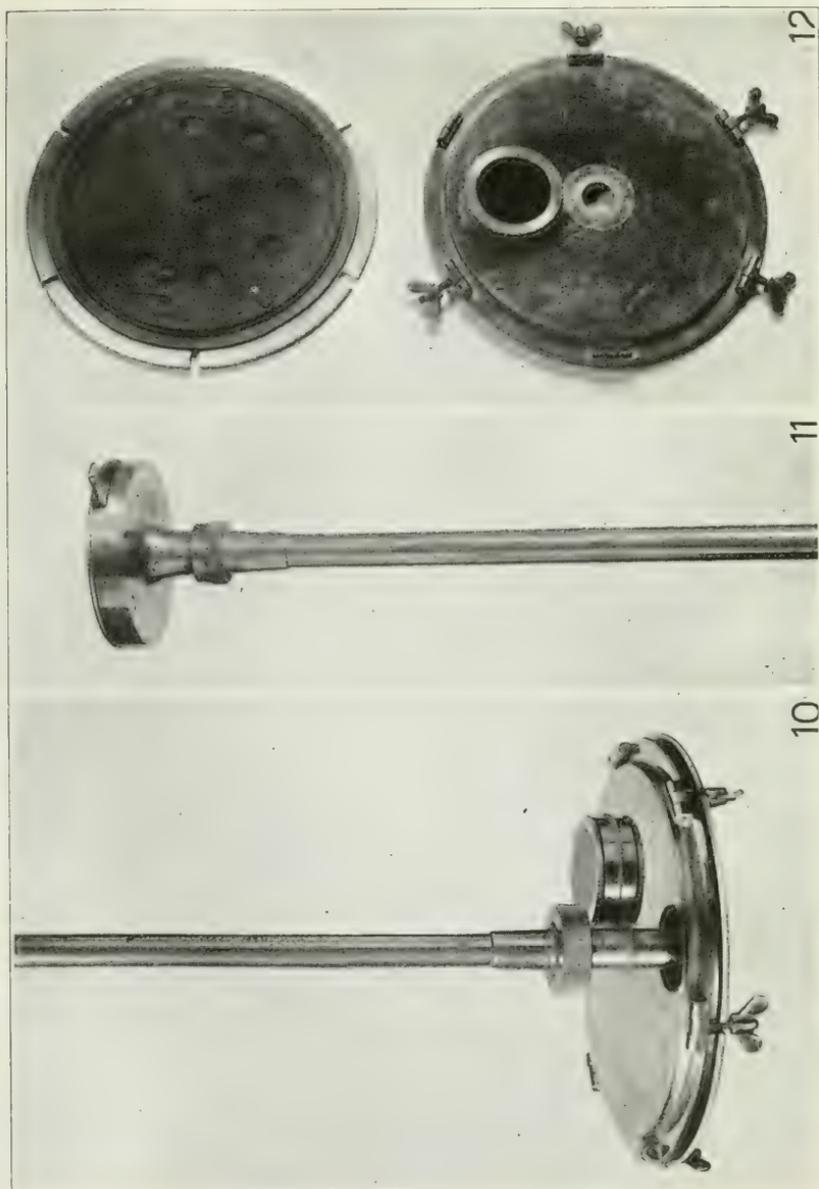


PLATE VIII

ANDERSEN AND WALKER







The following forms were found floating or attached almost exclusively in the upper two decimeters of water or in water not more than 2 dm. deep.

Clathrocystis aeruginosa	Microspora amoena
Coelosphaerium Kuetzingianum	Chaetophora cornu-damae
Nostoc muscorum	Chaetophora elegans
Nostoc humifusum	Bulbochaete sp.
Nostoc minutum	Oedogonium fragile
Nostoc zetterstedtii	Coleochaete orbicularis
Phormidium tenue	Gongrosira debaryana
Chara contraria	

Mixed with these were most of the unicellular forms of the yellow-green algae and most of the desmids listed for this lake.

Such forms as the following predominated in the deeper water, 2-4 dm. from the surface: *Rivularia natans*, *Rivularia pisum*, *Nostoc glomeratum*, and *Nostoc austini*. With these were associated *Merismopedium aeruginum*, *Oscillatoria subtilissima*, *Dictyosphaerium pulchellum*, *Gloeocystis gigas*, *Scenedesmus quadricauda*, and *S. bijugatus*. The distribution of unicellular free floating forms, however, was hard to determine because of the constant stirring of the water. It will be noted that few yellow-green algae are characteristically found in the lower zone.

Below a depth of 4 dm. many algae were found but not in any characteristic formation. Evidently they were there because crowded out elsewhere and they were not growing as well as the same species at higher levels.

As to grouping of the algae in these zones it was found that in any given location one or two species were dominant and others grew rather indiscriminately among them. The dominant species was apparently determined by the support on which it grew. In a bed of *Scirpus*, one species of *Chaetophora* and *Nostoc glomeratum* were almost universally the dominating forms, the *Chaetophora* dominating the upper and *Nostoc* the lower zone. On submerged mosses, *Potamogeton*, etc., *Gongrosira debaryana*, *Nostoc glomeratum*, or *Rivularia pisum* dominated, while at the margin of the lake a free floating form as *Nostoc pruniforme* or *Rivularia natans* might dominate. *Clathrocystis aeruginosa* or *Anabaena flos-aquae* usually

dominated the groups floating at the surface of the water, tho the constant winds kept these forms fairly well mixed.

#### SEASONAL DISTRIBUTION

Careful records of dates of collection were made to determine, so far as possible, the grouping of algae by seasons. As the study extended only from the middle of June until the middle of October, the observations included only summer and fall forms. Only the most general results were obtained. Of the species found in Hackberry lake the members of the Oscillatoriaceae were found only in the early summer, *Merismopedium* and *Coelosphaerium* only in July. Other members of the Myxophyceae were found in early stages in the first part of the season but reached full maturity and maximum abundance in October.

Most of the green algae were found throughout the period observed but they reached their maximum in midsummer except in a few cases. The species of *Gloeocystis* were found early in the summer. *Bulbochaete* was found late in the season (August–October) and the specimens were not fruiting. Nearly all the desmids were found during July. Only a few were present earlier or later in this lake, tho in places near by they occurred at other times in abundance.

The following table shows the forms found in the early, middle, and later summer and suggests something of the seasonal occurrence of the forms.

June 27–July 10	July 10–July 25	July 25–August
<i>Nostoc humifusum</i>	<i>Nostoc zetterstedtii</i>	<i>Nostoc muscorum</i>
<i>Nostoc glomeratum</i>	<i>Nostoc pruniforme</i>	<i>Nostoc minutum</i>
<i>Nostoc austinii</i>	<i>Nostoc humifusum</i>	<i>Nostoc caeruleum</i>
	<i>Nostoc glomeratum</i>	<i>Nostoc pruniforme</i>
		<i>Nostoc glomeratum</i>
<i>Anabaena flos-aquae</i>	<i>Anabaena flos-aquae</i>	<i>Anabaena flos-aquae</i>
<i>Oscillatoria subtilissima</i>		
<i>Phormidium tenue</i>		
<i>Rivularia pisum</i>	<i>Rivularia pisum</i>	<i>Rivularia pisum</i>
<i>Rivularia natans</i> (young)	<i>Rivularia natans</i>	<i>Rivularia natans</i>
	<i>Merismopedium aerugin- neum</i>	
	<i>Coelosphaerium kuetzing- ianum</i>	

June 27-July 10	July 10-July 25	July 25-August
Clathrocystis aeruginosa	Clathrocystis aeruginosa	Clathrocystis aeruginosa
	Staurastrum gracile	
	Spirotaenia obscura	
	Netrium digitus	
	Euastrum oblongum	
Cosmarium vexatum	Cosmarium subcrenatum	
Cosmarium obtusatum	Cosmarium retusifforme	
Cosmarium kjellmani grande	Cosmarium obtusatum	
	Cosmarium laeve	
	Cosmarium granatum	
	Cosmarium formosulum nathorstii	
	Cosmarium elfingii	
Closterium lanceolatum	Closterium pritchard- ianum	Closterium moniliferum
	Closterium leibleinii	
Chara contraria	Chara contraria	Chara contraria
Coleochaete orbicularis	Oedogonium fragile	Bulbochaete sp.
	Gongrosira debaryana	
Chaetophora elegans	Chaetophora elegans	Chaetophora elegans
Chaetophora cornu-damae	Chaetophora cornu-damae	Chaetophora cornu-damae
Gloeocystis vesiculosa	Microspora amoena	
Gloeocystis gigas		
Scenedesmus bijugatus	Scenedesmus quadricauda	
	Scenedesmus obliquus	
	Dictyosphaerium pulchel- lum	
	Pediastrum tetras	
	Pediastrum boryanum	
	Tetraedron trigonum	

A very interesting group of algae was found in a small ditch carrying water from a spring into the northwest end of Hackberry lake. *Closterium lunula* was so dominant here that at first it seemed to be the only species present. Closer examination, however, showed the following species associated with it.

Eremosphaera viridis	Cosmarium umbilicatum
Cylindrospermum majus	Euastrum verrucosum
Cosmarium angulosum concinnum	Pleurotaenium trabecula
Cosmarium circulare	Staurastrum meriani
Cosmarium pachydermum	Staurastrum punctulatum

*Phormidium tenue*, *P. valderianum*, and *Spirulina major* were found on the lake shore just above the water's edge, making the sand green at many places.

The following algae were found in this lake:

	<i>Chroococcaceae</i>	<i>Closterium</i> pritchardianum
Clathrocystis aeruginosa		<i>Cosmarium</i> elfingii
Coelosphaerium kuetzingianum		<i>Cosmarium</i> formosulum nathrostitii
Merismopedium aeruginosum		<i>Cosmarium</i> granatum
	<i>Oscillatoriaceae</i>	<i>Cosmarium</i> kjellmani grande
Oscillatoria subtilissima		<i>Cosmarium</i> laeve
Phormidium tenue		<i>Cosmarium</i> obtusatum
	<i>Nostocaceae</i>	<i>Cosmarium</i> retusifforme
Anabaena flos-aquae		<i>Cosmarium</i> subcrenatum
Cylindrospermum majus		<i>Cosmarium</i> vexatum
Nostoc austinii		<i>Euastrum</i> oblongum
Nostoc caeruleum		<i>Netrium</i> digitus
Nostoc glomeratum		<i>Spirotaenia</i> obscura
Nostoc humifusum		<i>Staurastrum</i> gracile
Nostoc minutum		
Nostoc muscorum		<i>Tetrasporaceae</i>
Nostoc pruniforme		<i>Dictyosphaerium</i> pulchellum
Nostoc zetterstedtii		
	<i>Rivulariaceae</i>	<i>Pleurococcaceae</i>
Rivularia natans		<i>Scenedesmus</i> bijugatus
Rivularia pisum		<i>Scenedesmus</i> obliquus
	<i>Bacillariaceae</i>	<i>Scenedesmus</i> quadricauda
Cocconeis placentula		<i>Tetraedron</i> trigonum
Cymbella cymbiformis		<i>Urococcus</i> insignis
Eunotia lunaris		
Fragilaria capucina		<i>Protococcaceae</i>
Gomphonema acuminatum		<i>Gloeocystis</i> gigas
Gomphonema parvulum		<i>Gloeocystis</i> vesiculosa
Navicula anglica		<i>Hydrodictyaceae</i>
Navicula cuspidata		<i>Pediastrum</i> boryanum
Navicula dicephala		<i>Pediastrum</i> tetras
Navicula sculpta		
Navicula subcapitata		<i>Ulotrichaceae</i>
Navicula viridis		<i>Microspora</i> amoena
Nitzschia spectabilis		
Nitzschia tryblionella		<i>Chaetophoraceae</i>
Surirella ovalis pinnata		<i>Chaetophora</i> cornu-damae
	<i>Desmidiaceae</i>	<i>Chaetophora</i> elegans
<i>Closterium</i> lanceolatum		<i>Gongrosira</i> debaryana
<i>Closterium</i> leibleinii		
<i>Closterium</i> moniliferum		<i>Oedogoniaceae</i>
		<i>Bulbochaete</i> sp.
		<i>Oedogonium</i> fragile
		<i>Coleochaetaceae</i>
		<i>Coleochaete</i> orbicularis
		<i>Characeae</i>
		<i>Chara</i> contraria

## CLEAR LAKE

This lake (Fig. 1, 2, 13) is a little wider and deeper than Hackberry lake and is about two-thirds of a mile distant from it. The water, however, is very alkaline (see discussion of alkalinity) and of a characteristic yellowish color. About the edge in places were rushes but there was little other vegetation in the lake. The algal flora was scarce in species but rich in individuals. Throughout the season the water was full of *Closterium aciculare*.<sup>1</sup> With this were found a few specimens of *Cosmarium obtusatum* and *Pediastrum boryanum*, and a few diatoms in fair abundance but that was all. Since in Clear lake the algae were all free floating, and the species were limited, no distribution studies were attempted. The same species were found throughout the summer. *Closterium aciculare* was the only characteristic form and was present throughout the season. The few other forms present with it were characteristic of the springs flowing into the lake and probably all came there by chance. It is probable that they could not have continued to live in so alkaline a water. Only one factor, alkalinity, could have influenced the algal flora of this lake.

In some places on the shore of the lake were springs whose water contained the richest algal flora found in the lake region. This was especially true at the west end (x in Fig. 13). Here the springs were on the sloping bank and the water seeped slowly down through the boggy soil. The surface was covered with grass and ferns forming sod enough to nearly support a hundred weight or more. Cattle coming to the spring for water had tramped over this sod forming holes from the size of a footprint to two or three feet in diameter. In these holes (Fig. 14) the water stood undisturbed for long periods with the thick grass sheltering the surface from the wind. The result was a large number of small aquaria. The water in these was kept uniformly fresh by seepage from the springs. While no chemical analysis was made, it was noticeable that the alkalinity was low.

<sup>1</sup> This form in size fits the description of the species as given by West and West (51) but may be the form referred to by West (50) as *Closteriopsis longissima* tho some of the specimens were over  $600\mu$  long and  $7\mu$  wide. He suggests that *Closteriopsis* may be a degenerate of *Closterium aciculare* var. *subpronum*.

The temperature was fairly uniform throughout the summer period varying from about 68° F. in the early morning to about 80° F. at 3 P.M. So far as was observed the ecological conditions in the various pockets were remarkably uniform in every way. Each was dominated by some one species, the usual dominants being *Anabaena torulosa*, various species of *Nostoc*, *Spirogyra*, *Scytonema*, *Oedogonium*, and *Mougeotia*. With these were mixed in various proportions the other species found. Attempts were made to determine whether there was any uniformity as to the species present with a given dominant but there seemed to be none.

The only ecological groupings of forms observed were seasonal. As will be seen from the accompanying list some forms were found only early in the summer, others late in the summer, and still others in the fall. The greatest variety of species was found in the middle of the summer. In the case of unicellular forms this list can only be looked upon as suggestive. Only a few specimens are found for some of them and chance in collecting may have caused others to be overlooked in one period or another. In this table early summer means from the middle of June to the middle of July; midsummer from the middle of July to the middle of August; fall is represented by collections made in October. Here fresh water, uniform temperature, protection from wind, and very shallow water form a habitat as uniform in every respect as is possible. Here the dominant forms found in a given water pocket can be due only to one of two things, seasonal periodicity which was very evident and the chance dominance of certain forms. Often the soil beneath a mass of *Spirogyra*, *Mougeotia*, or other such form was covered with desmids or diatoms. It is apparent that smaller forms, especially unicellular ones, may be shaded by the dominant species and hence their presence would be determined by their light relation. However, which of these shade loving forms should be associated with a given dominant species was chance so far as was determined. At other places on the lake shore were springs where a few algae were found but they were of so little consequence that the species were included only in the general list of species.

## CLEAR LAKE PUDDLES

	Early summer	Mid- summer	October
<i>Chroococcaceae</i>			
Aphanothece prasina.....		x	
Clathrocystis aeruginosa.....		x	
Gloeocapsa arenaria.....		x	
<i>Oscillatoriaceae</i>			
Oscillatoria formosa.....		x	
Oscillatoria limosa.....	x		x
Phormidium tenue.....		x	
Phormidium valderianum.....		x	
<i>Nostocaceae</i>			
Anabaena flos-aquae.....	x	x	
Anabaena oscillarioides.....		x	
Anabaena torulosa.....	x	x	x
Cylindrospermum comatum.....			x
Nodularia harveyana.....		x	
Nostoc linckia.....	x		x
Nostoc minutum.....		x	
Nostoc muscorum.....	x	x	
Nostoc spongiaeforme.....	x		
<i>Scytonemaceae</i>			
Scytonema crispum.....	x	x	x
<i>Rivulariaceae</i>			
Rivularia natans.....			x

*Bacillariaceae\**

Achnanthes lanceolata	Cystopleura zebra
Cyclotella meneghiniana	Eunotia diodon
Cymbella amphicephala	Eunotia lunaris
Cymbella cuspidata	Eunotia major
Cymbella naviculiformis	Fragilaria construens
Cymbella subaequalis	Gomphonema acuminatum
Cystopleura gibba	Gomphonema constrictum
Cystopleura gibba ventricosa	Gomphonema gracile
	Gomphonema montanum

\* No seasonal studies were made of diatoms.

Melosira varians	Navicula pupula
Navicula ambigua	Navicula sculpta
Navicula anglica	Navicula sphaerophora
Navicula bacilliformis	Navicula stauroptera
Navicula brebissonii	Navicula viridis
Navicula cuspidata	Nitzschia brebissonii
Navicula dicephala	Stauroneis anceps
Navicula elliptica	Stauroneis anceps amphicephala
Navicula gibba brevistriata	Stauroneis phoenicenteron
Navicula hilseana	Surirella ovalis ovata
Navicula major	Synedra rumpens
Navicula mesolepta	Synedra ulna
Navicula iridis	

	Early summer	Mid- summer	October
<i>Desmidiaceae</i>			
Arthrodesmus convergens.....	x	x	x
Closterium cynthia.....	x	x	x
Closterium didymotocum.....	x		
Closterium jenneri.....	x		
Closterium lanceolatum.....			x
Closterium lunula.....	x	x	x
Closterium moniliferum.....	x	x	x
Closterium parvulum.....		x	x
Closterium pritchardianum.....			x
Closterium siliqua.....			x
Closterium striolatum.....	x		
Closterium turgidum.....	x		
Cosmarium abruptum.....		x	
Cosmarium angulosum.....			x
Cosmarium angulosum concinnum.....		x	x
Cosmarium boeckii.....	x		x
Cosmarium blyttii.....		x	x
Cosmarium botrytis tumidum.....	x		
Cosmarium circulare.....	x		x
Cosmarium connatum.....	x	x	x
Cosmarium crenatum.....	x		
Cosmarium cyclicum.....	x		
Cosmarium cymatopleurum.....	x		
Cosmarium formosulum nathrostitii.....		x	x
Cosmarium galeritum.....		x	
Cosmarium granatum.....		x	x

	Early summer	Mid- summer	October
<i>Cosmarium holmiense</i> .....	x		x
<i>Cosmarium jkellmani grande</i> .....		x	
<i>Cosmarium meneghinii</i> .....		x	
<i>Cosmarium microsphinctum</i> .....	x		
<i>Cosmarium notabile</i> .....	x		
<i>Cosmarium obtusatum</i> .....	x		
<i>Cosmarium ochthodes</i> .....		x	
<i>Cosmarium pachydermum</i> .....		x	x
<i>Cosmarium pachydermum aethiopicum</i> ...	x		x
<i>Cosmarium phaseolus elevatum</i> .....		x	
<i>Cosmarium phaseolus forma minor</i> .....	x		
<i>Cosmarium portianum</i> .....	x		x
<i>Cosmarium pygmaeum</i> .....	x		
<i>Cosmarium pyramidatum</i> .....		x	x
<i>Cosmarium rectangulare hexagonum</i> .....		x	x
<i>Cosmarium sportella</i> .....		x	
<i>Cosmarium subrenatum</i> .....	x	x	
<i>Cosmarium subtumidum</i> .....		x	
<i>Cosmarium subundulatum</i> .....	x		
<i>Cosmarium taxichondrum</i> .....		x	
<i>Cosmarium tetraophthalmum</i> .....		x	
<i>Cosmarium trilobulatum</i> .....		x	
<i>Cosmarium tumidum</i> .....		x	
<i>Cosmarium vexatum</i> .....		x	
<i>Euastrum attenuatum</i> .....			x
<i>Euastrum bidentatum</i> .....		x	
<i>Euastrum binale</i> .....		x	x
<i>Euastrum dubium</i> .....		x	
<i>Euastrum oblongum</i> .....		x	x
<i>Euastrum verrucosum</i> .....	x	x	x
<i>Euastrum verrucosum alatum</i> .....		x	x
<i>Micrasterias pinnatifida</i> .....		x	x
<i>Micrasterias rotata</i> .....			x
<i>Netrium digitus</i> .....		x	x
<i>Netrium interruptum</i> .....			x
<i>Penium libellula intermedium</i> .....			x
<i>Penium naegeli</i> .....		x	
<i>Penium spirostriolatum</i> .....		x	x
<i>Pleurotaenium coronatum</i> .....	x	x	x
<i>Pleurotaenium nodulosum</i> .....		x	
<i>Pleurotaenium trabeculata</i> .....	x		

	Early summer	Mid- summer	October
<i>Spirotaenia condensata</i> .....		x	x
<i>Spirotaenia trabeculata</i> .....		x	
<i>Staurastrum alternans</i> .....			x
<i>Staurastrum dickiei</i> .....		x	x
<i>Staurastrum dilatatum</i> .....			x
<i>Staurastrum dispar</i> .....		x	
<i>Staurastrum hirsutum</i> .....		x	x
<i>Staurastrum margaritaceum</i> .....		x	x
<i>Staurastrum meriani</i> .....			x
<i>Staurastrum muticum</i> .....		x	
<i>Staurastrum orbiculare</i> .....	x	x	x
<i>Staurastrum orbiculare ralfsii</i> .....		x	
<i>Staurastrum paxilliferum</i> .....			x
<i>Staurastrum polytrichum</i> .....	x		
<i>Staurastrum punctulatum</i> .....	x	x	x
<i>Staurastrum saxonicum</i> .....		x	
<i>Staurastrum teliferum</i> .....		x	
<i>Zygnemaceae</i>			
<i>Spirogyra arcta catenaeformis</i> .....	x		
<i>Spirogyra crassa</i> .....	x		
<i>Spirogyra dubia</i> .....	x	x	x
<i>Spirogyra lutetiana</i> .....	x		
<i>Spirogyra neglecta</i> .....			x
<i>Spirogyra varians</i> .....			x
<i>Zygnema</i> sp.....	x	x	x
<i>Mesocarpaceae</i>			
<i>Mougeotia robusta</i> .....			x
<i>Mougeotia scalaris</i> .....			x
<i>Mougeotia viridis</i> .....	x		
<i>Volvocaceae</i>			
<i>Volvox aureus</i> .....		x	
<i>Tetrasporaceae</i>			
<i>Dictyosphaerium pulchellum</i> .....		x	x
<i>Tetraspora gelatinosa</i> .....	x		

	Early summer	Mid- summer	October
<i>Pleurococcaceae</i>			
<i>Crucigenia rectangularis</i> .....			x
<i>Eremosphaera viridis</i> .....		x	x
<i>Nephrocytium naegelii</i> .....	x		
<i>Oocystis solitaria</i> .....		x	x
<i>Rhaphidium polymorphum falcatum</i> .....		x	x
<i>Scenedesmus antennatus</i> .....	x		
<i>Scenedesmus bijugatus</i> .....	x		x
<i>Tetraedron reticulatum</i> .....			x
<i>Urococcus insignis</i> .....			x
<i>Protococcaceae</i>			
<i>Gloeocystis vesiculosa</i> .....	x		
<i>Ophiocytium capitatum</i> .....			x
<i>Hydrodictyaceae</i>			
<i>Coelastrum sphaericum</i> .....		x	
<i>Pediastrum angulosum</i> .....			x
<i>Pediastrum boryanum</i> .....	x	x	x
<i>Pediastrum tetras</i> .....		x	
<i>Ulotrichaceae</i>			
<i>Microspora pachyderma</i> .....		x	
<i>Microspora stagnorum</i> .....		x	
<i>Chaetophoraceae</i>			
<i>Stigeoclonium glomeratum</i> .....	x		
<i>Oedogoniaceae</i>			
<i>Bulbochaete</i> sp.....			x
<i>Oedogonium</i> sp.....	x	x	x
<i>Oedogonium capilliforme australe</i> .....		x	
<i>Oedogonium cardiacum</i> .....	x		
<i>Oedogonium crispum uruguayense</i> .....	x		
<i>Oedogonium fragile</i> .....		x	
<i>Oedogonium varians</i> .....			x
<i>Cladophoraceae</i>			
<i>Rhizoclonium hieroglyphicum</i> .....	x		

	Early summer	Mid- summer	October
<i>Vaucheriaceae</i>			
Vaucheria sp. ....			x

## BIG ALKALI LAKE

Big Alkali Lake (Fig. 1) is a little larger than Clear lake. While it is more alkaline than Hackberry, Dewey, or Watts, it is far less so than Clear lake. The water had the characteristic yellow color of the alkaline lakes of this region. Here again alkalinity must be given as the factor governing the algal flora of the lake. One visit only was made here. At first sight the water seemed absolutely barren but further investigation showed the following forms to be present. The Chara was quite abundant forming very much dwarfed patches on the bottom near the shore.

<i>Chroococcaceae</i>	Cosmarium granatum
Clathrocystis aeruginosa	Cosmarium meneghinii
Merismopedium glaucum	Cosmarium sexnotatum
Merismopedium tenuissimum	Staurastrum gracile
<i>Oscillatoriaceae</i>	Staurastrum paradoxum
Oscillatoria limosa	Staurastrum polymorphum
<i>Bacillariaceae</i>	<i>Tetrasporaceae</i>
Amphora ovalis	Dictyosphaerium pulchellum
Campylodiscus clypeus	<i>Pleurococcaceae</i>
Cymbella cistula	Scenedesmus bijugatus
Cystopleura gibba	Scenedesmus obliquus
Navicula cryptocephala veneta	Scenedesmus quadricauda
Navicula gastrum	<i>Hydrodictyceae</i>
Navicula oblonga	Pediastrum boryanum
Navicula sculpta	Pediastrum duplex clathratum
<i>Desmidiaceae</i>	<i>Characeae</i>
Cosmarium angulosum	Chara foetida rabenhorstii

## DEWEY LAKE

Dewey lake (Fig. 1) is situated about half a mile from Hackberry lake. It is less alkaline, nearly three times as large and proportionally deeper. Otherwise the conditions were much the same. Temperatures taken in the region of algal growth showed little variation from those in Hackberry lake.

The algae were found along the margins of the lake and attached to submerged plants near the surface. As no attempt was made to study conditions in this lake no explanation of the conspicuous difference in the forms found can be given unless it was the larger amount of water and difference in alkalinity. Collections were made here at irregular intervals and the list must not be looked upon as complete.

- |                               |                                 |
|-------------------------------|---------------------------------|
| <i>Chroococcaceae</i>         |                                 |
| Clathrocystis aeruginosa      | Navicula lanceolata             |
| Merismopedium glaucum         | Navicula major                  |
| <i>Oscillatoriaceae</i>       | Navicula oblonga                |
| Beggiatoa alba                | Navicula pupula                 |
| Lyngbya aerugineo-caerulea    | Staureneis acuta                |
| Oscillatoria amphibia         | Stauroneis smithii              |
| Oscillatoria formosa          | Stauroneis tenuissima           |
| Oscillatoria subtilissima     | Synedra rumpens                 |
| Phormidium fragile            | Synedra ulna                    |
| Phormidium tenue              | <i>Desmidiaceae</i>             |
| Phormidium valderianum        | Closterium pritchardianum       |
| <i>Nostocaceae</i>            | Cosmarium blyttii               |
| Anabaena flos-aquae           | Cosmarium boeckii               |
| Nostoc linckia                | Cosmarium formosulum nathorstii |
| Nostoc pruniforme             | Cosmarium impressulum           |
| <i>Scytonemaceae</i>          | Cosmarium obtusatum             |
| Tolypothrix distorta          | Cosmarium ochthodes var.        |
| <i>Rivulariaceae</i>          | Cosmarium subcrenatum           |
| Rivularia echinulata          | Cosmarium turpinii podolicum    |
| Rivularia natans              | Cosmarium vexatum               |
| <i>Bacillariaceae</i>         | Penium margaritaceum            |
| Amorpha ovalis                | Stauration orbiculare           |
| Brebissonia vulgaris          | <i>Pleurococcaceae</i>          |
| Cocconeis placentula          | Scenedesmus bijugatus           |
| Cymbella cistula              | Scenedesmus obliquus            |
| Cymbella cuspidata            | Tetraedron trigonum             |
| Cymbella lanceolata           | <i>Protococcaceae</i>           |
| Encyonema turgidum            | Characium ambiguum              |
| Eunotia lunaris               | Characium subulatum             |
| Fragilaria capucina           | Gloeocystis vesiculosa          |
| Fragilaria construens binodis | <i>Hydrodictyceae</i>           |
| Gomphonema constrictum        | Pediastrum boryanum             |
| Gomphonema gracile            | <i>Ulotrichaceae</i>            |
| Gomphonema montanum           | Hormiscia subtilis variabilis   |
| Gomphonema parvulum           | <i>Chaetophoraceae</i>          |
| Navicula cuspidata            | Chaetophora elegans             |
|                               | Gongrosira debaryana            |
|                               | Stigeoclonium aestivale         |

Stigeoclonium glomeratum  
*Oedogoniaceae*  
 Oedogonium grande

Oedogonium vaucherii  
*Helminthocladaceae*  
 Batrachospermum vagum

### WATTS LAKE

Watts lake (Fig. 1) is about half the size of Hackberry and only about one-third of a mile from it. Conditions in the lake were very similar to those in Hackberry in every respect. It was, however, slightly less alkaline than Dewey lake.

Collections were made here about once a week but other data were not taken. No boat was available on this lake and that may account for some of the difference in the reports for Watts and Hackberry lakes. No reason for a difference in species could be given unless it were the slight difference in alkalinity. The following species were found which it will be noted are nearly all included in those found in Hackberry and Dewey lakes and the springs on the shore of Clear lake.

*Chroococcaceae*  
 Clathrocystis aeruginosa  
 Coelosphaerium kuetzingianum  
 Merismopedium tenuissimum  
 Microcystis marginata  
*Nostocaceae*  
 Nostoc pruniforme  
*Bacillariaceae*  
 Achnanthes lanceolata  
 Amphora ovalis  
 Coconeis placentula  
 Gomphonema gracile  
 Gomphonema montanum  
 Homoeocladia amphioxys  
 Navicula gastrum  
 Navicula lanceolata  
*Desmidiaceae*  
 Cosmarium boeckii  
 Cosmarium formosulum nathorstii

Cosmarium geminatum  
 Cosmarium granatum  
 Cosmarium obtusatum  
 Cosmarium phaseolus  
 Cosmarium pseudopyramidatum  
 Cosmarium subcrenatum  
 Staurastrum gracile  
 Staurastrum margaritaceum  
*Tetrasporaceae*  
 Dictyosphaerium pulchellum  
*Pleurococcaceae*  
 Oocystis solitaria  
 Scenedesmus bijugatus  
 Scenedesmus obliquus  
 Scenedesmus quadricauda  
 Tetraedron minimum  
*Hydrodictyaceae*  
 Pediastrum boryanum  
*Characeae*  
 Chara contraria

### OTHER LAKES

One visit was made to each of the following lakes in the early summer. Trout and Dad's lakes (Fig. 1) are among the larger of the

group while *Phalaris* (Fig. 1) is one of the smaller. The Snake Creek Falls, about 15 miles distant, were visited once. These are falls in a creek which flows through the sandhill region. These collections were made so superficially that the lists must stand only as representing some species found in these localities.

The only form found which was sufficiently conspicuous to need special mention was *Nostoc verrucosum* which was extremely abundant on rocks in the cataract below the falls in Snake Creek.

The forms found in these localities are as follows:

### PHALARIS LAKE

#### *Chroococcaceae*

*Coelosphaerium kuetzingianum*  
*Merismopedium tenuissimum*  
*Dactylococcopsis raphidioides*

#### *Bacillariaceae*

*Amphora ovalis*  
*Cocconeis placentula*  
*Cystopleura gibba*  
*Cystopleura turgida*  
*Gomphonema montanum*  
*Homoeocladia amphibia*  
*Navicula cryptocephala veneta*  
*Navicula cuspidata*  
*Navicula elliptica*  
*Navicula gastrum*  
*Navicula oblonga*  
*Navicula sculpta*  
*Navicula sphaerophora*  
*Sceptroneis fibula*

#### *Desmidiaceae*

*Cosmarium angulosum*  
*Cosmarium blyttii*  
*Cosmarium formosulum nathorstii*  
*Cosmarium granatum subgranatum*  
*Cosmarium obtusatum*  
*Cosmarium regnellii*

#### *Tetrasporaceae*

*Dictyosphaerium pulchellum*

#### *Pleurococcaceae*

*Oocystis solitaria*  
*Pediastrum boryanum*  
*Scenedesmus bijugatus*  
*Scenedesmus quadricauda*  
*Tetraedron minimum*

#### *Characeae*

*Chara contraria*  
*Chara evoluta*  
*Chara fragilis*  
*Chara sp.*

### TROUT LAKE

#### *Chroococcaceae*

*Clathrocystis aeruginosa*

#### *Nostocaceae*

*Anabaena flos-aquae*  
*Nostoc zetterstedtii*

#### *Hydrodictyaceae*

*Pediastrum angulosum*

#### *Characeae*

*Chara sp.*

### DAD'S LAKE

#### *Chroococcaceae*

*Clathrocystis aeruginosa*

#### *Desmidiaceae*

*Closterium acerosum*

#### *Tetrasporaceae*

*Dictyosphaerium pulchellum*

#### *Hydrodictyaceae*

*Pediastrum boryanum*  
*Pediastrum duplex clathratum*

## SNAKE FALLS

	<i>Chroococccaceae</i>	Navicula humilis
Merismopedium glaucum		Navicula iridis
	<i>Oscillatoriaceae</i>	Navicula lanceolata
Arthrospira jenneri		Navicula limosa
Oscillatoria brevis		Navicula gibba brevistriata
Oscillatoria formosa		Navicula mesolepta
Phormidium retzii		Navicula pupula
	<i>Nostocaceae</i>	Navicula radiosa
Nostoc pruniforme		Navicula sculpta
Nostoc verrucosum		Navicula viridis
	<i>Rivulariaceae</i>	Homoeocladia amphibia
Calothrix parietina		Homoeocladia brebissonii
	<i>Bacillariaceae</i>	Homoeocladia amphioxys
Achnanthes lanceolata		Homoeocladia palea
Amphora ovalis		Rhoicosphenia curvata
Cocconeis placentula		Sceptroneis pacifica
Cymbella amphicephala		Stauroneis anceps
Cymbella cuspidata		Stauroneis phoenicenteron
Cymbella ehrenbergii		Surirella robusta
Cymbella gastroides		Surirella spiralis
Cystopleura gibba		Synedra ulna
Cystopleura ocellata		Tetracyclus lacustris
Cystopleura turgida		
Cystopleura zebra		<i>Desmidiaceae</i>
Denticula elegans		Closterium striolatum
Encyonema turgidum		Cosmarium microsphinctum
Eunotia major		Cosmarium portianum
Fragilaria mutabilis		Cosmarium sportella
Gomphonema acuminatum		Cosmarium undulatum wollei
Gomphonema herculeanum		Euastrum oblongum
Lysigonium crenulatum		Euastrum verrucosum
Lysigonium distans		Penium margaritaceum
Navicula ambigua		Staurastrum orbiculare hibernicum
Navicula anglica		
Navicula appendiculata		<i>Pleurococcaceae</i>
Navicula brebissonii		Scenedesmus obliquus
Navicula cuspidata craticula		
Navicula dicephala		<i>Protococcaceae</i>
Navicula elliptica		Chlorococcum humicola
		<i>Cladophoraceae</i>
		Cladophora glomerata

## CONCLUSION

It appears even from so brief a study as the one just described that the occurrence of algae in a given body of water at a given time is

due, to a certain extent, as Transeau (44), West (49), and others have said, to seasonal periodicity.

It is also evident that West (48 and 50), Oltmanns (34), Brannon (12), Wipple and Parker (53), Chambers (15), and many others are correct in their decisions that the mineral and gas content of water has much to do with its algal flora. Of these factors, alkalinity is probably to a great extent, the explanation for the wide difference in the algal flora of lakes so close together and so uniform in all other factors.

In a given lake the distribution of species may be explained by the one factor only that is variable, namely light intensity. Means for measuring this factor were entirely inefficient and only the crudest estimates can be made.

In small bodies of water where even the light is not variable to any measurable degree the dominant species and its associates are determined merely by chance except that forms lying beneath other forms are more shaded. This exception does not affect the dominant species but may affect the forms associated with it.

### ALGAE FOUND IN CHERRY COUNTY

#### Chroococcaceae.

- Aphanothece prasina A. Braun
- Clathrocystis aeruginosa (Kuetz.)  
Henfrey
- Coelosphaerium kuetzingianum Naeg.
- Dactylococcopsis raphidioides Hansg.
- Gloeocapsa arenaria (Hassall) Rabenh.
- Merismopedium aerugineum Bréb.
- Merismopedium glaucum (Ehrb.)  
Naeg.
- Merismopedium tenuissimum Lem-  
mERM.
- Microcystis marginata (Menegh.)  
Kuetz.

#### Oscillatoriaceae.

- Arthrospira jenneri (Kuetz.) Stiz.
- Lyngbya aerugineo-caerulea (Kuetz.)  
Gom.
- Oscillatoria amphibia Ag.
- Oscillatoria brevis (Kuetz.) Gom.
- Oscillatoria formosa Bory.

- Oscillatoria limosa. (Roth) Ag.
- Oscillatoria princeps Vauch.
- Oscillatoria sancta (Kuetz.) Gom.
- Oscillatoria subtilissima Kuetz.
- Oscillatoria tenuis Ag.
- Phormidium fragile (Menegh.) Gom.
- Phormidium retzii (Ag.) Gom.
- Phormidium tenue (Menegh.) Gom.
- Phormidium valderianum (Delp.)  
Gom.
- Spirulina major Kuetz.

#### Nostocaceae.

- Anabaena flos-aquae (Lyngb.) Bréb.
- Anabaena oscillarioides Bory.
- Anabaena torulosa (Carmich.) Lager-  
heim
- Cylindrospermum comatum Wood
- Cylindrospermum majus Kuetz.
- Nodularia harveyana (Thwaites) Thuret
- Nostoc austini Wood
- Nostoc caeruleum Lyngbye

- Nostoc commune* Vaucher  
*Nostoc glomeratum* Kuetz.  
*Nostoc humifusum* Carmichael  
*Nostoc linckia* (Roth) Bornet  
*Nostoc minutum* Desm.  
*Nostoc muscorum* Ag.  
*Nostoc pruniforme* (L.) Ag.  
*Nostoc spongiaeforme* Ag.  
*Nostoc verrucosum* (L.) Vauch.  
*Nostoc zetterstedtii* Areschoug  
 Scytonemaceae.  
*Scytonema crispum* (Ag.) Bornet  
*Tolypothrix distorta* (Hofm. B.) Kuetz.  
 Rivulariaceae.  
*Calothrix parietina* (Naeg.) Thur.  
*Rivularia echinulata* (Smith) Born  
*Rivularia natans* (Hedw.) Welw.  
*Rivularia pisum* Ag.  
 Bacillariaceae.  
*Achnanthes lanceolata* (Bréb.) Gr.  
*Amorpha ovalis* (Bréb.) Kuetz.  
*Brebissonia vulgaris* (Thwait) Kunze  
*Campylodiscus clypeus* Ehr.  
*Cocconeis placentula* Ehr.  
*Cyclotella meneghiniana* Kuetz.  
*Cymbella amphicephala* Naeg.  
*Cymbella cistula* (Hempr.) Kirchn.  
*Cymbella cuspidata* Kuetz.  
*Cymbella cymbiformis* (Kuetz.) Bréb.  
*Cymbella ehrenbergii* Kuetz.  
*Cymbella lanceolata* (Ehr.) Kirch.  
*Cymbella naviculiformis* Auersw.  
*Cymbella subaequalis* Grun.  
*Cystopleura gibba* (Ehr.) Kunze  
*Cystopleura zebra* (Ehr.) Kunze  
*Encyonema turgidum* (Greg.) Grun.  
*Eunotia diodon* Ehr.  
*Eunotia lunaris* Grun.  
*Eunotia major* (W. Sm.) Rabenh.  
*Fragilaria capucina* Desmaz.  
*Fragilaria construens* (Ehr.) Grun.  
*Fragilaria construens binodis* (Ehr.) Grun.  
*Gomphonema acuminatum* Ehr.  
*Gomphonema constrictum* Ehr.  
*Gomphonema gracile* Ehr.  
*Gomphonema herculeanum* Ehr.  
*Gomphonema montanum* Schum.  
*Gomphonema parvulum* Kuetz.  
*Homoeocladia amphibia* (Grun.) Kunze  
*Homoeocladia amphioxys* (Ehr.) Kunze  
*Homoeocladia brebissonii* (H. Sm.) Kunze  
*Homoeocladia palea* (Kuetz.) Kunze  
*Lysigonium crenulatum* (Kuetz.) Kunze  
*Lysigonium distans* (Kuetz.) Kunze  
*Lysigonium varians* (Ag.) D.T.  
*Navicula ambigua* Ehr.  
*Navicula anglica* Ralfs  
*Navicula appendiculata* (Ag.) Kuetz.  
*Navicula bacilliformis* Grun.  
*Navicula brebissonii* Kuetz.  
*Navicula cryptocephala veneta* (Kuetz.) Rabenh.  
*Navicula cuspidata* Kuetz.  
*Navicula dicephala* Ehr.  
*Navicula elliptica* Kuetz.  
*Navicula gastrum* Ehr.  
*Navicula gibba* (Ehr.) Kuetz.  
*Navicula gibba brevistriata* Grim.  
*Navicula hilseana* Jan.  
*Navicula humilis* Donk.  
*Navicula iridis* Ehr.  
*Navicula lanceolata* Kuetz.  
*Navicula limosa* Kuetz.  
*Navicula major* Kuetz.  
*Navicula mesoleptata* Ehr.  
*Navicula oblonga* Kuetz.  
*Navicula pupula* Kuetz.  
*Navicula radiosa* Kuetz.  
*Navicula sculpta* Ehr.  
*Navicula sphaerophora* Kuetz.  
*Navicula stauroptera* Grun.  
*Navicula subcapitata* Greg.  
*Navicula viridis* Kuetz.  
*Nitzschia brebissonii* W. Sm.  
*Nitzschia spectabilis* (Ehr.) Ralfs  
*Nitzschia tryblionella* Hantzsch.  
*Rhoicosphenia curvata* Grun.  
*Sceptroneis fibula* (Bréb.) Schuett

- Scoptroneis pacifica* (Grun.) Elmore (In press)  
*Stauroneis anceps* Ehr.  
*Stauroneis anceps amphicephala* Kuetz.  
*Stauroneis acuta* W. Sm.  
*Stauroneis phoenicenteron* Ehr.  
*Stauroneis smithii* Grun.  
*Surirella ovalis ovata* (Bréb.) V.H.  
*Surirella ovalis pinnata* (Bréb.) V.H.  
*Surirella robusta* Ehr.  
*Surirella spiralis* Kuetz.  
*Synedra rumpens* Kuetz.  
*Synedra ulna* (Nitzsch.) Ehr.  
*Tetracyclus lacustris* Ralfs
- Desmidiaceae.
- Arthrodesmus convergens* Ehrenb.  
*Closterium acerorum* (Schrank) Ehrenb.  
*Closterium aciculare* Tuffen West  
*Closterium cynthia* DeNot.  
*Closterium didymotocum* Corda  
*Closterium jenneri* Ralfs  
*Closterium lanceolatum* Kuetz.  
*Closterium leibleinii* Kuetz.  
*Closterium lunula* (Muell.) Nitzsch.  
*Closterium moniliferum* (Bory.) Ehrenb.  
*Closterium parvulum* Naeg.  
*Closterium pritchardianum* Arch.  
*Closterium siliqua* West and G. S. West  
*Closterium striolatum* Ehrenb.  
*Closterium turgidum* Ehrenb.  
*Cosmarium abruptum* Lund.  
*Cosmarium angulosum* Bréb.  
*Cosmarium angulosum concinnum* (Rabenh.) West and G. S. West  
*Cosmarium blyttii* Wille  
*Cosmarium boeckii* Wille  
*Cosmarium botrytis tumidum* Wolle  
*Cosmarium circulare* Reinsch.  
*Cosmarium connatum* Bréb.  
*Cosmarium crenatum* Ralfs  
*Cosmarium cyclicum* Lund.  
*Cosmarium cymatopleurum* Nordst.
- Cosmarium elfingii* Racib.  
*Cosmarium formosulum nathorstii* (Boldt) West and G. S. West  
*Cosmarium galeritum* Nordst.  
*Cosmarium geminatum* Lund.  
*Cosmarium granatum* Bréb.  
*Cosmarium granatum subgranatum* Nordst.  
*Cosmarium holmiense* Lund.  
*Cosmarium holmiense integrum* Lund.  
*Cosmarium impressulum* Elfv.  
*Cosmarium kjellmani grande* Wille  
*Cosmarium laeve* Rabenh.  
*Cosmarium meneghinii* Bréb.  
*Cosmarium microsphinctum* Nordst.  
*Cosmarium notabile* Bréb.  
*Cosmarium obtusatum* Schmidle  
*Cosmarium ochthodes* Nordst. var.  
*Cosmarium pachydermum* Lund.  
*Cosmarium pachydermum aethiopicum* West and G. S. West  
*Cosmarium phaseolus* Bréb.  
*Cosmarium phaseolus elevatum* Nordst.  
*Cosmarium phaseolus minor* Boldt  
*Cosmarium portianum* Arch.  
*Cosmarium protractum* (Naeg.) De-Bary  
*Cosmarium pseudopyramidatum* Lund.  
*Cosmarium pygmaeum* Arch.  
*Cosmarium pyramidatum* Bréb.  
*Cosmarium rectangulare hexagonum* (Elf.) West and G. S. West  
*Cosmarium regnellii* Wille  
*Cosmarium retusifforme* (Wille) Gutw.  
*Cosmarium sexnotatum* Gutw.  
*Cosmarium sportella* Bréb.  
*Cosmarium subcrenatum* Hantzsch  
*Cosmarium subtumidum* Nordst.  
*Cosmarium subundulatum* Wille  
*Cosmarium taxichondrum* Lund.  
*Cosmarium tetraophthalmum* Bréb.  
*Cosmarium trilobulatum* Reinsch  
*Cosmarium tumidum* Lund.  
*Cosmarium turpinii podolicum* Gutw.  
*Cosmarium umbilicatum* Luetkem.

- Cosmarium undulatum wollei* West  
*Cosmarium vexatum* West  
*Euastrum attenuatum* Wolle  
*Euastrum bidentatum* Naeg.  
*Euastrum binale* (Turp.) Ehrenb.  
*Euastrum dubium* Naeg.  
*Euastrum oblongum* (Grev.) Ralfs  
*Euastrum verrucosum* Ehrenb.  
*Euastrum verrucosum alatum* Wolle  
*Micrasterias pinnatifida* (Kuetz.) Ralfs  
*Micrasterias rotata* (Grev.) Ralfs  
*Netrium digitus* (Ehrenb.) Itzigs and  
 Rothe  
*Netrium interruptum* (Bréb.) Luetkem.  
*Pleurotaenium coronatum* (Bréb.)  
 Rabenh.  
*Penium libellula* (Focke) Nordst.  
*Penium margaritaceum* (Ehrenb.)  
 Bréb.  
*Penium naegelii* Bréb.  
*Penium spirostriolatum* Barker  
*Pleurotaenium nodulosum* (Bréb.) De-  
 Bary  
*Pleurotaenium trabecula* (Ehrenb.)  
 Naeg.  
*Spirotaenia condensata* Bréb.  
*Spirotaenia obscura* Ralfs  
*Spirotaenia trabeculata* A. Br.  
*Staurastrum alternans* Bréb.  
*Staurastrum dickiei* Ralfs  
*Staurastrum dilatatum* Ehrenb.  
*Staurastrum dispar* Bréb.  
*Staurastrum gracile* Ralfs  
*Staurastrum hirsutum* (Ehrenb.) Bréb.  
*Staurastrum margaritaceum* Ehrenb.  
*Staurastrum meriani* Reinsch  
*Staurastrum muticum* Bréb.  
*Staurastrum orbiculare* (Ehrenb.) Ralfs  
*Staurastrum orbiculare hibernicum*  
 West and G. S. West  
*Staurastrum orbiculare ralfsii* West  
 and G. S. West  
*Staurastrum paradoxum* Meyen  
*Staurastrum paxilliferum* G. S. West  
*Staurastrum polymorphum* Bréb.
- Staurastrum punctulatum* Bréb.  
*Staurastrum saxonicum* Bulnh.  
*Staurastrum teliferum* Ralfs
- Zygnemaceae.
- Spirogyra arcta catenaeformis* Kirchn.  
*Spirogyra crassa* Kuetz.  
*Spirogyra dubia* Kuetz.  
*Spirogyra lutetiana* Petit  
*Spirogyra neglecta* (Hass.) Kuetz.  
*Spirogyra varians* (Hass.) Kuetz.  
*Zygnema* Ag. sp.
- Mesocarpaceae.
- Mougeotia robusta* (DeBary) Wittr.  
*Mougeotia scalaris* Hass.  
*Mougeotia viridis* (Kuetz.) Wittr.
- Volvocaceae.
- Volvox aureus* Ehrenb.
- Tetrasporaceae.
- Tetraspora gelatinosa* (Vauch.) Desv.  
*Dictyosphaerium pulchellum* Wood
- Pleurococcaceae.
- Crucigenia rectangularis* (A. Br.) Gay  
*Eremosphaera viridis* DeBary  
*Nephrocytium naegelii* Grun.  
*Oocystis solitaria* Wittr.  
*Rhaphidium polymorphum falcatum*  
 (Corda) Rabenh.  
*Scenedesmus antennatus* Bréb.  
*Scenedesmus bijugatus* (Turp.) Kuetz.  
*Scenedesmus obliquus* (Turp.) Kuetz.  
*Scenedesmus quadricauda* (Turp.)  
 Bréb.  
*Tetraedron minimum* Reinsch  
*Tetraedron reticulatum* (Reinsch)  
 Hansg.  
*Tetraedron trigonum* (Naeg.) Hansg.  
*Urococcus insignis* Hass.
- Protococcaceae.
- Characium ambiguum* Hermann  
*Chlorococcum humicola* (Naeg.)  
 Rabenh.  
*Characium subulatum* A. Br.  
*Gloeocystis gigas* (Kuetz.) Lagerh.  
*Gloeocystis vesciculosa* Naeg.  
*Ophiocytium capitatum* Wolle

## Hydrodictyaceae.

- Coelastrum sphaericum* Naeg.  
*Pediastrum angulosum* (Ehrenb.)  
 Menegh.  
*Pediastrum boryanum* (Turp.)  
 Menegh.  
*Pediastrum duplex clathratum* A. Br.  
*Pediastrum tetras* (Ehrenb.) Ralfs

## Ulotrichaceae

- Microspora amoena* (Kuetz.) Rabenh.  
*Microspora pachyderma* (Wille)  
 Lagerh.  
*Microspora stagnorum* (Kuetz.) Lagerh.  
*Hormiscia subtilis variabilis* (Kuetz.)  
 Kirchn.

## Chaetophoraceae.

- Chaetophora cornu-damae* (Roth) Ag.  
*Chaetophora elegans* (Roth) Ag.  
*Gongrosira debaryana* Rabenhorst  
*Stigeoclonium aestivale* (Hazen) Collins  
*Stigeoclonium glomeratum* (Hazen)  
 Collins

## Oedogoniaceae.

- Bulbochaete* Ag. sp.

*Oedogonium* Link (several species not in fruit.)

- Oedogonium capilliforme australe*  
 Wittr.  
*Oedogonium cardiacum* (Hass.) Kuetz.  
*Oedogonium crispum uruguayense*  
 Magn. and Wille  
*Oedogonium fragile* Wittr.  
*Oedogonium grande* Kuetz.  
*Oedogonium varians* Wittr. and Lund.  
*Oedogonium vaucherii* (LeCl.) A. Br.

## Coleochaetaceae.

*Coleochaete orbicularis* Pringsh.

## Cladophoraceae.

- Cladophora glomerata* (L.) Kuetz.  
*Rhizoclonium hieroglyphicum* (Ag.)  
 Kuetz.

## Vaucheriaceae.

*Vaucheria* D.C. sp.

## Characeae.

- Chara* Vaill. sp.  
*Chara contraria* A. Br.  
*Chara evoluta* Allen  
*Chara foetida rabenhorstii* T. F. Allen  
*Chara fragilis* Desv.

## Helminthocladiaceae.

*Batrachospermum vagum* Ag.

## PUBLICATIONS CONSULTED

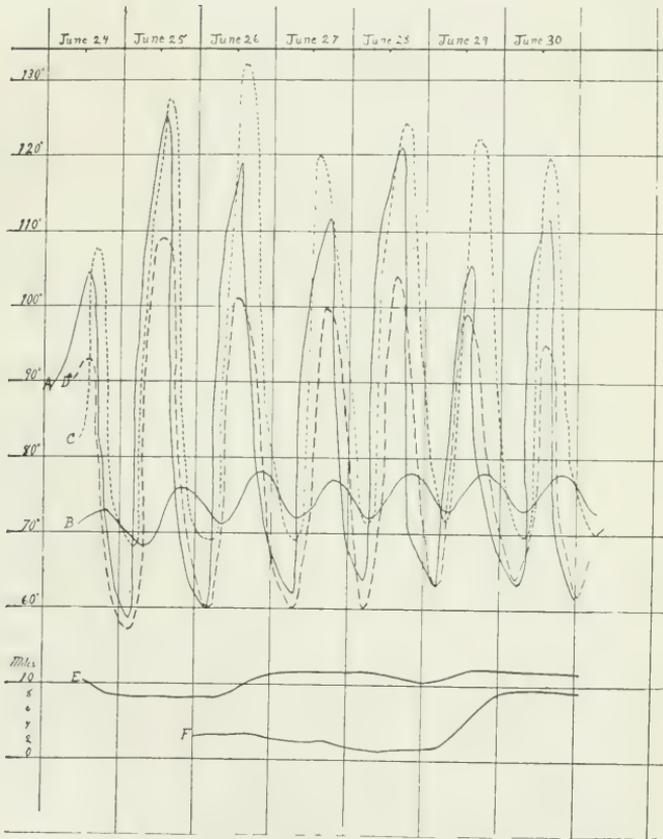
1. Agardh, J. G. *Species, genera, et ordines algarum.* 3 vols. 1848-1880.
2. Allen, T. F. *Characeae Americanae, illustrated and described.* Parts I and II. 1879.
3. ———. *The Characeae of America, Parts I and II.* 1880.
4. ———. *Characeae.* N. Y. State Museum Nat. Hist. 38th ann. report. 16, 15 Jan. 1885.
5. ———. *The Characeae of America.* Parts I and II, Fasc. 1, 2, and 3. 1888-1896.
6. Bessey, Charles E. *Supplementary list of recently reported species.* Webber's appendix to the catalogue of the flora of Nebraska, second edition. Contributions from the Bot. Dept. of the Uni. of Nebr. New series III:45-53, 1892.
7. Bessey, Charles E. and Webber, H. J. *Report of the botanist on the grasses and forage plants and the catalogue of plants.* Extracted from the report of the Nebraska State Board of Agriculture. 1889-1890.
8. *Botanical Survey of Nebraska, conducted by the Botanical Seminar, II, 1892, and III. 1893.*

9. Survey of Nebraska, conducted by the Botanical Seminar IV. Report on collections made in 1894-1895. 1896.
10. ————. Report on recent collections, Bot. Survey of Nebraska, pub. by Bot. Sem. 1901.
11. Birge, E. A. Absorption of the sun's energy by lakes. *Science*, 38:702. 1913.
12. Brannon, M. A. Factors influencing the flora of Devil's Lake, North Dakota. *Int. Rev. d. ges. Hydr. u. Hydro.* 4:291. 1911.
13. Brown, H. E. Algal periodicity in ponds and streams. *Bull. Tor. Bot. Club*, 35:223-248. 1908.
14. Brown, Wm. H. The plant life of Ellis, Great, Little, and Long's Lakes in North Carolina. *Contr. from the U. S. Nat. Herb.* 13:323-341. 1911.
15. Chambers, C. O. The relation of algae to dissolved oxygen and carbon-dioxide, with special reference to carbonates. 23rd ann. report Mo. Bot. Garden, pp. 171-207. 1912.
16. Collins, F. S. Green algae of North America. *Tufts College Studies*, vol. II, No. 3, pp. 79-480. 18 pls. 1909.
17. Comère, Joseph. De l'action du milieu considérée dans ses rapports avec la distribution générale des algues d'eau douce. *Mem. 25, Bull. Soc. Bot. France*, 16:1-96. 1913.
18. Cook, M. C. *British Desmids.* 1887.
19. ————. *British fresh-water algae exclusive of the Desmidiaceae and Diatomaceae.* 2 vols. 1882-1884.
20. Copeland, W. F. Periodicity in Spirogyra. *Bot. Gaz.* 47:9-25. 1909.
21. Danforth, C. H. Periodicity in Spirogyra with special reference to the work of Benecke. *Ann. Rep. Mo. Bot. Garden*, 21:49-59. 1910.
22. Dangeard, P. A. Determination of the rays concerned in chlorophyll synthesis. *Bul. Soc. Bot. France* 60:166-175. 1914.
23. DeToni, J. Bapt. *Sylloge algarum* I, 1889, V, 1907, IV, 1897-1905.
24. Engler, A. und Prantl, K. *Die natürlichen Pflanzenfamilien*, Teil I. 1897-1900.
25. Fritsch, F. E. and Rich, Florence. Studies on the occurrence and reproduction of British fresh-water algae in nature. *Ann. Bot.* 21:423-436. 1907.
26. Gomont, M. Maurice. *Monographie des Oscillariées.* (Nostocacees Homocystees). 1893.
27. Halstedt, P. D. Classification and description of the American species of Characeae. *Proc. Boston Soc. Nat. Hist.* XX. 1879.
28. Hassall, Arthur Hill. *A history of British fresh-water algae.* 1845.
29. Hirn, K. *Monographie und Iconographie der Oedogoniaceen.* *Acta Soc. Sci. Fennicae* 27. 1900.
30. Kützing, F. T. *Phycologia generalis oder Anatomie, Physiologie, und Systemkunde der Tange.* 1843.
31. Migula, W. *Kryptogamen-Flora von Deutschland, Deutsch-Österreich, und der Schweiz im Anschluss an Thome's Flora von Deutschland.* Band II: 1 Teil, 1907, und 2 Teil, 1909.
32. Murray, Sir John and Hjort, Dr. Johan. *The depths of the ocean.* 1912.
33. Needham, James G. and Lloyd, J. T. *The life of inland waters.* 1916.

34. Oltmanns, Dr. Friedrich. *Morphologie und Biologie der Algen*. 2 vols. 1905.
35. Petit, Paul. *Spirogyra des environs de Paris avec XII planches*. 1880.
36. Pool, R. J. A study of the vegetation of the sandhills of Nebraska. *Minnesota Botanical Studies*, Vol. 4, Part III. 1914.
37. Rabenhorst, Ludovico. *Flora europaea algarum aquae dulcis et submarinae*. 1864-1868.
38. Robbins, W. W. A preliminary list of the algae of Colorado. *Univ. of Colo. Studies*, 9:105. 1912.
39. Robinson, Ch. Budd. The Chareae of North America. *Bull. N. Y. Bot. Garden* 4: 244. 1906.
40. Saunders, D. and Woods, A. F. *Flora of Nebraska*, published by the Bot. Sem. Parts I and II. 1894.
41. Schmidle, W. Einige Algen aus Denver, Colorado, U. S. *Hedwigia* 34:84-85. Figs. 1, 2, 3a, and 3b. 1895.
42. Smith, J. G. and Pound, R. *Flora of the sandhill region of Sheridan and Cherry counties and lists of plants collected on a journey through the sandhills in July and August, 1892*. *Bot. Sur. Nebr.* II. 1893.
43. Tilden, Josephine E. The Myxophyceae of North America and adjacent regions including Central America, Greenland, Bermuda, the West Indies, and Hawaii. *Minnesota Algae I*. 1910.
44. Transeau, Edgar N. The periodicity of algae in Illinois. *Trans. Am. Mic. Soc.* 32:31. 1913.
45. Webber, H. J. Fresh-water algae of the plains. *Am. Nat.* 23:1011-1013. 1889.
46. ————. A second edition of Webber's appendix to the catalogue of the Flora of Nebraska. *Contributions from the Bot. Dept. of the Univ. of Nebr.* New series III:1-44. 1892.
47. ————. Appendix to the catalogue of the Flora of Nebraska. Contribution from the Shaw School of Botany. No. 9. *Trans. Acad. of Sc. St. Louis*, 6:1-47. 1892.
48. West, G. S. A treatise on British fresh-water algae. 1904.
49. ————. The algae of the Yan Yean reservoir, Victoria; a biological and ecological study. *Jour. Linn. Soc.* 39:1-80. 1909.
50. ————. *Algae*. Vol. I. 1916.
51. West, W. and West, G. S. A monograph of the British Desmidiaceae. 4 vols. 1904-1912.
52. ————. On the periodicity of the Phytoplankton of some British lakes. *Jour. Linn. Soc.* 40:395-432. 1912.
53. Whipple, G. C. and Parker, H. N. On the amount of oxygen and carbonic acid in natural waters and the effect of these gases upon the occurrence of microscopic organisms. *Trans. Am. Mic. Soc.* 23:103-144. 1902.
54. Wolle, Rev. Francis. *Desmids of the United States and list of American Pediatrums with 1,100 illustrations*. 1884.
55. ————. *Fresh-water algae of the United States, (exclusive of the Diatomaceae) complimentary to desmids of the U. S., with 2,300 illustrations*. 1887.

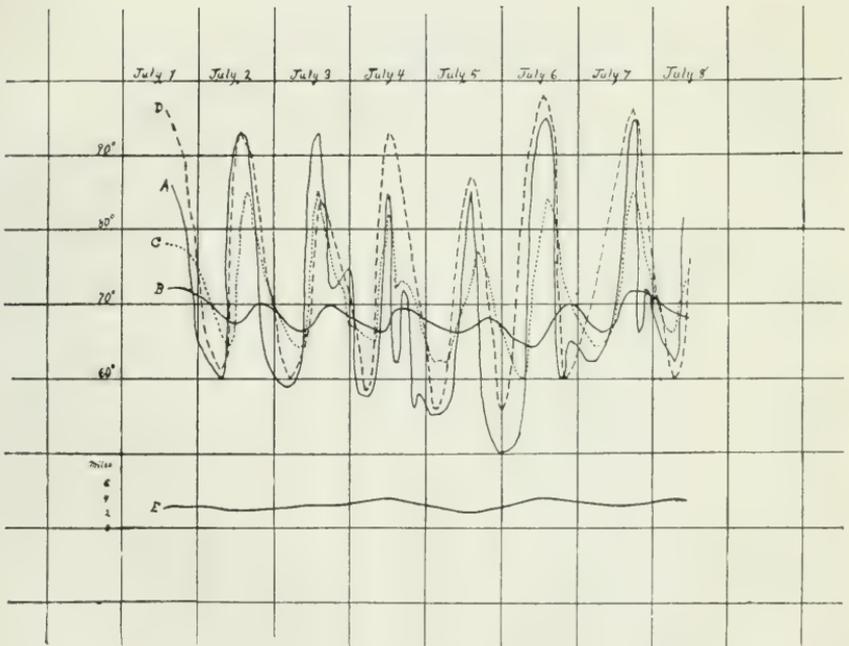
## EXPLANATION OF FIGURES

- Fig. 1. Map showing the lake region in Cherry County, Nebraska. Clear white areas represent water, dotted areas wet meadows, and  swamps. (From map by Dr. G. E. Condra).
- Fig. 2. A view taken from the top of a sandhill and showing at the front left a part of Clear lake, at the upper left a part of Dewey lake and in the distance at the right a narrow strip of White Water lake, also the "sandhills" and the meadows surrounding the lakes. (Photo by F. H. Shoemaker).
- Fig. 3. Hackberry lake from the northeast shore.
- Fig. 4. Taking water photometer records among the rushes on Hackberry lake.
- Fig. 5. Submerged moss stems covered with *Nostoc glomeratum*—Hackberry lake. (Photographed under water).
- Fig. 6. Section of *Scirpus* stem covered with *Chaetophora cornu-damae* and *Nostoc glomeratum*. (Photographed under water).
- Fig. 7. Water photometer records on solio paper. Upper row exposures made in air. Three lower lows exposures made under water.
- Fig. 8. Thermographs and anemometers in a blowout near the shore of Hackberry lake.
- Fig. 9. Thermographs and anemometers in the grassy meadow on the shore of Hackberry lake.
- Fig. 10. Lower end of water photometer showing water tight drum and window covered with ray filter. (Photo by F. H. Shoemaker.)
- Fig. 11. Upper end of water photometer showing lever by means of which successive areas of the photographic plate may be exposed to the window. (Photo by F. H. Shoemaker.)
- Fig. 12. Above, under side of upper half of drum showing perforated, revolving disk to which photographic plates are attached by means of two clips. (Photo by F. H. Shoemaker.)
- Fig. 12. Below, upper half of water tight drum with lower half and tube removed. (Photo by F. H. Shoemaker.)
- Fig. 13. Clear lake from a sandhill at its southwest end. At the right in the distance a narrow strip of Willow lake. (X) the location of springs shown in fig. 14.
- Fig. 14. Pockets of spring water on the southwest shore of Clear lake.
- Fig. 15. Chart showing: A, temperature of air; B, temperature of soil 8 inches below surface; and C, temperature of surface soil at a station in a blowout near the shore of Hackberry lake. Also D, temperature of air in shade of a building on the lake shore; E, wind velocity at the rim of the blowout; and F, wind velocity at the bottom of the same blowout. Numbers at the left indicate, above temperature in Fahrenheit and below miles per hour of wind velocity.
- Fig. 16. Chart showing: A, temperature of air; B, temperature of soil eight inches below the surface; and C, temperature of surface soil at a station in the grass near the lake shore; also D, temperature of air in the shade of a building on the lake shore; and E, wind velocity for the same period. Numbers at left indicate, above temperature in Fahrenheit and below miles per hour of wind velocity at the station.



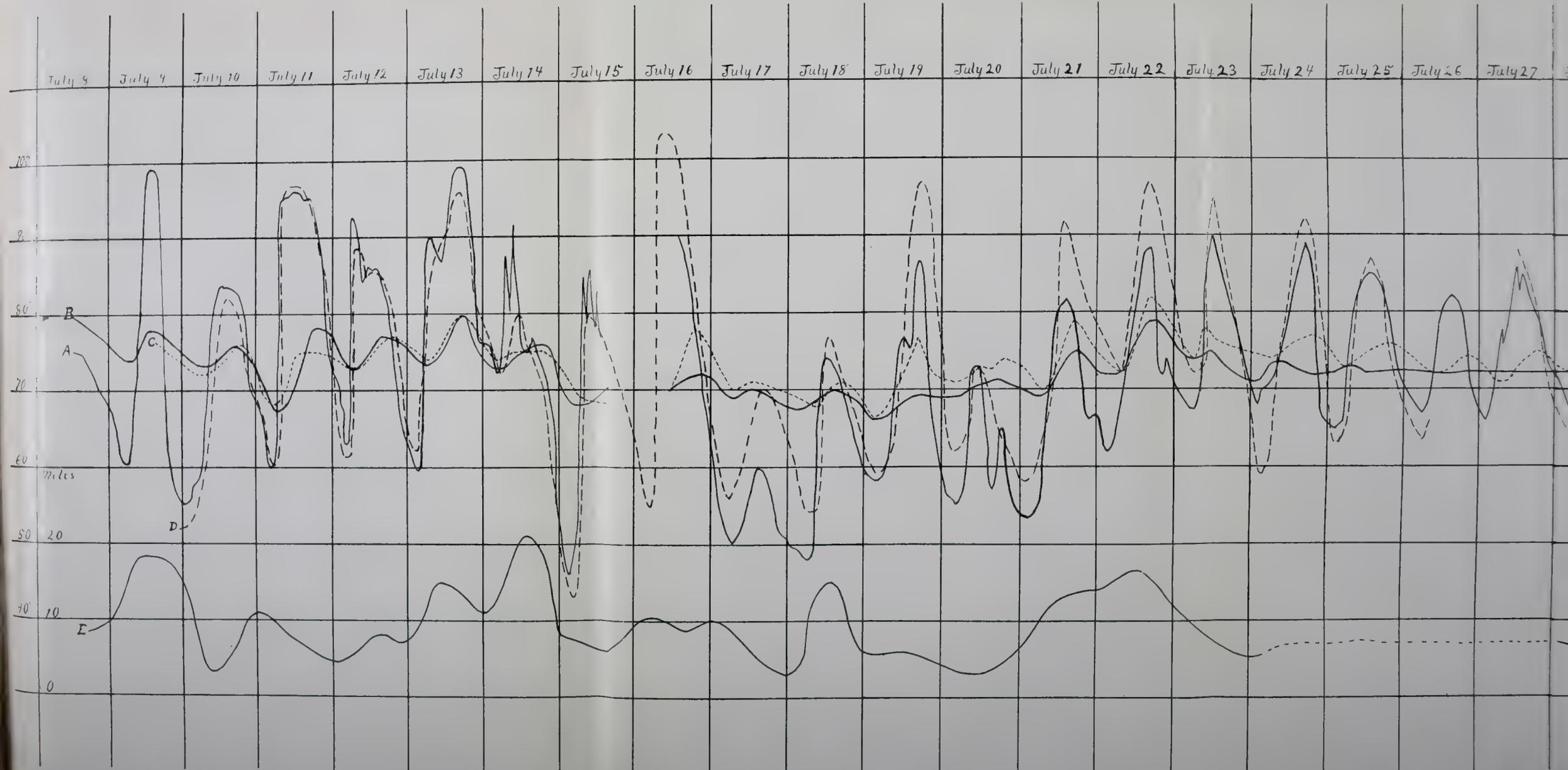


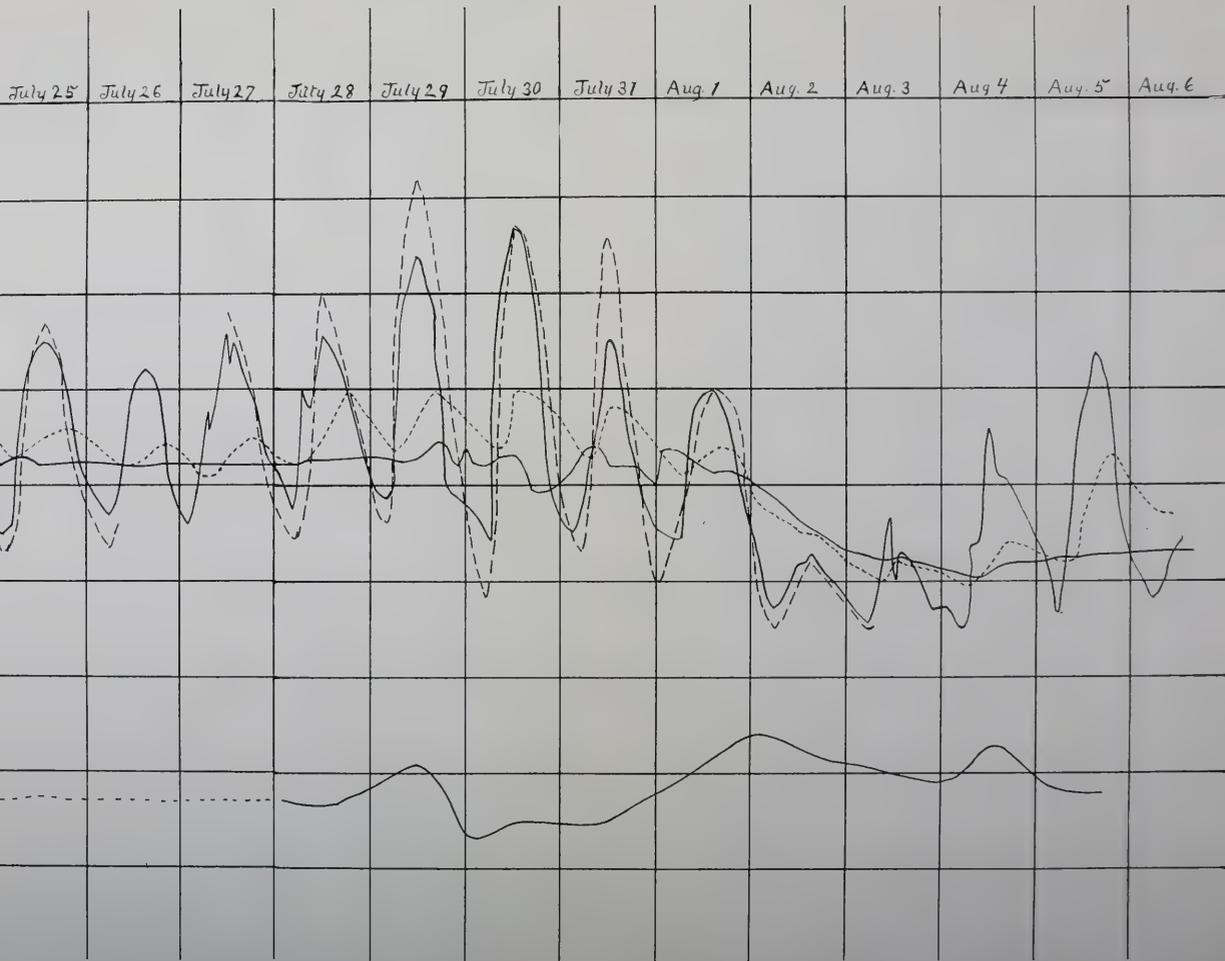
TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY  
VOL. XXXIX





74<sup>c</sup>





INSERT  
D-OUT  
R MAP  
HERE!



Fig. 17. Chart showing: A, air temperature; B, temperature of water at the bottom of lake (3 feet below surface the first week,  $4\frac{1}{2}$  feet below surface the second and third week, and 5 feet below surface the fourth week); C, temperature of water at surface of lake at a station located in a boat anchored in the lake; D, temperature of air in shade of a building on the lake shore; E, wind velocity at margin of lake. Numbers at left indicate above temperature in Fahrenheit and below miles per hour of wind velocity. July 24-27 anemometer readings were not taken. It was a period of very low wind and is indicated approximately by the dotted line.

*The University of Nebraska,  
Lincoln, Nebr.*

## 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.]

### LEECHES CONSIDERED AS OLIGOCHAETA MODIFIED FOR A PREDATORY LIFE

Michaelsen (Mitt. Zool. Mus. XXXVI, Hamburg, 1919) was led to a study of the relationships between these two groups of animals, by noticing a figure in a recent paper on Sudanese Hirudinea. The figure represented an organ that was interpreted by the author, as a diverticulum of the alimentary tract of the leech, opening to the exterior on the mid-dorsal surface of the 13th somite. Similar organs have been described in certain leeches from Sumatra, in which they are paired, and the external pores are ventrally situated. The figured organ strongly resembles the spermathecae of certain oligochaete species in the families of Enchytraeidae and Lumbriculidae, in which the spermathecae communicate internally with the alimentary tract. Similar relations have also been found in certain species of other families of Oligochaeta.

As a result of his studies, Michaelsen has reached the conclusion that the Hirudinea are, in reality, Lumbriculidae which have undergone special modifications in adaptation to a predatory mode of life. He believes that such a conclusion receives much support from a careful comparison of the structure of two intermediate types of worms: the Branchiobdellidae, and *Acanthobdella peledina* Grube. The former are parasitic in the gill chambers and on parts of the surface of crawfishes, and, as their name indicates, were formerly included with leeches; but recently their closer relationship with the Oligochaeta is generally admitted. *Acanthobdella peledina* is a peculiar leech-like parasite of certain fishes of the genus *Salmo*, in northeastern Europe, and in western Siberia. On the ventral surface

of several anterior somites, are paired bundles of setae, and the characters of the reproductive organs and of the body cavity are also nearer to those of the Oligochaeta than to those of the leeches. Michaelsen concludes that, although there is some justification for including these two groups in the family Lumbriculidae, it is nevertheless preferable to recognize them as two distinct families of Oligochaeta, Branchiobdellidae and Acanthobdellidae closely related to the Lumbriculidae. After making this disposition of these two groups, the author makes a comparison of the various structural characters of the Hirudinea and Oligochaeta.

Attention is called to the fact that there is a wide range of variation among different representatives of the Oligochaeta, and that most of the characters which one is accustomed to think of as typical of the Oligochaeta are not present in all members of the group, though they may be in a majority of the better known ones. It is also shown that many of the characters of Hirudinea which one is likely to assume as distinguishing them from Oligochaeta, may be found present in certain members of the latter group. Absence of setae occurs in a genus of the oligochaete family Enchytraeidae, as well as in Branchiobdellidae, and they are greatly reduced in numbers and size in various other representatives. As previously mentioned, four pairs of well developed setae are present on each of several anterior somites in *Acanthobdella peledina* which has previously, without question, been assumed to belong to the Hirudinea. The shortened body and thickened body wall of the leeches, with a correlated reduction of the body cavity, are already forecast in Chaetogaster and in certain species of Lumbriculidae, to say nothing of the Branchiobdellidae and Acanthobdella. They are natural accompaniments of a change of food, and assumption of a predatory mode of life.

There is great variation in the structure of the nephridia among the Oligochaeta, and absence of ciliated nephrostomes and of cilia in the excretory part of the ducts is found in species of diverse groups. The ventro-median position of the pores of the efferent ducts of the reproductive organs of leeches has a counterpart in certain species of Lumbriculidae and of the earthworm subfamily Eudrilinae.

The most significant character which distinguishes the Hirudinea, in general, from the Oligochaeta, is the position of the spermaries

in somites posterior to the one which contains the ovaries. This relative position of the two kinds of gonads is the opposite of that normally found in Oligochaeta, and in the connecting forms, Branchiobdellidae and Acanthobdella. To account for this reversal of relations, the author refers to instances where Oligochaeta are found with a considerable number of consecutive somites containing gonads; and also to papers by different writers, in which gonads of certain oligochaete species have been shown to produce one kind of germ cells at one time, and at other times to produce those of the opposite kind. From individuals with series of gonads of this type, he thinks it not improbable that there may have been derived descendants in which the relative position of the gonads of the two sexes is in the reverse order from that of the ancestors. For details of structure and references to the literature involved in these comparisons, the original paper must be consulted.

The author thinks it desirable to modify the outlines of classification, to fit these new views of relationship. He proposes a class Clitellata which is co-ordinate with the class Chaetopoda, and with three other classes which contain marine forms and are not involved. The class Clitellata includes two orders, Oligochaeta and Hirudinea; distinguished chiefly by the differences in the degree of development of the body cavity, and the relative order of the gonads. The class Chaetopoda includes two orders, Protochaeta and Polychaeta.

FRANK SMITH

*Department of Zoology,  
Univ. of Illinois*

# PROCEEDINGS OF THE AMERICAN MICROSCOPICAL SOCIETY

## MINUTES OF THE ST. LOUIS MEETING

The thirty-eighth annual meeting of the American Microscopical Society was held in affiliation with the A.A.A.S. at St. Louis, Mo., Dec. 31, 1919.

In the absence of President Griffin, Vice-President Whelpley acted as chairman.

The report of the Treasurer for the years 1918 and 1919 was accepted and referred to an auditing committee composed of Professors H. B. Ward and H. J. VanCleave.

The report of the Custodian was accepted, ordered printed, and referred to an auditing committee composed of Messrs. Edw. Pennock and Edw. P. Dolbey.

A vote of appreciation was extended to Professor T. W. Galloway, the retiring Secretary, for a most valuable service rendered to the Society during the past ten years.

The meeting voted approval of the action of the Executive Committee in appointing Mr. Wm. F. Henderson as Treasurer, and Professor Paul S. Welch as Secretary at dates in advance of the regular annual business meeting.

The following officers were duly nominated and elected for the constitutional periods: President, Professor T. W. Galloway, New York; First Vice-President, Chancey Juday, University of Wisconsin; Second Vice-President, Professor A. D. MacGillivray, University of Illinois; Secretary, Professor Paul S. Welch, University of Michigan; Treasurer, Mr. Wm. F. Henderson, James Millikin University.

Professor Frank Smith of the University of Illinois, Professor J. E. Ackert of the Kansas State Agricultural College, and Dr. B. H. Ransom of the Bureau of Animal Industry were chosen as the elective members of the Executive Committee for 1920.

Minutes of the last annual meeting were approved as printed.

Adjourned.

PAUL S. WELCH,  
*Secretary*

### CUSTODIAN'S REPORT FOR THE YEARS 1918 AND 1919 SPENCER—TOLLES FUND

Amount reported December 1917.....	5331.57
June 30, 1918 Dividends.....	159.93
Dec. 31, 1918 Dividends.....	164.73
June 30, 1919 Dividends.....	226.24
Dec. 31, 1919 Dividends.....	176.46
	727.36
	6058.93
less Grant No. 6.....	100.00
	5958.93
Net amount invested.....	5958.93
Increase during last two years \$627.36.	

## TOTALS

All contributions.....	800.27
All Sales of Transactions.....	878.38
All Life memberships.....	300.00
All Interest & Dividends.....	4270.28

## LESS

All Grants.....	250.00
All Dues for Life members.....	40.00
	<u>290.00</u>
	5958.93

Life members: (Robert Brown, dec'd.); J. Stanford Brown; Seth Bunker Capp; Harry B. Duncanson; A. H. Elliott; John Hatly.

Contributors of \$50 and over; John Aspinwall; Iron City Microscopical Society, Magnus Pflaum; Troy Scientific Society.

(Signed) M. PFLAUM,  
*Custodian.*

Philadelphia, Pa., January 10, 1920.

The undersigned having examined the foregoing report certify that we find the amount invested as shown therein \$5958.93—correct, as shown by the Pass-Book of the Keystone State B. & L. Association, the same being brought down to the 2nd instant inclusive.

(Signed) EDWARD PENNOCK,  
EDW. P. DOLBEY,  
*Auditing Committee.*

ANNUAL REPORT OF THE TREASURER OF THE AMERICAN  
MICROSCOPICAL SOCIETY

DECEMBER 22, 1917 TO DECEMBER 21, 1918

## RECEIPTS

Balance on hand from audit of 1917.....		\$770.57
Membership dues.....		548.84
Back volumes.....	\$ 34.84	
For 1918.....	226.00	
For 1919.....	286.00	
For 1920.....	2.00	
Subscriptions.....		\$ 370.40
Back volumes.....	\$ 50.00	
Volume 36.....	95.00	
Volume 37.....	140.20	
Volume 38.....	83.20	
Volume 39.....	2.00	
Initiation fees.....		\$ 15.00
Advertisers volume 36.....		55.00
volume 37.....		120.00
Sundries.....		1.09
		<hr/>
TOTAL RECEIPTS.....		\$1,880.90

EXPENDITURES

Publishing Transactions.....		\$877.82
Volume 36, number 4.....	\$293.82	
Volume 37, number 1.....	182.57	
Volume 37, number 2.....	181.65	
Volume 37, number 3.....	171.05	
Plates.....	48.73	
Postage and Express.....		\$59.48
Office Expenses.....		35.27
Secretary.....	\$23.17	
Treasurer.....	12.10	
Miscellaneous items.....		11.85
		<hr/>
TOTAL EXPENDITURES.....		\$ 984.42
Balance on hand.....		896.48
		<hr/>
		\$1,880.90

Respectfully submitted,

H. J. VAN CLEAVE, *Treasurer.*

REPORT OF OUTGOING TREASURER FOR THE PERIOD OF DEC. 22, 1918  
TO FEB. 28, 1919

RECEIPTS

Balance from 1918.....		\$896.48
Membership dues.....		72.00
Back volumes.....	\$10.00	
For 1919.....	60.00	
For 1920.....	2.00	
Subscriptions.....		47.40
Back volumes.....	\$ 4.00	
Volume 38.....	41.00	
Volume 39.....	2.40	
Initiation fees.....		9.00
Advertisements volume 37.....		25.00
		<hr/>
TOTAL RECEIPTS.....		\$1,049.88

EXPENDITURES	
Publication of Transactions Volume 37, number 4.....	\$267.14
Postage and Express.....	25.26
Office expenses.....	23.96
Secretary.....	\$ 5.55
Treasurer.....	18.41
Miscellaneous.....	3.50
<hr/>	
TOTAL EXPENDITURES.....	\$ 319.86
Balance transferred to new Treasurer.....	730.02
<hr/>	
	\$1,049.88

Respectfully submitted,

H. J. VAN CLEAVE, *Treasurer.*

REPORT OF AUDITING COMMITTEE ON TREASURER'S  
ACCOUNTS FROM DECEMBER 22, 1917 TO FEBRUARY 28, 1919

This is to certify that we have this day examined the accounts of H. J. Van Cleave, Treasurer, and have checked the vouchers against payments; we find the accounts correct and have verified the bank balance of \$730.02 to be transmitted to the incoming treasurer.

HENRY B. WARD

FRANK SMITH

*Audit Committee.*

ANNUAL REPORT OF THE TREASURER  
OF THE AMERICAN MICROSCOPICAL SOCIETY  
March 1, 1919 to December 24, 1919

RECEIPTS	
Balance received from former treasurer.....	\$ 730.02
Dues received from Volume 37 or before.....	36.00
Dues received from Volume 38.....	108.00
Dues received from Volume 39.....	292.10
Dues received from Volume 40.....	2.00
Initiation fees.....	66.00
Subscriptions for Volume 37 or before.....	16.00
Subscriptions for Volume 38.....	51.80
Subscriptions for Volume 39.....	34.00
Sales of Transactions, duplicates and back numbers.....	183.00
Donation from T. B. Magath for aid in publishing his paper.....	50.00
Advertising for Volumes 38 and 39.....	55.00
Sundries.....	4.50
<hr/>	
TOTAL.....	\$1,628.42

EXPENDITURES

Printing Transactions, Volume 38, No. 1 . . . . .	\$201.03
Printing Transactions, Volume 38, No. 2 . . . . .	265.21
Printing Transactions, Volume 38, No. 3 . . . . .	337.47
Plates for Volume 38, No. 3 . . . . .	76.30
Printing Author's Reprints . . . . .	20.54
Postage and Express for Secretary . . . . .	73.92
Postage and Express for Treasurer . . . . .	22.09
Office expenses of Secretary . . . . .	126.45
Office expenses of Treasurer . . . . .	19.70
Sundries . . . . .	5.67
Balance on hand . . . . .	480.04
<hr/>	
TOTAL CREDITS . . . . .	\$1,628.42

W. F. HENDERSON, *Treasurer*

This is to certify that we have examined the books and vouchers of the Treasurer and find them to be in good condition and to give a correct record of moneys received and expended as indicated.

HENRY B. WARD

H. J. VAN CLEAVE

*Auditing Committee*

March 24, 1920.



93<sup>m</sup>

TABLE OF CONTENTS

FOR VOLUME XXXIX, Number 2, April, 1920

Modern Dark-field Microscopy and the History of its Development, by Simon Henry Gage . . . . .	95
A New Bladder Fluke from the Frog, with Plate XIII, by John E. Guberlet	142
Labeling Illustrations, with Plates XIV to XVII, by Z. P. Metcalf . . . . .	149
Notes and Reviews: Position of Micropterygidae; Micropterygidae; Filariasis in U. S.; Polyembryony and Sex; Origin and Significance of Metamorphosis	163



TRANSACTIONS  
OF  
American Microscopical Society

(Published in Quarterly Instalments)

Vol. XXXIX

APRIL, 1920

No. 2

MODERN DARK-FIELD MICROSCOPY  
AND THE  
HISTORY OF ITS DEVELOPMENT

BY

SIMON HENRY GAGE

Professor of Histology and Embryology, Emeritus Cornell University

INTRODUCTION

In most work with the microscope the entire field of view is lighted and the objects to be studied appear as colored pictures or as shadows—in extreme cases, as silhouettes—on a white ground. As the field is always light, this has come to be known as Bright-Field Microscopy (Fig. 1).

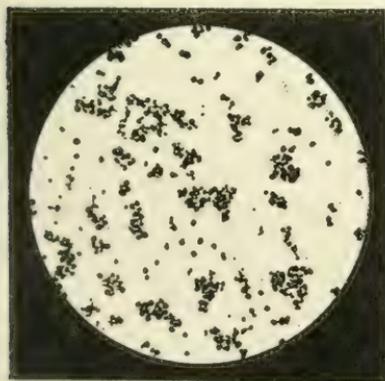


Fig. 1

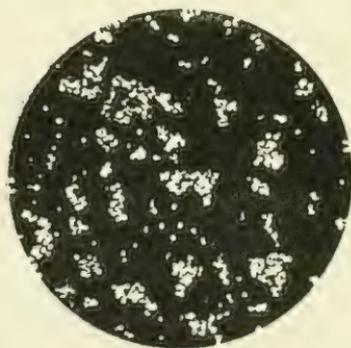


Fig. 2

Bright- and dark-field photo-micrographs of the same objects (starch grains).

In contrast with this is Dark-Field Microscopy in which the field is dark, and the objects appear as if they themselves emitted the light by which they are seen (Fig. 2).

The study of objects in a bright-field probably comprises 95% of all microscopic work, and is almost universally applicable. On the other hand dark-field microscopy has only limited applicability, and yet from the increased visibility given to many objects it is coming to be appreciated more and more.

*Definition.*—In its comprehensive sense, Dark-Field Microscopy is the study of objects by the light which the objects themselves turn into the microscope, and none of the light from any outside source passes directly into the microscope as with bright-field microscopy.

There are two principal cases: (A) The objects which are truly self-luminous like phosphorescent animals and plants; burning or incandescent objects, and fluorescent objects. (B) The objects which emit no light themselves, but which deflect the light reaching them from some outside source into the microscope.

These two groups are well represented in Astronomy. If one looks into the sky on a cloudless night, the fixed stars show by the light which they themselves emit, but the moon and the planets appear by the light from the sun which they reflect to the earth, the sun itself being wholly invisible at the time. As there is relatively very little light coming from the intervening space between the stars and planets, all appear to be self-luminous objects in a dark field. This reference to the sky at night will serve to bring out two other points with great clearness: (1) The enhanced visibility. Everybody knows that there are as many stars in the sky in the daytime as at night, but they are blotted out, so to speak, by the flood of direct light from the sun in the daytime, while at night when these direct rays are absent and no light comes from the back-ground the stars and the planets show again by the relatively feeble light which they send to the earth.

(2) The other point is that in dark-field microscopy the objects must be scattered, not covering the whole field (Fig. 2). If there were no intervening empty space the whole face of the sky would look bright

It will be seen from this that ordinary sections or other objects so large that they fill the whole field of the microscope cannot be studied advantageously by the dark-field method, for they would make the whole field bright. But for the liquids of the body, blood, lymph, synovial, and serous fluids, fluid from the cavities of the

nervous system, saliva, and all other mucous fluids, and isolated tissue elements where the solid or semi-solid substances are distributed in a liquid, the appearances given by this method are a revelation as was pointed out by Wenham and Edmunds and many others over fifty years ago. No less is the revelation coming from the study of bacteria, protozoa and other micro-organisms in the dark field.

#### DARK-FIELD AND ULTRA-MICROSCOPY

In both of these the objects seem to be self-luminous in a dark field, and no light reaches the eye directly from an outside source, but only as sent to the eye from the objects under observation.

The terms simply represent two steps, and merge into each other.

Dark-Field Microscopy deals with relatively large objects,  $0.2\mu$  or more in diameter, that is, those which come within the resolving power of the microscope.

Ultra-Microscopy deals with objects so small that they do not show as objects with details, but one infers their presence by the points of light which they turn into the microscope. This can be made clear by an easily tried-naked-eye observation. Suppose one is in a dark room, and a minute beam of brilliant light like sunlight or arc light is directed into the room. Unless one is in the path of this beam of light it will remain invisible, but if there are vapor or dust particles present they will deflect some of the light toward the eye and will appear as shining points. The character of the particles cannot be made out, but the points of light they reflect indicate their presence. As Tyndall used this method in determining whether a room was free from dust in his experiments in spontaneous generation, the appearance of the shining dust particles is sometimes called the "Tyndall effect."

The two forms are said to merge, because in studying objects like saliva, etc., with the microscope designed especially for dark-field work, some of the objects seen will show details, but some are so small that they show simple as points of light usually in the form of so-called diffraction discs. The larger objects in the saliva come in the province of dark-field microscopy, and the smallest ones, of ultra-microscopy, and in this case the instrument used might with equal propriety be called a dark-field or an ultra-microscope.

The great purpose of the dark-field microscope is to render minute objects or details of large objects plainer or actually visible

from the advantages offered by the contrast given between the brightly lighted objects and the dark background. For example, with the homogeneous immersion objective the study of fresh blood with the ordinary bright-field method enables one to see the red corpuscles with satisfaction, but the leucocytes are not easily found and the blood-dust (chylomicrons) and the fibrin filaments are not seen at all or very faintly. With the same microscope using the dark-field illumination the leucocytes are truly white cells, and the blood-dust is one of the striking features of the preparation, and the fibrin filaments seem like a delicate cobweb.

In this connection, perhaps a few words should be added on the terms Resolution and Visibility. Both came over from the ancient science of astronomy, and are properly used only when restricted as in astronomy.

By resolution is meant the seeing of two things as two, not blended. For example if two stars are close together they are resolved if they appear as two. When the telescope was invented it was found that many stars that appeared single were really two stars close together. If two lines are placed close together they appear as two to the naked eye when close up, but as one moves away the lines seem to fuse and make one. Visibility refers only to the possibility of seeing a thing. In the above examples the twin stars were visible to the naked eye but not resolved into two, and likewise the lines were long visible after they could be seen as two lines. Now the purpose of the ultra-microscope is solely to increase the visibility of small particles without reference to their details of structure. Dark-field microscopy, on the other hand, while it gives greatly increased visibility, also gives resolution of details.

As with bright-field microscopy the resolution of details of structure depends directly upon the numerical aperture (NA) of the objective, and the brightness upon the square of the aperture (NA<sup>2</sup>).

#### METHOD OF DARK-FIELD MICROSCOPY

In this article the ultramicroscope and the study of self-luminous objects will not be further considered, but the discussion will be limited to objects which must be lighted by some outside source.

There are two principal cases: (1) objects which are lighted from above the stage of the microscope or by so-called direct light (Fig. 3)

and, (2) objects which are lighted from below the stage, or by transmitted light (Fig. 4).

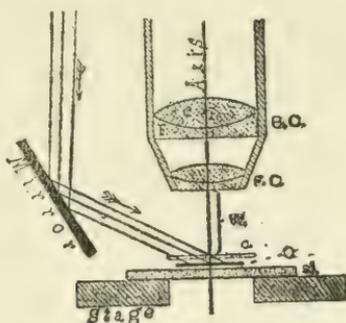


Fig. 3. Light from above the stage. (From *The Microscope*)

In both cases the light from the source is at such an angle that none of it can enter the objective directly but only as it is deflected or "radiated" by the objects in the microscopic field.

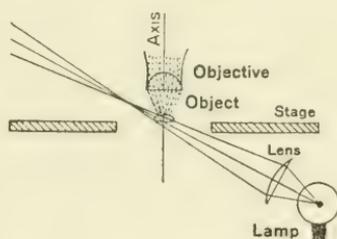


Fig. 4. Light from below the stage.

When the light upon the object is from above the stage the background must be non-reflecting. If the background were white there would be a kind of bright-field, not dark-field microscopy.

The black-background is secured either by placing the object directly upon some black velvet or other non-reflecting surface, or on a glass slide which in turn is placed upon black velvet, etc., or on a dark well. The simplest way to produce a dark-well is to turn the condenser aside and place a piece of black velvet over the foot of the microscope. Or the condenser can be lowered well and the velvet put over the top of the condenser.

Diffuse daylight from a window, or more satisfactorily, artificial light directed by a mirror or lens (bull's eye), is directed obliquely down upon the preparation (Fig. 3). Exactly the same preparation will answer for light from below the stage. In this case the condenser is turned out of the way, and some black-velvet put over the foot of the microscope to cut out stray light.

For a good naked eye demonstration showing the increased visibility due to the dark-field, some cotton may be placed on a piece of black velvet, and a similar tuft of cotton on a white card.

For the special methods of lighting microscopic objects from above the stage, see in the historical summary at the end of this paper.

*Dark-Field Microscopy by Transmitted Light.*—To make objects appear self-luminous in a dark field when illuminated by beams of light from below the stage, two things are necessary:

(1) The objects must be able to deflect in some way the light impinging upon them into the microscope.

(2) None of the light from the source must be allowed to pass directly into the microscope. These conditions are met when (a) the objects to be studied are of different refractive index from the medium in which they are mounted, and (b) when the transmitted light thrown upon the object is at such an angle that it falls wholly outside the aperture of the objective (Fig. 4-7).

The objects deflect the light into the microscope

- (1) By Reflection
- (2) By Refraction
- (3) By Diffraction

Any one of these will suffice, but any two or all of the ways may be combined in any given case.

For low powers where the aperture of the microscope objective is relatively small it is comparatively easy to make the transmitted beam of so great an angle that none of it can pass directly into the microscope. A simple experiment will show this: A 16 mm. or lower objective is used, the substage condenser is turned aside and on the stage is placed a clean slide with a little starch, flour, or other white powder dusted upon it. If now the mirror is turned to throw the light directly up into the microscope the field will be bright and the objects relatively dark, but if the mirror is turned at an angle suf-

ficient to throw the whole beam at a greater angle than the aperture of the objective will receive, the field will become dark and the starch or flour grains will stand out as if shining by their own light. If some black velvet is placed on the foot of the microscope so no light can be reflected upward into the microscope from the foot or the table, the field will be darker. This experiment succeeds by either natural or artificial light. If some water containing paramecium and other micro-organisms is put on the slide and put under the microscope, the organisms will appear bright and seem to be swimming in black ink.

It is readily seen that with the method just discussed the light is all from one side (Fig. 4). To light the objects from all sides, that is, with a ring of light, the simplest method, and the method utilized in all modern dark-field microscopy, is to use a hollow cone of light, the rays in the shell of light all being at so great an angle with the optic axis of the objective that none of them can enter the microscope directly (Fig. 4-7).

*With Refracting Condensers.* With the condensers of the achromatic or chromatic type used for bright-field microscopy a solid cone of rays is used. To get the dark-field effect the objects to be studied must be lighted only by the rays at so great an angle that they cannot enter the objective directly. This requires that the condenser shall have a considerably greater aperture than the objective. The ordinary method of making the hollow cone is to insert a dark stop—central stop—to block or shut off the central part of the solid cone of light. The object is then illuminated with a ring of light of an aperture greater than that of the objective (Fig. 6). Some of this light is turned by the objects into the microscope. As only a relatively small amount of the light is deflected by the objects into the microscope, it is evident that there must be a great deal of light to start with or there will not be enough passing from the object to the microscope to make it properly visible. The question also naturally arises how one is to determine the size of the central stop to be used with any given condenser and objective.

This is easily determined as follows: The field is lighted well as for ordinary bright-field observation and some object is got in focus. Then the object is removed and the iris diaphragm of the condenser opened to the fullest extent. If one then removes the ocular and looks down the tube of the microscope and slowly closes

the iris, when the full aperture of the objective is reached, that is, when the back lens of the objective is just filled with light, the opening in the iris represents the size of the central stop to use to cut out all the light which would pass into the microscope from the condenser; all the ring of light outside of this is of too great an angle for the aperture of the objective. One can measure the size of the opening in the iris with dividers and then prepare a central stop diaphragm.

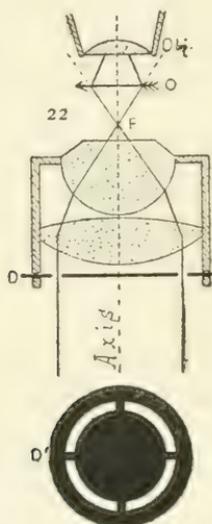


Fig. 5. Ordinary condenser with sectional and face views of the central stop (D). (From *The Microscope*)

A visiting card is good for this. It should be blackened with India ink. To be on the safe side it is wise to make the central stop a little greater in diameter than the iris opening (Fig. 5).

If now the microscope is lighted as brilliantly as possible, and then the iris opened to its full extent and the blackened central stop is put in the ring under the condenser, and a slide used with starch or flour on it, the flour or starch particles will be lighted with the ring of light, and they will deflect enough into the objective to make the objects appear bright as if shining by their own light, the background remaining dark. If the field looks gray or light instead of black it is because the central stop is too small or not centered or the particles used for objects are too numerous, not leaving enough blank space.

One can determine what is at fault thus: The ocular is removed. If the central stop is too small the back lens of the objective will show a ring of light around the outside. If the central stop is not centered there will be a meniscus of light on one side. If the objects are too numerous the whole field will be bright. To verify these statements one can use a specimen with flour or starch all over the slide. It will look dazzlingly light, with the ocular in place and the back-lens will be very bright when the ocular is removed.

For the meniscus of light when the central stop is decentered, purposely pull the ring holding the stop slightly to one side and the meniscus will appear in the back lens. To show the ring of light due to a too small size of the stop, the easiest way is to use a higher objective, say one of 3 or 4 mm. in place of the 16 mm. objective. While it is necessary to eliminate all the light which could enter the objective directly, the thicker the ring of light which remains to illuminate the objects the more brilliantly self-luminous will they appear, therefore one uses only the stop necessary for a given objective. If one makes central stops for the different objectives as described above it will be greatly emphasized that the objectives differ in aperture, in general the higher the power the greater the aperture, and consequently the larger must be the central stop, and the thinner the ring of light left to illuminate the object. As one needs more light for high powers instead of less than for low powers, the deficiency of light caused by the large central stop must be made good by using a more brilliant source of light for the high powers.

*Reflecting Condensers.* As was first pointed out by Wenham, 1850-1856, refracting condensers are not so well adapted for obtaining the best ring of light for dark-field work as a reflecting condenser, on account of the difficulty in getting rid of the spherical and chromatic aberration in the refracted bundles of such great aperture. He first (1850) used a silvered paraboloid and later (1856) one of solid glass as is now used. Within the last 10-15 years there has also been worked out reflecting condensers on the cardioid principle. The purpose of all forms is to give a ring of light which shall be of great aperture, and be as free as possible from chromatic and spherical aberration, and hence will form a sharp focus of the hollow cone upon the level where the objects are situated.

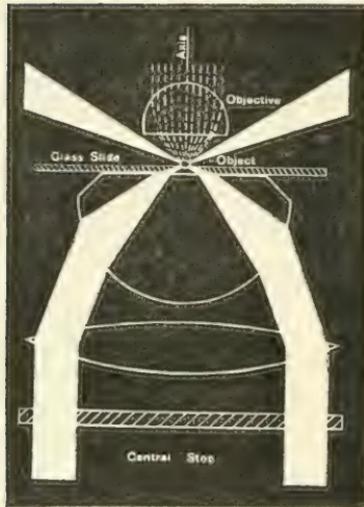


Fig. 6. Bright-field condenser with central stop to give dark-field illumination.

This is a sectional view showing the hollow cone of light focusing on the object and then continuing wholly outside the aperture of the objective.

The light deflected by the object into the aperture of the objective is represented by broken lines.

The glass slide is in homogeneous contact with the top of the condenser, and the medium beyond the object is represented as homogeneous with glass.

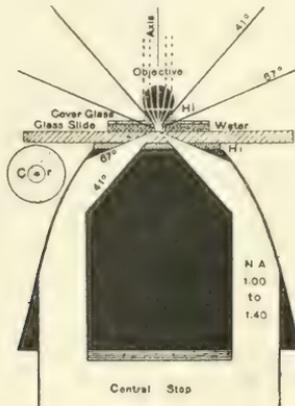


Fig. 7. Paraboloid condenser for dark-field illumination.

Axis—The principal optic axis of the microscope.

Central Stop—The opaque stop to cut out all light that would be at an aperture less than 1.00 NA.

Cover Glass—The cover for the object. For dry objectives it must conform to the objective, and with homogeneous objectives it must be less than their working distance in thickness.

C r—Face view of the top of the paraboloid showing the centering ring, the spot of white ink in the middle and the grains of starch for centering and focusing high powers.

Glass Slide—The slip of glass on which the object is mounted. It is connected with the top of the paraboloid by homogeneous liquid, and must be of a thickness to permit the focusing of the hollow cone of light upon the object.

Hi, Hi—Homogeneous liquid between the cover-glass and the objective and between the top of the condenser and the slide.

NA 1.00 to 1.40—The numerical aperture of the hollow cone of light focused on the object by the paraboloid. As indicated on the left this is represented by a glass angle of 41 to 67 degrees.

41° 67°—The limits of the angle of the rays in glass. Objective—The front lens of the objective. The light rays deflected by the object are indicated by white lines below and through the lens, then by broken, black lines above the front lens of the objective. Water—The mounting medium for the objects.

In this diagram the course of the rays from the paraboloid are indicated as if the objects were mounted in homogeneous liquid and that the rays passed beyond the focus into a medium homogeneous with glass.

TABLE SHOWING THE MAXIMUM ANGLE IN GLASS, AND THE CORRESPONDING NUMERICAL APERTURE OF THE LIGHT WHICH CAN PASS INTO MEDIA OF DIFFERENT REFRACTIVE INDEX ABOVE THE CONDENSER (FIG. 8-11)

	Angle in Glass	Numerical Aperture	Index of Refraction
1. Air over the condenser.....	41°	1.00	1.00
2. Water.....	61°	1.33	1.33
3. Glycerin.....	75° 15'	1.47	1.47
4. Homogeneous liquid.....	90°	1.52	1.52

In the reflecting as in the refracting condensers the central part of the light beam from the source is blocked out by a central stop and only a ring of light enters the condenser.

Immersion connection of condenser and glass slide bearing the specimen.—While the purpose of the reflecting condenser is to produce a very oblique beam of light for illuminating the objects, it is seen at once that the laws of refraction will prevent the light from passing from the condenser to the object unless the glass slide bearing the object is in immersion contact with the top of the condenser.

That is, for air (index 1.00) above the condenser, the rays in glass at  $41^\circ$ , NA 1.00 and less can pass from the condenser into the air and expand into a hemisphere of light in it (Fig. 8). Rays above  $41^\circ$  are totally reflected back into the condenser.

For water (index 1.33) above the condenser, rays in the glass at  $61^\circ$ , NA 1.33, and less can pass into the overlying water and make a complete hemisphere of light in it (Fig. 9). Rays above  $61^\circ$  are totally reflected back into the condenser.

For glycerin (index 1.47) above the condenser, rays in the glass at  $75^\circ 15'$ , NA 1.47 and less can pass from the glass into the overlying glycerin and form a hemisphere of light in it (Fig. 10). All rays at a greater angle are reflected back into the condenser.

For homogeneous liquid (index 1.52) over the condenser, there is no limit to the angle of light that can pass from the condenser to it (Fig. 11).

*Immersion Liquid between Condenser and Glass Slide.* While water or glycerin answers fairly well it is recommended that homogeneous liquid be used in all cases. At first glance this would seem unnecessary for, as just stated the aperture of the light is limited by the medium of least refractive index between the condenser and the object. Thus objects mounted in watery fluids, and especially those mounted in air would seem to have the illuminating ray that could reach them limited by an aperture of 1.33 in one case and of 1.00 in the other (glass angles of  $61^\circ$  and  $41^\circ$ ). This would be true if the objects were suspended in the water or in the air, but many of the particles are not suspended but rest on the glass slide, that is are in so-called *optical contact* with the slide. This being true, the angle of the light which can pass from the condenser to them depends upon their own refractive index, and not upon that of the mounting medium (air or water). This explains also why objects not in optical contact with the slide are rendered more visible by the homogeneous immersion contact of slide and condenser for the scattered light from the particles in optical contact helps to light up particles not in contact.

Another consideration also favors the use of the homogeneous immersion contact of slide and condenser, even for objects mounted in air. Physicists have found (see Wood) that beyond the critical angle, while all light is turned back into the denser medium, it does nevertheless pass one or more wave lengths into the rarer medium to

find, so to speak, an easier place to turn around in. If now any object is near enough the slide to fall into this turning distance of the totally reflected light it may be said to be in optical contact, and the light which meets it will pass into it instead of being totally reflected.

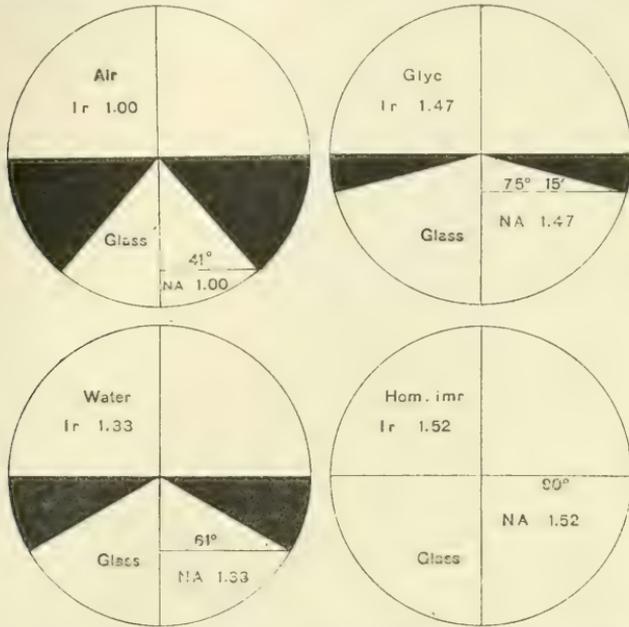


Fig. 8, 9, 10, 11. Diagrams showing the angle and numerical aperture of the light in glass to fill the entire hemisphere above, with overlying media of air, water, glycerin, or homogeneous immersion liquid.

As shown by the diagrams, the NA of the light in each case must equal the index of refraction (Ir) of the overlying medium to fill the overlying hemisphere with light. If the light is at a greater than the critical angle it is reflected back into the condenser. Such light is represented by black in 8, 9, 10. With homogeneous liquid (Hom. imr) above the condenser there is no critical angle.

It should be said in passing that the medium of least refractive index in the path of the light beam from the condenser determines the critical angle at which the light is wholly reflected, and hence determines the maximum angle of the illuminating pencil that can light the object, but this does not apply if the object is in optical contact with the glass (see below).

One can make a very convincing experiment to show the importance of remembering that some of the objects are in optical contact with the glass slide and hence may utilize light which could not pass

into the surrounding medium. If the upper face of the dark-field condenser is cleaned as perfectly as possible, and then lighted well, one can see no light emerging from the top except where the centering ring is situated or where there are some accidental scratches. If one dusts some starch, flour or other white powder on the clean surface, the particles which make optical contact with the glass will glow as if self-luminous. In case one wishes further evidence, the end of the condenser should be carefully cleaned, and a glass slide of the proper thickness connected with it by means of homogeneous liquid, then some flour or starch can be dusted on the slide and it will glow as did the particles on the top of the condenser. These demonstrations show well with the naked eye and with objectives up to 8 mm. (Fig. 7, Cr.)

*Aperture of the Ring of Light in the Condenser.* As the angle of the light illuminating the objects must be greater than can enter the objective employed it follows that the central part of the illuminating beam must be blocked out up to or beyond the aperture of the objective to be used. The greatest aperture rays possibly attainable depends upon the opticians ability to so design and construct the condenser that it will bring the remaining shell or ring of light to a focus. For those designed to be used with all powers, the aperture of this ring of light usually falls between 1.00 NA and 1.40 NA. As water and homogeneous immersion objectives have a numerical aperture greater than 1.00 NA. it follows that they could not be used for dark-field observation with their full aperture, because much of the light from the condenser could enter the objective, giving rise to a bright or at least a gray field.

*Reducing diaphragms for high apertured objectives.* As the lower limit in aperture of dark-field condensers is 1.00 NA, and sometimes even lower, it follows that a condenser for use with all objectives requires that none of them have an aperture over 1.00 NA. As all modern immersion objectives have an aperture greater than 1.00 NA, this aperture must be reduced by inserting a diaphragm in the objective.

The general law that the resolution varies directly with the aperture, and the brilliancy as the square of the aperture, holds with dark-field as with bright-field microscopy. In order to determine by actual experiment with various dark-field condensers the best aperture of the diaphragm to select, the writer requested, the Bausch & Lomb Optical Company and the Spencer Lens Company to supply

reducing diaphragms for their fluorite, homogeneous immersion objectives ranging from 0.50 NA. to 0.95 NA. As measured by me these diaphragms ranged from slightly above 0.50 NA, to 0.97 NA. These varying apertures were tested on each condenser, using the same light and as nearly as possible identical preparations (i.e., fresh blood mounted on slides of the proper thickness). It seemed to the

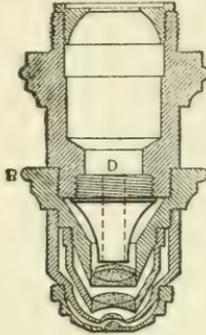


Fig. 12. Large aperture objective with diaphragm to reduce the aperture to less than 1.00 NA. (From Chamot)

D Funnel-shaped reducing diaphragm in the interior of the objective above the back lens.

writer that the law of aperture as stated above held rigidly. The question then is, which aperture shall be chosen if but one diaphragm is available? It seemed to the writer that the one of 0.80 NA should be chosen, at least for these fluorite objectives. If three are to be had the range should be 0.70, 0.80 and 0.90. The reason why one over 0.90 is not recommended is because some examples of the best of the dark-field condensers tested, seemed to have their lower limit somewhat below 1.00 NA, and hence the field could not be made completely dark with the diaphragm of 0.97 NA. With others, however, the field was as dark with this large aperture as with the lower apertured diaphragms.

A considerable range of reducing diaphragms for the homogeneous immersion objectives is recommended because all experience brings home to the worker with the microscope the conviction that some structures show better with the lower apertures and some with higher ones, and it is believed from considerable experience that the same fundamental principles hold in dark-field as in bright-field microscopy.

## LIGHTING FOR DARK-FIELD MICROSCOPY

As is almost self-evident, only a very small amount of the light passing through the condenser to the objects is deflected by the objects into the microscope, consequently the source of light must be of great brilliancy or there will not be enough to give sufficient light to render the minute details of the objects visible, when high powers are used. This visibility of minute details involves three things: (1) The aperture of the objectives; (2) The aperture of the illuminating pencil; (3) The intensity of the light.

The most powerful light is full sunlight. Following this is the direct current arc, the alternating current arc and then the glowing filament of the gas-filled or Mazda lamps.

The reflecting condensers are designed for parallel beams consequently the direct sunlight can be reflected into the condenser with the plane mirror of the microscope. If the arc lamp, a Mazda lamp, or any other artificial source is used a parallelizing system must be employed. The simplest and one of the most efficient is a plano-convex lens of about 60 to 80 mm. focus with the plane side next the light and the convex side toward the microscope mirror (Fig. 14) i.e., in position of least aberration. This is placed at about its principal focal distance from the source whether that be arc lamp, Mazda lamp, or any other source and the issuing beam will be of approximately parallel rays. These can then be reflected up into the dark-field condenser with the plane mirror.

## LAMPS FOR DARK-FIELD MICROSCOPY

Up to the present the small arc lamp (Fig. 13), using 4 to 6 amperes is practically the only one considered really satisfactory. There is no question of the excellence of the direct current arc. The alternating current arc has two equally bright craters which renders its use somewhat more difficult.

For most of the work in biology the arc gives more light than is comfortable to the eyes; but a still greater objection is that with the burning away of the carbons the source of light is constantly shifting its position, and hence the quality of the light varies from minute to minute. A third difficulty for hand-feed lamps is that one must stop observation frequently to adjust the carbons.

In spite of all these difficulties, however, the arc lamp is indispensable if one desires to attack all the problems for which the dark-field microscope is available.

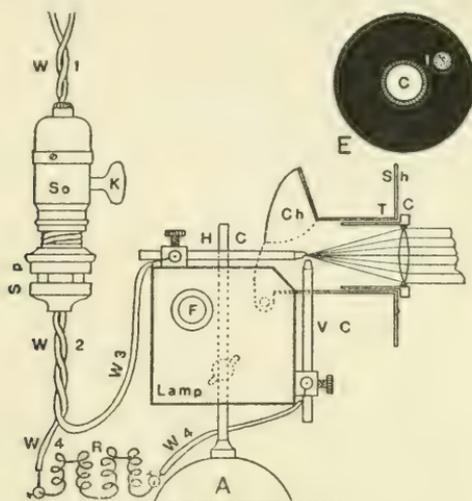


Fig. 13. Small arc lamp for dark-field illumination (From *Optic Projection*)

This figure is to show the wiring necessary and the arrangement of the arc and lens to give a parallel beam.

A—Heavy base of the lamp support. By means of a clamp the lamp can be fixed at any desired vertical height. HC and VC, the horizontal and vertical carbons. The HC must be made positive. F, the wheels by which the carbons are fed.

TC—The tube containing the condenser. The condenser in the inner tube can be moved back and forth to get a parallel beam. Sh, black shield, see E.

E—Black shield at the end of the lamp tube (Sh). It serves to screen the eyes and to show when the spot of light is thrown back by the mirror into the parallellizing lens.

W1, W2, W3, W4—The wires of the circuit passing from supply to the upper carbon (HC) and from the lower carbon (VC) to the rheostat, and from the rheostat back to the supply in W1. Never try to use an arc lamp without inserting a rheostat in the circuit. As shown, it forms a part of one wire. It makes no difference whether it is in the wire going to the upper or to the lower carbon, but it must be in one of them.

*6-Volt Headlight Lamp.*—Next to the arc lamp in excellence for dark-field work is the 6-volt gas-filled headlight lamp (Fig. 14). The reason of this excellence is that the filament giving the light is in a very close and small spiral not much larger than the crater of the small arc lamp, and hence approximates a point source of light.

The brilliancy is also very great as the filament is at about 2800° absolute. The two sizes that have been found most useful by the writer are the bulbs of 72 watts and those of 108 watts. For the bulb of 108 watts a mogul socket is essential; for the 72 watt bulb the ordinary socket is used.

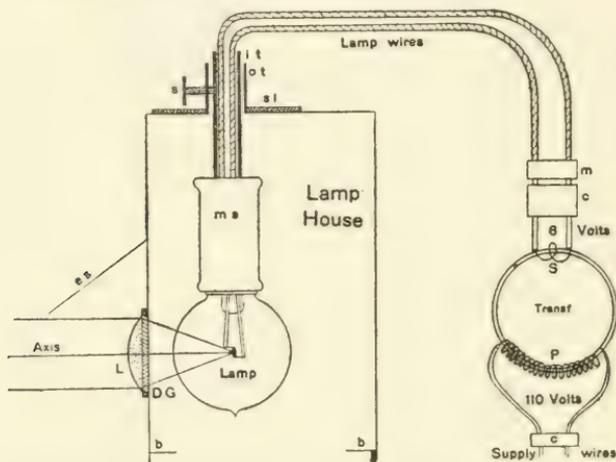


Fig. 14. Diagram of headlight lamp and transformer for dark-field illumination (About one-sixth natural size).

Axis—Axis of the parallel beam from the lens (L).

Lamp—The 6-volt, 108 watt headlight lamp with its very small, close filament centered to the axis of the lens. It is in a mogul socket (ms) and can be centered vertically and horizontally by the inner and outer tubes and set screw (it, ot, s), and the brass slide (sl).

Lamp House—The metal container for the lamp. (b b) Baffle plates near the bottom to help avoid stray light. At the left over the lens (L) is the sloping eye shade. L D G—Parallelizing lens cemented to polished daylight glass.

Lamp wires—The large wires from the transformer (Transf.) to the lamp (Double heater wires are good).

m c—Mistakeless connection between the lamp wires and the transformer (Transf.). This is a Manhattan stage connector, and is different from anything else in the laboratory and therefore the lamp can never be connected with a 110 volt circuit and burn out the lamp. Of course any other wholly different connection would answer just as well.

Transf.—Diagram of a step-down transformer. As there are 18 coils around the soft iron ring on the Primary (P) or 110 volt side, and but one coil around the Secondary (S) side, the voltage is stepped down 18 times, or from 110 to 6 volts. In an actual transformer the coils would be far more numerous, but in this proportion. If the transformer were connected wrongly, i.e., with the lamp wires connected with

the primary (P) side, and the 110 volt supply with the secondary (S) side, it would then be a step-up transformer, and raise the 110 volts 18 times—with disastrous results. C, separable connection for the 110 volt supply wires.

The only difficulty with these lamps is that as they are for a 6 volt circuit it is necessary to use a step-down transformer if one has an alternating current with a voltage of 110 or of 220, as is usual.

If one has a direct current of 110 or 220 voltage, then it is necessary to use a storage battery, in general like those used for the lighting and ignition systems of automobiles. As a transformer uses up but a very small amount of energy it will be readily seen that in stepping down the voltage the amperage is correspondingly raised from the general law that the wattage is the product of the voltage into the amperage, and knowing any two the third may readily be found.

For example with the 72 watt lamp, if the voltage is 6 the amperage must be  $72/6$  or 12 amperes. With the 108 watt bulb the amperage must be  $108/6 = 18$  amperes.

The heating of the filament is determined by the amperage, and also it must be remembered that the conductor of an electric current must be increased in due proportion for an increased amperage, consequently in the transformer the wires joining the 110 volt line is small because a very small amperage is necessary to give a large wattage; while from the transformer to the lamp the conducting wires must be large, to carry without heating the amperage necessary with the low voltage (6) to give the large wattage (108 or 72).

For the 18 amperes of the 108 watt bulb, the Fire Underwriters specifications call for wire of No. 12 or No. 14 Brown and Sharp Gauge, i.e., wire 1.6 to 2 mm. in diameter or a cable composed of smaller wire having the same conductivity. This specification is for continuous service. In wiring the headlight lamp from the transformer, so called *heater cable* is good, provided one uses a double cable, that is the entire cable for each wire. This is easily done by removing the insulation at the ends and twisting the two strands together, then it can be treated as one wire and the two thus treated used to join the lamp to the mistakeless connection (m c, Fig. 14, 15) of the transformer. As the resistance is small in these large conductors the full effect of the current remains to make especially brilliant the glowing lamp filament, and brilliancy is what is needed for this work.

It should be stated that the transformer for this purpose should be substantial and adapted to continuous service. It is known as a "Bell Transformer" as it is connected to ordinary house light systems for ringing door bells. The one used by the writer was obtained from the General Electric Co. in 1920 and costs at present seven dollars. It is marked: Transformer, type N D, Form P Volts 110 6. Capacity 108 KV-A, Cycles 60, Without taps in Primary." (For making the connections, see the explanation of Fig. 14.)

In comparing the two 6 volt lamps for dark-field work, the 72 watt lamp answers well for most purposes, but the 108 watt one approximates more nearly to the small arc lamp and is sufficient for probably 99% of all dark-field observation in biology. For the remaining 1% one could safely depend on sunlight.

*Stereopticon and Mazda lamps for dark-field.* In absence of the head-light lamps described above, one can get good results by using in the lamp-house (Fig. 14-15), a stereopticon lamp bulb of 100 to 250 watts. These bulbs have the filament arranged in a kind of ball, and hence fairly well concentrated. This filament must be centered with the parallelizing lens as described for the headlight bulbs. For the horizontal position, move the lamp back and forth by the brass slide until the front of the ball filament is in focus on the 10-meter screen. The microscope should then be placed from 15-25 cm. from the lamp-house. The rest of the procedure is exactly as for the headlight lamp.

If one has neither headlight lamp nor stereopticon lamp, still good work can be done in biology by using the Mazda C bulbs where the filament is in the form of a loop or C. This is centered and focused as for the other lamps (Fig. 18). If one has only a lamp similar to Fig. 18, the daylight glass can be removed and the microscope placed close to the lamp. Fairly good results can be obtained with a 100 watt mazda stereopticon or c bulb without a parallelizing lens.

The Spencer Lens Company recommend in addition to the small arc lamp, their small magic lantern (No. 394). This has either a 250 or a 400 watt stereopticon lamp bulb, and for parallelizing system, the two plano-convex lenses common with simple magic lanterns. The projection objective of the magic lantern is removed. This yields good results especially when a piece of clear daylight glass

is placed over the end of the cone left vacant by the removal of the objective.

A real advantage possessed by these different lights is that the lamps are connected directly with the 110 volt circuit, no transformer being required, as with the headlight lamps. But if one is to do much dark-field work the headlight lamps are much to be preferred.

*Daylight effects with the headlight or Mazda lamps.* For dark-field work as for work with the bright field, daylight effects are of the greatest advantage both for eye comfort and for the clearness with which details can be made out. The daylight effect is readily obtained by using a piece of daylight glass polished on both sides and cemented to the flat face of the parallelizing lens by means of Canada balsam (Fig. 14-15).

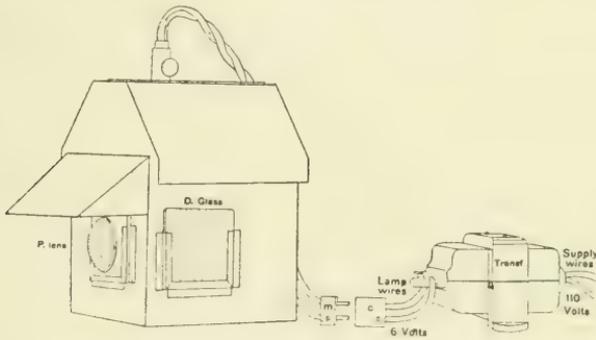


Fig. 15. Headlight lamp in its metal house, and the step-down transformer. (About one-eighth natural size)

D. Glass—The window of daylight glass on the side of the lamp-house to be used for bright-field work. With the glass removed the centering of the lamp is facilitated.

P. lens—Parallelizing lens of about 75 mm. focus. It is cemented to a piece of polished daylight glass.

m c—Mistakeless connection between the lamp wires and the transformer (Transf.). Such a connection prevents joining the lamp with the 110 volt circuit, and thus burning it out. This cannot be connected wrongly.

Transf.—Step-down transformer from 110 to 6 volts.

*Lamp-House with centering arrangement.* To avoid the non-utilized light, and to place the source of light in the most favorable position, there must be an opaque box to enclose and support the head-light or Mazda lamp. As the filament giving the light must be in the optic axis and practically in the focus of the parallelizing

lens, the lamp or the lens must be sufficiently movable to attain the end. In the lamp-house here figured (Fig. 14, 15) the lens is stationary and the lamp is movable horizontally and vertically, that is, it can be raised and lowered and moved toward and from the lens in the optic axis. For the most perfect centering there should also be arrangements for moving the lamp or the lens from side to side. In the one here shown the parallelizing lens can be shifted slightly to take care of the lateral centering.

*Centering the Lamp-filament.* As stated above the lamp-filament must be centered, that is, put in the principal optic axis of the parallelizing lens. This is most satisfactorily done by putting the parallelizing lens in position in the lamp-house and measuring the distance from the table to the middle point of the lens. The middle point of lamp filament should be placed at the same height from the table. This is easily accomplished by using the side window of the lamp-house and raising and lowering the lamp by means of the vertical adjustment (Fig. 14-15) until the filament is at the right height to be on the level of the optic axis. Then the lamp is turned until the spiral filament faces the lens. The two limbs of the fork holding the filament then face sidewise. Of course, they would make a shadow if they faced the lens.

*To get a parallel beam.* The most satisfactory way of doing this is to work at night or in a dark room. Having a white wall or white screen at about 10 meters distant, light the lamp and move it back and forth in the optic axis by means of the top slide (Fig. 14 sl) until the filament of the lamp is in focus on the screen, the filament will then be at about the principal focus of the parallelizing lens, that is, in a position to give approximately parallel light to the microscope. It is well to mark the position on the top of the lamp-house so that if it gets accidentally displaced it can be returned without trouble. It may be said in passing that the lamps are not all exactly alike so that when a new lamp is installed it is necessary to center and focus all over again.

*Focusing the crater of the small arc lamp.* The makers arrange the carbons and the lens tube so that the crater will be approximately in the optic axis (Fig. 13). Now to get the crater in the focus of the parallelizing lens one can proceed in principle as with the headlight lamp. In the arc lamp, the carbons are fixed and the lens movable. Work at night or in a dark room and with the lighted arc move the

lens back and forth until there is a sharp image of the crater on the 10-meter screen.

*Lighting the Microscope.* Assuming that the lamp filament or the crater of the arc lamp is centered with the parallelizing lens, one can find the best position for the microscope by holding some thick white paper in the path of the beam and slowly moving out along the beam. Where the spot of light is brightest and most uniform is the best place for the microscope mirror. With the headlight lamps and the arc light this is usually 20-30 cm. from the parallelizing lens.

To get the spot of light to fall on the  $45^\circ$  mirror properly, the center of the mirror must be at the level of the axis of the beam. This can be brought about either by raising the microscope on a block, by inclining the microscope, or by tipping the lamp-house over toward the microscope. If some white paper is put over the mirror one can tell easily when the cylinder of light falls upon it.

To get the light up through the condenser and into the objective it is necessary to so tip the mirror that an image of the source of light is directed back into the parallelizing lens. This image is reflected back from the flat top of the condenser to the mirror. With this arrangement of the mirror the microscope is almost always well lighted, and the mirror will need but a slight adjustment to give the best possible light. This will only be true however, when the source of light is centered to the parallelizing lens and the condenser to the axis of the microscope.

This method of lighting the microscope saves much time and worry. It is effective with the microscope vertical or inclined, with the lamp-house vertical or inclined, and finally it is unnecessary to have the microscope in line with the beam of light. It may be at right angles or at any angle provided the beam of light falls directly on the mirror and the image of the source can be reflected back to the parallelizing lens.

This method of lighting the microscope, so simple and generally applicable, has the one draw-back that the reflected image is rather faint and therefore not easily seen in a light room; at night or in a dark room it is very easily applied. If one is using the headlight lamp and the parallelizing lens is on the outside as shown in Fig. 14-15, one can tell easily when the image is reflected back into the lens from the bright image seemingly considerably nearer the

lamp filament than the blue image of the filament shown in the lens. To see these images one should look obliquely into the lens, that is, along a secondary not along the principal axis. One can also gain help in lighting by turning the mirror till a spot or ring of light appears on the upper end of the condenser. If the slide is in place with the oil for immersion, the spot of light will be bright. One must usually change the mirror slightly after the preparation is in focus to get the best light.

#### CENTERING AND FOCUSING THE DARK-FIELD CONDENSER

As can be seen by Fig. 6-7 the object must be in the focus of the dark-field condenser and this focus must be in the optic axis of the microscope.

The dark-field condenser must have a special mounting with centering screws, which is the common method; or if the microscope has a centering sub-stage arrangement the dark-field condenser need not have a special centering arrangement, but be put in the centering substage fitting. Ordinarily there is no centering arrangement on a microscope and hence the dark-field condenser must have a special centering arrangement of its own. The whole is then placed in the usual bright-field substage condenser ring and raised until it is at the level of the top of the stage. As a guide to centering, there is a circle scratched on the upper surface of the condenser (Fig. 7 c-r). With a low power (16 mm. objective or lower, and x5 ocular) one focuses down on the end of the condenser and if the small circle is not concentric with the circle of the field the centering screws are used with the two hands at the same time and adjusted until the circles are exactly even all around. Unfortunately this is not sufficient for the most satisfactory work, as it is rare that any two objectives will be exactly centered even though screwed into the same opening in the nose-piece, and much less likely to be centered if in different openings. To get the best results the objective to be used and the dark-field illuminator must be centered to each other. To accomplish this the following procedure has been found simple and certain: To start with the dark-field condenser is centered by the low objective as described above, and then with a crow-quill or other very fine pen one puts a very small point of Chinese white or other white ink in the middle of the little centering circle. This is

easily done if an objective of 20 to 40 mm. focus is used for centering the circle on the condenser.

Now for centering the oil immersion or other high power objective the field of which is less than the centering circle, the objective is put in place, but no immersion liquid need be used for the centering. The top of the condenser has dusted upon it some starch or flour or other fine white powder so that in focusing down upon the top of the condenser there will be some shining particles to focus on if the white ink in the center of the circle should happen to be entirely out of the field, which is often the case. When the objective is in focus the centering screws are used to shift the condenser until the minute spot of white ink in the center of the circle is exactly in the middle of the field. In this way any objective may be centered with the condenser, and so far as the centering is concerned, one can be sure of getting the best results of which the condenser is capable.

When the condenser is centered to the high objective, the starch particles and the white ink may be removed with a piece of moist lens paper or a soft cloth.

*Focusing the Condenser on the Object Level.* This is one of the most essential steps for good dark-field work. If the objects are not in the focus of the condenser they will not be sufficiently lighted so that they can radiate enough light into the microscope to show all their details.

One can proceed as follows, it being assumed that the preparation is mounted on a slide of the proper thickness for the given condenser:—Use a low power, 16 to 50 mm. objective and light the microscope as described in the preceding section. Look into the microscope and focus on a saliva preparation. Move the slide around until there are plenty of epithelial cells in the field and then make slight changes in the mirror until the most brilliant light is obtained. With the screw device for raising and lowering the condenser shift the position up and down slightly until the smallest and most brilliantly lighted point is found. When this is accomplished the condenser is in the optimum focus for that slide and will give the most brilliant light of which it is capable for the source of light used.

Any preparation for examination can have the condenser focused upon it as just described.

For experimental purposes a very satisfactory preparation for focusing the condenser is made as follows: A slide of the right thick-

ness is selected and cleaned and on one face near the middle is painted, with a fine brush, a very thin layer of Chinese white or other white ink. When this is dry, a drop of Canada balsam is put upon it and then a cover-glass. The white particles are very fine and serve admirably to show the focal point of the condenser. Such a slide can be kept as a standard and if the condenser is focused by its aid, it will be in the right position for any preparation mounted upon a slide of the same thickness as the standard. One must always remember, however, that many preparations have an appreciable thickness, and if the slide were of exactly the same thickness as the standard the light might be made more brilliant in a given case by focusing the condenser slightly upward for the higher levels of the preparation. This shows also that the slides selected for preparations should be somewhat under the maximum thickness allowable for the given condenser.

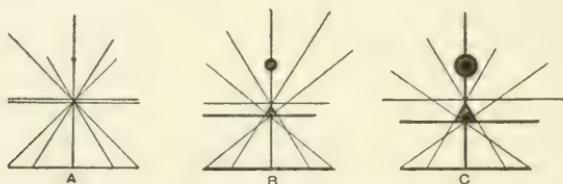


Fig. 16. Face and sectional views of the focus of the hollow cone of light from dark-field condensers

A—Sectional view of an optically perfect dark-field condenser in which the sun is represented as focused nearly to a point. No such condenser exists.

B—Sectional view of a possible condenser focus. It is drawn out somewhat and spreads laterally. The variation in the thickness of slide which might properly be used is shown by the two parallel lines enclosing the elongated focus.

C—Sectional view with a still more elongated focus. The parallel lines show that the variation in thickness of slide permissible is correspondingly increased.

The apparent size of the sun's image is shown on the axis above in each case. It is least sharp in C.

The black line above the letters (A, B, C) represents the top of the condenser.

*Thickness of glass-slide to use.* Mention has been made of glass slides of the proper thickness. What should this thickness be and how can it be determined are pertinent questions for one who is to get satisfactory results in dark-field work. The thickness of the slide with any given condenser is that which will bring the focus of the condenser—that is the image of the source of illumination—on the upper face of the glass slide where the object is located. Either

on the instrument or in the maker's directions for its use the thickness of slide which should be used with it is given. If such definite information is not available or if a person wishes to determine for himself the proper thickness of slide to use, it may be found out as follows: An arc lamp and a dark room are necessary. The light should preferably be parallelized as shown in Fig. 13. The tube of the microscope is removed, and a piece of uranium glass with plane faces is placed on the stage and connected with the top of the condenser by homogeneous immersion liquid. The uranium glass is strongly fluorescent and shows with great definiteness the exact path of the beams of light from the condenser. One can see exactly where the light comes to a focus above the condenser and then the diverging beams above the condenser. If the condenser were perfect the rays would focus very accurately at a point above the condenser face, Fig. 16 A. This focal point is where the object should be placed and its distance above the condenser face gives the thickness of the slide to use. One can see that with an optically perfect condenser the thickness should be very exact to get the most brilliant image. If the optical system is less perfect as shown in B Fig. 16 the rays do not all cross at one point, but over an appreciable thickness and anywhere within that elongated focus would give a brilliant illumination. In this case the thickness of the slide used could vary the length of this focus.

In Fig. 16 C the focus is much elongated and the slide might vary greatly in thickness and still give a brilliant image. Above the sectional view of the focus in each case is given a face view of the brightest point as described above in getting the focus of the condenser. One can readily see that the more perfect the focus at a point the smaller will be the point of light, and as all the rays are at that point it will be dazzlingly brilliant, while with B and C, where only part of the rays focus at any given level the circle of light will be less brilliant, but correspondingly greater in diameter. The larger circle of light has the advantage of giving a larger illuminated field, but the disadvantage of loss of brilliancy for the most exacting work. It should be mentioned also that as the focus gives an image of the source of light, the size of the source of light will also affect the size of the bright spot seen in looking down on the image. This is finely brought out by using the sun as a source and the arc light or the incandescent light.

One can see also from these figures that if the slide is too thin the objects will be partly in the dark space between the *converging* beams, and if the slide is too thick a part of the objects will be in the dark space between the *diverging* beams. If one sees the face view with a low power in either case there will be a ring of light and a central dark disc. and will look something like the central stop in Fig. 5 D

As the preparations (blood, saliva, etc.) usually studied by the dark-field method have an appreciable thickness it is better to use a slide somewhat thinner than the optimum where the object is almost exactly at the level of the upper surface. If the slide is somewhat thinner the various levels of the preparation can be focused on by the condenser by slightly raising and lowering it as the case demands. For example, if the optimum thickness is 1 mm. it is better to use slides of 0.90 or 0.95 mm. and if the optimum thickness is 1.55 mm. it is better to use one of 1.50 mm. for ordinary preparations.

*Thickness of cover-glass and tube-length.* These should be strictl in accordance with the construction of the objective. In all mode n objectives the makers state the tube-length and thickness of cover glass for which unadjustable objectives are corrected. As the dark-field illumination brings out very sharply any defects of correction in the objective, one should select a cover of the thickness, and the length of tube recommended by the maker of the objective. This applies particularly to dry, inadjustable objectives. If the objectives are dry and adjustable then corrections can be made for variations from the standard of cover thickness or tube-length.

If the objective being used is homogeneous immersion, the tube-length must be carefully attended to, but the thickness of the cover-glass is immaterial so long as it is thin enough to fall within the working distance of the objective; of course if it were thicker than that one would not be able to get the objective in focus (Bausch, '90; Gage, '87, 1912).

#### PRACTICAL APPLICATION OF DARK-FIELD MICROSCOPY

In the practical application of dark-field microscopy it is self-evident that it can be used successfully only with objects scattered, leaving a certain amount of blank or empty space between the objects. If the object being studied covered the whole field then it would all appear self-luminous and give a continuous bright appearance filling the whole field of the microscope.

In Biology, used in the comprehensive sense applied to it by Huxley, there come naturally the following groups of objects in which it is applicable, and likely to yield much information:—

(A) Unicellular organisms in both the plant and the animal kingdoms. This of course would include the Protozoan Animals, the Bacteria, and other unicellular plants.

(B) In the multicellular animals and plants it includes the natural fluid parts with their cellular and granular contents. In the vertebrates, including man, this would, for example, comprise the blood, and the lymph, with their cellular and granular contents; the tissue fluids, and the fluids in the natural cavities like the pericardial, the pleural and the peritoneal cavities, and the liquid found in the cavities of the central nervous system, the joint cavities and tendon sheaths. It is also of great service in the study of the liquids found in mucous containers, as milk, urine, bile, the saliva, the mucous in the nose, and other organs lined with mucous membrane.

Furthermore it is of help in the study of isolated elements of the body like ciliated cells, etc. In a word it is applicable to the study of all animal and vegetable structures—including the pathologic ones—that are naturally isolated, or that can be artificially separated so that there is sufficient blank space between the structural parts.

Dr. Chamot points out its help in the biological examination of water, in the study of foods, fibers, crystallization phenomena, sub-microscopic particles and colloids. He adds further (p. 40): "This method is invaluable for demonstrating the presence of very minute bodies or those whose index of refraction is so very nearly the same as that of the medium in which they occur as to cause them to escape detection when illuminated by transmitted light," i.e., by bright-field microscopy.

#### SUMMARY OF STEPS NECESSARY FOR SUCCESSFUL DARK-FIELD OBSERVATION

1. A powerful source of light must be available.
2. The dark-field condenser is put in place in the substage, and raised until the top is flush with the upper surface of the stage. The condenser is then accurately centered. If there is an iris diaphragm below the condenser it should be made wide open.
3. A homogeneous immersion objective with reducing diaphragm of about 0.80 N.A. is screwed into one of the openings of the nose-piece of the microscope.

4. Slides and cover-glasses of the proper thickness are made very clean, and put in position for rapid handling.

5. The preparation to be examined—blood, saliva, etc.—is mounted on the slide and covered; the cover-glass is sealed with mineral or castor oil, or with shellac cement.

6. The mounted preparation is held in the hand and one or more drops of homogeneous liquid put on the lower side of the slide opposite the cover-glass. The slide is then put upon the stage so that the homogeneous liquid makes immersion contact with the top of the condenser. The condenser may need to be raised or lowered slightly to make the contact perfect.

7. A drop of homogeneous liquid is put on the cover-glass.

8. The mirror is turned until there is a brilliant point of light in the homogeneous liquid on the cover. The objective is then lowered until it dips into the immersion liquid.

9. The microscope is then focused and the light made as brilliant as desired by turning the mirror.

10. Dark-field microscopy requires more accuracy of manipulation than does ordinary microscopy, but the increased visibility pays for all the trouble. A dimly lighted room is desirable for then the eyes are adjusted for twilight vision and can more easily make out the finest details.

*Method of Procedure.* As an example of the method to be followed in dark-field work, blood may be used. As pointed out nearly 50 years ago, by Dr. Edmunds, blood with dark-field illumination seems like a new structure, so many things are seen with the greatest distinctness that are wholly invisible or only glimpsed when seen by the bright-field method.

(1) Slides of the correct thickness for the condenser are selected and carefully cleaned.

Cover-glasses are also cleaned and placed where they can be easily grasped.

(2) For obtaining the fresh blood the part to be punctured should be cleaned well with 95% alcohol and then with a sterilized needle or Dr. Morre's Haemospast, the puncture is made. The drop of blood exuding can be quickly touched by a cover-glass, and the cover put on the center of one of the prepared slides. If a small amount adheres to the cover, it will spread out in a very thin layer when placed on the slide. At least one preparation should be made which

appears quite red. In making the preparations one should work rapidly so that the various corpuscles will be in their normal numbers, and the fibrin will be formed only after the preparation is on the slide.

If all the preparations are quite red, after a few minutes, one can be made thinner by pressing firmly on the cover by the ball of the thumb covered with gauze or lens paper. The gauze or paper absorbs the blood which runs out at the edge of the cover. In order to prevent evaporation and to help anchor the cover-glass so that it will not move by the pull of the viscid homogeneous immersion fluid, it is advisable to seal the cover by painting a ring of liquid vaseline (petroleum oil) or castor oil around the edge of the cover. One of the thick preparations should not be sealed, but kept for irrigation with normal salt to show especially the fibrin net-work. When ready to study the blood, put a large drop, or two large drops, of homogeneous liquid on the underside of the slide directly opposite the specimen, and place the slide on the stage of the microscope so that the immersion liquid will come over the face of the condenser. Then a drop of immersion liquid is put on the cover-glass and the objective run down into it. If the lighting is secured as explained above one soon learns to focus on the specimen. In general, the field all looks bright just before the objective gets down to the level for seeing the specimen.

(a) The erythrocytes will appear like dark discs with bright rims owing to the convex borders.

(b) The leucocytes appear as real white corpuscles owing to the granules within them which turn the light into the microscope. If the room is moderately warm—20 C or more—the leucocytes, some of them, will undergo the amoeboid movement, and the picture they present will be a revelation to those who never saw it or only with the bright-field microscope. From the clearness with which everything can be seen the minutest change can be followed, and also the most delicate pseudopod detected. Another striking feature will be noticed in the moving ones, that is, the vigorous Brownian movement of the granules in the part of the leucocyte with the amoeboid movement. In those showing no amoeboid movement there is usually no sign of the Brownian movement of the granules; also if a part of the leucocyte is not undergoing amoeboid movement the particles in it are usually motionless.

(c) The fibrin net-work will be seen like a delicate cob-web between the corpuscles. In different parts of the specimen one can find all the appearances of the fibrin shown in text-books on the blood.

(d) Chylomicrons appear everywhere like bright points in the empty spaces. They are in very active Brownian movement. These chylomicrons will probably be the most unusual part to those studying blood with the dark-field for the first time.\*

A very striking view of the fibrin net-work may be obtained by irrigating the thick blood preparation. If a drop of normal salt solution is placed on one edge of the cover-glass and a piece of blotting paper on the other the liquid is drawn through washing out many of the erythrocytes. If the washing out process is watched under the microscope the erythrocytes will be seen gliding over or through the fibrin net-work, or some of them will be anchored at one end and if the current is rapid the corpuscles will be pulled out into pear-shaped forms.

The leucocytes look like big white boulders in the stream, wholly unmoved by the rushing torrent around them.

#### HISTORY

Almost always in human progress two steps must be taken (1) The discovery of the fundamental principles involved, and (2) the development of knowledge in other fields to make the application of the principles possible. Often a long time, sometimes a very long time, intervenes between the first steps and the final rendering of the knowledge a part of the common knowledge of mankind. The development of Dark-Field Microscopy is a good illustration of both the statements made.

\*The term *chylomicron* is from two Greek words; *χῆλος*, juice or chyle, *μικρόν*, any small thing, technically the one-thousandth of a millimeter ( $\mu$ ). I have introduced this word to show the origin of these bodies from the chyle, and to indicate their general average size. Gulliver in 1840-1842, called these minute granules the *molecular base of the chyle* and showed that they were identical in the thoracic duct and in the blood vessels of the same animal. He gave their average size as  $1/36,000$  to  $1/24,000$  of an inch. They have been called by others free granules or granulations, elementary particles, etc. In 1896 H. F. Mueller described them as "A never-before observed constituent of the blood" and gave the name of haemoconia, literally, blood-dust. (See Gulliver, Lond. Edin. Phil. Mag. Jan. Feb. 1840; Appendix to Gerber's Anatomy, 1842, and notes in the Works of Hewson, 1846; Mueller, Centralblatt f. allg. Path. u. path. Anatomie, Bd. 7, 1896, pp. 529-539).

The ancient opticians, thousands of years ago, knew well that the principle of contrast was of the highest importance in rendering objects visible; but before this could be applied in microscopy, the microscope itself must be devised. This we see in its simplest form in the convex lenses of Roger Bacon (1266-1267) and in the now rarely used compound form of the Dutch spectacle makers, Jansen and Laprey (1590), composed of a convex objective and a concave ocular (Fig. 17). As a result of the Dutch Compound Microscope, Kepler was led to devise the modern form composed of a convex objective and a convex ocular (1610). But this Keplerian compound microscope has undergone many changes since its first conception and many modifications to render it suitable for giving ability to show the delicate structures in nature with their true appearance. Among these changes may be mentioned the preparation of achromatic lens combinations (Dolland 1757) for telescopes and applied to microscopic objectives between 1820-1830, put on the road to perfection by the introduction of the immersion principle (Hooke 1678, Brewster 1813, Amici 1840-1855) and by the aperture made available by the homogeneous immersion objectives of Tolles 1871-1874, and by the apochromatic objectives of Abbe. Condensers for lighting the object have also played a prominent part from that of Descarts (1637) to those recommended by Brewster (1831) and the homogeneous immersion condensers of Wenham, Tolles (1856 to 1871) and those now regularly made for homogeneous contact with the slide supporting the specimen.

Among the subsidiary discoveries were necessary the arc-light of Davy (1800) and the right-angled arc lamp of Albert T. Thompson (1894) (Fig. 13) and the electric generators now everywhere available. In these last days also the gas filled or Mazda lamps with their close filaments of Tungsten which approximate in brilliancy and compactness of source to the arc lamp and greatly excel it in convenience; and lastly of the production of a glass filter to give the light of the tungsten incandescent lamps true daylight quality, and make microscopic work by this artificial light as comfortable as the light from the northern sky (see Ives 1914, Gage 1915-1916).

The time also between the first appreciation of the dark-field for the study of microscopic objects by Lister (1830), Reade (1838), Wenham (1850), Edmunds (1877), and the appreciation of the microscopical worker in general, came only after the invention of the ultra-

microscope (1903) and the application of the dark-field method to the study and detection of pathologic micro-organisms especially the *Spirochaeta pallida* (1905). It now promises to give much help in working out the activities and minute details of microscopic structure in animals and plants from the lowest to the highest.

In the earliest stages of microscopic study the objects were seen by the light which they directed toward the microscope, and if over a dark background they appeared with varying degrees of brightness as if self-luminous; but even as early as 1637 (Fig. 17) Descartes microscope had provision for sending the light through the object. In this case much of the light did not reach the object at all, but passed on directly to the microscope. This mode of lighting showed the object more or less as a dark body on a brilliant background.

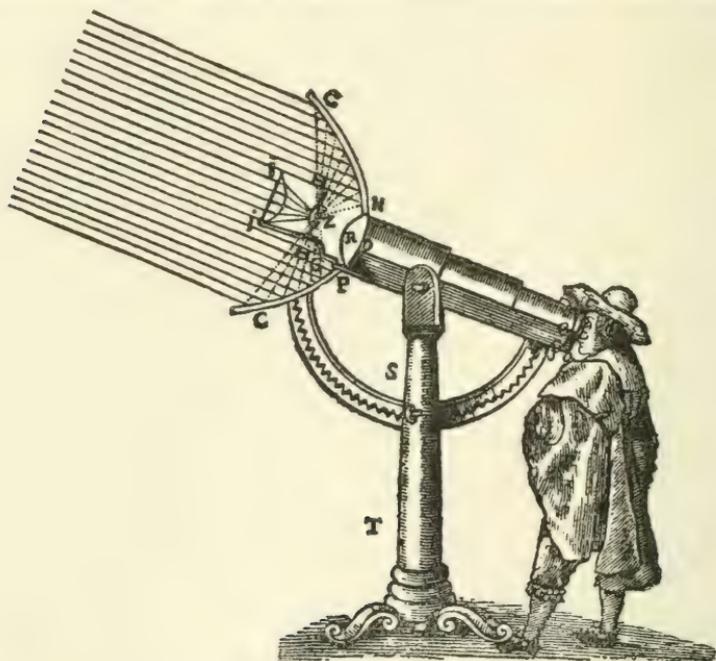


Fig. 17. Descartes Dutch compound microscope with a parabolic reflector and a condensing lens (From Descartes *Dioptrique*, 1637).

Ocular and Objective. The ocular is a plano-concave lens or amplifier, and the objective (N O P R) is a double convex lens.

Reflector and Condenser. For objects to be lighted from above, there is a parabolic mirror (c); for those to be lighted from below there is a condensing lens (i).

These two forms of lighting differed fundamentally in that with the first no light from the source passed into the microscope; but only that from the object, while with the second the light from the source as well as from the object got into the microscope.

The significance of this fundamental difference for the aperture of the objective and for dark-field microscopy were first appreciated by Lister (1830), Wenham (1854), and Gordon (1906), and was practically applied in the manufacture of dark-field apparatus by Zeiss (1904) and Leitz (1905). In a word, it was the appreciation, as stated by Lister (1830) that if the direct light from the source after it had reached the object, were prevented from entering the objective, by blacking the central part of the objective, then only the marginal part of the objective would be functional and that would receive only those rays from the object that were directed to it by the object itself, that is scattered light reflected, refracted, or diffracted, from the object, none of the light from the source getting directly into the microscope. As stated by Wright (p. 217) this is the method of dark-field microscopy by lighting the object with a solid cone of small aperture and, imaging it by hollow beams of large aperture. In practice this method has been discarded for the one by which the object is lighted by beams of light in such a direction with reference to the axis of the objective that none of them can enter the objective directly, and the light going to the microscope comes only from the objects themselves; they will therefore appear self-luminous on a dark background.

The two conditions are (a) where the light is directed upon the object from above and, therefore away from the objective, and (b) where the light is directed upon the object from below, and therefore toward the objective (Fig. 3-4).

If the light is directed upon the object from above and the object is over a non-reflecting background, the object will appear bright in a dark field. Of course, if it is on a light background that will also reflect light into the microscope and both object and background will appear light. It is assumed here that the object or objects cover only a part of the field, leaving plenty of empty space for background.

In striving after a truly non-reflecting background three distinguished men found the same thing, viz., that the only really black thing in nature is a black hole, that is, a space with black walls into which the light cannot enter directly. The dark walls absorb any

stray light, and the empty space gives no reflection. The first of these men devised for his microscopic purposes such a non-reflecting background by means of a small cup or well with the walls painted black. It is known as Lister's black well (1826). The second discoverer was Chevreul (1839), who found in his work on Contrasts that a black space gave the only non-reflecting background. Such a background was used by Marey for making moving pictures to show animal movements. Marey called it Chevreul's black. The third was J. H. Comstock (1901) who found in the study and photography of spider webs that no pigment or fabric was black enough for a background. He therefore devised a deep box with the inner walls covered with black velvet and placed it so that the light could not shine into it. Over the mouth of this box the web was placed and lighted at right angles to the opening of the box. The feeble light the webs reflected served well for photography.

These three men then absolutely independently found the same solution to their problem and doubtless many others have found also that Lister's, Chevreul's, and Comstock's black space is the only really black thing in nature.

From the time of Descartes (1637) the means for lighting objects from above the stage have been many. Some of them, like the bull's eye condenser (Fig. 4, lens) and the side reflector send the light only from one side, while with the circular mirror of Descartes (Fig. 17) and the somewhat similar Lieberkuhn reflector (1740) the light is reflected from all sides upon the object. If now the object is on a dark background, it will appear as if self-luminous.

From 1850 to the present two additional means have been devised for lighting from above. The first, following the suggestion of Riddell (1852) aims to make the objective its own condenser, the light being introduced into the side of the objective and reflected down by a small mirror or a prism (H. L. Smith 1865, Tolles 1866). (For a full discussion see W. A. Rogers, *Journal of the Royal Microscopical Society*, 1880, p. 754-758.)

The other method referred to is that of Prof. Alexander Silverman of the University of Pittsburgh. It consists of a circular electric lamp and reflector which surrounds the objective and shines down upon the object.

Of course all objects lighted from above the stage will give true dark-field effects only when there is a black background, and the objects are scattered, leaving empty space between them.

*Dark-Field Microscopy with Substage Illumination.* The first specific discussion of the possibility of dark-field microscopy with light from beneath the stage is found in a paper by the Rev. J. B. Reade of Cambridge University and is dated at Peckham, Nov. 1836, and is published as appendix No. 2 in the *Micrographia* of Goring and Pritchard, 1837. Reade says: p. 229: "To illustrate the two methods (Bright-field and dark-field) by reference to the telescope it may be observed that the discomfort of viewing spots on the sun not unaptly corresponds with the view of microscopic objects on an illuminated field; while the removal of all inconvenient and ineffective light from the field of the microscope corresponds with the clear and quiet view of stars on the dark blue vault of the firmament." He brings out very clearly in his paper that no light from the source shall pass directly into the microscope, only that from the object, and that the object appears "sparkling with exquisite lustre on a jet-black ground."

The first appearance of this method in the general literature of microscopy which was found occurs in John Quekett's *Practical Treatise on the Use of the Microscope*, 1st ed. 1848, pp. 178-179. He also furnishes a diagram to illustrate the method of lighting something like fig. 4 of the present article, and remarks: "The method consists in illuminating the object by a very powerful light, placed at such an angle with the axis of the microscope that none of the rays can enter it except those which fall directly upon the object, and are so far bent as to pass through it into the compound body," i.e., into the tube of the microscope.

It is referred to in the first edition of W. B. Carpenter's "The Microscope and its Revelations" (1856) as follows:

"Whenever the rays are directed (from below the stage) with such obliquity as not to be received into the object-glass at all, but are sufficiently retained by the object to render it (so to speak) self-luminous, we have what is known as the *black ground illumination*; to which the attention of microscopists generally was first drawn by the Rev. J. B. Reade in the year 1838 (1836-1837) although it had been practised sometime before not only by the author (Dr. Carpenter) but by several other observers."

In addition to the condensing lens of Reade for throwing the very oblique beam of light upon the object, the mirror was used for low powers, and for higher powers, prisms were used especially by Nacet and Shadboldt (1850). It was seen however, that light from only one side might give rise to false appearances.

In the third volume of the Transactions of the Microscopical Society of London, there appeared an epoch-making paper for dark-field microscopy. It is entitled "On the Illumination of Transparent Microscopic Objects on a New Principle." It was read by its author, F. H. Wenham, April 17, 1850. After discussing the prisms of Nacet and pointing out the defect of oblique light from one side only giving rise to false images, he proceeds to show how the defect may be obviated by using two prisms giving light from opposite sides, or, and this is the epoch making part of the paper for dark-field work, by using a truncated parabolic reflector to give a circle of light. A dark stop was present to cut out all but the rays which exceed the aperture of the objective "So that the light which enters the microscope shall be that which radiates only from the object, as if it were self-luminous." The parabolic speculum was truncated so that the light would focus on an object mounted upon the ordinary glass slide.

From this fundamental beginning, illumination by a hollow cone of light by the aid of the truncated parabola, all the advances in dark-ground illumination have proceeded. In 1851, Mr. Shadboldt says: "In order to obviate the objectional shadow (of lighting from one side only) as well as to procure a more brilliant illumination the parabolic condenser was projected by Mr. Wenham, to whom alone belongs the credit of having suggested the use of oblique illumination in *every azimuth*, so as to produce a black field." In this paper Mr. Shadboldt commends the use of a condenser made wholly of glass and depending upon internal reflections to take the place of the metallic parabolic mirror of Wenham. This he named a sphero-annular condenser. In considering the obliquity required to have all of the light going to the object of an angle to fall outside the aperture of the objective, it seems to Shadboldt highly desirable that each objective to be used in dark-field work should have its own special condenser. That he understood as perfectly as we the possibility of using a single condenser for all objectives is shown by the following quotation, p. 157, "It is highly desirable that the

condenser should be constructed specially with reference to the aperture of the object-glass with which it is intended to operate; and for a reason to be given immediately, it will be seen that cutting off some of the rays, in order to make a condenser work with objectives of very much larger aperture, although quite practicable and even generally in use with the parabolic condenser, is not nearly so advantageous as the use of a separate condenser for every object-glass . . . of high power at least."

In 1856 Mr. Wenham himself advocates the use of a truncated paraboloid of solid glass with a central stop to cut out all the central rays which would not be internally reflected from the upper surface of the paraboloid. He brings out in the clearest manner possible the need of using immersion contact with the paraboloid to permit the very oblique rays to pass out of the paraboloid into the overlying substance. If the object is in water, then water immersion and when the object is mounted in balsam, he advocates the use of an immersion liquid between the glass slide and the paraboloid of camphine, turpentine or oil of cloves as their refractive index is nearly the same as crown glass and permits the passage of the rays of great aperture to pass on into the slide and the balsam containing the objects. We now use cedar oil or other homogeneous liquid for the same purpose.

In 1877 Dr. James Edmunds presented before the Quekett Microscopical Club a paper on "A New Immersion Paraboloid Illuminator." It consisted of a paraboloid of glass cut off at an exactly calculated distance below the focus, this distance varying in the four lenses which constituted his set, and the plane top being made optically continuous, and as nearly as possible optically homogeneous with the substance of the slide, by means of a cementing fluid of high refractive index, such as anhydrous glycerine, castor oil, copaiba-balsam, oil of cloves, etc. The paraboloid lenses acted on the principle of total internal reflection, and each one was calculated for the thickness of the slide beneath which it was to be used ( $1/16$ th in  $1/100$  inch slides) so as to converge upon the object all of the light entering the base of the paraboloid. Parallel light should be thrown into the base of the paraboloid, and the most splendid effects were obtained by means of direct sunlight. Water immersion objectives of  $1/16$ th and  $1/8$ th inch focus were used. After speaking of some test objects he says, p. 19: "With bacterial fluids, the effect

was equally remarkable. Saliva, blood, etc., viewed by a good dry quarter of about  $95^\circ$  (NA 74), were seen almost as new objects when lighted up by this paraboloid."

As it was recognized from the time of Reade that to gain the dark-field effect the light going to the object must be of an obliquity so great that it could not enter the microscope directly; this involved either a paraboloid or other dark-field illuminator of such great range that it might be used with all objectives, or the suggestion of Shadboldt must be followed that each objective have a paraboloid especially constructed to give it the best possible effect. This question naturally became very insistant when the water immersion objectives of large aperture came into use, and especially when the homogeneous immersion objectives came into common use (1880-1890). It has finally been settled by adopting the first possibility, viz., the use of dark-field illuminators adapted for all objectives, the aperture of the objectives being reduced, where too great, to a point somewhat below 1.00 NA. This makes it possible to utilize a ring of light between 1.00 and 1.52 NA for the dark-field illumination, and this ring of light produced by the sun or the electric light has been found sufficient for practically all dark-field microscopy. It should be stated in passing that the ring of light produced by the dark-field illuminators usually falls between 1.00 NA, and 1.45 NA. Some fall below 1.00 NA and some only go to 1.30 or 1.35. The reducing diaphragms for homogeneous immersion objectives which have come to the writer with objectives have ranged from 0.40 NA to 0.80 NA.

From 1907-1910 papers were written describing and figuring reflecting condensers made on the cardioid principle to take the place of the truncated paraboloid in dark-field work. The effort was made to so figure the component segments of glass that the spherical and chromatic errors would be largely eliminated, and that the entire ring of light could be brought to a more perfect focus than is possible with the truncated paraboloid: that is, to be optically more like A than like B or C in Fig. 16. A simple plate form for use on the top of the stage has also been devised. When this is used the substage condenser is turned out so that the light can pass directly up from the plane mirror to the condenser. This form is not easy to keep accurately centered. From the writer's experience with quite a variety of these dark-field condensers in biological work

the paraboloids have proved the easiest to work with and the most generally satisfactory.

As a final word,—now that the means have been found for fuller microscopic revelations, it behooves biologists to make the most of them; and in the study of the finest details in living things by this dark-field lighting, perhaps a truer conception of structure and action can be gained than by a too exclusive dependence on dead material treated with the endless variety of fixers and stains.

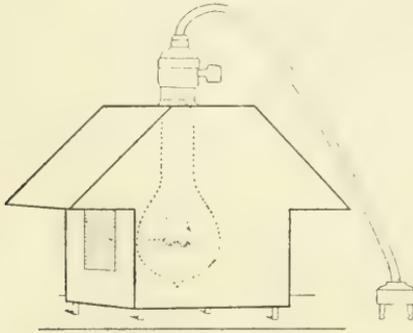


Fig. 18. Chalet microscope lamp for bright-field microscopy (Two-fifteenths natural size).

The lamp has two daylight-glass windows under the overhanging roof. The roof serves to shade the eyes. The source of light is a 100 watt Mazda C lamp bulb, the filament of which is centered with the windows.

#### BIBLIOGRAPHY

AKEHURST, S. C.

1914. Substage illumination by hollow cones. *Jour. Quekett Micr. Club*, Vol. XII, (1914) pp. 301-308. 3 pl.

BAUSCH, Edward

1890. The full utilization of the capacity of the microscope and means for obtaining the same. *Proc. Amer. Soc. Microscopists*, Vol. XII, 1890, pp. 43-49.

Among other matters Mr. Bausch gives a very thoughtful discussion of the effect of the cover-glass and of tube length.

BORELLUS, PETRUS

1655. *De vero Telescopii inventore, cum brevi omnium conspiciolorum historia. Ubi de eorum confectione, ac usu, seu de effectibus agitur, novaque quaedam circa ea proponuntur. Accessit etiam centuria observationum microscopiarum. Authore Petro Borello, regis christianissimi conciliario, et medico ordinario. Hagae-Comitum, ex typographia Adriani Vlacq, MDCLV (1655).*

Evidence from those with personal knowledge that telescopes and microscopes were made by the Dutch spectacle makers, Zacharias Jansen, and Hans Laprey, 1590.

CARPENTER, WILLIAM B.

1856. *The Microscope and its Revelations*. First edition 1856.

An admirable statement of dark-field microscopy is given with the apparatus devised up to that time for effecting it. Showing how greatly dark-field microscopy had been discarded in England one can compare the first and the 6th (1856-1881) editions of this work with the 8th edition, (1901).

CHAMOT, EMILE MONNIN

1915. *Elementary Chemical Microscopy*. New York, 1915.

This work is recommended not only for the account given of dark-field microscopy and its application, but for the ultra-microscope, the polariscope, the micro-spectroscope and indeed all other chemico-physical apparatus used with the microscope, and their application in chemical and physical investigations.

CHEVREUL, M. E.

1838. *De la Loi du Contraste simultané des Couleurs et de l'assortiment des objets colorés considéré d'après cette loi*. Paris, 1839. Work written 1835-1838. Third English edition, 1890. Part of Bohn's Scientific Library.

COMSTOCK, J. H.

1912. *The Spider Book*. A manual for the study of spiders and their near relatives, the scorpions, pseudoscorpions, whip-scorpions, harvestmen, and other members of the class Arachnida, found in America north of Mexico; with analytical keys for their classification and popular accounts of their habits. New York.

In this book are given pictures of the spider webs photographed against a black space, i.e., a deep box lined with black velvet. See p. 181. The first photographs made in this way were taken in 1901. They were exhibited before the Entomological Society of America at its first meeting, Dec. 28, 1906.

CONRADY, A. E.

1912. Resolution with dark-ground illumination. *Jour. Quekett Micr. Club*, Vol. 11, (1912) pp. 475-480.

He says: "To get the utmost resolving power with dark-ground illumination, the condenser must have not less than three times the NA of the objective. If the condenser has less than three times the aperture of the objective then the limit of resolution is found by taking  $\frac{1}{4}$  the sum of the apertures of objective and condenser: e.g., if cond. has NA of 1.40, and of obj. 1.00 NA, their sum is 2.40,  $\frac{1}{4}$  of 2.40 = 0.60 NA; 'limit in this case.'"

COX, HON. JACOB D.

1884. Robert B. Tolles and the angular aperture question. *Proc. Amer. Soc. Microscopists*, Vol. VI, (1884) pp. 5-39.

This very able address, one of the ablest our society ever had the fortune to hear from its president, brings out with absolute clearness and fairness the steps in progress and the role played by Robert B. Tolles in actually making possible the final step, and taking that step, in his homogeneous immersion objectives. That is not all, he published the formula by which the objectives were made. The reading of this address is most strongly recommended to our younger members.

## DESCARTES (LAT. CARTESIUS), RENÉ

1637. Oeuvres, Publiées par C. Adam et P. Tannery sous les auspices du ministère de l'instruction publique, Vols. I-XII. Paris, 1902.

The Dioptrique is in Vol. 6 of this edition, and the French and the figures are as in the original of 1637. In Cousin's edition the figures are often considerably modified and the French modernized.

## DOLLOND, JOHN

An account of some experiments concerning the different refrangibility of light. Read June 8, 1758. Philos. Trans. Roy Soc. Lond. 1758, pp. 733-743. This is the original paper on achromatic telescopes, etc.

## EDMUNDS, JAMES

1877. On a new immersion paraboloid illuminator. Jour. Quekett Micr. Club, Vol. V, (1877) pp. 17-21. Monthly Micr. Jour., Vol. XVIII, 1877, pp. 78-85.

The Paraboloid was made optically continuous and as nearly as possible, optically homogeneous with the slide by the use of anhydrous glycerin, castor oil, copaiba-balsam or oil of cloves. He says that saliva, blood, and bacterial fluids gave remarkable effects, and were almost like new objects when seen with this paraboloid.

## GAGE, S. H.

1917. The Microscope, an introduction to microscopic methods and to histology. 12th revised edition, Ithaca. 1917.

## GAGE, S. H. and H. P.

1914. Optic Projection. Principles, installation and use of the magic lantern, the projection microscope, etc. Ithaca, 1914.

## GAGE, S. H.

1887. I. Microscopical tube-length and the parts included in it by the various opticians of the world. II. The thickness of cover-glass for which unadjustable objectives are corrected. Proc. Amer. Soc. Microscopists, Vol. IX, 1887, pp. 168-172.

This paper gave the information that has led to greater uniformity.

## GAIDUKOV, N.

1910. Dunkelfeldbeleuchtung und Ultramikroskopie in der Biologie und in der Medizin. 5 plates, 81 pages. Jena, 1910.

There is a bibliography of books and papers covering 9 pages (202 titles).

## GORDON, J. W.

1907. The top-stop for developing latent power of the microscope. Jour. Roy. Micr. Soc., 1907, pp. 1-13. See also Wright, pp. 216-217.

The plan is to cut out all of the central beam by a stop at the eye point instead of by opaueing the central part of the objective.

## GORING AND PRITCHARD

1837. *Micrographia*, containing practical essays on reflecting, solar, oxy-hydrogen gas microscopes, micrometers, eye-pieces, etc. 231 p. Many figures in the text, one plate. Whittaker & Co., Ave-Maria-Lane, London, England. 1837. Rev. J. B. Reade on dark-field, pp. 227-231.

## HALL, JOHN CHARLES

1856. On an easy method of viewing certain of the Diatomaceae. *Quart. Jour. Micr. Sci.*, Vol. IV, (1856) pp. 205-208.

In this paper Dr. Hall figures natural size, the "spotted lens" of that time, i.e., a very thick, more than hemisphere of glass with the central part opaqued. (See Quekett, 3d. ed., p. 135 where it is said that it is the invention of Thomas Ross.) Hall used this spot lens for oblique light with the ordinary bright field microscopy. He expresses astonishment that this instrument, designed to give dark-field effects, should give bright ones. He did not consider the fact that the aperture of this spot lens was insufficient to throw all the light outside of the aperture of the objective. One would get the same effect if a wide-angled homogeneous immersion were used with a paraboloid, and no reducing diaphragm were put into the objective.

## HEIMSTÄDT, OSKAR

1907. Neuerungen an Spiegelkondensoren (Aus der optischen Werkstätte von C. Reichert in Wien). *Zeit. wiss. Mikr.*, Bd. XXIV, (1907) pp. 233-242.

## HEIMSTÄDT, OSKAR

1908. Spiegelkondensator und Paraboloid. *Zeit. wiss. Mikr.*, Bd. XXV, (1908) pp. 188-195. Erwiderung an Herrn O. Heimstädt, by Siedentopf, pp. 195-199.

Dr. Heimstädt objects to some of Dr. Siedentopf's statements in his paper, "Die Vorgeschichte der Spiegelkondensator." Perhaps the spirit of the polemic will best be brought out by a quotation from Heimstädt, p. 188. "Vol allem beeinträchtigt es den Wert und auch die Neuheit dieser Dunkelfeldbeleuchtung nicht im geringsten, dass dabei längst vergessene Methoden älterer englische Optiker wieder verwendet wurden." In a word, it is well brought out in these papers where the fundamental ideas came from.

## IGNATOWSKY, W. V.

1908. Ein neuer Spiegel-kondensator. *Zeit. wiss. Mikr.*, Bd. XXV, (1908) pp. 64-67 with figures of the substage and the plate form. See also Jentzsch, and Siedentopf. *Jour. Roy. Micr. Soc.*, London, 1911, pp. 50-55.

## JENTZSCH, DR. FELIX

1911. The reflecting concentric condenser. *Physikalische Zeitschrift*, Bd. XI, pp. 993-1000. See also Ignatowsky and Siedentopf, *Jour. Roy. Micro. Soc.*, 1911, pp. 50-55.

## KEPLER, JOHANNES

1604. *Opera Omnia*, Vol. II. Ad Vitellionem Paralipomena. (De modo visionis et humorum oculi usu.) 1604, pp. 226-229. 11 figs.

Correct dioptrics of the eye here given, and also the explanation of the effect of convex and concave spectacles.

1611. Dioptrica.—Demonstratio eorum quae visui et visibilibus propter conspiciam non ita pridem inventa accidunt, pp. 519-567. 35 figs., 1611.

The amplifier, real images, and erect images. The Keplerian microscope (Modern microscope.)

LISTER, JOSEPH JACKSON

1830. On some properties in achromatic object-glasses applicable to the improvement of the microscope. Philos. Trans. Royal Society London, Vol. 120 (1830) pp. 187-200.

On p. 191 he discusses the effect of a "Stop behind the object-glass" (retro-objective stop) by which only the outer zone of the objective is used, the central zone being stopped out. See Wenham, 1854.

MAREY, ETIENNE JULES

1901. The history of Chronophotography. Annual Report of the Smithsonian Institution for 1901, pp. 317-340.

On p. 320 Marey refers to Chevreul's method of obtaining perfect blackness.

MAYALL, JOHN, JUN.

1885. Cantor Lectures on the Microscope. Lectures delivered before the Royal Society of Arts, Nov. Dec. 1885.

On pp. 95-96 are given the facts regarding the working out and production of homogeneous immersion objectives. Tolles is given due credit.

MOORE, DR. V. A.

1897. The Hemospast, a new and convenient instrument for drawing blood for microscopic examination. Trans. Amer. Micr. Soc., Vol. XIX (1897) pp. 186-188.

After using this "spring needle lancet" individually and with large classes for many years I quite agree with Dr. Moore when he remarked to me the other day, "It is the most humane instrument I have ever seen for drawing blood." I would like to add to this: And one of the most efficient.

QUEKETT, JOHN

1848. A practical treatise on the use of the microscope including the different methods of preparing and examining animal, vegetable and mineral structures.

First edition, 1848. Reade's method given and illustrated pp. 178-179; Second edition, 1852, Reade's method illustrated pp. 194-195. Third edition, 1855, Reade's method, the method of Wenham, Spot-Lens method of Thomas Ross, the methods of Schadboldt and Nobert are all given.

READE, REV. J. B.

1837. On a new method of illuminating microscopic objects, pp. 227-231 of Goring and Pritchard's Micrographia, which see. (1837).

ROGERS, WM. A.

1880. On Tolles' interior illuminator for opaque objects. (With note by R. B. Tolles). Jour. Roy. Micr. Soc. London., Vol. III (1880) pp. 754-758.

In this paper Rogers gives the history of the devices for making the objective its own condenser by introducing light into its side and reflecting the light down upon the object.

## SHADBOLT, GEORGE

1851. Observations upon oblique illumination; with a description of the author's Sphaero-annular condenser. Trans. of the Micr. Soc. of London. Vol. III, pp. 132, 154.

This paper was read in 1851. As this condenser is like the glass paraboloids now used for dark-field work, they are often called the Wenham-Shadbolt paraboloids. Shadbolt discusses prisms in this volume.

## SIEDENTOPF, H.

1907. Paraboloid-Kondensator, eine neue Methode für Dunkelfeldbeleuchtung zur Sichtbarmachung und zur Moment-Mikrophotographie lebender Bakterien, etc. Zeit. wiss. Mikr., Bd. XXIV, (1907) pp. 104-108.
1907. Die Vorgeschichte der Spiegelkondensoren. Zeit. wiss. Mikr., Vol. XXIV (1907) pp. 382-395. 16 figures are given of early forms.
1908. Mikroskopische Beobachtungen beim Dunkelfeldbeleuchtung. (Mitteilung aus der optischen Werkstätte von C. Zeiss, Jena) Zeit. wiss. Mikr. Bd. XXV (1908), pp. 273-282. Two plates of photomicrographs of the rays above the different condensers. See also under Heimstädt.
1910. Cardioid-Condenser. Jour. Roy. Micr. Soc. Lond., 1910, pp. 515. See also Ignatowsky, and Jentsch, Jour. Roy. Micr. Soc. Lond., 1911, pp. 50-55, where will be found a statement concerning the historical relation of these different condensers.

## STEPHENSON, J. W.

1879. A catoptric, immersion illuminator. Jour. Roy. Micr. Soc. Lond., Vol. II (1879) pp. 36-37.

This condenser does not depend on internal reflection, but by a silvered surface around the central part. According to Siedentopf this is the condenser copied by Reichert; and according to Heimstädt Wenham's truncated paraboloid was copied by Zeiss (See under Heimstädt).

## WENHAM, F. H.

1850. On the illumination of transparent microscopic objects on a new principle. Trans. Micr. Soc. Lond., Vol. III. (1850) pp. 83-90.

This is the paper by Wenham in which dark-field illumination is produced by a hollow silvered parabolic speculum.

1854. On the theory of the illumination of objects under the microscope with relation to the aperture of the object-glass, and properties of light; with practical methods for special differences of texture and colour. Quart. Jour. Micr. Sci. Vol. II (1854) pp. 145-158.

In this paper Wenham refers to the method of Lister (1830) for darkening the central zone of the objective so that no light can enter the outer zone, unless, as Wenham says, it is "radiated" from the object (See his fig. 1, and pp. 149-150 of the article). On p. 153, in the reference to the effect that his paper of 1850 had had in the microscopical world he says, "As proof of the utility and correctness of my theory, I have only to mention the many applications of it that have since that time (between 1850 and 1854) come into general use, in the way of adapting central stops to the achromatic condenser, single (i.e., "spot lenses") and compound lenses, etc."

1856. On a method of illuminating opaque objects under the highest powers of the microscope. *Trans. Micr. Soc. Lond. in Quart. Jour. of Micr. Sci.*, Vol. IV, (1856) pp. 55-60.

It is in this paper that Mr. Wenham insists on making homogeneous contact with the slide and the top of the paraboloid. It will be noticed that in this paper he speaks of Opaque Objects, while in the paper of 1850 he speaks of Transparent Objects. By reading the two papers it will be seen that many of the objects mentioned in the two papers are identical. This gains an explanation from the fact that he has apparently given up the notion that the objects were visible by their own "radiated" light, but by the light they reflect to the microscope. Consequently he represents (Fig. 4) the light from the condenser going to the cover-glass and being reflected from it down upon the object and he says that it makes the most perfect kind of a Lieberkuhn reflector. One can see instantly that when homogeneous immersion objectives are used there can be no total reflection from the cover.

WOOD, ROBERT W.

1911. *Physical Optics*. New and Revised Edition, 1911.

On p. 373, he discusses, "Penetration of the disturbance into the second medium," and shows that going back to the time of Newton and Fresnel, it was known that while there was total reflection, the light seemed to pass for a minute distance into the rarer medium. This explains why one may get a brighter dark-field picture than is expected if objects are in optical contact with the slide.

WRIGHT, SIR A. E.

1907. *Principles of Microscopy*, being a handbook to the microscope. London and New York, 1907.

The writer has found this book the best and most thought-provoking of any that has been published on the microscope during the last 50 years.

## A NEW BLADDER FLUKE FROM THE FROG\*

BY  
JOHN E. GUBERLET

Bladder flukes have been reported a number of times from North American frogs but as yet very little work has been done on these forms in this country. The European species, however, have received more attention and their complete life histories have been worked out. In North America the studies on frog bladder flukes have been carried on by only four authors, namely Leidy (1851), Bensley (1897), Stafford (1902, 1905), and Cort (1912). The localities from which these were reported are Toronto, Canada; Rice Lake, Ontario, Canada; Urbana, Illinois; Bemidji, Minnesota; and North Judson, Indiana. The writer has at hand another species of frog bladder fluke from *Rana catesbiana* taken at Stillwater, Oklahoma.

In view of the fact that Cort (1912) has given a thorough review of the literature as well as a discussion of the nomenclature of this group, it is unnecessary to take up the history of the literature any farther at this time. The frog bladder distomes have been grouped into two genera by Looss and called *Gorgodera* (1899) and *Gorgoderina* (1902). The basis for this classification is on the number of testes which these animals have. The genus *Gorgodera* has nine testes while *Gorgoderina* has two. Of the latter genus there are known from North America three species, namely *Gorgoderina simplex* Looss, *G. translucida* Stafford and *G. attenuata* Stafford. Of the former genus there have been two species described, namely, *Gorgodera amplicava* Looss and *G. minima* Cort. The writer adds another species to the genus *Gorgodera*.

### GORGODERA CIRCAVA NOV. SP.

In the summer of 1918 the writer found in the urinary bladder of a large bull frog (*Rana catesbiana*) twenty trematodes (Figs. 1 and 2) which belong to a new species of the genus *Gorgodera*. In the early part of the summer of 1919 another bull frog yielded two specimens of the same species of trematode. These forms were so firmly attached to the wall of the urinary bladder by means of the acetabu-

\*Contribution from the Parasitology Laboratory of the Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma.

lum that it was necessary to tear the bladder apart in order to make the worms release their hold. The worms were killed by dashing hot corrosive acetic over them when they were well extended. In this way they were only very slightly contracted when killed.

It was thought at first that this form belonged to the species *Gorgoderia amplicava* Looss. Unfortunately, specimens of this species could not be obtained for comparison. From a study of the descriptions of *G. amplicava* in the literature on bladder flukes it was concluded that the species were not the same. The only other species of this genus known in North America is *Gorgoderia minima* Cort. That species is much smaller than the one to be described here. The European forms are all much larger than any of the American species of *Gorgoderia*.

This species of distome is similar in activity and habit to the others of this genus. The anterior portion of the body is very active and moves about freely while the posterior region is less active but not sluggish. The cuticle of the anterior part of the body is marked with minute longitudinal striations. These markings extend to or slightly beyond the acetabulum. The part of the body which is anterior to the acetabulum is cylindrical but becomes flattened near the acetabulum while the posterior portion is somewhat flattened and rather opaque. The opacity extends from the posterior end forward to the region of the ovary. That portion of the body occupied by the ovary, vitellaria and acetabulum is fairly transparent.

The length of the animal varies from 2.5 to 3.75 mm. with a width of .5 to .65 mm. in the region posterior to the ventral sucker. This form appears to be considerably smaller than *Gorgoderia amplicava* which has a length of 3 to 5 mm., and larger than *G. minima*, that form being 1 to 2 mm. in length. The individuals which measure 2.5 mm. in length have large numbers of eggs in the uterus while in those of the larger size this organ is entirely filled throughout giving it the appearance of being a mere egg sac. In individuals which are less than 2.5 mm. in length no eggs are developed.

The ventral sucker ranges from .60 to .75 mm. in diameter and is surrounded by a distinct circular sheath 0.05 to 0.135 mm. in width (Figs. 1 and 2, vss). This circular sheath around the acetabulum is very marked and is a rather distinct characteristic in this form. Therefore, I wish to propose the name *Gorgoderia circava* for this species.

The sheath around the sucker forms a distinct space or cavity between the wall of the sucker and structures of the body (Fig. 7). Small muscle bands (Fig. 7, mb) bind the tissues of the body to the ventral edge of the sucker. There are also a few muscle bands and connective tissue fibers extending across the cavity which connect the sucker with the internal parts of the body. From the external appearance of a normal animal the sheath is only slightly apparent from a side view and appears only as a slight bulge around the sucker. In an animal with both ends curved ventrally the sheath forms a distinct fold around the acetabulum (Fig. 4). The ventral sucker with the circular sheath produces a structure from .65 to .8 mm. in diameter, which is somewhat broader than the greatest breadth posterior to the sucker. The oral sucker has a diameter ranging from .30 to .37 mm. with an average of .33 mm. for ten specimens. The ratio of the oral sucker to the ventral sucker ranges from 1.8:1 to 2.3:1 with an average for ten specimens of 2.1:1. As stated by Cort (1912:162) the acetabulum of *G. amplivava* is 2.5 to 3 times the size of the oral sucker. Therefore, *G. circava* is different in this respect.

The mouth is situated in the oral sucker and appears as a triangular orifice in the posterior part of the sucker. The esophagus (Fig. 1, e) is a short narrow tube 0.14 mm. in length and 0.03 mm. in width. The intestinal ceca (Fig. 1 and 2, i) are about 0.055 mm. in width and are dorsal extending from the esophagus to within a short distance of the posterior end of the body. They are widely separated to give room for the reproductive organs which lie between as well as ventral to them. The ceca are dorsal and lateral to the testes. Some folds of the uterus pass to the lateral margins of the body and lie outside the ceca.

The reproductive system of *Gorgodera circava* is similar to that of the other species of this genus. The principal differences lie in the relative size and shape of parts, such as the number of vitellaria, shape of ovary, seminal vesicle and ejaculatory duct. There are nine testes, five on the same side with the ovary and four on the other. They are irregular in shape and the anterior ones are somewhat larger than those posterior. The shapes and sizes of the individual testes vary in different individuals but in general those which are anterior are proportionately broader than those posterior. With one exception the testes range about 0.23 mm. in length, 0.14

to 0.17 mm. in breadth and 0.22 mm. in thickness. The testis which is most posterior is usually much smaller than the others, measuring about 0.17 mm. in length by 0.12 mm. in breadth and 0.21 mm. thickness. The testes on either side are connected by minute tubules. From the dorso-anterior edge of the anterior testis on each side arises the vasa efferentia (Fig. 5, ve). These tubules extend anteriorly and unite in the region of the vitellaria to form the vas deferens which passes forward to the vesicula seminalis (Fig. 3, ves). The vesicula seminalis is a large pyriform sac dorsal to the anterior edge of the ventral sucker. It has a length of 0.15 to 0.2 mm., breadth of 0.14 mm. and thickness of about 0.15 mm. The shape and size is somewhat modified according to the degree of expansion or contraction of the worm. The vesicula seminalis is entirely filled with sperm cells. From the dorso-anterior edge of the vesicula seminalis the ejaculatory duct (Fig. 3, ed) arises and curves ventrad for some distance and then extends forward to the common genital pore (Fig. 3, g). This duct has a total length of 0.16 mm. and in the proximal region has a diameter of 0.015 mm. Around the distal portion of the duct are grouped the prostate glands (Fig. 3, p), a group of unicellular gland cells. In this region the ejaculatory duct is much enlarged forming a large pouch (Fig. 3, ep), or lumen in the midst of the prostate gland. This pouch or enlargement of the duct is 0.07 mm. in length and 0.05 mm. in diameter. The ejaculatory pouch as well as the duct is filled with sperms.

The vitellaria (Fig. 2, v) are immediately posterior to the ventral sucker and anterior to the ovary. They are made up of two groups of six to eight follicles each. One group lies toward each side of the animal and they are connected by a transverse vitelline duct. This duct becomes enlarged to form the vitelline reservoir in the median line of the body (Fig. 6, vr). From the dorsal surface of the vitelline reservoir arises a small median vitelline duct (Fig. 5, vd) which passes dorsal into Mehlis' gland where it unites with the ootype.

The ovary is a distinct three-lobed structure 0.27 mm. in length, 0.24 mm. in breadth, and 0.21 mm. in thickness. This organ lies toward the ventral side of the body. It may occur on either the right or left side as about half of the specimens studied showed it on one side and the other half on the other. The oviduct arises from the dorsal surface of the ovary as a funnel-shaped structure with the broad part of the funnel attached to the ovary. It extends

dorsad for some distance as it becomes narrow and then curves laterally or anteriorly, after which it enlarges immediately into the fertilization space (Figs. 5 and 6, f). It then becomes narrow again and passes forward near the dorsal surface of the animal to Mehlis' gland (Figs. 5 and 6, m) where it changes into the ootype. Mehlis' gland is a small group of unicellular gland cells located between the posterior edges of the vitellaria and dorsal to the transverse vitelline duct. Laurer's canal (Fig. 5 and 6, l) passes from the proximal region of the oviduct between the fertilization space and the ootype and makes a slight lateral curve. It then goes anteriorly and dorsally to the point where it opens on the dorsal surface of the body either dorsal or lateral to the ovary.

In passing from the ootype the uterus curves ventrad and bends back on itself (Fig. 5 and 6, u) in the median line of the body and goes posteriorly between the testes and finally reaches the posterior extremity of the body, where it fills with its numerous coils the region of the body posterior to the ovary and testes. The coils of the uterus become filled with eggs. Small masses of sperm cells are scattered throughout the coils of the uterus. The uterus finally emerges from the mass of coils in the region of the anterior testes (Fig. 2) and extends forward ventral to the ovary and vitellaria, passes dorsal to the ventral sucker and ventral, or slightly lateral to the vesicula seminalis to the genital pore (Fig. 3).

The eggs of *Gorgoderia circava* increase in size as they develop and pass from the ootype to the genital pore as in other species of the bladder flukes. In this case only the eggs in preserved specimens have been studied and no doubt there has been some shrinkage through the process of preservation. The eggs at the ootype measure about 0.016 mm. in length by 0.013 mm. in breadth; at the posterior end in the coils of the uterus 0.025 mm. in length by 0.019 mm. in breadth; while at or near the genital pore where they contain fully developed embryos, about 0.030 mm. in length by 0.023 mm. in breadth.

The chief differences between the American species of *Gorgoderia* lies in the size and shape of the animals; the structure, size and ratio in sizes of suckers; and the shape and relationship of the reproductive organs. *Gorgoderia minima*, described by Cort (1912) is the smallest of the three species, it being 1 to 2 mm. in length and its acetabulum is 1.6 to 2 times the size of the oral sucker. *Gorgoderia amplicava*

first described in this country by Bensley (1897), and reviewed by Stafford (1902), and again compared with *Gorgoderia minima* by Cort (1912), is considerably larger being 3 to 5 mm. in length and its acetabulum is 2.5 to 3 times the size of the oral sucker. *Gorgoderia circava* is 2.5 to 3.75 mm. in length and the acetabulum ranges from 1.8 to 2.3 times the size of the oral sucker. The acetabulum is also surrounded by a distinct circular sheath which is a distinctive characteristic of this species. In *Gorgoderia circava* the vitellaria are composed of six to eight follicles in each group while *Gorgoderia amplicava* has eight to ten in each and *Gorgoderia minima* has nine to eleven. The ovary of *Gorgoderia circava* is a distinct three-lobed structure while in *G. minima* it is only slightly lobed and in *G. amplicava* it has three to five irregular lobes with smaller or secondary lobes. The presence of the ejaculatory pouch in *Gorgoderia circava* is another structure not found in either of the other species. The differences in the reproductive organs and the presence of the circular sheath around the acetabulum clearly sets *Gorgoderia circava* off from the other species.

## LITERATURE CITED

BENSLEY, R. R.

1897. Two forms of *Distomum cygnoides*. Centr. f. Bakt., u. Infekt., 21:326-331.

CORT, W. W.

1912. North American frog bladder flukes. Trans. Amer. Mic. Soc., 31:151-166.

LEIDY, J.

1851. Contributions to Helminthology. Proc. Acad. Nat. Sci. Phila., 5:205-209.

LOOSS, A.

1899. Weitere Beiträge zur Kenntniss der Trematoden-fauna Aegyptens. Zool. Jahrb., Syst., 12:521-784.

1902. Ueber neue und bekannte Trematoden aus Seeschildkröten. Zool. Jahrb., Syst., 16:411-794.

STAFFORD, J.

1902. The American Representatives of *Distomum cygnoides*. Zool. Jahrb., Syst., 17:411-424.

1905. Trematodes from Canadian vertebrates. Zool. Anz., 28:681-694.

## EXPLANATION OF PLATE XIII

All drawings made with the aid of camera lucida.

Fig. 1. Dorsal view of *Gorgoderia circava*, X35.

Fig. 2. Ventral view of *Gorgoderia circava*, X35.

Fig. 3. Reconstruction from sagittal sections showing ends of reproductive organs and genital pore, X130.

Fig. 4. Outline drawing of small specimen which is bent ventrally at both ends causing the acetabular sheath to form fold around sucker, X35.

Fig. 5. Reconstruction of female genital organs from sagittal sections, X120.

Fig. 6. Reconstruction of female genital organs from frontal sections as seen from dorsal view, X120.

Fig. 7. Sagittal section through ventral sucker to show ventral sucker sheath, X35.

Abbreviations

<i>e</i>	esophagus	<i>ov</i>	oviduct
<i>ed</i>	ejaculatory duct	<i>p</i>	prostate gland
<i>ep</i>	ejaculatory pouch	<i>u</i>	uterus
<i>ex</i>	excretory pore	<i>v</i>	vitellaria
<i>f</i>	fertilization space	<i>va</i>	vas deferens
<i>g</i>	genital pore	<i>ve</i>	vasa efferentia
<i>i</i>	intestinal ceca	<i>ves</i>	vesicula seminalis
<i>l</i>	Laurer's canal	<i>vd</i>	median vitelline duct
<i>m</i>	Mehlis' gland	<i>vr</i>	vitelline reservoir
<i>mb</i>	muscle bands	<i>vs</i>	ventral sucker
<i>o</i>	ovary	<i>vss</i>	ventral sucker sheath
<i>os</i>	oral sucker	<i>t</i>	testes

147

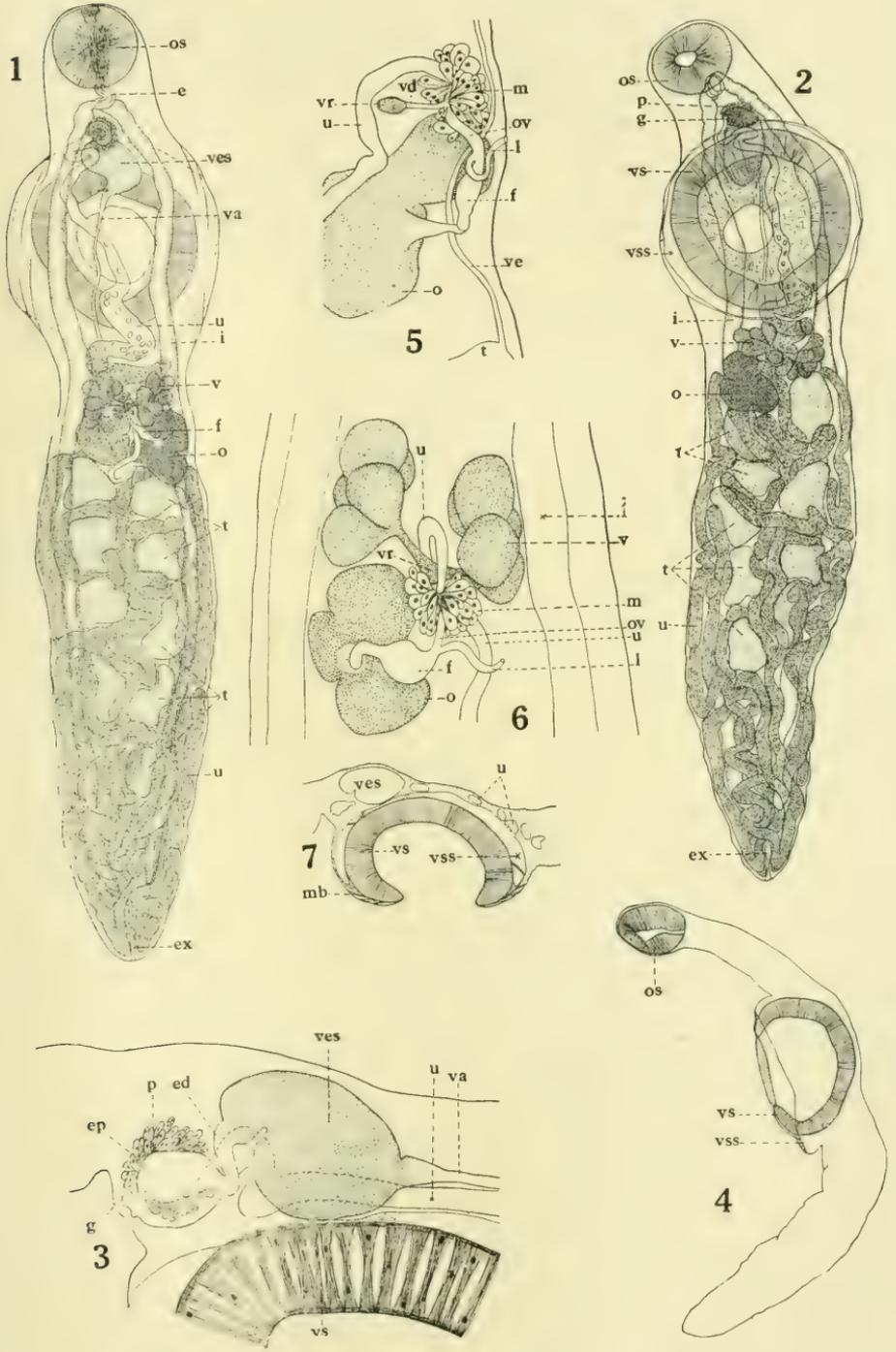


PLATE XIII

GUBERLET



## LABELING ILLUSTRATIONS

BY

Z. P. METCALF

North Carolina State College and Experiment Station

Sometimes really good illustrations are spoiled by faulty or poorly made labels and not infrequently biological illustrators do not do sufficient labeling to make their illustrations clear. The thought is frequently expressed by good draughtsmen that labeling is difficult and is therefore to be avoided, or they say they wish that they had been born with the ability to letter drawings properly thus expressing clearly that in their own opinions their drawings are not labeled as they should be. With these thoughts in mind it seems not amiss to outline the following methods that may be used for labeling biological illustrations.

It frequently becomes necessary to indicate separate parts on an illustration, hence it becomes necessary to label the drawing. This may be done in a variety of ways. If the person making the illustration has enough ability he may make the lettering free handed. Another method is to select letters or figures from printed matter. These may then be cut out and pasted on the illustration in the proper place. Still another method is to buy the cut-out letters and figures and paste these on the illustration. The sets desired may be written on the typewriter and cut out and pasted on the illustration. Labels may be printed directly on the illustration. And lastly guide lines may be drawn to the margins of the illustrations where they may be connected with type set up in the ordinary way when the illustration is printed. Regardless of the method of labeling selected care should be exercised to make the labels as neatly and accurately as possible. Care should also be exercised to see that the lettering is sufficiently large to stand the necessary reduction if the illustration is reduced.

The labels of biological illustrations are generally indicated in the following manner: (1) by Arabic numerals, (2) by the initial letters of the name of the parts of the object labeled, (3) by abbreviation of the names of the parts of the object labeled, (4) by a sequence of letters, and (5) by the full names of the parts of the object. In the first four methods it is necessary to print an explanation of the

labels. The fifth method requires no explanation. The method selected will depend upon the personal choice of the draughtsman. There is much to be said in favor of the fifth method provided there are not too many parts to label or the names are not too complicated. By this method the attention is not distracted by having to search through a long list of explanations. If it is preferred to abbreviate the names, then there is much to be said in favor of the second and third methods, for the initial letters and the abbreviation will indicate the real name of the part. If either of these methods is selected the explanations should be arranged alphabetically, so that it will not be necessary to look through the entire list to find the explanation for any abbreviation.

The labels may be placed at the margin of the illustration or directly on the illustration. The method we select will depend to a great extent on the nature of the illustration. If the parts are large enough so that the labels may be placed directly on the part without obscuring any details this is perhaps the best method. For many illustrations, however, this is not possible. The labels should then be placed at the margin. This latter method necessitates the use of guide lines. Guide lines may be simple straight lines or they may be brackets. Brackets are used to indicate areas of considerable extent which could not be indicated by a single line. Instead of the bracket, two lines drawn at an angle to each other may be used. The straight lines may be solid, dotted or dashed lines. If a dashed or dotted line is used care should be taken to get the dots of uniform size or the dashes of uniform length. As a general proposition guide lines should be straight and should run parallel to the main margin of the drawing if possible. On very light drawings the guide lines should be black. If the guide lines run over alternate dark and light areas it is advisable to use a double line, one part being black and the other part white. Care should be exercised to keep guide lines of uniform thickness. This may readily be accomplished by using a ruling pen. Ordinary liquid India ink may be used for black lines and any good grade of Chinese white for white lines. Care should be taken not to make the lines so thick that they will look unsightly when the drawing is complete. On the other hand, the lines should not be so delicate that they will not stand the necessary reductions.

Cut out letters or figures which are gummed on the back may be purchased in a wide variety of styles and sizes. These are very desirable for labeling drawings especially if the correct size and a suitable style are selected. Care should be taken to see that the separate letters are pasted in a straight line. This may readily be accomplished by drawing a faint pencil line to indicate the bottom of the letters, and then bringing the letters to this line. Cut out letters have the advantage that they may be pasted directly on the illustration, and will obscure only a minimum amount of detail.

Labels may be written on the typewriter using a good black record ribbon. In making labels on the typewriter only a new ribbon should be used and the type should be thoroughly clean so that good sharp impressions can be secured. These labels may then be cut out and pasted on the drawing as recommended above for printed labels. The chief difficulty with this method is that the labels are too small unless the illustration is not to be much reduced in reproduction. Type written labels may be enlarged by making a negative from them by any of the enlarging methods, and then printing a positive from this negative on a smooth gaslight paper. This method is advocated chiefly where we need a large number of labels of the same kind.

Labels may be selected from printed matter and pasted on the drawing. It is necessary to bear in mind the amount of reductions that will take place in reproducing the drawing. The ordinary book type is about 9 points, therefore if the drawing is reduced to one third the original labels should be 28 point. If the drawing is reduced to one fourth the original label will have to be 36 point. It is usually difficult to find letters of sufficient variety in these sizes. Special labels may be set up by the printer but this is usually a very expensive method. Plate XIV shows the various sizes of printed letters and may be used to determine the size of letters that it is necessary to use. Thus if the drawing is reduced to one third labels the size of 36 point will appear as 12 point or 18 point will appear as 6 point, etc. The various sized letters on this plate may be traced on thin tracing paper in India ink and pasted on the drawing.

#### HAND PRINTED LABELS FROM RUBBER TYPE

Labels may also be printed by hand from rubber type directly on the illustrations. For printing labels from rubber type we will

need a set of rubber type of the proper size, holder to hold the type, and a stamp pad filled with faint blue ink. The proper combinations of letters are set up in the holder, bearing in mind that the type are inverted and reversed. The type in the holder are then stamped in the pad and then on the drawing. The labels are then traced over with India ink. It is necessary to trace over labels made with a rubber stamp because the margins are not clear cut. The advantage of using the pale blue ink is that if the illustration is reproduced by ordinary photographic processes the blue will not show and need cause no trouble if slight errors are made. Rubber type may be secured in a variety of styles and sizes. A neat legible style should be selected and a size selected so that it will stand the necessary reduction. Sets of complete alphabets may be purchased or separate stamps may be produced from dealers in office supplies. The former has the advantage that any label may be readily set up. The latter is preferable if many labels of the same kind are to be printed at one time. The holders for rubber type are usually supplied with the sets of type. These holders are convenient and since they are adapted to the size type with which they are supplied they leave little to be desired. Stamp pads for this purpose should be inked with a faint blue ink as this color is not very active, photographically it does not bother the engraver. After the labels have been stamped with the faint blue ink they must be finished up with black India ink. This requires a fine pen and a little attention to details, but can be done with considerable rapidity after a little practice.

#### HAND PRINTED LABELS FROM METAL TYPE

Labels may also be printed from the regular metal type of the printer very much as the rubber type is used. To print labels from metal type we will need some black printers ink, a font of type of proper size, a holder for the type and a compositor's roll.

The ink used for hand printed labels of the metal type is the regular black printers ink. This usually requires thinning to work properly. Benzine, gasoline or xylol may be used for thinning the ink. For this purpose the ink is placed on a piece of glass and the solvent is added drop by drop while the ink is worked with a spatula, until it is of the proper consistency. Experience soon teaches when the ink is of the proper consistency. When it is thought that the ink is properly mixed a small amount of the prepared ink is spread on the

compositor's roll and the type in the holder is stamped on the roll and printed on a piece of paper. If the ink is in the proper condition and has been properly spread on the roll enough will adhere to the face of the type to make a good label. If the ink is too thin it will spread when we attempt to print a label. If it is too thick not enough of it will adhere to the face of the type to print a good label.

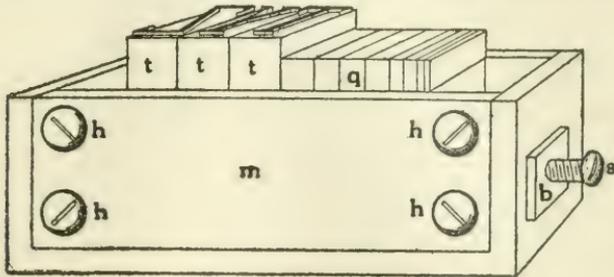


Fig. 1. Type holder for printing labels.

b, bur of the stove bolt which is soldered to the type box.  
 h, heads of stove bolts used to clamp the movable plate against the type  
 m, movable plate

q, quads which are used to fill out the line of type  
 s, stove bolt used to clamp the type in the line  
 t, type

The type holder (Fig. 1) used for hand printed labels is a shallow box made of brass. The height of the box is a little less than the height of the type. One side of the box is movable and is fastened to the opposite side by means of four set screws. This is used to lock the type in rows. One end of the box has a set screw which is used to lock the type. This holder is used very much as an ordinary rubber stamp is used. If only one figure or letter is to be printed in each label it is not necessary to use the type holder, but the individual types can be held in the hand very readily.

Type is set up in an inverted and reversed position. Each piece of type has little grooves called the nicks which indicate when the type is in a proper position. As soon as a line of type is set up a glance will reveal whether all of the characters are in the proper position. The characters to be used are picked out from the type box one at a time and placed in the holder which is held in the left hand in an inclined position so that the type will lay against the fixed side of the holder. As soon as the label is completely set up the rows of type are locked in place by tightening the set screws

which fasten the movable side and the end. At first the set screws are only set tight enough to hold the type firmly in place. A soft wooden block is then placed on the face of the type and the type is leveled up by pounding with a light hammer. The properly set up label is firmly pressed on to the inked compositor's roll and the inked type is then stamped on the illustration. Care being taken to press the type down firmly at all points without allowing it to move.

The advantages of hand printed labels are that they are very neat and accurate and that they may be made by any one without previous experience. The chief disadvantage is that it is somewhat laborious to set up the type. But with the large sized type used in printing labels for illustrations this is not a very large item. It must be remembered that it requires several hours for the printers ink to dry and care must be exercised in handling the illustrations or the labels may be ruined.

#### FREE-HAND LETTERING

Occasionally it is not possible to letter an illustration by any of the methods given above, in that case it is necessary to have recourse to free-hand lettering. Free-hand lettering is a special kind of free-hand drawing by means of which the draughtsman learns to draw the design of letters neatly and rapidly. There are no special tricks of the trade about lettering that cannot be learned by the biologist who will conscientiously try to master the subject.

The first consideration in free-hand lettering as in other kinds of free-hand drawing is to get the main proportions. After the main proportions are secured the details are added. The more important details are added first then the finer and finer details until the lettering is complete. Just as the individual letters are found to vary one from the other so in printing a line of lettering it will be found necessary to space the letters carefully with reference to each other, otherwise the lettering will not have a neat appearance when finished. In lettering each letter is influenced by the letters on each side of it so that no general rule can be laid down which will make it possible to always place a letter the proper distance from its neighbors. The only rule that can be given is that all letters should seem to have the same amount of space allotted to them. Obviously this amount of space will vary with the different letters and with the letters on each side of it. Thus a capital I requires less space than a capital M.

Then, too, a capital I will require more space if placed between a capital M and a capital N than if placed between a capital E and a capital T because these letters have a great amount of free space whereas the M and N have practically no free space. The letters in each word must be studied, therefore, in order to determine the proper spacing. Trials should be made in order to see just what spacing looks the best. If this is done critically gradually a proper conception of proper spacing of letters will be acquired.

The usual error made by beginners is to space the letters too far apart. Letters look better when they are crowded well together.

The letters in any design that is to be treated in free-hand lettering should be sketched in with a pencil complete before finishing up any of the letters. This is done in order to insure a proper balance of the words with each other and a proper spacing of the letters. In sketching letters it is usually advisable to draw two faint lines one for the top and the other for the bottom of the letters. Sometimes it is advisable to divide this space by a third line so that certain letters may be carefully drawn with rapidity. After the base lines are drawn the letters are sketched in spacing each letter with reference to the other letters, and indicating at first only the general outlines of the letters. Corrections should be made until the whole has a well balanced appearance. Some letters may then be carried forward and the principal minor details indicated, making any necessary corrections in the other letters in the line to maintain the balance. After experience has been gained letters may be sketched in ink free-hand, especially such forms as the Gothic. And any one having considerable lettering to do should practice this form of lettering, but the art of lettering is soon lost unless it is used day by day.

After the pencil sketch of a line of lettering is finished the letters may be finished with India ink on drawings or with black water color or oil color on paintings. In doing this the borders of the main letters are finished first and then the borders of the finer details; the body of the letters being filled in last of all. Care must be taken in finishing up the borders not to exceed the limits penciled and to keep all straight lines straight and all curved lines a true curve. In filling in the body of the letter care must be taken not to allow the color to run over the borders that have been finished. In finishing large letters a ruling pen and a straight edge may be used for the longer

straight lines on the borders of the letters but on the smaller letters and for all fine details a pen must be used free hand.

The idea is prevalent that the forms of letters are fixed but nothing is farther from the truth. There are certain broad general styles of letters such as the Roman and Gothic but the variations in these styles are as many as there are draughtsmen. A few of the more important styles are discussed below and plates showing standard letters are given, not with the idea that these should be copied slavishly but that these designs may be helpful in producing letters for drawing and may serve to indicate the main styles. All letters occur in two forms, capitals usually called caps and small letters called lower case. If there are only a few letters in a group as in the abbreviated signs used to label parts of a drawing either caps or lower case or both kinds of letters may be used, but if there is a series of words it is better to use lower case letter throughout as we are used to reading words printed in lower case types. Words in lettering should be well separated so that there is no doubt as to the limits of the separate words. The rule is that the words should be separated at least by a space equal to that occupied by the widest letter and slightly more space would be better.

The Gothic letter is the simplest letter because it is formed of lines of a uniform thickness throughout. Gothic letters exist in two forms, a vertical Gothic and an inclined Gothic. In the vertical Gothic alphabet the main axis of the letters is vertical and since the lines are all of a uniform thickness it is a fairly easy alphabet to letter in a free-hand manner. For convenience of discussion the letters are divided into the following groups, (1) letters composed of straight horizontal and vertical lines, (2) letters composed of horizontal or vertical lines with diagonal lines, (3) letters composed of straight and curved lines and (4) letters composed of curved lines only. Furthermore it is convenient to define a full bodied letter as a letter occupying as much horizontal as vertical space. For purposes of analysis the letters are drawn on cross section paper each letter occupying a vertical distance of five units. The thickness of the stroke is only  $\frac{2}{3}$  of a unit.

In the first group of letters we have the capitals E, F, H, I, L and T and the lower case letters i, l. In the capital E it will be noted that the letter is not a full-bodied letter as the foot occupies only  $4\frac{1}{2}$  units and the cap only  $\frac{1}{4}$  units. The tongue of the letter occupies

only  $2\frac{1}{2}$  units and is placed only slightly higher than the middle. The capital F is identical with the E except that the foot is omitted. Care should be taken not to extend the cap too much or the letter will look top-heavy. The capital H occupies about 4 units as otherwise it looks too broad. The tongue is placed on a level with the tongue in the E and F, that is slightly above the middle. The capital I needs no comments as it is simply a straight line with a thickness of  $\frac{2}{3}$  of a unit. The foot of the capital L is about  $3\frac{1}{2}$  units in length to prolong it makes it appear unwieldy. The full bodied lower case letter occupies only three-fourths the space allotted to the full bodied capitals and the width of stroke is only a half unit. The small lower case letters occupy only two-thirds of the vertical space occupied by the large lower case letters. Therefore the body of a small lower case letter like i would occupy only one-half of the vertical space allotted to a capital letter, hence  $2\frac{1}{2}$  units the dot being placed one full unit above the top of the letter. The lower case l would occupy  $\frac{3}{4}$  of the vertical space allotted to a capital.

To the second group belong the capitals A, K, M, N, V, W, X, Y and Z, and the lower case letters k, v, w, x, y and z. The capital A occupies the full width of five spaces below and slopes to the top line uniformly on both legs. The top does not end in a sharp point but in a point that is about one-half unit wide. The tongue of the A is placed about one and one-half units above the base line. The capital K is somewhat difficult as it is composed of two diagonal lines at different angles. The top diagonal is usually placed about three and one-half spaces from the vertical stroke and at such an angle that if it were projected the lower border of the diagonal would strike the base line one full unit to the left of the vertical stroke. The lower diagonal is placed four units from the vertical stroke and at such an angle that its top border projected would strike the top of the vertical stroke. The capital V is simply the capital A inverted and the tongue omitted. The capital M is simply the V with two vertical strokes added on each side. Note that these vertical strokes end in their full width and not reduced as in the case of the top of the A and the bottom of the V. The capital N consists of two vertical strokes four units apart connected by a diagonal running from the top of the left hand stroke to the bottom of the right hand stroke not the reverse as is frequently seen in lettered signs. The diagonal is placed at such an angle that the vertical strokes will end in full

width on both the base and top limiting lines. The capital W may be considered as two V's contracted to occupy only four spaces each and united so that the apex of the jointed diagonals shall occupy only half a unit each. The capital X is simply two diagonals which cross each other in the center. This letter is therefore a full bodied letter. The capital Y is composed of two arms which are six units apart and run at such an angle as to unite two and one-half units from the base line. The foot of the capital Z is four and one-half units long and the cap only four units long. The cap and the foot are connected by a diagonal placed at the proper angle. In the lower case letters the stem of the K occupies the full vertical unit for lower case letters and the diagonals of the letter bear the same relation to each other that they do in the capital K but they are reduced to one-half. The lower case v, w, x and z are the same as the caps except they are reduced to one-half. The lower case y is the same as the lower case v with the right diagonal extended below the base line, the full length allotted to lower case letters.

In the third group we have the capitals B, D, J, P, R and U; and the lower case letters a, b, d, e, f, g, h, m, n, p, q, r, t, and u. The capital B may be considered as a capital E with the ends of the cap and the foot connected to the tongue by arcs of circles. It will be noted that this makes the top part of the letter somewhat smaller than the bottom. The capital R may be considered as a capital F with the cap and tongue connected as in the B and a tail added to the lower part. The tail of the R should extend beyond the top part of the letter at least a full unit otherwise the letter will look top-heavy. The P is similar to the R without a tail but the top part of the P is made longer by dropping the tongue about one-half unit below its position in R. A capital D is produced by using a foot and cap similar to the foot and cap in the capital B and connecting these two horizontal lines by a regular curve. The capital J and U are similar to each other save that the J has a single vertical arm and the U a double arm. The J is somewhat narrower occupying only three and one-half units whereas the U occupies about four and one-half units. The vertical arms are in each case about three and one-half units long. In the lower case letters b, d, p and g all have the same form and the q is simply the g with the stem turned to the left to distinguish these two forms; and the a is quite similar but with a shorter stem. The lower case u may be taken as the type of another

group of letters. It is essentially like the capital U with the right arm extended to the base. The lower case n is simply the u turned upside down and reversed and the m is simply two ns contracted slightly and united. While the lower case h is simply the n with the left side prolonged the full length of lower case letters. The lower case f, j, r and t are essentially vertical lines with short curved tails added. The capitals O and Q are essentially complete circles which extend slightly above the top line and slightly below the base line. The capitals C and G are parts of circles and offer no special difficulties. The capital S is composed of two curves joined by a third curve and is one of the most difficult letters to handle. The upper and lower limbs are arcs of ellipses whose major axes lie in horizontal planes with the major axis of the upper ellipse slightly shorter than the major axis of the lower ellipse and with their minor axes about in the ratio of two to three. The lower case o, a and s are essentially the same as the corresponding capitals and lower case c is similar with a horizontal line across the upper two-thirds of the circle.

The Gothic numerals may be taken as standard just as we took the Gothic letters. It will be noted that the numerals 1 and 4 are the only ones composed of straight lines only. The numeral 1 is a straight line  $\frac{1}{2}$  unit wide, the numeral 4 has a total width of four units and is drawn so that the horizontal tongue is one and one-half units above the base line. The numerals 3 and 8 are essentially the same being composed of two broad ellipses joined together the upper ellipse having a shorter major axis than the lower ellipse. The minor axes of the two ellipses bear a relation of about 2 to 3 to each other. The numeral 0 is merely a flattened ellipse with a major axis of 5 units and a minor axis of 4 units. The numerals 6 and 9 are the same being simply placed in different positions. It will be noted that they are essentially the same as the numeral 0 except for the formation of the small ellipse at the bottom of the 6 and the top of the 9. Note further that the tail of the 9 is somewhat expanded being near the base line and that the tail of the 6 is somewhat contracted being near the top line. This preserves the proper balance. The numeral 5 is essentially the same as the 6 except that the tail is composed of a straight vertical and horizontal line. The top is somewhat contracted to preserve the proper balance. The numeral 7 is four units wide with the curved vertical stroke ending on the base line about one unit to the right of the point where the horizontal line starts on the

top limiting line. The numeral 2 is the most difficult in the whole series as it consists of a compound curve. The top curve is somewhat like the top curve of 3 but is flatter and the bottom curve is more pronounced than the curve in the numeral 7.

The inclined Gothic is the vertical Gothic inclined at about  $15^\circ$  from the perpendicular. We need not make any special analysis of the separate letters as that has been done for the vertical Gothic. This is a favorite alphabet for draughtsmen who do a great deal of lettering as it can be done with great speed and if carefully done it looks neat and is very legible. For these reasons it is especially valuable for large amounts of labeling.

The Roman Gothic is in many respects a more pleasing alphabet than the Gothic. The basis of the letters is the same as for the Gothic but certain lines called body strokes are shaded by being made thicker, while some lines are made thinner than in the Gothic letter. The shaded or body strokes are usually made one unit wide and the hair lines are usually made one-half unit wide. Roman Gothic letters of widely different appearances may readily be secured by varying the width of the hair line. It should be noted that the curved body strokes are slightly thicker than the straight body strokes. Where curved lines join straight lines the union is made very gradually so that the eye cannot detect the point of union.

The Roman letter is a further modification of the Roman-Gothic by the addition of serifs to the strokes so that no lines end with a line of uniform thickness. This is the type of letter used in most printing and is the most difficult letter for the draughtsman to handle. However, it is perhaps the neatest appearing letter and should be used more extensively than it is at the present time. The separate letters need not be analyzed separately because they have essentially the same form as in the Gothic alphabet. The body stroke is usually considered as one unit in width for the straight lines and slightly wider for the curved lines. Sometimes variation in this standard is made for some special purpose. The hair lines vary greatly in different styles of this letter from lines as thin as they can be drawn easily to lines at least half a unit in width. By varying the widths of the hair lines, letters of quite different appearances may easily be secured. The serifs demand special attention and must be drawn neatly and accurately or they will ruin the appearance of the lettering. Horizontal serifs are usually about one unit in length and are con-

nected to the main stroke by a gentle curve which is made tangent to the main stroke and to the serif. Vertical serifs are usually made about one and one-half units long and are connected to the main stroke by a gentle curve which is tangent to the serif but not tangent to the main stroke. Exception, however, must be made in the case of the double serifs found on the tongue of the E and F, which are smaller than the other vertical serifs. In letters like E, s and Z in which are two vertical serifs the upper one is made slightly shorter than the lower one for the sake of appearance. In the capital J and some of the lower case letters it will be noted that curved lines instead of ending in straight serifs end in curved comma shaped marks called kerns. Instead of filling in the body strokes of Roman letters solidly they may be indicated by two hair lines. This makes a neat appearing letter and is useful for display titles but is difficult to execute and therefore seldom employed in labeling biological illustrations.

## EXPLANATION OF PLATES

Plate XIV. Letters and figures of various sized type.

Plate XV. Vertical Gothic letters analyzed.

Plate XVI. Roman Gothic letters analyzed.

Plate XVII. Roman letters analyzed.

— 30 point —

ABCDEFGHIJK  
abcdefghijk  
1234567890

— 24 point —

ABCDEFGHIJKLMNO  
abcdefghijklmno  
1234567890

— 18 point —

ABCDEFGHIJKLMNO PQ  
abcdefghijklmnopq  
1234567890

— 12 point —

ABCDEFGHIJKLMNO PQRSTU VWXYZ  
abcdefghijklmnopqrstu vwxyz  
1234567890

— 8 point —

ABCDEFGHIJKLMNO PQRSTU VWXYZ  
abcdefghijklmnopqrstu vwxyz  
1234567890

— 6 point —

ABCDEFGHIJKLMNO PQRSTU VWXYZ  
abcdefghijklmnopqrstu vwxyz  
1234567890



EFHILLT i l 42

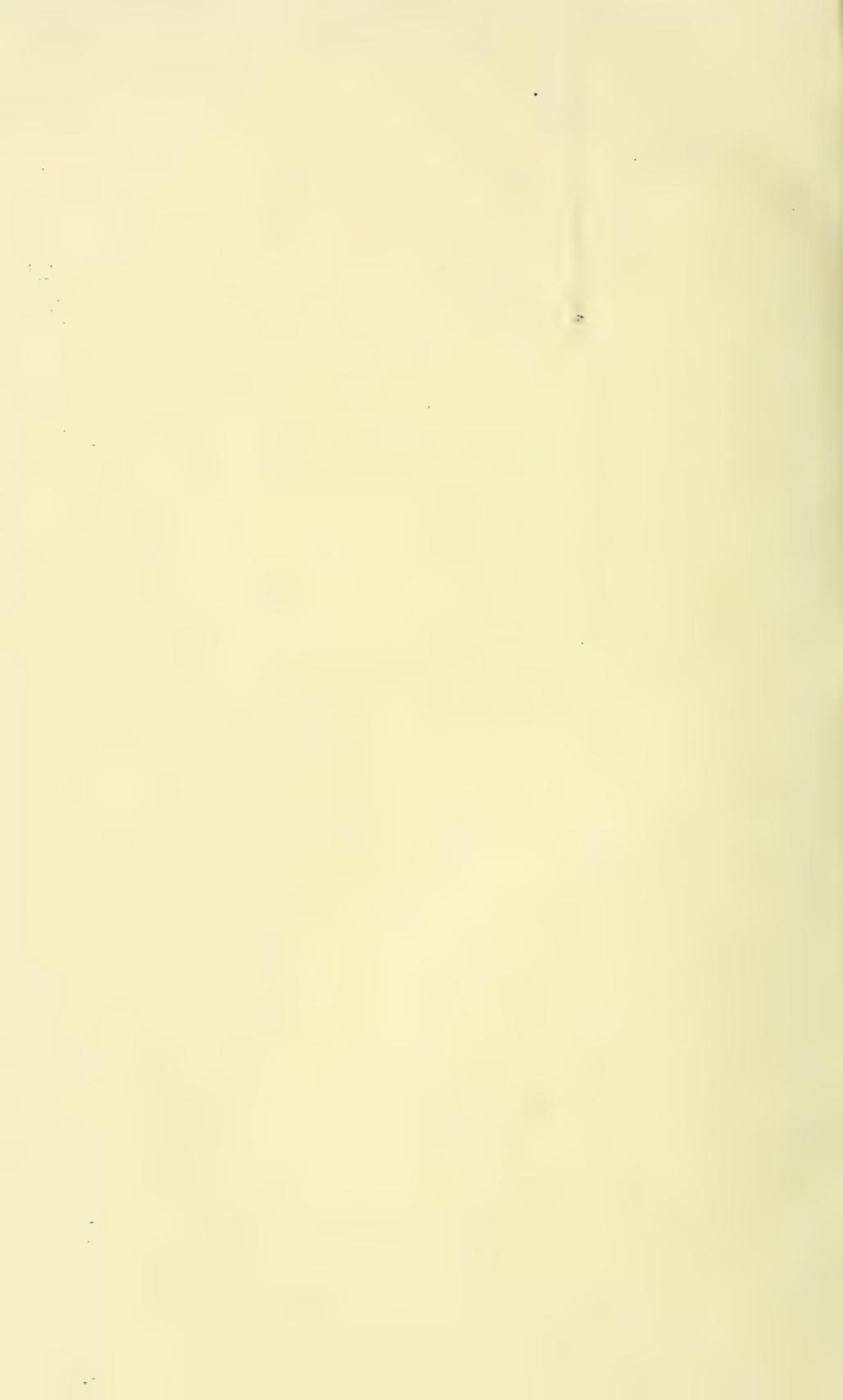
AKVMNWXy

YZ k v w x z 380

BRPDJUBdpp

g a u n m h f j r t 695

OOCC'S o c s e 7



EEHILLT;||42

AKVMNWXy

YZkvwXz380

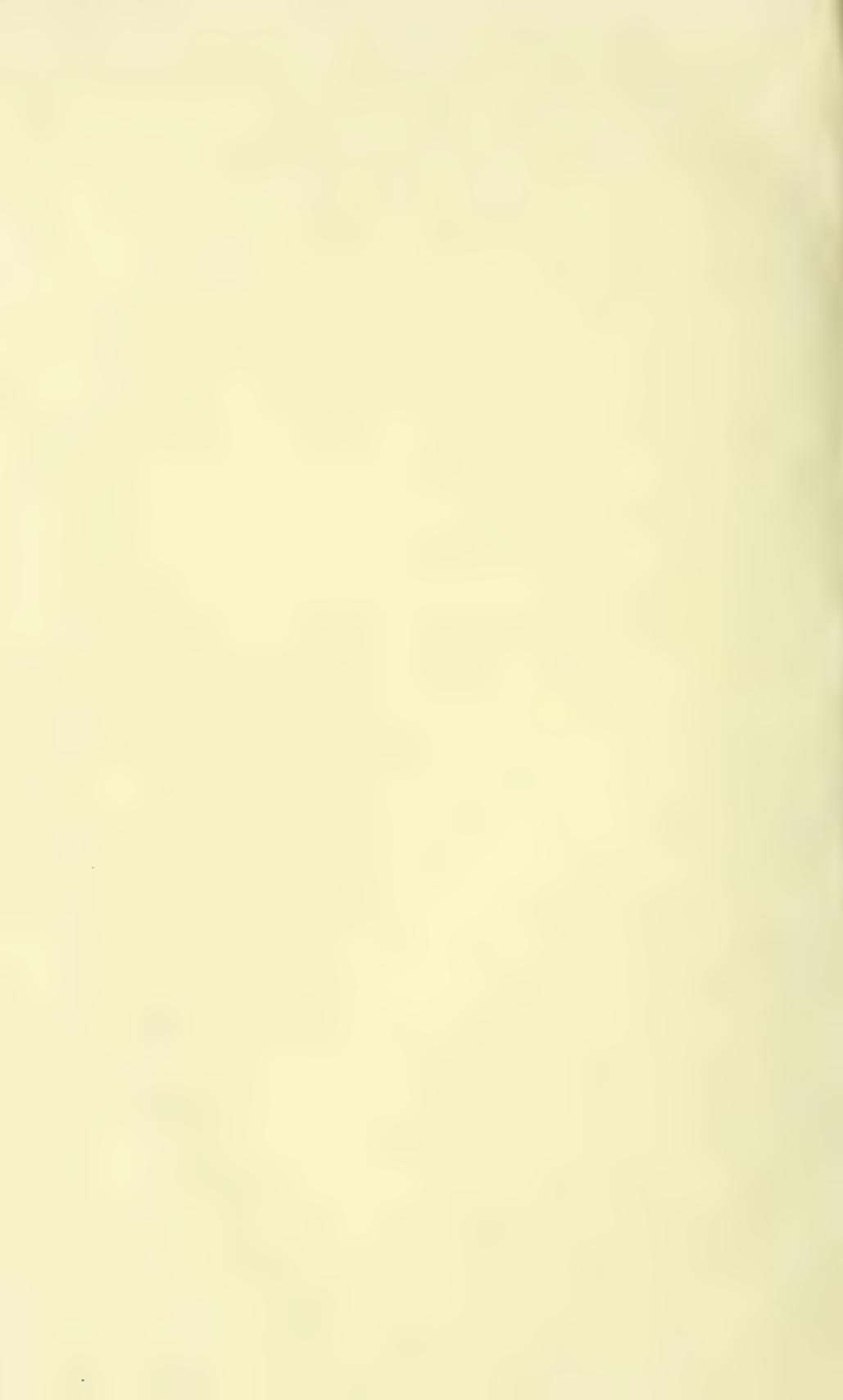
BRPDJUbdppq

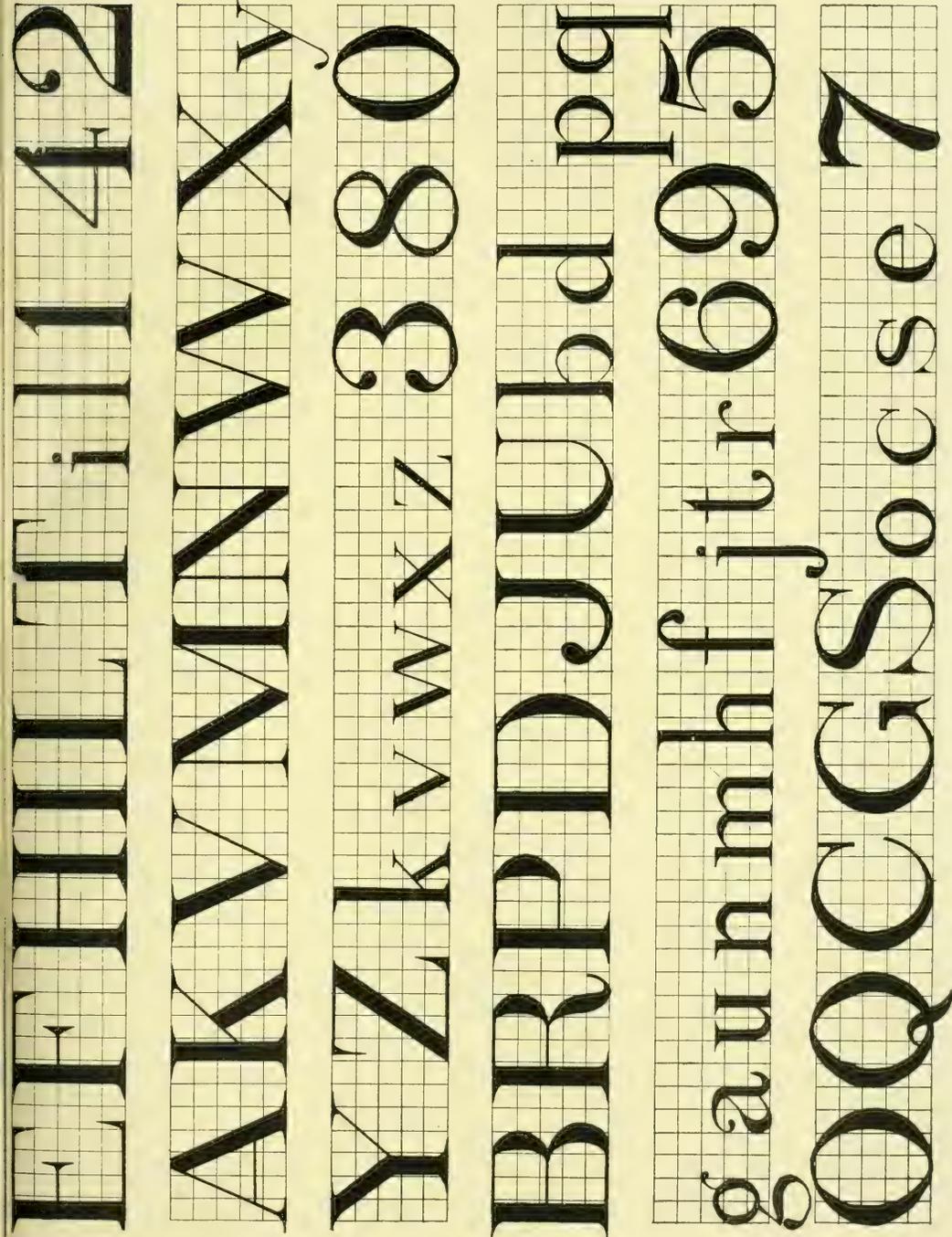
gaunmhfjtr695

OQCSocse7

PLATE XVI

METCALF







## 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.]

### ENTOMOLOGICAL ABSTRACTS

*Position of Micropterygidae*—Tillyard (1919, Proc. Linn. Soc. N. S. Wales, 44:95–136) has made an extensive study of the remarkable family of archaic moths, the *Micropterygidae*. Chapman (1917) removed the genus *Micropteryx* from the remainder of the family and proposed a new order, Zeugloptera, for its reception. Comstock (1918), on the other hand, removed the whole family *Micropterygidae* from the Lepidoptera and placed it as a new suborder of the Trichoptera. Tillyard finds no justification for either of these views. The proposed “Zeugloptera” is found to lack a single character not found in some other order. In all of the *Micropterygidae*  $M_4$  does not occur as a separate vein of the forewing; the characteristic trichopterous wing-spot is lacking; the pupal wing-tracheation is complete; the scales are broad and possess numerous striae; and functional frenula are present. These characters definitely rule out the possibility of these insects being Trichoptera, and necessitate the conclusion that that they must be archaic Lepidoptera.

*Micropterygidae*.—Braun (1919, Ann. Ent. Soc. Am., 12:349–367) has also attacked the problem of the position of the *Micropterygidae*. A study of wing structure in the primitive Lepidoptera shows, according to this writer, that while the *Micropterygidae* stand close to the common ancestor of Lepidoptera and Trichoptera they are true Lepidoptera and have given rise to all of the remainder of that order by several divergent lines, one represented by the Nepticulidae, another by the Hepialidae, and a third “much branched line includes the frenate Lepidoptera, of which some members such as the Prodoxidae, Incurvariidae, etc., conserve some of the trichopterous characters of their ancestry and must therefore be regarded as the most primitive of the Frenatae.”

*Filariasis in U. S.*—Francis (1919, U. S. Publ. Health Service, Hygienic Lab. Bull. No. 117) reports on a study of filariasis in Southern United States. *Filaria bancrofti* is the species concerned and one endemic focus has been located in this country at Charleston, S. C. Of 400 individuals examined in that city, 77 were infected with microfilaria, whereas of 1,470 examinations in nine southern cities outside of Charleston only 9 showed infection. The data indicated that cases outside of Charleston have derived the infection either from residence in Charleston or from residence at some place outside of the United States, as in Cuba. Transmission occurs only through the mosquito, but the certainty of the process is limited by the following facts: (1) No multiplication of the filaria in the mosquito; (2) The small number actually passing successfully through the mosquito; and the still smaller number which reach the lymph glands of man; (3) Male and female filaria must find lodgment in the same lymph gland of man in order that reproduction occur; (4) Infection of mosquitoes can occur only during a few hours before and after midnight; (5) The biting act of the mosquito only drops the microfilaria on the free surface of the skin of man whence it must penetrate the intact skin. The mosquito, *Culex fatigans*, was found to be the transmitter. The anatomy of the mosquito proboscis in relation to filaria transmission is discussed and the inward and outward courses of the filariae pointed out. The former is through the stilette bundle along with the ingested blood, while the latter is through the interior of the labium. Eight well executed plates, mostly in color, add to the value of the paper.

*Polyembryony and Sex.*—Patterson (1919, Journ. Heredity, 10:344-352) reports results of a study of the origin and development of mixed broods in polyembryonic Hymenoptera and the ratio in production of males and females. In 162 broods of *Copidosoma gelechiae*, 90 were female, 62 male and 10 mixed. The sex ratio was found to be approximately 3 females to 2 males. The great excess of females in four of the mixed broods suggested the possibility that both sexes might arise from a single fertilized egg. In *Paracopidosomopsis floridanus*, 1.7% of the broods were pure female, 11.3% pure male, and 87% mixed. The percentage of males varied from 0.06 to 72.07 and in over 58% of the broods less than 10% of the individuals in any brood were males. In *Platygaster rubi* not a single pure male brood was found. This, however, might be explained by the

prevailing conditions which make it unusual that an unfertilized female might escape. Only 6 of the 105 broods were pure female. In the 99 mixed broods, the number of females, in every brood, exceeded the number of males. In 53 broods only one male per brood appeared, 17 had 2 males each, and 13 had 3 each. The other broods showed a varying number, but not exceeding 10. That some mixed broods result from two parasitic eggs, one from a fertilized female and one from a virgin female, seems very probable but two difficulties stand in the way of the exclusive application of this application, namely, (1) simultaneous emergence of individuals of a mixed brood, and (2) striking predominance of females over the males in the great majority of broods. A *Paracopidosomopsis* female, in about 66% of the cases, deposits two eggs in the host egg at a single oviposition, and in the majority of cases both eggs were found to be fertilized. A host egg mass of 28 eggs exposed to a mixed brood of parasites yielded 14 with 1 parasitic egg, 11 with 2 each, and 3 with 3 each. Eight of the 11 indicated two ovipositions, while 3 seemed to represent one oviposition. In each of the 3 remaining eggs the three parasitic eggs apparently represented different ovipositions. Therefore the two-egg explanation seems inadequate for the mixed broods of *Platygaster*. It is proposed that some of the mixed broods may result by one fertilized egg giving rise to both sexes through abnormal behavior of the two sex chromosomes during early cleavage, as for example, somatic non-disjunction in which certain blastomeres receiving but one x chromosome would produce male embryos.

*Origin and Significance of Metamorphosis.*—Crampton (1919, Bull. Brooklyn Ent. Soc., 14:33-40; 93-101) considers critically the problems of origin and significance of metamorphosis in insects. Presence or absence of metamorphosis, although worthy of careful consideration, cannot be regarded as an important factor in determining the relationships of insects, according to this writer. An ancestral group, it is contended, may include some forms which have "developed the tendency towards a metamorphosis, to a marked degree, while other representatives of the same ancestral group do not exhibit any marked indications of such a tendency." Plecoptera, Embiidae, Dermaptera, Coleoptera and their allies constitute the "plecopteroid superorder" and are regarded as the ancestral group from which the higher insects were derived. This group contains forms exhibiting well marked metamorphosis and some which do not. The higher

forms are divided into two super orders: (1) the "psocoid superorder" containing the Psocodae, Mallophaga, Anopleura (Pediculidae, s.b.), Hemiptera, Homoptera and their allies—a group in which few members exhibit traces of metamorphosis; and (2) the "neuropteroid superorder" comprising the Neuroptera, Hymenoptera, Mecoptera, Diptora, Siphonaptera, Trichoptera, Lepidoptera and their allies, all being predominantly holometabolous. Thus it is suggested that we might expect the coleopterous representatives of the ancestral group to be somewhat nearer the derived holometabolous group, while the remaining representatives of the ancestral group would be nearer the derived non-metabolous group. To account for the origin of metamorphosis among some of the ancestral forms, it is thought that there arose a tendency (by mutation, etc.) of the immature stages to differ from the adults, resulting eventually in stages which could enter an environment untenable by the adult. Such forms, favored by natural selection, would tend to persist and thus there would appear a "propensity towards the production of complete metamorphosis." Against the claim of Handlirsch that *cold* produced metamorphosis, Crampton argues that "insects in which the tendency toward metamorphosis was *already well developed*, were better equipped than their less fortunate fellows, to penetrate the less favorable regions of winter-frost, etc., and there establish themselves." No support is found in embryology or palaeontology for the view that larval stages represent "free-living embryos." Disagreement with any view that environment *causes* metamorphosis is expressed. The pupal stage is regarded as the "making over" period necessitated when immature and adult stages come to differ so markedly that a great change must be involved in the transition. Larvae stages are regarded by this author as having some phylogenetic significance and may yield valuable hints as to relationships. Whether primitive types of larvae represent ancestral conditions more nearly than adults do seems uncertain. In some cases it seems to be true but in other instances the larvae have become far more specialized than the adult, thus involving secondary characters.

PAUL S. WELCH

*Department of Zoology,  
University of Michigan*

100

TABLE OF CONTENTS

FOR VOLUME XXXIX, Number 3, July, 1920

---

Protozoa of the Devil's Lake Complex, with two plates, by C. H. Edmondson . . .	167
Age, Growth, and Scale Characters of the Mulletts, <i>Mugil cephalus</i> and <i>Mugil curema</i> , with seven figures and seven plates, by A. P. Jacot. . . . .	199



127

# TRANSACTIONS OF American Microscopical Society

(Published in Quarterly Instalments)

Vol. XXXIX

JULY, 1920

No. 3

## PROTOZOA OF THE DEVIL'S LAKE COMPLEX, NORTH DAKOTA

BY

CHARLES HOWARD EDMONDSON, PH.D.

University of Oregon<sup>1</sup>

### CONTENTS

	PAGE
1. Introduction.....	167
2. Taxonomy.....	171
3. Experiments.....	190
4. Summary and Conclusions.....	193
5. Index.....	195

### 1. INTRODUCTION

Several reports previously issued<sup>2</sup> have described the physiographic and chemical characteristics of Devil's Lake situated in Ramsey County, North Dakota. In a recent paper Dr. R. T. Young,<sup>3</sup> of the University of North Dakota, has indicated something of the possibilities and limitations of the lake from a biological point of view, as well as the general scope of the work already accomplished in that direction. It will only be necessary, therefore, to set forth a few of the specific features of this water area which may have some bearing on the report to follow.

<sup>1</sup> The investigations included in this report were carried on at the State Biological Station of North Dakota.

<sup>2</sup> Biennial Report of the State Biological Station of North Dakota, 1911-12.

Pope, T. E. B. Devil's Lake, North Dakota, a study of physical and biological conditions, with a view to the acclimatization of fish. U. S. Bureau of Fisheries Document 634, 1908.

Simpson, H. E. The Physiography of the Devil's-Stump Lake Region, North Dakota. Sixth Biennial Report of the State Geological Survey of North Dakota, 1912.

Upham, W. The Glacial Lake Agassiz, Mon. 25, U. S. Geological Survey, 1895.

<sup>3</sup> Young, R. T. The Work of the North Dakota Biological Station at Devil's Lake. The Scientific Monthly, December, 1917.

Biological studies of Devil's Lake made by the United States Bureau of Fisheries in 1908 indicate the presence of four vertebrate inhabitants of the lake, namely: a stickleback, *Eucalia inconstans*; a minnow, *Pimephales promelas*; the hellbender, *Cryptobranchus alleghaniensis*; and the leopard frog, *Rana pipens*. Among the metazoan invertebrates reported are crustaceans, rotifers, nematodes, a flat worm, an arachnid and a number of species of insects. One may collect the shells of at least fifteen molluscs from the water line on the shore, but no living forms have been taken from the lake. Sponges, coelenterates, polyzoans and annelids are apparently entirely absent.

Investigations of the protozoan fauna of the Devil's Lake complex were undertaken as a part of the general biological survey of that water area. Although, in many respects, this fauna was found to be such as one might expect in a fresh water lake of similar depth, yet some very pronounced differences were disclosed. The almost total absence of shell-bearing rhizopods may possibly find its explanation in the chemical analysis of the water. *Arcella vulgaris* Ehrenberg, a very constant and usually abundant form in fresh water, was rarely observed and two species of *Diffugia*, which are among the most common protozoa in lakes where there is considerable ooze, were taken only in situations where the salinity of the water must have been materially reduced by the in-seepage of surface water. A species of *Euglypha* was taken in the overflow of the lake water from the fish tank. The only other shelled rhizopod observed was a single specimen of *Cyhoderia ampulla* Leidy, taken from the main lake.

The fact that the ooze at the bottom of the lake at times has been found to be entirely free from oxygen might also be a contributing factor to the scarcity of these usually common bottom-dwelling rhizopods of the shell-bearing type, although the presence of the larvae of a certain midge in this ooze as well as the work of Birge and Juday in Wisconsin,<sup>7</sup> where a considerable number of animals were found at the bottom of lakes in the absence of oxygen, would hardly seem to make this factor one of great importance.

Experiments of a preliminary character, recorded at the end of the taxonomic part of this report, indicate that certain protozoa having

<sup>7</sup> Birge and Juday, The Inland Waters of Wisconsin; Wisconsin Geological and Natural History Survey, 1911.

adjusted themselves to fresh water conditions are not, in all cases at least, readily adaptable to the waters of Devil's Lake.

The writer wishes to acknowledge his indebtedness to Dr. R. T. Young, Director of the State Biological Station of North Dakota, whose co-operation made this report possible, and to Mr. E. G. Moberg for his valuable assistance in collecting material.

## 2. TAXONOMY

SUBPHYLUM SARCODINA  
CLASS RHIZOPODA  
SUBCLASS AMOEBAE  
ORDER GYMMAMOEBIDA

FAMILY AMOEBIDAE

*Genus Amoeba Ehrenberg, 1831*

### *Amoeba proteus* (Rösel).

*Der kleine Proteus* Rösel, *Insecten Belustigung*, 1755, tab. 101.

*Amoeba proteus* Leidy, *Pr. Ac. Nat. Sc.*, 1878.

Occurrence.—Associated with *Ruppia* in Whipple Bay, Creel Bay, Minnewaukon Bay, Six-mile Bay, East Lake, and also taken from the east side of the main lake and from the overflow of lake water from the fish tank near the laboratory.

### *Amoeba radiosa* Ehrenberg.

*Amoeba radiosa* Ehrenberg, *Abh. Akad. Wiss.*, Berlin, 1830.

Occurrence.—Rarely observed. Taken with *Ruppia* from Minnewaukon Bay, also from Big Mission Lake.

### *Amoeba limax* Dujardin.

*Amoeba limax* Dujardin, *Histoire Naturelle des Zoophytes Infusoires*, Paris, 1841.

Occurrence.—Associated with *Ruppia* and algae at the head of Creel Bay, Big Mission Lake (numerous), Little Mission Lake (numerous), and the east side of the main lake (numerous).

### *Amoeba verrucosa* Ehrenberg.

*Amoeba verrucosa* Ehrenberg, *Die Infusionsthierchen als Vollkommene Organismen*, 1838.

Occurrence.—Observed but once, from material taken along the east side of Creel Bay.

*Amoeba guttula* Dujardin.

*Amoeba guttula* Dujardin, Histoire Naturelle des Zoophytes Infusoires, Paris, 1841.

Occurrence.—Taken from algae near Brannon's Island, from both ooze and floating algae in Creel Bay, from the east side of the main lake, and from sediment on rocks near the Station.

*Amoeba striata* Pénard.

*Amoeba striata* Pénard, Études sur les Rhizopodes d'eau douce. Mem. Soc. Phys. et Hist. Nat. Geneve, 1890.

Occurrence.—One specimen only observed in plant infusion from Stump Lake.

*Amoeba vitraea* (Hertwig and Lesser).

*Dactylophaerium vitraem* Hertwig and Lesser, Ueber Rhizopoden und denselben nahestehende Organismen. Arch. Mikr. Anat. Vol. 10, Suppl., 1874.

Occurrence.—Taken from the east side of Creel Bay.

#### ORDER TESTACEA

##### FAMILY ARCELLIDAE

##### *Genus Diffugia* Leclerc, 1815

*Diffugia pyriformis* Perty.

*Diffugia pyriformis* Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Occurrence.—Only observed from Big Mission Lake in a location where fresh water seeps into the lake.

*Diffugia constricta* Ehrenberg.

*Diffugia constricta* Ehrenberg, Abh. Akad. Wiss. Berlin, 1841.

Occurrence.—Taken from Big Mission Lake in the same situation as the preceding species, and also from the head of Creel Bay near the entrance of a sewer.

##### *Genus Arcella* Ehrenberg, 1830

*Arcella vulgaris* Ehrenberg.

*Arcella vulgaris* Ehrenberg, Abh. Akad. Wiss. Berlin, 1830.

Occurrence.—Taken in ooze from the head of Creel Bay and from near the station, also from Big Mission Lake near the in-seepage of fresh water; abundant in the latter locality.

## FAMILY EUGLYPHIDAE

Genus *Cyphoderia* Schlumberger, 1845*Cyphoderia ampulla* (Ehrenberg).*Diffugia ampulla* Ehrenberg, Bericht Preuss. Akad. Wiss., 1840.Occurrence.—One specimen only has been observed. Taken from Whipple Bay among *Ruppia*.Genus *Euglypha* Dujardin, 1841*Euglypha alveolata* Dujardin.*Euglypha alveolata* Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Taken from the overflow of lake water from the fish-tank near the Station. Observed but once.

## SUBCLASS HELIOZOA

## ORDER APHROTHORACIDA

Genus *Actinophrys* Ehrenberg, 1830*Actinophrys sol* Ehrenberg.*Actinophrys sol* Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.Occurrence.—Rarely observed, taken from among *Ruppia* in Minnewaukon Bay.

## SUBPHYLUM MASTIGOPHORA

## CLASS ZOOMASTIGOPHORA

## SUBCLASS LISSOFLAGELLATA

## ORDER MONADIDA

## FAMILY RHIZOMASTIGIDAE

Genus *Cercomonas* Dujardin, 1841*Cercomonas* sp. Figures 1–3, Plate XVIII.Probably *Cercomonas longicauda* Dujardin. Very plastic with caudal filament often developed. Diameter, when spherical, 10 $\mu$ 

Occurrence.—Observed in infusions from Stump Lake only.

## FAMILY HETEROMONADIDAE

Genus *Monas* Müller, 1786*Monas* sp. Figures 4, 5, Plate XVIII.Very plastic. Diameter, when spherical, 20 $\mu$ . May represent *Monas fluida* Dujardin.

Occurrence.—In the ooze from Creel Bay.

*Monas* sp. Figure 8, Plate XVIII.

Length  $9\mu$ ; body persistent in form, anterior region very granular. Corresponds in some degree to *Monas irregularis* Perty.

Occurrence.—In the ooze from Creel Bay. From a stale culture of *Ruppia*, Creel Bay.

*Monas* sp. Figure 7, Plate XVIII.

Body moderately plastic. Length, when extended,  $15-18\mu$ . Possibly same as figures 4 and 5.

Occurrence.—In the ooze from Creel Bay.

ORDER HETEROMASTIGIDA

FAMILY HETEROMITIDAE

*Genus Heteromita Dujardin, 1841*

*Heteromita globosa* (Stein).

*Bodo globosus* Stein, Der Organismus des Infusionthiere, Abth. 3, 1878.

Occurrence.—In dredged material from Creel Bay.

*Heteromita* sp. Figure 6, Plate XVIII.

But little of detail determined. Length  $5\mu$ . The form probably represents *Heteromita ovata* Dujardin.

Occurrence.—Taken from ooze on rocks near the Station.

ORDER POLYMASTIGIDA

FAMILY POLYMASTIGIDAE

*Genus Trepomonas Dujardin, 1841*

*Trepomonas agilis* Dujardin.

*Trepomonas agilis* Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Taken from Big Mission Lake, Whipple Bay, from the ooze of the main lake and from the east side of the main lake. Abundant in the latter locality.

ORDER EUGLENIDA

FAMILY EUGLENIDAE

*Genus Euglena Ehrenberg, 1830*

*Euglena viridis* Ehrenberg.

*Euglena viridis* Ehrenberg Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Observed from Minnewaukon Bay, Big Mission Lake, in the ooze from Creel Bay and from the east side of the main lake.

*Euglena desus* Ehrenberg.

*Euglena desus* Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Minnewaukon Bay, Six-mile Bay, near Brannon's Island, Big Mission Lake, Little Mission Lake, East Lake, and the ooze from the main lake.

*Genus Phacus Dujardin, 1841*

*Phacus pyrum* (Ehrenberg).

*Euglena pyrum* Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Minnewaukon Bay, Creel Bay, Big Mission Lake (numerous), and the east side of the main lake.

*Genus Eutreptia Perty, 1852*

*Eutreptia viridis* Perty.

*Eutreptia viridis* Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Occurrence.—From the surface among *Ruppia*, Big Mission Lake.

FAMILY ASTASIIDAE

*Genus Astasia Ehrenberg, 1830*

*Astasia tricophora* (Ehrenberg).

*Trachelius tricophorus* Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—Among *Ruppia* from Whipple Bay, from Creel Bay, in the ooze from Big Mission Lake, and among algae near Brannon's Island.

FAMILY PERANEMIIDAE

*Genus Petalomonas Stein, 1859*

*Petalomonas mediocanellata* Stein.

*Petalomonas mediocanellata* Stein, Der Organismus der Infusions-thiere, 1878.

Occurrence.—Taken from the surface of Big Mission Lake and from the ooze of the main lake.

*Petalomonas* sp. Figure 10, Plate XVIII.

Has some resemblance to *Petalomonas ervilia* Stein. Conspicuous groove entire length of the body. Length  $36\mu$ .

Occurrence.—From the ooze of Creel Bay.

*Genus Heteronema Dujardin, 1841*

*Heteronema acus* (Ehrenberg).

*Astasia acus* Ehrenberg, Abh. Akad. Wiss., Berlin, 1830.

Occurrence.—From Six-mile Bay and from the ooze of Creel Bay.

*Genus Anisonema Dujardin, 1841*

*Anisonema grande* (acinus) (Ehrenberg).

*Bodo grandis* Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

*Anisonema acinus* Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Among *Ruppia* and algae at the head of Creel Bay.

*Genus Notosolenus Stokes, 1884*

*Notosolenus* sp. Figure 9, Plate XVIII.

Length about  $15\mu$ .

Occurrence.—From Whipple Bay, Stump Lake and from the overflow of the fish-tank near the Station.

#### ORDER CHLOROFLAGELLIDA

##### FAMILY TETRAMITIDAE

*Genus Tetraselmis Stein, 1878*

*Tetraselmis cordiformis* (Carter).

*Cryptoglana cordiformis* Carter, Annals of Natural History 1858.

Occurrence.—Taken from Stump Lake only.

##### FAMILY POLYTOMIDAE

*Genus Polytoma Ehrenberg, 1838*

*Polytoma uvella* Ehrenberg.

*Polytoma uvella* Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Found at the head and along the east side of Creel Bay.

FAMILY TRIMASTIGIDAE

*Undetermined genus*

Undetermined species. Figures 11, 12, Plate XVIII.

Description.—Body elongate, somewhat compressed, slightly plastic, attenuated posteriorly; surface marked longitudinally by several conspicuous ridges; flagella three in number arising from the anterior extremity, equal and equalling the body in length; nucleus and contractile vacuole unobserved. Length 20 $\mu$ .

Occurrence.—Numerous among *Ruppia* from Creel Bay.

FAMILY CHLAMYDOMONADIDAE

*Genus Chlamydomonas Ehrenberg, 1833*

*Chlamydomonas pulvisculus* Ehrenberg.

*Chlamydomonas pulvisculus* Ehrenberg, Abh. Akad. Wiss., Berlin, 1833.

Occurrence.—Taken from the head of Creel Bay.

SUBCLASS DINOFLAGELLIDA

ORDER DINIFERIDA

FAMILY PERIDINIIDAE

*Genus Glenodinium Ehrenberg, 1832*

*Glenodinium pulvisculus* Ehrenberg.

*Glenodinium pulvisculus* Ehrenberg, Die Infusionsthierchen als Vollkommene Organismen, 1838.

Occurrence.—Taken from the surface and from the ooze at the bottom of Creel Bay.

SUBPHYLUM INFUSORIA

CLASS CILIATA

ORDER HOLOTRICHA

FAMILY ENCHELINIDAE

*Genus Holophrya Ehrenberg, 1831*

*Holophrya ovum* Ehrenberg.

*Holophrya ovum* Ehrenberg, Die Infusionsthierchen als Vollkommene Organismen, 1838.

Occurrence.—Among algae from Creel Bay.

*Holophrya* sp. Figure 13, Plate XVIII.

Resembling *Holophrya ovum* Ehrenberg but much smaller. Length 30-40 $\mu$ .

Occurrence.—In the ooze from Creel Bay.

*Genus Urotricha Claparède and Lachmann, 1858*

*Urotricha labiata*, new species, Figure 14, Plate XVIII.

Description.—Body ovate, about twice as long as broad, equally rounded at both extremities. Cilia covering the entire body, arranged in longitudinal rows and vibrating independently. A very fine seta, nearly as long as the body, extending from the posterior extremity. Mouth anterior, subterminal, beneath a prominent, lobe-like lip. Nucleus central. Contractile vacuole posterior. Reproduction by transverse fission. Length of body about 30 $\mu$ .

Occurrence.—Taken from numerous localities in Devil's Lake.

*Genus Prorodon Ehrenberg, 1833*

*Prorodon teres* Ehrenberg.

*Prorodon teres* Ehrenberg, Die Infusionsthierchen als Vollkommene Organismen, 1838.

Occurrence.—Among *Ruppia* and algae of Big Mission Lake and the main lake.

*Prorodon edentatus* Claparède and Lachmann.

*Prorodon edentatus* Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Infusions of *Ruppia* from Big Mission Lake and Minnewaukon Bay.

*Prorodon griseus* Claparède and Lachmann.

*Prorodon griseus* Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Taken from Stump Lake only.

*Genus Enchelys Ehrenberg, 1838*

*Enchelys* sp. Figure 15, Plate XVIII.

Length from 15-20 $\mu$ .

Occurrence.—Ooze from the main lake and from the overflow of lake water from the fish-tank.

*Genus Spathidium Dujardin, 1841*

*Spathidium spatula* Dujardin.

*Spathidium spatula* Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Among algae from the head of Creel Bay.

*Spathidium* sp. Figure 16, Plate XVIII.

A very long, narrow and flattened form. Length 120 $\mu$ .

Occurrence.—Taken from infusions from the head of Creel Bay.

*Spathidium* sp. Figure 17, Plate XVIII.

A much shorter form than the preceding, with a conspicuous collar about the oral extremity. Length 30 $\mu$ .

Occurrence.—From the ooze of the main lake.

*Undetermined Genus*<sup>8</sup>

Undetermined species. Figures 1, 2, Plate XIX.

Description.—Body elongate, plastic, slightly compressed dorso-ventrally, inflated posteriorly, narrow anteriorly, rounded at both extremities; cilia of uniform length arranged in longitudinal rows, covering the entire surface; aperture a narrow slit diagonally placed, sub-terminal; contractile vacuole posterior; nucleus concealed; endoplasm completely filled with green chloroplasts. Length 90 $\mu$ .

Occurrence.—From the surface of the main lake and from among *Ruppia* and algae.

*Genus Chaenia Dujardin, 1841*

*Chaenia teres* Dujardin.

*Chaenia teres* Dujardin, Histoire Naturelle des Zoophytes Infusoires. 1841.

Occurrence.—Among algae from the head of Creel Bay.

*Genus Mesodinium Stein, 1862*

*Mesodinium pulex* (Claparède and Lachmann).

*Halteria pulex* Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—A common form on the surface and in the ooze of the main lake.

<sup>8</sup> The form is treated here with doubt as to its taxonomic position.

*Genus Didinium Stein, 1859*

*Didinium nasutum* (Müller).

*Vorticella nasutum* Müller, *Animalcula Infusoria Fluvialia et Marina*, 1786.

Occurrence.—Among *Ruppia* from Minnewaukon Bay, Whipple Bay, and from the east side of the main lake.

*Genus Lacrymaria Ehrenberg, 1830*

*Lacrymaria olor* Ehrenberg.

*Lacrymaria olor* Ehrenberg, *Abh. Akad. Wiss.*, Berlin, 1830.

Occurrence.—Among *Ruppia* in Creel Bay.

*Lacrymaria truncata* Stokes.

*Lacrymaria truncata* Stokes, *Ann. and Mag. Nat. Hist.*, June, 1885.

Occurrence.—Among *Ruppia* from the north end of the main lake.

*Lacrymaria cohnii* Kent.

*Lacrymaria cohnii* Kent, *A Manual of the Infusoria*, 1881-1882.

Occurrence.—In an infusion from Stump Lake.

*Lacrymaria lagenula* Claparède and Lachmann.

*Lacrymaria lagenula* Claparède and Lachmann, *Études sur les Infusoires et les Rhizopodes*, 1858.

Occurrence.—In ooze from the main lake.

## FAMILY TRACHELINIDAE

*Genus Lionotus Wrzesniowski, 1870*

*Lionotus fasciola* (Ehrenberg).

*Amphileptus fasciola* Ehrenberg, *Die Infusionsthierchen als Vollkommene Organismen*, 1838.

Occurrence.—Abundant in many parts of the main lake, also taken from Stump Lake and Big Mission Lake.

*Lionotus* sp. Figure 3, Plate XIX.

A very small species. Length about 40 $\mu$ . Often seen in conjugation.

Occurrence.—Among algae from Creel Bay.

*Genus Amphileptus Ehrenberg, 1830*

*Amphileptus meleagris* (Ehrenberg).

*Trachelius meleagris* Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

*Amphileptus meleagris* Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Taken in Stump Lake and from algae at the head of Creel Bay.

## FAMILY CHLAMYDODONTIDAE

*Genus Nassula Ehrenberg, 1838*

*Nassula rubens* (Perty).

*Cyclogramma rubens* Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

*Nassula rubens* Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—From the overflow of lake water from the fish-tank near the Station.

*Nasula ornata* Ehrenberg.

*Nasula ornata* Ehrenberg, Die Infusionsthierchen als Vollkommene Organismen, 1838.

Occurrence.—Taken from Lake "N" only.

*Genus Chilodon Ehrenberg, 1833*

*Chilodon cucullulus* (Müller).

*Colpoda cucullulus* Müller, Animalcula Infusoria Fluvialia et Marina, 1786.

Occurrence.—Infusions of algae from Creel Bay, Big Mission Lake, and Whipple Bay.

*Chilodon caudatus* Stokes.

*Chilodon caudatus* Stokes, Am. Jour. Sci. 29, April, 1885.

Occurrence.—Among *Ruppia* from Minnewaukon Bay.

*Genus Aegyria Claparède and Lachmann, 1858*

*Aegyria pusilla* (?) Claparède and Lachmann.

*Aegyria pusilla* Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Among algae near the Station.

## FAMILY CHILIFERIDAE

*Genus Glaucoma Ehrenberg, 1830**Glaucoma scintillans* Ehrenberg.*Glaucoma scintillans* Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—In algae infusion from near Brannon's Island.

*Glaucoma margaritaceum* (Ehrenberg).*Cyclidium margaritaceum* Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.*Cinetochilum margaritaceum* Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Occurrence.—Very abundant. From the ooze of Creel Bay, the surface of Creel Bay, Stump Lake, and near Brannon's Island in the main lake.

*Genus Leucophrys Ehrenberg, 1830**Leucophrys patula* (Müller).*Trichoda patula* Müller, Animalcula Infusoria Fluvialia et Marina, 1786.

Occurrence.—One specimen only observed, from the east side of the main lake. A very typical specimen.

*Genus Frontonia Ehrenberg, 1838**Frontonia leucas* Ehrenberg.*Frontonia leucas* Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Taken from the east side of the main lake and from East Lake. Abundant in Six-mile Bay and Minnewaukon Bay.

*Genus Loxocephalus Eberhard, 1868**Loxocephalus granulatus* Kent.*Loxocephalus granulatus* Kent, A Manual of the Infusoria, 1881-1882.

Occurrence.—Taken only in the ooze of Big Mission Lake near the in-seepage of fresh water.

*Genus Uronema Dujardin, 1841*

*Uronema marinum* Dujardin.

*Uronema marinum* Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—One of the most common species in the lake. Abundant everywhere both at the surface and in the ooze.

*Genus Colpidium Stein, 1868*

*Colpidium putrinum* Stokes.

*Colpidium putrinum* Stokes, Ann. and Mag. Nat. Hist. Feb., 1886.

Occurrence.—From algae at the east side of Creel Bay.

*Genus Tillina Gruber, 1879*

*Tillina saprophila* Stokes.

*Tillina saprophila* Stokes, Am. Nat., Feb., 1884.

Occurrence.—Taken only in the overflow of lake water from the fish-tank near the station.

## FAMILY PARAMAECIDAE

*Genus Paramaecium Müller, 1786*

*Paramaecium trichium* Stokes.

*Paramaecium trichium* Stokes, Am. Naturalist, 19, May, 1885.

Occurrence.—From near the mouth of a sewer at the head of Creel Bay, and from ooze near the rock pile in the main lake.

*Paramaecium caudatum* Ehrenberg.

*Paramaecium caudatum* Ehrenberg. Die Infusionsthierchen als Vollkommene Organismen, 1838.

Occurrence.—Taken from Big Mission Lake near the in-seepage of fresh water.

## FAMILY PLEURONEMIDAE

*Genus Cyclidium Ehrenberg, 1838*

*Cyclidium glaucoma* Ehrenberg.

*Cyclidium glaucoma* Ehrenberg, Die Infusionsthierchen als Vollkommene Organismen, 1838.

Occurrence.—Abundant everywhere, at the surface and in the ooze in all parts of the lake.

*Cyclidium litomesum* Stokes.

*Cyclidium litomesum* Stokes, Am. Monthly Micro. Jour., 6, Dec. 1884.

Occurrence.—Numerous in infusions from the head of Creel Bay and in the ooze from the main lake.

*Genus Pleuronema Dujardin, 1841*

*Pleuronema chrysalis* (Ehrenberg).

*Paramacium chrysalis* Ehrenberg, Die Infusionsthierchen als Vollkommene Organismen, 1838.

*Pleuronema crassa* Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Observed in infusions from Stump Lake only.

#### ORDER HETEROTRICHA

##### FAMILY PLAGIOTOMIIDAE

*Genus Metopus Claparède and Lachmann, 1858*

*Metopus sigmoides* (Müller).

*Trichoda sigmoides* Müller, Animalcula Infusoria Fluvialia et Marina, 1786.

Occurrence.—Common in dredged material from Minnewaukon Bay, Creel Bay, and the main lake. Abundant in East Lake.

*Genus Spirostomum Ehrenberg, 1835*

*Spirostomum ambiguum* Ehrenberg.

*Spirostomum ambiguum* Ehrenberg, Abh. Akad. Wiss., Berlin, 1835.

Occurrence.—Observed in dredged material from Creel Bay.

##### FAMILY HALTERIDAE

*Genus Halteria Dujardin, 1841*

*Halteria grandinella* (Müller).

*Trichoda grandinella* Müller, Animalcula Infusoria Fluvialia et Marina, 1786.

*Halteria grandinella* Dujardin, Histoire Naturelle des Zoophytes Infusoires, 1841.

Occurrence.—Common in infusions of *Ruppia* and algae from Whipple Bay and Creel Bay and in the ooze of the main lake.

## ORDER HYPOTRICHA

## FAMILY OXYTRICHIDAE

*Genus Uroleptus*<sup>9</sup> Ehrenberg, 1831

*Uroleptus agilis* Englemann.

*Uroleptus agilis* Englemann, Zeit. Wiss. Zool., Bd. 11, 1861.

Occurrence.—From the ooze of the main lake, also from Six-mile Bay.

*Uroleptus rattulus* (?) Stein.

*Uroleptus rattulus* Stein, Der Organismus der Infusionsthier, 1859.

Occurrence.—Among *Ruppia* from Whipple Bay.

*Genus Oxytricha*<sup>9</sup> Ehrenberg, 1830

*Oxytricha fallax* Stein.

*Oxytricha fallax* Stein, Der Organismus der Infusionsthier, 1859.

Occurrence.—Among algae from Creel Bay.

*Oxytricha pellionella* (Müller).

*Trichoda pellionella* Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

*Oxytricha pellionella* Ehrenberg, Die Infusionsthierchen als Volkommene Organismen, 1838.

Occurrence.—Taken from *Ruppia* near the Station, Big Mission Lake, Whipple Bay, north end of Creel Bay, and the ooze from the fish-tank after being flooded by lake water.

*Oxytricha parvistyla* Stein.

*Oxytricha parvistyla* Stein, Der Organismus der Infusionsthier, 1859.

Occurrence.—Among *Ruppia* near the Station.

*Oxytricha bifaria* Stokes.

*Oxytricha bifaria* Stokes, Ann. and Mag. Nat. Hist., Aug., 1887.

Occurrence.—Abundant in Creel Bay, also taken from Whipple Bay.

<sup>9</sup> Further study would, no doubt, result in the determination of other species of the genus than those listed.

*Genus Histrio Sterki, 1878***Histrio erethysticus** Stokes.

*Histrio erethysticus* Stokes, Proc. Am. Philos. Soc. 24; 126, 1887.  
Occurrence.—Among *Ruppia* from near the Station.

*Genus Stylonychia Ehrenberg, 1830***Stylonychia notophora** Stokes.

*Stylonychia notophora* Stokes, Ann. and Mag. Nat. Hist. June, 1885.

Occurrence.—With algae from Creel Bay.

*Genus Holosticha Wrzesniowski, 1877***Holosticha vernalis** (?) Stokes.

*Holosticha vernalis* Stokes, Ann. and Mag. Nat. Hist., Aug., 1887.

A form bearing considerable resemblance to Stokes' species was occasionally observed. Length 140 $\mu$ .

Occurrence.—Among *Ruppia* from the main lake.

*Genus Pleurotricha Stein, 1859***Pleurotricha lanceolata** (Ehrenberg).

*Stylonychia lanceolata* Ehrenberg, Die Infusionsthierchen als Vollkommene Organismen, 1838.

*Pleurotricha lanceolata* Stein, Der Organismus der Infusionsthierchen, 1859.

Occurrence.—Taken at the head of Creel Bay.

*Genus Tachysoma Stokes, 1887***Tachysoma parvistyla** Stokes.

*Tachysoma parvistyla* Stokes, Ann. and Mag. Nat. Hist. Aug., 1887.

Occurrence.—Observed in infusions from Stump Lake only.

## FAMILY EUPLOTIDAE

*Genus Euplotes Ehrenberg, 1831***Euplotes charon** (Müller).

*Trichoda charon* Müller, Animalcula Infusoria Fluviatilia et Marina, 1786.

*Euplotes charon* Ehrenberg, Die Infusionsthierchen als Vollkommene Organismen, 1838.

Occurrence.—Abundant among infusions of *Ruppia* and algae from many parts of the main lake, and also from East Lake.

*Euplotes patella* (Müller).

*Kerona patella* Müller, *Animalcula Infusoria Fluvialia et Marina*, 1786.

*Euplotes patella* Ehrenberg, *Die Infusionsthier als Vollkommene Organismen*, 1838.

Occurrence.—Found in Stump Lake, Big Mission Lake, East Lake and in numerous localities in the main lake.

*Genus Aspidisca Ehrenberg, 1830*

*Aspidisca costata* (Dujardin).

*Coccludina costata* Dujardin, *Histoire Naturelle des Zoophytes Infusoires*, 1841.

Occurrence.—Taken in Whipple Bay; numerous among *Ruppia* in Minnewaukon Bay and also on the east side of the main lake.

#### ORDER PERITRICHIA

##### FAMILY VORTICELLIDAE

*Genus Vorticella Linnaeus, 1767*

*Vorticella telescopica* Kent.

*Vorticella telescopica* Kent, *a Manual of the Infusoria*, 1881–1882.

Occurrence.—Among *Ruppia* at the north end of the main lake.

*Vorticella convallaria* Linnaeus.

*Vorticella convallaria* Linnaeus, *Systema Naturae*, Ed. 12, 1767.

Occurrence.—Attached to diatoms in the main lake, also among *Ruppia* in Big Mission Lake.

*Vorticella octavo* Stokes.

*Vorticella octavo* Stokes, *Ann. and Mag. Nat. Hist.*, June, 1885.

Occurrence.—Among *Ruppia* at the north end of the main lake.

*Vorticella microstoma* Ehrenberg.

*Vorticella microstoma* Ehrenberg, *Die Infusionsthierchen als Vollkommene Organismen*, 1838.

Occurrence.—Taken at the east side of the main lake.

*Vorticella* sp. Figure 4, Plate XIX.

A very common form, resembling *Vorticella rabdostyloides* Kellicott but is considerably smaller and the body is transversely striated. Length of stalk  $12\mu$ , with the diameter of the body nearly the same.

Occurrence.—Attached to floating diatoms.

*Vorticella* sp. Figure 5, Plate XIX.

A species with more elongate body than the preceding but also transversely striate. Length of body  $28\mu$ , stalk  $68\mu$ .

Occurrence.—Attached to floating diatoms.

*Genus Gerda Claparède and Lachmann, 1858**Gerda annulata*, new species. Figure 10, Plate XIX.

Description.—Body elongated, cylindrical, of nearly equal diameter throughout, curved when extended; surface finely striate transversely; a prominent annular ridge present usually about one-fourth the distance from the posterior extremity; peristome border revolute, disc slightly elevated; contractile vacuole conspicuous; nucleus not observed. Length of body, extended,  $80\mu$ .

Occurrence.—Among algae and *Ruppia* from the north end of the main lake.

*Genus Epistylis Ehrenberg, 1830**Epistylis plicatilis* Ehrenberg.

*Epistylis plicatilis* Ehrenberg, Die Infusionsthierchen als Vorkommene Organismen, 1838.

Occurrence.—From the east side of Creel Bay.

*Epistylis branchiophila* Perty.

*Epistylis branchiophila* Perty, Zur Kenntniss kleinster Lebensformen in der Schweiz, 1852.

Occurrence.—Among algae near the head of Creel Bay.

*Genus Carchesium Ehrenberg, 1838**Carchesium epistylidis* Claparède and Lachmann.

*Carchesium epistylidis* Claparède and Lachmann, Études sur les Infusoires et les Rhizopodes, 1858.

Occurrence.—Among algae from Creel Bay.

*Genus Zoothamnium Ehrenberg, 1838*

*Zoothamnium alterans* Claparède and Lachmann.

*Zoothamnium alterans* Claparède and Lachmann, *Études sur les Infusoires et les Rhizopodes*, 1858.

Occurrence.—Among *Ruppia* and algae from Stump Lake.

*Zoothamnium* sp. Figure 6, Plate XIX.

Stalk very stout, zooids smooth, usually 2-8 in a colony. Length of stalk  $216\mu$ , of zooid  $64\mu$ .

Occurrence.—From Stump Lake, East Lake, Creel Bay, Whipple Bay, and from the main lake. Attached to algae or *Ruppia*. A fairly common form.

*Genus Vaginocola Lamarck, 1816*

*Vaginocola crystallina* Ehrenberg.

*Vaginocola crystallina* Ehrenberg, *Die Infusionsthierchen als Vollkommene Organismen*, 1838.

Occurrence.—Numerous among algae from East Lake, also taken from Stump Lake and from the north end of the main lake.

*Genus Cothurnia Ehrenberg, 1831*

*Cothurnia imberbis* Ehrenberg.

*Cothurnia imberbis* Ehrenberg, *Die Infusionsthierchen als Vollkommene Organismen*, 1838.

Occurrence.—Commonly attached to floating diatoms, from dredged material and also among *Ruppia* in Creel Bay. Also taken from Stump Lake.

*Cothurnia curva* Stein.

*Cothurnia curva* Stein, *Der Organismus der Infusionsthierchen*, 1859.

Occurrence.—Among *Ruppia* at the north end of the main lake.

## CLASS SUCTORIA

## FAMILY PODOPHRYIDAE

*Genus Podophrya Ehrenberg, 1838*

*Podophrya libera* Perty.

*Podophrya libera* Perty, *Zur Kenntniss kleinster Lebensformen in der Schweiz*, 1852.

Occurrence.—Numerous at east side of the main lake.

*Podophrya* sp. Figure 9, Plate XIX.

Bears some slight resemblance to *Podophrya cyclopus* Claparède and Lachmann. The lobulated border may have represented a reproductive phase or possibly was abnormal. Total height  $60\mu$ , stalk  $20\mu$ .

Occurrence.—Attached to algae from the main lake. Several specimens were observed by Dr. R. T. Young.

*Genus Sphaerophrya Claparède and Lachmann, 1858*

*Sphaerophrya magna* Maupas.

*Sphaerophrya magna* Maupas, Arch. de Zoologie Experimentale, tom 9, Nov., 1881.

Occurrence.—From Stump Lake and the east side of the main lake.

#### FAMILY ACINETIDAE

*Genus Acineta Ehrenberg, 1838*

*Acineta* sp. Figure 7, Plate XIX.

Body triangular in broad view, compressed; endoplasm very granular, nucleus concealed. Total height  $50\mu$ , stalk  $20\mu$ . This species resembles, in some degree, *Acineta lemnae* Stein.

Occurrence.—From floating material in the main lake and also among algae from Stump Lake.

*Acineta* sp. Figure 8, Plate XIX.

Body oval, slightly broader distally, greatly compressed; endoplasm granular concealing the nucleus and contractile vacuole.

Total height  $60-72\mu$ , stalk about  $15\mu$ .

Occurrence.—Attached to algae from Stump Lake. Commonly feeding on *Uronema*.

### 3. EXPERIMENTS

Preliminary experiments in transferring protozoa from fresh water to the concentrated water of Devil's Lake and vice versa.

In order to test the reactions of certain protozoa taken from other sources to the more concentrated waters of Devil's Lake a series of simple experiments were carried out by which forms of protozoa common to fresh water were transferred directly into the more saline water of the lake.

Infusions from a small body of fresh water near the southern boundary of the main lake were prepared and certain protozoa which readily appear in cultures were used in the tests.

By placing a drop of the fresh water culture on one end of a microscopic slide and a drop of lake water near the middle of the slide and, with a needle, drawing out from each drop toward the other a narrow channel of water until the two met, the protozoa were conducted from the fresh water drop into that of the lake water. To eliminate possible influence of the fresh water a series of drops of lake water were used and the organisms rapidly transferred from one to the other until they reached a pure medium of lake water.

The waters from the two sources were kept at a uniform temperature and the effect of the change of environment thus brought about was carefully noted by the activity of the organisms.

In similar manner the transference of certain protozoa from lake water to fresh water was accomplished and the effect of such change observed as hereinafter noted.

#### A. Transference of protozoa from fresh water to lake water.

1. *Paramecium* sp. A specimen of a species, probably *Paramecium caudatum* Ehrenberg, commonly occurring in the fresh water was removed to the pure lake water with the following results: An immediate change occurred in the organism. The body became greatly compressed dorso-centrally with erratic movements at first which soon gave way to a more steady, forward movement with slow rotations on the long axis. A noticeable change also occurred in the contractile vacuoles. The normal rhythmic collapse of the vacuoles ceased after a few minutes and they became greatly dilated and distorted. After ten minutes of rotary movements the organism became quiet with the cilia of the periphery and the oral groove still active. Many non-contractile vacuoles filled the endoplasm. Death occurred at the end of twelve minutes.

A second specimen, after showing the same flattening of the body, moved in circles for six minutes then assumed the forward movement with rotations on the long axis. In eighteen minutes the organism became quiet with a highly vacuolated endoplasm and the cilia of the oral groove vibrating feebly. Death occurred in twenty-six minutes.

A third specimen after exhibiting similar physical and physiological changes came to complete rest in twenty-two minutes. Death resulted in twenty-five minutes.

A fourth specimen showed similar responses and died in fifteen minutes.

Seven specimens were then transferred at the same time. Six of these, after exhibiting similar responses as the preceding, were dead at the end of ten minutes. One, after reacting in like manner, died at the end of eighteen minutes.

2. *Stylonychia* sp. Several tests with a species of *Stylonychia* were carried out. Unusual responses were less quickly manifested by *Stylonychia* than *Paramecium* when brought into contact with the lake water. Commonly after five or six minutes of normal movements a rapid whirling over and over of the body occurred gradually subsiding into complete rest. Death occurred in all specimens in from sixteen to thirty-two minutes.

Reactions of similar character were obtained from *Paramecium* and *Stylonychia* by the introduction of small quantities of NaCl into the fresh water in which they were normally living.

3. *Metopus* sp. A short type of *Metopus*, common in fresh water, was transferred to the saline lake water. The most noticeable change was an almost immediate flattening of the body. Normal rotary movements continued for eight minutes when the organism came to rest with the cilia of the surface still more or less active. Death occurred at the end of fifteen minutes.

Numerous individuals of this species were used in successive experiments with reactions similar in each case. Death resulted in all specimens in from eleven to eighteen minutes.

B. Transference of protozoa from the concentrated lake water to fresh water.

1. *Uroleptus* sp. The form used was one of the elongated types. More than sixty specimens were used in the tests. With few exceptions but with considerable degree of variation, the following reactions were very evident: After a period of from ten to fifteen minutes contact with the fresh water, during which time more or less normal activities were maintained, the organisms came to rest with the cilia still in motion. The cell bodies became shortened and dilated, in

many instances assuming a spherical form. After enduring this state of depression for from ten to fifteen minutes the organisms showed signs of recovery. The bodies gradually assumed an elongated form and normal activities reappeared. Within a period of one hour and twenty-five minutes from the time the organisms were first introduced into the fresh water all, with the exception of a few which failed to survive the state of depression, had fully recovered and were responding in a normal manner.

Considerable variation in the effect of the change was noted. Of those surviving some were slightly affected and wholly recovered in forty-five minutes, some in sixty minutes, while others required the longer time noted above.

2. *Euplotes patella* (Müller). Numerous individuals of this species were transferred as in the preceding experiment. The effect in this case was an immediate one. As soon as contact was made with the fresh water the cell bodies became swollen and distorted, losing the longitudinal striations and all resemblance to normal individuals. During this state of depression the organisms were at rest with the cirri in feeble motion. After a period of fifteen minutes the cells began to resume movements although in a distorted condition. In fifteen minutes more the longitudinal striations reappeared and soon after normal responses were entirely restored.

3. *Uronema marinum* Dujardin. The transference of this species from the lake water to fresh water resulted in no apparent state of physical depression and no diminished or unusual responses to stimuli could be detected. The species is commonly recognized as both a marine and fresh water form.

#### 4. SUMMARY AND CONCLUSIONS

##### SUMMARY OF THE GROUPS OF PROTOZOA RECORDED

Sarcodina.....	13 species
Mastigophora.....	22 "
Infusoria.....	76 "
<hr/>	
Total.....	111 species

##### Conclusions

1. The proportion of the number of species of the three groups of protozoa recognized in Devil's Lake corresponds favorably with the same in a typical fresh water lake.

2. A most noticeable feature of the study of this fauna is the apparent total absence of numerous forms universally found in fresh water. The dearth of shell-bearing rhizopods was mentioned in the introduction. Many common species of flagellates and ciliates were, at no time during the survey, observed in the concentrated waters of the lake.

3. The subdivisions of the classes of protozoa are fairly well represented in Devil's Lake. Two new species are described in the report but with the exception of the facts mentioned in the preceding paragraph, the protozoan fauna of Devil's Lake cannot be considered an unusual one.

4. Experiments of the interchange of protozoa between fresh water and the lake water seem to indicate that the organisms of the lake may adjust themselves to fresh water conditions with more readiness than can the forms accustomed to a fresh water environment accommodate themselves to the concentrated water of the lake.

5. INDEX

	PAGE		PAGE
Acineta.....	190	Chaenia.....	179
<i>Acineta lemnae</i> .....	190	<i>Chaenia teres</i> .....	179
Acineta sp.....	190	Chiliferidae.....	182
Acinetidae.....	190	Chilodon.....	181
Actinophrys.....	173	Chilodon caudatus.....	181
Actinophrys sol.....	173	Chilodon cucullulus.....	181
Aegyria.....	181	Chlamyodontidae.....	181
<i>Aegyria pusilla</i> .....	181	Chlamydomonadidae.....	177
Amoeba.....	171	Chlamydomonas.....	177
Amoebae.....	171	Chlamydomonas pulvisculus.....	177
<i>Amoeba guttula</i> .....	172	Chloroflagellida.....	176
<i>Amoeba limax</i> .....	171	Ciliata.....	177
<i>Amoeba proteus</i> .....	171	<i>Cinetochilum margaritaceum</i> .....	182
<i>Amoeba radiosa</i> .....	171	<i>Coccudina costata</i> .....	187
<i>Amoeba striata</i> .....	172	Colpidium.....	183
<i>Amoeba verrucosa</i> .....	171	Colpidium putrinum.....	183
<i>Amoeba vitrea</i> .....	172	<i>Colpoda cucullulus</i> .....	181
Amoebidae.....	171	Cothurnia.....	189
Amphileptus.....	181	<i>Cothurnia curva</i> .....	189
<i>Amphileptus fasciola</i> .....	180	<i>Cothurnia imberbis</i> .....	189
<i>Amphileptus meleagris</i> .....	181	<i>Cryptobranchus alleghaniensis</i> .....	170
Anisonema.....	176	<i>Cryptoglena cordiformis</i> .....	176
<i>Anisonema acinus</i> .....	176	Cyclidium.....	183
<i>Anisonema grande</i> .....	176	Cyclidium glaucoma.....	183
Aphrothoracida.....	173	Cyclidium litomesum.....	184
Arcella.....	172	<i>Cyclidium margaritaceum</i> .....	182
<i>Arcella vulgaris</i> .....	172	<i>Cyclogramma rubens</i> .....	181
Arcellidae.....	172	Cyphoderia.....	173
Aspidisca.....	187	Cyphoderia ampulla.....	173
<i>Aspidisca costata</i> .....	187		
Astasia.....	175	Dactylosphaerium vitraem.....	172
<i>Astasia acus</i> .....	176	Didinium.....	180
<i>Astasia tricophora</i> .....	175	Didinium nasutum.....	180
Astasiidae.....	175	Diffugia.....	172
		<i>Diffugia ampulla</i> .....	173
<i>Bodo globosus</i> .....	174	Diffugia constricta.....	172
<i>Bodo grandis</i> .....	176	Diffugia pyriformis.....	172
		Diniferida.....	177
Carchesium.....	188	Dinoflagellida.....	177
<i>Carchesium epistylidis</i> .....	188		
Cercomonas.....	173	Enchelinidae.....	177
<i>Cercomonas longicauda</i> .....	173	Enchelys.....	178
<i>Cercomonas</i> sp.....	173	Enchelys sp.....	178

	PAGE		PAGE
Epistylis.....	188	Heteronema acus.....	176
Epistylis branchiophila.....	188	Heterotricha.....	184
Epistylis plicatilis.....	188	Histrio.....	186
<i>Eucalia inconstans</i> .....	170	Histrio erethysticus.....	186
Euglena.....	174	Holophrya.....	177
Euglena deses.....	175	Holophrya ovum.....	177
<i>Euglena pyrum</i> .....	175	Holophrya sp.....	178
Euglena viridis.....	174	Holosticha.....	186
Euglenida.....	174	Holosticha vernalis.....	186
Euglenidae.....	174	Holotricha.....	177
Euglypha.....	173	Hopotricha.....	185
Euglypha alveolata.....	173		
Euglyphidae.....	173	Infusoria.....	177
Euplotes.....	186		
Euplotes charon.....	186	<i>Kerona patella</i> .....	187
Euplotes patella.....	187		
Euplotidae.....	186	Lacrymaria.....	180
Eutreptia.....	175	Lacrymaria cohnii.....	180
Eutreptia viridis.....	175	Lacrymaria lagenula.....	180
		Lacrymaria olor.....	180
Frontonia.....	182	Lacrymaria truncata.....	180
Frontonia leucas.....	182	Leucophrys.....	182
		Leucophrys patula.....	182
Glaucoma.....	182	Lionotus.....	180
Glaucoma margaritaceum.....	182	Lionotus fasciola.....	180
Glaucoma scintillans.....	182	Lionotus sp.....	180
Glenodinium.....	177	Lissoflagellata.....	173
Glenodinium pulvisculus.....	177	Loxocephalus.....	182
Gerda.....	188	Loxocephalus granulatus.....	182
Gerda annulata.....	188		
Gymnamoebida.....	171	Mastigophora.....	173
		Mesodinium.....	179
Halteria.....	184	Mesodinium pulex.....	179
Halteria grandinella.....	184	Metopus.....	184
<i>Halteria pulex</i> .....	179	Metopus sigmoides.....	184
Halteridae.....	184	Metopus sp.....	192
Heliozoa.....	173	Monadida.....	173
Heteromastigida.....	174	Monas.....	173
Heteromita.....	174	<i>Monas fluida</i> .....	173
Heteromita globosa.....	174	<i>Monas irregularis</i> .....	174
<i>Heteromita ovata</i> .....	174	Monas sp.....	174
Heteromita sp.....	174		
Heteromitidae.....	174	Nassula.....	181
Heteromonadidae.....	173	Nassula ornata.....	181
Heteronema.....	176	Nassula rubens.....	181

	PAGE		PAGE
Notosolenus.....	176	Prorodon griseus.....	178
Notosolenus sp.....	176	Prorodon teres.....	178
Oxytricha.....	185	<i>Rana pipens</i> .....	170
Oxytricha bifaria.....	185	Rhizomastigidae.....	173
Oxytricha fallax.....	185	Rhizopoda.....	171
Oxytricha parvistyla.....	185	Sarcodina.....	171
Oxytricha pellionella.....	185	Spathidium.....	179
Oxytrichidae.....	185	Spathidium spatula.....	179
Paramaecidae.....	183	Spathidium sp.....	179
Paramaecium.....	183	Sphaerophrya.....	190
Paramaecium caudatum.....	183	Sphaerophrya magna.....	190
<i>Paramaecium chrysalis</i> .....	184	Spirostomum.....	184
Paramaecium sp.....	191	Spirostomum ambiguum.....	184
Paramaecium trichium.....	183	Stylonychia.....	186
Peranemiidae.....	175	<i>Stylonychia lanceolata</i> .....	186
Peridiniidae.....	177	Stylonychia notophora.....	186
Peritricha.....	187	Stylonychia sp.....	192
Petalomonas.....	175	Suctoria.....	189
<i>Petalomonas ervilia</i> .....	176	Tachysoma.....	186
Petalomonas mediocanellata.....	175	Tachysoma parvistyla.....	186
Petalomonas sp.....	176	Testacea.....	172
Phacus.....	175	Tetramitidae.....	176
Phacus pyrum.....	175	Tetraselmis.....	176
<i>Pimephales promelas</i> .....	170	Tetraselmis cordiformis.....	176
Plagiotomiidae.....	184	Tillina.....	183
Pleuronema.....	184	Tillina saprophila.....	183
Pleuronema chrysalis.....	184	Trachelinidae.....	180
<i>Pleuronema crassa</i> .....	184	<i>Trachelius meleagris</i> .....	181
Pleuronemidae.....	183	<i>Trachelius tricophorus</i> .....	175
Pleurotricha.....	186	Trepomonas.....	174
Pleurotricha lanceolata.....	186	Trepomonas agilis.....	174
Podophrya.....	189	<i>Trichoda charon</i> .....	186
<i>Podophrya cyclopus</i> .....	190	<i>Trichoda grandinella</i> .....	184
Podophrya libera.....	189	<i>Trichoda patula</i> .....	182
Podophrya sp.....	190	<i>Trichoda pellionella</i> .....	185
Podophryidae.....	189	<i>Trichoda sigmoides</i> .....	184
Polymastigida.....	174	Trimastigidae.....	177
Polymastigidae.....	174	Undetermined genus.....	177,179
Polytoma.....	176	Undetermined species.....	177,179
Polytoma uvella.....	176	Uroleptus.....	185
Polytomidae.....	176	Uroleptus agilis.....	185
Prorodon.....	178		
Prorodon edentatus.....	178		

	PAGE		PAGE
Uroleptus rattulus.....	185	<i>Vorticella nasutum</i> .....	180
Uroleptus sp.....	192	<i>Vorticella octavo</i> .....	187
Uronema.....	183	<i>Vorticella rabdostyloides</i> .....	188
Uronema marinum.....	183	<i>Vorticella sp.</i> .....	188
Urotricha.....	178	<i>Vorticella telescopica</i> .....	187
Urotricha labiata.....	178	Vorticellidae.....	187
Vaginocola.....	189	Zoomastigophora.....	173
Vaginocola crystallina.....	189	Zoothamnium.....	189
Vorticella.....	187	Zoothamnium alterans.....	189
Vorticella convallaria.....	187	Zoothamnium sp.....	189
Vorticella microstoma.....	187		

## EXPLANATION OF PLATES

## PLATE XVIII

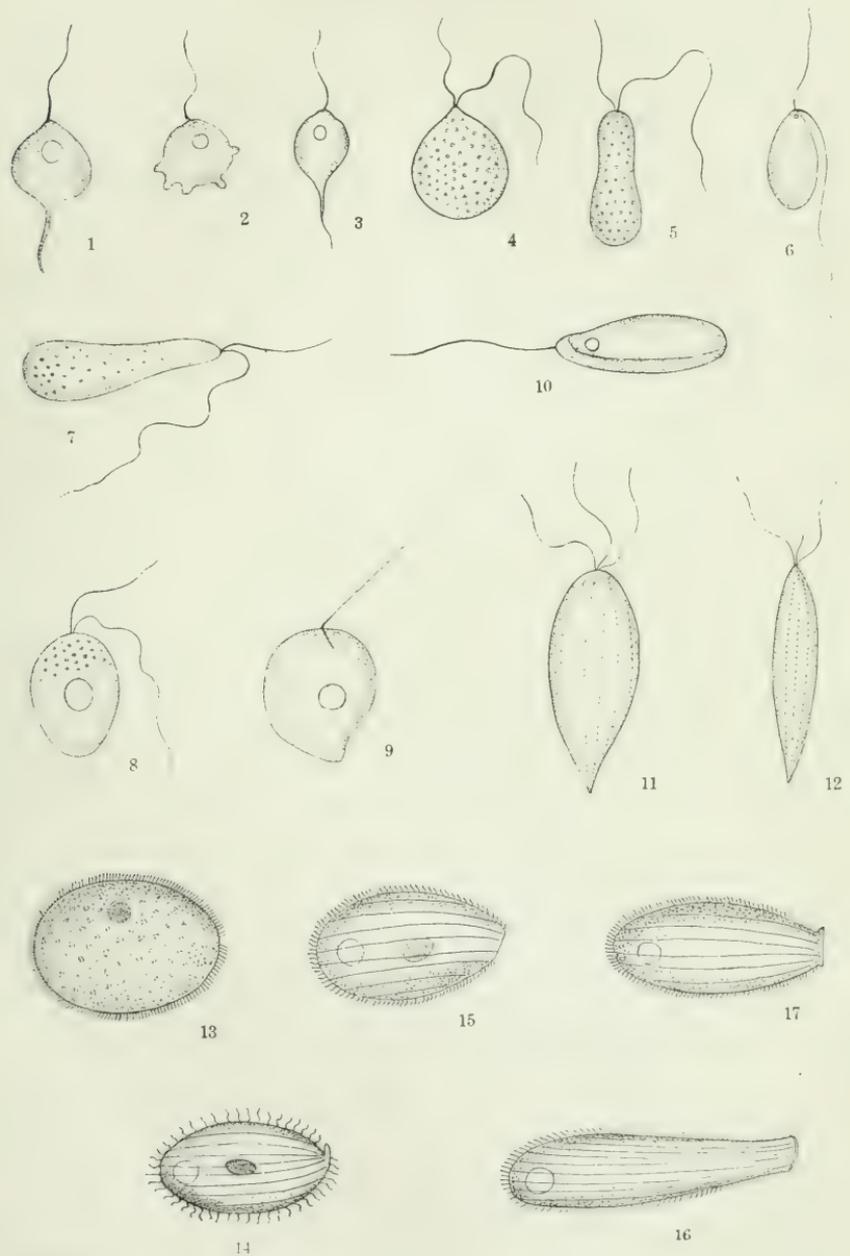
- Figs. 1-3. *Cercomonas* sp. x 1000.  
 Figs. 4, 5. *Monas* sp. x 750.  
 Fig. 6. *Heteromita* sp. x 2000.  
 Fig. 7. *Monas* sp. x 1200.  
 Fig. 8. *Monas* sp. x 1500.  
 Fig. 9. *Notosolenus* sp. x 900.  
 Fig. 10. *Petalomonas* sp. x 600.  
 Figs. 11, 12. Undetermined genus and sp. x 1250.  
 Fig. 13. *Holophrya* sp. x 550.  
 Fig. 14. *Uronema labiata*, new sp. x 750. (Posterior seta omitted in figure.)  
 Fig. 15. *Enchelys* sp. x 1200.  
 Fig. 16. *Spathidium* sp. x 300.  
 Fig. 17. *Spathidium* sp. x 900.

## PLATE XIX

- Figs. 1, 2. Undetermined genus and sp. x 380.  
 Fig. 3. *Lionotus* sp. x 800.  
 Fig. 4. *Vorticella* sp., including stalk. x 800.  
 Fig. 5. *Vorticella* sp., including stalk. x 350.  
 Fig. 6. *Zoothamnium* sp., including stalk. x 250.  
 Fig. 7. *Acineta* sp., including stalk. x 400.  
 Fig. 8. *Acineta* sp., including stalk. x 280.  
 Fig. 9. *Podophrya* sp., including stalk. x 370.  
 Fig. 10. *Gerda annulata*, new species. x 500

1950

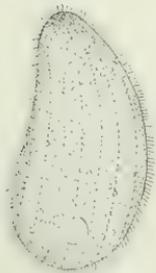
TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY  
VOL. XXXIX





1956

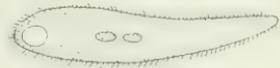
TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY  
VOL. XXXIX



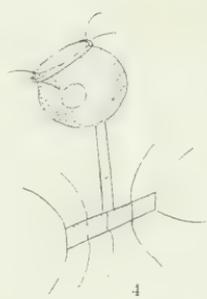
1



2



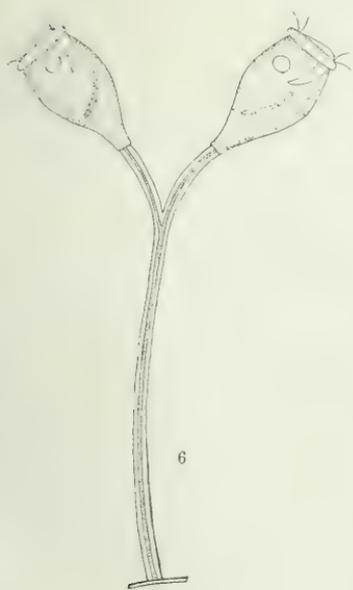
3



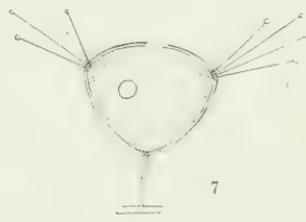
4



5



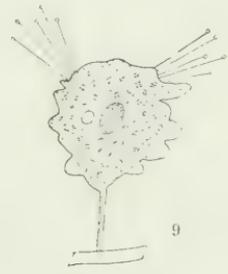
6



7



x



9



10

PLATE XIX

EDMONDSON



AGE, GROWTH AND SCALE CHARACTERS OF THE  
MULLETS, *MUGIL CEPHALUS* AND *MUGIL  
CUREMA*

BY  
ARTHUR PAUL JACOT

CONTENTS

	PAGE
Introduction.....	199
Differentiation of species.....	200
<i>Mugil cephalus</i> Linné.....	204
Determination of young.....	204
Development of young.....	204
Migration.....	214
Second age-group.....	220
Adults.....	221
<i>Mugil curema</i> Cuv. & Val.....	223
Young.....	223
Migration.....	226
Adults.....	226
Summary.....	226
Bibliography.....	227
Explanation of Plates.....	229

INTRODUCTION

During the summers of 1915 and 1916 the writer was given the opportunity of studying the rate of growth and development of the mullet of the Atlantic coast of the United States. The collecting was done at and about Beaufort, N. C. Of the two species under consideration, the striped mullet (*Mugil cephalus*) has far the more economic importance. It ranges through all tropic and warm waters of the globe and has long been used as food. On our south Atlantic and Gulf coasts it has been sought so constantly and taken in such quantities that its numbers have noticeably decreased so that the supply continually falls short of the demand. For this reason the artificial propagation of this species is very desirable and it is towards this end that the present investigation has been made.

*Differentiation of Species*

Along the Atlantic coast from New England to Florida, two species of mullet may be encountered, *Mugil curema* and *Mugil cephalus*, the latter being much the more common. Commercially, no distinction is made although the fishermen seem to be aware of two species, calling the former the "silverside" or white mullet and the other the "jumping" or striped mullet. Other common names are locally used. When asked wherein they differ, the fishermen give a variety of more or less accurate answers, and generally end with some statement to the effect that the "silverside" is very seldom caught. A review of the fisheries literature on these species shows a lumping of the two, so that no accurate information concerning their respective habits can be secured.

Technically the silverside mullet (*M. curema*) differs from the jumping mullet (*M. cephalus*) in having more heavily scaled second dorsal and anal fins, nine rays in the anal fin in contrast to eight in *M. cephalus*, and 38 versus 42 scales in the lateral series and 12 versus 14 scales in transverse (diagonal) series. The field marks are the scalation of the anal and second dorsal fins and a lack of the longitudinal stripes of *M. cephalus*.

Because of the unreliability of single characters in species determination, and because of the possible difference in coloration of adult and young, a study of the variation of specific characters was necessary. Relative measurements in these two species are impracticable as the slight difference in ratios is repeatedly exceeded by individual variation. The amount of scalation on the second dorsal and anal fins is a fairly good character for both old and young fish, but is relative only. The *total* number of rays and spines in the anal fin is not constant. Specimens of *M. curema* with a total of 13 anal fin supports (rays and spines), the last of which may be bi- or tripartite, are not rare, while specimens with 11 supports are rare. *M. cephalus* has ten supports more often than twelve. Thus, though quite constant, this character cannot be wholly relied upon. This leaves the scalation of the two species to be considered.

The mullet is an unusually favorable subject for lepidology because of the relatively large scales and the presence of the lateral line groove (without the pore) which is found on nearly every scale, and which materially aids in the alignment of the scale rows. The



forty-first scale. The other scales of the last row become more evident dorsad<sup>1</sup> and ventrad. In the figure the numerals on the lateral median line designate the number of the transverse row, those on the body designate the number of scales in the transverse rows of that area except where a numeral appears above or below the body, in which case the numeral designates the total number, but as one of them is situated on the dorsal or ventral median line, that scale belongs as much to one side as to the other. All numerals include the lateral median line (except those upon it). The numerals in parentheses are the corresponding figures for *M. curema*, (where omitted they are not given). The reason for the diminution of the number of scales in the transverse rows caudad or cephalad is shown in figure 2

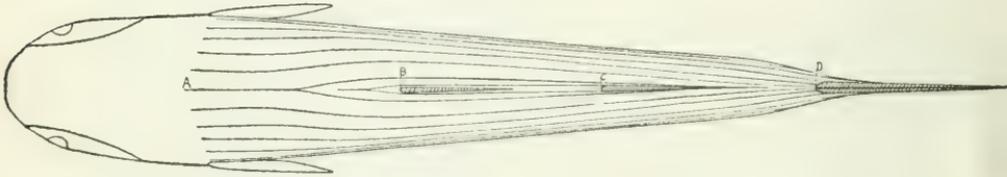


Figure 2. Scalation of the mullet on the back.

which is a dorsal aspect of figure 1 with the lateral line grooves connected by continuous lines. Thus when two lines run together, two scale rows become one row, and where a single line ends, a scale row becomes crowded out. A similar condition obtains on the venter. It therefore seems that reduction of scale rows occurs on the dorsal and ventral median lines—a condition very different from that in the Ophidia (Ruthven 1908). The exact location of the termination of a lateral row varies with the individual so that figure 2 is but individual and the area between *C* and *D* varies in appearance with each specimen. Likewise, there is variation in appearance between points *A* and *B* and the corresponding section on the venter. The area between points *B* and *C* down to, and including the venter, may be definitely relied upon as constant. The transition band on the ventral section (the area between 10(9) and 9(8)) is liable to shift caudad or cephalad a scale or two, but this should cause no confusion.

<sup>1</sup> The termination *-ad* as explained by Wilder and Gage (1882) signifies "towards," "in the direction of," etc.

From this analysis it should be evident that any variation in number of scales in the horizontal row will shift the *limits* of the various areas caudad or cephalad, depending on the individual, and this in turn means that only the middle area is unshifting. This middle area is also so broad that the desired flexibility in counting is given and the possibility of the complete loss of a row is greatly minimized.

A total of forty-four catches made between December 22 and September 4, during several years were plotted on co-ordinate paper using the abscissae for the length of the fish and the ordinates for the date of capture. Simple lines are used for *M. cephalus*, and railroad lines for *M. curema* and *harengus* (see fig. 3). The length of speci-

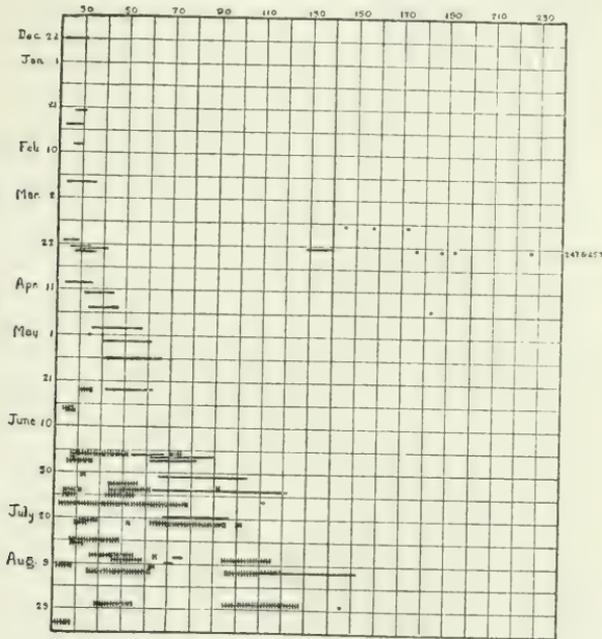


Figure 3. Record of non-linead mullets:  
 — • *Mugil cephalus*.  
 —•—•—• *Mugil curema*.

mens herein given is the greatest possible length and all measurements unless otherwise stated are in millimeters.

## MUGIL CEPHALUS LINNÉ

*Determination of Young*

Before anything can be done with the young of *M. cephalus*, it will be necessary to go back to *Myxus harengus* of Günther. In 1883 this species was established as the type of a new genus *Querimana* (Jordan) which differs from the genus *Mugil* in having the following characters—a serrated preorbital, thin lips, no adipose eyelid, stronger teeth and two instead of three anal spines. Bean (1903) has described the development of a third anal spine from the first ray. Further investigation has brought out the fact that the adult also has a serrated preorbital, as will later be described. The condition of the lips, teeth and adipose eyelid will, in the proper place, be shown to be but juvenile characteristics. Thus the genus *Querimana*, consisting of juvenile mullet, becomes a synonym of *Mugil*.

The specimens of *M. cephalus* ranging from 23mm. up to 40 or 50mm. were very carefully examined and were found to be juvenile *M. cephalus*, a heretofore undescribed *Querimana* (having the "Querimana" formula of A. II, 9; scales 42-14).

The specimens running into the *M. curema* group answer perfectly to the description given for *Q. harengus*. The description of *Q. harengus* (Jordan, 1896), gives it thirty-eight scales in the lateral series, twelve in transverse series and an anal fin formula of II, 10. Adulting (changing to adult condition) this fin formula according to the evidence given by Bean (1903) we have A. III, 9. This agrees with *M. curema*. The development of *Q. harengus* further shows it to be the young of *M. curema*, as will be shown below and not a distinct species.

*Development of Young*

The juvenile stage of *M. cephalus* begins with individuals as small as 23mm. Their first appearance is in the form of well developed fish without the slightest larval appearance. As already described by various writers, they form compact schools, swimming near the surface of the water. They may be found in deep water, or more often in water but a few inches in depth. The time of the year during which they are to be found may best be seen by consulting figure 3. They might easily be mistaken for the young of *M. curema*, because

of the similar coloration. Collections made from December to March consist of slim silvery individuals of small size. There seems to be very little growth during this time. The sides are devoid of pigment, being sharply defined from the dark brownish-green back. In later March and early April this dark dorsal band is extended down the sides by the gradual appearance of pigment cells. By mid-April these pigment cells have so increased as to merge the dark back into the silvery venter. With this advance in color, the fish rapidly increases in length and the abdomen is bulged by the developing intestine. Besides these external evidences of a turning point in the life history of the species, the growing parts of the fish show this change, though some more strongly than others. In the following the juvenile characteristics of this species through the development of the fish to its adult form, this turning point has been noted and the reason sought. The lengths of the fish as given below are used for the purpose of correlating the development of the various parts with the fish as a whole, and are of typical specimens. The silvery (or juvenile) stage is found in specimens from 23 to 32mm. in length, while those from 30 to 35mm. are somewhat difficult to distinguish as silvery or dusky because of the merging of the two forms at this size.

The *preorbital* in the juvenile stage has some 10 or 12 points, teeth or serrations, of fair size. As the fish grows these points become more and more numerous, less slender and less distinct. In older fish they become blunt and stocky until in a large individual (502mm.) there were 53 teeth on the margin, crowded so as to place about four to a millimeter.

The *adipose eyelid* shows no marked acceleration in rate of growth at the end of the silvery stage. It is entirely lacking in the smallest specimens, but by the time the fish has reached a length of 28mm., with the aid of a high power binocular microscope, a slight translucent growth can be detected just anterior to the eye. In describing the state of transparency of the eyelid, it must be remembered that only alcoholic specimens are being described, in life the adipose eyelid being perfectly transparent at any age. For the sake of convenience the eyelid has been divided into three parts: (a) the ring, which is situated about the rim of the orbit, (b) the anterior lobe, and (c) the posterior lobe. When the fish is about 30mm. long, the anterior lobe

having slightly thickened, has become semi-translucent and has stretched backward over the eye. At 32mm. length, the anterior lobe has further thickened and become slightly more opaque, stretching farther back over the eye and merging into the ring which has just become visible. By the time the fish is 36mm. long, the anterior lobe has become opaque, while the ring, which has very slightly stretched posteriorly, has become semi-translucent. From this stage, the gradual development of the growth can be easily followed without the aid of the microscope. At 39 mm. the posterior lobe has become quite definite while the anterior has thickened and become more nearly opaque. On 42mm. specimens, the anterior lobe is visible to the unaided eye. The ring has assumed an opaque cast at its inner edge and stretched out over the rim of the nearest scales. The posterior lobe has, by now, spread out over the preopercle but is still translucent. The growth of the orbital ring is now very slow, its chief expansion being inward over the eye. In a specimen 47mm. in length the posterior lobe has become so opaque as to become visible to the unaided eye. Its lateral growth takes it not farther up or down than the outer diameter of the ring, while its chief growth is posteriorly over the preoperculum. The anterior lobe grows no farther forward, having reached a point just anterior to the nostril, but it slowly grows out over the eye. At 54mm., the more rapid growth of the lobes has caused them to overrun the ring anteriorly and posteriorly, so that the ring now assumes an elliptical shape, the long axis of the ellipse being vertical. Sixty millimeter specimens show this ellipse more strongly developed, the posterior lobe much lengthened posteriorly and the whole eyelid in its typical form, so that it is now only a matter of slow growth before the adipose eyelid has assumed its maximum development in the full-grown adult. Thus it is seen that there is no break between the juvenile stage and the adult.

The *thin lips* given as a characteristic of the genus *Querimana* do not appear disproportionally thin for so small a specimen. If there is any relative thickening of the lips beyond normal development, it is so gradual as to be imperceptible.

That *the teeth* are slightly stronger in the juvenile young than in the adult cannot be considered a generic difference in itself, as it may well be due to a retrograde modification due to change in food habit; as there seems to be grounds to suspect is the case.

The development of the *third anal spine* from a ray was described by Bean (1903) but deserves further comment. The ray is simple and has about four articulations. At the close of the juvenile stage

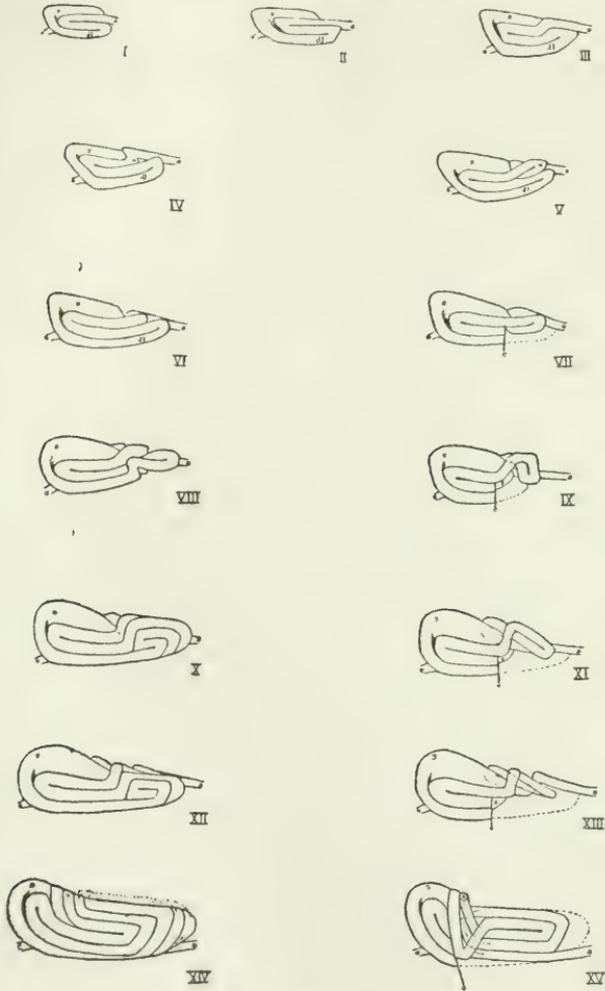


Figure 4. Development and convolutions of the intestine of *Mugil cephalus* when from 23 mm. to 40 mm. in total length. s=stomach; c=esophagus; a=anus; c=line of cut of duodenal loop, see p. 208. At figure v the fish is 32 mm. long and transforming into the dusky stage.

this ray ceases growing with the same rapidity as the true rays and becomes heavier basally, continuing to become relatively heavier and stiffer, until it is about one-third of the space between the tips of the second spine and the first ray, longer than the second spine (Fig. 1). This relative length is maintained throughout life. As this spine continues growing and thickening, the articulations become obliterated until lost so that in adults the third spine is basally as heavy as the second and quite equal to it as a spine. This development should be of much interest to the morphologist and systematist.

The *reproductive organs* are so rudimentary as to be invisible throughout any part of the season or at any point in the juvenile stage. This might be sufficient reason in itself for discarding the genus *Querimana*.

The *development of the intestine* gives further evidence of the relations of the two forms. Owing to the difficulty of describing this development a series of outline sketches (Fig. 4) have been prepared, illustrating, by a lateral aspect, each successive change.

The most simple form (fig. 4, I) consists of a duodenal loop and a "straight-away" to the anus. When the figure V stage is reached the fish is passing into the dark or dusky stage, the kink *n* having lengthened into a loop whose lower member has twisted upward and over its upper one to form a loop. At the next figure (VI) the spleen appears as a yellowish body, 25mm. in diameter, and from then on becomes a factor in influencing the convolutions of the intestine. At the figure VIII stage the duodenal loop makes a kink which soon becomes a loop and thus destroys the duodenal loop in its typical form. Each odd figure from VII to XV shows the convolutions on the further or inside by the cutting away of the duodenal loop or its modification at line c. Beyond the stage shown in figures XIV-XV the convolutions become so intricate that their study would surpass the scope of this paper, the length of the fish at this time averaging about 40mm. Thus, in the lengthening of the intestine, there is a marked acceleration in the rate of growth at the time when the fish is about 32mm. long, i.e., when the fish is passing into the young or dusky stage.

Besides this development in length and complexity of the intestine proper, the whole abdominal cavity is eloquent of the change exter-

nally noticeable. To appreciate this change, it is necessary to begin with the earliest individuals. All December specimens examined had their entire viscera and the walls of the abdomen colored orange, while the peritoneum in many cases was grayish with dark spots, otherwise it was of a semi-translucent blackish color. The length of the intestine was at times half that of the individual itself, though generally about three-quarters its length. In January, the coloring of the intestine was the same as for the previous month with the exception of a few individuals in which it was yellowish while the peritoneum averaged darker, and the walls of the abdomen a little lighter. The length of the intestine showed a slight increase over specimens of corresponding lengths of the previous month. In February the viscera were yellowish to pale, a very few individuals having traces of vegetable matter in the intestine. The peritoneum showed no special change, while the walls of the abdomen were pale. The intestine, on an average, had increased in length to a slight extent, but in no cases equaled the length of the individual. In late March, quite a few specimens had entirely lost their internal orange or yellow color and the intestine had traces of dark matter. The peritoneum was black and the flesh about the viscera had assumed the more natural dark coloration. These specimens showed marked increase in length of intestine, it being considerably longer than the individual. These fish were passing into the dusky stage. The majority of specimens, however, were much like those of the previous month. The viscera of April specimens are rarely orange or yellow tinged, the great majority having the intestine more or less filled with dark matter. The length of the intestine had also correspondingly increased. These fish were well into the dusky stage, their intestine appearing as represented in VII to XV of figure 4. It was from these slowly developing individuals that material for figure 4 was taken. From this time on the growth of the fish is very rapid in comparison with that of the previous month. Thus the increased length of the intestine can be directly correlated with its own color.

An explanation for the change in visceral coloration described in the preceding paragraph was sought by examination of stomach contents. The silvery-sided individuals (juvenile fish) showed an almost exclusive diet of crustacea, mainly copepods, and as alcohol almost invariably turns this form of life a salmon red, the coloration of the

viscera is accounted for. The intestine as well as the stomach were filled with this food, the latter not yet having reached the gizzard development. In the most immature individuals the stomach's form was that of a simple sack. The stomach contents of the dusky stage consisted, roughly, of 40% sand and mineral matter and 60% vegetable and animal matter. This latter consisted of 50% diatoms, 35% algae and other soft vegetable matter and 15% animal miscellaneous. This seems to be the usual ration of the fish during the remainder of its life, it being known as a mud feeder (See also Linton 1913).

From the above, two things should be apparent, namely (a) that the first form (the juvenile or silvery stage) develops into the second (the dusky stage), (b) that the juvenile stage is one of slow growth and development which is more rapid after the fish has changed diet, (made evident by the change in the color of the viscera). Because the intestine, the stomach and the whole fish acquire an acceleration in rate of growth and development at the time of change of diet, we conclude that this period of change is due to change of diet.

A study of *the development of the scale* along with the development of the individual is essential to the correct understanding and interpretation of the adult scale. The simplest form procurable is found in the juvenile or silvery-sided individuals (figs. 8-9). Text figure 5 represents one of these scales (in its natural position) divided into areas using Masterman's (1913) method. Rather than deal with the axes, greater convenience is found in using the areas formed by these axes as designated in figure 5. The terms "dorsal" and "ventral" have not been used as no need was found for this differentiation of sides. In the scale work here presented the scales were taken from the lateral median line on the two rows originating at the latero-anterior edge of the first dorsal fin (scales 10 and 11 of figure 1), except in the very young, where this was done as nearly as possible. For convenience, the appearance of the scale is described by means of a formula in which *a*, *l* and *p* stand for the anterior, lateral and posterior areas respectively, while the number following each of these refers to the number of circuli in that area. Thus, the formula for the scale of figure 5 would be a. 11; l. 0; p. 22. The two lateral areas generally differ in number of circuli when these are present; the average has then been taken. In the more advanced stages of growth the number of

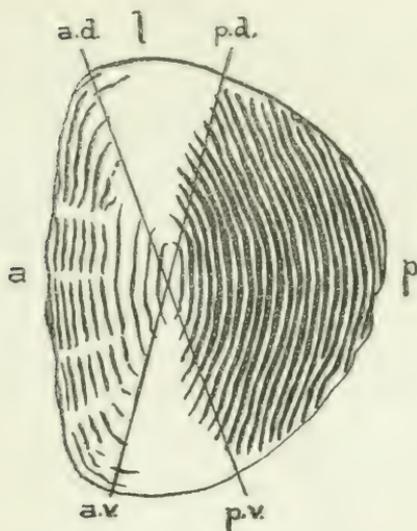


Figure 5. Advanced silvery stage scale, x45 divided into areas. a. v.=antero ventral axis; p. v.=postero-ventral axis; a. d.=antero dorsal axis; p. d.=postero-dorsal axis; l.=lateral areas; a.=anterior area or basal end; p.=posterior area or apical end. Formula=a. 11, l. o, p. 22.

circuli on the lateral area was computed by finding the average from two or three scales (taken from the place above mentioned from one or both sides of the fish). This does not affect the general result as whatever variation is shown in these four scales is about as great as the difference between the scales taken from corresponding places of two different fish of the same size from the same school. In other words, it was found that the average number of lateral circuli of scales 10 and 11 from fish number one was more constant than the average number of lateral circuli of scales 10 and 10 from fish number one and two (both being of the same size and from the same school). With this, it must be remembered that the number of circuli does not represent the number of "days" of growth, but that they testify to the approximate *development* of the individual. The formula, then, is useful in conveying a fairly good idea of the size and amount of development of the fish from which it was taken.

The smallest fish have a scale already well developed, a 23mm. specimen having a circuli formula a. 7; l. 0; p. 15 (fig. 8). This being

the type of scale showing the least growth of any procurable, it is inferred that the greater portion of this scale was formed at the spawning grounds. Notice on this scale, (a) that the circuli on the posterior area are much closer together than those of the anterior area, (b) that the lateral areas are without circuli and (c) that the circuli nearest the center are farther spaced than those further out. As the scale enlarges, more circuli form on its anterior and posterior edges, until the scale has reached a maximum development of a. 11; l.0; p. 22 on a fish of 32mm. length. This type of scale may be found on any silvery-sided individual, i.e., during the months of December, January, February, many in March, and a few in April. The addition of circuli during this season is very slow, so that the scale in three months' time, shows no more advance than illustrated in figure 9. Very often the scale shows less development than this before the juvenile stage comes to a close. From this point, the method of growth of the scale completely changes. Figure 10 shows a scale whose formula at the silvery-side stage was a. 10; l.0 p. 20. A little later two *closely spaced* anterior circuli were added, and, while this was going on, the tenth anterior circulus stretched back along the outer rim of the scale, thus forming a lateral circulus, so that the formula of the complete scale has become a. 10+2; l.0+1; p. 20, and the posterior edge of the scale shows a narrow border without circuli. In the next figure (11) three anterior circuli have been added to the juvenile scale, two of which have become lateral; the posterior border is quite wide but without definite circuli. Note the shallow depression just posterior to the center. This is the beginning of what is, in some fish, known as the lateral line groove, and in this paper will be referred to by this term. Figure 12 has two more anterior circuli, an additional lateral circulus, a broken or fragmentary posterior circulus with suggestions of a second, and a larger lateral line groove. Note the fine reticulations at the anterior edge of the posterior circuli. The fish from which the scale of figure 11 was taken had a length of 38mm. The circulation is still further advanced giving the formula a. 9+10; l.0+4 or 5; p. 19+1(or 2). The lateral line groove almost obliterates the first few posterior circuli and the reticulation or veining is more extensive and better developed. This series should clearly show the way the scale changes its habit of sculpture (habit of growth of the configuration of the surface). Figure 14 shows a scale from a 45mm. individual and

gives the effect of this new development. The juvenile scale is seen to be completely encircled by the later more rapid addition of circuli which *tend* to be continuous. Thus, the scale is of the cycloid type. Note, (a) that the anterior circuli are almost twice as numerous as the lateral, their ends terminating near the anterior axes, (b) that the anterior circuli of the outer series curve in the opposite direction to those of the juvenile scale, thus making a definite demarkation between the two scales, (c) that in the outer series the closely spaced circuli are anterior while they are posterior in the juvenile scale, and vice versa with the widely spaced circuli, so that in this respect the habit of sculpture is reversed. This change, although taking place at the same time as a change in diet, and occurring during the months of March and April, is not due to seasonal or dietal change, for the scale of *M. curema* passes through the same change at a different season (during the summer) and unaccompanied by a change of food. It is therefore inferred that this change is due to some previous change in habit of growth of the scale, i.e., the change is phylogenetic.

During this development the juvenile scale which is designated by various authors as the nuclear area, nucleus,<sup>2</sup> centrum, initial field, etc., occasionally passes through a process of deterioration of surface face sculpture. This begins with the veining just anterior to the posterior circuli (figs. 11-14) spreading farther and farther until the lateral areas are covered (figs. 17-19). When the lateral areas are fairly well filled in, the posterior circuli are gradually replaced by the veining so that the veined area is linear or ovate in shape and not the shape of the scale. A process giving a similar aspect has been described and accounted for by Dahl (1911, p. 11-13).

The addition of circula continues more or less regularly for a longer or shorter time according to the individual. Figure 15 illustrates a scale taken from a 60-70mm. specimen, and serves to show the nature of growth of the apical or posterior circuli. Note the way in which the posterior circuli are bending out toward the apex of the scale. With the bending out of these circuli the scale grows more rapidly at the apex and on this posterior lobe narrow, pointed, posteriorly directed cteni gradually rise from the surface. These cteni are firm and strong, much longer than wide, slightly bent to give

<sup>2</sup> It seems preferable to reserve the term "nucleus" for the structural center of the scale as used by Cockerell (1913).

more rigidity, and sharply pointed (figs. 20, 25, 27). These cteni continue to form row after row, the scale taking on the appearance of the one illustrated by figure 20. This scale (removed from a fish taken on the 23rd of August) contains all the characteristics of the species although the individual was but 145mm. long and not yet one year old. The lateral line groove has extended backward to the posterior margin of the juvenile scale and forward as a narrower channel to its anterior margin and to the posterior end of one of the basal radii. This linear shape is that assumed by the lateral line in the adult. In figure 22, although the scale shows nearly the same amount of growth, the cteni have not as yet begun to form. Before the further development of the scale is noted, it will be necessary to review what is known of the migration of this fish.

### *Migration*

The earliest reliable information we have concerning the migration of the mullet is a note left by Dr. Yarrow (Smith 1907) on the fish in the Beaufort region in 1871. The substance of this note relative to migration is that small-sized individuals appear in May, and that in later August fish commence to school preparatory to migration. He says:

The schools appear to come from the northward through Albermarle, Pamlico, and Core sounds, gradually working their way southward. Their departure through the various inlets seems to depend upon a favorable state of the wind, which should be from the northward, for it has been noticed frequently that when the wind hauled, the schools of mullet already without the harbor have suddenly turned, re-entering the inlet, and pursued their course southward through Bogue Sound.

A few years later the U. S. Commission of Fish and Fisheries sent out Mr. Ravenel (1887) to find out what he could about the mullet. The method pursued was to visit various fishing centers and consult the fishermen. The only reliable information we need note is that at Beaufort three "runs" were noted as follows:

small mullet	4-5 inches	June-Aug. 30.
fat	"	Sept.-Oct. 10.
roe	"	Oct. 10-Nov. 15.

The same year the Commission issued its comprehensive work on the fishery industries in which there are two papers on the mullet. The first one (Goode 1887) treating of the natural history will not

be considered as it is based almost entirely on hearsay but on the second (Earll 1887) which is much more comprehensive and reliable. From under its caption "movements" the following general notes have been extracted:

. . . Small sized individuals are scattered about on the feeding grounds in the grassy bays and marshes bordering the coast. Here they remain till late in July, when they proceed to the deeper channels of the larger bays, where they gather in schools of small size. Little is known of the whereabouts of the large mullet at this season. Later the migrations begin, the fish of medium size moving southward. Their places are soon filled by large fish. . . . These (roe mullet) remain until the first cold storm occurs, when they start for the south, moving rapidly along the outer shore, or through the inland passage. These fish are followed by smaller individuals known as "frost mullet," which remain through the greater part of the winter. The movement seems to be general along the entire coast, all fish along the Atlantic seaboard being reported as traveling southward, while those rounding Florida Keys continue their coastwise migrations, gradually working northward and westward towards the Texas line. No return movement is reported at any season along the Atlantic. . . .

In N. J. waters the mullet make their appearance in schools about the first of September, gradually working southward and entirely disappearing by the last of October. The same is true for the coast between Cape May and Cape Henry, including the waters of Chesapeake Bay.

The small fish are seen in June on the N. C. coast, these gradually increasing in numbers until the first of August, when the schools have attained considerable size, but thus far no tendency to migration is noticeable. A little later a southern movement begins, and school after school passes, the size of the individuals constantly increasing till the first of September when the old or roe mullet arrive. . . . If the weather continues pleasant they remain along the shores until the eggs have become well developed before moving southward, but at the approach of the first cold storm they are off and other smaller individuals follow in their wake, so that by the first of January the greater part have disappeared. Comparatively few are seen from that date until the following June, though scattering ones may be taken at any time.

At Wilmington [N. C.] small mullet are occasionally taken at any season, though they are abundant from June to September only, and large ones are seen only in the fall. As at Beaufort, the migration begins about the middle of August. The first schools are composed of fish of medium size. . . . By the first of September these have entirely disappeared, and their places have been taken by the "fat mullet." These are very abundant for several weeks, the roe mullet arriving about the middle of October, before they have entirely disappeared. "Frost" or "inch" [the distance between the eyes] mullet, as they are sometimes called, follow in large, compact schools, the last disappearing about the middle of December. Smaller fish, called "winter-mullet," are abundant till spring. . . .

At Charleston the run is somewhat similar to that at Wilmington.

In East Florida, especially the St. John's River, fish of all sizes may be seen at any time. . . .

In the Gulf of Mexico it is claimed that the mullet are even more abundant than along our Atlantic coast. . . . They are never entirely absent, though, as on the Atlantic coast, they are much more abundant in the fall than at any other season. . . .

From the evidence at hand it is clear that the mullet fisheries for different parts of West Florida continue from the middle of August to the first of January, though the height of the season, for most localities, is in October and November. Farther west the fish seem less inclined to migrate, remaining more constantly in any given locality, and on the Texas coast it is said that there is no special time of abundance, but that mullet are equally plentiful at any season.

Notes on Wood's Hole (Smith 1897) state that *M. cephalus* is "Found from September to end of October, going in large schools about October 1." For the same region Sumner (1911) reports *M. cephalus* as "Present from July to December; most common in the fall." In summary, Bean (1903) states that about New York the earliest appearance of *M. cephalus* is in August when they are few, that in September they are found in the New York markets and that "the great schools were absent till October."

The two most striking facts brought out by this literature are those of a fall migration and the almost complete absence of large mullet on our coast during the later winter, spring and early summer. This migration seems to begin at the northern extremity of the range of the species and extends southward with the migrating fish. The migration seems to be orderly, deliberate, and in series, each series being made up of a certain age group, almost the whole coast load of mullet slipping around the peninsula of Florida and along the gulf coast before all have scattered through the more torrid water which is the real home of the mullet. Thus, there can be no question about a definite fall migration down the Atlantic coast to warm water. Another thing to be noticed and borne in mind is that the migration is slow and leisurely, taking at least three months, so that it would seem that each individual had time to live at its leisure on the way south. Finally, notice should be taken of the lack of any noticeable northward migration. Thus nothing is known of the fish from the time it reaches the gulf until it reappears in late summer. There can be no doubt that the fish does not return north during the winter, but that it is living in southern waters where it can feed unrestrain-

edly. After the winter therefore, in spring or early summer, this species must return north. For a possible record of this period of the life history of the fish, the scale may again be studied.

At the time that the young are from 40 to 60mm. long or about the beginning of May, individuals of another age-group, as small as 120mm. in length and up, make their appearance. These individuals keep increasing in size and numbers throughout the summer so that by the end of August they are very common and range from 220-370mm. in length. Their scales are all characterized by the single "line" or break in the continuity of the circuli typically illustrated in figure 25. The fish from which this scale was removed was taken on July 2 (1915) and had a total length of 218mm. Notice (a) the deterioration of the sculpture in the center, (b) the ctenoid area and the position of the sharpest and the most worn teeth, (c) the unpored lateral line groove, (d) the continuity of the "line" from the lateral area posteriorly to and into the ctenoid area, and anteriorly across the anterior area, (e) that the "line" is formed (1) laterally by the termination of the circuli in exactly the same way as they are terminated at the outer edge of the scale, and (2) anteriorly by the termination of the circuli in exactly the same way as they terminate at the anterior edge of the scale, (f) that this "line" is the so-called "winter-line" and (g) that the circuli within the "line" are all equally spaced. With the last three points (d, e, f) in mind as well as the fact that this is a south wintering fish, let us consult the scales of fish which remain in cold northern waters during the winter. Good illustrations of such scales have been published by Gilbert (1913), Mastermann (1913), Lea (1913), Nilsson (1914) and Hjort (1914) of the salmon, herring, mackerel and cod respectively. In the scales of the salmon and cod, close examination will reveal that the winter area is formed by the crowding together of the circuli (the circuli of the cod are broken into dashes). The mullet scale is entirely lacking in the crowding of circuli, testifying to undiminished feeding during the winter. The herring and mackerel scales, due to non-concentric circuli on the older section of the scale show an entirely different type of "winter line." In this case it is formed by the pinching out of the circuli. Thus they cannot be used to compare with the mullet. Now, since the mullet is not affected by winter conditions and does not show the typical winter crowding of the circuli, another cause

must be sought for the break in the sequence of the circuli which does occur. As already pointed out, this break is exactly similar to the break caused by cessation of life. The break is sudden and complete. We advance the hypothesis that this line is caused by a spring migration differing from the fall migration in being made (typically) by a continuous run and not by a slow gradual shifting as in the fall. Various types of these "lines" or *linea*<sup>3</sup> may be encountered. Figure 25 illustrates its more typical and usual appearance, i.e., when the *linea* is similar to the periphery. An occasional type of *linea* consists of a straight but wide space between some two lateral circuli. In one scale examined practically all the pre-migratory lateral circuli had slightly shifted laterally and continued posteriorly as post-migratory circuli. This may have been due to a migration of such a nature that growth was retarded, not entirely stopped. Figure 24, if closely examined, will show two closely spaced *lineae*, the outermost being the most distinct. Such a form is occasional and may be due to a second migration several weeks after the first, the fish going still further north. Thus, the actual number of *lineae* cannot be absolutely relied upon for the age of the individual. Furthermore, one cannot consider every *linea* a migration line as any cessation in feeding or growth for any reason whatever, might cause the interruption and renewed growth of the scale necessary to form a *linea*. Therefore, though the actual number of *linea* is not always reliable for the determination of the number of seasons which the individual has passed through, the *linea* may be relied upon for age determination when properly understood. Before this can be done, however, the development of the scale of the species must be studied along with the development and life history of that species.

The above mentioned hypothesis seems to be further substantiated when one notices that specimens from 129 to 257mm. long (clearly of a second age-component (fig. 3) having as many as 70 lateral circuli outside the juvenile scale) were taken in March. The lateral circuli of the scales of these individuals were all evenly spaced

<sup>3</sup> From the Latin *linea*, -ae, f; using the term in its more figurative application. I introduce this new term to specifically label the definite feature of the scale typically illustrated in figure 23 and explained above, restricting the terms *peronidia*, *annuli*, *winter band*, *annular ring*, etc., to the area of circuli between the *lineae* or between the first *linea* and the juvenile scale.

and no linea of any kind could be detected, yet they were passing through or had just passed through the winter, evidently at or in the general vicinity of Beaufort. Thus, all mullet do not leave our coast, those here in winter having probably come from much farther up the coast. Furthermore, no fish with a single linea were taken before late April which could at all be considered of this second age group (or younger). The fish scale from which figure 23 was taken was removed from an individual taken on the 28th of April. The linea is some three circuli from the margin of the scale, thus setting the date of migration during the earlier part of April. Other specimens taken during the spring have the following number of post-linea circuli:

- May 2—2 and 4
- May 4—4
- May 11—7
- May 12—0, 3, 6, 6, 7, 7, 7, 10
- May 25—3 and 10

Allowing an average accretion of five circuli per month, this data gives early April as the norm of migration. That it is general and fairly definite is brought out by figure 6, which is the record of the



Figure 6. Record of Postmigratory annuli of Jumping Mullet caught July 10, 1915.

number of post-lineal circuli of a catch made on the tenth of July of one linead mullet. The fish being all taken on the same date, any variation of the date of migration should be shown by the number of circuli. The abscissae give the number of circuli and the ordinates the length of the specimens; the points of greatest magnitude designate three specimens recorded at that point, etc. Although there is a variation of 14 circuli, or nearly three months, it is not necessarily all due to difference in date of migration, for individuals vary in rapid-

ity of accretion of circuli, i.e., in a given time one individual may acquire  $x$  circuli while another would acquire  $x+3$  or 4. However great or small a variation in time there may be, figure 6 clearly shows that there is a definite time of migration which, as has already been shown, takes place in earlier April and normally consists of a single continuous run from southern feeding ground to more northern waters. That the fish migrate in deep water off the coast seems evident from the fact that the fishermen are unaware of such a movement and that the fish is practically neglected by them until the fall migration.

### *Second Age-Group*

The arrival of the jumping mullet in April marks the beginning of its second season on our Atlantic coast; its age ranges from 14 to 17 months and its size from 120 to 200mm. (5 to 8 inches) (see figs. 3

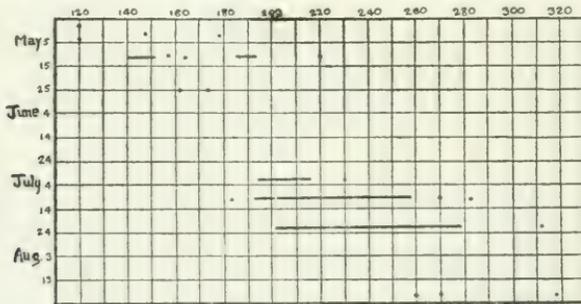


Figure 7. Record of one linead mullet.

and 7). "The individuals are scattered about on the feeding grounds in the grassy bays and marshes bordering the coast" (Earl 1887). They can be found in small numbers over any mud bottom, mud flats, etc., where vegetable plankton is abundant. Specimens may be secured at any time during the spring and summer but they are so scattered as to make fishing for mullet alone an expensive proposition. Living thus they grow to a length of from 225 to 325mm. by mid-August. Their flesh is very soft and oily, hence their name "fat mullet." By this time they have begun to gather into schools of ever increasing size, the social instinct becomes dominant as the reproductive organs rapidly develop. By late September the southward

migration has begun and as the fish move down the coast and the roe ripens, they spawn. Out of a batch of ten roe mullet purchased at the Beaufort market on October 9, 20 and 25, four had but a single linea as follows:

Length of fish 410, Scale formula	1.68+50 <sup>4</sup>
414	1.63+45
426	1.57+57
431	1.69+57

The remaining six each had two migration lines giving the following scale formulas:

Length of fish 426, Scale formula	1.42+60+28
440	1.51+44+21
454	1.43+45+24
473	1.40+50+28
483	1.47+55+24
493	1.60+46+23

The small number of circuli acquired during the third year indicates that the rapid growth of the fish had been partially checked by having attained sexual maturity—as is usually the case. If the series examined was typical, and every effort was made to make a very general choice, this would mean that the jumping mullet generally attains maturity and spawns for the first time in its second year. That this is not always the case is evident from the scales of a male 392mm. long with testes 34mm. long and 7mm. wide, taken on July 12 (1915). The average number of lateral circuli for two or three scales reads 1.38+72+8, which means that it was spawned late in the season, that it probably migrated north early the first year, late in the second and in its third year was maturing early. No other fish was taken with reproductive organs so far advanced, so early in the year; the time of the year for organs of that size normally being in mid August.

#### *Adults*

According to scale evidence, the majority of jumping mullet breed for the first time during their second year. At this time they average

<sup>4</sup> As it is unnecessary to mention the number of circuli in the anterior area or in the juvenile scale, the formula for older fish need include only the number of circuli in the lateral area using a + sign for the linea.

less than a foot and a half, and constitute the great bulk of the mullet fishery. The largest mullet that has come under our observation is a 502mm. (20 inch) roe mullet whose scale (fig. 26), shows it to have been in its fifth year. Some individuals are reputed to attain a length of two and a half feet and a weight of ten pounds. What may be said concerning the average adult mullet applies equally well to the larger individuals. Those which return in the spring pass the time in the marshes, mud flats, and mud bottoms of the wide shallow estuaries, sounds, etc., so characteristic of our sunken and inundated Atlantic slope, at least as far north as Cape Cod. The colder temperature and rugged coast extending from Maine northward forms a highly efficient barrier for such a highly specialized fish as the mullet. In its spring and summer feeding grounds it can thrive secure from man for it is so scattered as to make seining unprofitable and it is in a practically inaccessible locality due (a) to the soft muddy bottom in which it seeks cover and in which man sinks so as to make seining impossible, (b) to the reeds and grasses over which the lead line will continually rise and allow the fish to run under—not to mention those which clear the floats with three to eight feet to spare, and (c) to the inaccessibility of the locality to power boats. Such is the choice feeding ground of the mullet, and such is the locality from which this fish returns to deeper water, fat and full, to enjoy a more gregarious and social life. As the schools increase in size and the temperature of the water lowers, their reproductive organs having developed, they move slowly down the coast *en masse* both outside and inside the Banks, spawning as necessity demands. At Beaufort roe mullet are rare in September, common in October, abundant in late October and early November, and rare in December; they are caught both inside and outside the banks, though (according to the fishermen) never with the eggs running (prime ripe); while spent mullet are found wherever mullet are to be found. Some of the fishermen attribute this lack of "running" roe mullet to their going out to sea to spawn while others claim that they spawn in fresh water because the young are found there (although they are equally abundant all the way out to well beyond the shore line). Thus nothing is known of the spawning grounds of this species and therefore of its eggs or larvae, the earliest stage known being the already well developed young described at the beginning of this paper.

## MUGIL CUREMA CUV. &amp; VAL.

*Young*

The young of the white mullet, as above shown, is the so-called *Querimana harengus*, and is undoubtedly found as far north as Wood's Hole. At Beaufort they have not been recorded earlier than May 25th, but there is reason to believe that they could be found even as early as late April. In habitat and habit they are similar to *M. cephalus*.

The *development* of this species is, in general, like the former, but without a definite silvery stage and with a constant rate of development of the various parts and of the individual. The smallest specimens normally procurable are 20 to 21mm. long and as much developed as are 23mm. specimens of *M. cephalus*. At this least size the alimentary canal contains no trace of the crustacean diet so characteristic of the other species, their stomachs being filled with the dark mud matter on which they continue to feed. Aside from this difference the two species are similar in their juvenile characteristics, i.e., they have cyclid scales, no adipose eyelid, and but two anal fin spines.

The *development of the scale* though mainly similar to that of the striped mullet is interestingly different. The juvenile scale differs from that of *M. cephalus* in a tendency toward one pair less of basal radii and in tending to have lateral circuli connecting anterior and posterior circuli (figs. 16-19). The lowest formula found was a 10, 1.0, p. 14, thus being more advanced than corresponding *M. cephalus*. As these juvenile fish acquire their adult characters the habit of sculpture of the basal area of the scale changes in the same way as does that of *M. cephalus*. The development of the apical portion of the scale, on the other hand, is strikingly different. In *M. cephalus* the lateral circuli generally extend backward following the contour of the juvenile scale until they meet and thus form about it a close fitting frame. This is so foreign to *M. curema* that it only rarely occurs and then only with the first circulus. The second one in extending backward tends to diverge from the first, the third from the second though possibly less, and so on (figs. 16-19). This occasionally occurs in *M. cephalus* scales (fig. 15) but only with a few circuli. The typical lateral circulation for *M. curema* scales is this

divergent type but without the close-fitting lateral circulus, the very first one forming an acute angle and terminating very briefly at the edge of the juvenile scale or continuing through it as an apical circulus. Meanwhile each apical circulus has done one of two things, it has entirely stopped growing or it has continued to grow. If all apical circuli cease growing at the same time another apical circulus may form about them as above described (figs. 16-19) and thus very definitely mark off the juvenile scale as in the other species, but, unlike it, this new circulus is close to the juvenile scale and immediately followed by others so that the apical circuli are much more closely spaced than in the jumping mullet. (Compare figs. 16-19 with figs. 12-15). If all the apical circuli of the juvenile scale continue to grow in full strength and unchanged direction (of very rare occurrence) the apical boundary of the juvenile scale is undiscernible. Although these circuli will continue to extend across the transition line between the juvenile and young scale, until they meet lateral circuli or reach an equivalent distance, they generally become thin at the transition line, or, in rare cases, become obsolete at that point, (figs. 16, 17). Accompanying this weakness of growth the circuli will often become curved or more widely or irregularly spaced at the transition line, so that the boundaries of the juvenile scale are plainly discernible. The first few apical circuli of the juvenile scale never run beyond it, extending only to the line of the posterior axes where they occasionally turn and become lateral circuli. In the great majority of juvenile scales all apical circuli do not pursue the same course, so that the scales present an enormous amount of variation on the transition line (figs. 16-19). For this reason it is very rare when the juvenile scale is not set off from the remainder of the scale posteriorly, while it is always discernible anteriorly. When apical circuli meet lateral circuli they do so at an acute angle thereby forming a type of circulation quite characteristic of the scale of this species (figs. 17-19, 21). Figure 16 shows such an angle just formed, another about to form, and another some distance from forming. Thus, although there is not so striking a transition in the scale of *M. curema* as in *M. cephalus*, yet there is a change so marked as to be unexplainable. It is certain that this change in the scale sculpture is not due to migration for all stages of the change, and scales some time before the change would take place,

are procurable as long as juvenile fish are obtainable, and further, the change is not merely a seeming cessation of growth for a short period, but a complete change in *sculpture habit*; nor is it due to change in diet for the intestine contents of the fish before and after the change, in the scale, are alike. Thus again the change seems to be recapitulatory or phylogenetic. A factor in the destruction of the central sculpture, and more so than in the other species, is the spreading of the lateral line groove (figs. 17, 18).

After a various number of apical circuli have been formed (generally more than in *M. cephalus*) a break appears at the apical center in which cteni are formed (figs. 18, 19, 21). These cteni are added and develop much as in *M. cephalus*, but have an entirely different appearance. Instead of being narrow, slightly curved, keeled, and sharply pointed as in *M. cephalus*, the cteni of this species are wide and flat with a very inconspicuous keel at the apical end (figs. 27, 28). Moreover, the cteni in *M. curema* practically all appear in a well defined projecting band while in *M. cephalus* they gradually merge back into the old worn stubs of former teeth called by Cockerell (1913) "apical marginal elements" (herein, for brevity, called ctenobasii), and do not project as a well-defined band beyond the normal outline of the scale except in very advanced scales (fig. 26). In figure 27 notice how the ctenobasii seem in places to be broken up circuli and in others worn down cteni, as though the cteni were modifications of the circuli. In *M. curema* (fig. 28) the transition is not so gradual, the fringe of cteni seeming quite segregated from the remainder of the scale. The ctenobasii, however, are present in even greater numbers than in the other species and although they do not seem to be worn down cteni they occupy an area once covered by them (figs. 18, 19, 21). They must therefore be considered deteriorated cteni and noted as another difference between the two species. The cteni are added row after row along with the circuli throughout the summer until the fish have reached a maximum size of 230mm. in September when they migrate south. Figure 21 is from a scale of a 121mm. fish taken on the 23rd of August, and shows all the characteristics of the scale of this species. Compared with figure 20 (the corresponding scale of the other species) the radii are seen to be fewer in number. This is constantly the case. Both these scales having been taken from the same position on the fish's body, this difference is a real specific

difference. The lateral circuli are also more closely spaced in *M. curema* than in *M. cephalus* in scales of equal size. This does not mean that one species accrues circuli at a greater rate than the other.

### *Migration*

In the fall the scattered individuals and small schools gather over the sandy bottoms in schools of ever increasing size, much as do the other species, and each school in its turn migrates leisurely south. During the winter this mullet is very rarely if ever found in the Beaufort region but with the approach of summer an occasional individual may be taken. It is, however, so uncommon in its second season or older, that the fishermen consider it a matter of curiosity or note when one is caught. Several specimens about eight inches long were taken on the 27th of June. From the scale formula of an individual 184mm. long ( $1.67+26$ ) there seems to be little doubt that this fish migrated in the early spring. Three other scales from fish bearing no data show a similar linea, but situated farther from the edge of the scale.

### *Adults*

Due to the scarcity of this species at Beaufort no true adults were procured so that practically nothing is known concerning their habits. From figure 3 it is evident that the spawning period must be rather protracted and, if an estimate of the time can be made from the dates when the smallest fish are procurable the season would be (conservatively) from mid-April to mid-August, the height of the season probably being in May.

### SUMMARY

#### *Mugil cephalus* Linné

1. To the synonymy of the genus *Mugil* should be added *Querimana*.
2. To the synonymy of the species *M. curema* should be added *Q. harengus*, its juvenile young.
3. *M. cephalus* spawns in October and November (September to December).
4. The juvenile young pass the winter without much growth.

5. In the spring the juvenile change diet and grow very rapidly until fall when they school and migrate south not to return until spring.

6. In the spring, the young, at that time from five to eight inches long, return north by a (typically) continuous run.

7. By the second fall the fish have reached a length of a foot or more and attained maturity.

8. In October and November these two-year-old fish migrate south spawning as they go.

9. Jumping mullet may attain an age of five or six years, spawning each year after maturity.

*Mugil curema* Cuv. & Val.

1. *M. curema* spawns in May and June (April to August).

2. The young are abundant in the bays and estuaries of our Atlantic coast and develop rapidly.

3. In the fall the young school and migrate south.

4. After their first year, white mullet are but seldom caught north of Florida.

BIBLIOGRAPHY

BEAN, T. H.

1913. Catalogue of the Fishes of N. Y., N. Y. State Museum Bulletin No. 60, p. 366.

COCKERELL, T. D. A.

1913. Observations on Fish Scales. Bulletin U. S. Bureau of Fisheries, vol. 32, 1912, pp. 117-174.

DAHL, KNUT

1911. Age and Growth of Salmon and Trout in Norway as Shown by Their Scales. Salmon Trout and Association. London. pp. 1-141.

EARLL, R. E.

1887. The Mullet Fishery. The Fishery Industries of the U. S., U. S. Commission of Fish and Fisheries, Section V, vol. I, pp. 555-582.

GILBERT, C. H.

1913. Age at Maturity of the Pacific Coast Salmon of the Genus *Oncorhynchus*. Bulletin U. S. Bureau of Fisheries, vol. 32, pp. 1-22, pls. I-XVII.

GOODE G. B.

1887. Food Fishes of the U. S. The Fishery Industries of the U. S., U. S. Commission of Fish and Fisheries, Section I, vol. I, pp. 449-456.

HJORT, JOHAN

1914. Fluctuations in the Great Fisheries of N. Europe. Conseil Permanent International pour l'Exploration de la Mer. Rapports et Proces-verbaux, vol. XX, p. 122, pl. 3.

## JORDAN, D. S. &amp; EVERMANN, B. W.

1896. The Fishes of North & Middle America. Bulletin U. S. National Museum, No. 47, pt. I, p. 809.

————— & GILBERT, C. H. .

1883. Notes on a Collection of Fishes from Charleston, S. C. Proceedings U. S. National Museum, vol. V, pp. 580-590 (588).

1884. Description of Ten New Species of Fishes from Key West, Florida. Proceedings U. S. National Museum, vol. VII, pp. 24-32 (26).

————— & SWAIN, J.

1884. A Review of the American Species of Marine Mugilidae. Proceedings U. S. National Museum, vol. VII, p. 274.

## KENDALL, W. C.

1892. See under Smith, H. M. 1892, but p. 192, footnote.

## SMITH, H. M. &amp; KENDALL, W. C.

1892. Extension of the Recorded Range of Certain Marine and Fresh-water Fishes of the Atlantic Coast of the U. S. Bulletin U. S. Fish Commission, vol. XIV, pp. 15-21 (21).

## LEA, EINAR

1913. Further Studies Concerning the Method of Calculating the Growth of Herrings. Conseil Permanent International pour l'Exploration de la Mer. Publications de Circonstance No. 66.

## LINTON, E.

1904. Parasites of Fishes of Beaufort. Bulletin U. S. Bureau of Fisheries, vol. XXIV, pp. 321-428 (361).

## MASTERMANN, A. T.

1913. Report on Investigations upon the Salmon with Special Reference to Age Determination by Study of Scales. Board of Agriculture and Fisheries of England, Fisheries Investigations, Series I, vol. I, p. 12.

## NILSON, DAVID

1914. A Contribution to the Biology of the Mackerel. Conseil Permanent International pour l'Exploration de la Mer. Publication de Circonstance, No. 69.

## RAVENEL, W. DEC.

1887. Information Bearing upon the Artificial Propagation of the Mullet. Bulletin U. S. Fish Commission, vol. VII, pp. 197-202.

## RUTHVEN, A. G.

1908. Variations and Genetic Relationships of the Garter-Snakes. U. S. National Museum, Bulletin 61.

## SMITH, H. M.

1892. Report on a Collection of Fishes from the Albemarle Region of N. C. Bulletin U. S. Fish Commission, vol. XI, pp. 185-200 (192).

1897. Fishes Found in the Vicinity of Woods Hole. Bulletin U. S. Fish Commission, vol. XVII, pp. 85-111 (94).

1907. Fishes of North Carolina. N. C. Geological and Economic Survey, p. 181.

STARKS, E. C.

1900. Osteological Characters of the Fishes of the Suborder Percesoces. Proceedings U. S. National Museum, vol. 22, pp. 1-10 (7), pl. I-III.

SUMNER, F. B., OSBURN, R. C. & COLE, L. J.

1911. A Biological Survey of the Waters of Woods Hole and Vicinity. A Catalogue of the Marine Fauna. Bulletin U. S. Bureau of Fisheries, vol. XXXI, pt. II, p. 747.

WILDER, B. G. & GAGE, S. H.

1882. Anatomical Technology. Barnes & Co., p. 27, & 47.

#### EXPLANATION OF PLATES

##### PLATE XX

Fig. 8. Juvenile scale from smallest fish normally obtainable (23 mm. fish), x 45.

Fig. 9. Juvenile scale with maximum amount of development (32 mm. fish), x 45.

Figs. 10-14. Juvenile scale being enclosed by the more advanced type of scale (29 mm.—45 mm. fish), x 45.

##### PLATE XXI

Fig. 15. Scale of a 60-70 mm. mullett with cteni first forming, x 45.

Figs. 16-17. Juvenile scale being enclosed by the more advanced type of scale, x 45.

Figs. 18-19. Development of cteni on scale of the white mullett, x 45.

##### PLATE XXII

Fig. 20. Typical scale of advanced first season jumping mullett, x 25.

Fig. 21. Typical scale of advanced first season 121 mm. white mullet taken August 23, x 21.

##### PLATE XXIII

Fig. 22. Scale of a 117 mm. mullet with unusual amount of circulation, x 30.

Fig. 23. Scale of 120 mm. jumping mullet taken April 28 with linea very near margin, x 25.

Fig. 24. Scale of jumping mullet, with a double linea, x 30.

##### PLATE XXIV

Fig. 25. Typical scale of second season jumping mullet. x 30.

##### PLATE XXV

Fig. 26. Scale of a five year jumping mullet 502 mm. (20 inches) long. x 13. Development of scale of *M. curema*.

##### PLATE XXVI

Figs. 27-28. Ctenoid area of scales of adult *M. cephalus* and *M. curema*, highly magnified.





11



12



10



13



9



14



8

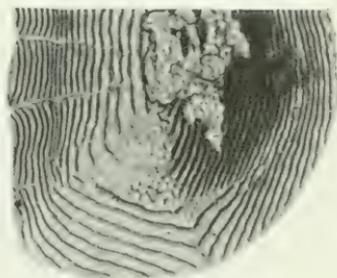




19



18



17



15



16





21



20





24

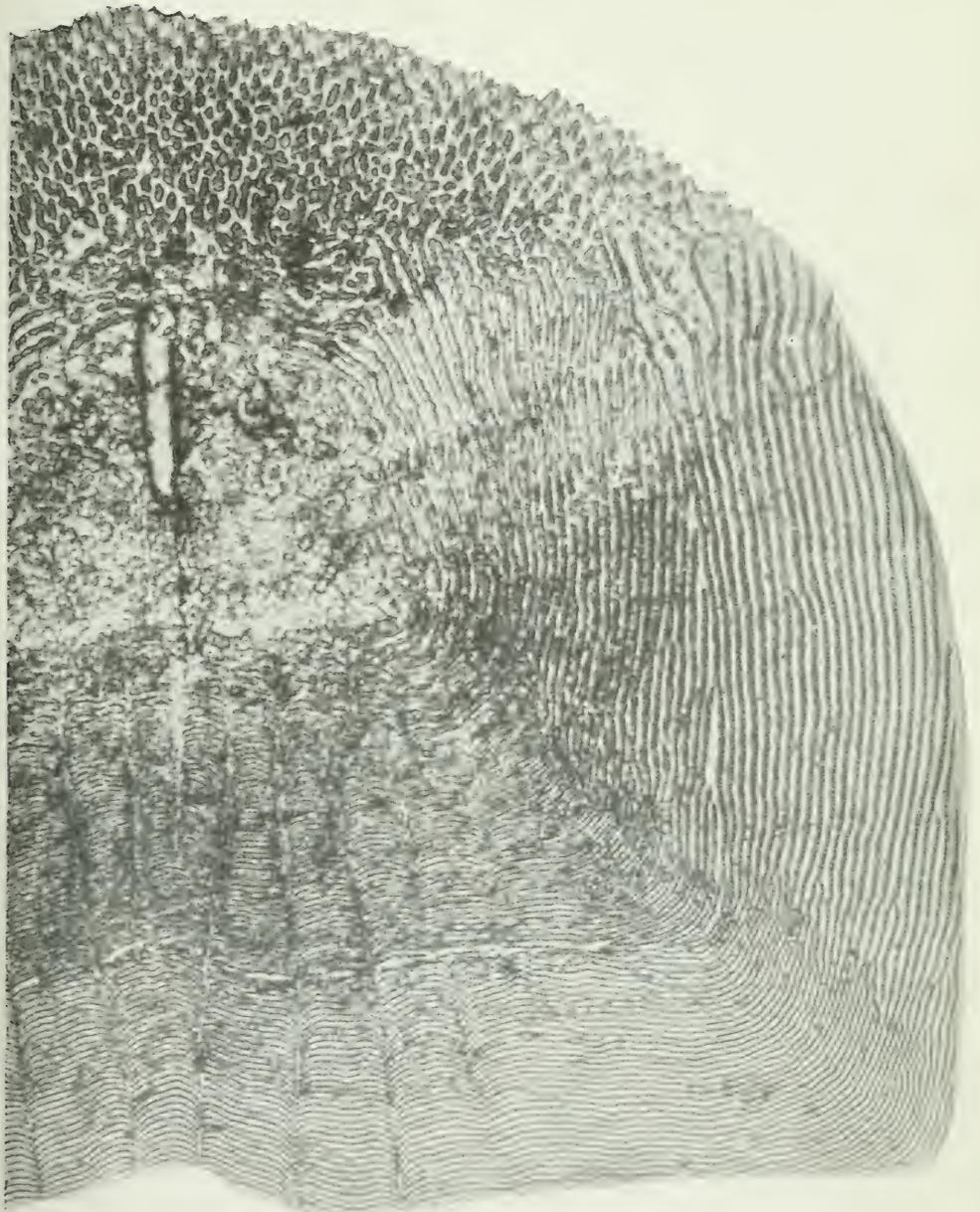


22

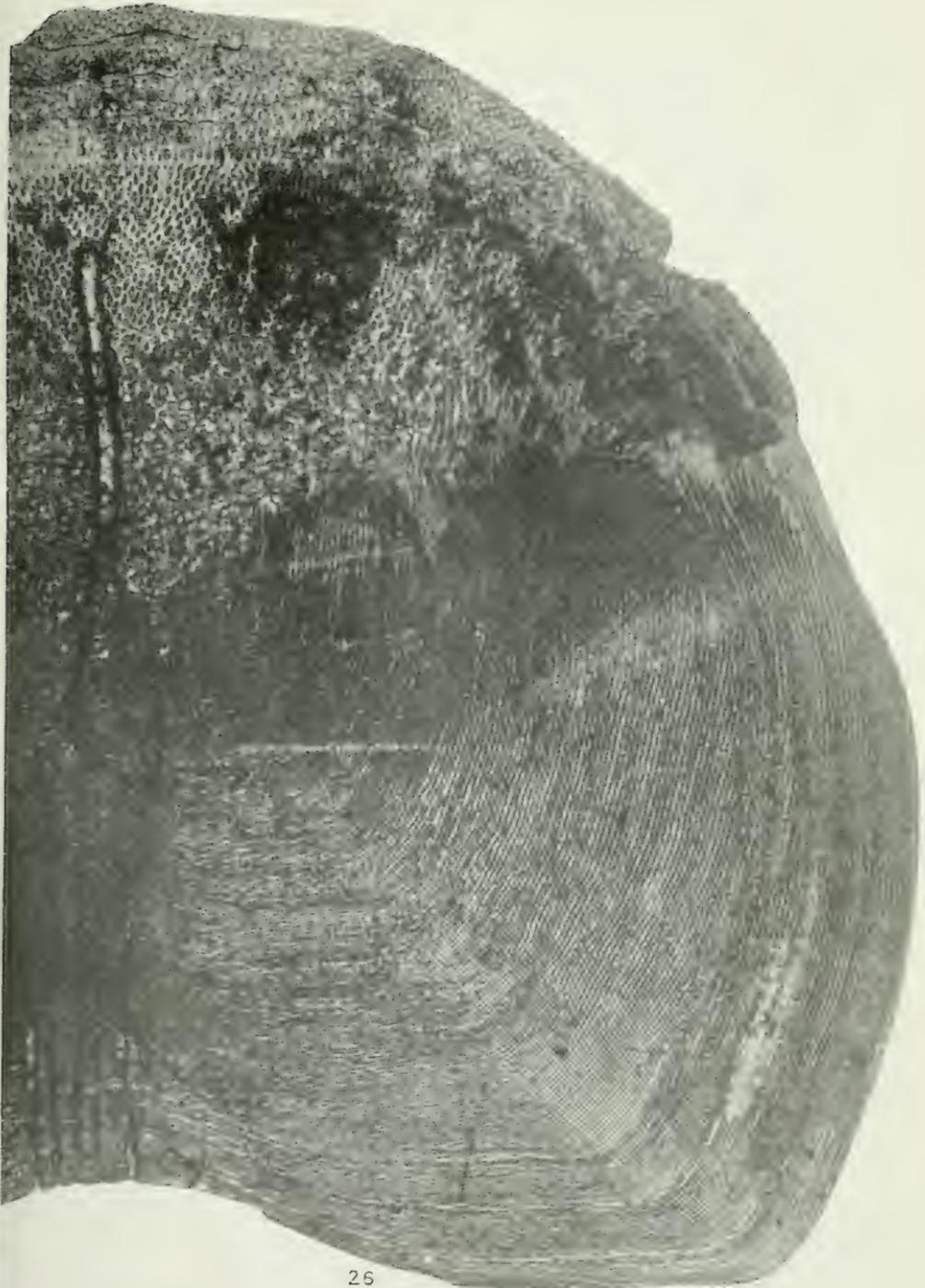


23

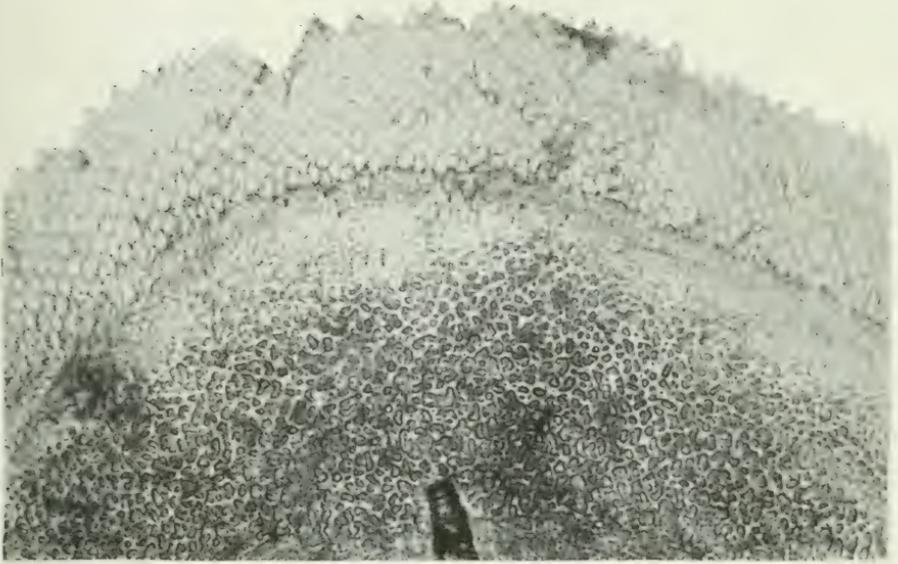




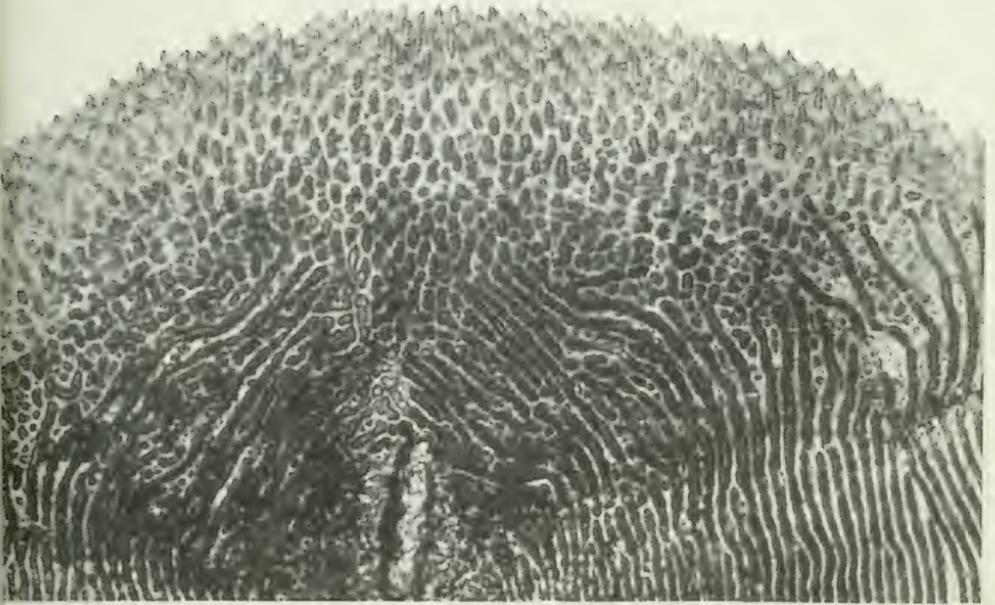








28



27



351

# TRANSACTIONS OF American Microscopical Society

(Published in Quarterly Instalments)

Vol. XXXIX

OCTOBER, 1920

No. 4

## MICRO-TECHNIQUE

### SUGGESTIONS FOR METHODS AND APPARATUS

N. A. COBB

United States Department of Agriculture

#### I

#### SYSTEMATICALLY EXAMINING LARGE SERIES OF MICROSCOPICAL OBJECTS

There are various methods of recording the position and character of each member of a large series of objects mounted on a microscope slide. One of the commonest methods involves the use of a recording, mechanical stage. Each object on the slide receives a record-number consisting of two separate readings from scales engraved on the mechanical stage. The following method, however, is successful without a mechanical stage or finder of any sort, and is characterized by simplicity and expedition. It may be called the method of charting.

The method consists in making a camera lucida drawing or chart, at low magnification, of all the objects of which it is desired to make record. The chart is diagrammatic; each object is represented on the chart by a simple, characteristic diagram, and the diagrams are then numbered in series. The sheet that carries the chart may also carry a series of printed numbers with corresponding spaces for records. (See Figure 1.) Where the objects belong to a few great groups, such as land-inhabiting, fresh-water, and marine, the printing of the blank sheets in correspondingly assorted colors is an advantage.

The chart is made by using a camera lucida and an objective of about five-inch focus.<sup>1</sup> In order to reduce the magnification, the objective may be screwed into the end of the draw-tube of the microscope barrel. A low power eye-piece is used with the objective, so

<sup>1</sup> A very strongly magnifying spectacle lens will serve the purpose.

that all the objects on the slide can be seen at one time. A chart having a magnification of five diameters is of convenient size. The suitable illumination is secured by using a concave mirror without sub-stage condenser. The light may be direct, in which case the objects are seen as dark bodies on a light background, or a dark-ground effect can be produced by inserting between the concave mirror and the objects a small opaque disc. A suitable disc may be made by stripping the barbules from a dark-colored six-inch wing or tail feather so

<p>Soil - Imported roots of plants, +                  Brazil - Diff. #510                  No. 7                  1-11</p>	<table border="0"> <tr><td>1</td><td>Tylenchus spiralis</td><td>26</td><td>See. 11</td></tr> <tr><td>2</td><td>Cephalobus ?</td><td>27</td><td>" "</td></tr> <tr><td>3</td><td>Doryl. stytracturus</td><td>28</td><td>" "</td></tr> <tr><td>4</td><td>Achromadora brazil.</td><td>29</td><td>" "</td></tr> <tr><td>5</td><td>Doryl. caudatus?</td><td>30</td><td>Mononchus minor</td></tr> <tr><td>6</td><td>Elassonema - two</td><td>31</td><td>" fragment minor</td></tr> <tr><td>7</td><td>Tylenchus perfectua</td><td>32</td><td>Rhabditia</td></tr> <tr><td>8</td><td>Doryl. additicius</td><td>33</td><td>Y. Doryl. protrudens</td></tr> <tr><td>9</td><td>" protrudens</td><td>34</td><td>See. 11</td></tr> <tr><td>10</td><td>" "</td><td>35</td><td>Y. Doryl.</td></tr> <tr><td>11</td><td>?</td><td>36</td><td>Achromadora</td></tr> <tr><td>12</td><td>Tropiconema tenuicollis</td><td>37</td><td>See. 11</td></tr> <tr><td>13</td><td>See. 11 Egg</td><td>38</td><td>Rhabditia</td></tr> <tr><td>14</td><td>Achromadora-papillae?</td><td>39</td><td>Elassonema</td></tr> <tr><td>15</td><td>Fibre</td><td>40</td><td>Y. Doryl.</td></tr> <tr><td>16</td><td>Mononchus</td><td>41</td><td>See. 11</td></tr> <tr><td>17</td><td>Achromadora</td><td>42</td><td>Fibre</td></tr> <tr><td>18</td><td>Rhabditia</td><td>43</td><td>See. 11</td></tr> <tr><td>19</td><td>Ironus</td><td>44</td><td>Doryl. poor</td></tr> <tr><td>20</td><td>Elassonema</td><td>45</td><td>Rhabditia</td></tr> <tr><td>21</td><td>"</td><td>46</td><td>" "</td></tr> <tr><td>22</td><td>Rhabditia</td><td>47</td><td>Doryl. sl. tl.</td></tr> <tr><td>23</td><td>Tylenchus</td><td>48</td><td>?</td></tr> <tr><td>24</td><td>Mononchus minor</td><td>49</td><td>Doryl. sl. Eggs</td></tr> <tr><td>25</td><td>Rhabditia</td><td>50</td><td></td></tr> </table>	1	Tylenchus spiralis	26	See. 11	2	Cephalobus ?	27	" "	3	Doryl. stytracturus	28	" "	4	Achromadora brazil.	29	" "	5	Doryl. caudatus?	30	Mononchus minor	6	Elassonema - two	31	" fragment minor	7	Tylenchus perfectua	32	Rhabditia	8	Doryl. additicius	33	Y. Doryl. protrudens	9	" protrudens	34	See. 11	10	" "	35	Y. Doryl.	11	?	36	Achromadora	12	Tropiconema tenuicollis	37	See. 11	13	See. 11 Egg	38	Rhabditia	14	Achromadora-papillae?	39	Elassonema	15	Fibre	40	Y. Doryl.	16	Mononchus	41	See. 11	17	Achromadora	42	Fibre	18	Rhabditia	43	See. 11	19	Ironus	44	Doryl. poor	20	Elassonema	45	Rhabditia	21	"	46	" "	22	Rhabditia	47	Doryl. sl. tl.	23	Tylenchus	48	?	24	Mononchus minor	49	Doryl. sl. Eggs	25	Rhabditia	50	
1	Tylenchus spiralis	26	See. 11																																																																																																		
2	Cephalobus ?	27	" "																																																																																																		
3	Doryl. stytracturus	28	" "																																																																																																		
4	Achromadora brazil.	29	" "																																																																																																		
5	Doryl. caudatus?	30	Mononchus minor																																																																																																		
6	Elassonema - two	31	" fragment minor																																																																																																		
7	Tylenchus perfectua	32	Rhabditia																																																																																																		
8	Doryl. additicius	33	Y. Doryl. protrudens																																																																																																		
9	" protrudens	34	See. 11																																																																																																		
10	" "	35	Y. Doryl.																																																																																																		
11	?	36	Achromadora																																																																																																		
12	Tropiconema tenuicollis	37	See. 11																																																																																																		
13	See. 11 Egg	38	Rhabditia																																																																																																		
14	Achromadora-papillae?	39	Elassonema																																																																																																		
15	Fibre	40	Y. Doryl.																																																																																																		
16	Mononchus	41	See. 11																																																																																																		
17	Achromadora	42	Fibre																																																																																																		
18	Rhabditia	43	See. 11																																																																																																		
19	Ironus	44	Doryl. poor																																																																																																		
20	Elassonema	45	Rhabditia																																																																																																		
21	"	46	" "																																																																																																		
22	Rhabditia	47	Doryl. sl. tl.																																																																																																		
23	Tylenchus	48	?																																																																																																		
24	Mononchus minor	49	Doryl. sl. Eggs																																																																																																		
25	Rhabditia	50																																																																																																			

Fig. 1. Record chart used in tabulating large numbers of microscopic objects arranged on a series of slides. As printed the chart was 5x8 inches, and carried only the two columns of figures 1 to 50 inclusive. At the left is seen the camera lucida drawing, or chart, recording the form, size, and relative position of forty-nine microscopic objects, —in this particular case, nemas. Immediately above the chart are seen the data relating to the particular slide charted, which was No. 7 in a series of eleven slides (1-11), and which carried a collection of forty-nine nemas gathered from soil attached to the roots of plants imported from Brazil. Names and other notes with regard to the nemas were typewritten opposite the appropriate numbers. Nos. 2, 7, 12, 13, 14, 23, 48, and 49 were encircled to indicate that these specimens were of especial interest. One-half size.

as to leave only a small fan-shaped tip at the end, from one-half to three-fourths of an inch across. With scissors, this is trimmed so as to have a somewhat rounded contour. While the right hand is engaged in making the chart, the left hand can flirt this little disc in and out between the objects and the concave mirror and so produce a

is sawed from a sheet of German silver about one two-hundredth of an inch thick. The edges of the central aperture are beveled so that the mixture frozen on it becomes dove-tailed to the plate. In a similar way, the small, washer-shaped piece of German silver fastened to the top of the dome, as shown in Fig. 3, *r*, is also beveled.

The German silver wheel is soldered throughout to a round sheet of exceedingly thin brass or German silver. Then into six marginal perforations in the German silver wheel, brass pins are soldered, giving to the whole affair the appearance of a six-legged table. The heads of the pins are filed off so as to give clearance for the microtome knife. The pins serve to fasten the plate to a perforated cork, being thrust into the cork as shown in the illustration. The rim of the dome of thin sheet metal is somewhat similarly stiffened by soldering to it a ring of German silver which is perforated and supplied with six brass pins in the manner just described.

Though the dome-form is somewhat more difficult to construct than the flat, it is more efficient for three reasons: It is more rigid, it gives a better clearance for the microtome knife, and it contains less material.

In the case of small and moderate sized objects of which only a few sections are required, the method is extraordinarily expeditious. Objects of such a size that they can be imbedded in a few drops of the freezing mixture placed on the control part of either of these metal supports can be frozen in a few seconds by applying an ordinary ether spray to the under side of one of these thin metal supports. The exceeding rapidity of the congelation gives rise to a consistency favorable to section cutting.

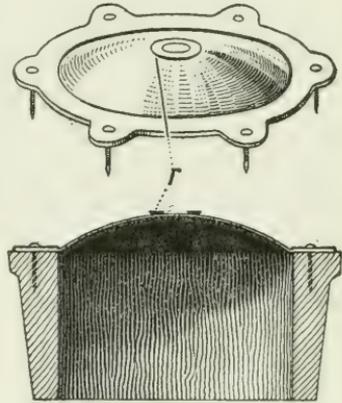


Fig. 3. Perspective view and longitudinal section of a freezing-microtome object-holder mounted on a cork cylinder. The holder is made of metal only about  $2/1000$  of an inch thick. The edges of the ring (*r*) are beveled so that the imbedding mixture when frozen is dovetailed to the holder.

## III

TO OBTAIN AN END VIEW OF A NEMA, ROTIFER, OR OTHER  
SIMILAR SMALL OBJECT

Suppose the object is a nema of which an end view of the head is required: decapitate the nema behind the pharynx with the aid of an eye knife, or similar very small tool, having a very slender, thin blade. The smallest and most slender-bladed knife used by oculists in operations on the eye is a very suitable tool, and it must have the degree of sharpness characteristic of surgical instruments in good order. Bring the nema by appropriate methods into glycerine; the decapitation should be done in a drop of glycerine placed on the surface of a transparent piece of celluloid. Push the nema to the bottom of the glycerine and against the celluloid; decapitate by pressing the edge of the knife against the nema as the latter rests on the celluloid. The celluloid is sufficiently soft so that the edge of the knife will not be dulled. If the knife is sharp, the cut will be clean, and the object satisfactory. If the knife is dull, the nema will be more or less crushed at the point of section and the preparation may prove unsatisfactory.

Mount the head in melted glycerine jelly, using sufficient jelly so that the object may stand on end after being covered in. Place the mount on the stage of a microscope, bring the object into focus, and with a dissecting needle gently shove the cover-glass slightly back and forth until the object is seen to be on end. Allow to remain on the stage of the microscope until the jelly sets, watching from time to time to see that the object maintains the desired position.

According to my experience, this is a better method of obtaining end-on and sectional views of the heads of free-living nemas and other similar small organisms than that of sectioning and imbedding. The trouble with the method of sections is that the microtome knife very seldom cuts the object to advantage. It is quite likely to cut in the wrong place. If the ends of the setae or the surfaces of the lips are removed in the first cut, it is a very troublesome matter to obtain a good view or good sketch of the structures. Even if some of the parts should not be lost or offer difficulty in mounting, there are so many chances that the microtome blade will cut through at a disadvantageous place that, as a rule, a very considerable number of nemas will have to be sectioned before a good preparation is secured.

The method of sections has the further disadvantage that the following of such small objects through the various dehydrating and

staining fluids, and the final orientation of them, is a tedious and difficult matter. Moreover in the case of nemas, there is considerable difficulty in properly imbedding the object. The cuticle of nemas is so impenetrable that unless special precautions are taken, the paraffine will not thoroughly penetrate the tissues, and the results will be unsatisfactory.

End views may be obtained by mounting the nemas in a microscopic well made from a thin section of thermometer tubing. The tubing should be like that used in the most delicate medical thermometers, that is to say, with the smallest aperture procurable. This tubing may be bought under the name thermometer, or barometer tubing. It is well to have on hand ground sections of varying thickness, from one-quarter of a millimeter thick to one millimeter or more. The discs are cemented to a glass microscope slide at the time of using by means of smoking hot wax or other suitable cement. Before cementing the disc to the slide, fill the capillary aperture in the disc with mounting fluid. This may be easily done by placing on the slide a very tiny drop of the mounting fluid, and laying the disc onto the small drop. The mounting fluid will enter the aperture by capillarity. If it be desired to look at the head end of a nema, it is placed in the microscopic well, tail down. If the nema is too long for the well, it may be cut to fit it. The point is, to see that the object has about the same length as the depth of the well, so that the end portion of the object it is desired to view will come close to the under side of the cover-glass when this latter is placed on the top of the well, or rather on the disc of the glass containing the well. In placing the nema in the well, a suitable tool is a small, curved hair cemented to the end of a dissecting needle. Human eye-brow hairs are suitable for this purpose. Using this method, the specimen can be examined in clove oil, cedar oil, or any mixture of these or any other similar thin mounting fluid. Cedar oil, having the same refractive index as the glass composing the well, has advantages in connection with illumination. The illumination in aqueous media is less satisfactory.

When the glass discs are not in use, it is best to keep them in absolute alcohol in a glass-stoppered bottle. They should not be allowed to become dry with mounting fluid in the capillary orifice, otherwise they will be very troublesome to clean out.

## IV

DESTAINING OF NEMAS OR OTHER SMALL  
OBJECTS IN THE DIFFERENTIATOR

In handling a mass of small organisms by the differentiator method, there is sometimes considerable difficulty in securing satisfactory destaining. There is little difficulty in getting a mass of organisms thoroughly impregnated with the stain, no matter how varied they may be in species and in size; it is simply a matter of time. The trouble comes in destaining. If the destaining process is carried on until the largest of the objects, or the most impenetrable ones, are sufficiently destained, it will generally happen that smaller specimens, or those more easily penetrated, are deprived of too much of their color. It is therefore a matter requiring considerable experience and judgment to successfully destain such a miscellaneous collection. The difficulty is considerably increased by the fact that when enclosed in the differentiator tube, the specimens are not very easy to examine critically by any ordinary method. If the differentiator be held toward a strong light, the organisms may be examined by the aid of an ordinary pocket lens, but not very critically. The most satisfactory piece of apparatus for this work is what is sometimes known as the chemical microscope, in which the objective is below the stage and the light that passes through it from above is reflected by a prism placed below so as to pass obliquely upward through a barrel carrying an eye-piece. If the differentiator tube containing the destained nemas is laid on a glass stage over the objective of such an inverted microscope, and a little water, or still better, cedar oil, be placed between the differentiator tube and the glass stage, it will be found that the nemas or other objects will sink to the bottom of the fluid in the differentiator tube so as to come as near as possible to the objective of the microscope. If the glass stage is thin, there is no difficulty in using a one-half to two-thirds inch objective. In this way, the nemas may be examined more critically with regard to the extent of the destaining.

If it is desired to use a lens of higher power, it is sometimes possible to do so by resorting to another method. Place a cover-glass on a horizontal surface, and on the cover-glass a good-sized drop of cedar oil. Lay the differentiator tube into this drop of cedar oil in such a way that the nemas come opposite the cover-glass. It will now be

dark-ground effect as desired. To do this the feather "disc" must be materially smaller than the mirror.

The charts are nothing more than rude camera lucida drawings of the objects, and with practice can be made with great rapidity. A lot of fifty nemas mounted under a three-quarter inch round cover-glass can be drawn in two to three minutes with sufficient accuracy to make a very useful chart. (See Figure 1.) Each nema-diagram on the chart has four very distinct properties, (1) Position, (2) Form, (3) Size, (4) Orientation. For the most satisfactory work, it is desirable that a certain optimum number of objects exist on the slide. This optimum is determined by the number of them that will appear in a single field of the lens afterward used in searching. Suppose a sixteen millimeter objective is used as a searching objective, and a four millimeter for the examination; then the optimum number of objects under the cover-glass is that number which brings into each field of the sixteen millimeter objective one to three objects.

After the chart is made, the short, crooked lines, representing the nemas, say, are numbered in transversely arranged groups. Each transverse group of the series constitutes a band of nemas running across the mount and having such width as comes fairly well within the scope of a single field of the 16 mm. objective. These imaginary bands are illustrated in Figure 1. It will be seen that there are four such bands. The nemas are numbered more or less consecutively. Proceeding in this manner, on reaching the end of the first band, one numbers the second band, also more or less consecutively, and so on to the end.

In recording, begin with No. 1, placing it in the field of the 16 mm. objective. It is recognized by its size, form and orientation. Having recorded No. 1 and examined it with the 4 mm. objective, a glance at the chart will indicate at what distance, and in what direction, No. 2 lies from No. 1. Revolving to the 16 mm. objective and looking through the microscope at Nema No. 1, the slide is moved in the indicated direction until No. 2 is found and recognized. After recording No. 2, No. 3 is found in the same way, and so throughout. The novice will be surprised to find how easy it is, with a little practice, to follow the series through without error.

The drawings should be so made and numbered that the chart and the objects as seen under the microscope will resemble each other.

If no care be taken in this respect, the chart may be found to be "left-handed." Securing a "right-handed" chart is merely a matter of properly arranging the paper at the time the chart is drawn. Diagrams should be so made with reference to the printed matter that when it is right side up, the objects as viewed through the microscope will have the same orientations as the diagrams.

This completes the description of this method, except to explain that in the example illustrated, the numbers encircled are so marked in order to indicate that those particular specimens present noteworthy features.

The method may be elaborated in a variety of ways for the recording of nemas, rotifers, protozoa, desmids and a vast array of other microscopic objects. If the charts are of card-system size, say 5x8", they lend themselves to all sorts of convenient methods of filing. By using thin paper, carbon copies can be made at the original draft.

The charts can be made and used by a grade of assistant that might hardly be intrusted with the use of a recording mechanical stage, and who may lack training in the accurate reading of scales and the recording of numbers. Floating of the objects, of course, disarranges them. Newly made slides are sometimes subject to this disadvantage. The difficulty is avoided by keeping the slides always in a horizontal position.

## II

### OBJECT SUPPORT FOR A FREEZING MICROTOME

In this freezing microtome attachment, the object is to reduce the metal parts to a minimum and to concentrate the effects of the freezing mixture as much as possible upon the object to be frozen.

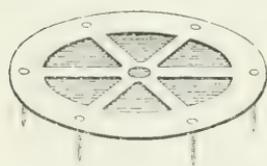


Fig. 2

To this end the object is placed on a thin metal plate, only about one to three thousandths of an inch thick, to which the necessary rigidity is imparted either by soldering it to a radiating framework in the form of a flat wheel sawed from somewhat thicker metal, or, preferably, by giving to the metal the form of a dome. These metal supports

are illustrated in Fig. 2 and Fig. 3 in which they are shown full size. A six-spoked wheel, having a hub-hole one-eighth of an inch across,

found that the cover-glass will adhere to the differentiator by capillarity, so long as the differentiator is held in a horizontal position. If the chemical microscope stage has a large aperture, it will be possible to lay the differentiator across the stage, cover-glass downward. In this way, if the differentiator tubing is thin, it will be possible to use even quarter-inch objectives of long focus.

Where considerable work is done with differentiators, a chemical microscope used in this way is a valuable accessory.

## V

### COMPRESSORIUM FOR CHROMOSOMES

When chromosomes or other similar minute bodies are so massed together that one lies behind another and is thus liable to be missed in counting, the compressorium described below may prove useful in overcoming the difficulty, which none of the ordinary compressoria will do.

When such a mass of chromosomes is flattened out by pressure, the individual chromosomes behave somewhat as would the seeds of a pulpy fruit under similar circumstances. They appear to be of a different consistency from the material in which they lie, and behave under pressure as if harder and more compact than the surrounding matter. Under moderate pressure they do not show much tendency to break in pieces, but rather to accommodate themselves to the narrower quarters by rearranging themselves more nearly in one plane. So far as enumeration of the chromosomes is concerned, this new arrangement has two advantages: 1st, they may all be more readily brought into a single view, that is, all brought into focus at one time; 2nd, in the flattening-out process, they slip one over another somewhat, and recede from each other—for instance, as the seeds inside a grape will do, when similarly pressed.

The compressorium I have devised to secure this effect is constructed as follows: Take a safety-razor blade—one of the thinnest kind, having perforations an eighth of an inch in diameter—and

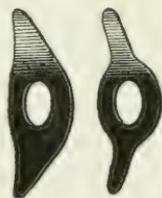


Fig. 4. Two curved, perforated, steel springs made from thin, safety-razor blades, as described in the text. These two forms, while of the same length, nearly one inch, are of different degrees of springiness; that at the left being the weaker.

soften it by heating it to a red heat. With shears, cut a somewhat diamond-shaped piece from the softened blade, so that the "diamond" is about three to four times as long as wide, and has one of the round apertures in its center; bend this elongated "diamond" into a symmetrical bow whose depth is one-eighth of an inch or more. See Fig. 4. Heat the bow in a flame to a cherry-red and plunge it into cold oil or water to harden it. This will result in a springy piece of metal that can be utilized to exert pressure on a small cover-glass under which are mounted cells containing the chromosomes it is desired to scatter. The length of the piece of springy steel may conveniently be made to be about one inch, so that it will just reach across an ordinary three-by-one glass microscope slide. Bind the slide in a piece of thin metal having a three-quarter inch perforation at the back—that is to say, so bend a piece of thin sheet metal that an ordinary slide will slip into it through grooves along the two sides of the folded piece of metal. See Fig. 5. This metal should simply pass around the edges of the slide and lap over about a sixteenth of an inch at each edge leaving one face of the slide uncovered. The grooves should be a little wider than the thickness of the slide—at least enough wider so as easily to admit the thin perforated metal spring. Place the cells, the chromosomes of which are to be studied, on the slide opposite the middle of the three-quarter inch aperture. Use very little mounting medium; cover the cellular tissue to be treated with a small round cover-glass. Tuck the ends of the bowed piece of springy perforated

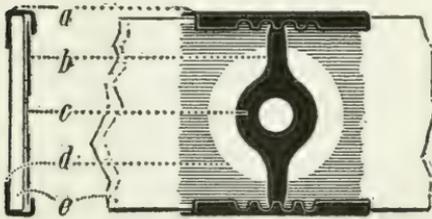


Fig. 5. Portion of a 3x1 inch glass microscope slide enwrapped with thin metal as described in the text. *a*, thin metal wrapper; *b*, one of the springs shown in Fig. 4, placed in position on the slide so as to press the small round cover-glass, *c*, against the slide, *e*; *d*, aperture in the back of the metal wrapper, *a*. The ends of the spring, *b*, enter through the notches on the edges of the wrapper, *a*, so that in being applied the spring does not need to be rotated more than a few degrees.

steel under the edges of the metal slide-case or holder, holding the spring against the small cover-slip in such a way that the cells to be compressed lie opposite the center of the small perforation. Press and lock the spring in the same way as in the case of the springs at the back of an ordinary photographic printing frame. The cells will now be under pressure at or near the center of the perforation in the steel spring. The entire contrivance will differ but very

little in form and size from an ordinary microscope slide and can be placed on any microscope stage in the same way as a slide. The piece of springy steel is so thin that it in no way prevents the use of a high-power immersion objective. Needless to say, it is for this reason that it is made from such thin metal. The spring may be manipulated with the aid of matches or wooden toothpicks.

Ordinary slides and cover-glasses are almost never perfectly flat. Better results will be obtained by this method if the slide has its convex surface up and the cover has its convex surface down, so that the cellular tissues to be treated lie between two very slightly convex surfaces. It will be found that in this way very compact groups of chromosomes and other similar objects can sometimes be scattered so as to be counted, when otherwise they could not be counted.

There seems to be comparatively little danger of exerting too much pressure. The beginner's tendency at first is to exert, if anything, too little pressure. The greatest difficulty arises from sliding the glasses on each other, since much of this ruins the preparation. To overcome this difficulty, a series of three or four notches, close together, may be filed in the edges of the metal holder before it is folded about the slide,—or rather about the metal core on which it is bent, or formed, and which naturally has a little greater width and thickness than the slide. If now the bowed spring has a length a little less than the distance between the bottoms of the notches in the edges of the slide-holder, it will be found when it is pressed down that the pointed ends can be tucked through the notches and under the edges of the holder without materially sliding or rotating the spring. The accompanying illustrations will assist in understanding this simple and effective device.

The particular cells to be compressed are prepared and searched out in the usual way, then dissected out together with as little of the surrounding tissue as possible, an operation performed with the aid of an ordinary dissecting microscope. It may be advisable to look at the group of chromosomes from both sides. To do this, the metal holder, instead of having a three-quarter inch perforation, should have a much smaller perforation, say about one-eighth of an inch. Instead of using a three-by-one glass slide, cement to the inside of the metal holder a thin cover-glass several sizes larger than that to be

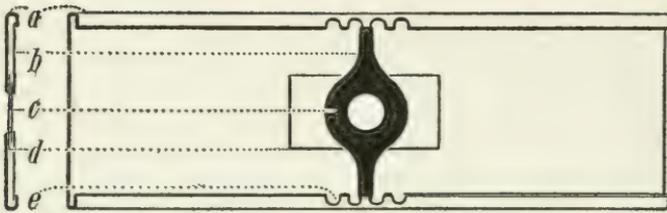


Fig. 6. A metal holder for clamping a microscopic object between two thin cover-glasses. *a*, metal holder; *b*, steel spring as illustrated in Figs. 4 and 5; *c*, small, round cover-glass; *d*, rectangular cover-glass underneath the round cover-glass; *e*, notches in the metal holder for the reception of the spring. This holder enables the microscopist to look at the object with an immersion lens from either direction.

placed over the object. As the metal holder, in order to be stiff enough, has to be several times thicker than the bowed spring, it may be advisable to bevel the edge of the round aperture in the holder, so that it will interfere as little as possible with the use of an immersion objective. On a slide constructed in this manner, the object is held between two cover-glasses, and hence may be viewed from either side with equal ease. Such a slide furthermore permits the use of an immersion lens as a condenser, a proceeding that has advantages.

## LIST OF MEMBERS

### HONORARY MEMBERS

- CRISP, FRANK, LL.B., B.A., F.R.M.S.,  
5 Lansdowne Road, Notting Hill, London, England  
PFLAUM, MAGNUS.....2334 S. 21st St., Philadelphia, Pa.

### LIFE MEMBERS

- BROWN, J. STANFORD, Ph.B., A.M.....P.O. Box 38, Far View, Black Hall, Conn.  
CAPP, SETH BUNKER.....P.O. Box 2054, Philadelphia, Pa.  
DUNCANSON, PROF. HENRY B., A.M.....R.F.D. 3, Box 212, Seattle, Wash.  
ELLIOTT, PROF. ARTHUR H.....52 E. 41st St., New York City  
HATELY, JOHN C.....Chicago Beach Hotel, Chicago, Ill.

### MEMBERS

The figures denote the year of the member's election, except '78, which marks an original member. The TRANSACTIONS are not sent to members in arrears, and two years' arrearage forfeits membership. (See Article IV of By-Laws.)

### MEMBERS ADMITTED SINCE THE LAST PUBLISHED LIST

- |                  |                   |
|------------------|-------------------|
| ARNOLD, L. P.    | JACKSON, F. S.    |
| ASHLEY, F. M.    | JUDD, H. D.       |
| BRUNQUIST, E. H. | KENYAN, W. A.     |
| CASHEN, DOROTHY  | KOSTIR, W. J.     |
| CLOVIS, MABEL C. | KUDO, R.          |
| DE PUY, P. L.    | MANCHEÉ, E. D.    |
| ELMORE, C. J.    | MC COLLOCH, J. W. |
| ELLIS, C.        | McCULLOCH, IRENE  |
| ENBURG, J. M.    | MUTTKOWSKI, R. A. |
| GRAVELLE, P. O.  | PICKETT, F. L.    |
| GROSS, F. O.     | RYE, L. E.        |
| HAYES, W. P.     | SCOTT, HELEN M.   |
| HUDSON, D. V.    | SIMON, C. L.      |
| HUBERT, H. E.    | TUERS, R. V.      |
| HICKMAN, J. R.   | TAYLOR, W. R.     |
| HISAW, F. L.     | WALTON, A. S.     |
| HOTTES, C. F.    | WARREN, SHIELDS   |
| HOPKINSON, D.    | YOSHIDA, SADA O   |
| JACOT, A. P.     |                   |

- ACKERT, JAMES EDWARD, Ph.D., '11. . . . . Kas. State Agr. Col., Manhattan, Kans.  
 ADAMS, FREDERICK, C.E., '19. . . . . Plaza Necaxa, Colonia Cuauhtenac, Mexico, D.F., Mexico.  
 ALLEN, HARRISON SANBORN, M.A., '15. . . . . 442 Farmington Ave., Waterbury, Conn.  
 ALLEN, WM. RAY, M.A., '15. . . . . 525 So. Park Ave., Bloomington, Ind.  
 ALLEN, WYNFRED E., A.M., '04. . . . . Scripps Inst., La Jolla, Cal.  
 ANDERSEN, EMMA N., '16. . . . . Station A, Lincoln, Nebr.  
 ANDRAS, J. C., B.A., '12. . . . . 540 S. Main St., Manchester, Ill.  
 ARNOLD, FRANK, '13. . . . . 408 House Building, Pittsburg, Pa.  
 ARNOLD, L. P., O.D., '20. . . . . Vulcans Temple, Carlisle, Ark.  
 ARNOLD, WM. T., '17. . . . . 21 Park Rd., Wyomissing, Pa.  
 ASHLEY, FRANK M., M.E., '20. . . . . Tribune Building, New York City, N. Y.  
 ATCHISON, MRS. W. S., A.M., '16. . . . . 263 Walnut Ave., Elgin, Ill.  
 ATHERTON, PROF. L. G., A.B., M.S., '12. . . . . State Normal School, Madison, S. D.  
 ATWOOD, H. F., '78. . . . . 16 Seneca Parkway, Rochester, N. Y.  
 BALDWIN, HERBERT B., '13. . . . . 927 Broad Street, Newark, N. J.  
 BARKER, FRANKLIN D., Ph.D. '03. . . . . University of Nebraska, Lincoln, Neb.  
 BARRE, H. W., B.Sc., M.A., '12. . . . . Clemson College, S. C.  
 BASS, C. C., M.D., '13. . . . . 3515 Prytania Street, New Orleans, La.  
 BAUSCH, EDWARD, '78. . . . . 179 N. St. Paul St., Rochester, N. Y.  
 BAUSCH, WILLIAM, '88. . . . . St. Paul St., Rochester, N. Y.  
 BEAN, A. M., M.A., '15. . . . . 2811 Benvenue Ave., Berkeley, Cal.  
 BECK, WILLIAM A., M.Sc., '16. . . . . St. Mary College, Dayton, Ohio  
 BENNEHOFF, J. D., M.S., '13. . . . . Alfred College, Alfred, N. Y.  
 BETTS, JOHN B., '11. . . . . 111 Market St., Camden, N. J.  
 BIRGE, PROF. E. A., Sc.D., LL.D., '99. . . . . 744 Langdon St., Madison, Wis.  
 BLACK, J. H., M.D., '12. . . . . 530 Wilson Bldg., Dallas, Texas  
 BOOTH, MARY A., F.R.M.S., F.R.P.S., '82. . . . . 60 Dartmouth St., Springfield, Mass.  
 BOYER, C. S., A.M., '92. . . . . 6140 Columbia Ave., Philadelphia, Pa.  
 BROOKOVER, CHAS., A.B., M.S., '05. . . . . Univ. of Louisville, Louisville, Ky.  
 BROWN, ALICE L., '19. . . . . Kans. St. Ag. Col., Manhattan, Kans.  
 BROWN, F. R., A.B., '12. . . . . William Nast College, Kiukiang, China  
 BRUNQUIST, E. H., A.M., '19. . . . . Dept. Zoology, Northwestern Univ., Evanston, Ill.  
 BRUUN, CHARLES A., LL.B., '16. . . . . 314 Reliance Bldg., Kansas City, Mo.  
 BRYANT, PROF. EARL R., A.M., '10. . . . . Muskingum College, New Concord, O.  
 BULL, JAMES EDGAR, ESQ., '92. . . . . 141 Broadway, New York City  
 BULLITT, PROF. J. B., M.A., M.D., '12. . . . . Chapel Hill, N. C.  
 BUNKER, GEO. C., B.S., '17. . . . . Gatun, Canal Zone  
 BUSWELL, A. M., M.A., '16. . . . . Univ. of Ill., Urbana, Ill.  
 CABALLERO, PROF. GUSTAV A., '16. . . . . Fordham Univ., New York City  
 CARLSON, C. O., A.B., '13. . . . . Doane College, Crete, Nebr.  
 CARTER, PROF. CHARLES, '11. . . . . Parsons College, Fairfield, Ia.  
 CARTER, JOHN E., '86. . . . . 5356 Knox St., Germantown, Philadelphia, Pa.  
 CASHEN, DOROTHY, A.M., '19. . . . .  
 . . . . . Dept. Botany, Kans. State Agr. College, Manhattan, Kans.

- CHESTER, WAYLAND MORGAN, M.A., '15..... Colgate University, Hamilton, N. Y.  
 CHICKERING, A. M., A.M., '16..... Albion, Mich.  
 CLARK, GEORGE EDW., M.D., '96..... Towson, Md.  
 CLARK, HOWARD W., A.M., '12..... Fairport, Iowa  
 CLEMENTS, MRS. F. E., Ph.D., '03..... Tucson, Ariz.  
 CLOVIS, MABLE C., M.D., '20..... Ohio Valley General Hospital, Wheeling, West Va.  
 COBB, N. A., Ph.D., '14..... Falls Church, Va.  
 COGHILL, PROF. GEORGE E., Ph.D., '11..... R.F.D. 9, Lawrence, Kans.  
 COLTON, HAROLD S., Ph.D., '11..... Zoological Lab., Univ. of Pa., Philadelphia  
 CONE, ALBERT, '12..... Editorial Staff, "*Lumberman*," Chicago, Ill.  
 CONGER, ALLEN C., M.A., '15..... P.O. Box 663, East Lansing, Mich.  
 CONLON, JAMES J., Ph.D., '14..... 717 Hyde St., San Francisco, Cal.  
 COOPER, ARTHUR R., A.M., Ph.D., '16..... College of Medicine, Univ. Ill., Chicago, Ill.  
 CORNELL UNIV. LIBRARY (PROF. S. H. GAGE)..... Ithaca, N. Y.  
 CORT, W. W., Ph.D., '11.....  
 ..... J.H.U. School of Hygiene, 310 W. Monument St., Baltimore, Md.  
 COTT, GEORGE F., '11..... 1001 Main St., Buffalo, N. Y.  
 COVEY, GEORGE W., '11..... College View, Nebr.  
 DARBAKER, LEASURE KLINE, Ph.D., M.D., '11.....  
 ..... 7025 Hamilton Ave., Homewood Sta., Pittsburg, Pa.  
 DAVIS, PROF. H. S., Ph.D., '12..... University of Florida, Gainesville, Fla.  
 DEERE, EMIL OLAF, A.M., S.M., '13..... Bethany College, Lindsborg, Kans.  
 DEMETER, THEODORE E., '19..... P.O. Box 313, Scottsdale, Pa.  
 DE PUY, PERCY LEROY, B.S., '19..... 1725 Leavenworth St., Manhattan, Kans.  
 DEWITT, CHARLES H., M.S., '11..... 355 College Ave., Valparaiso, Ind.  
 DISBROW, WILLIAM S., M.D., Ph.G., '01..... 151 Orchard St., Newark, N. J.  
 DODGE, CARROLL W., Ph.D., '14..... Brown Univ., Providence, R. I.  
 DOLBEY, EDWARD P., '06..... 3613 Woodland Ave., Philadelphia, Pa.  
 DOUBLEDAY, ARTHUR W., M.D., '16..... 220 Marlborough St., Boston, Mass.  
 DRESCHER, W. E., '87..... Care Bausch & Lomb Opt. Co., Rochester, N. Y.  
 DUNCAN, PROF. F. N., Ph.D., '16..... So. Methodist Univ., Dallas, Tex.  
 EDDY, MILTON W., '11..... U. S. Ammonium Plant, Perryville, Md.  
 EDDY, SAMUEL A., '15..... Tower Hill, Ill.  
 EDMONDSON, CHARLES H., Ph.D., '15..... College of Hawaii, Honolulu  
 EGGLESTON, H. R., M.A., '13..... Marietta College, Marietta, Ohio  
 EIGENMANN, PROF. C. H., '95..... 630 Atwater Ave., Bloomington, Ind.  
 ELLIOTT, FRANK R., M.A., '15..... 324 Kinsey St., Richmond, Ind.  
 ELLIS, PROF. M. M., Ph.D., '12..... Dept. Physiol., University of Mo., Columbia, Mo.  
 ELLIS, CARLETON, B.S., '20..... 92 Greenwood Ave., Montclair, N. J.  
 ELMORE, PROF. C. J., '19..... Grand Island College, Grand Island, Nebr.  
 ELROD, PROF. MORTON J., M.A., M.S., '98..... University of Montana, Missoula, Mont.  
 ENBURG, J. M., '20..... 5207 Baltimore St., Philadelphia, Pa.  
 ESSENBERG, MRS. CHRISTINE, M.S., '16..... Scripps Institute, La Jolla, Cal.  
 ESTERLY, CALVIN O., '15..... Occidental College, Los Angeles, Cal.

- EYRE, JOHN W. H., M.D., M.S., F.R.M.S., '99..... Guy's Hospital, London, S. E., England
- FARMER, ERMIE I., A.B., '19..... Beloit College, Beloit, Wis.
- FATTIG, PROF. P. W., B.S., M.S., '12..... P.O. Box 315, Gainesville, Fla.
- FELLOWS, CHAS. S., F.R.M.S., '83..... 107 Cham. of Comm., Minneapolis, Minn.
- FERNANDEZ, FR. MANUEL, B.S., '16..... San Juan de Latran College, Manila, P. I.
- FINDLAY, MERLIN C., A.M., '15..... Park College, Parkville, Mo.
- FITZ-RANDOLPH, RAYMOND B., F.R.M.S., '11..... State Laboratory of Hygiene, Trenton, N. J.
- FOOTE, J. S., M.D., '01..... Creighton Medical College, Omaha, Nebr.
- FURNISS, H. W., M.D., Ph.D., '05..... 56 Brazos St., Hartford, Conn.
- GABRIELE, H. J., '16..... 2659 California St., San Francisco, Cal.
- GAGE, PROF. SIMON H., B.S., '82..... 4 South Ave., Ithaca, N. Y.
- GALLOWAY, PROF. T. W., A. M., Ph.D., '01..... 105 West 40th St., New York, N. Y.
- GILBERT, E. M., Ph.D., '19..... Biology Building, U. of Wis., Madison, Wis.
- GOLDSMITH, G. W., B.A., '13..... Lafayette, La.
- GOWEN, FRANCIS H., '14..... R. D. 1, Box 14, Exeter, N. H.
- GRAFF, JOHN H., '19..... Research Dept., Brown Company, Berlin, N. H.
- GRAHAM, CHARLES W., M.E., '11..... 447 W. 14th St., New York City
- GRAHAM, JOHN YOUNG, Ph.D., '14..... University, Alabama
- GRAVELLE, P. O., '19..... 114 Prospect St., South Orange, N. J.
- GRIFFIN, LAWRENCE E., '13..... Reed College, Portland, Ore.
- GROSS, F. O., M.D., '19..... 1816 Erie Ave., Philadelphia, Pa.
- GUBERLET, JOHN E., Ph.D., '11..... A. & M. College, Stillwater, Okla.
- GUYER, MICHAEL F., Ph.D., '11..... University of Wisconsin, Madison, Wis.
- HAGELSTEIN, ROBERT, '16..... 165 Cleveland Ave., Mineola, Nassau Co., N. Y.
- HAGUE, FLORENCE, A. M., '16..... Nat. Hist. Bldg., Urbana, Ill.
- HALL, F. GREGORY, B.A., '17..... Biology Building, Univ. of Wis., Madison, Wis.
- HANCE, ROBERT T., B.A., '13..... Zool. Lab., U. of Pa., Philadelphia, Pa.
- HANKINSON, T. L., '03..... New York College of Forestry, Syracuse, N. Y.
- HANSEN, JAMES, '15..... St. Johns Univ., Collegeville, Minn.
- HARDY, EUGENE H..... 1230 So. Keystone Ave., Indianapolis, Ind.
- HARMAN, MARY T., '13..... Kansas State Agr. College, Manhattan, Kansas
- HAYES, W. P., M.S., '19..... 319 N. 18th St., Manhattan, Kans.
- HEALD, F. D., Ph.D., '06..... Wash. State College, Pullman, Wash.
- HEATH, ROY FRANKLIN, M.Sc., '18..... P.O. Box 270, Billings, Montana
- HEIMBURGER, HARRY V., A.B., '14..... Fennville, Mich., R.F.D. 2.
- HENDERSON, WILLIAM, '11..... Mellon Inst., Univ. of Pittsburg, Pittsburg, Pa.
- HICKMAN, J. R., A.B., '19..... Bristol, West Virginia
- HILTON, WILLIAM A., Ph.D., '15..... Claremont, Cal.
- HISAW, F. L., M.S., '19..... Kans. State Agr. College, Manhattan, Kans.
- HJORTH, LUDVIC C., '12..... Meadowdale, Snohomish County, Washington
- HOLY CROSS COLLEGE, PROFESSOR OF BIOLOGY..... Worcester, Mass.
- HOPKINS, FRANK B., B.S., '19..... North Salem, Ind.
- HOPKINSON, D., M.D., '20..... 1008 Third St., Milwaukee, Wis.
- HOSKINS, WM. '79..... 49 6th St., LaGrange, Ill.

- HOTTES, C. F., Ph.D., '20.....Nat. Hist. Bldg., Univ. of Ill., Urbana, Ill.  
 HOWLAND, HENRY R., A.M., '98.....217 Summer St., Buffalo, N. Y.  
 HUBERT, H. E., B.S., '20.....3615 Melpomene St., New Orleans, La.  
 HUDSON, D. V., B.S., '20..... Johns Hopkins Medical School, Baltimore, Md.  
 HUGHES, SALLY P., '15..... Grinnell College, Grinnell, Ia.  
 IVES, FREDERIC E., '02.....1201 Race St., Philadelphia, Pa.  
 JACKSON, F. S., M.D., '19.....McGill University, Montreal, Canada  
 JACOT, A. P., A.B., '19.....No. China Language School, Peking, China  
 JEFFS, PROF. R. E., '11.....Univ. of Okla., Norman, Okla.  
 JENNER, E. A., M.A., '12.....Science Hall, Indianola, Ia.  
 JOHNSON, B. J., '12.....Joplin, Mo., R.F.D. 4-147  
 JOHNSON, FRANK S., M.D., '93.....Hotel Darby, Los Angeles, Cal.  
 JORDAN, PROF. H. E., '12.....University Place, Charlottesville, Va.  
 JUDAY, CHANCEY, '00.....Biology Bldg., U. of Wis., Madison, Wis.  
 JUDD, H. D., Opt.D., '19.....460 W. Philadelphia Ave., Detroit, Mich.  
 KERNALL, MORRIS J., A.M.....Bismarck, No. Dak.  
 KENYAN, W. A., A.B., '20.....Milton, Wis.  
 KINCAID, TREVOR, A.M., '12.....University of Washington, Seattle, Wash.  
 KING, INEZ, B.S., '14.....Phillips Univ., Enid, Okla.  
 KIRSCH, PROF. ALEXANDER M., M.G., '16.....Notre Dame (Univ.), Ind.  
 KLINEDINST, HERMAN, '19.....836 So. George St., York, Pa.  
 VON KLEINSMID, PRES. R. B.....Univ. of Arizona, Tucson, Arizona  
 KNIGHT, F. P. H., '11.....1015 Blondeau St., Keokuk, Ia.  
 KOFOID, CHARLES A., Ph.D., '99. University of California, 2616 Etna St., Berkeley, Cal.  
 KOSTER, W. J., M.A., '20.....Dept. Zoology, Ohio State Univ., Columbus, Ohio  
 KOTZ, A. L., M.D., '91.....32 S. Fourth St., Easton, Pa.  
 KRECKER, FREDERIC H., Ph.D., '15.....Ohio State University, Columbus, Ohio  
 KUDO, R., Ph.D., '20.....Dept. Zoology, Univ. of Ill., Urbana, Ill.  
 LAMBERT, C. A., '12...Bank of New South Wales, Warwick, Queensland, Australia  
 LARUE, GEORGE R., Ph.D., '11.....University of Michigan, Ann Arbor, Mich.  
 LATHAM, MISS V. A., M.D., D.D.S., F.R.M.S., '88.....  
 .....1644 Morse Ave., Rogers Park, Chicago, Ill.  
 LATIMER, HOMER B., M.A., '11.....Univ. of Minn., Minneapolis, Minn.  
 LEWIS, IVEY FOREMAN, Ph.D., '18.....University, Va.  
 LEWIS, MRS. KATHERINE B., '89.....Bellwood Farms, Geneva, N. Y.  
 LITTERER, WM., A.M., M.D., '06.....Nashville, Tenn.  
 LOMB, ADOLPH, '92.....289 Westminster Road, Rochester, N. Y.  
 LONGFELLOW, ROBERT CAPLES, M.S., M.D., '11.....1611 22nd St., Toledo, Ohio  
 LOWDEN, HUGH B., '16.....1312 York St., Denver, Colo.  
 LOWREY, ELEANOR C., '19.....1826 D. St., Lincoln, Nebr.  
 LYON, HOWARD N., M.D., '84.....828 N. Wheaton Ave., Wheaton, Ill.  
 MACGILLIVRAY, ALEXANDER D., '12.....603 W. Michigan Avenue, Urbana, Ill.  
 MACK, MARGARET ELIZABETH, A.M., '13.....Univ. of Nevada, Reno, Nev.  
 MAGATH, T. B., M.S., Ph.D., '13.....Mayo Clinic, Rochester, Minn.  
 MANCHEÉ, E. D., '19.....200 Glen Cairn Ave., Toronto, Can.

- MARR, GEORGE HENRY, M.E., '11.....94 Silver St., Waterville, Maine  
MARSHALL, COLLINS, M.D., '96.....2507 Penn. Ave., Washington, D. C.  
MARSHALL, RUTH, Ph.D., '07.....Rockford College, Rockford, Ill.  
MARSHALL, W. S., Ph.D., '12.....139 E. Gilman St., Madison, Wis.  
MARTLAND, HARRISON S., A.B., M.D., '14.....1138 Broad St., Newark, N. J.  
MATHER, E., M.D., Ph.D., '02.....228 Gratiot Ave., Mt. Clemens, Mich.  
MAY, HENRY GUSTAV, Ph.D., '15.....  
.....Agr. Exp. Sta., Rhode Island State College, Kingston, R. I.  
MAYHEW, ROY L., B.S., '15.....Wesleyan College, Warrentown, Mo.  
MAYWALD, FREDERICK J., '02.....222 Grand Ave., Nutley, N. J.  
MC COLLOCH, J. W., B.S., '19.....Kans. Agr. Exp. Sta., Manhattan, Kans.  
MCGREERY, GEO. L., '13.....110 Nevada St., Carson City, Nev.  
MCCULLOCH, IRENE, Ph.D., '20.....  
.....Dept. Biology, Sophie Newcomb Memorial College, Tulane Univ., New Orleans, La.  
MCEWAN, A., '15.....Fifth Ave. Guarantee Building, 522 Fifth Ave., New York, N. Y.  
MCKAY, JOSEPH, '84.....259 Eighth St., Troy, N. Y.  
MCKEEVER, FRED L., F.R.M.S., '06.....P.O. Box 210, Penticton, B. C.  
MCLAUGHLIN, ALVAH R., M.A., '15.....Pullman, Wash.  
MCWILLIAMS, JOHN, '14.....Lock Box 62, Greenwich, Conn.  
MERCER, A. CLIFFORD, M.D., F.R.M.S., '82.....324 Montgomery St., Syracuse, N. Y.  
MERCER, W. F., Ph.D., '99.....200 E. State St., Athens, Ohio  
METCALF, PROF. ZENO P., B.A., '12.....Col. A. & M. A., W. Raleigh, N. C.  
MILLER, CHARLES H., '11.....Med. School, Johns Hopkins U., Baltimore, Md.  
MILLER, JOHN A., Ph.D., F.R.M.S., '89.....44 Lewis Block, Buffalo, N. Y.  
MOCKETT, J. H., SR., '01.....2302 Sumner St., Lincoln, Nebr.  
MOELLER, H., M.D., '07.....341 West 57th St., New York, N. Y.  
MONTGOMERY, CHARLES S., '19.....420 Riverside Drive, New York, N. Y.  
MOODY, ROBERT P., M.D., '07.....Hearst Anat. Lab., U. of Cal., Berkeley, Cal.  
MORGAN, ANNA HAVEN, Ph.D., '16.....Mt. Holyoke Coll., So. Hadley, Mass.  
MUTKOWSKI, R. A., Ph.D., '19.....Univ. of Idaho, Moscow, Idaho  
MYERS, FRANK J., '13.....15 S. Cornwall Place, Ventnor City, N. J.  
NESBIT, ROBT. A., '16.....Biology Building, Univ. of Wis., Madison, Wis.  
NORRIS, PROF. HARRY WALDO, '11.....816 East St., Grinnell, Iowa  
NORTON, CHARLES E., M.D., '11.....118 Lisbon St., Lewiston, Me.  
OGLEVEE, C.S., B.S., Sc.D., '12.....1006 N. Union St., Lincoln, Ill.  
OSBORN, PROF. HERBERT, M.S., '05.....Ohio State University, Columbus, O.  
OTT, HARVEY N., A.M., '03.....Spencer Lens Co., Buffalo, N. Y.  
PAGE, IRVINE HEINLY, '17.....810 University, Ithaca, N. Y.  
PATRICK, FRANK, Ph.D., '91.....1500 Linwood Blvd., Kansas City, Mo.  
PEASE, FRED N., '87.....P.O. Box 503, Altoona, Pa.  
PENNOCK, EDWARD, '79.....3609 Woodland Ave., Philadelphia, Pa.  
PETERSON, NIELS FREDERICK, '11.....Plainview, Nebr.  
PICKETT, F. L., Ph.D. '20. Dept. Botany, State College of Washington, Pullman, Wash.  
PIATT, H. S., Ph.D., '19.....561 W. 141st St., New York, N. Y.  
PITT, EDWARD, '11.....Brandhock, Gerrard's Cross, Bucks, England

- PLOUGH, HAROLD H., A.M., '16. . . . . Dept. Biology, Amherst Coll., Amherst, Mass.  
 POHL, JOHN C., JR., '17. . . . . 204 N. 10th St., Easton, Pa.  
 POOL, RAYMOND J., Ph.D., '15. . . . . Station A, Lincoln, Nebr.  
 POUND, ROSCOE, A.M., Ph.D., '98. . . . . Harvard Law School, Cambridge, Mass.  
 POWERS, E. B., A.B., '12. . . . . Univ. of Nebraska, Lincoln, Nebr.  
 PRAEGER, WM. E., M.S., '14. . . . . 421 Douglas Ave., Kalamazoo, Mich.  
 PROCTER, WILLIAM, Ph.B., '19. . . . . 149 Broadway, New York, N. Y.  
 PURDY, WILLIAM C., M.Sc., '16. . . . . 3rd & Kilgour Sts., Cincinnati, Ohio  
 QUILLIAN, MARVIN C., A.M., '13. . . . . Wesleyan Col., Macon, Ga.  
 RANKIN, WALTER M., '13. . . . . Princeton University, Princeton, N. J.  
 RANSOM, BRAYTON H., '99. . . . . U. S. Bureau of Animal Industry, Washington, D. C.  
 RECTOR, FRANK LESLIE, M.D., '11. . . . . 227 Fulton St., New York City  
 REESE, PROF. ALBERT M., Ph.D. (Hop.) '05. . . . . W. Va. Univ., Morgantown, W. Va.  
 RICHARDS, AUTE, Ph.D., '12. . . . . Dept. Zoology, Univ. of Oklahoma, Norman, Oklahoma  
 RILEY, C. F. CURTIS, M.S., '15. . . . . Univ. of Manitoba, Winnipeg, Can.  
 ROBERTS, E. WILLIS, '11. . . . . 65 Rose St., Battle Creek, Mich.  
 ROBERTS, H. L., '14. . . . . State Normal School, Cape Girardeau, Mo.  
 ROBERTS, J. M., '11. . . . . 460 E. Ohio St., Chicago, Ill.  
 ROBINSON, J. E., M.D., '15. . . . . Box 405, Temple, Texas  
 ROE, G. C., A.B., '17. . . . . 113 R Street, N. W., Washington, D. C.  
 ROGERS, WALTER E., '11. . . . . Lawrence College, Appleton, Wis.  
 ROSS, LUTHER SHERMAN, S.M., '11. . . . . 1308 27th St., Des Moines, Ia.  
 RUSH, R. C., M.D., '12. . . . . Hudson, Ohio  
 RYE, L. E., A.B., '20. . . . . 62 Laureston St., Brockton, Mass.  
 SCHEAR, E. W., E., '19. . . . . 107 West Park, Westerville, Ohio  
 SCOTT, HELEN M., A.M., '19. . . . . 26 Whites Place, Bloomington, Ill.  
 SCOTT, J. W., '12. . . . . Univ. of Wyo., Laramie, Wyo.  
 SHANTZ, H. L., Ph.D., '04. . . . . Bureau Plant Industry, Washington, D. C.  
 SHEARER, J. B., '88. . . . . 809 Adams St., Bay City, Mich.  
 SHEERAR, LEONARD F., '19. . . . . 158 W. State St., Wellsville, N. Y.  
 SHELDON, JOHN LEWIS, Ph.D., '15. . . . . Morgantown, W. Va.  
 SHULTZ, CHAS. S., '82. . . . . Box 135, Hoboken, N. J.  
 SIMON, C. L., M.D., '20. . . . . 1734 Linden Ave., Baltimore, Md.  
 SISTER MAGNA, O.S.B., M.A., '16. . . . . St. Benedict's College, St. Joseph, Minn.  
 SITLER, IDA, B.S., '16. . . . . Lake Erie College, Painesville, Ohio  
 SMITH, PROF. FRANK, A.M., '12. . . . . 1005 W. California Ave., Urbana, Ill.  
 SMITH, GILBERT MORGAN, Ph.D., '15. . . . . 1606 Hoyt St., Madison, Wis.  
 SMITH, J. C., '96. . . . . 131 Carondelet St., New Orleans, La.  
 SOAR, C. D., F.R.M.S., '07. . . . . 37 Dryburgh Road, Putney, London, S. W., England  
 SPAULDING, M. H., A.M., '13. . . . . 720 W. Babcock St., Bozeman, Mont.  
 SPURGEON, CHARLES H., A.M., '13. . . . . Sheridan, Ind.  
 STEVENS, PROF. H. E., M.S., '12. . . . . Agricultural Experiment Station, Gainesville, Fla.  
 STEWART, THOMAS S., M.D., '17. . . . . 18th and Spruce Sts., Philadelphia, Pa.  
 STONE, G. E. . . . . 1725 LeRoy Ave., Berkeley, Cal.  
 STONE, GRACE A., A.M., '16. . . . . Teacher's College, New York City

- STUNKARD, HORACE W., Ph.D., '13. . . . . New York Univ., Univ. Heights, New York City  
 SUMMERS, PROF. H. E., '86. . . . . Ames, Iowa  
 SWEZY, OLIVE, Ph.D., '15. . . . . East Hall, University of California, Berkeley, Cal.  
 SWINGLE, PROF. LEROY D., '06. . . . . Univ. of Utah, Salt Lake City, Utah  
 TAYLOR, W. R., Ph.D., '20. . . . . 1340 N. 12th St., Philadelphia, Pa.  
 TERRELL, TRUMAN C., M.D., '16. . . . . 1301 Eighth St., Fort Worth, Tex.  
 THOMAS, ARTHUR H., '99. . . . . 12th and Walnut Sts., Philadelphia, Pa.  
 TIMMINS, GEORGE, '96. . . . . 1410 E. Genesee St., Syracuse, N. Y.  
 TINSLEY, RANDOLPH WORD, B.S., '15. . . . . Georgetown, Texas  
 TODD, JAMES C., B.A., M.D., '11. . . . . Boulder, Colo.  
 TUCKER, O. C., Ph.G., '19. . . . . 898 S. Clarkson St., Denver, Colo.  
 TUERS, R. V., '20. . . . . Dept. Biology, N. Y. Univ., New York, N. Y.  
 VAN CLEAVE, HARLEY J., Ph.D., '11. . . . . 300 N. H. Bldg., Urbana, Ill.  
 VAN COTT, HARVEY A., '17. . . . . 11 West 45th St., Bayonne, N.J.  
 WAITE, FREDERICK C., Ph.D., '11. . . . .  
 . . . . . Medical Department, Western Reserve Univ., Cleveland, Ohio  
 WALKER, ELDA R., Ph.D., '07. . . . . University of Nebraska, Lincoln, Neb.  
 WALKER, LEVA BELLE, '13. . . . . Station A, Lincoln, Nebr.  
 WALTON, A. S., '19. . . . . 2819 W. Girard Ave., Philadelphia, Pa.  
 WARBRICK, J. C., '12. . . . . 306 E. 43rd St., Chicago, Ill.  
 WARD, HENRY B., A.M., Ph.D., '87. . . . . University of Illinois, Urbana, Ill.  
 WARNER, E. A., M.D., Ph.G., '17. . . . . Nevada, Iowa  
 WARREN, D. T., '18. . . . . 1805 Patterson Ave., Roanoke, Va.  
 WARREN, S., A.B., '19. . . . . 28 Hawthorne Rd., Brookline, Mass.  
 WATERWORTH, W. A., '15. . . . . 286 Lambton Quay, Wellington, N. Zealand  
 WEESE, A. O., '14. . . . . Univ. of N. M., Albuquerque, N. M.  
 WELCH, PAUL S., Ph.D., '11. . . . . Univ. of Michigan, Ann Arbor, Mich.  
 WELSH, LIEUT. B. C., '14. . . . . 24 Upper Mountain Ave., Montclair, N. J.  
 WESTON, WILLIAM H., JR., Ph.D., '16. . . . . Fed. Hort. Board, Washington, D. C.  
 WHEELER, E. J., Ph. D., '00. . . . . 79 Chapel St., Albany, N. Y.  
 WHELPLEY, H. M., M.D., Ph.G., '09. . . . . 2342 Albion Pl., St. Louis, Mo.  
 WHITING, WILLIAM J., '15. . . U.S. Naval Gun Factory, Optical Shop, Rochester, N. Y.  
 WILLIAMSON, WM., F.R.S.E., '07. . . . . 79 Morningside Drive, Edinburg, Scotland  
 WILSON, CHARLES EARL, A.M., '15. . . . . R. R. 1, Box 137, Brazil, Indiana  
 WILSON, RAY W., '18. . . . . 2051 Seneca, Buffalo, N. Y.  
 WISMER, NETTIE M., '19. . . . . Kans. St. Agri. Coll., Manhattan, Kans.  
 WOODSEDALEK, JERRY EDWARD, Ph.D., '15. . . . . Moscow, Idaho  
 WOLCOTT, ROBERT HENRY, A.M., M.D., '98. . . . . Univ. of Nebraska, Lincoln, Neb.  
 WOOD, ARTHUR KING, '14. . . . . 61 E. 65th St., New York, N. Y.  
 YOSHIDA, SADA0, '20. . . Pathological Department, Osaka Medical College, Osaka, Japan  
 ZOOK, DAVID L., B.S., '05. . . . . 965 Holliston, Ave., Pasadena, Cal.

## SUBSCRIBERS

- ACADEMY OF NATURAL SCIENCES. . . . . Logan Square, Philadelphia, Pa.  
 AGRICULTURAL EXP. STA. LIBRARY. . . . . Knoxville, Tennessee  
 ALFRED COLLEGE LIBRARY. . . . . Alfred, N. Y.

- AMERICAN MUSEUM OF NATURAL HISTORY.....  
 ..... 77th St. and Central Park, New York, N. Y.
- AMHERST COLLEGE LIBRARY..... Amherst, Mass.
- BABCOCK SCIENTIFIC LIBRARY..... Plainfield, N. J.
- BALL, MISS F. D..... Junior College, Grand Rapids, Mich.
- BELOIT COLLEGE LIBRARY..... Beloit, Wis.
- BIBLIOTHECA DE LA FACULTAD DE MEDICINE..... Montevideo, Uruguay
- BICKFORD BIOLOGICAL LIBRARY..... Bates Col., Lewiston, Me.
- BOSTON PUBLIC LIBRARY..... Boston, Mass.
- BOSTON SOCIETY OF NATURAL HISTORY..... Berkeley St., Boston, Mass.
- BROWN UNIVERSITY BIOLOGICAL LIBRARY..... Providence, R. I.
- BUREAU OF SCIENCE LIBRARY..... Manila, P. I.
- CARNEGIE FREE LIBRARY..... Allegheny, Pa.
- CARNEGIE LIBRARY..... Periodical Div., Schenley Park, Pittsburg, Pa.
- CHEMISTS' CLUB LIBRARY, A. H. ELLIOTT..... 52 East 41st St., New York City
- CHICAGO UNIVERSITY LIBRARY..... Chicago, Ill.
- CLARKE, T. J..... 417 Third Ave., Brooklyn, N. Y.
- CLEVELAND PUBLIC LIBRARY..... Cleveland, Ohio
- COBURN LIBRARY OF COLORADO COLLEGE..... Colorado Springs, Colo.
- COLBY COLLEGE LIBRARY..... Waterville, Me.
- COLLEGE OF CITY OF NEW YORK LIBRARY (Biological Laboratories).....  
 ..... St. Nicolas Terrace and 139th St., New York City
- COLLEGE OF PHYSICIANS LIBRARY..... 19 S. 22nd St., Philadelphia, Pa.
- COLORADO AGRICULTURAL COLLEGE LIBRARY..... Fort Collins, Colo.
- COLORADO STATE NORMAL LIBRARY..... Greeley, Colo.
- COLUMBIA UNIVERSITY LIBRARY..... New York, N. Y.
- CORNISH BROTHERS..... 39 New Street, Birmingham, England
- DECATUR TEACHERS' PEDAGOGICAL LIBRARY..... Public Schools, Decatur, Ill.
- DE PAUW UNIV., ALFRED DICKEY BIOL. LIBRARY..... Greencastle, Ind.
- DEPT. AGRIC. LIBRARY, UNIV. FARM..... St. Paul, Minn.
- DETROIT PUBLIC LIBRARY..... Detroit, Mich.
- DOANE COLLEGE LIBRARY..... Crete, Nebraska
- DRAKE UNIVERSITY LIBRARY..... Des Moines, Ia.
- DULAU & Co..... 34-36 Margaret St., Cavendish Square, London, England
- EARLHAM COLLEGE LIBRARY..... Earlham P.O., Richmond, Indiana
- FARGO COLLEGE LIBRARY..... Fargo, N. Dak.
- FORDHAM UNIVERSITY LIBRARY..... Fordham, N. Y.
- FRANKLIN & MARSHALL COLLEGE LIBRARY..... Lancaster, Pa.
- GEORGE WASHINGTON UNIVERSITY LIBRARY..... Washington, D. C.
- GROSVENOR LIBRARY..... Buffalo, N. Y.
- HAMAKER, J. J..... Randolph-Macon Woman's College, Lynchburg, Va.
- ILLINOIS NAT. HIST. SURVEY LIBRARY..... Urbana, Ill.
- INDIANA STATE LIBRARY..... Indianapolis, Ind.
- IOWA STATE TEACHERS' COLLEGE LIBRARY..... Cedar Falls, Iowa
- JAMES MILLIKIN UNIVERSITY LIBRARY..... Decatur, Ill.

JOHN CRERAR LIBRARY.....	Chicago, Ill.
JOHNS HOPKINS UNIV. LIBRARY.....	Baltimore, Md.
KANSAS CITY PUBLIC LIBRARY.....	Kansas City, Mo.
KANSAS STATE AGR'L COLLEGE LIBRARY.....	Manhattan, Kans.
KNOX COLLEGE LIBRARY.....	Galesburg, Ill.
LELAND STANFORD JR., UNIV. LIBRARY.....	Stanford, Cal.
L'INSTITUTO OSWALDO CRUZ (CHEZ MR. A. SCHLACHTER).....	46 Rue Madame, Paris, France
LOS ANGELES PUBLIC LIBRARY.....	Los Angeles, Cal.
MARIETTA COLLEGE LIBRARY.....	Marietta, Ohio
MASS. AGRICULTURAL COLLEGE LIBRARY.....	Amherst, Mass.
MCGILL UNIVERSITY LIBRARY.....	Montreal, Can.
MICHIGAN STATE NORMAL COLLEGE LIBRARY.....	Ypsilanti, Mich.
MILWAUKEE PUBLIC LIBRARY.....	Milwaukee, Wis.
MINNESOTA UNIV., FARM LIBRARY.....	St. Paul, Minn.
MISSOURI BOTANICAL GARDEN.....	St. Louis, Mo.
MISSOURI VALLEY COLLEGE LIBRARY.....	Marshall, Mo.
MONTANA STATE COLLEGE OF AGRICULTURE LIBRARY.....	Bozeman, Mont.
MOUNT HOLYOKE COLLEGE LIBRARY.....	South Hadley, Mass.
MUSEUM COMPARATIVE ZOOLOGY (HARVARD).....	Cambridge, Mass.
MUSKINGUM COLLEGE LIBRARY.....	New Concord, Ohio
NEW HAMPSHIRE STATE LIBRARY.....	Concord, N. H.
NEW YORK ACADEMY OF MEDICINE.....	17 W. Forty-third St., New York City
NEW YORK MICROSCOPICAL SOCIETY.....	463 West St., New York City
NEW YORK PUBLIC LIBRARY.....	476 Fifth Ave., New York City
NEW YORK STATE LIBRARY.....	Serial Section, Albany, N. Y.
NORTHWESTERN COLLEGE LIBRARY.....	Naperville, Ill.
OBERLIN COLLEGE LIBRARY.....	Oberlin, Ohio
OHIO STATE UNIVERSITY LIBRARY.....	Columbus, Ohio
OHIO WESLEYAN UNIVERSITY LIBRARY.....	Delaware, Ohio
OMAHA PUBLIC LIBRARY.....	Omaha, Nebr.
OREGON AGRICULTURAL COLLEGE LIBRARY.....	Corvallis, Ore.
OTTEROOM, DR. ANDREW.....	Fargo Clinic, Fargo, N. Dak.
PAMMEL, PROF. L. H.....	Department of Botany, Iowa State College, Ames, Ia.
PRINCETON UNIVERSITY LIBRARY.....	Princeton, N. J.
PUGET SOUND BIOL. STATION LIBRARY.....	Univ. of Washington, Seattle, Wash.
PURDUE UNIVERSITY LIBRARY.....	Lafayette, Ind.
QUEEN'S UNIVERSITY LIBRARY.....	Kingston, Ontario
RICE INSTITUTE LIBRARY.....	Houston, Texas
ROCKFORD COLLEGE LIBRARY.....	Rockford, Ill.
RUTGERS COLLEGE LIBRARY.....	New Brunswick, N. J.
SCRIPPS INSTITUTION LIBRARY.....	La Jolla, Cal.
SMITH COLLEGE LIBRARY.....	Northampton, Mass.
SOUTH DAKOTA COLL. AGR. AND MECH. ARTS LIBRARY.....	Brookings, S. D.
SYRACUSE PUBLIC LIBRARY.....	Syracuse, N. Y.

TENNESSEE AGR. EXPERIMENT STATION.....	Knoxville, Tenn.
TEXAS CHRISTIAN UNIVERSITY LIBRARY.....	Fort Worth, Tex.
TRINITY COLLEGE LIBRARY.....	Durham, N. C.
TRINITY COLLEGE LIBRARY.....	Washington, D. C.
U. S. DEPT. OF AGRICULTURE LIBRARY.....	Washington, D. C.
UNIVERSITY OF ARIZONA LIBRARY.....	Tucson, Ariz.
UNIVERSITY OF ARK. MEDICAL DEPT. LIBRARY.....	Little Rock, Ark.
UNIVERSITY OF CALIFORNIA LIBRARY.....	Berkeley, Cal.
UNIVERSITY OF IOWA LIBRARY.....	Iowa City, Iowa
UNIVERSITY OF KANSAS LIBRARY.....	Lawrence, Kans.
UNIVERSITY OF MICHIGAN LIBRARY.....	Ann Arbor, Mich.
UNIVERSITY OF MINNESOTA LIBRARY.....	Minneapolis, Minn.
UNIVERSITY OF MISSOURI LIBRARY.....	Columbia, Mo.
UNIVERSITY OF MONTANA LIBRARY.....	Missoula, Mont.
UNIVERSITY OF NEBRASKA LIBRARY.....	Lincoln, Neb.
UNIVERSITY OF OREGON LIBRARY.....	Eugene, Oregon
UNIVERSITY OF PENNSYLVANIA LIBRARY.....	Philadelphia, Pa.
UNIVERSITY OF PORTO RICO LIBRARY.....	Rio Pedras, P. R.
UNIVERSITY OF THE SOUTH LIBRARY.....	Sewanee, Tenn.
UNIVERSITY OF SOUTHERN CALIFORNIA LIBRARY.....	Los Angeles, Cal.
UNIVERSITY OF TEXAS LIBRARY.....	Austin, Texas
UNIVERSITY OF TORONTO LIBRARY.....	Toronto, Canada
UNIVERSITY OF UTAH LIBRARY.....	Salt Lake City, Utah
UNIVERSITY OF VIRGINIA LIBRARY.....	Charlottesville, Virginia
UNIVERSITY OF WASHINGTON LIBRARY.....	Seattle, Wash.
UNIVERSITY OF WISCONSIN LIBRARY.....	Madison, Wis.
UNIVERSITY OF WYOMING LIBRARY.....	Laramie, Wyo.
VASSAR COLLEGE LIBRARY.....	Poughkeepsie, N. Y.
WASHINGTON AND LEE BIOLOGICAL DEPT. LIBRARY.....	Lexington, Va.
WASHINGTON STATE COLLEGE LIBRARY.....	Pullman, Wash.
WESLEYAN UNIVERSITY LIBRARY.....	Middletown, Conn.
WESTERN COLLEGE FOR WOMEN LIBRARY.....	Oxford, Ohio
YALE COLLEGE LIBRARY.....	New Haven, Conn.

## INDEX

- A
- Age, Growth and Scale Characters of Mulletts, 199.
- Algae, An Ecological Study of, 51
- Andersen, Emma N. and Walker, Elda R., An Ecological Study of the Algae of some Sandhill Lakes, 51
- B
- Bladder Fluke, from the Frog, 142
- Braun, Micropterygidae, 163
- C
- Cestodarian Parasite, *Glaridacris catostomi* gen. nov., sp. nov., 5
- Cobb, N. A., Micro-technique, 231
- Cooper, A. R., *Glaridacris catostomi* gen. nov., sp. nov.: A Cestodarian Parasite, 5
- Crampton, Origin and Significance of Metamorphosis, 165
- Custodian's Report for the Years 1918 and 1919, 89
- D
- Dark-field Microscopy, Modern, 95
- Devil's Lake, Protozoa of, 167
- E
- Edmondson, C. H., Protozoa of the Devil's Lake Complex, North Dakota, 167
- Enchytraeidae, The Genera of, 25
- Entomological Abstracts, 163
- F
- Filariasis in U. S., 164
- Fluke, from the Frog, 142
- Francis, Filariasis in U. S., 164
- Frog, New Bladder Fluke from, 142
- G
- Gage, S. H., Modern Dark-field Microscopy, 95
- Glaridacris catostomi* gen. nov., sp. nov.: A Cestodarian Parasite, 5
- Growth of the Mulletts, 199
- Guberlet, J. E., A New Bladder Fluke from the Frog, 142
- H
- Henderson, W. F., Report of the Treasurer, 92
- I
- Illustrations, Labeling, 149
- J
- Jacot, A. P., Age, Growth and Scale Characters of the Mulletts, 199.
- L
- Labeling Illustrations, 149
- Leeches, Considered as *Oligochaeta* Modified for a Predatory Life, 86
- M
- Meeting, Minutes of the St. Louis, 89
- Metamorphosis, Origin and Significance of, 165
- Metcalf, Z. P., Labeling Illustrations, 149
- Micropterygidae, 163
- Micro-technique, Methods and Apparatus, 231
- Microscopy, Dark-field, 95
- Minutes of the St. Louis Meeting, 89
- Mugil cephalus*, 199
- Mugil curema*, 199
- Mulletts, Age, Growth and Scale Characters of, 199
- O
- Oligochaeta*, Leeches considered as, 86
- Oligochaeta*, The Genera of the Enchytraeidae, 25
- P
- Patterson, Polyembryony and Sex, 164
- Pflaum, M., Custodian's Report, 89
- Polyembryony and Sex, 164
- Protozoa of the Devil's Lake Complex, North Dakota, 167

## R

- Report of Auditing Committee on Treasurer's Accounts, 92  
 Report of the Treasurer, H. J. Van Cleave, 90  
 Report of the Treasurer, W. F. Henderson, 92

## S

- Sandhill Lakes, An Ecological Study of the Algae of, 51  
 Scale Characters of the Mulletts, 199  
 Sex, Polyembryony and, 164  
 Smith, F., Leeches Considered as Oligochaeta, 86  
 Spencer-Tolles Fund, Custodian's Report, 89  
 St. Louis, Minutes of the Meeting, 89

## T

- Tillyard, Position of Micropterygidae, 163

## V

- Van Cleave, H. J., Report of the Treasurer, 90

## W

- Walker, Elda R., Andersen, Emma N., and, An Ecological Study of the Algae of Some Sandhill Lakes, 51  
 Welch, P. S., Entomological Abstracts, 163  
 Welch, P. S., Genera of the Enchytraeidae (Oligochaeta), 25  
 Welch, P. S., Minutes of the St. Louis Meeting, 89



TRANSACTIONS  
OF THE  
American  
Microscopical Society

ORGANIZED 1878

INCORPORATED 1891

---

PUBLISHED QUARTERLY

BY THE SOCIETY

---

EDITED BY THE SECRETARY

PAUL S. WELCH

ANN ARBOR, MICHIGAN

---

VOLUME XL

NUMBER ONE

---

Entered as Second-class Matter August 43, 1918, at the Post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in Section 1103, of the Act of October 3, 1917, authorized Oct. 21, 1918

---

The Collegiate Press  
GEORGE BANTA PUBLISHING COMPANY  
MENASHA, WISCONSIN  
1921

TABLE OF CONTENTS

FOR VOLUME XL, Number 1, January, 1921

Acanthocephala from the Eel, with one plate, by H. J. VanCleave. . . . .	1
A Brief Study of the Range of Error in Micro-Enumeration, by W. E. Allen. . . . .	14
Department of Methods, Reviews, Abstracts, and Briefer Articles	
Remarks on the Life-history and the Scale Characters of American Mulletts, by C. L. Hubbs. . . . .	26
Spring Migration in the Crayfish, <i>Cambarus argillicola</i> Faxon, by H. Cummins. . .	28
Preparing Collections of the Mollusca for Exhibition and Study, with five figures, by F. C. Baker. . . . .	31
Proceedings of the American Microscopical Society. . . . .	47

1

TRANSACTIONS  
OF  
American Microscopical Society

(Published in Quarterly Instalments)

Vol. XL

JANUARY, 1921

No. 1

ACANTHOCEPHALA FROM THE EEL<sup>1</sup>

BY

H. J. VAN CLEAVE

Introduction

Materials and Acknowledgments

Records of Acanthocephala infesting *Anguilla chrysypa*

Descriptions of Species

*Tanaorhamphus ambiguus* n. sp.

*Neoechinorhynchus cylindricus* (Van C., 1913)

*Echinorhynchus coregoni* Linkins in Van C., 1919

*Echinorhynchus thecatus* Linton, 1891

Uncertain Identifications of Species

*Neoechinorhynchus agilis* (Rud.)

*Koleops anguilla* Lockwood

*Echinorhynchus claviceps* Zeder

*Echinorhynchus globulosus* Rud.

Conclusions

Acanthocephala from *Anguilla vulgaris*

Literature Cited

Explanation of Plate

INTRODUCTION

Because of their migratory habits, eels offer some fascinating problems to investigators interested in the geographical distribution of parasitic organisms. The migrations of most other animals are between places of essentially similar surroundings and for that reason little evidence is available regarding the source of infestations borne by the wanderers. Very little is known of the definite limitations to the distribution of parasites of purely limnetic and terrestrial organisms. On the other hand most of the larger groups of parasitic organisms include species or even genera which are distinctively limited to either a marine or a fresh-water habitat. One of the most interesting and perplexing features of the parasitism of migratory organisms is the fact that they may carry throughout their range mature parasites the larval stages of which have been acquired in a very narrowly restricted locality. Because of the fairly sharp contrast

<sup>1</sup> Contributions from the Zoological Laboratory of the University of Illinois, No. 175.

between the marine and the fresh-water faunas, organisms like the eel which wander from one to the other offer exceptional opportunity for the study of the influence of migration upon the parasitic fauna of the host. In such migrants it becomes possible to recognize the source of a parasitic infestation with much greater certainty than is possible when the migratory movements are between localities of approximately the same physical environment.

The investigations of Zschokke upon the parasites of salmon stand as probably the most comprehensive work in the literature upon the parasites of migratory fishes. In his studies he included observations upon individuals ascending the Rhine and upon others taken from the Baltic Sea. From the former he recorded twenty species of parasitic worms all of which were apparently acquired by the salmon before they left the ocean. The lack of parasites of limnetic origin is obviously correlated with the fact that all evidence seems to indicate that the Rhine salmon commonly refuses to take food after entering the river. In the Baltic salmon, however, Zschokke found some typically fresh-water species of parasites occurring with the ostensibly marine species. Zschokke's work has been summarized by Ward (1910:1160) in the following manner: "The Rhine salmon shelters a purely marine parasitic fauna, while the Baltic salmon reckons many limnetic forms among its parasitic guests. This remarkable condition finds explanation in the continued feeding of the latter type, even in fresh water, and the resulting enrichment of its parasitic fauna with limnetic forms when it returns to the sea."

Unfortunately, the parasites of the eels have not been investigated as thoroughly as have those of the salmon and little effort has been directed toward interpretation of the isolated observations. In the present paper attention will be confined to the Acanthocephala infesting the eel. A number of investigators have compiled lists of the parasites of the European eel, *Anguilla vulgaris*, but to the present time very little material has been available regarding the Acanthocephala of the North American species, *Anguilla chrysypa*.

Any attempt to determine the origin of the parasitic fauna of the eel must take into account both the pelagic and the limnetic periods in the life cycle of an individual. Regarding the source of infestation of the adult eel found in fresh-water, at least four possibilities must be considered:

1. The parasites may have been acquired in the marine habitat and are retained for a longer or shorter period of time after the host enters fresh-water. If the host remains in fresh-water for a period longer than the life of the individual parasites constituting the original infestation, the intestine would ultimately be freed from its parasites;

2. Eggs or larvae of parasites acquired while the host was in the ocean when discharged into fresh-water may succeed in becoming established in the new habitat through their adaptability to entirely new primary hosts;

3. The parasites may represent an entirely new infestation of typically fresh-water species acquired after the loss of any marine species that might have been carried at the time of leaving the ocean;

4. Marine and fresh-water species may become commingled in the body of the same host individual.

A number of the earlier records of Acanthocephala from *Anguilla chrysypa* have contained apparent misidentifications of species. In some of these instances the present writer has had the opportunity of examining the materials and has found that in one instance of a reputed occurrence of a marine European species from *A. chrysypa* the specimens really belong to a typically fresh-water species probably restricted to North America. In the present paper the writer hopes to analyse the earlier records of Acanthocephala from the eel and to add a considerable bulk of original data which has accrued from the study of several important collections.

#### MATERIALS AND ACKNOWLEDGMENTS

In the investigation of this problem I have had the privilege of examining specimens and of incorporating data from a number of important parasite collections. Specimens from the U. S. National Museum collected by E. Linton and by A. Hassall have been especially interesting in this connection. Through the courtesy of C. C. Adams specimens secured by H. S. Pratt and F. C. Baker in the course of investigations by the New York State College of Forestry and the U. S. Bureau of Fisheries upon the fauna of Oneida Lake, New York, have been available for study.

In all the writer has examined four species of Acanthocephala from the intestine of *Anguilla chrysypa*, of which one represents a new species described here for the first time. In attempting to make a correct disposition of the species mentioned in the earlier works the writer has been extremely fortunate in being able to examine collections which have verified surmised incorrect determinations and have made corrections in the identification possible.

For data concerning the Acanthocephala of *Anguilla vulgaris* the writer has found it necessary to utilize the published records of European investigators. In a number of instances, where records are the result of compilation from various sources, there are probably errors in the determination of the species. Through the kindness of Professor K. M. Levander of Finland, I have been permitted to study the Acanthocephala encountered in the course of his investigations upon the food and parasites of the eel.

RECORDS OF ACANTHOCEPHALA INFESTING *Anguilla chrysypha*

The first reference to the occurrence of an acanthocephalan in the American eel is that given by Samuel Lockwood in 1872. In a highly entertaining but superficial manner he described a new genus and new species of acanthocephalan from a cyst in the intestine of an eel ascribing to this new form the name *Koleops anguilla*. Unfortunately his description and his figures, based upon the study of a single living specimen, are so generalized that they possess but little of scientific value. Apparently the acanthocephalan from the hog is the only other species of these worms that had ever come to his attention. Both his specific description and his generic diagnosis consist in simple enumeration of a few points of difference between his specimen and "Echinorhynchus gigas." All of these differences, with the possible exception of the poorly described proboscis, might apply equally to any one of numerous genera belonging to the family Echinorhynchidae. Consequently both the genus and the species stand as unrecognizable.

Joseph Leidy in his extensive pioneer researches on fish parasites has made no mention of ever encountering Acanthocephala in the American eel.

Edwin Linton, in various reports, has given notice of the occurrence of Acanthocephala in this host. He identified (1889 and 1901) as *Echinorhynchus agilis* specimens which I have determined (Van Cleave, 1913) as *Neoechinorhynchus cylindratu*s. Other specimens (1901) he believed to belong to the European species *E. globulosus*. According to his statement these last named specimens were from the collection of the U. S. National Museum. I have examined specimens from this same collection which bear a label indicating that they were identified as *E. globulosus* by Linton and seem to be the same individuals referred to in his paper just cited. A thorough study of these specimens has demonstrated that they represent an undescribed species of the genus *Tanaorhamphus* which is described later in this same paper.

Data concerning the parasites of fishes for A Biological Survey of the Waters of Woods Hole and Vicinity (Sumner, Osborn, and Cole, 1913) were furnished by Linton. Under *Anguilla rostrata* (= *A. chrysypha*) but two species of Acanthocephala were mentioned, namely, "*E. clavaiceps* and *E. globulosus*." The first of these is apparently a renaming of what Linton had earlier identified as "*E. agilis*" and what I have more recently shown to be *Neoechinorhynchus cylindratu*s.

In the investigation of parasites of fishes from the Illinois River eight eels were examined by the writer (Van Cleave, 1919) but no Acanthocephala were discovered. In addition the writer encountered numerous negative

records as the result of the examination of large numbers of eels taken from salt water at Woods Hole, Mass., during the month of August.

#### DESCRIPTIONS OF SPECIES

In the light of the present investigation four valid species of Acanthocephala are to be attributed to *Anguilla chrysypa*. Of these one species is new and two of the remaining ones are reported for the first time from this host.

##### *Tanaorhamphus ambiguus* n. sp.

*Definition.* With the characters of the genus. Type female 7.9 mm. long; maximum diameter 0.67 mm.; diameter of posterior region of body about 0.35 mm. Proboscis cylindrical, 0.77 mm. long and 0.19 mm. in diameter, armed with twenty longitudinal rows of about sixteen hooks each. Hooks near middle of proboscis 41 to 47 $\mu$  long, with the hook at base of each row about 24 $\mu$  long. Proboscis receptacle 0.96 mm. long with wall composed of a single muscular layer. Central nervous ganglion at base of proboscis receptacle. Lemnisci 2.3 mm. long, cylindrical. In the type female each lemniscus contains three giant nuclei. Subcuticular giant nuclei arranged five in mid-dorsal line of body and one in mid-ventral line.

Males have not been studied. Embryos not fully formed in specimens under observation.

Type host: *Anguilla chrysypa*.

Type female collected by Albert Hassall at Baltimore, Maryland, May 30, 1891. This specimen with others of *N. cylindricus* and *E. coregoni* was deposited in the U. S. National Museum under catalog number 6301 of the Hassall Collection. The writer has examined two additional individuals belonging to this same species from the National Museum. These have the catalog number 6471 and Linton's identification as *E. globulosus*. Neither of these was in as good condition for study as the one selected as type. One specimen, apparently a male of this species, had at some time become dried out and for that reason an accurate determination of internal structure is impossible. Linton (1901:435) states that there are three specimens in the National Museum collection, but it is entirely probable that one specimen was entirely lost at the same time that the damage was done to the male. In the reference just cited, Linton states that this species was numerous in collections made August 7 and 28, 1899.

One of the marked peculiarities of the type of this species lies in the number of nuclei within the lemnisci. All other species of Neoechinocephalidae from North America examined by the writer agree in possessing two giant nuclei in one lemniscus and one in the other. The lemnisci of the only other specimen of this species are entirely obscured by the develop-

ing egg masses within the body cavity. In figure 5 the three nuclei of one lemniscus are shown while two of those in the other lemniscus occur in the region where the two organs overlap. Until additional specimens are secured for study it is impossible to determine whether this deviation from the customary number of nuclei is an individual abnormality or a character of this species.

But one other species has been described for the genus *Tanaorhamphus* and it is apparently very sharply restricted in its occurrence, having been found in but one host, the gizzard shad (*Dorosoma cepedianum*) from the Illinois River.

*Neoechinorhynchus cylindratu*s (Van Cleave, 1913)

*Echinorhynchus agilis* of Linton 1889 and 1901

*Echinorhynchus clavaiceps* of Linton in Sumner, Osborn and Cole, 1913.

*Definition.* With the characters of the genus. Body almost cylindrical except in immature forms which have the posterior region gradually attenuated. Proboscis approximately globular, slightly broader than long (0.172 by 0.150 mm.), provided with three circles of six hooks each. Hooks of terminal circle 79 to 97 $\mu$  long, each bearing a root 58 $\mu$  long and 29 $\mu$  wide inside the tissue of the proboscis wall. Hooks of middle circle about 37 $\mu$  long, without reflexed root. Basal hooks 21 to 25  $\mu$  long, simple, thorn-like. Embryos within body cavity of gravid female 49 to 51 $\mu$  long and 15 to 21 $\mu$  broad.

Specimens collected by Professor Linton at Woods Hole, Mass., were identified as belonging to this species by the writer in 1913 (p. 188). Additional records of the occurrence of this species in the eel have been discovered since that time. Six juvenile specimens were taken from the intestine of an eel from Oneida Lake, New York, in the materials collected by F. C. Baker and H. S. Pratt. Three other eels from the same locality were found to harbor *Acanthocephala* of another species but none of *N. cylindratu*s. Two individuals of this species were encountered in an eel examined by A. Hassall at Baltimore, Md., along with the type of *T. ambiguu*s and two individuals of *E. coregoni*.

*N. cylindratu*s is distinctively a fresh-water species, the development of which is unknown. Without much question this species gains entrance into the eels after they enter fresh-water. The infrequency of its occurrence in the eel and the lightness of individual cases of infestation would indicate that the eel does not serve as an important definitive host for this species of parasite.

*Echinorhynchus coregoni* Linkins in Van Cleave, 1919

*Definition.* With the characters of the genus. Proboscis cylindrical, carrying twelve to fifteen longitudinal rows of ten or eleven hooks each.

Hooks not crowded on proboscis. Basal hooks 28 to  $53\mu$  long; those on middle of proboscis 65 to  $80\mu$  long; those near anterior tip smaller and weaker than those near middle of proboscis. Ventral hooks slightly larger and stronger than dorsal hooks. Lemnisci not longer than proboscis receptacle. Cement glands of male in a compact mass. Embryos within body cavity of gravid female 51 to  $91\mu$  long by 17 to  $20\mu$  wide, with an approximately globular prolongation of the middle membrane at each pole.

This species was originally described from the Great Lakes but present indications seem to suggest that its distribution is fairly broad. The writer has recently (1920:6) recorded its occurrence in fresh-water hosts from the arctic regions of this continent. Two individuals of this species were taken from an eel at Baltimore, Md., by Hassall. As indicated earlier in this paper this species was found associated with *N. cylindricus* and *T. ambiguus*.

*Echinorhynchus thecatus* Linton, 1891

*Definition.* With the characters of the genus. Proboscis when fully extended approximately perpendicular to main axis of the body or forming an acute angle with the axis. Proboscis usually about 1 mm. long. Proboscis receptacle long and slender, about 1.5 times the length of the proboscis. Hooks alternate in arrangement: in twelve longitudinal rows of twelve or thirteen hooks each; those at base of proboscis 41 to  $53\mu$  long; near middle of proboscis  $71\mu$  long; each hook surrounded by a conspicuous cuticular elevation which frequently completely ensheathes the basal hooks of each row. Lemnisci long and slender, about 1.5 times the length of the receptacle. Embryos within body cavity of gravid female 80 to  $110\mu$  long by 24 to  $30\mu$  broad.

This species is distinctively a member of the fresh-water group. It has been reported from fishes taken from the ocean but these hosts frequent fresh-water habitats. The general outline of the life cycle of this species has been worked out (Van Cleave, 1921) thereby offering additional evidence of its close association with the fresh-water fauna where it occurs as one of the most characteristic acanthocephalan parasites of fresh-water fishes of North America. In the collections from Oneida Lake, referred to above, this species was found in fairly large numbers in the intestine of all of the eels carrying acanthocephalan infestation. This constitutes the first record of the occurrence of *E. thecatus* from *Anguilla chrysoptera*.

UNCERTAIN IDENTIFICATIONS

Among the earlier American workers in parasitology there was a marked tendency to ascribe to specimens of Acanthocephala found in American hosts the names of European species. Thus much of the older

literature is replete with instances of the recorded occurrence of European species in American hosts, whereas in the cases that have been investigated carefully it has been shown that with few exceptions the acanthocephalan fauna is distinctively different on the two continents. From point of view of biology and of distribution of the individual species it is extremely desirable that these errors in determination be cited and corrected if possible. In the following section the writer has attempted to analyse some of these older records in the light of more recent investigations.

*Neoechinorhynchus agilis* (Rudolphi)  
*E. agilis* Rud.

The writer has examined some of Rudolphi's type specimens (Van Cleave, 1919:246) of this species and is confident that all records of the occurrence of this species on the American continent are based upon erroneous identifications. It seems probable that *N. agilis* is rather sharply restricted in distribution to the Mediterranean region.

As indicated previously, Linton (1889 and 1901) identified under this name specimens from the eel which unquestionably belong to the species *N. cylindratus*, the commonest representative of the genus in North American fishes. Furthermore *N. agilis* is a marine species while *N. cylindratus* is definitely associated with the fresh-water habitat.

*Koleops anguilla* Lockwood

In the description of this genus and species not a single diagnostic character is given. Characters which are discussed are all contrasted with the conditions found in "*Echinorhynchus gigas*." Apparently this last named species is the only other species of the group with which the describer was acquainted. Supposed peculiarities of the new form are in reality common to all acanthocephalans except members of the family Gigantorhynchidae. Even the drawings are not available as a supplement to the meagre description for no detail of structure, either external or internal, is shown.

*"Echinorhynchus clavaceps"* Zeder

This species has been considered by modern writers as a synonym of *Neoechinorhynchus rutili* (Müller) which infests fresh-water fishes of central Europe. In his later works Linton apparently uses this name to replace his earlier identifications of *E. agilis*. It is highly probable that specimens identified as *E. clavaceps* belong in reality to the species *N. cylindratus*.

*Echinorhynchus globulosus* Rudolphi

Linton (1901:435) identified acanthocephalans from the eel which were deposited in the U. S. National Museum as belonging to this species.

These same specimens have been found to belong to a previously undescribed species of the genus *Tanaorhamphus* which is described elsewhere in this paper as *T. ambiguus*.

#### CONCLUSIONS

Four valid species of *Acanthocephala* are known to occur in the intestine of *Anguilla chrysypha*. All four of these are distinctively American species, of which three are known to occur only in fresh-water fishes. The fourth belongs to a genus which includes but one other species and it is restricted to a single species of fresh-water host. In all of the instances that have been examined carefully there is no evidence of marine species of *Acanthocephala* inhabiting the intestine of *Anguilla chrysypha*. In this connection a comparison with data from records of infestation of the European eel is interesting.

#### ACANTHOCEPHALA FROM *Anguilla vulgaris*

Lack of unanimity in determining the synonymy of many species of *Acanthocephala* infesting fishes makes direct comparisons between records of European investigators extremely difficult. Before the time of Lühe many specific names, the authors of which recognized them as synonyms of earlier workers, had come into very general usage. Thus, for instance, Rudolphi (1802:53) gave the new name *Echinorhynchus angustatus* to *Ech. lucii* O. F. Müller (1778). In spite of this obvious renaming most European workers have, until very recently, failed to recognize the priority of the name *E. lucii* and have continued to use the synonym instead of the valid name of the species. Numerous instances of similar nature are encountered in the literature dealing with the *Acanthocephala* of fishes. In the following paragraphs names of commonly recognized synonyms have been replaced by the valid names of the species in question.

In his "Register der Acanthocephalen und parasitischen Plattwürmer," Lühe (1911:91) has compiled a list of eighteen species of parasitic worms (exclusive of nematodes) which have been recorded from the eel in central Europe. Only six of these are recognized as strictly marine, while all seven species of the *Acanthocephala* included in his list are typically fresh-water forms with the possible exception of the encysted larvae of an undetermined species of the genus *Corynosoma*.

K. M. Levander (1909) in his admirable contribution to the knowledge of the food and parasites of the fishes of Finland has utilized the names current in the literature for the species of *Acanthocephala* encountered in his investigations. His data upon the parasites of *Anguilla vulgaris* include three species of *Acanthocephala* from this host. Since the time of the publication of his work the names *Echinorhynchus angustatus*, *E.*

*globulosus*, and *E. claviceps* have become recognized as synonyms of *Acanthocephalus lucii* (Müller), *Ac. anguillae* (Müller), and *Neoechinorhynchus rutili* (Müller) respectively. Professor Levander very kindly sent me specimens from *Anguilla vulgaris* and I have been able to confirm his determinations with changes to the valid forms of the names mentioned above.

Porta (1905) listed nine species of Acanthocephala from the eel of which two, *Echinorhynchus gadi* and *Acanthocephalus propinquus*, are supposedly of marine origin. However, Porta's records in this work are the result of promiscuous compilation and it is entirely possible that either or both of these species have been misidentified.

The following table gives some of the more important contributions to the knowledge of the acanthocephalan fauna of the European eel.

TABLE I  
Acanthocephala Reported from *Anguilla vulgaris*

Species	Porta 1905	Lühe 1911	Levander 1909	Stossich 1885-1898
<i>Neoechinorhynchus rutili</i>	+	+	+	
<i>Acanthocephalus lucii</i>	+	+	+	+
<i>Acanthocephalus anguillae</i>	+	+	+	
<i>Acanthocephalus propinquus</i>	+			+
<i>Echinorhynchus gadi</i>	+			
<i>Echinorhynchus clavula</i>	+	+		
<i>Echinorhynchus lateralis</i>	+			+
<i>Echinorhynchus salmonis</i>		+		
<i>Pomphorhynchus laevis</i>	+	+		
<i>Echinorhynchus miliaris</i> (encysted)	+			+
<i>Corynosoma sp?</i> (encysted)		+		

In the above table are listed eleven species of Acanthocephala from *Anguilla vulgaris*. Two of these are larval forms thus leaving nine species as recorded from the intestine of this host. One of these nine species, *A. propinquus* is restricted to marine fishes while another, *E. gadi*, is considered by European investigators as a typically marine species which is brought into fresh-water only through the agency of migratory fishes. Thus of the species of Acanthocephala infesting *Anguilla vulgaris* two are apparently acquired during the marine phase of its existence and the remaining seven are introduced into the intestine of the eel while it is an inhabitant of fresh-water. From the foregoing it will be seen that the two species of eel differ not only in regard to the species of Acanthocephala harbored but likewise differ in the origin of their infestations. The Acanthocephala of *Anguilla chrysope* seem to be entirely of fresh-water origin

while those of *A. vulgaris* comprise a mixture of marine and fresh-water species.

## LITERATURE CITED

- LEVANDER, K. M.  
 1909. Beobachtungen über die Nahrung und die Parasiten der Fische des Finnischen Meerbusens. Finnländische Hydrographisch-Biologische Untersuchungen. No. 5.
- LINTON, E.  
 1889. Notes on Entozoa of Marine fishes of New England, with Descriptions of several New Species. Rep. Comm'r. U. S. Comm. Fish and Fisheries for 1886; 453-511.  
 1901. Parasites of fishes of the Woods Hole region. Bul. U. S. Fish Comm. 19:405-492.  
 1905. Parasites of fishes of Beaufort, North Carolina. Bul. U. S. Bur. Fish. 24: 321-428.
- LOCKWOOD, S.  
 1872. A new entozoon from the eel. Amer. Nat. 6:449-454.
- LÜHE, M.  
 1911. Acanthocephalen. Die Süßwasserfauna Deutschlands, Heft 16. Jena.
- PORTA, A.  
 1905. Gli Echinorinchi dei Pesci. Arch. Zoologico 2:149-214.
- STOSSICH, M.  
 1885. Brani di elmintologia Tergestina, II serie. Bollett. Soc. Adriat. di scienze naturali in Trieste, IX.  
 1886. Brani di elmintologia Tergestina, Serie Terza. Ibid. 9:1-7.  
 1898. Saggio di una fauna elmintologica di Trieste e provincie contermini. Programma della Civica Scuola Reale Superiore, 1898:1-162.
- SUMNER, F. B., OSBORN, R. C., and COLE, L. J.  
 1913. A Biological Survey of the Waters of Woods Hole and Vicinity. Part II, Section III. Bul. U. S. Bureau of Fish. 31.
- VAN CLEAVE, H. J.  
 1913. The genus *Neorhynchus* in North America. Zool. Anz. 43:177-190.  
 1919. Acanthocephala from the Illinois River, with descriptions of species and synopsis of the family Neoechinorhynchidae. Bul. Ill. Nat. Hist. Survey Vol. 13, No. 8.  
 1920. Acanthocephala of the Canadian Arctic Expedition, 1913-1918. Rep. Can. Arct. Expedit. 1913-1918 Vol. 9, Part E.  
 1921. Notes on the Life Cycle of Two Species of Acanthocephala from Freshwater Fishes. Jour. Parasitol. 6:167-172.
- WARD, H. B.  
 1910. Internal Parasites of the Sebago Salmon. Bul. Bureau of Fish. 28:1151-1194.
- ZSCHOKKE, F.  
 1889. Erster Beitrag zur Parasitenfauna von *Trutta salar*. Verhandl. d. Naturforsch. Gesellsch. i. Basel, 8:761-795.  
 1891. Die Parasitenfauna von *Trutta salar*. Centralbl. f. Bakt. u. Parasitenk. 10: 694-699; 738-745; 792-801; 829-838.  
 1902. Marine Schmarotzer in Süßwasserfischen. Verhandl. d. Naturforsch. Gesellsch. i. Basel, 16:118-157.

## EXPLANATION OF PLATE

Each figure is accompanied by a scale which indicates the magnification. The scales accompanying figures 3 and 5 have the value of 1 mm., all others on this plate represent 0.05 mm.

All figures were drawn from stained whole mounts in damar with the aid of a camera lucida.

Fig. 1. Profile view, portion of a single row of hooks from proboscis of *E. thecatus*, showing characteristic cuticular prominence around each hook.

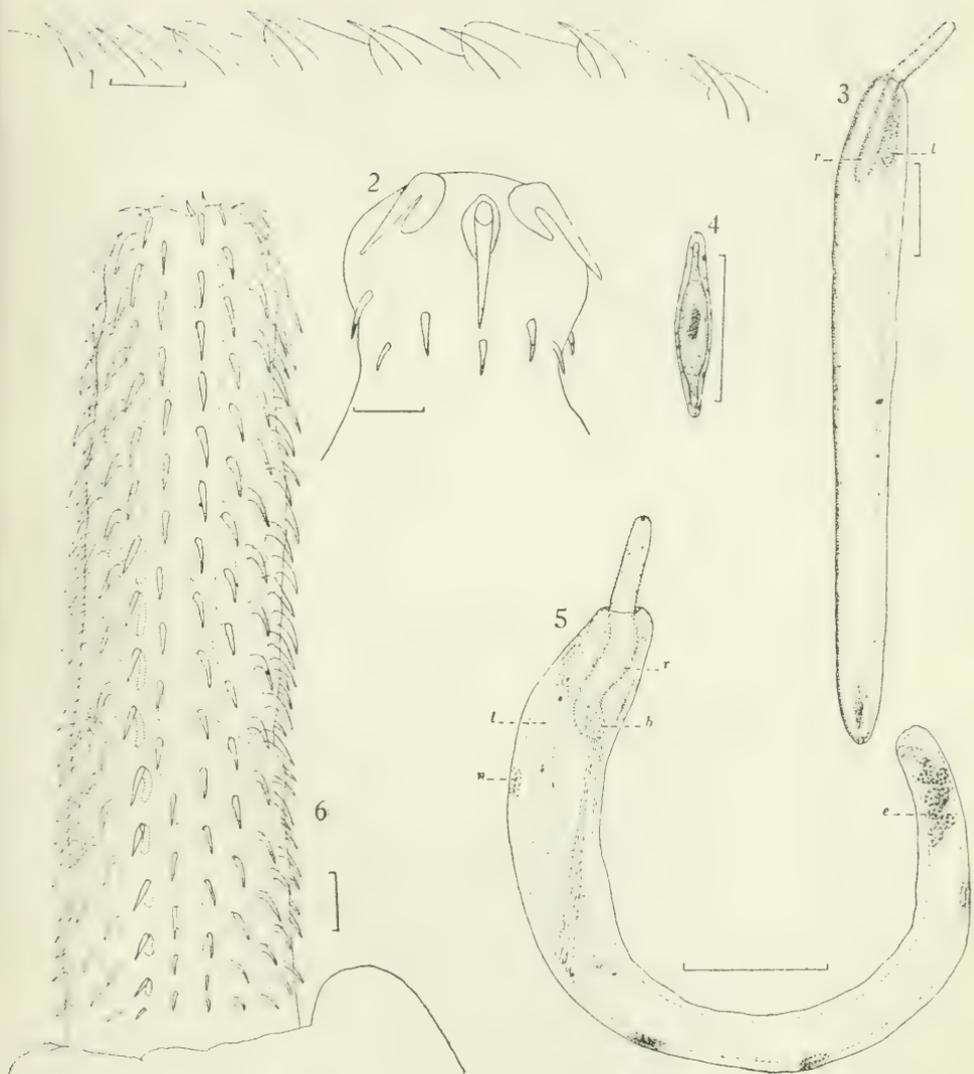
Fig. 2. Proboscis of *N. cylindratu*s, surface view, showing characteristic arrangement of hooks.

Fig. 3. Female of *E. coregoni* showing general arrangement of organs. Note especially the lemniscus (*l*) which is shorter than the receptacle of the proboscis (*r*).

Fig. 4. Embryo from body cavity of female of *E. coregoni* from *A. chrysypa*.

Fig. 5. *Tanaorhamphus ambiguus*, type female, showing general arrangement of organs: *b*-brain, *c*-egg mass, *l*-lemniscus, *n*-subcuticular giant nucleus, *r*-receptacle of the proboscis.

Fig. 6. Proboscis of type female of *T. ambiguus*.



## A BRIEF STUDY OF THE RANGE OF ERROR IN MICRO-ENUMERATION

BY  
W. E. ALLEN

Like many other people who have undertaken statistical study of microscopic organisms, I worked for a long time without any very great effort to determine the accuracy of enumeration. This was probably due to five reasons: first, various authorities state that by counting into so many hundreds or thousands, the limits of accuracy are reached, second, because two or three trial counts indicated substantial agreement, third, because the series of counts seemed to follow a normal sequence, fourth, because the insistent demands of routine work made it difficult to undertake a study of this sort, fifth, because it seemed that if great care were observed in handling and counting there was no great probability of improving matters by making such a study.

But it so happened that my co-worker in the Scripps Institution, the late Mr. E. L. Michael, when looking over the manuscript of my paper on the plankton of the San Joaquin River, raised the question as to the accuracy of my counts. We discussed the matter at various times and he always remained sceptical as to my guess that my counts were not in error more than plus or minus ten per cent. So, finally, when I got settled down to my regular program of work on marine phytoplankton, after adopting the measured water method of collecting, it became necessary to get more definite information concerning the accuracy of the counts.

I have made no thorough search of plankton literature for a record of such studies, but I have had access to the most important European and American papers, which I have scanned rather hastily without finding any indication of such a record. Hence, it seemed to me that my experience might be of some value to other workers in this or similar lines. I also thought it might lead some one to make a more thorough study of this interesting problem.

To one who has not given any serious thought to the matter, it may appear that the counting of microscopic organisms is quite similar to the counting of any common objects such as beans or apples. In the case of the plankton organisms, this is not true for several reasons. In the first place, there is usually a certain amount of dirt or débris likely to hide some individuals. Then there is the fact that if one wishes to be sure of getting a required number of the organisms, he must (because he cannot see them) filter a sufficient amount of water to give an actual excess over what he is able to count. He must then (except in the use of one or two highly

specialized methods) take a fractional part of his catch and estimate the total from the number found in this fractional part. The extraction of the fractional part from the whole and the even spreading of this under the microscope for counting is an important phase in the routine of plankton counting. One can take a pint of beans and after counting the number contained, compute fairly accurately the number in a bushel or a car, but he cannot take the individual organisms one by one from his fractional measure and make such an exact estimate. Furthermore, the microscopic things are necessarily handled in fluid through which they should be nearly uniformly spread for count. If one had to take beans mixed in four or five or one thousand times their volume of water and make the count while they were in the mixture, he might have a little better idea of the difficulty of microscopic counting. Furthermore, there is the matter of eye fatigue and the difficulty of recording the count as it progresses.

A few days after beginning the work of collecting by the measured water method on September 1, 1919, I made a beginning at a study of accuracy of enumeration which I was obliged to discontinue. I did, however, make eight counts of a catch (7728) taken in the forenoon of September 6. The results of these counts are partly summarized in table I.

TABLE I  
Eight counts by non standard method, of Catch 7728

No. of Count	1		2		3		4		5		6		7		8		Average
	Total	Per cent of Deviation															
Diatom cells. . . . .	1	91	17	40	8	33	5	41	11	8	3	25	24	100	27	150	12
Dinoflagellate cells. . . . .	350	6	334	1	228	14	292	10	275	17	308	23	406	24	294	11	329
Diatom and Dinoflagellate cells	351	3	351	3	296	13	297	12	286	16	401	15	430	21	321	6	341

The series was not very good because the conditions of counting were not nearly enough alike, first because the first four counts were made on the same day and the other four at intervals of one to four days, second, because the material was kept in the mixing tube throughout the series, merely being shaken up after return of the fractional amount from the slide after each count. Furthermore, the number of diatoms was so small

as to make that part of the count unreliable and none of the counts were carried quite so far as necessary to give sufficiently dependable results. In spite of these deficiencies the table shows that the series was sufficiently good to be considered statistically significant. Thus it appears that five out of the eight counts of dinoflagellates showed deviations from the mean of less than fifteen per cent and the highest per cent of such deviation was twenty-four. The showing for total numbers of cells is even better, a fact which calls attention to the general probability that a deviation of count in one group may be largely obscured by the count of another group when the two are combined for a total.

Although the table gives great emphasis to the point that the count of such a few individuals as those of the diatoms is valueless as a basis of generalization, it should not be forgotten that such a count may be worth recording because of its positive indication of presence of organisms. Furthermore, the system of random sampling to which we are usually forced, may sometimes lead to just as great differences in estimating the plankton population as is represented here. The significance of both errors becomes rapidly less with increase in numbers of samples.

Constantly harassed by the feeling that I ought to still further improve my basis of judgment as to the values of individual counts, I finally returned to a study of the problem on January 21, 1920, and gave it a large part of my time for the next two months. First I took some care in the selection of a catch for study and finally decided on the one (8102) for 8 P. M. on January 11, 1920, because it showed fairly good representation of both diatoms and dinoflagellates and also because it was relatively free from dirt. First I made ten counts of this catch at intervals of one day or more, the slide being emptied into the mixing tube each time, but the whole being left there instead of being returned to the bottle. The summary of results for this series is shown in table II.

TABLE II  
Ten counts of Catch 8102

Number of Count.....	Percentage of deviation from the mean									
	1	2	3	4	5	6	7	8	9	10
Total Diatom Cells.....	29	15	10	75	28	5	10	20	2	12
Total Dinoflagellate Cells.....	4	18	29	3	9	2	33	15	21	18
Above totals combined.....	23	11	14	58	24	3	11	11	6	13

In this series a few of the more abundant organisms were only counted on one tenth or one twentieth of the slide although most species were

counted on one fourth. At any rate, there is some probability that the counts of those forms which were most abundant were not carried quite far enough to yield really satisfactory results. Even so, the table shows that in only three counts out of the ten was there more than fifteen per cent deviation from the mean in total numbers of organisms and of diatoms and that there was similar deviation in only four out of the ten totals of dinoflagellates. Stated in another way, the showing is that sixty to seventy per cent of the counts deviated from the mean by not more than plus or minus fifteen per cent.

A momentary inspection of table II shows that the fourth count was the only one in which the deviation exceeded thirty-three per cent and that the enormous deviation in that case was due to some difference in the count of diatoms. Three possible causes of this great deviation

TABLE III  
Two counts each of ten successive catches

Catch Number		Régular Count	Recount	Average	Per cent of Deviation
8102	Total Diatoms.....	1112	1814	1463	24
	Total Dinoflagellates...	468	576	522	10
	Combined totals.....	1580	2390	1985	20
8104	Total Diatoms.....	1572	2460	2016	22
	Total Dinoflagellates...	276	440	358	23
	Combined totals.....	1848	2900	2374	22
8105	Total Diatoms.....	2098	2011	2054	2
	Total Dinoflagellates...	358	404	381	6
	Combined totals.....	2456	2415	2435	1
8107	Total Diatoms.....	2898	2132	2515	15
	Total Dinoflagellates...	256	568	412	38
	Combined totals.....	3154	2700	2927	8
8108	Total Diatoms.....	3298	2336	2817	17
	Total Dinoflagellates...	372	394	383	3
	Combined totals.....	3670	2730	3200	15

TABLE III (Continued)  
Two counts each of ten successive catches

Catch Number		Regular Count	Recount	Average	Per cent of Deviation
8110	Total Diatoms.....	1602	912	1257	28
	Total Dinoflagellates...	324	426	375	14
	Combined totals.....	1926	1338	1632	12
8111	Total Diatoms.....	682	728	705	3
	Total Dinoflagellates...	164	178	171	4
	Combined totals.....	846	906	876	3
8113	Total Diatoms.....	1516	1386	1451	4
	Total Dinoflagellates...	316	272	294	7
	Combined totals.....	1832	1658	1745	5
8114	Total Diatoms.....	988	1652	1320	25
	Total Dinoflagellates...	424	460	442	4
	Combined totals.....	1412	2112	1762	20
8116	Total Diatoms.....	2760	3348	3054	9
	Total Dinoflagellates...	158	110	134	18
	Combined totals.....	2918	3458	3188	8

require particular mention, first, it is extremely difficult to secure even distribution of the diatoms in the counting cell, second, there was an insufficient count of the more abundant diatoms, third, there may have been a personal error in keeping the tally of the count. My own opinion was that this particular deviation was mainly due to difference in the evenness of spread of the diatoms through the suspending fluid and to insufficient count.

For further test of the matter before making any very definite change in method, I then took ten consecutive catches and made two counts of each. The results are partly shown in table III.

In this table it may be noted that there was only one deviation from the mean of as much as thirty per cent. The fact that this one deviation of thirty-eight per cent was in the count of dinoflagellates might lead

one to think that dinoflagellates could not be any more readily mixed through the fluid than diatoms. My detailed record of the count shows, however, that this deviation was mainly due to differences in the count of extremely minute forms which I have been including under the name *Gymnodinium* sp. The difficulty of seeing these forms is quite sufficient to account for this error under the circumstances. It appears, then, from this particular series that the deviation in the count of the fairly visible forms is usually well inside of thirty per cent.

In order to have some basis of judgment as to what increase in accuracy might be expected if counts were made covering the whole slide instead of a fractional part, I then made eight counts of a single catch using four different mounts. For each mount I made one count over the whole slide and one count over one fourth of the slide. The most important results are summarized in table IV.

TABLE IV  
Counts of four mounts of Catch 8102

Number of mount .....	Percentage of deviation from mean			
	1	2	3	4
	Percentage of deviation in full slide counts			
Diatom colonies.....	18	5	3	25
Diatom cells.....	11	8	3	0
Dinoflagellate cells.....	5	3	3	5
Total cells.....	10	7	2	2
	Percentage of deviation in fourth of slide counts			
Diatom colonies.....	47	30	12	16
Diatom cells.....	30	53	25	25
Dinoflagellate cells.....	8	12	12	8
Total cells.....	25	35	20	33

While the four counts of each kind are not enough for definite conclusions, they are quite suggestive. It was not practicable to carry the series further because of the great amount of time required. As it is there is strong indication that under usual conditions the count covering the full slide is much more likely to approach the mean than is the count made over some part only.

After giving the matter a good deal of thought, I came to the conclusion that by standardizing mixing processes, much could be done toward reducing the errors of the fractional counts. I, therefore, adopted the practice of shaking the storage bottle for one minute before pouring the contents into a mixing tube, and of reversing twenty times each mixing tube used. All other manipulations had already been made as nearly uniform as possible.

I then selected for study catch number 8104 of 8 A. M., January 12, because of its close resemblance to 8102 which had become somewhat unreliable from repeated handling. Twenty counts were made of samples from this catch. At least twenty-four hours intervened between each two counts and the total catch was returned to the storage bottle after each count so that the sampling might be done in approximately the same way each time. With the first ten counts a test was made of the method of selecting fractional areas in the cell. In one case the areas were selected at intervals around the margin and in the other a median zone lengthwise of the cell and covering one fourth of its area was selected. The second ten counts were made by the median zone-method but record was kept of the numbers at areas of one fifth as well as of one fourth of the slide. The results are summarized in tables V and VI.

Table V shows the percentage of deviation from the mean by marginal (twentieth to fourth of slide) and median (fourth of slide) counts in the first ten counts, calculated from the mean for this ten, by fifth and fourth of slide counts in the second ten calculated from the mean for that ten and by fourth of slide counts in the twenty counts calculated from the mean for the whole twenty. Without attempting extended analysis of the tables, I may call attention to the fact that the deviations shown by ten counts do not indicate very much difference in most cases between the marginal count (which varied from  $1/20$  to  $1/4$  of the slide) and the fourth of slide count, nor between the fifth of slide and fourth of slide counts, but that there is a much greater range of deviation in the marginal counts. I also note the fact that there is a better approximation to the mean in the fourth of slide counts in the case of *Gonyaulax polyedra*, which is a dinoflagellate of sub-globular form. Such a difference in count of this organism might be expected because its shape would favor fairly even distribution in mixing and handling while most other organisms are sufficiently irregular in form to lead one to expect them to be more erratic in any distribution undertaken by shaking or stirring of the surrounding fluid. In the twenty count series it may be noted that the difference between *Gonyaulax* and total dinoflagellates tends to disappear but that the difference between both and diatoms is accentuated. The close resemblance of *Gonyaulax* to total dinoflagellates is attributable largely to the fact that *Gonyaulax* contributed about two thirds of the total.

The increased difference in range of deviation between Gonyaulax and the total diatoms is explicable on the basis of what has just been said as to differences in distribution due to form.

TABLE V—Catch 8104—Percentages of deviation from the mean

	Diatom colonies			Diatom cells			Dinoflagellate cells			Total cells			Gonyaulax polyedra		
	Median $\frac{1}{4}$														
	No. of counts			No. of counts			No. of counts			No. of counts			No. of counts		
	Marginal count	10	20												
1		6	9		19	1		8	16		6	6		10	0
2	12	12	22	17	10	18	21	3	1	12	8	16	21	3	1
3	30	13	1	15	5	4	15	10	6	49	6	3	11	1	5
4	7	0	11	9	1	10	12	7	11	10	2	11	23	14	18
5	6	1	11	12	8	1	34	12	15	16	6	3	27	3	7
6	4	3	8	0	11	1	23	3	7	3	10	0	5	16	12
7	7	12	21	0	16	24	0	8	3	0	14	21	13	7	11
8	24	14	23	37	29	36	0	10	13	33	27	33	5	12	16
9	7	5	16	11	15	23	4	7	2	10	12	20	5	5	0
10	31	38	23	35	56	42	15	9	5	28	50	37	19	5	0
	Median $\frac{1}{5}$ of slide	10													
11	0	1	10	14	15	26	4	5	10	13	14	24	13	12	17
12	2	1	10	4	1	8	6	0	3	4	1	6	18	7	3
13	13	12	3	6	7	0	10	2	6	4	6	1	10	2	6
14	7	5	17	9	1	10	5	0	3	7	1	9	1	2	2
15	8	4	15	3	2	7	5	6	2	3	2	6	4	4	0
16	11	10	21	12	5	14	12	13	9	10	3	12	1	7	3
17	8	13	4	1	6	3	5	3	1	2	5	2	16	13	9
18	5	3	8	0	4	4	4	0	4	0	4	4	8	2	6
19	13	6	18	19	8	17	4	10	14	18	8	17	1	7	12
20	5	5	17	4	8	0	10	6	9	2	6	2	13	10	13

Table VI covers some of the same ground as table V but in a different way. In this table enumeration totals are shown instead of percentages, with the addition of a list of numbers of *Gonyaulax polyedra* in each of the twenty counts and a list of numbers of both cells and colonies of *Nitzschia seriata* in each of the twenty counts. It also includes a statistical summary which the late Mr. E. L. Michael very kindly prepared for me. The series is too short for statistical treatment but the summary has some interest in a suggestive way.

This summary indicates that the extreme deviation is not only more than twice as great in the case of diatom cells as it is in the case of *Gonyaulax* and total dinoflagellates but that the same thing is true of both cells and colonies of *Nitzschia seriata*, the most abundant diatom in the catch. *Nitzschia seriata* is a slender spindle-shaped diatom occurring very largely in colonies of two to six individuals. Its form would lead me to expect it to be quite erratic in distribution by any possible method of mixing. This is also to be expected of the other numerous diatoms, which belong mainly to the *Chaetoceras* group. It is also interesting to note that the wide range of deviation in diatoms is due to the tenth and eleventh counts and that in count ten the numbers of both dinoflagellates and *Gonyaulax* are very close to the mean, though *Gonyaulax* approaches the extreme deviation in the eleventh count.

This last point is important because of its indication that the error lies in the mixing and distributing of the organisms rather than in the method of counting. The normal count of the less erratic *Gonyaulax* indicates that there was no serious mistake in counting, computing or recording, while the known erratic distribution of the diatoms does indicate considerable variability in results of mixing. In spite of the large extreme deviation due to diatoms, the mean variability for total cells is only 12.2%, a fact which gives ground for thinking that totals of most counts are within a range of error of less than ten per cent.

A point which can be verified by the reader in table VI, but not in the others (though true of all), is that the deviations are fairly evenly distributed on both sides of the mean. This is an indication in this type of study that the fluctuations are normal and that they appear approximately according to expectation.

Although this study as a whole is distinctly brief and fragmentary it seems to give a good practical basis for the following provisional conclusions: First, that by very great care the extreme deviation (in total numbers of diatoms and dinoflagellates) could probably be kept within twenty-five per cent; second, that the mean deviation can be easily kept within ten per cent; third, that diatoms are more variable in the counts than dinoflagellates; fourth, that the causes of variability are to be found in the processes of mixing, sampling and spreading on the slide, rather

TABLE VI

Catch S104

Enumeration totals, deviations, etc.

Count	Total Diatom Colonies	Total Diatom Cells	Total Dinoflagel- late cells	Total Cells	Gonyaulax polyedra	Nitzschia seriata
1st	696	2152	304	2456	220	Col. 280 Cells 660
2nd	676	2100	332	2432	212	188 372
3rd	872	2464	356	2820	204	292 560
4th	768	2300	300	2600	176	232 436
5th	764	2532	284	2816	200	252 604
6th	792	2604	312	2916	240	220 552
7th	680	1948	348	2296	192	160 372
8th	664	1652	292	1944	180	240 536
9th	728	1984	344	2328	216	264 424
10th	1060	3644	352	3996	216	308 648
11th	948	5232	368	3600	252	304 796
12th	952	2770	348	3118	208	264 532
13th	840	2596	356	2952	228	232 508
14th	1008	2828	348	3176	220	224 384

TABLE VI (Continued)

Catch 8104

Enumeration totals, deviations, etc.

Count	Total Diatom Colonies	Total Diatom Cells	Total Dinoflagel- late cells	Total Cells	Gonyaulax polyedra	Nitzschia seriata
15th	992	2760	328	3088	216	336 608
16th	1048	2940	304	3244	208	316 632
17th	832	2640	340	2980	196	252 564
18th	932	2676	348	3024	228	284 556
19th	1016	3016	384	3400	240	304 738
20th	1008	2592	368	2960	244	304 616
Extreme deviation	246=30.2%	1059=41%	52=15.5%	1089=37.4%	39=18.2%	103=39.1% 242=43.7%
Average	814	2585	336	2907	215	263 554
Standard deviation	142	463	27	410	20	44 112
Average deviation	124=15.2%	358=13.9%	23=6.9%	354=12.2%	16=7.5%	37=14% 87=15.7%

than in the counting; and fifth, that the range of error in counting is at worst far less for microplankton material than is the range of error in locating, catching and preserving material.

It seems fair to regard these results as suggestive for microscopic material in general, e. g., enumeration of blood corpuscles might be expected to show a range of error somewhat similar to that of *Gonyaulax* and direct enumeration of bacilli to give results resembling those from diatoms.

As regards my own use of the study, I may say that it has led me to decide on the mixing procedure already mentioned, and in counting to

carry all enumerations to fifty individuals (or fifty colonies) or to a very close approach to fifty at a convenient computing point, except that all enumerations are stopped when one eighth of the slide has been covered.

I may say frankly that for a single count or for a very short series of counts, this number limit and area limit are too small. But in handling large numbers of catches in large series and working through long periods of time, one must give close attention to the law of diminishing returns. Would the counting of a larger number of abundant forms or the counting of all over a larger area give enough greater approach to accuracy to compensate for the greater effort and use of time? It has not seemed to me that it would for present purposes. With the lens combination on a monocular microscope which was used in making this study, it was convenient to work over the area of one fourth of the slide. Later when using a different lens combination on a binocular microscope, it was found that an area of one eighth of the slide was more convenient. In fact some counts are so fatiguing and so time consuming at one fourth slide as to be impracticable in a long series. With my present standardized procedure I should expect the one eighth slide counts to show about the same range of error as indicated for the one fifth slide counts in table V. I have not yet had time to verify this assumption. At worst the range of error in careful work will certainly not be as great as that due to other factors as far as microplankton is concerned.

Finally, I may say that although the results which I have obtained are inadequate for definite conclusions, they do indicate that with standardized procedure the microscope phase of plankton study is much more nearly accurate than some of the other phases.

## DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

---

---

### REMARKS ON THE LIFE-HISTORY AND THE SCALE CHARACTERS OF AMERICAN MULLETTS

BY

CARL L. HUBBS

Museum of Zoology, University of Michigan

Mr. Arthur Paul Jacot, in the July issue of these TRANSACTIONS for 1920 (pp. 199-229), has presented the results of his investigations on scales of two American mulletts: *Mugil cephalus* and *M. curema*. He has discovered a number of facts bearing significantly upon problems in several of the zoological sub-sciences. These facts, and, more particularly the author's interpretations of them, are discussed in this brief note.

Mr. Jacot's discovery that the scales of these species of *Mugil* are ctenoid proves an unexpected confirmation of the view recently held by Jordan and Hubbs<sup>1</sup> that the group Percosoces, comprising the Mugilidae and related families, is derived from the typical Acanthopterygii (which is characterized in part by ctenoid scales), and hence is not transitional between the cycloid-scaled malacopterygian fishes and the more specialized spiny-rayed types. The wide differences found in the character of the ctenii on the scales of the species of *Mugil* studied are also of considerable taxonomic interest.

The detailed account given of the development of scale structure, and the final proof of the transformation of the first soft-ray of the anal fin of the juvenile or Querimana stage into the third anal spine of the adult *Mugil*, are valuable contributions from the standpoint of the comparative anatomy of these structures. This juvenile metamorphosis of *Mugil* has long been in need of the detailed study which Mr. Jacot has accorded it.

The sharply defined mark on the scales of *Mugil cephalus*, which the author appears to have interpreted as a metamorphic annulus, is obviously the first winter annulus; apparently intensified, it is true, by the fact the adult characters appear first in the spring, synchronously with the resumption of the growth of the scale and of the fish, following the cessation of growth significantly demonstrated to occur during the winter. The portion of the scale within this first winter annulus therefore corresponds with the

<sup>1</sup> A monographic review of the family of Atherinidae or Silversides (Stanford Univ. Publ.), 1919.

brief period of initial growth<sup>2</sup> between hatching and winter. The similarity existing between the first annulus developed on the scales of *Mugil curema* to that of *M. cephalus* indicates that this species likewise breeds in the fall, rather than during the summer as Jacot supposed.

This altered conception of the nature of the first annulus involves a different interpretation of the age at maturity of *Mugil cephalus*: the first spawning fish appear to be just two years old (rather than in their second year); similarly, the oldest individual examined was six years old.

The second and succeeding line-like annuli developed on the scales of these species of *Mugil* being typical of the winter marks developed on the scales of marine fishes of temperate waters, and of the coregonine fishes of the Great Lakes, it is, to say the least, unnecessary to follow Jacot in interpreting these marks as migratory rather than as winter annuli. The fact that an annulus was not evident near the margin of scales of mullets taken at Beaufort in early spring indicates merely that the spring growth of these fishes had not yet commenced, and not that these fishes were exceptional non-migratory individuals. Indeed, it is not at all certain that the mullets actually do migrate southward during the winter, for a growing body of evidence is indicating that in this season many shore-fishes of the Temperate Zone merely retreat into deeper water and become less active, and hence appear absent because not caught.

These altered interpretations bring Mr. Jacot's facts into much better agreement with the results of studies made on the life-history of other fishes, and in the opinion of the writer, enhance the value of his contributions.

Mr. Jacot has introduced some new terms, none of which will probably be adopted. Of these "adulting (changing to the adult condition)" and "circulation" (referring to the course of the circuli on the scales), require no further comment. The term "linea (from the Latin *linea*, ae, f.; using the term in its more figurative expression)," is unnecessarily substituted for *annulus* or "winter band"; a similar statement might be applied to "ctenobasii."

<sup>2</sup> It is probable that the juvenile mullets pass during this period through a pelagic stage, for which the Querimana characters are well adapted. *Labidesthes sicculus*, a fresh-water fish related to *Mugil*, passes through such an initial pelagic stage.

SPRING MIGRATION IN THE CRAYFISH, *CIMBARUS ARGIL-  
LICOLA FAXON*

BY

HAROLD CUMMINS  
Tulane University

Incidental to a study of the migration of frogs into their breeding ponds, carried out near Ann Arbor, Michigan, in the spring of 1914, some interesting observations were made upon migratory activities of this burrowing crayfish. So little is published regarding the habits of crayfishes that even these few notes seem to be worthy of publication.

The location and character of the pond and the method of obtaining migration data by trapping are described in another paper.<sup>1</sup> Briefly it may be said that the pond is at the edge of a cultivated field, bounded partially by a wood which adjoins the field. About this pond a trap was constructed, extending approximately two-thirds of its circumference. The trap consisted of a cloth fence, provided with leaders of similar construction extending radially outward from the main fence. At the junction of each leader and the main fence a pail half-filled with water was sunk in the earth with its top at ground level. Crayfishes migrating toward the pond came in contact with this cloth barrier, and as they edged along it in an attempt to enter the pond were entrapped in the pails. Since the pond was not completely enclosed by the trap the number of crayfishes taken does not necessarily represent the total number of migrants; some may have gained entrance where the fence was incomplete.

Whité's Wood, at the edge of which the pond is located, fulfills the habitat requirements of this species, and so far as the writer's collections indicate, *C. argillicola* is the only crayfish that occurs there. In addition to the observation pond there are four small ponds in the wood, two of which like the observation pond are usually not dried in the summer. The remaining two always dry during the summer. All of them are frequented by crayfishes. The burrows, usually with chimneys, form a characteristic feature of the habitat; sometimes they are found at some distance from the ponds, but usually near them, and when the ponds dry numbers of chimneys are thrown up on the exposed mud.

After their winter torpidity was dissipated by warm weather, crayfishes reappeared in the pond, not only from the bottom of the pond itself but also from outside sources. The first individuals to appear were those which had spent the winter in burrows in the pond bottom. On March 23 the burrows were first opened to the surface, numbers of them being

<sup>1</sup> Cummins, Harold, The rôle of voice and coloration in spring migration and sex recognition in frogs. *Jour. Exp. Zool.*, v. 30, no. 3, April 1920.

observed for the first time on that date. Each opening was circular, averaging 1.5 cm. in diameter. Bordering the opening of each burrow was an approximately circular area, averaging 9 cm. in diameter, of light-colored sand, apparently brought up from a lower level in the process of opening the burrow. The sand was not thrown into a high convex mass, but rather was so small in quantity as to be not appreciably elevated from the level of the pond bottom. Unfortunately no attempt was made to collect crayfishes from the pond before March 23, therefore it is impossible to state whether or not all individuals spent the winter in burrows. Data on the reappearance of crayfishes which were outside of the pond during the winter, presumably in their burrows, were obtained from the trap. The results of the trap are presented in the accompanying chart.

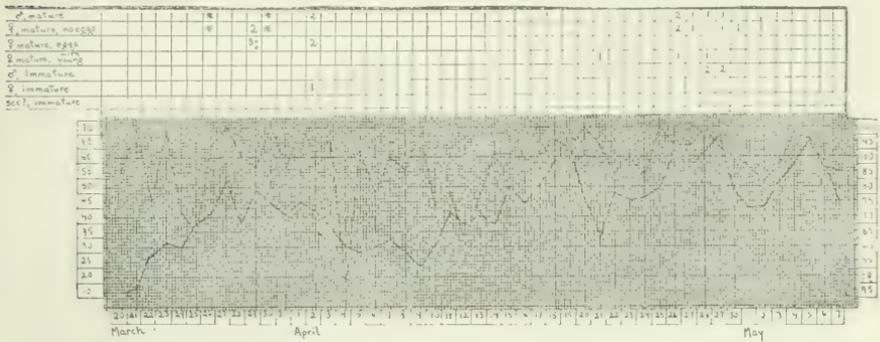


Chart showing the trap catch from March 26 to April 30, inclusive, and temperature and humidity records from March 20 to May 7, inclusive. Temperatures in the Fahrenheit scale are indicated at the left, and the average temperature for the 24 hours ending 7 A. M. of each day is plotted in a continuous line on the graph. Degrees of relative humidity are indicated at the right, and the average for the 24 hours ending 7 A. M. of each day is plotted in a broken line on the graph. In several instances the number of trapped crayfishes was recorded in the field notes as "several"; such records are here shown by the asterisk (\*).

It is evident from the chart that migration occurs at irregular intervals. There is a broken migration wave from March 26 to April 2, inclusive, and an unbroken wave from April 26 to 30, inclusive, while in the period from April 3 through April 25 but one crayfish was trapped. The migration waves occurred during periods of relatively high temperature and humidity. The lowest temperature with which a migration is coincident is 33.6 degrees (April 21), but with only one individual. A temperature more favorable to migration, if judgment can be based on the trap catch, is 42 degrees or over. All the catches are coincident with high humidities, the lowest being 87 (March 29), the others ranging between 90 and 100.

With the exception of the female carrying young, captured on April 21, all the collections were made in the morning, representing crayfishes

migrating during the preceding night. The single exception is noteworthy in demonstrating that spring migratory activity may occur in the daytime. This migrant was not actually trapped, but was noted at 10 A. M. walking in the grass near the edge of the pond. In view of the remaining migrations having been nocturnal, probably this record represents a crayfish which migrated during the preceding night, and, coming in contact with the fence, walked away instead of alongside as did others.

The small number of immature individuals is suggestive. One trapped April 30 was only an inch in length, and therefore unquestionably not sexually mature. The remaining six were about two-thirds the size of average adults. Whether there is some stimulus controlling the migration of adults, which is usually lacking in the young, or whether the young will migrate later are questions which cannot be answered with the data at hand. The facts of an early beginning of migration among adults and the retardation of migration of all but one of the immature crayfishes leads to the inference that migratory impulses occur both in young and adults, but begin to function earlier in the season in the latter.

A total of ten plus "several" females with eggs were captured on March 29 and April 2, and none appeared thereafter. During the first migration wave several females without eggs (two plus two lots of "several" each) were trapped, and in the second wave five without eggs appeared. Three females carrying young appeared in the second wave. The bearing of these data upon the time and place of egg-laying and hatching is important, but difficult of interpretation. If those females bearing eggs and young furnish a standard of comparison, we must assume either that there is a prolonged period for the egg-laying and hatching of the last five females or that their eggs already had hatched. The same question does not arise in connection with the females without eggs which were captured early. It seems that a migratory movement of adult females in the spring would prove advantageous to the young, for they would hatch in water which presumably provides a more favorable environment for them than the burrows.

PREPARING COLLECTIONS OF THE MOLLUSCA  
FOR EXHIBITION AND STUDY<sup>1</sup>

BY

FRANK COLLINS BAKER

Curator, Museum of Natural History, University of Illinois

The Mollusca form a large group of the Animal Kingdom and members of this phylum are used for economic or biologic study by many biologists, zoologists, geologists, ecologists, and others interested in the study of animal life. Collections are also made for their beauty or interest by amateur students. Whatever the cause of interest it is important that the collections made should be properly prepared and preserved for future consultation. The good appearance and permanence of a collection of mollusks depend very largely upon the care taken in cleaning and preparing the individual specimens. The modus operandi varies with the size and the kind of mollusk.

## CLEANING THE SPECIMENS

*Mussels or River Clams.* The river mussels, when only the shells are to be preserved, should be placed in boiling water which will cause the valves to open slightly. The adductor muscles may be cut with a thin-bladed knife and the animal matter removed. Care should be taken to remove all of the animal matter from the region of the muscles where it is strongly fastened. During this process the collector must avoid breaking or injuring the edge of the shell where the substance is very thin, the new shelly matter as well as the epidermis or periostracum being newly formed at this part of the shell. This is especially true of the thin-shelled mussels like *Anodonta*. After removing the animal parts the shells should be washed carefully to remove the mucus and any parts of the animal remaining. Care must be exercised to avoid breaking the ligament which holds the two valves of the shell together. When thoroughly cleaned the two valves may be tied together with *white* string (*never* use colored string for it will mark the shells) and the shells laid on boards or other objects to dry in a warm place. Never allow the sun to shine on specimens of this kind for they will then dry too quickly and the epidermis will peel off. A few shells of each lot should be broken apart so that the interior, especially the hinge structure, may be studied.

Many shells will be marred by incrustations of lime or other matter. This may be removed with muriatic or oxalic acid, which may be applied with a small camel's hair brush. As these acids, especially muriatic acid, readily attack that part of the shell not protected by the horny epidermis,

<sup>1</sup>Contribution from the Museum of Natural History, University of Illinois, No. 17.

The specimens should be washed carefully and quickly after using the acid. Many shells may need to be scrubbed with a small scrubbing brush or a nail brush to remove the extraneous matter. In some cases, however, it may be desirable to preserve the shells in their natural state, with all the incrustations and other foreign matter attached, to indicate the character of the water or bottom in which the animals lived. This may be necessary in some ecological studies. After the shells are thoroughly dry the strings may be removed and the surface of the shells rubbed with vaseline. This will usually prevent the epidermis from peeling or cracking and will give the shell the appearance it had when living in the water. Great care should be used to see that all of the surplus vaseline is removed or the surface will become sticky and unsightly. A soft rag may be used to rub the shells perfectly dry and clean.

*Finger-nail Clams-Sphaeriidae.* The smaller bivalves—*Sphaerium*, *Musculium*, *Pisidium*—are usually too small for the animal to be removed from the shell and they may be killed in 70 per cent alcohol from which they may be removed and dried in a few days. In the case of the larger *Sphaerium* the animal may be removed, after having been killed by boiling or by preservation in alcohol for a few days. As the valves of the shell are liable to open after being cleaned, and as they are usually too small to be tied together, they may be wrapped tightly in a plain piece of tissue paper until dry, when the paper may be removed. No oil or other preservative should be used for these shells.

*Fresh Water Univalves or Snails.* The larger fresh water snails may be killed by boiling or by preservation for a few days in 70 or 80 per cent alcohol. The animals are then easily extracted with a dissecting needle. A needle with a curved or twisted point is more effective in removing the animal from the inner whorls than one with a straight point. In the large *Lymnaea*, *Planorbis*, and *Physa*, the animals are easily removable, but in the *Pleurocera*, *Campeloma*, and related genera, the animals must be removed with great care as the upper part of the animal, containing the liver and part of the sexual organs, is liable to break off and remain in the shell. When removing these animals, get a firm hold of the body with the dissecting needle and then by a slow, careful, twisting motion remove the animal. All animal matter should be removed from the large shells.

If there are incrustations or other foreign material on the surface of the shells this may be taken off with a brush, scraped off with a knife, or removed with the acids mentioned for mussel shells, oxalic acid being the best. The acids must be used with care that the fine texture of the shells may not be injured. In those species having an operculum, like *Campeloma* and *Pleurocera*, the opercula of a few individuals of each lot should be removed from the foot of the snail, dried, and placed inside the aperture of the shell, which may then be closed with a piece of fine cotton. It is

not a good idea to glue the operculum to the cotton because the inner side which bears the muscle scars for its attachment to the operculigerous lobe of the animal may be needed for study. All shells should be thoroughly dried before placing them in the cabinet and before placing the operculum in the aperture. It is well in the larger *Campeloma* and *Vivipara*, to wipe the surface gently with the rag used for vaselining the mussel shells, using the same care as recommended for that group in this particular. The smaller snails, *Ammnicola*, *Valvata*, small Lymnaeas (*Galba*), *Ancylus*, etc., may be killed in 70 per cent alcohol, from which they may be removed in a few days and dried. The little fresh water limpets (*Ancylus*) should have the animal carefully removed with the point of the dissecting needle. As these small limpets are usually coated with foreign matter they may be effectively cleaned by being allowed to float, upside down, on the surface of a small quantity of oxalic acid, after which they may be washed and carefully wiped with a camel's hair brush. The shell is thus easily cleaned if held, aperture downward, on the tip of the index finger.

*Land Shells.* The larger land snails or Helices should be placed in warm water which should be quickly brought to the boiling point to kill the animals. It is of importance to be certain that the water is boiling for hot water will not kill the animal at once and it will then be difficult to remove from the shell. Land shells cannot be left too long in the boiling water because the fore part of the body is liable to break away from the part containing the liver, which will then remain in the upper whorls of the shell and be very difficult to remove. If not killed quickly by boiling, the columella muscle will not be loosened from the pillar lip and the animal cannot be pulled out without breaking in pieces. The larger species must be boiled for fully a minute but the smaller species, the size of *Polygyra hirsuta*, will be ready to have the animal removed in 10 or 15 seconds. To prevent loss in a large tin or pot it is well to place the snails to be boiled in a wire dipper which may be obtained in any 10 cent store.

To insure successful extraction of the animals it is necessary to use great care and plenty of time. The same curved dissecting needle mentioned previously is well suited for removing the animals of land snails, and the same twisting motion is necessary as described under fresh water snails. If the animal breaks during the operation, leaving a portion in the upper whorls of the shell, the remaining part may be removed with jets of water from a small syringe, preferably a fine-pointed dental syringe. It may be well sometimes to place the shell in alcohol for a day or two in order that the part of the animal left in the shell may be loosened, after which the syringe will usually remove the matter. Sometimes a vigorous shaking, or, with the hand holding the shell, striking the other hand or the thigh, will aid in loosening the refractory matter. Much

patience and some ingenuity is necessary in removing the animals from their shells in which the aperture is restricted or contracted by teeth or folds, and in these cases the fine syringe will be found useful to start the body from the shell. All shells should be washed out inside with the syringe and scrubbed on the outside with a tooth brush, or other small brush, to remove all traces of mucus, dirt, or other foreign matter. A gentle flow of water from a tap or faucet is very effectual in removing mucus and dirt from the interior of large shells. If the mucus is unusually adhesive, as is sometimes the case, it may be necessary to use a small piece of sponge or cotton attached to the curved dissecting needle, or held with a pair of curved forceps, to remove the unsightly material. Land shells do not require vaseline for the preservation of the epidermis as suggested for fresh water mussels and large water snails. When perfectly clean the shells may be laid on boards or other objects and laid in a convenient place to dry. Never allow shells to dry in the sun for they will crack and be spoiled for cabinet purposes. Too strong emphasis cannot be laid on the injunction to remove *all* animal matter from the larger land shells, which have a peculiarly offensive odor all their own if placed in a cabinet only partly cleaned.

The small land snails, especially the members of the Pupillidae and those snails having teeth or folds in the aperture, cannot well have the animals removed. If these are kept for a few days in a dry place the animal will retract well within the shell and they may then be placed in 30 or 40 per cent alcohol for twenty-four hours, after which they may be dried and no offensive odor will be retained. Vermin will not usually attack a shell that is thus well soaked in alcohol. When dirt of any kind remains attached to these small shells they may be effectually cleaned by being put in a bottle with fine, clean sand, and a vigorous shaking will remove the dirt. This process should not be used for fragile shells. It is especially effective with the Pupillidae.

*Marine Shells.* The directions given above for land and fresh water shells apply equally well for marine mollusks. The snails from the sea, however, are more difficult to prepare because of the more powerful columellar muscle by which the animal is attached to the shell. For the larger species of sea snails the curved dissecting needle will hardly be adequate to extract the animal. For this purpose nothing is better than a stout fish hook which has been heated and then bent in the form of a partial spiral. Plunging in cold water after shaping will return the temper of the steel sufficiently for the purpose for which it is made. The shank may be firmly fastened in a wooden handle made in convenient shape to fit the hand, and the result is a very useful implement. In extracting the larger animals from their shells, it is important that the hook be deeply and firmly buried in the large, tough muscle attached to the columella

pillar or axis of the shell. A strong, steady pull will usually bring the animal.

Bivalve shells, clams, may be treated in a similar manner to Unionidae mentioned on a previous page. Boring clams, like *Pholas*, *Teredo*, and others, will require special attention to preserve the extra pieces of shelly matter connected with shell. Small clams may be treated in the same manner as mentioned under finger-nail shells. The same may be said of the small snails which should be treated as the small fresh water or land snails. Marine shells may be killed in boiling water or by preservation in alcohol. As in the case of land and fresh water mollusks, formalin is not a good preservative on account of its action on the shells.

Many marine snails are encrusted with limy matter, the tubes of worms, the hard shelly bases of corallines, and the dried remains of sponges. These may be removed with an old file the end of which has been ground to a point. Little chisels and punches like engraver's tools are also excellent for this purpose. With care and experience the collector will be able to scale off the greater part of this extraneous matter without harming the shell beneath. The judicious use of muriatic acid will also help in the final cleaning process, but this reagent must be used with great discretion in order not to mar the surface of the shell.

#### PREPARATION FOR ANATOMICAL STUDY

It is frequently desirable that some of the material collected should be preserved for the study of the animal. Fresh water pulmonates, such as *Lymnaea*, *Planorbis*, *Physa*, may be placed directly in 30 per cent alcohol, where they may remain for twenty-four hours. They should then be placed in 50 per cent alcohol for another twenty-four hours, and finally preserved in 75 or 80 per cent alcohol. The fresh water operculate snails may be preserved in the same manner, as may also most of the marine snail shells.

Land shells, however, must be killed in osmic acid or by drowning, the latter being the best, causing the animal to die in a fully expanded condition. For drowning, the writer has obtained the best results by placing the snails in a large, wide-mouthed bottle, filling the bottle level full with water and placing a heavy piece of glass over the water to exclude all air bubbles. In twelve to twenty-four hours the animals will be fully expanded and quite dead and may then be removed to 30, 50, and 80 per cent alcohol as recommended above for fresh water snails. Care must be exercised that the snails are not taken from the drowning water too soon, for in this case they will contract badly when placed in alcohol.

Final preservation may be made in a 2 per cent solution of formaldehyde, but alcohol is better for the flexibility of the animal, which has a

tendency to harden and become brittle in formaldehyde. Even in a weak solution of formaldehyde the shells gradually soften and easily break when handled. A recent examination of some molluscan material in a research collection of a well-known laboratory was found to be time wasted because the material had been preserved in formaldehyde, and the shells had softened and curled up, almost entirely losing their original character. Valuable material upon which scientific conclusions are based is thus liable to be ruined for future study and examination.

Slugs (*Limax*, etc.) and snails with small or very thin shells may be preserved as mentioned for the animals of land shells. The eggs of all mollusks, fresh water as well as land, should be preserved in alcohol, after passing through the different grades of the preservative. Some eggs, as those of *Pyramidula* and *Polygyra*, have a more or less hard shell and may be dried and preserved in bottles. In the case of large eggs of the *Bulimi* and other large land shells, they must be treated in the same manner as birds' eggs and the contents removed by means of an egg blow pipe. They may then be dried and placed in the collection.

For bringing out details of the surface structure of snails a 1 per cent solution of chromic acid has been found to be a good reagent. Müller's fluid is also an excellent fixing reagent. These reagents, however, harden the body to such an extent that it is often difficult to make gross anatomical examinations and the alcohol method described above is the best for all purposes. When using the fixing reagents mentioned it is highly important that the animals be washed thoroughly in running water before being transferred to the different grades of alcohol. Twelve to forty-eight hours will be necessary for this purpose, depending upon the size of the specimen treated. No specimens should be placed in strong alcohol at once as this reagent extracts the water so rapidly that the internal organs are shrunken and distorted. For sectioning and some histological purposes the hardening methods mentioned are excellent.

#### PRESERVATION FOR STUDY OR EXHIBITION

The method of preserving and arranging a collection of mollusks will depend wholly upon the purpose for which it was made. All collections may be roughly divided into two types, those for display and those for study. Each of these types requires a different treatment.

*Collections for Display.* Collections of this kind will probably be confined almost exclusively to museums of one kind or another. An exhibition collection of the Mollusca, even in a public museum, should be more or less synoptic in character, and arranged to show the principal features of classification, as well as facts relative to different kinds of habitats—ponds, rivers, swamps, shallow water, deep water, rocky shores,

sandy shores, forests, plains, and valleys—in short, the ecology of this type of animals. The geographic distribution—Arctic, temperate, tropic, island, continental, etc.—should be indicated by charts; the variation of individuals and the economic use made of certain species should also be clearly indicated. For some of this display, models may be used to illustrate ecology, geographic variation, and methods of life. Features of this kind add much to the value of a collection and are always interesting to those persons visiting a museum that are not particularly interested in the general subject of mollusks. Such economic displays as pearl buttons and the clams from which they are made, both fresh water and marine, shell money as used by the native tribes of this and other countries, mollusks used for food, injurious snails, pearls, and other topics of like nature, are very interesting, useful, and highly educational.

For exhibiting mollusks a strong, durable, attractive tablet is essential. Such an one can be made of heavy binder's board (no. 20) cut into convenient sizes and covered with such material as will give the best effect to the collection. Many shells will look well on a black background and these may be mounted on tablets that have been covered with a dull black paper. Dark shells look better on a light background, and for these the writer has used an ivory-colored cardboard known as Royal Worcester Bristol Board, a material that withstands the fading power of light better than any other paper used. For these light backgrounds the cardboard is cut just a trifle smaller than the tablet, the edges of which have previously been passépartouted with a dead black paper used for binding together lantern slides, and the light cardboard is glued to the tablet (glue being used only about the margin of the card), leaving a border of black. This method produces a handsome tablet that is both durable and attractive. When the label is attached (which should be made of the same cardboard used for the center of the tablet) the whole has a pleasing appearance. The sizes of these tablets, as used by the writer in his museum work, and found to be the most useful, may be 3 x 2, 3 x 3, 3 x 4, 3 x 6, 3 x 9, 6 x 6, 9 x 9, and 12 x 12 inches. All of these are multiples of the small unit, 3 x 2 inches.

To make an exhibit collection of the greatest value from a teaching standpoint, many drawings of structure and development, maps of distribution, and labels describing the function of organs, as well as notes of interest concerning the animals or shells, should be freely used. A famous museum man, Dr. G. Brown Goode, once said that a museum was a collection of labels illustrated by specimens, and while this axiom is pretty strong and the matter may be somewhat overdone, the fact nevertheless remains true that a collection for public exhibition must be largely explained or interpreted by means of illustrations, models, and descriptive labels. Perhaps the statement of the great British museum administrator,

Sir William H. Flower, more nearly describes the use and function of a museum, who says: "It is not the objects placed in a museum that constitute its value, so much as the method in which they are displayed and the use made of them for the purpose of education."

Printed labels are the best for permanent display, but as these are expensive the next best are typewritten labels which may be printed on a typewriter having a platen such as is used by the librarians for card catalog work. The ribbon should be black carbon. Where large shells or series of shells illustrating some feature of structure or variation are to be exhibited a uniform black or ivory-colored background may be employed, using a large sheet of dull black paper or a sheet of the bristol board mentioned above for tablets.

*Cases.* Nearly all molluscan shells are best displayed in horizontal or flat cases. Shelving in an upright case can be used, but this method of installation is not as attractive nor as easy to install as in a flat case. Very large specimens or material preserved in alcohol or other fluid (these should be flat-sided glass jars) are best shown in upright cases or in the A-cases that are now used in many museums. In some museums the space beneath the flat cases is utilized for the purpose of storing the study series in drawers. In some of the older museums these drawers have (or had) glass tops and the contents could be seen by the visitor by simply pulling out the drawer. Excepting where the matter of space is vital this should not be done. The open museum halls are poor places for the proper storage of a research series which must be consulted in the presence of curious visitors who greatly bother the student. These collections should be stored in drawer cabinets kept in rooms especially reserved for research collections and made convenient for their study. This subject is more fully treated on a later page.

For holding cards and labels in an upright position the writer has found the pins and ticket holders sold by stationers to serve the purpose admirably. For attaching specimens to tablets it is better to use wax than glue, the former being easy to remove if the shell is needed for examination, while glue is difficult to remove without injury to the shell. Bright, polished shells, like *Cypraea* and *Oliva*, are difficult to attach to the tablets on account of their smoothness. The prepared clay known as 'plastene,' 'modelit,' and 'permodello' has been used to some extent by the writer and has been found excellent for this purpose, if the mixture does not have too large a percentage of oil, which discolors the tablet. By drying the clay a trifle the amount of oil may be reduced. This clay usually provides a mold in which the shell may be held in any desired position. The clay is made in several colors among which gray-green, terra cotta, or dark brown are the best. If the shells are of the common kind and are not likely to be needed for study the liquid glues will prove

the best medium for fastening the specimens to the tablets. The shells may be propped in any desired position until the glue hardens.

### COLLECTIONS FOR STUDY

While there are several ways in which a collection of mollusks can be installed for exhibition, there is but one good method of caring for a study or research series of these animals. The dry specimens should be kept in drawer-cabinets. In considering the size of the cabinet the dimensions of the primary unit, the individual tray containing the specimens must first be decided upon.

*Pasteboard Trays.* These should be made as multiples of the smallest unit. This unit may be 1 x 2 inches for the smallest species and 3 x 2 inches for the larger series, the unit of width here being three inches. If one desires to carry out the 1 x 2 unit for the entire series the larger trays may be multiples of two inches. The various sizes that are the most useful, as learned from experience, are as follows: a. 1 x 2, 2 x 2, 2 x 3, 2 x 4, 4 x 4, 4 x 6, 6 x 6, 6 x 9, 9 x 9, 12 x 12. b. 1 x 3, 2 x 3, 3 x 3, 3 x 4, 3 x 6, 3 x 9, 6 x 6, 9 x 9, 12 x 12. The depth of the trays should be one-half inch, except in the largest size which should be three-fourths of an inch in depth. The trays may be covered with black or white glazed paper which gives them a pleasing appearance. These trays can be made by any box manufacturer. Ingenious students may be able to make their own trays if they have the time. To do this pieces of cardboard should be cut as shown in figure 1 and the four pieces indicated by the dotted line, folded together and attached by adhesive paper. Trays of this kind can be quite economically made in a short time.

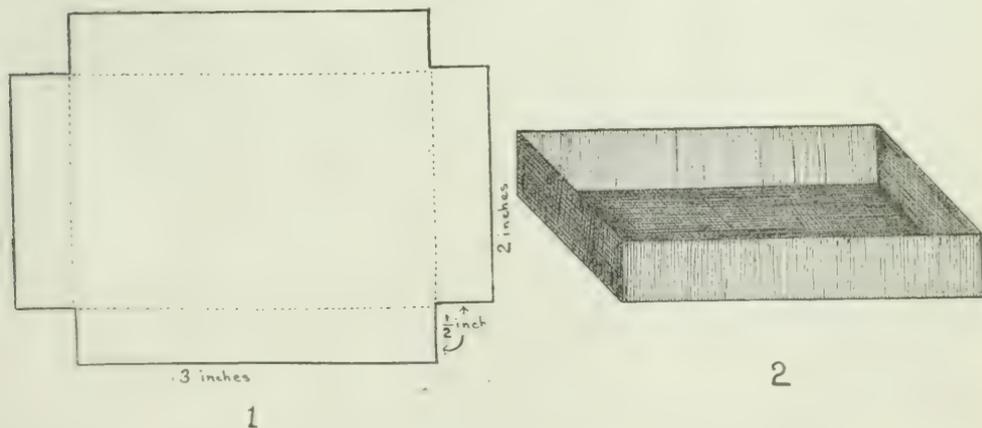


Fig. 1. Method of making a pasteboard tray for holding specimens.

*Drawers.* Having selected the size of the individual tray the next step is the dimension of the individual drawer. This should be made of a

size to contain the trays in a manner that there may be no waste space. A convenient size measurement, *inside*, is  $15\frac{1}{4} \times 21\frac{1}{4}$  inches. This allows five rows of the three inch unit trays which fit snugly in both dimensions of the drawer. If the two inch is used the drawer should be an inch wider or  $16\frac{1}{4}$  inches. This will hold eight rows of the two-inch unit. The depth of the drawer will depend upon the character of the specimens it will contain. The smallest specimens need a drawer not over an inch in depth while the largest may require a depth of five or six inches. For an all around depth the writer uses a drawer two inches in depth and when larger specimens are installed the space of two drawers is used for one. This method has been found quite satisfactory and does not on the average take more room than when drawers are made of varying depth. It is seldom that specimens of greatly different size will be placed in the same cabinet, if room is wisely left for expansion, as should be done in the larger collections. The drawers mentioned above, which are in use in the University of Illinois Museum, are made of three-eighths inch material for the sides and ends and compo board is used for the bottom. These drawers require no handles and are very inexpensive.

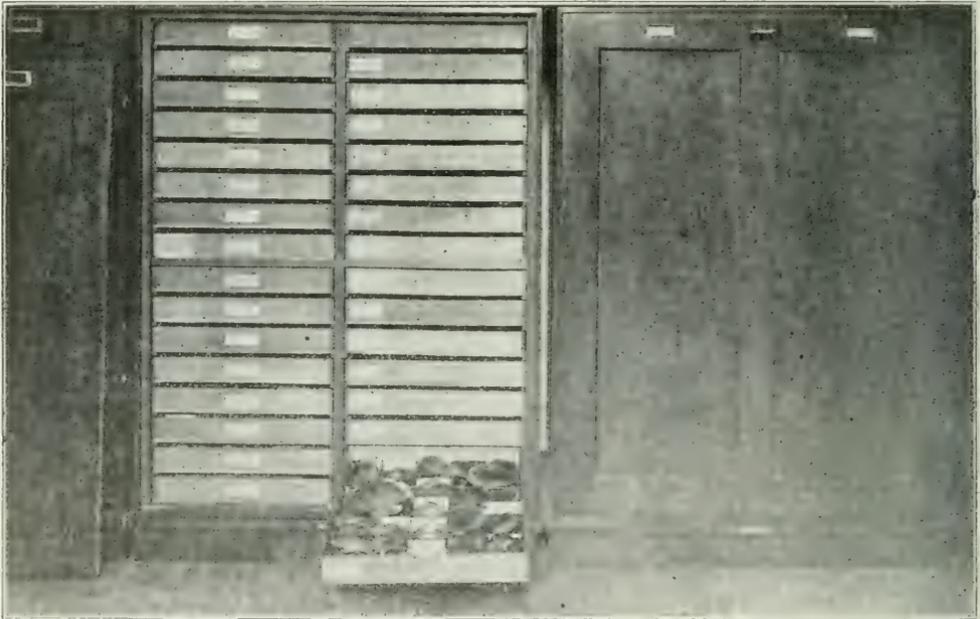


Fig. 2. Storage cabinet of drawers, 32 in each cabinet. An open drawer at the bottom shows the method of attaching labels to the back of the trays, as described in the text. University of Illinois Museum.

*Cabinets.* Having determined the sizes of the trays and the drawers the next thing is the size and style of the cabinet to contain the drawers. This should also be of the unit pattern so that several may fit together. The cabinets that are in use in the University of Illinois Museum, and which the writer has used in other museums he has had charge of, are shown in figure 2. These have the following dimensions:

Height 46, width  $35\frac{1}{2}$ , depth 25 inches, outside measurements.

Drawer space  $16\frac{1}{8}$  inches wide,  $2\frac{3}{8}$  inches between the drawer runners.

Drawer runners  $\frac{3}{8}$  inch pieces sunk in the sides of case  $\frac{3}{16}$  inch.

Each case holds 32 drawers, 8 in each of four sections.

If the drawers are to be one inch deep the space between the runners should be  $1\text{--}5\frac{1}{16}$  inches and the case would hold sixteen drawers in each section or sixty-four in each cabinet. The drawers are really  $\frac{3}{8}$  of an inch deeper than the dimensions given, this extra space being occupied by the runners. This space allows for extra large shells which may be included with the smaller ones. It is usually essential that all cabinets be of the same size and contain the same size of drawer so that additions and rearrangements may be made without unduly changing the contents of the drawers. This is very important when large additions are made necessitating the rearrangement of a large part of the collection. The drawers may be made of whitewood or basswood and simply shellaced or varnished. The cabinets are best if made of oak and finished in some dark color. The door of the cabinet should be made with a groove which extends entirely around the inner margin. This should fit into a tongue in the sides of the cabinet which also extends entirely around the cabinet. A piece of plush or felt fitted into the groove in the door will keep out the dust very effectually. Rubber has been used but this substance soon loses its resiliency and becomes worthless. The door should be made so that it may be entirely removed from the cabinet so that it will not be in the way when the collection is being studied. The photograph, fig. 2, indicates these points.

For smaller species, as the Pupillidae, Valloniidae, Sphaeriidae, Amnicolidae, as well as many groups of minute marine shells, the writer has used a case made to hold legal blanks which has proved very convenient and satisfactory. The dimensions of the drawers are:

Length  $14\frac{1}{4}$ , width 9, depth 1 inch; height of case of ten drawers 14 inches.

Each drawer holds 56 of the 1 x 2 inch unit trays or 560 trays in the cabinet. These cases are admirably adapted for holding these small shells, the drawers not being large enough to be cumbersome as is the case with a large drawer filled with these small trays. Several legal blank cases may be installed in one of the larger cabinets if it is desired to keep the cabinets perfectly uniform. The only possible criticism may be that

these legal blank cases are not perfectly dust proof and for permanent installation they should, perhaps, be enclosed in a cabinet as suggested above. Each drawer should be labelled with the name of the contents and each cabinet should have the name of the group it contains.

*Bottles and Vials.* For the safety of the collections glass bottles or vials should be provided for all shells under  $\frac{3}{4}$  inch diameter. These should be made in different diameters but only of two lengths to fit the two unit widths of trays, two and three inches wide. Convenient sizes are as follows:

$1\frac{3}{4} \times \frac{3}{8}$ ,  $1\frac{3}{4} \times \frac{1}{2}$ ,  $1\frac{3}{4} \times \frac{3}{4}$ ,  $1\frac{3}{4} \times 1$  inch.

$2\frac{1}{2} \times \frac{3}{4}$ ,  $2\frac{1}{2} \times \frac{1}{2}$ ,  $2\frac{1}{2} \times \frac{3}{4}$ ,  $2\frac{1}{2} \times 1$  inch.

Occasionally a larger size will be needed and a vial of  $1\frac{1}{2}$  inch diameter will be found useful. Only a few of this size will usually be required. The bottles known as shell vials, obtainable through almost any druggist, are especially adapted for the preservation of molluscan material and can be made of any of the sizes mentioned above. Short corks may be used but these should be rolled or squeezed to soften them so that the fragile tubes may not be broken when the cork is forced into the mouth of the vial. Rolling with a hard piece of wood or metal or pressing between a pair of large flat-nosed pliers will be found to accomplish this purpose admirably. Homeopathic vials may be used but these are not as good for dry specimens as the shell vials. Where expense is a serious item very good containers may be made by rolling a piece of stiff paper over a lead pencil or other round object the size of the required container and then gluing or pasting the edges together and closing one end with cotton. A cotton cork may also be used for the other end. The *modus operandi* of this method is indicated in figure 3. The cylinders may be made in lengths of legal blanks and then cut off in lengths to fit the trays.

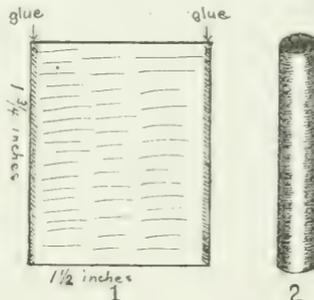


Fig. 3. Method of making paper shell tubes for holding small specimens.

#### STORAGE OF ALCOHOLIC MATERIAL

The proper storage of material preserved in alcohol is a matter requiring considerable attention. For this purpose homeopathic vials are better

than shell vials, because they are thicker and the strong, reinforced opening makes it possible to press a cork in very tightly, which retards evaporation of the liquid contents. Fairly large vials should be used even for small specimens in order that the storage may be uniform and liquid enough provided for the specimens to obviate frequent filling of the containers. Several sizes of bottles, 2, 4, 6 ounce, will be required to preserve the larger specimens. For mussels or series of specimens the clamp-top, all glass fruit jars (Atlas for example) are excellent for storage purposes. The old-fashioned Mason jar is not good because the liquid evaporates quickly and the metal screw top cannot be made permanently tight, besides becoming very unsightly in a short time. All of the jars should be of glass, except the rubbers, and these will need to be changed at intervals. The quart and pint jars have been found the most useful for purposes of storage, although the two quart size may be necessary at times.

Professor Frank Smith, of the Department of Zoology, University of Illinois, has made use of a method, first devised by the United States National Museum, for the preservation and storage of small specimens in vials which has much to commend it. The small vials, after being filled with alcohol or other preservative and having a wad of cotton placed in the mouth of the vial, are stored, bottom upward, in a large jar, of two liter capacity or larger, which is then filled with alcohol or other fluid. By this means the smaller vials may be kept without adding new liquid for a long time. Also, the large jar will become empty before the small vials and thus a warning is given before any damage can be done to the specimen. It often happens when valuable material is stored in many small vials that lack of proper attention permits the vials to become empty of fluid and the specimens dry out and are thus ruined.

Many zoologists will prefer the single bottle method, however, on account of the accessibility of the material, and for such the storage should be in standard racks, which may be stored on compo board shelves in the unit cabinets described previously. These racks will vary in width but should be of the same length. Convenient dimensions are as follows:

Vials, length 22, width  $1\frac{3}{8}$ , height side  $2\frac{1}{4}$ , height front 3 inches.

Bottles, length 22, width  $2\frac{1}{4}$ , height side  $2\frac{1}{4}$ , height front 3 inches.

The general form used is indicated in figure 4. The stock should be



Fig. 4. Standard rack for holding alcoholic material, vial size.

$\frac{3}{8}$  inch for ends and  $\frac{3}{16}$  inch for bottom and sides. The large jars are perhaps best stored on shelves, although a rack similar to the one suggested for the vials and bottles, but made large enough to hold the jars, may be used. These racks may be made by any good carpenter or the lumber can be cut in a mill and the collector can put the racks together himself.

#### REGISTRATION AND LABELING

Every set of specimens should have with it a label giving the name of the species, its locality, the principal ecological conditions under which it was found, the name of the collector, and the name of the authority who determined the species, as well as the date of collection. For this purpose cardboard labels just the width of the inside of the unit tray, 1 x 2 or 1 x 3 inches, may be used. The writer has found by experience that an excellent method of attaching the label to the tray is to glue the upper edge of the label to the upper margin of the unit width of the tray, at the back. When a whole drawer is arranged with the labels affixed in this manner the different species and their localities may easily be read. The specimens or vial of specimens lie in front of this label, as shown in figure 2. Genera or group divisions may be indicated on labels fastened to the bottom of the 1 x 2 or 1 x 3 inch trays. A sample of label in use in the Museum of the University of Illinois is shown below.

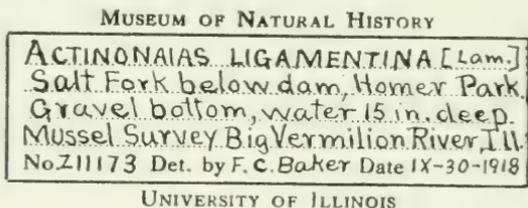


Fig. 5. Sample of label.

A catalog number should be given each set of specimens and this number should be placed in the vial containing the specimens or in the case of large specimens, written on the shell. For mussels both valves should be numbered. The best quality of indelible carbon ink should be used for this purpose. The writer has found Higgin's eternal ink (water-proof) to be the best for all purposes. The alcoholic material should have a label placed in each bottle written with the same kind of ink. Cardboard labels for this purpose are good. A permanent cloth known as 'mapstock' velum, sold by Jos. Bancroft & Sons Co., Rockford near Wilmington, Delaware, has been found admirable for this purpose.

A serial catalog kept in a book and a card catalog are invaluable for the proper recording and convenient classification of a collection. The book should be arranged to contain the serial numbers of the collection.

This volume may be made as elaborate as the pocket book of the collector will permit, varying from a simple note book to a large printed folio. For museums and large collections of private individuals the large folio is by all means the best. This may be arranged with the headings suggested below.

Cat. No.	Name	Locality	No. of specimens	Received from	Collected by	Date	Remarks

Other entries, such as original no., identified by, dry, alcoholic, etc., may be used if it is desired to elaborate further. For large institutions an accession catalog is necessary, in which is recorded the material by lots as received. In such cases an entry, accession number, is usually made room for after catalog number in the species catalog.

The card catalog should contain the references to all of the lots of one species, showing the different places from which they came, on one card, or each lot of a species may have all of the information recorded on one card. The first is more convenient for a small collection but the latter is perhaps better for a large institution or collection, giving all of the known data concerning each species lot on one card. The cards should be arranged alphabetically under the genera, the names of which should appear on guide cards, as is done for library card catalogs. Experience will suggest many ways in which the cataloging may be so arranged as to make the collection most useful, which is its legitimate function.

In closing let me say that a collection of mollusks is valuable principally for the information which it may contain. It is of paramount importance, therefore, that the data or information concerning each lot of specimens be made as accurate and complete as possible. This should be done in the field if possible and not left until later when memory may play one tricks as to the exact habitat of some specimens in a large lot. There are many questions still unsettled regarding the classification, geographic distribution, ecological habitat, and economic importance of this class of animals and any conscientious collector may add real scientific knowledge concerning some common species by exercising care and intelligence in making collections.

#### SOME PAPERS RELATING TO THE COLLECTING AND PREPARATION OF MOLLUSCA FOR BOTH EXHIBITION AND STUDY

BAKER, FRANK C.

1898. Mollusca of the Chicago Area. The Pelecypoda. Section VI, Instructions for Collecting Mollusks, pp. 25-32.

1900. A new Museum Tablet. Amer. Nat., XXXIV, pp. 283-284.

1902. The Descriptive Arrangement of Museum Collections. *The Museums Journal* (English), II, pp. 106-110.
1904. The Arrangement of the Collection of Mollusca in the Chicago Academy of Sciences. *Museums Journal* (English), II, pp. 354-360.
1909. Suggestions for an Educational Exhibit of Mollusks. *Proc. Amer. Assoc. Museums*, III, pp. 56-59.
1910. Same title, *Museums Journal*, IX, pp. 394-397.
- DALL, WILLIAM H.
1892. Instructions for Collecting Mollusks and other Useful Hints for the Conchologist. *Bull. U. S. Nat. Museum*, No. 39, Part G, pp. 1-56.
- STERKI, VICTOR.
1916. Some Directions and Suggestions for Collecting the Sphaeriidae and Aquatic Gastropods. *Annals Carnegie Museum*, X, pp. 478-486.
- WALKER, BRYANT.
1902. Hints on Collecting Land and Fresh-water Mollusca. *Journ. of Applied Microscopy and Laboratory Methods*, V, No. 9, pp. 1954-1961.

# PROCEEDINGS OF THE AMERICAN MICROSCOPICAL SOCIETY

## MINUTES OF THE CHICAGO MEETING

The thirty-ninth annual meeting of the American Microscopical Society was held in affiliation with the A.A.A.S. at Chicago, Ill., Dec. 29, 1920.

In the absence of President Galloway, Vice-President Juday acted as chairman.

The report of the Treasurer for the year 1920 was read by the Secretary and was referred to an auditing committee composed of Professors F. H. Krecker and J. W. Kostir.

The report of the Custodian was read by the Secretary and referred to an auditing committee composed of Messrs. Edw. Pennock and Edw. P. Dolbey. The meeting voted unanimously to send greetings to the Custodian, Mr. Magnus Pflaum, and to congratulate him on the growth of the Spencer-Tolles fund.

The Secretary presented a general report on the affairs of his office.

The following officers were nominated and elected for the constitutional periods: President, Professor Frank Smith, University of Illinois; First Vice-President, Professor J. E. Ackert, Kansas State Agricultural College; Second Vice-President, Professor Ruth Marshall, Rockford College.

Professor E. M. Gilbert, University of Wisconsin, Dr. B. H. Ransom, Bureau of Animal Industry, Professor Chancey Juday, University of Wisconsin, were chosen as the elective members of the Executive Committee for 1921.

Adjourned.

PAUL S. WELCH, *Secretary*

### CUSTODIAN'S REPORT FOR THE YEAR 1920

#### SPENCER-TOLLES FUND

Balance reported for the year 1919.....		\$5,958.93
May 17, Sale of Transactions.....	\$ 150.00	
June 30, Dividend.....	238.32	
Dec. 6, Sale of Transactions.....	80.00	
Dec. 31, Dividends.....	190.41	658.73
		<hr/>
Net amount invested.....		6,617.66
Increase during the year \$658.73.		

#### TOTALS

##### *Receipts*

All contributions.....		800.27
All Sales of Transactions.....	1,108.38	
All Life-members.....	300.00	
All interest and dividends.....	4,699.01	
		<hr/>
		6,907.66

##### *Disbursements*

All grants.....	250.00	
All dues for life-members.....	40.00	290.00
		<hr/>
		6,617.66

Life-members: (Robert Brown, dec'd); J. Stanford Brown; Seth Bunker Capp; Harry B. Dunkanson; A. H. Elliott; John Hately.

Contributions of \$50 and over: John Aspinwal; Iron City Microscopical Society; Magnus Pflaum; Troy Scientific Society.

MAGNUS PFLAUM,

*Custodian.*

Philadelphia, Pa., Jan. 15, 1921.

The undersigned having examined the foregoing report certify that we have found the same true and correct.

EDWARD PENNOCK,  
EDW. P. DOLBEY,  
*Auditing Committee.*

ANNUAL REPORT OF THE TREASURER OF THE AMERICAN  
MICROSCOPICAL SOCIETY  
DECEMBER 24, 1919 TO DECEMBER 17, 1920

RECEIPTS

Balance on hand, December 24, 1919.....	\$ 480.04
Dues received for Volume 38 or before.....	52.10
Dues received for Volume 39.....	186.00
Dues received for Volume 40.....	272.10
Initiation fees.....	90.00
Subscriptions for Volume 38 or before.....	38.00
Subscriptions for Volume 39.....	193.90
Subscriptions for Volume 40.....	28.80
Sales of Transactions, duplicates and back numbers.....	325.70
Advertising, Volume 38.....	192.50
Advertising, Volume 39.....	14.00
Grant from Spencer-Tolles Fund.....	100.00
Messrs. Anderson and Walker, plates.....	76.15
Mr. A. Jacot, plates.....	60.00
Sundries.....	3.12
TOTAL.....	\$2,112.41

EXPENDITURES

Printing Transactions, Volume 38, No. 4.....	\$ 152.75
Plates for Volume 38, No. 4.....	23.00
Printing Transactions, Volume 39, No. 1.....	397.91
Printing Transactions, Volume 39, No. 2.....	262.51
Printing Transactions, Volume 39, No. 3.....	243.82
Plates for Volume 39, No. 3.....	116.30
Printing Authors' Reprints.....	45.90
Postage and Express for Secretary.....	43.16
Postage and Express for Treasurer.....	8.77
Office expenses of Secretary.....	56.46
Office expenses of Treasurer.....	32.64
Office expenses of Custodian.....	5.00
Secretary, Trip to St. Louis Meeting.....	50.00
Spencer-Tolles Fund.....	230.00
Balance on hand.....	444.19
TOTAL CREDITS.....	\$2,112.41

W. F. HENDERSON, *Treasurer.*

The accounts of W. F. Henderson, Treasurer of the American Microscopical Society, for the year of Dec. 24, 1919, to Dec. 17, 1920, have been examined by the Auditing Committee and found to be correct.

Respectfully submitted,  
F. H. KRECKER,  
W. J. KOSTER,  
*Auditing Committee.*

TRANSACTIONS  
OF THE  
American  
Microscopical Society

ORGANIZED 1878

INCORPORATED 1891

---

PUBLISHED QUARTERLY

BY THE SOCIETY

---

EDITED BY THE SECRETARY

PAUL S. WELCH

ANN ARBOR, MICHIGAN

---

VOLUME XL

NUMBER TWO

---

Entered as Second-class Matter August 13, 1918, at the Post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in Section 1103, of the Act of October 3, 1917, authorized Oct. 21, 1918

---

The Collegiate Press  
GEORGE BANTA PUBLISHING COMPANY  
MENASHA, WISCONSIN  
1921

TABLE OF CONTENTS

FOR VOLUME XL, NUMBER 2, April, 1921

Larval Flukes from Georgia, with two plates, by E. C. Faust.....	49
On the Nature of Structures Characteristic of Cnidosporidian Spores, by R. Kudo....	59
DEPARTMENT OF SUMMARIES	
Recent Advances in Parasitology, by E. C. Faust.....	75
DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES	
A Method for Orienting and Mounting Microscopical Objects in Glycerine, by Charles Bullard.....	89
A Method of Demonstrating the Sheath Structure of a Desmid, with one figure, by W. R. Taylor.....	94

TRANSACTIONS  
OF  
American Microscopical Society

(Published in Quarterly Instalments)

Vol. XL

APRIL, 1921

No. 2

LARVAL FLUKES FROM GEORGIA<sup>1</sup>

(With two Plates)

By

ERNEST CARROLL FAUST

Parasitologist, Union Medical College, Peking

In a previous study (Faust 1919) I have reviewed the cercariae described from the United States. I discussed the regions from which forms had been reported and suggested that the Southeastern United States offered an unexplored field where conditions were eminently favorable for their existence.

From October 1918 to May 1919 I had the opportunity to examine several hundred mollusks from the region of Rome, Georgia, and in two of these species, *Goniobasis carinifera* Lamarck and *Anculosa carinata* Brug, I have discovered the larval flukes described in this paper. I wish to acknowledge my indebtedness to Mrs. Lola Swift Faust for the collection of the material and to Mr. Bryant Walker for the determination of the hosts.

The larvae represented in this investigation are all distomes and fall into the following groups:

Allocreadiine larvae . . . . .	1
xiphidiocercariae . . . . .	3
microcercous cercaria . . . . .	1
echinostome larva . . . . .	1
furcocercariae . . . . .	2
cystocercous cercaria . . . . .	1

All of these forms are new to this region. Two have been described in a previous paper (Faust 1919a).

*Cercaria thalia* nov. spec.

(Figs. 1, 2)

Host: *Goniobasis carinifera* Lamarck.

This interesting larva for which I propose the name *Cercaria thalia* was found in *Goniobasis carinifera* Lamarck, collected from Rotary Lake,

<sup>1</sup> Contributions from the Department of Pathology, Union Medical College, Peking, China.

Rome, Georgia. The redia and cercaria stages were dissected out of the digestive gland of the snail, while the agamodistomes were found in the lung sac. Both the redia and the cercaria were characterized by a graceful movement.

In the cercaria a good share of the organs are rendered opalescent by the sub-integumentary cells which are muciferous rather than cystogenous in structure. The larva is distinguished by a pair of eye-spots just lateral to the pharynx. The body measures 0.4 mm. in length by 0.15 mm. in width. The tail is slightly longer and has a proximal diameter of  $57\mu$ . The oral sucker measures  $71\mu$  in section while the acetabulum when extended has a diameter of  $90\mu$ . No spines have been observed on the body or tail. There is a prepharynx and a small pharynx with a short esophagus showing constrictions at intervals along its course. The ceca terminate about one-third the body distance from the posterior end. There are three pairs of mucin glands, with ducts opening on the dorsal aspect of the orifice.

The excretory bladder has a long median shank and slender cornua so that the entire appearance is that of a furculum with a supporting stem. I have observed a triplet group of flame cells at the anterior extremity of the body and a similar number at the posterior limit, but have been unable to make out the number of groups intermediate. From the group formula which I have previously shown to exist for this family of trematodes (Faust 1919a:334) four other flame cell units are to be expected. A median collecting tubule runs almost the entire distance of the tail.

The agamodistome (Fig. 2) lacks the pigmented eyespots and, indeed, shows few of the larval characters. The most readily recognizable common feature is the excretory bladder. On the other hand this fluke shows evidences of rapid maturity. The shape is that of a mature worm rather than that of a larva. The ceca have enlarged and what is especially noteworthy, the genital organs have reached a high degree of complexity. The testes and ovary are in their relative positions, the vitelline follicles are well formed, the seminal vesicle and the seminal receptacle are each conspicuous and Laurer's canal is distinguishable. So well developed are all of these organs that it is a simple matter to place the worm in the Allocreadinae. Thus a considerable share of the life history of this animal is at hand, even tho only the first larval host is known.

The cercaria is produced in a redia with a small orange-colored gut, a short prepharyngeal region, a small pharynx, and a small but conspicuous birth pore. Moreover, both daughter rediae and cercariae develop within the same parent redia. It is noteworthy that the cercaria possesses eyespots, a common feature of the larval allocreadid form, but lacks a stylet.

*Cercaria camilla* nov. spec.

(Fig. 3)

Host: *Goniobasis carinifera* Lamarck.

The stylet cercaria for which I propose the name *Cercaria camilla* is a rapidly moving larva with an oblong-ovate body 0.16 mm. long by 0.066 mm. wide and a tail 0.11 mm. long and  $18\mu$  in diameter at the base. Both body and tail are spinose. The oral sucker is  $26\mu$  in diameter while the acetabulum measures only  $13\mu$  in transection. The latter lies midway between the anterior and posterior limits of the body.

Inserted into the roof of the anterior end is a typical quilled stylet. The oral sucker leads into an enormous prepharyngeal pocket  $53\mu$  in diameter, with a thick, semi-muscular, semi-mucoid wall. This in turn leads directly into a minute muscular pharynx. Beyond the pharynx a short esophagus connects with a part of short blunt ceca. The mucin glands consist of three pairs. Two of these pairs are granular, acidophilic, while one pair is densely muciferous, basophilic in reaction. The contents of each gland passes thru a long duct to empty at the side of the stylet.

The excretory bladder has the shape of an inverted truncated pyramid, from which emerge delicate collecting tubules. These tubules when traced to their sources reveal on each side of the body four pairs of flame cells posteriorly disposed. A single collecting tubule runs down the middle part of the tail. The flame-cell formula has an identical common denominator with that of *Allocreadium isoporum* Looss (Faust 1919a:327, 334), namely

$$(2 \ 2+2+2) \ (2+2)$$

The parthenita of *C. camilla* is a very simple sac-shaped sporocyst containing eight to twelve cercariae. Encystment of the cercariae has not been observed.

*Cercaria tabitha* nov. spec.

(Fig. 4)

Host: *Goniobasis carinifera* Lamarck.

This cercaria for which I suggest the name *Cercaria tabitha* has an ovoid body 0.15 mm. long by 0.088 mm. wide and a blunt tail 0.1 mm. long by 0.017 mm. in diameter at the base. The body is covered with heavy spines and the oral sucker is provided with a blunt stylet. The large oral sucker,  $32\mu$  in diameter, is provided internally with a thick mucoid substance resembling that of the stylet. Behind this is a small pharynx. There are four mucin glands on each side of the body, one of which contains a basophilic substance. They all empty far laterad at the margin of the oral sucker. The remainder of the digestive tract has not been traced. The acetabulum is in the middle of the ventral side of the body. It measures  $21\mu$  in diameter.

The excretory bladder is cup-shaped. The collecting tubules emerge from the anterolateral aspect of the bladder while the pore is posteriad.

The sporocyst is a simple, sacculate structure, containing up to twenty-four larvae.

The tail of the cercaria is dropped readily under a cover glass, but encystment has not been observed.

*Cercaria pandora* nov. spec.

(Fig. 8)

Host: *Goniobasis carinifera* Lamarck.

This little larva for which I suggest the name *Cercaria pandora* is oblong-ovate with a body measurement of 0.145 mm. in length by 0.054 mm. in width and a tail not more than half as long by  $17\mu$  in diameter at the base. The anterior sucker is large, measuring  $38\mu$  in diameter. The acetabulum is mesad and very small ( $14\mu$ ). A small stylet with a sharp, delicate point is inserted into the dorsal wall of the oral sucker. A small pharynx is located just behind the orifice. It leads into a forked gut densely surrounded with gland cells. The mucin glands consist of four pairs, the posterior one of which has a large nucleus and gives an acidophilic reaction.

The excretory bladder is a roughly truncated cone with conspicuous lateral cornua. The caudal excretory canal has several tributaries but no observable flame cells.

The larva develops in large numbers in simple sacculate sporocysts.

*Cercaria medea* nov. spec.

(Fig. 7)

Host: *Goniobasis carinifera* Lamarck.

This larval fluke for which I suggest the name *Cercaria medea* has a long, slender body and a short, stubby tail. The latter is so limited in extent as to place the larva in the group of the microcercous cercariae.

The animal measures 0.22 mm. in length by 0.065 mm. in width and has a tail only  $21\mu$  long. The latter structure is semiglandular, the products of which are poured into a common atrium. The cells are chromophobic and have irregular shaped nuclei. The anteriormost part of the body together with the acetabulum bear small sharp spines. The oral sucker has a diameter of  $25\mu$  and the acetabulum of  $27\mu$ . Inserted in the dorsal wall of the oral sucker is a minute, simple-pointed stylet (See fig. 7a).

Behind the oral sucker is a small pharynx. A long, narrow esophagus runs back from this to the anterior aspect of the acetabulum, whence the ceca continue posteriad to the subcaudal region of the body. A group of twelve to fifteen mucin glands is situated on each side of the body posterior

to the acetabulum. A very delicate bundle of ducts conveys the products of these glands to the region of the stylet.

The excretory bladder is long and bag-shaped, giving rise to a pair of cornua laterad just behind the acetabulum. The main collecting tubule on each side of the body bifurcates just anterior to the acetabulum. A median tubule extends into the tail and opens into the mucin pocket.

Two strings of germ cells extend longitudinally across the acetabulum.

The sporocyst in which the cercariae develop varies greatly in size. It has a muscular anterior end and at irregular intervals has constrictions. The movement of both the cercaria and the sporocyst is slow.

*Cercaria penthesilia* nov. spec.

(Fig. 9)

Host: *Goniobasis carinifera* Lamarck.

This cercaria for which I propose the name *Cercaria penthesilia* is an echinostome larva which is probably immature, in which the circlet of collar spines has not yet developed. It measures 0.2 mm. in length by 0.084 mm. in width, and has a tail 0.135 mm. long by  $14\mu$  in diameter at the base. The body is covered with many short spines closely studded together. The oral sucker has a diameter of  $30\mu$  and the acetabulum of  $32\mu$ . The entire body has a thick subintegumentary lining of long rhabditiform cystogenous granules, which bear evidence of the animal's future encystment. A fluted keel is found in the distal third of the tail.

There is a short prepharyngeal region of the digestive tube followed by a small pharynx. The esophagus forks almost immediately to form ceca which extend far caudad. Paired groups of acidophilic mucin glands run mesad to the ceca.

The excretory bladder is spheroidal with a large opening dorsocaudad. The cornu on each side is quite inconspicuous. From it a dilated collecting tube is traced which becomes narrow in the region of the pharynx. Several flame cells have been found but the exact number has not been worked out. Running posteriad from the bladder is a collecting tube for the tail. Half-way down the tail it divides to form a pair of tubules opening laterad.

The genital system is represented by two groups of germ cells lying longitudinally across the acetabulum. The nervous system is conspicuous, with especially large ventral trunks.

The redia in which the cercaria develops is provided with the pharynx, gut, birthpore and lateral appendages found in the echinostome group.

*Cercaria quattuor-solenata* Faust 1919

and

*Cercaria furcicauda* Faust 1919

(Figs. 5, 10)

These furcicaudous species, *Cercaria quattuor-solenata*, and *C. furcicauda*, were originally described in connection with a study of the excretory

system of distome cercariae (Faust 1919a: 337, 338) and are included here for the sake of completeness. The host of both species is *Anculosa carinata* Brug.

*Cercaria stephanocauda* nov. spec.  
(Fig. 6)

This interesting larva which I have designated as *Cercaria stephanocauda* represents a group which has recently received considerable attention (Ward 1916, Pratt 1919, Faust 1918). Five species have been described from North America. While in many respects the structure of the body of the immature larva resembles that of the described species, the tail is unique.

The worm has a body 2 mm. long and 1.2 mm. wide. The shank of the tail is 4 mm. long, while the lamellate furcae measure 1.1 mm. long and 0.5 mm. wide. The anterior fourth of the tail shank consists of a collared region with about nine definite ringed constrictions, running around the tail. At the posterior end of this collar there are numerous tubercles in a single row which are the only traces of mammilations anywhere on the body. Behind this collar the tail proceeds toward the distal portion with gradual constriction. The tail is attached to the body proximally by a number of powerful longitudinal muscles.

The oral sucker measures  $430\mu$  in diameter and the smaller ventral sucker,  $360\mu$ . The pharynx just behind the oral sucker has a diameter of  $200\mu$ . It connects with the ceca by a very short esophagus. The ceca proceed directly laterad almost to the posterior margin of the body. They are slightly convoluted.

The excretory system consists of a minute bladder and large collecting tubules. The latter reach to the region of the oral sucker, then are reflexed and break up into capillaries. A large collecting tubule extends the length of the tail, forking into the furcae and opening outward at the distal end of each furca.

The genital glands are very immature as contrasted with the condition in *Cercaria macrostoma* (Faust 1918). The germ glands are connected by a chain of cells which lie in the median line, with one gland offside to the left.

The sporocyst is large, being a simple sac with undifferentiated tissue.

#### SUMMARY

A study of cercariae taken from snails at Rome, Georgia, shows new species with interesting relations to previously described forms.

## REFERENCES CITED

FAUST, E. C.

1918. Two new cystocercous cercariae from North America. *Jour. Parasit.*, 4:148-153, 1 pl.

1919. A biological survey of described cercariae in the United States. *Am. Nat.*, 53:85-92.

1919a. The excretory system in Digenea. II. Observations on the excretory system in distome cercariae. *Biol. Bull.*, 36:322-339, 10 figs.

PRATT, H. S.

1919. A new cystocercous cercaria. *Jour. Parasit.*, 5:128-131, 2 figs.

WARD, H. B.

1916. Notes on two free-swimming larval trematodes from North America. *Jour. Parasit.*, 3:10-20, 1 pl.

## DESCRIPTION OF PLATES

- Figs. 1, 2.—*Cercaria thalia* 1, cercaria, ventral view, X 126; 2, agamodistome, ventral view, showing precocious development of genital organs, X 40.
- Fig. 3.—*Cercaria camilla*, ventral view, X 400.
- Fig. 4.—*Cercaria tabitha*, ventral view, X 285.
- Fig. 5.—*Cercaria quattuor-solenata*, ventral view, X 200.
- Fig. 6.—*Cercaria stephanocauda*, ventral view, X 28.
- Fig. 7.—*Cercaria medea*, ventral view, X 90; 7a, detail of stylet.
- Fig. 8.—*Cercaria pandora*, ventral view, X 285.
- Fig. 9.—*Cercaria penthesilia*, ventral view, X 276.
- Fig. 10.—*Cercaria furcicauda*, ventral view, X 200.

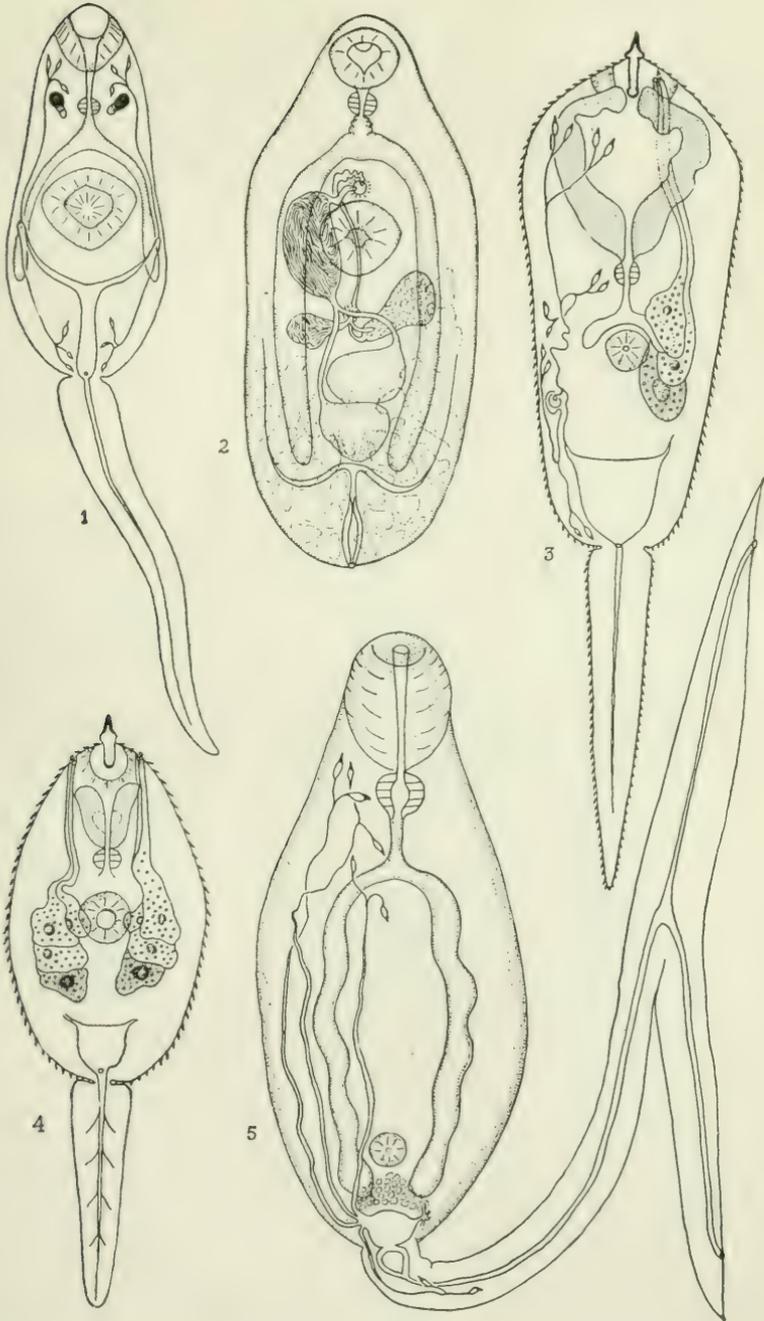


PLATE II.

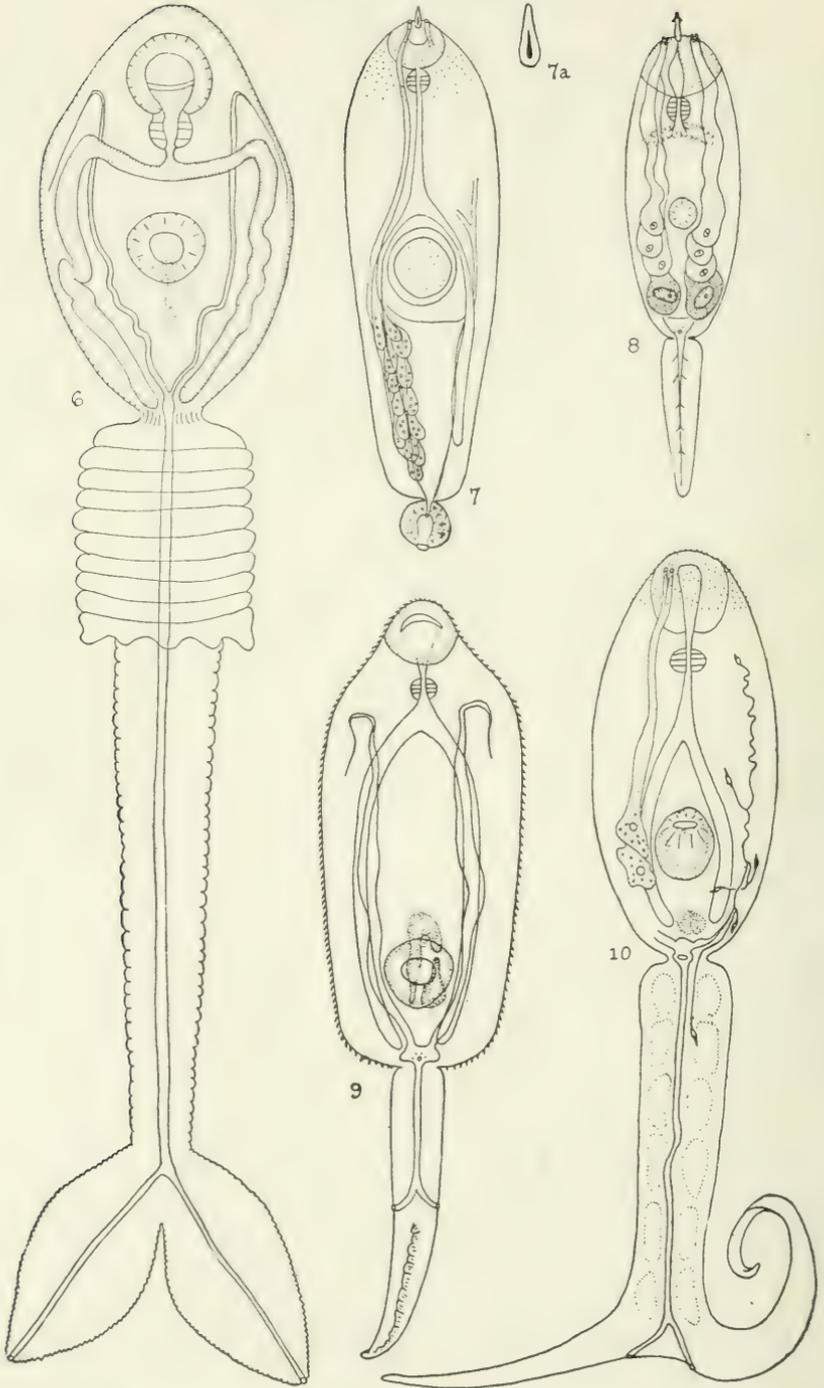


PLATE III.

ON THE NATURE OF STRUCTURES CHARACTERISTIC  
OF CNIDOSPORIDIAN SPORES<sup>1</sup>

By  
R. KUDO

CONTENTS

Introduction.....	59
Material and methods.....	60
The spore membrane.....	60
The polar filament.....	65
The iodophilous vacuole.....	70
Summary.....	73
Bibliography.....	73

INTRODUCTION

Although our knowledge of the morphology and development of Cnidosporidia has recently been greatly increased, little is known about the chemical nature of the different parts which compose the spores. In the study of artificial cultivation of Cnidosporidia, it becomes necessary to obtain a definite view regarding this point. Unfortunately the majority of opinions advanced by several authors are not accompanied by any definite experimental data. This is possibly due to the fact that because the organisms have never been found in direct contact with higher vertebrates as their parasites, they have not interested such a large number of investigators as did other parasitic Protozoa which are directly responsible for serious diseases among mammals, and that the number of organisms found at one time is not generally large so that only little is left when the study of their morphology and development is completed.

Erdmann (1917:317-318) has recently expressed a view that the polar filaments of the spore of *Chloromyxum leydigi* were probably composed of glycogen and plastin, and further suggested, in referring to my paper on the polar filament of the spore of *Nosema bombycis* (Kudo, 1913), that her method might be useful for the study of the nature of polar filaments of the Microsporidian.

I have been working for some time on the subject by using several species of Microsporidia and Myxosporidia. The results thus far obtained in regard to the polar filaments are entirely different from those of Erdmann's. Besides, as I believe the results of observations upon the nature of spore membrane and the so-called iodophilous vacuole seem to be

<sup>1</sup> Contribution from the Zoological Laboratory of the University of Illinois, No. 182.

<sup>2</sup> I am greatly indebted to Professor Henry B. Ward who kindly placed the material at my disposal.

more or less interesting, they are summarized and presented in the following pages.

#### MATERIAL AND METHODS

For the study of spore membrane of Myxosporidia, *Henneguya salminicola* Ward<sup>2</sup> was chosen. This species, as was described by Ward (1920), forms numerous large cysts in the host tissue. Moreover, it was not only obtainable in a very large number, but was also favorable for the study due to the presence of the posterior process characteristic of the genus. The Myxosporidian had been preserved in formol since 1914 together with the host tissue. The cysts were isolated, washed thoroughly with distilled water, and the cyst wall was punctured. The emulsion thus obtained was used to make numerous smears. For Microsporidia, spores of *Nosema bombycis* Nägeli and *Nosema apis* Zander were selected. Although they are much smaller than such a form as *Thelohania magna* Kudo (Kudo, 1920), the enormous number that could be procured favored their selection as representatives of Microsporidia. The infected silk-worm moths and the infected ventriculus and intestine of honey bees were emulsified with distilled water, and were used as stock emulsions.

The nature of the polar filaments was studied by using the fresh spores of *Myxobolus mesentericus* Kudo, *Mitraspora elongata* Kudo, *Leptotheca ohlmacheri* (Gurley) Labbé, *Nosema bombycis*, *Nosema apis* and *Thelohania magna*.

The following four species of the family Myxobolidae were used for the study of iodophilous vacuole. They were fixed either in formol or sublimate alcohol as are indicated below: *Myxobolus discrepans* Kudo and *Myxobolus mesentericus* Kudo fixed in sublimate alcohol, and preserved in 95% alcohol; *Myxobolus aureatus* Ward and *Henneguya salminicola* Ward fixed and preserved in formol. Besides, the following five species from other genera were also studied for the comparison: *Wardia ovinocua* Kudo, *Mitraspora elongata* Kudo, *Leptotheca ohlmacheri* (Gurley) Labbé, *Chloromyxum wardi* Kudo and *Myxidium americanum* Kudo.

The experiments were conducted both in smears and section preparations. In former case, the smears were first allowed to dry before the application of chemicals or stains especially for the determination of spore membrane. Detailed descriptions of methods used will be given in the corresponding chapters.

#### THE SPORE MEMBRANE

The strong resistance of spores of *Nosema bombycis* against certain chemicals has long been known by the studies of Frei and Lebert (1856) and Haberlandt and Verson (1870), as the parasite is the cause of the well known pebrine disease of silk worms. These authors, however, attacked

the problem with the aim of destroying the spores rather than the determination of its nature.

As to the spore membrane of Myxosporidia, Bütschli seems to be the first who studied the effect of concentrated sulphuric acid upon the spores of *Myxobolus mülleri* Bütschli. He (Bütschli, 1881:634) states as follows: "Die Schalensubstanz besitzt eine recht ansehnliche Widerstands-fähigkeit gegen Reagentien; dennoch ist die Angabe Balbiani's, dass sie auch in erhitzten Mineralsäuren sich erhalte, nicht begründet. Erstmaliges Erhitzen in konzentrierter Schwefelsäure liess zwar die Schalen nur in ihre beiden Klappen zerfallen, zerstörte dagegen die sogleich zu erwähnenden Polkapseln völlig; nochmaliges Erhitzen bewirkt jedoch auch völlige Zerstörung der Schalen." Balbiani (1883:202) on the other hand states that the boiling sulphuric acid does not affect the Myxosporidian spore membrane. Gurley (1894:83) agreed with Bütschli, writing as follows: "This (shell) substance is thin, very transparent, insoluble in the strongest acids and alkalies in the cold, certainly in some, and probably in most species destroyed by (soluble in ?) concentrated sulphuric acid at its boiling temperature."

It is my opinion that the controversy of the results of experiments among these authors are probably due to the difference in the concentration of the acid used. Although none of the investigators mentioned their exact technique, it is almost certain that they added concentrated sulphuric acid to the emulsion of the spores they had. Consequently they did not observe the effect of the truly concentrated sulphuric acid, but that of more or less diluted acid which varied from a stronger concentration (Bütschli and Gurley) to a weaker one (Balbiani) according to the relative amounts of water and the acid. In order to avoid this error in the present experiments, the smears of spore emulsions were dried on the slides before they were subjected to the action of reagents.

None of the above mentioned three authors, however, has expressed an opinion in regard to the chemical nature of the membrane. It was Thélohan who advanced his observations concerning this point. Yet in his valuable work on Myxosporidia, Thélohan (1895:260) simply states as follows: "Je n'ai pas déterminé la nature chimique de la substance qui constitue l'enveloppe. Elle ne présente en tout cas aucun des caractères de la cellulose."

Although a large number of papers on Cnidosporeidia has appeared lately, none touches this problem. Davis (1917:210) states that "surrounding the spore is a thin, tough, transparent membrane, the sporocyst, which is probably of a chitinoid nature." He, however, does not give any experimental datum to support this statement. Auerbach (1910:17) in his monograph wrote negatively as follows: "Die chemische Zusammensetzung der Schale ist meines Wissens noch nicht sicher bekannt."

Thus the opinions of a few investigators regarding the chemical nature of the Cnidosporidian spore membrane may be summarized as follows: The spore membrane of Myxosporidia does not give a positive cellulose reaction, and seems to be of chitinoid nature.

The results of my experiments will be reported here.

a) Tests for albuminoid substances:—Spores of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis* are not affected by boiling potassium hydrate solution (35 per cent), and do not give any recognizable positive Millon's reaction. It may therefore be said that the spore membrane is not composed of albuminoid substances.

b) Tests for cellulose:—For the control of the cellulose tests, a filter paper was used.

1) Ammoniacal solution of copper oxide. Six fibers of the filter paper were taken out, and were mounted on two slides, each containing three fibers. To one distilled water was added, while to the other ammoniacal solution of copper oxide. Both were covered with cover glasses. The water emulsions of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis* were smeared on slides, were dried, and were treated in the same way as the fibers of filter paper. The preparations were kept in a moist chamber. The results of observations are as follows:

	Soon after the treatment	16 hours later	36 hours later
Cellulose with water	Outline of fibers sharp	No change	No change
Cellulose with ammon. solution of copper oxide	Outline of fibers less sharp	Outline faint	More invisible
<i>Henneguya salm.</i> , <i>Nosema bombycis</i> and <i>N. apis</i> with water	Outline of spores sharp	No change	No change
<i>Henn. salminicola</i> , <i>Nosema apis</i> , and <i>N. bombycis</i> with amm. sol. of copper oxide	Outline of spores sharp	No change	No change

From the above, it is clear that the ammoniacal solution of copper oxide does not dissolve the spore membrane of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis*.

2) Lugol solution and sulphuric acid. Small pieces of filter paper were treated with Lugol solution, washed with distilled water, and were dried. They were mounted on slides in distilled water and in 50 per cent sulphuric acid respectively. Dried smears of spore emulsion of *Henneguya*

*salminicola*, *Nosema apis* and *Nosema bombycis* were treated in a similar way. The results of observations are as follows:

	Soon after preparation
Cellulose: water and H <sub>2</sub> SO <sub>4</sub>	No coloration
Cellulose: Lugol	Deep brown
Cellulose: Lugol and H <sub>2</sub> SO <sub>4</sub>	Violet
<i>Henn. salm.</i> : water and H <sub>2</sub> SO <sub>4</sub>	No coloration
<i>Henn. salm.</i> : Lugol	Slightly yellowish
<i>Henn. salm.</i> : Lugol and H <sub>2</sub> SO <sub>4</sub>	Slightly yellowish
<i>Nosema apis</i> and <i>bombycis</i> : water and H <sub>2</sub> SO <sub>4</sub>	No coloration
<i>Nosema apis</i> and <i>bombycis</i> : Lugol	Almost unstained
<i>Nosema apis</i> and <i>bombycis</i> : Lugol and H <sub>2</sub> SO <sub>4</sub>	Almost unstained

3) Zinc chloride-iodine-potassium iodide mixture. Fibers of a filter paper and dried smears of spores of *Henneguya salminicola*, *Nosema bombycis*, and *Nosema apis* were treated with the following mixture: Zincum chloratum pur. sicc. 20 gr., potassium iodide 6.5 gr., iodine 1.3 gr., and distilled water 10.5 cc. The results of observations are as follows:

	Soon after preparation	16 hours later
Cellulose with water	No staining	No staining
Cellulose with mixture	Violet blue	Violet blue
The spores with water	No staining	No staining
The spores with mixture	Slightly yellowish; iodophilous vacuole of <i>Henneguya</i> brownish	No change

As will be seen from the above experiments, none of the cellulose tests gives positive reaction. It may therefore be stated as was remarked by Thélohan (1895) that the spore membrane of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis* is not of cellulose nature.

c) Tests for chitin:—For the control of chitin test, I have prepared chitin from the wings of honey bees.

1) Alkalies and acids. Dried smears of spores of *Henneguya salminicola*, *Nosema bombycis*, and *Nosema apis*, were treated with potassium hydrate solution and mineral acids. The results are as follows:

	Chitin	Spore membrane of <i>Henn. salminicola</i>	Spore membrane of <i>Nosema apis</i> and <i>Nosema bombycis</i>
Boiling KOH (35%)	Insoluble	Insoluble	Insoluble
Boiling dilute HNO <sub>3</sub>	Soluble	Spore becomes more or less swollen; contents attacked	Spore becomes greatly swollen and hardly visible
Boiling conc. HNO <sub>3</sub>	Soluble	Spore becomes larger; spore membrane of uniform thickness; less refractive	Spore becomes extremely enlarged; invisible.
Conc. HCl (room temp.)	Soluble	Slightly soluble; outline irregular	Spore enlarged and invisible
Boiling conc. HCl	Soluble	Attacked	Disintegrates rapidly
Dilute H <sub>2</sub> SO <sub>4</sub>	Insoluble	Insoluble	Insoluble
Conc. H <sub>2</sub> SO <sub>4</sub> (room temp)	Soluble	Membrane becomes thinner; outline irregular; valves split; caudal filament broken	Greatly enlarged and invisible
Boiling conc. H <sub>2</sub> SO <sub>4</sub>	Soluble	Completely dissolved	Completely dissolved

2) Zinc chloride and Lugol solution. The spore emulsions of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis* were mixed with potassium hydrate solution (35 per cent), and washed thoroughly by centrifugation. After being partly dried, 1 cc. of a mixture of 33 $\frac{1}{3}$  per cent aqueous solution of zinc chloride (10 cc.) and of strong Lugol solution (5 drops) was added, and observed. The results follow

	Color reaction
Chitin	Dark brown
<i>Henneguya salminicola</i>	Spore membrane very slightly yellowish
<i>Nosema bombycis</i> and <i>Nosema apis</i>	Spore membrane unstained

3) Potassium iodide and sulphuric acid. Chitin and dried smears of *Henneguya salminicola*, *Nosema bombycis* and *Nosema apis* were boiled at

160°C with potassium hydrate solution (35 per cent) for thirty minutes, washed thoroughly with 90 per cent alcohol, and then with distilled water. They were then treated with a weak solution of potassium iodide which had been acidified with sulphuric acid, and were examined. The results are as follows:

	Color reaction
Chitin	Bluish violet
<i>Henneguya salminicola</i>	No visible staining of the membrane
<i>Nosema bombycis</i> and <i>Nosema apis</i>	No visible staining of the membrane

As will be seen from the above experiments, the staining reaction gives very ambiguous results. On the other hand, the effect of mineral acids upon the spore membrane seems to be decisive. The spore membrane of *Nosema bombycis* and *Nosema apis* behaves very much like chitin under the influence of mineral acids, while that of *Henneguya salminicola* is more or less different in this respect.

#### THE POLAR FILAMENT

Thélohan (1890:207) expressed an opinion that the substance composing the wall of the polar capsule was identical with that composing the spore membrane, as both stained in the same way with safranin. This view probably led Minchin (1912:399) to state that "a polar capsule is a hollow, pearshaped body with a tough envelope, probably chitinous in nature. . . . Coiled up within the capsule is a delicate filament, often of great length, probably of the same nature as the capsule, and continuous with it." Minchin does not give any evidence to support this view. Davis (1917:210) possibly referred to Minchin, although he did not make it clear, when he stated as follows: "Surrounding the capsule is a tough, refractive envelope, probably chitinous. . . . Coiled up within the capsule is a delicate filament, usually of comparatively great length, which is probably of the same material as the capsule."

Erdmann (1917:317), by studying the developing polar filament of *Chloromyxum leydigi*, came to the conclusion that the polar filament is composed of glycogen and plastin. She writes as follows: "Die vier Polkapseln füllen jetzt ihre Mutterzelle aus, die Plastinscheiben werden zu Plastinringen, die durch Glykogen verbunden sind. Der Polfaden ist

entstanden. . . . Dagegen kann ich die Umwandlung des Chromatins and des Plasmas in eine stark färbare Substanz, nach meinen Befunden, Glykogen, bestätigen." To support this view, Erdmann further remarked the difficulty in extruding the polar filament of spores of the species of the genus *Chloromyxum* as follows "Ausgestreckte Polfäden von *Chloromyxum* sporen sind kaum an Präparaten beobachtet. Thélohan (Taf. IX, Fig. 100c) bildet die Polfäden einer frischen Spore von *Chloromyxum quadratum* ab, Erdmann *Chloromyxum leydigi* mit kurzem Polfäden. Auerbach ist es nicht gelungen, bei *Chloromyxum dubium* sie zu zeigen. Lebzelter erwähnt sie nicht bei *Chloromyxum thymalli*. Durch eine von mir ausprobierte Methode der Fixierung (100 Proz. Alkohol bis auf 40 Grad erhitzt) gelingt es leicht, die Polfäden zum Austreten zu bringen und zu fixieren. Glykogenfärbung nach Fixierung zeigt, dass der Polfaden aus Glykogen und einer platinähnlichen Substanz zusammengesetzt ist (Taf. 14, Fig. 27)." I have, however, had no difficulty in causing the extrusion of the polar filament from the fresh spores of three out of five species of *Chloromyxum* which I have studied up to date. In *Chloromyxum misgurni* Kudo (Kudo 1916, Fig. 3e), *Chloromyxum fujitai* Kudo (drawings were omitted due to the lack of space) and *Chloromyxum trijugum* Kudo (Kudo 1919: Fig. 181), I have caused the filament extrusion. The other two species, *Chloromyxum catostomi* Kudo (Kudo 1919) and *Chloromyxum wardi* Kudo (Kudo 1919) were studied only in fixed specimens, and no attempt was made to cause the filament extrusion. Erdmann has probably studied a small number of spores.

Regarding the various methods which had been reported by several investigators as successful in extruding the polar filaments of various Cnidosporidian spores, I already summarized them in one of my papers (Kudo 1918). Of many methods which I have tried since that date, the following gave always the best results. For Myxosporidian spores, potassium hydrate solution or perhydrol will always bring out satisfactory results. This is especially true in the case of tissue infecting forms. When the spores are found in the gall bladder or urinary bladder, the best results are obtained by centrifuging the spore containing bile or urine followed by repeated washing with distilled water before the spores are subjected to the influence of the chemicals, although this is not of absolute necessity. Yet when the number of spores present in the bile or urine is very small, the treatment is favorable as the addition of potassium hydrate solution to the bile produces a large amount of precipitation which hinders the observation greatly. In most cases, no staining of the filament of Myxosporidian spores seems to be necessary, due to the favorable thickness and distinctness even in unstained state. For staining, Fontana's method of staining spirochaetes, or Giemsa's solution gives beautiful results. The

latter has its advantage over the former in bringing out the differentiation of nucleus and sporoplasm beside the filament, although in some cases the filament does not take the stain for unknown reasons. For Microsporidia, mechanical pressure or perhydrol gives beautiful preparations of extruded filaments. As I did not describe the detail of the method used (Kudo 1913), I have recently described the exact technique for the application of mechanical pressure elsewhere (Kudo 1920).

Although Erdmann was apparently unaware of it, the filament extrusion under the effect of absolute alcohol had been described by Ohlmacher (1893) in the case of *Leptotheca ohlmacheri* (Gurley) Labbé. In the section preparations of kidneys of *Bufo lentiginosus* fixed with absolute alcohol, Ohlmacher saw a number of spores with extruded polar filaments. Ohlmacher was of the opinion that "it is, of course, evident that they (polar filaments) must have been thrown out from spores before the organisms were killed by the alcohol employed in fixing." According to my own observations on a large number of section preparations obtained from *Rana clamitans*, it is clear that in Ohlmacher's preparation, the fixation with absolute alcohol which caused vigorous shrinkage of the spore membrane and sporoplasm, was only responsible for the presence of spores with extruded polar filaments. It is certain that the absolute alcohol method of Erdmann is according to my comparative study on various methods far inferior in having incompleteness and irregularity in its action.

The results of my observations are as follows:

1) The effect of water upon the polar filament. To determine whether the extruded polar filaments of spores of *Nosema bombycis*, *Nosema apis*, *Myxobolus mesentericus* and *Leptotheca ohlmacheri* are soluble in water or not, fresh spores were subjected to mechanical pressure. After removing the coverglasses, the smears were covered with distilled water, and were kept in a moist chamber. The examinations were done under a dark field microscope, and also in Fontana preparations. The results were similar in four species, which are as follows:

	Results of Observations
Control: soon after the application of mechanical pressure	Extruded polar filaments
One day in water	Polar filaments unchanged
Two days in water	Polar filaments unchanged
Four days in water	Polar filaments unchanged
Eight days in water	Polar filaments unchanged

The experiments were repeated many times on other species than mentioned above, but always giving the same results. From these experiments it may be concluded that the polar filaments of the spores of Cnidosporidia mentioned above are insoluble in distilled water at room temperature.

2) The effect of filtered saliva upon the polar filaments. To determine whether the extruded polar filaments of spores of the species mentioned above are soluble in filtered saliva or not, fresh spores were pressed mechanically. A drop of filtered saliva was added to each smear, and the smears were kept in a moist chamber. The examinations were done as in the preceding experiments, and revealed the following results which were practically the same in the four species:

	Observations
Control: soon after the extrusion	Extruded polar filaments
10 minutes in saliva	Polar filaments unchanged
30 minutes in saliva	Polar filaments unchanged
1 hour in saliva	Polar filaments unchanged
3 hours in saliva	Polar filaments unchanged
16 hours in saliva	Polar filaments unchanged
32 hours in saliva	Polar filaments unchanged
3 days in saliva	Polar filaments unchanged
6 days in saliva	Polar filaments unchanged

From the experiments, it may be said that the extruded polar filaments of the spores of *Leptotheca ohlmacheri*, *Myxobolus mesentericus*, *Nosema bombycis* and *Nosema apis*, are insoluble in filtered saliva at room temperature.

3) Staining with Lugol solution. The extruded polar filaments of spores of *Nosema bombycis*, *Nosema apis*, *Thelohanian magna*, *Leptotheca ohlmacheri* and *Myxobolus mesentericus* stain uniformly as light yellowish as their spore membrane by Lugol solution, and do not take any deeper color.

4) Staining after Best's method. The extruded polar filaments of spores of *Nosema bombycis*, *Nosema apis*, *Thelohanian magna*, *Leptotheca*

*ohlmacheri* and *Myxobolus mesentericus* remain unstained by Best's method.

5) Staining after Lubarsch's method. The extruded polar filaments of spores of *Nosema bombycis*, *Nosema apis*, *Thelohania magna*, *Leptotheca ohlmacheri* and *Myxobolus mesentericus* stain uniformly slightly bluish-violet by Lubarsch's method. The polar capsules of the latter two species and the spore membranes frequently stain deep violet.

6) Staining by Löffler's method. The extruded polar filaments of spores of *Nosema bombycis* (Kudo, 1913), and of *Nosema apis*, *Thelohania magna*, *Leptotheca ohlmacheri* and *Chloromyxum trijugum* are stained deep violet by Löffler's method. The spore membrane is also stained in the same color.

7) Staining with Giemsa's stain. The extruded polar filament of *Nosema bombycis* (Kudo, 1916), and of *Nosema apis* and *Chloromyxum trijugum* (Kudo 1920, Fig. 181) have been stained in deep, dark red. The polar capsules of the latter species and the spore membrane also frequently stain the same color.

8) Staining with Fontana's mixture for staining spirochoetes. The extruded polar filaments of *Nosema bombycis*, *Nosema apis*, *Thelohania magna*, *Thelohania illinoisensis*, *Leptotheca ohlmacheri*, *Chloromyxum trijugum* stain in from yellowish to dark brown color by Fontana's method. The spore membrane takes the stain in the similar manner.

From these experiments, it is clear that the polar filaments of spores of various species of Cnidosporidia, which have been listed in the above, are not composed of glycogen as was thought by Erdmann in the case of *Chloromyxum leydigi*. The only means which led Erdmann to the already quoted conclusion regarding the chemical nature of the polar filament is the results of Lubarsch's staining. My experiments have shown clearly that while this staining brings out more or less bluish stained filaments, other tests for its glycogenous nature proved to be negative. The staining effect of Löffler's method is similar to that on the flagella of *Bacillus typhosus*, and that of Fontana's method is exactly the same as that on various spirochoetes.

As to its true nature, I am, however, still unable to determine. It has been noted by many investigators in numerous species of Myxosporidia, and by myself in *Leptotheca ohlmacheri* and *Thelohania magna* that the nucleus for the polar capsule becomes nebulous or diffused during the formation of the polar filament. The chromatic substance of the nucleus breaks up into numerous small granules and a large part of it unites with a peculiar substance or substances which become differentiated in the capsulogenous cell, first in a retort shape and then in rounded form. The polar filament is apparently formed from this mixture.

## THE IODINOPHILOUS VACUOLE

In the mature spores of Myxosporidia belonging to the family Myxobolidae, there exists regularly a more or less conspicuous rounded space which is generally known as an iodophilous vacuole because of its behavior toward iodine.

Müller (1841) seems to be the first to notice this peculiar structure and figured vacuoles in his drawings. Bütschli (1881:636) observed the vacuole in the spore of *Myxobolus mülleri* which had previously been seen by Müller, and designated it as a nucleus. He described the structure as follows: "Von besonderem Interesse erscheint das unzweifelhafte Vorhandensein eines Zellkerns in der plasmatischen Inhaltmasse der Sporen. Häufig ist dieser Kern schon in frischem Zustand ohne Weiteres als kreisförmiger bis ovaler, heller Fleck recht deutlich sichtbar (Fig. 1n). Besser tritt er jedoch nach Behandlung mit verdünnter Essigsäure oder Jodtinktur hervor und zeigt dann eine dunkle, etwas granulirt erscheinende Hülle (Fig. 2n) und eine Anzahl ziemlich blasser Granula, welche durch den Inhalt zerstreut sind. Leider setzten sich dem Versuch, den Kern zu färben, sehr energische Hindernisse entgegen, da das Färbungsmittel nicht in die Sporenschale eindringt; jedoch kann dieser Umstand nicht gegen Kernnatur des fraglichen Gebildes angeführt werden, da auch das Plasma der Färbung widerstand. Dennoch beobachtete ich einige Fälle deutlicher Kernfärbung bei Anwendung von Alaunkarmin."

That Bütschli's view was not correct was shown by Thélohan (1899: 919-920) who studied the structure more closely with the following statements: "Si, en effet, on traite ces spores par différents réactifs, on acquiert bientôt la certitude que la tache claire observée par M. Bütschli, et décrite et figurée par lui comme un noyau, est en réalité une formation d'ordre tout différent. Peu visible à l'état frais, à-cause de la transparence du protoplasma, elle apparaît plus nettement par l'action de l'alcool, des acides acétique, azotique, osmique ou du nitrate d'argent à 2 pour 100. On la voit alors entourée par le reste de la masse plasmique, qui, coagulée sous l'influence de ces liquides, se distingue par son aspect finement granuleux et sa moindre réfringence. Elle a tous les caractères d'une vacuole creusée au sein de cette masse et remplie d'une substance particulière, remarquable par sa résistance aux réactifs colorants caractéristiques de la substance nucléaire. Seul l'iode se-fixe sur elle, et, tandis que, sous son influence, le reste de la spore prend une coloration d'un jaune pâle, on voit cette vésicule devenir d'un rouge brunâtre qui rappelle absolument la teinte que prend la matière glycogène par l'action de ce réactif. Comme celle-ci, cette substance est insoluble dans l'alcool et garde sa réaction vis-à-vis de l'iode dans les spores conservées dans ce liquide. Comme elle encore, elle est soluble dans les alcalis. Les acides la modifient, et après leur action elle ne se colore plus. Toutefois, je n'ai pu, dans ces circonstances,

obtenir la réduction de la liqueur cupro-potassique." Gurley (1894:209) is in entire accord with the observations of Thélohan quoted above.

Keysseltz (1908:264) is the only other investigator who studied rather closely the structure under consideration. He remarks as follows: "Die Vacuole hat eine rundliche Form; ihre Grösse ist nicht ganz konstant. An der lebenfrischen Spore kann man sie nicht oder kaum bemerken. Nach Behandlung mit *Argentum nitricum*, Alkohol, Osmiumsäure (Vgl. Thélohan) Aqua destillata, gewöhnlichem Wasser (bei einzelner Sporen) beim Erhitzen sowie beim Antrocknen tritt sie deutlicher als heller Bezirk hervor. Sie ist gegen das umgebende Plasma nicht durch eine deutliche Membran abgesetzt. Beim Zusatz von wässriger oder alkoholischer Jodlösung, färbt sich ihr Inhalt mahoganibraun, eine Reaktion, die für die Sporen der *Myxobolen* spezifisch zu sein scheint. Er erscheint dann zuweilen fast homogen, häufiger bemerkt man verschwommene dunklere und hellere Flecke verschiedener Grösse und Form. Der Inhalt scheint mir eine zähflüssige Substanz zu sein, die in der Zelle gleichsam suspendiert ist. In konservierten, mit Farbstoffen behandelten Sporen tingiert sich die Vacuole nicht. Sie imponiert als heller Fleck in der Copula. Durch das Jod wird in der Regel auch in den zwischen den Polkapseln befindlichen Räume ein kleiner nicht scharf umgrenzter Bezirk mahoganibraun gefärbt." Auerbach (1910:16) simply states that "die Vacuole färbt sich bei Zusatz von Jodtinktur braun."

To summarize the views advanced by previous authors, the so-called iodophilous vacuole is stained with iodine mixtures, and therefore is of glycogenous nature.

My observations gave the following results.

1) The effect of distilled water upon the vacuole. In order to determine whether the vacuole is affected by distilled water in partly exposed conditions or not, fresh spores of *Myxobolus mesentericus* and *Henneguya mictospora* were crushed under the cover glass, and were kept in distilled water. After six hours, the smears were treated with Lugol solution, which gave the following results on examination:

	Observations
Control spores without being pressed	Typical coloration of the vacuole
Spores crushed	No visible staining of vacuole-like structure

The experiments show that the contents of the vacuole disappear when placed in contact with distilled water.

2) Treatment with Lugol solution. Smears and section preparations of *Henneguya salminicola*, *Myxobolus aureatus*, *Myxobolus discrepans* and *Henneguya mictospora* were treated with Lugol solution. The vacuoles stained in brownish orange which on warming disappeared.

3) Staining by Lubarsch's method. Section preparations of *Henneguya salminicola* were stained in Lubarsch's mixture, the iodophilous vacuole, the polar capsules as well as spore membrane were stained in deep bluish violet.

4) Staining by Best's method. Section preparations of *Henneguya salminicola* were stained by Best's method. The vacuole took a faint pink color.

5) Staining with Delafield's haematoxylin and Lugol solution. Section preparations and smears of *Henneguya salminicola* and *Myxobolus mesentericus* were first stained with Delafield's haematoxylin. After being washed thoroughly, they were mounted in gum and Lugol mixture. The iodophilous vacuole stained in reddish brown.

From the experiments mentioned above, it is certain that the so-called iodophilous vacuole of spores of the family Myxobolidae contains a substance similar to glycogen in characters.

In the section preparations of the cysts of *Henneguya salminicola*, one sees the appearance of the vacuole as the spore formation proceeds. The glycogenous substance in the sporoplasm remains inconspicuous while the spore is in the pansporoblast, although one can trace the gradual concentration of the substance in it. When the spore matures and separates itself from the other spore, the vacuole becomes sharply outlined. The vacuole reaches its maximum size when the spore is completely formed.

No particular body that corresponds to the iodophilous vacuole was found in the spores of species belonging to families other than Myxobolidae, although I have tested several species repeatedly.

It is generally understood without any experimental evidence that the glycogen occurring in the spores of the family Myxobolidae is probably used for the future development of the sporoplasm. Then it is strange to notice the fact that the spores of other families which are essentially the same in habitat and in many other respects, do not contain the glycogen in such a conspicuous way as in this particular family. The majority of species belonging to the family Myxobolidae attack the tissue of the host, yet some species of the genera *Chloromyxum*, *Myxidium* and *Sphaerospora* and all the species of the family Myxosomatidae which do not show any iodophilous vacuole in the spore at any stage of its development, inhabit also the tissue of the host. Therefore the occurrence of the iodophilous vacuole does not seem to be correlated with the tissue infesting characters of the Myxosporidia, as was suggested by Gurley (1894).

## SUMMARY

1) The spore membrane of *Nosema apis* and *Nosema bombycis*, taken as representatives of Microsporidia, is proved to be composed of a substance similar to chitin in its chemical reaction.

2) The spore membrane of *Henneguya salminicola*, taken as a representative of Myxosporidia, is proved to be composed of a substance, the chemical reactions of which are less similar to those of chitin compared with the microsporidian spore membrane.

3) The polar filaments of cnidosporidian spores are not composed of glycogen as was suggested by Erdmann. They are formed by the mixture of a part of the nucleus and a substance differentiated in the capsulogenous cell.

4) A review of the methods which cause the filament extrusion in Cnidosporidian spores is presented.

5) The so-called iodophilous vacuole of the spores of the family Myxobolidae contains a substance, the chemical reactions of which are similar to those of glycogen.

## BIBLIOGRAPHY

- AUERBACH, M.  
1910. Die Cnidosporidien. Leipzig. 255 pp.
- BALBIANI, G.  
1883. Myxosporidies ou psorospermies des poissons. Journ. micr., 7:197-204.
- BÜTSCHLI, O.  
1881. Beiträge zur Kenntnis der Fischpsorospermien. Zeit. wiss. Zool., 35:627-651.
- DAVIS, H. S.  
1917. The Myxosporidia of the Beaufort region. Bull. Bur. Fish., 35:203-243.
- ERDMANN, RH.  
1917. *Chloromyxum leydigi* und seine Beziehungen zu anderen Myxosporidien. Teil II. Arch. Protist., 37:276-326.
- GURLEY, R. R.  
1894. The Myxosporidia, or psorosperms of fishes, and the epidemics produced by them. Rep. U. S. Fish Comm., 5:65-304.
- KEYSSELITZ, G.  
1908. Die Entwicklung von *Myxobolus pfeifferi* Thélohan. Arch. Protist., 11:252-308.
- KUDO, R.  
1913. Eine neue Methode die Sporen von *Nosema bombycis* Nägeli mit ihren ausgeschnittenen Polfäden dauerhaft zu präparieren und deren Länge genauer zu bestimmen. Zool. Anz., 41:368-372.  
1916. On the structure and life history of *Nosema bombycis* Nägeli. Bull. Imer. Seric. Exper. Stat., 1:31-51.  
1916a. Notes on Myxosporidia found in some fresh water fishes of Japan, with the descriptions of three new species. Jour. Parasit., 3:3-9.  
1918. Experiments on the extrusion of polar filaments of cnidosporidian spores. Jour. Parasit., 4:141-147.

1920. On the structure of some microsporidian spores. *Jour. Parasit.*, 6:178-182.
- 1920a. Studies on Myxosporidia. *Ill. Biol. Monogr.*, 5:243-503, 25 pl. and 2 figs.
1921. Notes on *Nosema apis* Zander. *Jour. Parasit.*, 7:85-90.
- MINCHIN, E. A.
1912. An introduction to the study of the Protozoa. London. 517 pp.
- MÜLLER, J.
1841. Ueber eine eigenthümliche krankhafte parasitische Bildung mit specifisch organisirten Samenkörperchen. *Arch. Anat. Phys. Med.*, 5:466-488.
- OHLMACHER, A. P.
1893. Myxosporidia in the common toad with preliminary observations on two chromophile substances in their spores. *Jour. Amer. Med. Assoc.*, 20:561-567.
- THÉLOHAN, P.
1889. Sur la constitution des spores des Myxosporidies. *C. R. acad. sc.*, 109:919-922.
1890. Contributions à l'étude des Myxosporidies. *Ann. microgr.*, 2:193-213.
1895. Recherches sur les Myxosporidies. *Bull. sc. Fr. Belg.*, 26:100-394.
- WARD, H. B.
1920. Notes on North American Myxosporidia. *Jour. Parasit.*, 6:49-64.

**DEPARTMENT OF SUMMARIES**  
**DEVOTED TO DIGESTS OF PROGRESS IN BIOLOGY**

---

---

**RECENT ADVANCES IN PARASITOLOGY<sup>1</sup>**

BY ERNEST CARROLL FAUST

Parasitology has made extraordinary progress during the last decade. In writing on this topic ten years ago Ward (1910) states increasing interest in problems of medical zoology had made the mass of material at that time so vast that a review of it was difficult. Since then stupendous progress has been made.

These advances have been due in part to the more general recognition of the importance of this science and the relation of its development to human welfare and in part to the stimulus of the World War. While individual investigators have contributed a great wealth of valuable data to the science, the most outstanding discoveries have come as the result of the work of commissions and bureaus, undertaking fundamental problems of parasitology on a comprehensive scale. Such accomplished facts as the eradication of yellow fever and the minimization of malaria in the Panama Canal Zone, the Hookworm and Tuberculosis campaigns of the International Health Board, and results of the Bilharzia Mission in Egypt are outstanding monuments of progress during this decade.

In attacking the problems in hand the life history of the parasite has been frequently worked out. As in the solution of previous protozoan and helminth diseases, a knowledge of the life history has not only been valuable but in most cases the essential factor in the eradication of the evil. Such a knowledge has shown the most practicable way of breaking the vicious cycle.

As a result of the World War world problems have developed in parasitology from what were formerly matters of Oriental or tropical concern. Troops coming from countries subject to tropical diseases, returning home, have brought infections with them. Such is quite likely the case in such protozoan diseases as amebiasis which require no intermediate host for part of their life cycle. But, in addition, the added impetus which has resulted from the study of such infections as World War problems, has made it evident that the pre-war infection in England and America, for example, was much higher than had previously been believed.

The period has been marked by the development of new laboratories and intensive study of parasitic problems in new fields. In part this

<sup>1</sup> Contributions from the Department of Pathology, Peking Union Medical College, Peking, China.

work has been done by investigators, who, native to the region, have gained distinction in such problems. In part it has been accomplished by commissions which have been sent into the country to make these researches. In part it has been brought about by the efforts of those, who, distant from the field, have diligently sought out a solution to the problems, brought to them by explorers.

The progress in parasitology has been stimulated and cooperation of investigators secured by the appearance of several new Journals devoted entirely or for a major part to parasitology. First must be mentioned the *Tropical Disease Bulletin*, London, 1913, a review of all the important literature on the subject of tropical parasitology and medicine. The establishment of the *Journal of Parasitology* (1914) in America affords opportunity for publications of investigations of a type midway between *Parasitology of Cambridge* and the *Annals of the Liverpool School*. More recently the *Kitasato Archives of Experimental Medicine* has entered the field, affording opportunity for workers in the Orient to publish near at home. Just recently a long-felt want has been filled by the appearance of the *American Journal of Hygiene* and the *American Journal of Tropical Medicine*.

Continuing the task of placing in the hands of investigators a dependable and indispensable index of Medical and Veterinary Zoology, Stiles and Hassall (1912) have published their *Index-Catalogue on Cestoda and Cestodaria*. Its value over the *Trematode Catalogue* of the series consists in the more thoro analysis of specific and sub-specific citations with the page reference for each and in the fewer number of errors which inevitably creep into a work of such scope. The long-awaited companion volume on *Roundworms* has recently been issued (1920) and meets the expectations of the most critical reviewer. Along this line one cannot commend too highly the synopses of important papers relating to medical parasitology appearing in the *Tropical Disease Bulletin*. It is to be regretted, however, that the reviewers of this Bulletin have not seen fit to include certain other reviews which, altho technically non-medical, are fundamentally related to medical problems.

New species and new systematology in helminths are brought together in a most workable digest in the chapters on *Platyhelminthes* and *Nemathelminthes* in Ward and Whipple's *Fresh-Water Biology* (1918). Progress in American helminthology is shown in the fact that many of these species are described for the first time and in the introduction of a considerable portion on cercariae to the subchapter on trematodes. The data are made especially valuable by their relation to one another in the form of a key, and are made the more workable by ample illustrations.

The most comprehensive treatise on the subject of human parasitology which has appeared within the decade is *Fantham, Stephens, and Theo-*

bald's "Animal Parasites of Man" (1916), a book which has no equal in point of completeness and in up-to-the-minute information on human entozoa. The writing of a brief review of progress in this field gives an insight into the monumental character of this book. While strides have been made in the science ever since the publication of "Animal Parasites" it remains the reliable compendium and guide to the researcher or practitioner encountering entozoic ailments. In their Manual of Tropical Medicine (1919) Castellani and Chalmers have not only contributed greatly to the knowledge of tropical protozoa, helminths and arthropods from data largely drawn from their own wealth of experience in the Tropics, but they have likewise secured the permanent cooperation of the practitioner in problems of parasitology by presenting the clinical and pathological pictures of these parasitic infections. The manual stands as a lasting memorial to the junior author, who gave his life for the work.

Perusal of the literature of parasitology of the period which is covered in this review reveals a vast wealth of investigation, the major part of which falls within the group of the protozoa. Workers on protozoa have been many and a considerable share of their contributions significant. Certain problems like amebiasis have been studied in new fields. In other cases the life history has been elucidated. In many cases, however, mere symptomatology and diagnosis have been set down, where the lack of new data hardly warrants more than a statement of the case.

Foremost among workers in protozoology are those of the English Schools, comprising Stephens, Fantham, Nuttall, Yorke, Macfie, Wenyon and Porter. With these investigators life histories have played an important rôle. With them, too, detailed descriptions of morphological features have not been neglected. One is most convinced of the thoroughness of the work of the Liverpool School in reviewing the elaborate and most detailed methods which have been followed in the experimental treatment of malaria.

Work on the Continent of Europe of a high character has been done by Laveran, Leger, França, Negri and Galli-Valerio. In the Americas Craig, Kofoid, Darling, Hadley, Chagas and Magalhaes have made noteworthy contributions, while Cleland's solution of dengue in Australia and Miyajima's studies on the tsutsugamuchi deserve the highest praise.

In the words of Wenyon (1915) our knowledge of trypanosomiasis and malaria has reached something like full fruition. Hardly so much can be said of the majority of protozoon infections, partly because the circumstances have not been favorable, partly because the investigations have been side tracked.

In 1911 Novy touched upon the progress that had been made in our knowledge of protozoan infections and their treatment. The life history of *Trypanosoma brucei* had just been demonstrated (1909) and remained

one of the outstanding discoveries of the decade. Little was known of the spirochaetes and their pathogenicity aside from the studies on treponema. The life history of the malarial plasmodium had been well authenticated, but other hematozoon forms were little known. Since then many groups have been carefully studied. Nuttall (1913) has found the life cycle of *Babesia* in dogs, horses and cattle to pass thru certain ticks as intermediate hosts and has discovered curative salts for these infections. Stephens (1914) describes a new tertian malarial parasite, *Plasmodium tenue*, from the Central Provinces, India. Yakimoff (1917) contributes to the knowledge of *Piroplasma*, *Theileria*, *Nuttallia* and *Anaplasma* infections of domestic and semi-domesticated animals of Russian Turkestan. Fantham (1910) and Hadley (1911) have given a clear morphological analysis of *Eimeria avium*.

Again, the studies of Ross and Thomson (1916) on Egyptian sand amebae show the necessity of preventing contamination of dry sand with fecal matter.

Wenyon and O'Conner (1917) have helped to solve the practical treatment of the protozoan infections of man in Egypt. They have been able to standardize treatment of amoebiasis. Three new human Parasites, *Waskia intestinalis* and *Tricercomonas intestinalis* and *Entamoeba nana* have been found in these studies.

Craig (1917) has established a basis for classification of amebae parasitic in man which allows one to profit from his numerous investigations in this field. He recognizes as valid species, *Craigia hominis* and *C. migrans*, *Endamoeba coli*, *E. histolytica* and *E. gingivalis*, and *Vahlkampfia lobospinosa*. A more conservative standard is presented by Dobell in his monograph on the amoebae (1919), a treatise which for its thoroughness commands the attention and admiration both of the theoretical and the practical parasitologist.

Von Prowazek (1913) has published an important paper on *Balantidium coli*. He has carefully reviewed the geographical distribution of the species, described its histology in minute detail and methods of propagation, and has worked out its pathogenicity.

Work of the character of Fantham and Porter's (1914) contribution to the life-history of *Nosema bombi* has been of increasing importance in elucidating the general knowledge of protozoan life cycles and thus contributing indirectly to a knowledge of related human forms where experimental infections are obviously less possible. In reviewing the work on protozoa one is struck by the mass of such work of an excellent character of which lack of space unfortunately does not even permit mention.

Watson's monograph (1916) on Gregarines constitutes a well organized synopsis of new and described species of the group, many species of which had previously been investigated only piecemeal

Dobell (1918) has contributed a valuable memoir in his study of human coccidia. Following up the work of Wenyon (1915) he has described three definitely known species infecting man (*Isospora hominis* Riv. 1878, *Eimeria wenyoni* n. sp. and *E. oxyspora* n. sp.), in addition to throwing doubt on the identity of a third Eimeria species as that of the rabbit (*E. stiedae*).

Moroff (1915), after a searching investigation, places the sarcosporidia close to the gregarines and coccidian forms in the subclass Telosporidia, along with the Haemosporidia.

Wolback's work on the Rocky Mountain spotted fever (1918) has shown that the causal agent of the disease is a minute parasite, present in the blood of infected mammals and in ticks which are capable of transmitting the disease.

Kudo's monograph on the Myxosporidia (1920) is memorable not only as a collation of the work of earlier investigators, but as a survey of the large number of myxosporidian forms studied by Kudo himself.

The work of Poche (1913) on the System of Protozoa is a comprehensive treatment of nomenclature of the group. It is notable for the large number of new orders, suborders and families proposed, many of which are readjustments of rank justified by the increase in number of the group. With the wealth of knowledge of morphology and life histories of the Protozoa careful systematic readjustments of this type are increasingly necessary.

Work on the helminths has been continued by many of the investigators who have already established a name for themselves among parasitologists. In Europe Odhner has contributed further studies to his work on phylogeny and systematology, among the most interesting of which are those on Schistosome and Holostome groups. Goldschmidt has extended his investigations on cytology most successfully. Kossack has monographed the monostomes, while Monticelli and Lühe have contributed much to the knowledge of trematodes. Fuhrmann, Leon and von Ratz have studied the cestodes while Railliet and Henry and Seurat have made notable contributions to the nematodes. The most brilliant work of the younger helminthologists in Europe is undoubtedly that by Leiper.

In America such studies have been continued by Ward, Ransom and Young on Cestodes, by Ward on Trematodes, and by Ward and Ransom on nematodes. In addition there has arisen in the United States a considerable group of younger investigators, of whom La Rue, Cort, Boeck and Van Cleave deserve prominent mention.

In Japan Katsurada, Fujinami and Goto have produced work of high merit. Yoshida, Okanama, Kobayashi and Miyairi have added much to life-history problems.

The contributions on Australian helminths count among their number the investigations of Nicoll, Cleland, S. J. Johnston, T. H. Johnston,

Breinl and Sweet. This summary of important contributors to the science would not be complete without mention of Ssinitzin, Skrjabin and Yaki-moff for Russia and Southwell for India.

Ward (1917) has emphasized the necessity of rearranging forms "so as to express better their correct relationships in the light of more perfect knowledge of their structure." But he adds the essential corollary that it has been his fixed principle never to make any change until he was personally familiar with the form discussed or had acquired such acquaintance with its structure as to know that some change was inevitable and that the proposed modification was defensible on morphological grounds. On this basis he has made fundamental but conservative changes in trematode and acanthocephalan groups and has established order in the nematode group where previously taxonomy was confined most usually to mere descriptions of new species.

The period has experienced an advance in helminthology from an almost purely zoological science to one ministering to the needs of comparative bionomics and medicine.

The outstanding morphological and systematic contribution to our knowledge of the Cestoda during recent years is La Rue's Monograph on the Family Proteocephalidae (1914). Provided with a wealth of American material, supplemented by more than an ordinary amount of types of the group described by European and other workers, La Rue has been enabled to mold the material into a comprehensive and practically exhaustive treatise. His descriptions and drawings are detailed, yet clear, his types are well defined and the amount of material collected, the amount used in study and the location of each specimen in the collection are minutely set down. Added to this are valuable synoptic tables and a workable natural key to the group. The contribution as a whole is such as to place the author immediately in the rank of the foremost helminthologists.

Recently Cooper (1919) has monographed another group of cestoda from fishes which contributes to our knowledge in that group.

Among other contributions on cestode anatomy and phylogeny the work of Douthitt (1915) on Anoplocephalidae is worthy of mention. Because of the care which this investigator used in working over his material and the gradual way in which he built up a natural classification of the group the monograph will serve as a lasting memoir to his efforts.

Ransom (1913) has made possible the statement that *Cysticercus ovis* is the intermediate stage of a dog tapeworm, *Taenia ovis* (Cobbold) Ransom and in working out the life-history of this cestode experimentally has solved a problem of long standing. This species in the bladder-worm stage has thus been proved to be distinct from *Cysticercus cellulosae* and the adult from *Taenia tenella*, *T. solium*, *T. hydatigena*, and *T. marginata*. Treatment of dogs for the tapeworm is found not only to eradicate this per-

plexing economic problem of sheep measles but rids them of other worms of equally serious pathogenicity.

Beddard (1911-1914) has contributed studies from time to time, making known to science a large number of cestodes parasitic in animals in the Zoological Society Gardens (London). Likewise Skrjabin (1914) has contributed to our knowledge of the Cestoda of birds of Russian Turkestan. Fuhrmann (1918) in a detailed survey of the avian cestodes from New Caledonia and Loyalty Isle adds materially to the knowledge of the families Tetrabothriidae, Anoplocephalidae, Davaineidae, Dilepididae, Hymenolepidae, Acoleidae and Amabiliidae.

Thus the comparative work on cestodes has been greatly advanced.

The striking advances in our knowledge of the trematodes have come as life-history problems. Members of the medical profession have been especially sympathetic to this work because it was concerned with flukes most of which affected man. It is particularly noteworthy that all of these without exception have borne out the principle established for *Fasciola hepatica*, that the miracidium penetrates a mollusk, and from the mollusk the cercaria emerges which reaches its definitive host (immediately or intermediately according to the group to which the species belongs) and there becomes mature. Faust (1918) following Ssinitzin's work (1911) has found the sporocyst and the redia stages to be true parthenitae.

A problem which Looss had repeatedly attempted to solve in Egypt and on which Katsurada and Fujinami have contributed much in Japan was the schistosome life history. Credit for the first solution of the life cycle is due to Miyairi and Suzuki (1914) in Japan and later to Leiper and Atkinson for Japanese species and Leiper (1915) for the two Egyptian species. A clear understanding that the miracidium enters a gasteropod and that by change of cycle the cercaria emerges from the snail and directly infects man thru the skin or the mouth has made possible methods for preventing the disease. It has also made possible a clear restatement of the thesis that "The larval metamorphosis of all digenetic trematodes occurs without known exception in the bodies of molluscs belonging to the classes Gasteropods and Lamellibranchia." Leiper (1918) has shown that when once infected the patient is practically incurable. He has found from his Egyptian Researches that

"(1) Transient collections of water are quite safe after recent contamination.

"(2) All permanent collections of water, such as the Nile, canals, marshes and birkets, are potentially dangerous, depending upon the presence of the essential intermediary host.

"(3) The removal of infected persons from a given area would have no effect, at least for some months, in reducing the liability to infection, as the intermediate hosts discharge infective agents for a prolonged period.

“(4) Infected troops can not reinfect themselves or spread the disease directly to others. They could only carry the disease to other parts of the world where a local mollusc could efficiently act as a carrier.

“(5) Infection actually takes place both by the mouth and through the skin.

“(6) Infection in towns is acquired from unfiltered water which is still supplied, even in Cairo, in addition to filtered water, and is delivered by a separate system of pipes.

“(7) Eradication can be effected without the cooperation of infected individuals by destroying the molluscan intermediaries.”

Nakagawa (1916) has unravelled the life cycle of *Paragonimus Westermanni*, showing that the cercaria is developed in *Melania* species and the encysted larva in *Potamon*. These infected crabs when fed to puppies gave rise to typical pulmonary paragonimiasis. Moreover the route of infection has been found to be from the intestinal wall in the vicinity of the jejunum, thru the abdominal cavity, thru the diaphragm and pleural lining, where it bores thru the lung tissue and encysts. Yoshida, working on the same problem entirely independently in Japan, was able to substantiate Nakagawa's results. Kobayashi's work on this fluke in Korea (1918) has hardly as convincing data as those of his colleagues. On the other hand the latter investigator (1915) has clearly shown experimentally that the encysted larva found in several species of Japanese fresh-water fish develops into the human fluke, *Clonorchis sinensis*. Thus far, however, he has not worked out the cercarial phase of the life-history of this worm. Nakagawa (1921) has just published his experimental work on *Fasciolopsis buski*, which he finds to infect the hog as the encysted post-cercarial distomule.

Recently interest in larval trematodes has been revived and the field for study of this group in America has been studied by Cort and Faust, who have shown that specific marks of discrimination in cercariae are important even tho they are minute. These investigators have added data on the larvae which should facilitate life history studies on flukes. Among these studies are those on flame-cell constancy and homology, including the use of this system as a basis for systematic relations. Of importance both to pure science and to medical parasitology, Cort's monograph (1919) on the cercaria of the Japanese blood fluke sets a record for careful study and detail in this group. Furthermore, Cort's study on the stages of development of the schistosome in the definitive host (1921) makes a valuable addition to the ontogeny of the fluke.

One can not overlook the researches of Ssinitzin (1911) in this field. This investigator has not only presented data on many interesting and unique larval flukes, but has presented theories of their phylogenetic relations which are at least extremely suggestive and stimulating.

A morphological paper which has done much to show the necessity of exactness in differentiation of closely related species is that of Ward and Hirsch (1915) on the species of *Paragonimus*. These authors have found the type, size and group relationships of the spines to be distinctly diagnostic, and this fact, coupled with the importance of one of these species to medical science in the Orient makes the work especially significant.

Comparatively few investigators have made important studies during the past decade on the morphology and systematology of parasitic nematodes. Railliet and Henry in France and Ward and Magath in America have published researches which constitute marked exceptions to this lack of such investigation in this group. The first significant analysis of the parasitic Nematodes in America is embodied in Ward's chapter on these worms in Ward and Whipple's *Fresh-Water Biology* (1918).

A most important contribution to the morphology of the nematode is embodied in Magath's monograph of *Camallanus americanus* (1919). This thesis constitutes the most significant work on a single nematode species since the researches of Looss on *Ancylostoma duodenale*. The writer describes in detail the organs and systems of the worm and arrives at the conclusion that formulae for measurement are not dependable but that where doubt arises in systematology there remains only the accurate morphological description of every organ and part of the form in question.

The most widespread campaign ever undertaken by governmental or private interest for the eradication of a particular disease is that which was undertaken by the Rockefeller Foundation for the banishment of hookworm from the earth. In 1909 the Rockefeller Sanitary Commission was created to combat the hookworm in the United States. The findings of this Commission of the prevalence of the worm, the "arrest of physical, mental and moral growth, great loss of life, and noticeable decrease in economic efficiency," together with the success which attended treatment of hookworm infection, led to the establishment in 1913 of the International Health Commission (afterwards known as the International Health Board) with the purpose in view of "extending to other countries the work of eradicating hookworm disease as opportunity should offer" and, so far as practicable, to follow up the treatment and cure of this disease with the establishment of agencies for the promotion of public sanitation and the spread of the knowledge of scientific medicine.

Forthwith this commission proceeded to determine 1) the geographic distribution and the approximate degree of infection, 2) to examine microscopically the cases and cure those infected, and 3) to establish sanitary conditions which would prevent soil-pollution.

At the close of 1918 the Board had solely or cooperatively attacked the problem in the Southern United States, Central Mexico, Cuba, Porto Rico, Jamaica, a considerable share of South America, Egypt, Ceylon,

Siam, The Malay, South China, New Guinea, Papua, Java, Guam, and Queensland, Australia, and new work was under way in the Madras Presidency, India.

While the intensive method of microscopic examination and treatment of patients within a limited area was utilized, the more fundamental purpose of the campaign has been to develop an education propaganda for better sanitary conditions so that the sources of infection will be eliminated.

One of the fundamental life-history problems which has engaged the attention of investigators in several geographically different centers is that of *Ascaris*. Captain Stewart of the Indian Medical Service (1917, 1918) has shown that *Ascaris lumbricoides*, and *A. mystax* can be developed to a certain larval stage in the mouse and rat.

Ransom and Foster (1917, 1919, 1920) have been able to produce individuals more nearly mature in the sheep and goat. The latter writers have shown, however, that these stages of development in animals other than the hog and man do not necessarily imply that the mouse, or rat, sheep or goat serve as intermediate hosts for these parasites. Yoshida (1919), working on guinea-pigs, was able to trace the life history as follows: "The ascarid larvae escape from the egg shell in the intestine of the host and proceed to the abdominal cavity by boring through the wall of intestine. Thence they pierce the diaphragm to enter the pleural cavity, finally penetrating into the lungs from their surface. . . . Furthermore, the lungs are the only necessary and important organ to be passed by the larvae in the course of their development. . . . (They) continue their development and migrate to the mouth cavity through the trachea, again passing down the alimentary canal to the intestine of the host.

Work on the Acanthocephala has been relatively meager. Lühe's digest of the group (1911) has given a basis for Continental investigations while Van Cleave's numerous studies on American species constitute marked progress in methods and thoroughness of investigation.

Almost the entire amount of our knowledge of insects in the rôle of carrier and intermediate hosts of parasitic disease has come within the last few years. It is within this period that the life histories of the trypanosome, the piroplasmas and the spirochaetes have been shown to develop in specific flies, fleas, bugs or lice as the case may be. Moreover, certain tapeworms, especially those of poultry, have been just recently shown to develop as larvae within insect hosts. Finally there is further proof of the importance in the spread of parasitic disease when the insect acts merely as a vector. For these reasons important campaigns against these several insects have been carried on by private forces and by government agencies, foremost of which is the all but complete eradication from the Western Hemisphere of yellow fever by controlling the mosquito transmitting the

disease, with plans under way for a campaign on this insect in the remaining locus of infection.

Thus a considerable share of the problems which confronted parasitology at the beginning of the decade have been carried to completion while others are being gradually sifted out. In their place, however, have come still others which require the greater skill and the wider point of view for their full solution. All of these signs of progress in parasitology indicate that this science is rapidly coming to assume the place which it deserves as the companion of bacteriology and gross pathology.

## IMPORTANT LITERATURE ON PARASITOLOGY

## General

CASTELLANI, A. and CHALMERS, A. J.

1919. Manual of Tropical Medicine. 2436 pp. London.

FANTHAM, H. B., STEPHENS, J. W. W., and THEOBALD, F. V.

1916. The Animal Parasites of Man. 900 pp. London and New York.

STILES, C. W., and HASSALL, A.

1912. Index Catalogue of Medical and Veterinary Zoology. Subjects: Cestoda and Cestodaria. Hyg. Lab. Bull., No. 85, 467 pp.

1920. *Ibid.* Subjects: Roundworms. Hyg. Lab. Bull., No. 114, 886 pp.

WARD, H. B.

1910. Recent Progress in Parasitology. Trans. Am. Micr. Soc., 29:119-158.

1917. On the Structure and Classification of North American Parasitic Worms. Jour. Parasit., 4:1-12.

WARD, H. B. and WHIPPLE, G. C.

1918. Fresh-Water Biology. Chapters on "Parasitic Flatworms" and "Parasitic Nematodes." 1111 pp. New York.

## Protozoa

CRAIG, C. F.

1917. The Classification of the Parasitic Amoebae of Man. Jour. Med. Research 35:425-442.

DOBELL, C.

1918-1919. A Revision of the Coccidia Parasitic in Man. Parasit., 11:147-197, 1 pl.

1919. The Amoebae Parasitic in Man. 155 pp., 4 pl. London.

FANTHAM, H. B.

1910. The Morphology and Life History of *Eimeria (Coccidium) avium*, a Sporozoon Causing a Fatal Disease among Young Grouse. Proc. Zool. Soc. London, 1910: 672-691, 4 pl.

FANTHAM, H. B. and PORTER, ANNIE.

1914. The Morphology, Biology and Economic Importance of *Nosema bombi* n. sp., Parasitic in Various Humble Bees (*Bombus* spp.) Ann. Trop. Med. Parasit., 8:623-638, 1 pl.

HADLEY, P. B.

1911. *Eimeria avium*, a Morphological Study. Arch. Protistenkde., 23:7-50, 2 pl.

KUDO, R.

1920. Studies on Myxosporidia. Ill. Biol. Monogr., 5:244-503, 25 pl.

MOROFF, TH.

1915. Zur Kenntnis der Sarkosporidien. Arch. Protistenkde., 35: 256-315.

NOVY, F. G.

1911. Recent Achievements in Parasitology. 13th Rept. Mich. Acad. Sci., 18-32.

NUTTALL, G. H. F.

1913. The Herter Lectures. III. Piroplasmiasis. Parasit., 6:302-320, 14 figs.

POCHE, F.

1913. Das System der Protozoa. Arch. Protistenkde., 30:125-321, 1 fig.

VON PROWAZEK, S.

1913. Zur Kenntnis der Balantidiosis. Arch. Schiffs-Trop. Hyg., Beihefte 17:369-390, 2 pl.

ROSS, R. and THOMSON, D.

1916. Studies on Egyptian Sand Amoebae. Proc. R. Soc. Med., Sec. Epiderm. and St., Med., 9:38-48.

STEPHENS, J. W. W.

1914. A New Malarial Parasite of Man. *Ann. Trop. Med. Parasit.*, 8:119-128, 3 pl.

WATSON, M. E.

1916. Studies on Gregarines. *Ill. Biol. Monogr.*, 2:213-468, 15 pl.

WENYON, C. M.

1915. Leishmania Problems. *Jour. Trop. Med.*, 18:241-247.

WENYON, C. M. and O'CONNOR, F. W.

1917. An Inquiry into Some Problems affecting the Spread and Incidence of Intestinal Protozoal Infections of British Troops and Natives in Egypt. *Jour. R. Army Med. Corps*, 28:1-34, 151-187, 346-370; 4 pl.

WOLBACK, S. B.

1918. The Etiology and Pathology of Rocky Mountain Spotted Fever. *Jour. Med. Research*, 32:499-508.

YAKIMOFF, W. L.

1917. Parasites du sang des animaux en Transcaucasie. *Bull. Soc. Path., exot.*, 10: 98-99.

#### Cestoda

BEDDARD, F. E.

1911-1914. Contributions to the Anatomy and Systematic Arrangement of the Cestoidea. I-XV. *Proc. Zool. Soc. London*.

COOPER, A. R.

1919. North American Pseudophyllidean Cestodes from Fishes. *Ill. Biol. Monogr.*, 4:295-541, 13 pl.

DOUTHITT, H.

1915. Studies on the Cestode Family Anoplocephalidae. *Ill. Biol. Monogr.*, 1:353-446, 6 pl.

FUHRMANN, O.

1918. Cestodes d'oiseaux de la Nouvelle-Caledonie et des Iles Loyalty. In Sarasin and Roux's *Nova Caledonia, Zoologie*. 2: (Lief, 4) No. 14. 2 pl, 78 figs. Wiesbaden.

LA RUE, G. R.

1914. A Revision of the Cestode Family Proteocephalidae. *Ill. Biol. Monogr.*, 1:1-350, 12 pl.

RANSOM, B. H.

1913. *Cysticercus Ovis*, the Cause of Tapeworm Cysts in Mutton. *Jour. Agr. Research*, 1:15-58, 3 pl., 13 figs.

SKRJAKIN, K. J.

1914. Beitrag zur Kenntnis einige Vogelcestoden. *Centralbl. Bakt. Parasit.*, (I) Orig., 75:59-83.

#### Trematoda

CORT, W. W.

1919. The Cercaria of the Japanese Blood Fluke, *Schistosoma japonicum* Katsurada. *Univ. Calif. Pub., Zool.*, 18:485-507, 3 figs.

1921. The Development of the Japanese Blood Fluke, *Schistosoma japonicum* Katsurada, in its Final Host. *Am. Jour. Hyg.*, 1:1-38, 4 pl.

FAUST, E. C.

1918. Life History Studies on Montana Trematodes. *Ill. Biol. Monogr.*, 4:1-120, 9 pl.

KOBAYASHI, H.

1915. On the Life History and Morphology of *Clonorchis sinensis*. *Centralbl. Bakt. Parasit (I) Orig.*, 75:299-318, 4 pl.

1918. Studies on the Lung-Fluke in Korea. *Mitt. Med. Fachschule, Keijo*, 1918, pp. 97-115, 2 pl.

LEIPER, R. T.

1915. Report on the Results of the Bilharzia Mission in Egypt, 1915. Jour. Roy. Med. Corps, 25:253-267.

MIVAIRI, K. and SUZUKI, M.

1914. Der Zwischewirt des *Schistosomum japonicum* Katsurada. Mitt. Med. Fak. Univ. Kyushu, Fukuoka, 1:187-197, 2 pl.

NAKAGAWA, K.

1916. The mode of Infection in Pulmonary Distomiasis. Jour. Infect. Dis., 18:131-142, 2 maps, 4 pl.

ODHNER, T.

1910. Results of the Swedish Zoological Expedition to Egypt and the White Nile 1901. No. 23A. Nordostafrikanische Trematoden. I. Fascioliden. 170 pp. 6 Taf. 14 figs. Uppsala.

- 1911-1913. Zur natürlichen System der digenen Trematoden. I-VI. Zool. Anz., 37: 181-191, 237-253; 38: 97-117, 513-531; 41: 54-71; 42: 289-318.

SSINITZIN, D. TH.

1911. La Génération parthénogénétique des Trématodes et sa descendance dans les mollusques de la Mer Noire. Mem. Acad. Sci. St. Petersburg, (8) 30:1-127, 6 pl. (Russian).

WARD, H. B. and HIRSCH, E. F.

1915. The Species of Paragonimus and their Differentiation. Ann. Trop. Med. Parasit., 9:109-162, 5 pl.

#### Nematoda and Acanthocephala

MAGATH, T. B.

1919. *Camallanus americanus*, nov. spec. Trans. Am. Micr. Soc., 38:43-107, 10 pl.

RAILLIET, A. ET HENRY, A.

1914. Essai de Classification des "Heterakidae." IX Congr. Int. Zool., Monaco, pp. 674-682.

1916. La Famille des Thelziidae. Jour. Parasit., 2:99-105.

RANSOM, H. B. and FOSTER, W. D.

1917. Life History of *Ascaris lumbricoides* and Related Forms. Jour. Agr. Research, 9:395-398.

1919. Recent Discoveries Concerning the Life History of *Ascaris lumbricoides*. Jour. Parasit., 5:93-99.

1920. Observations on the Life History of *Ascaris lumbricoides*. U. S. Dept. Agr. Bull. 817. 47 pp.

STEWART, F. H.

1917. On the Development of *Ascaris lumbricoides* Lin. and *Arcaris suilla* Duj. in the Rat and Mouse. Parasit. 9:213-227, 2 pl.

1918. On the Development of *Ascaris lumbricoides* and *A. mystax* in the mouse. Parasit., 19:189-196, 1 pl.

YOSHIDA, S. O.

1919. On the Development of *Ascaris lumbricoides* L. Jour. Parasit., 5:105-115.

LÜHE, M.

1911. Acanthocephalen. Süßwassfauna Deutschlands. 116 pp., 27 figs., Jena.

VAN CLEAVE, H. J.

1918. The Acanthocephala of North American Birds. Trans. Am. Micr. Soc. 37:19-48, 5 pl.

1919. Acanthocephala from the Illinois River, with Descriptions of Species and a Synopsis of the Family Neoechinorhynchidae. State Ill. Nat. Hist. Survey, 13:225-257, 7 pl.

## DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

---

### A METHOD FOR ORIENTING AND MOUNTING MICRO- SCOPICAL OBJECTS IN GLYCERINE

BY  
CHARLES BULLARD

The object of this paper is to describe a method of mounting desmids or similar microscopical objects in glycerine, so that they may not only be drawn in different positions with the camera lucida, but may form the basis of an herbarium of mounts which may be regarded as permanent, since the oldest preparations made by this method, now about twenty-five years of age, show no signs of deterioration. The account herewith presented is offered for the purpose of enabling others to utilize it, as well as to answer certain inquiries that have been made in regard to the subject. In its details the procedure presents no novel features. Its principles are those used in the laboratory of Professor Thaxter for mounting the lower fungi and other of the more delicate Thallophytes in glycerine, or glycerine and eosin, and sealing with King's Cement.<sup>1</sup> This method is especially well adapted for mounting desmids for study from the point of view of the systematist; since, in the vast majority of species only the well developed empty cells and semi-cells are useful for this purpose. Those species of *Cosmarium* or of *Spirotaenia*, for example, the cell-contents of which have to be studied, require more exact fixation methods, with which this paper is not concerned.

In mounting a sufficiently large object, no "finding ring" is needed. But for marking the position of a small or large series of minute objects, it is often essential. A ring of Brunswick Black has been found most serviceable for this purpose, and should be prepared months in advance, so as to become well seasoned. It may be placed centrally on the slide by means of the turntable, and need not be larger than the field of the low power.

#### THE MATERIALS AND THE METHOD OF THEIR USE

Since the procedure here described involves the use of a weak glycerine jelly as a means of orientation, it is necessary, in order to avoid the diffi-

<sup>1</sup> King's Cement was an invention of the Rev. J. D. King of Cottage City, Massachusetts who did not publish the formula or method of preparation. There is an antiquated recipe published on page 235 in Rev. A. B. Hervey's translation of Behren's "Guide to the Microscope in Botany" (S. E. Cassino Co., Boston 1885). Dr. Hervey assures me that it is genuine, as he received it directly from King. If the more modern form of this cement cannot be obtained from a dealer, it can be bought of its present maker, Professor R. E. Schuh, Howard University, Washington, D. C.

culties inevitably associated with the process in warm weather, to perform the manipulations indicated in the cooler part of the year. After the objects are oriented, the weak jelly may be satisfactorily set by placing the slide-box containing the preparations outside a window where it will be chilled. The drawing can be done in the warm part of the year, when the light is also best. This weak jelly is made from any good clear glycerine jelly, such as that prepared by Kaiser's formula. A few drops of melted jelly in a small vial is reduced by the addition of boiled or distilled water, until the mixture will just set at the ordinary temperature of the room. It should be perfectly limpid. The cork may be furnished with a dropper, by pushing into its lower end a piece of platinum wire of such length that a small loop at the lower end nearly touches the bottom of the vial. The vial should be kept well corked.

Objects may be lifted, transferred and oriented by means of a fine needle such as "No. 12 Sharps." The needle is pushed eye first into the end of a large match of straight grained wood, until a quarter of an inch, or less, of the pointed end, which is thus as rigid as possible, remains projecting. A smaller instrument may be made by the addition of a proper bristle, for which purpose a carefully selected whisker of a cat or dog answers admirably; since it combines stiffness and elasticity with an extremely delicate point. This bristle should be cemented to the mounted needle, and bound in place by means of a long human hair, or a fine waxed silk thread, in such a position that the point of the bristle projects slightly. It is important in order to obtain the necessary rigidity that the free portion of the bristle should be as short as possible consistently with convenient use.

The half inch circular cover glass is best adapted for general use, and in mounts of this nature, it is necessary to employ a shallow cell. This may be readily made by supporting the cover at one side by either a somewhat compressible, or an entirely rigid support. The latter results in a better mount mechanically, the former is easier to work with. The more flexible support, consists in fibers of blue blotting paper completely picked and teased out, and then felted together again into a ball by means of the forceps. This material has the advantage of compressibility, which permits one to vary a little the amount of glycerine used for the mounting medium. It has a certain disadvantage, however, from the fact that, as it is not a rigid support, care must be taken that a mass of sufficient size is used, to prevent the cover from touching the object, after the cement has dried and contracted. A rigid cover glass support may be made by selecting a very thin cover glass and placing it in an elongated folded paper. If this is pressed against the edge of the table and drawn back and forth, the glass within will be finely comminuted. A minute fragment of this may then be

used to support the cover. One must learn by experience the approximate amount of glycerine needed to fill the space beneath the cover.

The cement may be kept in an ounce, or half-ounce wide mouthed bottle. Into the bottom of the cork a match, bearing the ringing brush is inserted; the point of the brush nearly touching the bottom of the bottle. The ringing brush itself should be the smallest obtainable with not many but rather long hairs. A match may be pushed into the quill and firmly bound with silk, and the whole fitted to the cork as mentioned.

For a mounting medium it is best to use only chemically pure glycerine, filtered if necessary. It may be kept in a vial to the cork of which is fitted a platinum loop, or a properly selected mourning pin, by means of which the glycerine may be conveniently applied.

#### MANIPULATIONS

The details of the procedure for mounting large and small objects are the same, but the smaller species are obviously the more difficult. A slide is prepared for the reception of the objects by placing a minute smear, or streak of the weak jelly, within the ring previously prepared; or if desirable a series of minute drops may be used. It is convenient to employ an ordinary dissecting stand, with a x12 aplanatic triplet, on which the slide thus prepared is left, with the focus and light exactly regulated. The material to be mounted having been spread out in glycerine on a slide, the particular individual desired is selected under the compound microscope, and pushed about with the needle beyond the edge of the glycerine until freed from glycerine and all extraneous matter. In this condition it will readily stick to the needle, and can thus be lifted from the slide, transferred to the surface of the weak jelly, and there left until all the individuals desired for this mount have been transferred. The weak jelly should then be liquefied by breathing gently upon it, and the slide at once placed under the low power of the compound microscope. It will then be found that, with a little practice, the objects can be easily and systematically arranged with the needle. They may be set up in lines, or curves, in whatever order may prove most convenient. Should the jelly harden too rapidly, it may again be liquefied as above described. In a short time, one acquires skill in setting up objects, such as desmid semi-cells, in different positions under the compound microscope, without disturbing those already in position. For drawing care should be taken so to set up a symmetrical object that its vertical axis coincides with the optical axis, or nearly so. The horizontal axes may be pointed in any direction, by merely revolving the slide on the stage. After placing and orienting the specimens, the slide may be put away upside down in a slide box, until two or three more are brought to the same state of preparation. In this con-

dition they may be left outside a window all night in order to harden the jelly.

When the preparation is ready to mount and seal, the support of blotting paper, or cover chip, already mentioned, should be placed near the edge where it will be just included by the cover as it is lowered. A clean drop of glycerine should then be placed on the object, sufficient to fill the cell as exactly as possible. If blotter shreds are used, and the glycerine does not quite fill up the cell after the cover is placed in position, cautious pressure over them with the point of the needle will spread the glycerine, and fill the cavity completely. It may then be carefully sealed. If more glycerine must be added to fill a cell, a small drop should be placed at a short distance from the edge of the cover, and a narrow streak of it drawn with the needle to the edge of the cover; so that a little will flow beneath it. After repeating this process with the needle till the cell is completely filled, the surplus glycerine must be carefully wiped off.

The complete removal, before sealing, of this surplus is absolutely essential for the preparation of a permanent mount; since this is the only way to prevent subsequent leakage. A very little cement under the edge within the mount serves to make it stronger. In order to remove all trace of glycerine, it must be very carefully wiped off by means of an old, much washed handkerchief, folded over the end of the forefinger in such a manner as to form a point, which is moistened with alcohol. This can safely be pushed up till it touches the edge of the cover, and then repeatedly renewed and worked around it until all glycerine is removed. In case a still larger surplus of glycerine must be removed, it is convenient to use small strips of blotter folded  $\wedge$  shape. One end is moistened with alcohol and pushed up against the edge of the cover. Several pieces about the cover edge absorbing simultaneously will gradually remove most of the excess, after which, the slide must be very carefully cleaned with the handkerchief, as before mentioned, and the mount at once sealed.

When there is only one specimen, and different aspects of it must be drawn, the preparation is demounted after the first figure is made, and before sealing the mount. The cover is lifted off, the glycerine drained away, the jelly again liquefied as before, and the object transferred to another prepared slide, remounted in a new position and drawn again. The last remount, showing the object in its most characteristic position, may be sealed.

To seal a perfectly cleaned mount, it may be held in the unsupported left hand, while with the right, a light ring of cement is applied, after resting the right hand on the left. With a full brush, a drop of cement is then started on the edge of the cover, the hands being held as before, and led around the circle by means of the brush in such a manner that the

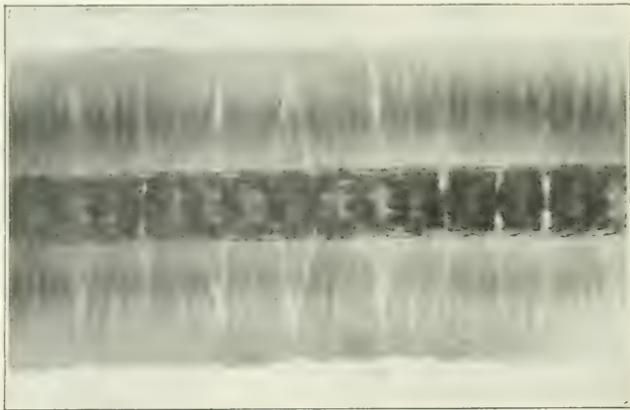
cement ring is partly on the cover and partly on the slide. The first ring thus helps to keep the second under better control. Additional rings may be applied on the turntable, when the sealing rings are hard. It has been found convenient to finish with two coats of Brunswick Black. This is soluble in turpentine, and offers more resistance to the solvent action of alcohol used to clean immersion oil from the cover.

In glycerine mounts of most objects, collapsed specimens regain their turgescence; and air bubbles, if present, disappear in a few days. Since these mounts are delicate, they must be handled with care, and always kept horizontal. Any necessary modifications of this method may be made in order to mount other microscopical objects needing orientation for camera drawing, and thus make it possible to obtain more accurate figures. But the fact that mounts thus sealed have shown no signs of leakage for so many years, indicates that the correct principles have been applied at this critical point, and should not be lightly changed.

*Cambridge, Mass., April, 1921*

## A METHOD OF DEMONSTRATING THE SHEATH STRUCTURE OF A DESMID

The structure of the cell wall in the Desmids is intimately concerned with the method of formation of the mucilaginous sheath, which in many members of the group is found to surround the cell. In the Saccodermæ the wall is believed to be continuous thruout, having no pores communicating with the exterior. On the other hand, the Placcodermæ in addition to other distinguishing characteristics frequently show pores connecting the protoplast with the surrounding medium.<sup>1</sup> In the Placcodermæ it is considered that the mucus exudes thru the pores, and may accumulate outside the cell wall, so forming the sheath. It is not usually possible to observe directly evidence of this extrusion, but in the filamentous desmid *Hyalotheca dissiliensis* (Sm.) Bréb. the sheath shows under reduced illumination striae radiating from a zone around the ends of each cell.



HYALOTHECA DISSILIENSIS (Sm.) Bréb.

Showing sheath stained with Methylene Blue and Picric Acid. Magnification 415 diameters. Photomicrograph with 100 watt condensed filament lamp, Wratten K<sub>3</sub> and B screens,  $\frac{1}{3}$ " Objective, X10 Ocular, field and sub-stage condensers.

The usual methods of staining algal cells, when applied in this case with the hope of more clearly demonstrating the structure of this sheath, caused much distortion. In the summer of 1919 at the Marine Biological Laboratory, Woods Hole, Massachusetts, the writer worked out the following method for the use of the students, and as it has been tried out on subsequent occasions with uniformly satisfactory results, it is offered

<sup>1</sup> In this respect see Lütkemüller, J., Die Zellmembran der Desmidiaceen. Beiträge zur Biologie der Pflanzen, (Cohn), 8:347-414. 1902.

as being suited for use with classes. The great abundance in which *Hyalotheca dissiliensis* (Sm.) Bréb. often occurs makes it peculiarly convenient, but the method is no doubt adaptable for use with other forms.

Fresh living material is placed in a .05% aqueous solution of Methylene Blue for 45 to 60 seconds. It is then removed, rinsed in distilled water and placed in a  $\frac{1}{10}$  saturated aqueous solution of Picric Acid. This serves to fix the stain and brings out in a most striking manner the striations in the sheath. The material may be examined in the Picric Acid solution, or removed after a minute or two to water. Preparations are best used soon after staining, as the sheath begins to disintegrate after a few hours.

WM. RANDOLPH TAYLOR.

*Botanical Laboratory,  
University of Pennsylvania*



720

TRANSACTIONS  
OF THE  
American  
Microscopical Society

ORGANIZED 1878

INCORPORATED 1891

---

PUBLISHED QUARTERLY

BY THE SOCIETY

---

EDITED BY THE SECRETARY

PAUL S. WELCH

ANN ARBOR, MICHIGAN

---

VOLUME XL

NUMBER THREE

---

Entered as Second-class Matter August 13, 1918, at the Post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in Section 1103, of the Act of October 3, 1917, authorized Oct. 21, 1918

---

The Collegiate Press  
GEORGE BANTA PUBLISHING COMPANY  
MENASHA, WISCONSIN  
1921

## TABLE OF CONTENTS

FOR VOLUME XL, Number 3, July, 1921

Observations on the Distribution and Life History of <i>Cephalobium microbivorum</i> Cobb and of its Host, <i>Gryllus assimilis</i> Fabricius, with one plate and three figures, by J. E. Ackert and F. M. Wadley.....	97
A Sarcophagid Parasite of the Common Field Cricket, by C. A. Herrick.....	116
Fresh Water and Marine Gymnostominan Infusoria, with four plates and three figures, by L. A. Hausman.....	118
Copper: Its Occurrence and Rôle in Insects and Other Animals, by R. A. Muttkowski..	144
DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES	
Microscope Illumination with Reference to Brownian Movement and Combination Lighting, by A. Silverman.....	158

# TRANSACTIONS OF American Microscopical Society

(Published in Quarterly Instalments)

Vol. XL

JULY, 1921

No. 3

## OBSERVATIONS ON THE DISTRIBUTION AND LIFE HISTORY OF *CEPHALOBIMUM MICROBIVORUM* COBB AND OF ITS HOST, *GRYLLUS ASSIMILIS* FABRICIUS<sup>1</sup>

By

JAMES E. ACKERT AND F. M. WADLEY

### CONTENTS

Introduction .....	98
The Parasite, <i>Cephalobium microbivorum</i> Cobb .....	98
Description .....	98
Habitat .....	100
Methods of Procedure .....	101
Removal of Nematodes .....	101
Culturing of Nematodes .....	101
Observations on Development .....	102
Early Cleavage to Coiled Embryo .....	102
Hatching .....	103
Factor Affecting Development .....	103
The Host, <i>Gryllus assimilis</i> .....	104
Distribution .....	104
Habits .....	104
Habitats .....	105
Proportion of Male and Female Crickets in Nature .....	105
Distribution of <i>Cephalobium microbivorum</i> .....	105
Geographical Distribution .....	105
Distribution in the Cricket Hosts .....	106
Proportion of Sexes and Parthenogenesis .....	107
Effect of Nematode on Host .....	107
Life History of <i>Cephalobium microbivorum</i> .....	108
Life Cycle .....	108
Seasonal Endurance .....	109
Other Parasites of the Cricket .....	109
Summary .....	110
Literature Cited .....	112
Explanation of Plate .....	114

<sup>1</sup> Contribution No. 52 from the Zoology Department, Agricultural Experiment Station of the Kansas State Agricultural College.

## INTRODUCTION

While securing gregarines from black field crickets for class use, in October, 1918, the senior writer found heavy infestations of small nematodes, some of which were sent to Dr. N. A. Cobb for identification. Determining that these nematodes represent a new genus, Doctor Cobb suggested that studies be made on the distribution and life history of it. Observations on its distribution have been made at Woods Hole, Mass.; Falls Church, Va.; Douglas Lake, Mich.; Rockford, Ill.; and Manhattan, Kan. The studies on its life history and that of the crickets were made at Manhattan. Further work on certain phases of these studies would be very desirable, but as this cannot be done for some time, it seems best to put the present findings on record. The writers wish to express their indebtedness to Doctor Cobb for suggesting this nematode study, and to Director Frank R. Lillie of the Marine Biological Laboratory, Woods Hole, Mass., and Director George R. LaRue of the University of Michigan Biological Station for the privilege of using equipment at the respective stations.

THE PARASITE, *CEPHALOBIMUM MICROBIVORUM* COBB

## DESCRIPTION

These nematodes which are from 2 to 3 mm. in length were identified as *Cephalobium microbivorum* n. g., n. sp., by Dr. N. A. Cobb, who submits this description.

The following characterizations and description, with figures, are taken from "Contribution to a Science of Nematology," No. IX; "One Hundred New Nemas," N. A. Cobb, 1920.

The characters other than specific are assembled from Cobb's Keys.

## PHYLUM Nematodes

SUBPHYLUM Laimia: Nemas having a more or less distinct pharynx.

CLASS Anonchia: Nemas lacking onchia.

SUBCLASS Anodontia: Nemas lacking odontia.

ORDER Polyaimia: Nemas having an unarmed pharynx, composed of two or more successive chambers more or less distinctly separated from each other.

GENUS *Cephalobium*

Cavity of the pharynx more or less prismoid or cylindroid (not conoid or very irregular), and containing a glottoid organ at its base. Oesophagus with median bulb and posterior swelling. Amphids none so far as known. Seta-like labial papillae 6. Single lateral wing present; striae fine, plain. Spinneret absent.

Preanal and postanal papillae present on the male. Tail conoid or subconoid; terminus acute, unarmed. Bursa none. Spicula two, equal, more or less arcuate; not jointed; their width not uniform. Accessories (gubernaculum) present. Inner ends of spicula cephalated by constriction. Length of the spicula  $1\frac{1}{4}$  times as great as anal body diameter.

54. *Cephalobium microbivorum*, n. sp. The single wing begins near the head and ends near the terminus. Its optical expression is either a pair of lines or a single line in the middle of a field one-twelfth as wide as the body. The contour of the body may become crenate in the anal region. There are about thirteen lateral organs on each side connected with pores



## HABITAT

To ascertain the part of the cricket inhabited by *C. microbivorum* some crickets were carefully dissected. In each case the thin-walled ileum readily revealed the writhing nematodes which appeared in bold contrast to the dark fecal contents. None of these parasites was found in the coelom, nor in any organ outside of the digestive tract. In one case two dead adults were taken from the colon of a freshly dissected cricket, whose ileum contained several live specimens. To facilitate subsequent discussion a brief description and a diagram are given of the alimentary canal of the cricket host.

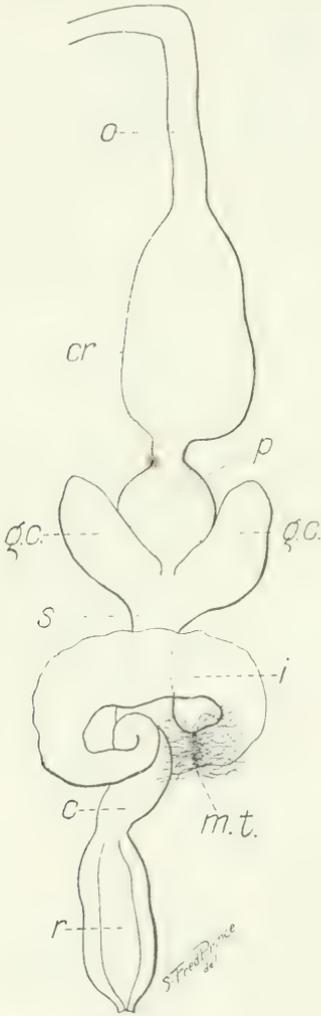


FIG. 1

Figure 1. Showing digestive tract of *Gryllus assimilis*. *c*, colon; *cr*, crop; *g.c.*, gastric caecum; *i*, ileum; *m.t.*, malpighian tubes; *o*, oesophagus; *p*, proventriculus; *r*, rectum; *s*, stomach.  $\times 4$ .

The digestive tract (fig. 1) of this black field cricket, *Gryllus assimilis*, bears many resemblances to that of the large and nearly wingless western cricket, *Anabrus*, as shown by Packard (1878-79, p. 175). The oesophagus (*o*) connects with the mouth and after making a sharp bend proceeds through the posterior part of the head to open into the spacious crop (*cr*) which occupies the thoracic and anterior abdominal portion of the coelom. After greatly narrowing, the crop opens into the strong proventriculus (*p*) which is much larger than the corresponding organ in *Anabrus*. In *G. assimilis* the diameter of this organ exceeds three times that of the junction with the crop, whereas in *Anabrus* the proventriculus is considerably reduced in size.

The proventriculus opens by a very narrow canal into the true stomach (s) which immediately gives off anteriorly two large, flattened gastric caeca (g.c.), situated one above the other. The stomach is surprisingly slender. After passing backward a short distance it makes an abrupt turn upward, narrows slightly and terminates, giving off numerous Malpighian tubes (m. t.) where it joins the intestine. Like *Anabrus* the remainder of the digestive tract is distinctly divisible into three portions: the ileum, colon, and rectum. Unlike the large western species, however, the stomach of *G. assimilis* is considerably shorter and the ileum (i) longer and much more capacious. The walls of the stomach are thick and translucent, while those of the ileum are thin and transparent. So thin are the walls of the latter that not only are the enclosed, motile nematodes visible, but also the eggs in the females' bodies. Before terminating, the ileum narrows considerably, makes a pronounced twist and then opens into the larger, thick-walled colon (c). Continuing posteriorly, the colon with a slight constriction connects with the still larger rectum (r) with which the anus communicates.

#### METHODS OF PROCEDURE

##### REMOVAL OF NEMATODES

The principal method employed for the removal of the nematodes is here briefly described: After excising the head of the cricket an incision was made in the posteroventral wall of the abdomen with fine scissors. By cutting forward through the median ventral surface, being careful not to cut deeply, nearly the whole lower body wall could be laid open without disturbing the digestive tract. On inserting fine forceps into the mesothoracic region the crop could be seized, and with slow, sustained effort the entire digestive tract withdrawn. In this extended condition the food tube was placed upon an ordinary glass slide, the rectum being excised at the anus. After covering with a few drops of normal salt solution, the intestine was teased with needles. The optical examinations were made with the aid of dissecting, binocular, and compound microscopes.

##### CULTURING OF NEMATODES

The culturing of the nematodes and their eggs was carried on for a time with fair success, but this phase of the problem should be continued. Having the nematodes on regular microscopic slides made it easy to remove the substances not wanted in the culture, and to add materials desired. With a little care the culture could be held to a restricted area on the slide by the surface tension of the solution. To prevent drying, the culture slides were placed in Petri dishes containing a few drops of distilled water. Subsequent microscopic examinations of the cultures were made either in or out of these small moist chambers.

Culture fluids used included normal salt solution alone, with fecal material, with peptone, and with both fecal material and peptone. Eggs hatched in each fluid, but growth of young nematodes appeared to occur only when a few drops of 0.8% peptone (in distilled water) were added. Dilute peptone was one of the successful solutions used by Welch and Wehrle (1918, p. 151) in their extensive nematode cultures.

In normal saline solution adult nematodes lived from one to six days, eggs developed and hatched, but even vigorous free embryos failed to increase in length and died within three days. When a few drops of 0.8% peptone were added to normal saline, embryos hatched and lived six days. The best results were obtained with equal volumes of normal saline and 0.8% peptone solution and a trace of cricket feces. In this medium several free embryos lived eight days, and a few thirteen days, the latter increasing their body lengths  $16\frac{2}{3}$  per cent. However, before the young nematodes had developed markedly, the culturing had to be abandoned on account of failure to secure nematodes. The adult crickets which had been collected prior to a cold wave succumbed in a few days, thus destroying the source of supply.

#### OBSERVATIONS ON DEVELOPMENT

##### EARLY CLEAVAGE TO COILED EMBRYO

The observations on development were upon living material, no attempt being made to trace the formation of germ layers or organs. The translucency of the dividing cells made it possible to follow the individual blastomeres until they formed a more or less spherical mass, such as shown in fig. 6. Whether such developing forms as represented in figs. 6 and 7 were hollow is uncertain, owing to the growing opacity of the embryos. For convenience in description the terminology of Martin (1913) is partly followed. For the uncertain stages shown in figs. 6 and 7, a morula rather than *the* morula is used. Likewise, when the vermiform shape of the embryo is first attained (figs. 8, 9) the term *curved* embryos is employed, whereas, the fully attenuated enclosed embryo (fig. 11) is designated as a *coiled* embryo.

These few observations on the development of the external form of *C. microbivorum* are given in the hope that someone may find opportunity to work out the embryology of this nematode. Such a study would be valuable and would be greatly facilitated by the thin, elastic egg shells.

In the present studies fertilized eggs, which can be distinguished by their clear nuclei, were mounted under cover slips in normal saline solution and studied under low and high powers of the microscope, a few drops of distilled water being added occasionally to compensate for evaporation. In other cases the live females containing eggs were so mounted, and in

this way external development was traced from the fertilized egg through hatching.

Under these conditions the early cleavage stages, as shown in figs. 2 to 5, develop somewhat rapidly, each cell division occurring in from ten to sixteen minutes. As segmentation proceeds and the bulk of the embryo increases, the thin, elastic shell expands accordingly. Eggs in an early cleavage stage (fig. 5) at 5 p. m. were in a morula stage with large blastomeres (fig. 6) at 8 p. m. Eighteen hours afterward development had proceeded to a morula with small blastomeres (fig. 7), in twenty more hours to a curved embryo (fig. 9), and six hours later to a coiled, motile embryo (fig. 11).

#### HATCHING

The stages represented in figs. 2-11 inclusive were observed in the uteri living worms mounted in normal salt solution under cover slips. Hatching occurred only after the eggs were ejected. Emergence from the egg was accomplished by repeated thrusts of the anterior end of the embryo against the thin shell which soon began to give way (fig. 12), finally rupturing (fig. 13) and liberating the writhing embryo.

#### FACTOR AFFECTING DEVELOPMENT

As noted above, eggs in nematodes which were in culture media developed somewhat rapidly, attaining the coiled, motile stage in approximately two days. But in the body of the live host uterine eggs do not appear to develop beyond the four-cell stage, as numerous examinations have shown. However, such eggs in a dead and somewhat macerated cricket were in more advanced stages of development. Also in the voided feces of an infested cricket, eggs in a morula stage were found. Thus it appears that the failure of uterine eggs to develop beyond the four-cell stage in the living cricket is due to an inhibiting factor. This factor the writers believe to be lack of sufficient oxygen. In the higher animals it is well known that the intestines contain enormous numbers of bacteria which must take much of the free oxygen, especially in the colon and adjacent regions where anaerobic bacteria thrive. In the ileums of these crickets there were numerous bacteria. The cases of advanced development of uterine eggs of the nematodes in the macerated cricket would favor this interpretation, as the thin wall of the disintegrating ileum quickly ruptures, admitting oxygen. Likewise, eggs which developed up to hatching in the uteri of cultured nematodes would be in the presence of more oxygen than when in the ileum of the cricket. An ample supply of oxygen to eggs in the separate fecal pellets is obvious. That additional oxygen accelerates development in nematode eggs has been determined by the senior writer

who observed more rapid development in cultured (normal saline) eggs of *Ascaridia perspicillum* on the addition of 10% hydrogen peroxide (unpublished results).

#### THE HOST, *GRYLLUS ASSIMILIS* DISTRIBUTION

In October, 1919, when the cricket examinations were at their height some adults were sent to Mr. James A. G. Rehn, who identified them as *Gryllus assimilis* Fabricius. Rehn and Hebard (1915, pp. 295, 296) regard this species as the common black field cricket of the Americas, which ranges from Canada to Argentina and from the Atlantic to the Pacific.

In the vicinity of Manhattan there are two races of this species, one maturing in August and September, the other in April and May. The fall adults lay their eggs in October, depositing them in the soil, under stones, and in other protected places. These eggs hatch the following spring according to Bruner (1886, p. 194), and the young mature in August and September. Concerning the occurrence and behavior of the spring adults, a few notes from the senior author's records for another problem are given, "About the last of May, 1915, adult crickets were found in nature mating. Several pairs of these were placed in cages containing sterilized earth, some carefully selected stems of alfalfa, and a small block of wood. Care was taken not to introduce any other animals." These records show that every second day the caged crickets were given one of the following foods: green alfalfa, fresh apple, algae, and small bits of fresh beef. Young crickets hatched in three weeks. By October the nymphs were approximately half-grown, averaging one-half the length of the adults.

From these and other observations it appears that in the vicinity of Manhattan, Kan., *G. assimilis* produces only one brood per year, but is represented by two races, the fall adults, laying their eggs in the autumn, passing the winter in this stage, hatching in the spring, and maturing in late summer or autumn; and the spring adults, depositing their eggs in the spring, hatching in early summer, passing the winter in a nymphal stage, and maturing the following spring. These respective findings are in close accord with the observations of McNeill (1891, p. 5) for *G. abbreviatus* in Illinois and of Blatchley (1901, p. 439) for *G. pennsylvanicus* in Indiana.

#### HABITS

Concerning the habits of the common black cricket, Blatchley (1901, p. 436) states that it is nocturnal, omnivorous and cannibalistic. The present studies indicate that these crickets in nature are largely nocturnal, but that they may stridulate, move about, and feed to some extent in the day time.

That they are omnivorous in nature is amply confirmed by these observations. Plants on which they have been seen feeding include alfalfa (*Medicago sativa*), bluegrass (*Poa pratensis*), bindweed (*Convolvulus spp.*), crabgrass (*Syntherisma sanguinale*), and Bermuda grass (*Cyniopsis dactylon*). Decomposing plant and animal tissues appear not to be distasteful, as the crickets have been seen feeding on both. Portions of dead crickets and other arthropods have been taken in preference to wilted grass, and in a few instances the animal tissues were in an advanced stage of decomposition.

Cannibalism is of frequent occurrence among the common black crickets, but apparently they seldom attack each other in life. In ordinary captivity mortality is high; some of the captives usually survive and frequently feed upon their deceased mates. The senior writer in connection with another problem reared crickets from eggs in large life history cages, making almost daily observations for months. Crickets were often seen dying, and sooner or later others began to devour them. In a single instance, a live cricket was observed to approach a dying one, lying on its back, and begin feeding on the latter's hind femur. At no other time, either in 1915-16 or during the present studies, has one cricket been seen to feed upon another living one.

#### HABITATS

The wide distribution of *G. assimilis* is doubtless due in part to its omnivorous feeding habits and to its varied habitats. Among the habitats from which it has been taken are the following: at edges of side walks, in holes in the ground and chinks in walls of buildings, under old hardened ox feces, sticks, boards, logs, stones, and stone walls, and among various kinds of vegetation.

Besides proximity to food and a reasonable amount of protection the diurnal habitat of this cricket must afford an atmosphere of comparatively high humidity. In artificial rearing the mortality was exceedingly high until water was sprinkled into the cages, when the percentage of survivals markedly increased.

#### PROPORTION OF MALE AND FEMALE CRICKETS IN NATURE

The writers found the number of male and female crickets to be approximately equal in nature, except during late October after the breeding season is over. At this time the adult females, with their abdomens distended with eggs, far outnumbered the surviving adult males.

#### DISTRIBUTION OF CEPHALOBIMUM MICROBIVORUM GEOGRAPHICAL DISTRIBUTION

Examinations of black field crickets for *C. microbivorum* have been made in five states: Kansas, Massachusetts, Virginia, Michigan, and

Illinois, but to date these nematodes have been found only in Kansas and Virginia. From April to June, 1919, Dr. N. A. Cobb examined a few black field crickets at Falls Church, Va., and found *C. microbivorum* in nearly every cricket. At Manhattan, Kan., these nematodes are known to have been of common occurrence in the adult black crickets during the autumns of the last three years (1918, 1919, 1920).

#### DISTRIBUTION IN THE CRICKET HOSTS

A study of the data collected during the search for *C. microbivorum* in the local black field crickets reveals some interesting points in the distribution of these nematodes in their hosts. Most of the examinations of the crickets were made during the periods between September 19 and October 31, in 1919 and 1920. From Table I it is seen that the number of female crickets examined exceeds that of the males. This was due to certain collections made late in October after the breeding season and after the consequent heavy mortality of male crickets. Collections made in September included nearly equal numbers of males and females.

TABLE I. SHOWING NEMATODE INFESTATION OF ADULT CRICKETS EXAMINED BETWEEN SEPTEMBER 19 AND OCTOBER 31, 1919 AND 1920, AT MANHATTAN, KAN.

	No. Crickets Examined	No. Crickets Infested	Per cent Infested	Total No. Nematodes	Average Infestation per Cricket	Range of Infestation
Male Crickets	14	10	71.4	217	21.7	2 to 51
Female Crickets	33	30	90.9	822	27.4	3 to 91
All Crickets	47	40	85.1	1,029	25.7	2 to 91

From Table I it is seen that over eighty-five per cent of the crickets examined by the writers between September 19 and October 31 were infested with this nematode. Approximately seventy per cent of the males and ninety per cent of the females contained these parasites. Table I likewise shows that both the range and average infestation of the females exceed that of the males. That the intestine of the male cricket furnishes a suitable environment for these parasites is evident from infestations amounting to as many as fifty-one *C. microbivorum*. Consequently the explanation of these phenomena must be sought elsewhere. In October the females' bodies are usually gorged with eggs, and are larger than those of the males. Obviously, to afford this greater development, more food

would be required than for the males, thus increasing the chances of the females ingesting a larger number of nematode eggs or larvae. The large, distensible crop (fig. 1, cr) is adapted for receiving quantities of food, and the numerous fecal pellets voided daily by these females are evidences of large appetites. Thus, to the writers, the most plausible explanation of the higher percentage, average, and range of nematode infestations in the females is that the engorged females take more food, and thus, on the average, swallow more eggs or larvae.

Occasional examinations of three or four specimens of *G. assimilis* were made during June, August, and September, 1920. No specimens of *C. microbivorum* were found in any of these crickets until August 21 when one was taken from an adult female. Of six mature crickets—three males and three females—examined on this date, two of the females contained nematodes, the other infestation consisting of two immature specimens. In September the recorded infestations ranged from seven to thirty-one nematodes, and in October from seven to as many as ninety-one.

In addition to the examinations of adult crickets shown in Table I, some half-grown black crickets were dissected on November 3, 1920. In the ileum of one of these nymphs were two mature specimens of *C. microbivorum*. The significance of this observation will be discussed later.

#### PROPORTION OF SEXES AND PARTHENOGENESIS

The nematodes observed included noticeably more females than males, and in one case males were entirely lacking. This case will be discussed presently, but concerning the presence of more female nematodes than males, the writers have no explanation to offer. Merrill and Ford (1916, p. 127) likewise found the females more numerous in the two species of nematodes they studied.

In the case just mentioned in which males were lacking, the infestation consisted of three females each containing fertilized eggs. These females may have been fecundated by males which had already left the host, as two dead nematodes were found in the colon of a cricket immediately after killing. Or, the females in question may have been parthenogenetic as Welch and Wehrle (1918, p. 159) and others have observed in small nematodes. It is possible also that they may have been protandric hermaphrodites, but the almost constant occurrence of males in the infestations favors the view that the females were probably fecundated by males which subsequently passed from the cricket.

#### EFFECT OF NEMATODE ON HOST

The effect of *C. microbivorum* on the host does not appear to be serious, as apparently normal crickets often harbored thirty or more of these nematodes. On the other hand, it seems probable that so many compar-

atively large entozoa must be detrimental to the host. Flury (1912) has shown in the cases of nematodes parasitic in higher animals that they cause injury not only by taking food material and by stoppage, but that on account of their imperfect digestive system their excreta contain toxins which are absorbed by the host. Some of these injuries would be likely to occur in the infested crickets.

#### LIFE HISTORY OF CEPHALOBIUM MICROBIVORUM LIFE CYCLE

As stated elsewhere, *C. microbivorum* matures in the intestine of the field cricket and eggs normally develop to the four-cell stage. When such eggs are ejected and kept moist development proceeds to the coiled, motile embryo stage in about two days. Cricket feces voided during the night and examined the following morning contained eggs of *C. microbivorum* in a morula stage. To ascertain the probable fate of such eggs in nature, four adult female crickets were placed in a lantern globe cage over some moist, sterilized earth. They lived from four to six days, the dead ones being removed before they were attacked by their mates. Two of these crickets were subsequently found to be infested with mature female nematodes containing eggs. The earth in the cage was moistened nearly every day, but as it was kept uncovered in the laboratory, evaporation was rapid and the culture dried out several times. Ten days after the infested crickets were placed over the sterilized earth examinations of portions of the latter were made which revealed two dead larvae, slightly larger than newly hatched embryos.

These observations indicate that in nature the eggs pass from the body of the cricket in early cleavage stages, and since the diurnal habitat of the cricket must have an atmosphere of comparatively high humidity, as shown elsewhere, a fair percentage of the nematode eggs voided in the daytime would be protected by the humidity of the cricket habitat. Since in culturing, these nematode eggs hatched in each separate medium, it is logical to infer that they would hatch in the moist débris of a cricket habitat. The thin elastic shell which bursts after a few thrusts would probably not long confine the embryos. Another reason which leads the writers to think that these young nematodes pass a period free in nature is that while immature stages of *C. microbivorum* are found in the ileum of the cricket, these nematode larvae are always much larger than the newly hatched embryos. If infection were caused by the cricket's ingestion of nematode eggs, one would expect occasionally to find in the crickets young nematodes the size of newly hatched ones, but this has failed to occur in the removal of over one thousand of these nematodes. The writers made no attempt to infest crickets by giving them hatched embryos or free larvae, but Merrill and Ford (1916, p. 127) succeeded in infecting termites with free larvae of the nematode, *Diplogaster acrivora* Cobb.

The indiscriminate feeding habits of the crickets would give ample opportunity for the ingestion of larvae of *C. microbivorum*, for as stated elsewhere, they feed upon decomposing plant and animal tissues, and these substances are commonly found in the moist diurnal habitats. Once in the ileum of the cricket the young *C. microbivorum* evidently thrive, for they occur in active stages ranging from one-third to normal lengths.

#### SEASONAL ENDURANCE

In the light of the information available, this nematode's problem of enduring the seasons seems relatively simple. It has been noted that in the vicinity of Manhattan, Kan., two races of *G. assimilis* occur, one spending the winter in the egg stage, and the other in the nymphal stage. The finding in November of mature specimens of *C. microbivorum* in nymphal black field crickets which live over the winter here and elsewhere in protected places indicates one way in which the cold season may be endured. These nymphal crickets mature in May, and deposit their eggs early in June, thus making it possible to shelter these adult parasites until summer.<sup>2</sup> By this time the eggs of the other race are hatched, furnishing possible hosts for the young *C. microbivorum* liberated as eggs from the winter-enduring nymphal crickets. Protection of the larvae against sudden desiccation and high temperatures would be afforded deeper in the habitat. At any rate, the fact that the fall infestations are the heaviest indicates that this nematode's problem of enduring the summer is not a serious one.

Another possible means of *C. microbivorum* enduring the winter might be afforded by the habit of the fall adult crickets crawling into the ground and into other protected places when their life work is finished. If such infested crickets were killed by freezing and remained congealed throughout the winter, the spring season might be well advanced before maceration of the crickets' bodies proceeded far enough to admit sufficient oxygen for the development of the enclosed nematode eggs. The plausibility of this method is strengthened by the fact that many nematodes can withstand some freezing. It is possible, of course, that larvae of these nematodes pass the winter free in the soil. Further studies in connection with the culturing of *C. microbivorum* will doubtless settle this and other points in its life problems.

#### OTHER PARASITES OF THE CRICKET

While searching for *C. microbivorum* in the crickets certain other parasites were encountered; viz., gregarines, gordiacea larvae and dip-terous larvae. In July, 1919, gregarines were present in many of the common black crickets examined at Woods Hole, Mass. At Douglas

<sup>2</sup> The writers made no examinations of the spring adults, but Doctor Cobb's examinations from April to June were of adults of this race in nearly all of which he found *C. microbivorum*.

Lake, Mich., these protozoan parasites were of frequent occurrence in crickets in August, 1920, and larval gordiacea were occasionally found.

Of the 106 mature or nearly mature crickets examined gregarines were found in thirty-seven per cent, and gordiacea larvae in 9 per cent of them. The crickets were taken from four localities; viz., Douglas Lake shore line about seventy-five yards wide; Burt Lake shore line approximately ten yards in width; Sedge Pool shore line about eight yards in width; and an upland pasture one and one-half miles from water.

The only gordiacea infestations occurred in the Sedge Pool locality, which was also the most favorable for gregarine infestations. This pool, which is separated from Douglas Lake by a narrow ledge, is approximately 200 feet long by 120 feet wide. It is protected by two to four foot banks and by a substantial growth of timber on three sides, leaving the east and southeast sides open to the direct rays of the sun. Most of the crickets were taken on the narrow ledge at the east side of the pool within ten to twenty feet of the protected water's edge. The infested crickets contained gordiacea larvae in later stages of development, some of them having attained the dark adult coloration. Two that escaped from a cricket in a bottle of water were readily identified as *Paragordius varius* (Leidy). This was the only species obtained by May (1919), who examined several hundred crickets from the east shore of Douglas Lake in connection with his studies on the life history of this species. The lowest percentage (30 per cent) of gregarine infestation was in the upland pasture one and one-half miles from Douglas Lake, while the highest (45 per cent) occurred at Sedge Pool. This indicates that these protozoan parasites thrive better under moister conditions.

Records for gregarine infestation of the crickets examined at Manhattan, Kan., are not complete, but of twenty crickets taken from nature between June 24 and October 21, 1920, eleven, or fifty-five per cent of them, were infested. The infestations ranged from one to as high as 517 gregarines, the average being slightly over sixty-two. Not a specimen of *Paragordius* was found here, but from two crickets examined by Herrick (1921) a few sarcophagid larvae were taken, this apparently being a new case of parasitism in *G. assimilis*.

#### SUMMARY

1. In the autumns of 1918, 1919, 1920 black field crickets, in the vicinity of Manhattan, Kan., were infested with a new species of nematode which has been identified as *Cephalobium microbivorum* Cobb.

2. In the body of the living cricket development of the eggs has not been observed to exceed the four-cell stage. This is attributed to lack of sufficient oxygen.

3. In culturing, eggs hatched in all moist media used, but young nematodes grew only when 0.8% peptone was added, two specimens increasing their lengths 16 $\frac{2}{3}$  per cent in thirteen days. In early cleavage, each cell division was accomplished in from ten to sixteen minutes; and in approximately two days the embryo was fully formed. Hatching is accomplished by repeated thrusts of the anterior end of the embryo against the thin elastic shell which soon ruptures and liberates the embryo.

4. The parasitic habitat of this nematode is the spacious ileum of *Gryllus assimilis* Fabricius, which is the common black field cricket of the Americas, ranging from Canada to Argentina and from the Atlantic to the Pacific. In the vicinity of Manhattan, Kan., this species is represented by two races, one maturing in August and September, the other in April and May; each race produces one brood of crickets per year. The race maturing in the spring winters here in the nymphal stage, while the adult fall race spends the cold season in the egg stage. Eggs of *G. assimilis* hatched (June) in three weeks after deposition. Late in October they were half grown, and by the last of the following May they were mature and mating.

5. These crickets are omnivorous, feeding on various kinds of plant and animal tissues, both fresh and decomposed. Cannibalism is of common occurrence among them. They are known to devour their dead and dying, but not to attack each other in normal condition. Their diurnal habitats, which may include a variety of situations, must furnish some protection and sustain a certain amount of moisture.

6. The numbers of adult male and female crickets observed in nature were about equal, except in the autumn after the breeding season, when more females survived.

7. Of crickets examined in five states, only those from Kansas and Virginia have been infested with *C. microbivorum*. At Manhattan, Kan., about 85 per cent of the fall adult crickets examined were infested; 70 per cent of the males and 90 per cent of the females. The females also contained a larger number of these parasites. Both the higher percentage of parasitism and the heavier infestations of the females are attributed to their greater voracity.

8. Infestations of fall adults were first found in August, the parasites being young and few. By September adult nematodes were taken and the size of infestations increased to 31. In October both young and adults were numerous, a maximum infestation amounting to 91 of these nematodes.

9. Nymphal, black field crickets of the race which winters in this stage were infested with mature nematodes in November.

10. Female nematodes were more numerous than males; for this phenomenon no explanation is offered. One cricket contained 3 female nematodes, each having fertilized eggs. Death of the males after fecundation is deemed more probable than parthenogenesis or protandric hermaphroditism.

11. No positive deleterious effect of the parasites on the host was observed, but this does not preclude possible injury.

12. The life cycle of *C. microbivorum* appears to be as follows: The nematode matures in the ileum of the common field cricket. Its eggs are deposited in early cleavage stages and passed from the body of the cricket. Under moist conditions furnished by the diurnal habitat the eggs soon hatch, and in the presence of nutritive substances the liberated embryos grow. Sooner or later the larval nematodes are swallowed by the omnivorous cricket in whose ileum they mature.

13. The nematode's problem of enduring the seasons is apparently solved by the occurrence of the two races of *G. assimilis*, the winter nymphs sheltering some of the mature nematodes through the colder months and the young of the fall adults ingesting larval nematodes during the warmer ones.

This method is probably supplemented by the infested bodies of certain fall adult crickets which, though dead, pass the winter in a somewhat congealed condition, the macerating bodies later liberating mature nematodes and eggs.

14. Other parasites encountered in the crickets examined included gregarines, gordiacea larvae and dipterous larvae. Gregarines were found generally, *Paragordius varius* larvae only at Douglas Lake, Mich., and sarcophagid larvae only at Manhattan, Kan.

#### LITERATURE CITED

BLATCHLEY, W. S.

1901. Orthoptera of Indiana. Rept. State Geol. Ind., 27:123-471.

BRUNER, L.

1886. Second Contribution to a Knowledge of the Orthoptera of Kansas. Bull. Washburn College, Lab. Nat. Hist., 1:193-200.

FLURY, F.

1912. Zur Chemie und Toxikologie der Ascariden. Arch. exper. Path., 62:273-390.

HERRICK, C. A.

1921. A Sarcophagid Parasite of the Common Field Cricket. Trans. Amer. Micr. Soc., 40:115-116.

MARTIN, A.

1913. Recherches sur les Conditions du Developpement Embryonnaire des Nematodes Parasites. Ann. Sci. Nat. (Zool.), Paris (9), 18:1-151.

MAY, H. G.

1919. Contributions to the life histories of *Gordius robustus* Leidy and *Paragordius varius* (Leidy). Ill. Biol. Monographs, 5:1-118.

MCNEILL, J.

1891. A List of the Orthoptera of Illinois. I. Psyche, 6:3-9.

MERRILL, J. H., AND FORD, A. L.

1916. Life History and Habits of Two New Nematodes Parasitic on Insects. Jour. Ag. Res., 6:115-127.

PACKARD, A. S.

1878-79. The Western Cricket. Second Report U. S. Ent. Commission, Washington, pp. 163-178.

REHN, J. A. G., AND HEBARD, M.

1915. The Genus Gryllus (Orthoptera) as found in America. Proc. Acad. Nat. Sci. Phila., 67:293-322.

WELCH, P. S., AND WEHRLE, L. P.

1918. Observations on Reproduction in Certain Parthenogenetic and Bisexual Nematodes reared in artificial Media. Trans. Amer. Micr. Soc., 37:141-176.

## EXPLANATION OF PLATE

All drawings were made with the aid of a camera lucida and are of the same magnification, X 400. The figures show stages in the embryological development of the external form of *Cephalobium microbivorum* Cobb.

Figs. 2 to 5. Eggs in early cleavage.

Fig. 6. Morula with large blastomeres.

Fig. 7. Morula with small blastomeres.

Figs. 8, 9. Eggs containing curved embryos.

Figs. 10, 11. Eggs containing coiled embryos.

Figs. 12, 13. Eggs in process of hatching.

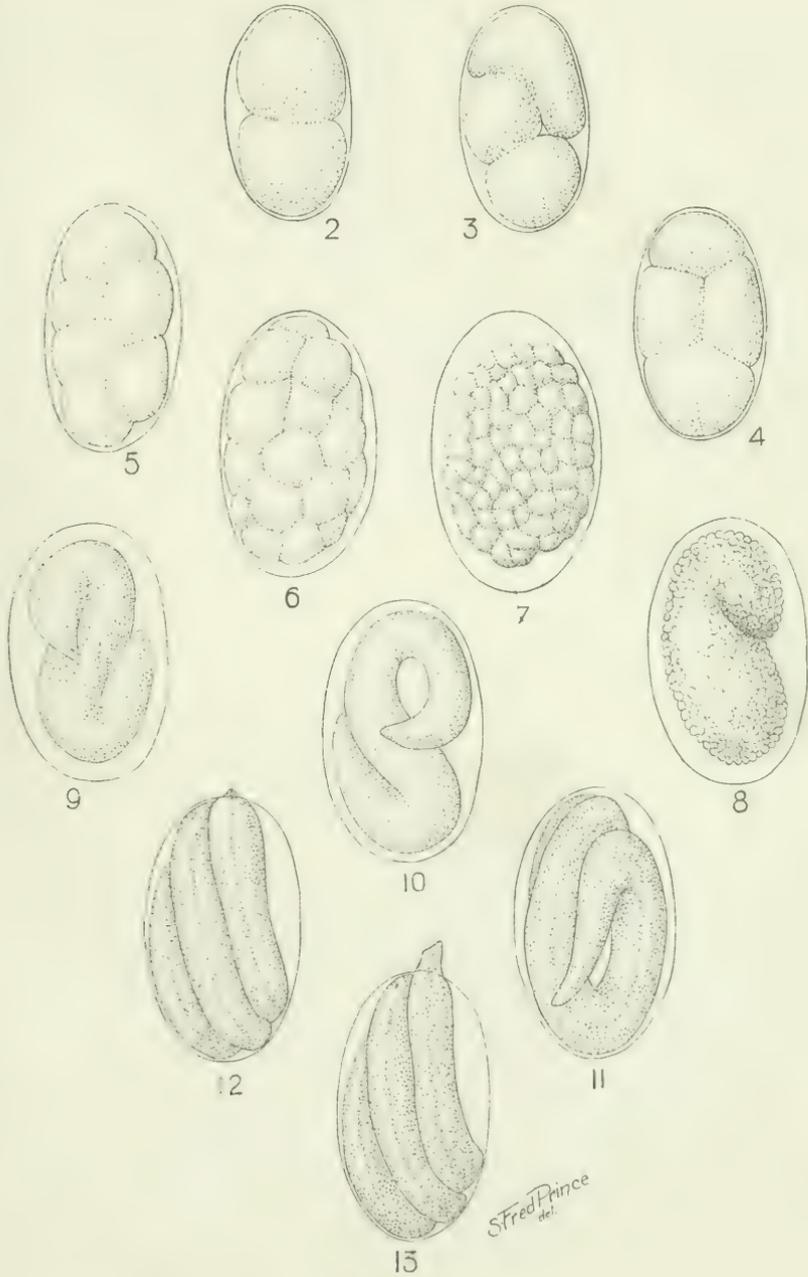


PLATE IV

# A SARCOPHAGID PARASITE OF THE COMMON FIELD CRICKET<sup>1</sup>

BY

CHESTER A. HERRICK

The purpose of this article is to record what seems to be a new case of parasitism in the cricket, *Gryllus assimilis* Fabricius, which, according to Rehn and Hebard (1915, pp. 295, 296), is the common black field cricket, not only of Kansas but of America. The cricket hosts of this parasite were taken in an alfalfa field near a stone wall. This wall and the débris along the south side of it afforded excellent protection for the congregated crickets. Numerous dead crickets were in the wall and under the débris near it and may have attracted the sarcophagids.

While examining these black field crickets for nematodes under the direction of Dr. J. E. Ackert, two insect larvae were found on Sept. 29, 1920. From the method of examination it is apparent that these larvae were in the body-cavity of the crickets. After excising the extreme posterior end of the cricket the thorax and abdomen were gently separated, and by sustained effort the whole intestine was removed from the abdomen. While the intestine was being examined the active larval parasites escaped through the large open end of the abdomen. One larva was 3 mm. in length, while the other, which was further developed, was 14 mm. long. The large larva was placed in a small covered tin box where, within 24 hours, it had pupated and become cemented to the floor of the box.

In this container the pupa was kept at laboratory temperatures, which ranged from 55° to 96° F., with an average temperature of 72° F. On the nineteenth day after pupation the adult fly emerged. It was sent to the Bureau of Entomology of the United States Department of Agriculture, where Dr. J. M. Aldrich identified it as *Sarcophaga kellyi* Aldrich.

This sarcophagid was first seen by Kelly (1914), who discovered it as a parasite of grasshoppers at Wellington, Kan., in mid-summer. His attention was attracted to certain flies that struck flying grasshoppers and caused them "to drop to the ground as if shot." On examining such grasshoppers he found tiny larvae crawling toward the base of the unfolded hind wing. Similar observations were subsequently made in New Mexico by Smith (1915), who found that this fly chose healthy, freshly molted, or inactive grasshoppers for the deposition of its larvae. He states (p. 8) that, "The female (*Sarcophaga kellyi*) upon locating a suitable victim was observed to alight upon the dorsum of the thorax and quickly deposit sev-

<sup>1</sup> Contribution No. 29 from the Zoology Department, Agricultural Experiment Station of the Kansas State Agricultural College.

eral living maggots, which, encountering only the soft tender membrane, speedily made their way into the body cavity of their host. The maggots are capable, however, of entering a host which is fully dried out and hardened." This observer found as many as sixteen of these larvae in the body-cavity of a single grasshopper. He found that the larvae usually escaped through the wall of the thorax immediately behind the anterior coxa, but that others either bored through the abdominal wall or escaped through the anus.

Kelly (p. 438), who reared large numbers of *S. kellyi*, found that the larvae deposited in the fall, on escaping from the grasshopper host, penetrated the ground to a depth of from one-half an inch to 2 inches, where they remained throughout most of the winter. Pupation in nature occurred early in March and adults emerged from late March until the last of May. In warm weather Kelly (p. 439) found that the life cycle was completed in much shorter periods. By June or July a second generation had matured, and from this time "until November no distinction could be made between generations on account of overlapping. However, judging from the rapidity of their development, there were probably three or four additional generations, making about five or six for the season." According to this author, *S. kellyi* has been reared from grasshoppers at Wellington, Kan., Washington, D.C., and points in New Mexico, Arizona, and Utah.

#### SUMMARY

1. Larvae of *Sarcophaga kellyi* Aldrich were found inhabiting the body-cavity of black field crickets, *Gryllus assimilis* Fabricius at Manhattan, Kan., in September, 1920.
2. This seems to be a new case of parasitism in the black field cricket.
3. The larvae of *Sarcophaga kellyi* have been reported from grasshoppers in Kansas, Washington, D.C., New Mexico, Arizona, and Utah.

#### LITERATURE CITED

- ELLIOT, F. O. C.  
 1914. A New Sarcophagid Parasite of Grasshoppers. Jour. Agr. Research, 2:435-446.
- REHN, J. A. G., AND HEBARD, M.  
 1915. The Genus *Gryllus* (Orthoptera) as found in America. Proc. Acad. Nat. Sci. Phila., 67:293-322.
- SMITH, H. E.  
 1915. The Grasshopper Outbreak in New Mexico During the Summer of 1913. U. S. Bur. Ent., Bul. 293:1-12.

# FRESH WATER AND MARINE GYMNOSTOMINAN INFUSORIA

LEON AUGUSTUS HAUSMAN, PH.D.  
Biological Laboratory, Cornell University

## INTRODUCTION

The present contribution to the survey of the protozoa deals with the characteristic appearance, habits, and habitats of members of one of the largest and most important groups of the protozoa, namely, the *Gymnostomina*. Within this suborder are included many of the largest of these unicellular forms of animal life; forms which constitute one of the most, if not, indeed, the most important source of food supply for the smaller aquatic organisms, which in their turn form the bulk of the food of fishes. Their presence in ponds and streams is of great importance, for they convert refuse matters which might pollute the water into an available source of food for higher forms of life. A study of the protozoan faunas of waterways should, it seems, go hand in hand with a study of the problems of water purification, and of the preservation and utilization of our aquatic resources.

The majority of the species of *Gymnostomina* treated in this paper are fresh water. Several marine species are also included.

The water samples of which examination was made were secured from various portions of New York, Connecticut, Massachusetts, and Mississippi, and over 1,000 were examined. They were taken from open lakes, ponds, roadside pools, rivers, brooks, rills, marshes, watering troughs, and the like.<sup>1</sup> The marine samples were secured from the Connecticut shore of Long Island Sound and from tidal estuaries and embayments, in the vicinity of New Haven.

## METHODS OF STUDY

Methods of collecting material containing protozoa, in the field, are too well known to need much discussion here. The methods used in the present investigation underwent no decided original modifications from the methods commonly employed.<sup>2</sup>

Half a dozen pint fruit jars, fitted into a small, suit-case-like conveyance, together with a small silk plancton net, a large, long handled cooking spoon, and several glass tubes of various lengths (with detachable compression bulbs for "sucking"), comprised the entire field equipment. The jars were labelled, and a record kept of the nature of the locations from

<sup>1</sup> See Hausman, L. A. Observations on the Ecology of the Protozoa, Am. Nat., vol. 4, 1917, p. 157.

<sup>2</sup> See Hausman, L. A. A Contribution to the Life History of *Amoeba proteus*, Leidy, Biol. Bull., No. 5, May, 1920, p. 340.

which the samples were taken, for future reference. Likewise each precise spot whence samples came was indicated on a topographic map. Possibly this may be found useful at some later time.

Upon arrival at the laboratory the samples were transferred to wide, open-mouthed jars. An examination was made of each sample immediately, and for a week or so, on each succeeding day, with the view of keeping record of the new species which emerged from encystment with the gradual stagnation and putrefication of the water, for except in a very few cases the samples contained algae or other vegetal matter. The small bolting silk net shown in Fig. 3 was used for concentrating the infusoria content of one or more pipettefuls of water from the middle or bottoms of the samples where the water was usually more or less clear. No concentration methods were needed in the examination of the surface scum of the putrescing material.

All measurements were made with an ocular micrometer, or from a ruled millimeter slide, from retarded living, or freshly killed specimens.

The characters which are, perhaps, the most satisfactory for use in the identification of the living animals are: the contour of the body, the positions of the buccal cavity and of the largest contractile vacuole, and the disposition of the cilia. Killing and staining, or *intra vitam* staining may make apparent the structure of the pharynx and also of the nuclear elements. This treatment may sometimes be necessary for bringing out of the cilia. Methods of *post mortem* and of *intra vitam* staining will be discussed later.

For the first examination of samples a small drop of water was taken from the top scum, or from concentrated material (the results of straining) and mixed with an equal volume of very viscous gelatine solution, and the whole thoroughly stirred together on the slide with a curved needle.<sup>3</sup> Or often several drops were mixed with an equal part of the gelatine in a watch crystal and used on the slide when needed. The drop on the slide was now carefully flattened out and examined *without a cover glass* under low power (16 mm. objective and 4x eyepiece) to ascertain if the solution were of a viscosity great enough to check sufficiently the movement of the protozoa. If not, it was allowed to concentrate still more by evaporation, until properly viscous, and a cover glass applied. Magnification with the 16 mm. objective and the 4x and 10x eyepieces, and with the 4 mm. objective, and the same two eyepieces was usually found of sufficient strength for the determination of the species described in this paper. A word of caution is to be given here concerning the clarity of the gelatine solution. The gelatine used must be of the best grade and the solution must be

<sup>3</sup>See Hausman, L. A. The Manipulation and Identification of the Free-Swimming Mastigophora of Fresh Waters, *Am. Nat.*, vol. 44, 1920, p. 333.

perfectly fresh. It was found that gelatine which had stood for some time became cloudy in appearance and stringy in texture, due to the growth of colonies of mould plants and bacteria.

Another method of quieting the movements of the protozoa, which was developed, consisted in chilling the slide and its supported water drop on a small block of ice.<sup>3</sup> As the temperature decreased the motions of the protozoa became slower and slower, though never so slow as those incarcerated within the gelatine mixture. This method was devised more in the spirit of curiosity than in any hope that it would be as great an aid as the gelatine method of quieting movement.

Permanent mounts of the infusoria are believed to be very unsatisfactory, with the exception of those made of *Difflugia*, *Arcella*, *Euglypha*, the *Foraminifera*, and others whose bodies secrete a protective shell or test. And here it is the test and not the creature itself which is preserved in its original form. During the process of killing, of staining, and of mounting, the body form is more or less distorted, and the cilia deformed or lost. The most convincing demonstration of the poverty of the mounted slide can be had by examining together a living *Paramoecium* retarded in the gelatine solution, or one freshly stained *intra vitam*, and a mounted slide, of the same creature, of the best manufacture obtainable. For optimum results in the study of gross anatomy, at least, or for the needs of the systematist, nothing, I think, can equal the *intra vitam* staining, with the creature hampered in its movements in the gelatine solution. The movements of the cilia or of the contractile vacuoles are often of the greatest aid in determining their position and form. In fact the presence and form of the pharynx in its entire length can often be made out, in certain species, only by means of the cilia vibrating within it.

The stains<sup>4</sup> most frequently used were methyl blue, and gentian violet. Safranin, methyl green, and iodine were also used. Safranin, it was found, stained the deepest, and methyl blue the least. For certain forms, therefore, the one was used, and for others, the other. In the case of each stain, a 95% alcoholic solution of the dry stain was made and kept in a small bottle ready to be diluted before applying to the slide. The staining set holder (Fig. 1) was designed to contain in a compact and convenient form the requisite number of stains, and other reagents, together with solid glass dipping rods for each. Thus any mixture of reagents was prevented. The labels (shown underneath the holes for containing the dipping rods) bore the names of the reagents. A great deal of comfort was derived from this very simple piece of apparatus.

The killing and staining was accomplished in two ways; either by killing first and staining afterwards, or by performing both operations simultane-

<sup>4</sup> See formulary of reagents at end of paper.

ously. The killing fluids used were: a 10% aqueous solution of tannic acid, a 1% aqueous solution of copper sulphate, a 2% aqueous solution of osmic acid, a 4% aqueous solution of acetic acid, a 3% aqueous solution of mercuric chloride, a 1% aqueous solution of formaldehyde. The osmic acid and copper sulphate solutions seemed to be the best killers, killing the animals at once, and without apparent distortion. Neither did disintegration set in with such rapidity as was the case when some of the other killing reagents were employed. These killing reagents can be used in other strengths than those given here but these percentages seemed to give the best results.

The killing was done either with a large amount of the material in a watch crystal, or underneath the cover glass, and the staining was accomplished in the same way. Where the protozoa were extremely abundant, as they were usually in surface scums or infusions of decaying marine algae, the watch crystal "mass" staining or killing was found to be the most satisfactory, as well as the easiest and quickest. This method had also to commend it the fact that both the killing reagent and the stain could be most readily controlled. Several watch crystals full of material were placed side by side and very delicate gradations of color secured.

As has been previously stated, the *intra vitam* staining gave by far the best results. This was accomplished either under the cover glass, or in the watch crystals, following the methods noted above, after the gelatine had been added and the proper degree of viscosity secured.

#### PREPARATION OF CULTURES

In order that a large number of individuals of a given species may be available for examination, it is necessary to depend upon cultures. For convenience in designation, there have been recognized in this paper the following types: (1) natural cultures, that is, those in which large numbers of a species appear, in natural conditions in the field and without any artificial manipulation of the medium in which they occur, (2) indirect cultures, or those which result from merely collecting the material and allowing it to stand and to decompose in the laboratory, and (3) artificial cultures, or those which are prepared with a definite nutritive medium (determined by experimentation) and inoculated with the desired species.

There is little or no exercise of technique involved in securing either natural or indirect cultures. One soon learns to recognize good natural culture environments such as greenish duck ponds, for *Euglenae* of various species; boggy water supporting growths of *Sphagnum*, for *Prorodon niceus* and *armatus*; watering troughs with *Spirogyra* or other *Chlorophyceae*, for species of *Chilodon* and *Holophrya*; clear, cold waters for *Astasia*, etc.

For indirect cultures one has merely to allow the collected water and vegetation to stand in the warmth and light of a south-exposed laboratory window, and make regular examinations day by day.

Where, however, but few individuals of a desired species occur, it becomes necessary to aid their propagation artificially. Results which gave earnest of better ones with further experimentation, were obtained by what is here termed artificial culturing. This was accomplished by segregating desired individuals, and then introducing into the jar of water in which they were placed some favorable nutritive substance. The methodology of preparing such cultures has been well enough developed at the present time, possibly, to make an account worth while, though many problems of detail still await solution.

For capturing individual protozoa under the microscope, there was devised what is here called an *isolation pipette*, shown in Fig. 4. A soft glass tube is drawn out to a hair-like degree of fineness at one end, and inserted into a thin walled rubber tube at the other. The opposite end of the rubber tube is tightly closed by means of a sealed glass tube. The hair-like point of the pipette is first dipped into clear water to allow capillary attraction to draw as much as it will up into the bore. The forefinger of the left hand is laid lightly upon the rubber tube near to its closed extremity, compressing it slightly and thereby driving out a small drop of the water from the tip of the glass pipette. The latter is now inserted with the right hand underneath the objective and into the uncovered drop on the slide. Release of the pressure of the left forefinger results in the withdrawal into the hairlike bore of the pipette a small quantity of water, the amount of which can be delicately regulated.

After the desired animal has been thus captured it is forced out into the water in the isolation jar, and there is added the proper nutritive substance. Thereafter the whole is set in a warm, light place to "ripen." It was found advisable, from the standpoint of ease of handling, to make the culture in small 3 or 4 cm. stender dishes. To inoculate such small cultures it sufficed, on several occasions, to introduce but a single individual. This, however, it must be confessed, was because we could secure no more, and the successes resultant from this meagre inoculation were regarded merely as fortunate accidents.<sup>5</sup>

Several inoculations, aggregating some half dozen, or preferably more, individuals are usually necessary. There was no certain way of determining, save after the anticipated development of the culture, whether the

<sup>5</sup> Single individuals can be removed by means of the isolation pipette, and introduced, for long-continued observations, into a device termed the *micro-aquarium* (See Hausman, L. A., *The Vibratile Oral Membranes of *Glaucoma scintillans*, Ehr., Am. Nat., vol. 44, 1920, p. 427.*

animals had actually been introduced into the isolation jar and inoculation actually accomplished. Rather clumsy attempts, yet in several instances not unsuccessful ones, were made to make as certain as possible the incarceration of single individuals within the isolation jar by first ejecting the captured animal into a drop of clear water on a slide while under the microscope, noting the presence of the creature, and then washing it off carefully into the material in the isolation jar with a fine stream of clear water.

Tubes of different tip diameters were used, and it was noted that the most success attended the use of the finest of these which it was possible to use for a given species. It is well to give the tip a slight turn when drawing it out, as shown in the figure. This seemed to make it easier to manipulate under the microscope.

It was found practicably impossible to manipulate the pipette and to capture the protozoa under any power greater than that afforded by the use of the 10x eyepiece and the 16 mm. objective. To insure the best results the drop of water must be well flattened out, and first freed from annoying débris.

For maturing the cultures rapidly, and under conditions which could be regulated and tabulated for further referene, a culture oven, such as is illustrated in Figure 2 was devised. A large aquarium jar was equipped with perforated tin shelves hung by copper wires from the upper rim of the jar; heated with a carbon filament lamp, placed on a copper wire platform to prevent it coming into contact with the glass bottom of the jar, and covered with a cardboard cover bearing a thermometer. This had the advantage of furnishing to the stender dish cultures placed on the shelves, at once the requisite amounts of heat and light. The temperature of the interior of the oven was regulated by raising or lowering the cardboard cover, propping it up with little wooden blocks.

Samples were dessicated in this oven by removing the cover and allowing the cultures to remain until they had dried. In this way cultures of *Holophrya*, *Prorodon*, and *Loxophyllum* were kept and resuscitated at pleasure. This method of keeping material by dessication might be a useful one for class requirements. The dried material could be removed from the dishes and placed in labelled envelopes, and filed away in a card catalogue tray. More experimentation along this line might reveal the fact of its being possible to have on file any quantity of protozoa material which could be revived at will for class room use!

#### KEY TO THE FAMILIES AND SUBFAMILIES

- I. Protozoa possessing, at some stage of the life cycle, locomotor appendages in the form of cilia, either single or fused into membranes.  
Macro and micro nucleus present. CLASS INFUSORIA

II. Cilia present during the entire life cycle; buccal cavity and anal orifice normally present; contractile vacuole often connected with an excretory tube system.

SUBCLASS CILIATA

III. Cilia more or less alike in form and distribution over the entire body, having a tendency to lengthen (or in some cases to be present only) on the oral or aboral side. Buccal cilia usually a trifle longer than the others.

ORDER HOLOTRICHIDA

IV. Lacking undulating membranes about the buccal cavity, the latter being closed except during the ingestion of food.

SUBORDER GYMNOSTOMINA

A. Body outline usually oval or extended; neither oral nor aboral sides flattened

*Family Encheliniidae*

AA. Body outline sometimes oval or extended, more often, however, with oral side either flattened or concave.

B. Buccal cavity terminal or nearly so

*Family Tracheliniidae*

BB. Buccal cavity not terminal

C. Gullet with pronounced curve

*Family Enchelyiidae*

CC. Gullet without pronounced curve

D. Body entirely and evenly ciliated

*Subfamily Nassulinae*

DD. Cilia longer on, or confined to aboral side

*Subfamily Chilodontidae*

DDD. Cilia confined to oral side

*Subfamily Erviliinae*

KEY TO THE GENERA MENTIONED IN THIS PAPER

A. Body ovoid, ellipsoidal, or almost spherical

B. Body distinctly ovoid or spherical

C. Posterior spinous process present.....UROTRICHA

CC. Posterior spinous process not present

D. Possessing spiral band of longer cilia.....PERISPIRA

DD. Not possessing spiral band of longer cilia

E. Cilia restricted to one or two circles about the body

F. One midway circle of cilia present.....MESODINUM

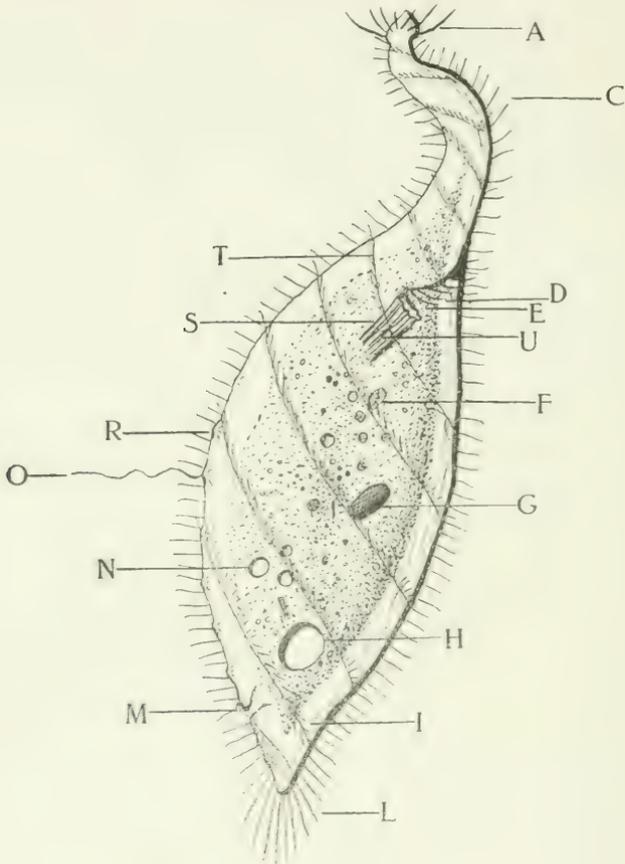
FF. Two such circles present.....DIDINIUM

EE. Cilia not restricted to one or two circles about the body

F. Cilia restricted to one side of body.....TROCHILIA

FF. Cilia not restricted to one side of the body

- G. Buccal cavity anteriorly terminal
  - H. Nucleus long and curved.....ENCHELYODON
  - HH. Nucleus usually ovoidal.....HOLOPHRYA
- GG. Buccal cavity not anteriorly terminal
  - H. With a short neck like, or lip like projection from anterior end.....TRACHELIUS
  - HH. Without such a projection.....NASSULA
- BB. Body not ovoid or nearly spherical, but drawn out into the form of an elongated ellipsoid
- C. Body armored with rectangular plates
  - D. Cilia arising from middle of rectangular plates..... COLEPS
  - DD. Cilia not arising from middle of plates.....TIARINA
- CC. Body not armored with rectangular plates
  - D. Cilia restricted to ventral surface
    - E. Body about five times as long as, or longer than, greatest diameter.....LIONOTUS
    - EE. Body less than five times as long as its greatest diameter.....LIONOTOPSIS
  - DD. Cilia not restricted to the ventral surface
    - E. Body longitudinally furrowed.....PLAGIOPOGON
    - EE. Body not longitudinally furrowed.....PRORODON
- AA. Body not ovoid, ellipsoid, or almost spherical
- B. Body purse or flask shaped
- C. With long flexible neck or proboscis
  - D. With circle of longer cilia about the anterior extremity of the proboscis.....TRACHELOCERCA
  - DD. Without such a circle of cilia.....LACRYMARIA
- CC. With short neck
  - D. Neck obliquely truncated
    - E. Ciliation entire
      - F. Body capable of distortion at will.....ENCHELYS
      - FF. Body not capable of distortion.....SPHATHIDIUM
    - EE. Ciliation not entire.....PHASCOLODON
  - DD. Neck not obliquely truncated
    - E. Tentacular process arising from the buccal cavity in the anterior end of neck.....ILEONEMA
    - EE. Without such a tentacular process TRACHELOPHYLLUM
- BB. Body not purse or flask shaped
- C. Body ribbon or leaflike
- D. Body very elongate
  - E. Anterior end rounded.....AMPHILEPTUS
  - EE. Anterior end not rounded.....FLEXIPHYLLUM



An ideal composite gymnostominan ciliate, to show the various anatomical divisions and organs of the body.

- a. proboscis cilia
- c. proboscis
- d. buccal cilia
- e. buccal cavity opening into pharynx
- f. food vacuoles
- g. nucleus
- h. principal contractile vacuole
- i. hyaline border of body
- l. caudal cilia
- m. anus
- n. smaller contractile vacuole
- o. discharged trichocyst
- r. undischarged trichocyst
- s. pharynx
- t. cilia band
- u. pharyngeal rods

- DD. Body not very elongate  
 E. With anterior border crenulate.....LOXODES  
 EE. Without crenulations on the anterior border  
 F. Neck elongated and constricted.....DILEPTUS  
 FF. Neck not elongated and constricted..LOXOPHYLLUM  
 CC. Body not leaflike; usually kidney or bean shaped, or nearly so  
 D. Pharynx long and curved.....TILLINA  
 DD. Pharynx short and straight.....CHILODON

## OBSERVATIONS ON THE RECORDED SPECIES

Genus *Holophrya*

This genus, a very common one, and widely distributed, possesses either an enormous number of distinct species, or a much smaller number, many of which are very variable in size and form, and often, indeed, in coloration. From our limited observations we are inclined to take the latter view. Little attempt has heretofore been made to accord all of these diverse forms specific names.

The species here called Sp. 1 and 6 were commonly found in brackish tidal estuary water about a mile from Long Island Sound among detached, floating *Fucus* and green Sea Lettuce, the first day after having been brought into the laboratory. All of the individuals of these species observed (and there must have been hundreds seen) varied but little away from an average. Later on, however, considerable variation occurred as the numbers became greater in the slowly putrescing material. It is difficult to say whether this was due, however, to an increasing variation among the individual members of the species, or whether new species were making their appearance.

Species 5, 6, and 7 were present in enormous numbers in a four days old infusion of fresh cabbage leaves, and in an infusion of dried corn leaves of the same age.

Species 2, 3, and 4 were met with occasionally in almost all samples, both marine and fresh water, particularly in those from ponds.

The globularity of body, and the small anterior or anterior-lateral buccal cavity, together with the uniform length and distribution of the cilia, appear to be constant characteristics of the members of the genus. Among such small, globular forms, mutilated individuals seemed not to be very common. We are of the opinion that figures of very irregular forms assigned to this genus were made from such mutilated individuals.

*Holophrya* sp. 1 to 7 shown in Figs. 5-11.

Genus *Urotricha*

Members of *Urotricha* may easily be recognized by the presence of the caudal spine or seta, and by their curious habit of swimming slowly and

evenly and then suddenly jerking ahead, or to the right or left, as though shot by a spring, a motion resulting from a quick lateral snap of the rigid caudal seta.

Kent has observed that the walls of the pharynx are surprisingly elastic, and that this enables the creatures often to take in food, the bulk of which may equal their own bodies!

If the posterior spine is not easily seen, staining makes it easily visible. *Urotricha farcta* and *platystoma*, Figs. 12 and 13.

#### Genus *Perispira*

The *Perispira ovum* (Fig. 14), of Stein, which we have recorded from stagnant pond waters, may be the same as the *Holophrya ovum*, of Ehrenberg. If this be so then it is an aberrant form of *Holophrya*, for it possesses a spiral band of longer cilia characteristic of *Perispira*. Since even ciliation is characteristic of *Holophrya*, this form had best be placed in *Perispira*.

#### Genus *Enchelys*

This genus is hardly distinguishable from *Holophrya*, the presence of the laterally opening buccal cavity of *Enchelys* being apparently the sole point of difference. And when the members of the latter assume a globular form, which they do with apparent volition, the buccal cavity becomes almost exactly anteriorly terminal, much like that of many species of *Holophrya*. This assumption of globularity occurs, often, when the animal is gorged with food granules. The young, soon after division has been completed, also take on a globular body.

The smaller species figured is regarded as the *Enchelys farcimen* (Fig. 16) of Ehrenberg. *E. pupa* (Fig. 15) was met with several times in pond water.

#### Genus *Enchelyodon*

The ovate-elongate body and the terminal buccal cavity, together with the large size, should serve to distinguish *Enchelyodon farctus* (Fig. 17) from forms in the genera *Prorodon* and *Enchelys*. Note that the cilia are very short. We were unable to see them in the unstained animal. This form was rarely found in the waters of bogs, ponds, slowly moving streams, etc.

#### Genus *Spathidium*

The chief difference between this genus and *Enchelys*, from which it is separated only with difficulty, appears to be the possession of a longer pharynx, usually furnished with pharyngeal rods. The latter, however, are not easily visible.

The species figured, which seems to be *Spathidium spathula* (Fig. 18), was found in pond and slow stream waters.

Genus *Prorodon*

Both *Prorodon armatus* (Fig. 19) and *P. ovum* (Fig. 20) were rarely found in pond waters. The buccal cavities of both are distinct, and the prominent pharyngeal rods of the latter were very good as an identification characteristic.

Genus *Lacrymaria*

*Lacrymaria olor* (Fig. 21) is a common form in infusions of leaves both of deciduous trees and of aquatic plants. Like *Trachelocerca olor* (Fig. 22) it often lies with its lenticular body concealed among a mass of debris and shoots forth its long serpentine neck in all directions. Whether this is a deliberately willed concealment for the purpose of protection, or for the advantage which it secures for the seizure of prey is uncertain. I have not seen this habit mentioned elsewhere, and yet I found it to be a very common one among the many individuals observed.

Its size is extremely variable, but the constant body form offers a ready means for identification.

Genus *Trachelocerca*

To be distinguished from *Lacrymaria* chiefly by the smaller size of its members. *Trachelocerca olor* (Fig. 22) and *Lacrymaria olor* are almost identical in habits. The movements of the smaller form are, however, the more rapid. *Trachelocerca olor* is found commonly among the smaller aquatic vegetation in small quiet pools and coves.

*Trachelocerca phoenicopterus* (Fig. 23) is a marine species occurring among algae along the shore, as well as in putrifying infusions. Its length seems to be very variable.

Genus *Ileonema*

*Ileonema dispar* (Fig. 24) occurs among *Spirogyra*, *Zygnemea*, *Oscillatoria*, and can probably be found among any of the fresh water filamentous algae. It is not a common form, and usually disappeared soon from fresh material.

The cilia are sparse and apparently weak.

Genus *Plagiopogon*

*Plagiopogon coleps* (Fig. 25) which we figure from Kent, we believe to have found in salt water among decaying *Fucus* and other algae. It closely resembles *Coleps hirtus* (Fig. 26) though the longitudinal furrows of the body and the absence of armor plates are apparent under high powers. It seems to be a species of fairly constant form and size.

Genus *Coleps*

*Coleps hirtus* (Fig. 26) is a very common form of ciliate, the commonest of its genus, among decaying vegetation and in old infusions, and can be

readily identified from its size and armored body. In swimming it twirls rapidly on its longitudinal axis and pursues a rapid, wavering reckless course. It is an exceedingly voracious species and appears to feed on both animal and vegetable tissue, and the bacteria which are disintegrating them.

#### Genus *Tiarina*

*Tiarina fusus* (Fig. 27), a marine form from among decaying algae, resembles *Coleps* in structure very closely. The form of the body is, however, different. The form which we figure we take to be *Tiarina fusus*, (Fig. 27) which is apparently the same as the *Coleps fusus* of Claparède and Lachmann.

#### Genus *Didinium*<sup>6</sup>

*Didinium nasutum* (Fig. 28), not an uncommon form in decaying and fresh aquatic vegetation, is one of the largest of the ciliates. Its two zones of cilia offer an easy character for identification. This form appears freely where an adequate supply of smaller ciliates appear, for it is upon these that it feeds. The habits of this species have been exhaustively studied by S. O. Mast (22) and recorded in one of the most interesting of the recent papers on protozoan habits.

The natatory movements of this species are much like those of *Urotricha*, namely a slow gliding progression interrupted frequently by spasmodic jerks.

#### Genus *Mesodinium*

*Mesodinium cinctum* (Fig. 30) is not an uncommon form in salt water and when swimming rapidly looks very much like a minute replica of *Didinium nasutum*. The constricted median line and the single zone of median cilia make it easy to identify when at rest.

The smaller *Mesodinium* (Fig. 29), which is found rather rarely associated with the preceding species, I consider to be the *Mesodinium pulex* of Claparède and Lachmann.

#### Genus *Tillina*<sup>7</sup>

*Tillina magna* (Fig. 31) were found frequently in a ten day's old infusion of dried corn leaves associated with various species of *Holophrya*, *Chilodon*, and *Colpoda*. Its distinguishing characteristics are the irregular, asymmetrical body and the curved, ciliated pharynx.

#### Genus *Amphileptus*

Fig. 32 I have called provisionally, *Amphileptus gutta*. It seems to occur in both marine and fresh water infusions. They bear either many smaller contractile vacuoles distributed over the posterior two-thirds of the body,

<sup>6</sup> See Mast, S. O., The Reactions of *Didinium nasutum*, etc., Biol. Bull., vol. 16., 1908, p. 91.

<sup>7</sup> See Gregory, L. H. Observations on the Life History of *Tillina magna*, Jour. Exp. Zool., vol. 6, 1909.

or occasionally, yet not so frequently, one single large vacuole, situated in the posterior half or posterior end, or slightly to one side. Because of a lack of very definite characteristics forms like this are difficult to place with certainty.

#### Genus *Lionotus*

Members of this genus are among the most graceful ciliates. Viewed from above, the apparently slender neck is seen to be broad and leaflike. Figures of these species should, therefore, indicate this and not lead to the impression that the neck is of the same type as that possessed by *Lacrymaria* or *Trachelocerca*.

*Lionotus wrzesniewski* (Fig. 36) is a large form, found in pond waters amid living and dead aquatic vegetation, where often occurs, also, *Lionotus fasciola* (Fig. 34). The latter species is also found in salt water with *Fucus* or other marine algae. A similar form, entirely restricted to fresh water, is *Lionotus pleurosigma* (Fig. 35). This species can be distinguished from *fasciola* only by its clear, deep, hyaline border.

The smallest of the species figured (Fig. 33) was found in brackish water in a tidal estuary among detached, floating marine algae.

#### Genus *Lionotopsis*

Fig. 37 is, perhaps, the *Lionotopsis anser* of Conn, drawn from but a few poorly defined individuals found in pond water. The position of the buccal cavity could not be determined.

#### Genus *Loxophyllum*

Members of this genus can usually be recognized by the gracefully flexible way in which they glide about over and through débris or wrap their pliant and leaflike bodies about it. The ease with which the curved anterior portion of the body is used for the examination of possible food substances reminds one of the sensitive exploratory gropings of the tip of an elephant's trunk. The deep, clear, hyaline border possessed by all the *Loxophylla* is constantly characteristic.

*Loxophyllum setigerum* (Fig. 39) and *rostratum* (Fig. 40) were found quite abundantly in brackish water. The latter appeared in great abundance in an eight days old infusion of green Sea Lettuce and *Fucus* in salt water.

*Loxophyllum* sp. 1 occurred in fresh water among aquatic vegetation (Fig. 38).

#### Genus *Trachelophyllum*

Fig. 41 has been called *Trachelophyllum tachyblastum*, from a single specimen found in pond water.

#### Genus *Flexiphyllum*

*Flexiphyllum elongatum* (Fig. 42) is frequently met with in pond water among growing vegetation. Its motion is a graceful and sinuous gliding

and it makes rapid progress through the water. We have found that it prefers to move concealed amid débris.

#### Genus *Trachelius*

*Trachelius ovum* (Fig. 43) possibly the most common species, can be distinguished by its large size, its curious little neck, and its deliberate motions. The buccal cavity and gullet are quite prominent in most individuals. The size and shape of the neck is apparently subject to considerable variation. Within the body the number of contractile vacuoles is normally very large.

#### Genus *Dileptus*

*Dileptus gigas* (Fig. 44), fairly common form, is of unusual variability in size and shape. It is entirely carnivorous and possessed of a voracious appetite. The prey is stung and rendered helpless by the discharge of the trichocysts located along the border of the long neck like process, and if too large to be swept into the buccal cavity by the lashings of the buccal cilia is forced in by the writhings of the neck. The body often rotates on its longitudinal axis during progression through the water.

Individuals have been reported which measured 800  $\mu$ .

#### Genus *Loxodes*

*Loxodes rostratum* (Fig. 45) was found only rarely in pond water among fresh and decaying vegetation. It is reported to occur also commonly, in infusions.

#### Genus *Nassula*

This is a beautiful genus, its members being symmetrically ovoid, and many of them iridescent. *Nassula microstoma* (Fig. 48) is a very pretty species. It is usually brownish or yellowish, the color depending upon its contained food. Under strong light, as it revolves through the water, it scintillates brightly, reminding one of a small, ovoid, minutely faceted epidote. This was a very common species in brackish tidal estuary water.

*Nassula ornata* (Fig. 46 and *Sp. 1* (Fig. 47) were found in pond waters among fresh and decaying vegetation.

#### Genus *Chilodon*

*Chilodon*, much like *Holophrya*, is a genus containing a great number of species of considerable variability of form and size. Of all the species which vary in this way among themselves, *Chilodon cucullulus* (Fig. 52), the commonest, is the most flagrantly disregarding of maintaining its proper dimensions and contour! In the same infusion we have found no less than a dozen differently shaped and sized specimens! Calkins says of this species that it is "extremely variable . . . and has received so many different names that it hardly pays to enumerate them all." It is "one of the most common and widely spread ciliates known."



depths of color. When used in aqueous solutions, very dilute, they make good intra vitam stains.)

- |                  |   |
|------------------|---|
| 1. methyl blue   | 6. gentian violet   |
| 2. methyl green  | 7. iodine, with potassium iodide (a killing stain, either with water or alcohol). |
| 3. Lichtgrün     |   |
| 4. Bismark brown |   |
| 5. Safranin      |   |

## BIBLIOGRAPHY

- BLOCHMANN, F.  
1895. Die Mikroskopische Tierwelt des Süßwassers, Abt. I. Protozoa, Hamburg.
- BÜTSCHLI, O.  
1880-1889. Protozoa, in Bronn's Klassen und Ordnungen des Thierreichs, Bd. I, Th. I-III, Leipzig u. Heidelberg.
- CALKINS, G. N.  
1901. Marine Protozoa of Woods Hole, Bull. U. S. Fish Comm. vol. 21.
- CALKINS, G. N.  
1901. The Protozoa, N. Y.
- CALKINS, G. N.  
1909. Protozoology, N. Y.
- CARTER, H. J.  
1856. Notes on the Freshwater *Infusoria* of the Island of Bombay, No. I Organization Ann. and Mag. of Nat. Hist., 2nd Ser. vol. 18, p. 115.
- CLAPARÈDE ET LACHMANN  
1868. Études sur les Infusoires et les Rhizopodes, Genève et Bale.
- COCKERELL, T. D.  
1911. The Fauna of Boulder County, Colo., Univ. of Colo. Studies, vol. 8.
- CONN, H. W.  
A Preliminary Report on the Protozoa of the Fresh Waters of Connecticut, Conn. State Geol. and Nat. Hist. Survey, Bull. 2.
- DOFLEIN  
1909. Lehrbuch der Protozoenkinde, Jena.
- EDMONSON, C. H.  
1906. The Protozoa of Iowa, Pro. Davenport Acad. Sci. vol. II, p. 1.
- GREGORY, L. H.  
1909. Observations on the Life History of *Tillina magna*, Jour. Exp. Zool., vol. 6, no. 3.
- GRUBER  
1882. Neue Infusorien, Zeit. Wiss. Zool. vol. 33, p. 439.
- GRUBER  
1884. Die Protozoen des Hafens von Genua, Nov. Act. des K. Leop. Car. Deutsch. Akad. der Naturfor., vol. 46, p. 475.
- HAUSMAN, L. A.  
1917. Observations on the Ecology of the Protozoa, Am. Nat., vol. 4, p. 157.
- HENDERSON, W. D.  
1905. Notes on the Infusoria of Freiburg im Bressgau, Zool. Anz., vol. 29, p. 1.
- JENNINGS, H. S.  
1899. A Report on the Protozoa of Lake Erie, etc., Bull. U. S. Fish Com., p. 105.
- KENT, W. S.  
1880-1881. A Manual of the Infusoria, 3 vols., Lond.

- KOFOID, C. A.  
1897-1901. Plancton Studies II, Bull. Ill. State Lab. Nat. Hist., vol. 5, p. 273.
- LANDACRE, F. L.  
1908. Protozoa of Sandusky Bay and Vicinity, Ohio Acad. Sci., vol. 4, p. 421. (This paper contains an excellent bibliography, chiefly of American writers, brought up to the date, 1904)
- LANKESTER, E. RAY (Editor)  
1903. A Treatise on Zoology, Part I, Introduction and Protozoa, 2nd fascicle, Lond.
- LIEBERKUHNS, N.  
1856. Contributions to the Anatomy of the *Infusoria*, Ann. and Mag. of Nat. Hist. 2nd Ser., vol. 18, p. 319.
- MAST, S. O.  
1908. The Reactions of *Didinium nasutum*, etc., Biol. Bull., Woods Hole, Vol. 16, p. 91.
- MERESCHKOWSKY, C.  
1881. On Some New or Little Known *Infusoria*, Ann. and Mag. of Nat. Hist. 5th Ser., vol. 7, No. 39, p. 209.
- MEUNIER, A.  
1910. Microplankton des Mers de Barents et de Kara, Duc d'Orleans Campagne Arctique de 1907, Brussels.
- MINCHIN, E. A.  
1907. Protozoa, in Allbutt and Rolleston's A System of Medicine, Lond.
- MINCHIN, E. A.  
1912. An Introduction to the Study of the Protozoa, Lond.
- PRATT, H. S.  
1916. A Manual of the Common Invertebrate Animals, etc., Chicago.
- ROUX, J.  
1901. Faune Infusorienne des Eaux Stagnantes des Environs de Genève, Genève.
- ROUX, J.  
1902. Note sur les Infusoires Ciliés du Lac Lemman, Revue Suisse Zool., T. 8, fasc. 3, p. 459.
- SMITH, I. F.  
1914. A Preliminary Report on the *Infusoria* of Kansas, Kansas Univ. Sci. Bull., vol. 9, No. 13.
- STEIN, F.  
1854. Die Infusionsthier, auf Ihre Entwicklungsgeschichte, Leipzig.
- STEIN, F.  
1859. Der Organismus der Infusionsthier, 3 vols., Leipzig.
- STEVENS, N. M.  
1901. Studies on Ciliate *Infusoria*, Proc. Calif. Acad. Sci., 3rd Ser., vol. 3.
- STOKES, A. C.  
1885. Some New *Infusoria*, Am. Nat., vol. 19, No. 5, p. 433.
- STOKES, A. C.  
1887. Notices of New Fresh Water *Infusoria*, Pro. Am. Phil. Soc., vol. 24, p. 244.
- STOKES, A. C.  
1888. A Preliminary Contribution Towards a History of the *Infusoria* of the Fresh Waters of the United States, Jour. Trent. Nat. Hist. Soc., vol. 1, no. 3.
- STOKES, A. C.  
1918. Aquatic Microscopy for Beginners, 4th ed., N. Y.
- WARD AND WHIPPLE  
1918. Fresh Water Biology, N. Y.

## EXPLANATION OF PLATE

- Fig. 1. Stain or reagent set holder.  
Fig. 2. Culture oven *a*, switch, *c*, cardboard cover, *d*, tin shelf, *e*, lamp rack, *f*, copper wire for suspending shelves, *g*, thermometer.  
Fig. 3. Strainer for concentrating samples.  
Fig. 4. Isolation pipette  
Fig. 5. *Holophrya* sp. 1, 15–25 $\mu$   
Fig. 6. *Holophrya* sp. 2, 15–25 $\mu$   
Fig. 7. *Holophrya* sp. 3, 15–25 $\mu$   
Fig. 8. *Holophrya* sp. 4, 15–25 $\mu$   
Fig. 9. *Holophrya* sp. 5, 45–55 $\mu$   
Fig. 10. *Holophrya* sp. 6, 30–35 $\mu$   
Fig. 11. *Holophrya* sp. 7, 40–45 $\mu$ .  
Fig. 12. *Urotricha farcta*, 15–25 $\mu$   
Fig. 13. *Urotricha platystoma*, 35–45 $\mu$   
Fig. 14. *Perispira ovum*, 80–100 $\mu$   
Fig. 15. *Enchelys pupa*, 80–100 $\mu$   
Fig. 16. *Enchelys farcimen*, 25–50 $\mu$   
Fig. 17. *Enchelyodon farctus*, 175–225 $\mu$   
Fig. 18. *Spathidium spathula*, 60–80 $\mu$   
Fig. 19. *Prorodon armatus*, 25–30 $\mu$   
Fig. 20. (See Plate II)  
Fig. 21. (See Plate II)  
Fig. 22. *Trachelocerca olor*, 320–380 $\mu$

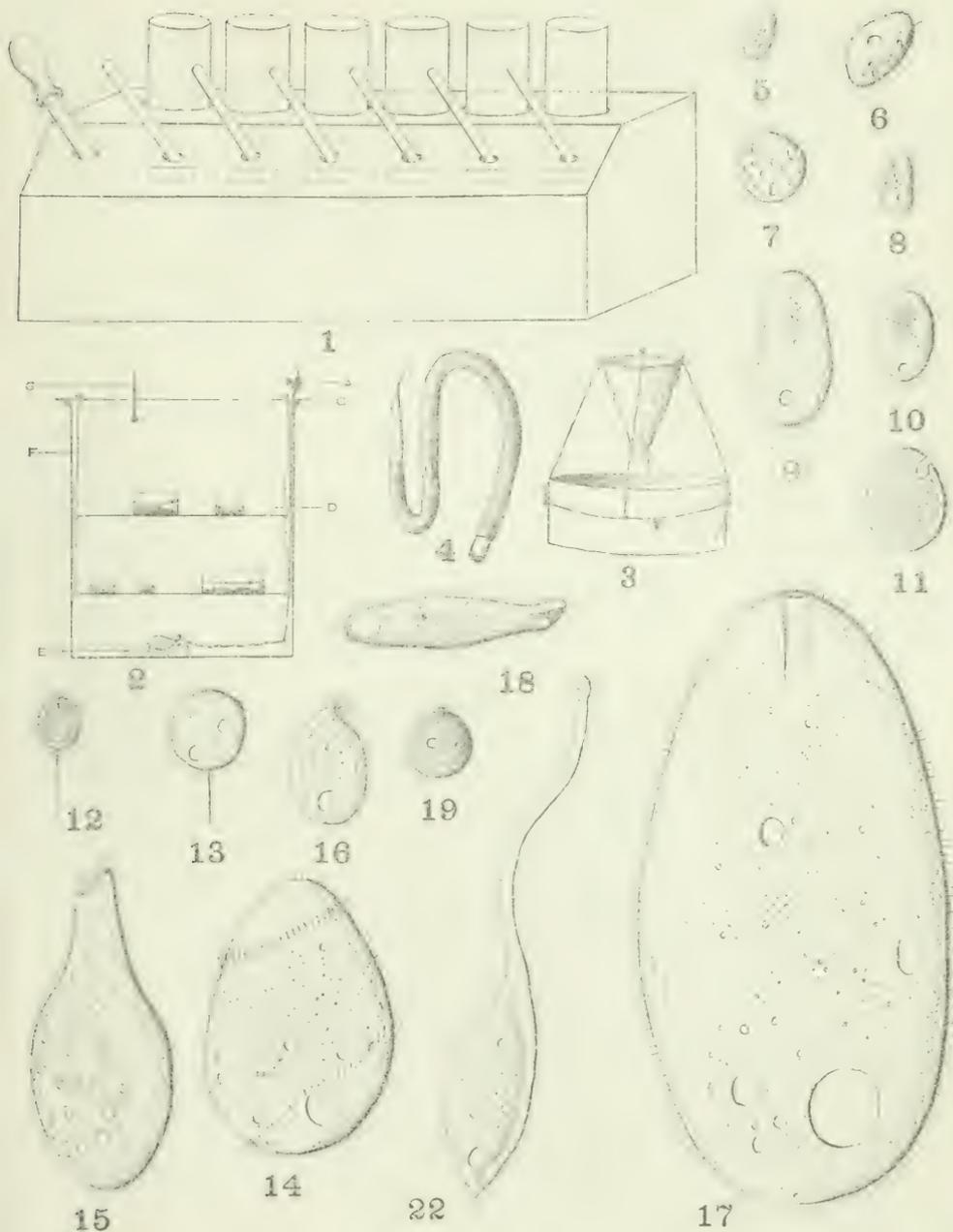


PLATE V

## EXPLANATION OF PLATE

- Fig. 20. *Prorodon ovum*, 100–130 $\mu$ .  
Fig. 21. *Lacrymaria olor*, 320–380 $\mu$ .  
Fig. 23. *Trachelocerca phoenicopterus*, 450–1000 $\mu$ .  
Fig. 24. *Ileonema dispar*, 115–125 $\mu$ .  
Fig. 25. *Plagiopogon coleps*, 75–90 $\mu$ .  
Fig. 26. *Coleps hirtus*, 45–55 $\mu$ .  
Fig. 27. *Tiarina fusus*, 75–80 $\mu$ .  
Fig. 28. *Didinium nasutum*, 850–1000 $\mu$   
(the only species not drawn to scale. If represented in its relative proportions, it would be more than twice and a half as large as *Trachelius ovum*, Fig. 43, Plate IV).  
Fig. 29. *Mesodinium pulex*, 10–20 $\mu$ .  
Fig. 30. *Mesodinium cinctum*, 30–45 $\mu$ .  
Fig. 31. (See Plate III).  
Fig. 32. *Amphileptus gutta*, 40–60 $\mu$ .  
Fig. 33. *Lionotus* sp. 1, 25–35 $\mu$ .  
Fig. 34. *Lionotus fasciola*, 75–125 $\mu$ .  
Fig. 35. *Lionotus pleurosigma*, 110–125 $\mu$ .  
Fig. 36. *Lionotus wrzesniowski*, 175–200 $\mu$ .  
Fig. 37. *Lionotopsis anser*, 75–100 $\mu$ .  
Fig. 38. *Loxophyllum*, sp. 1, 45–50 $\mu$ .

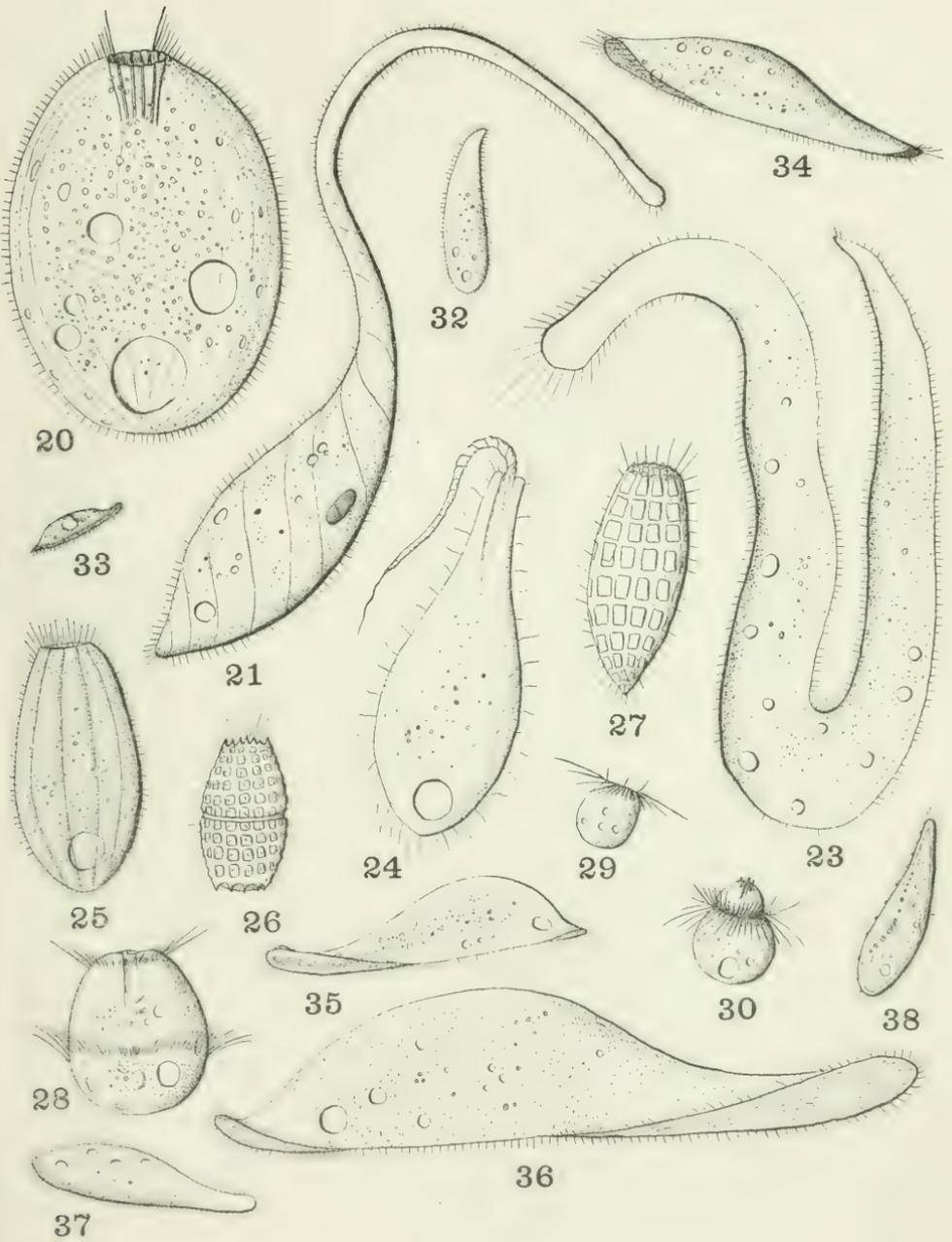
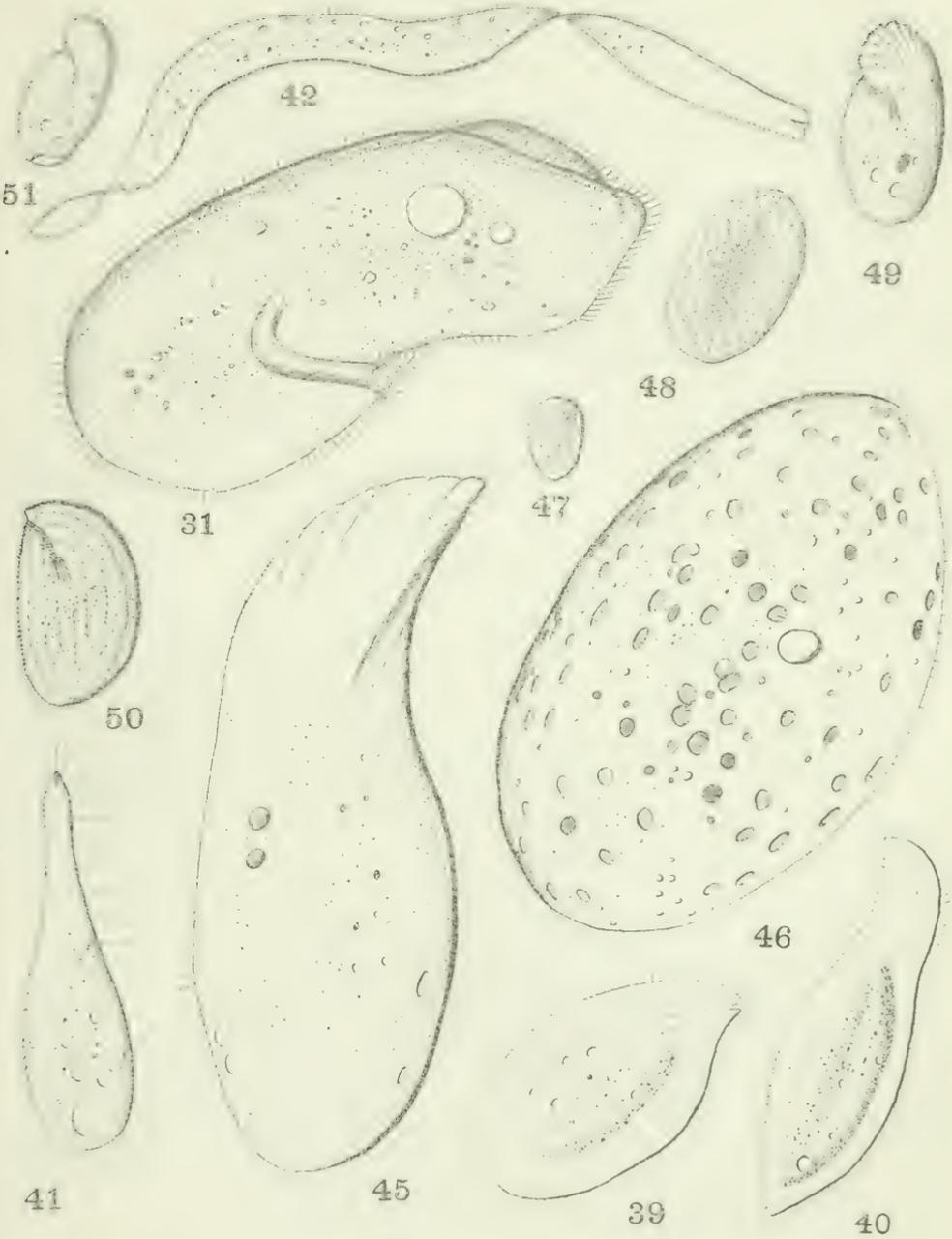


PLATE VI

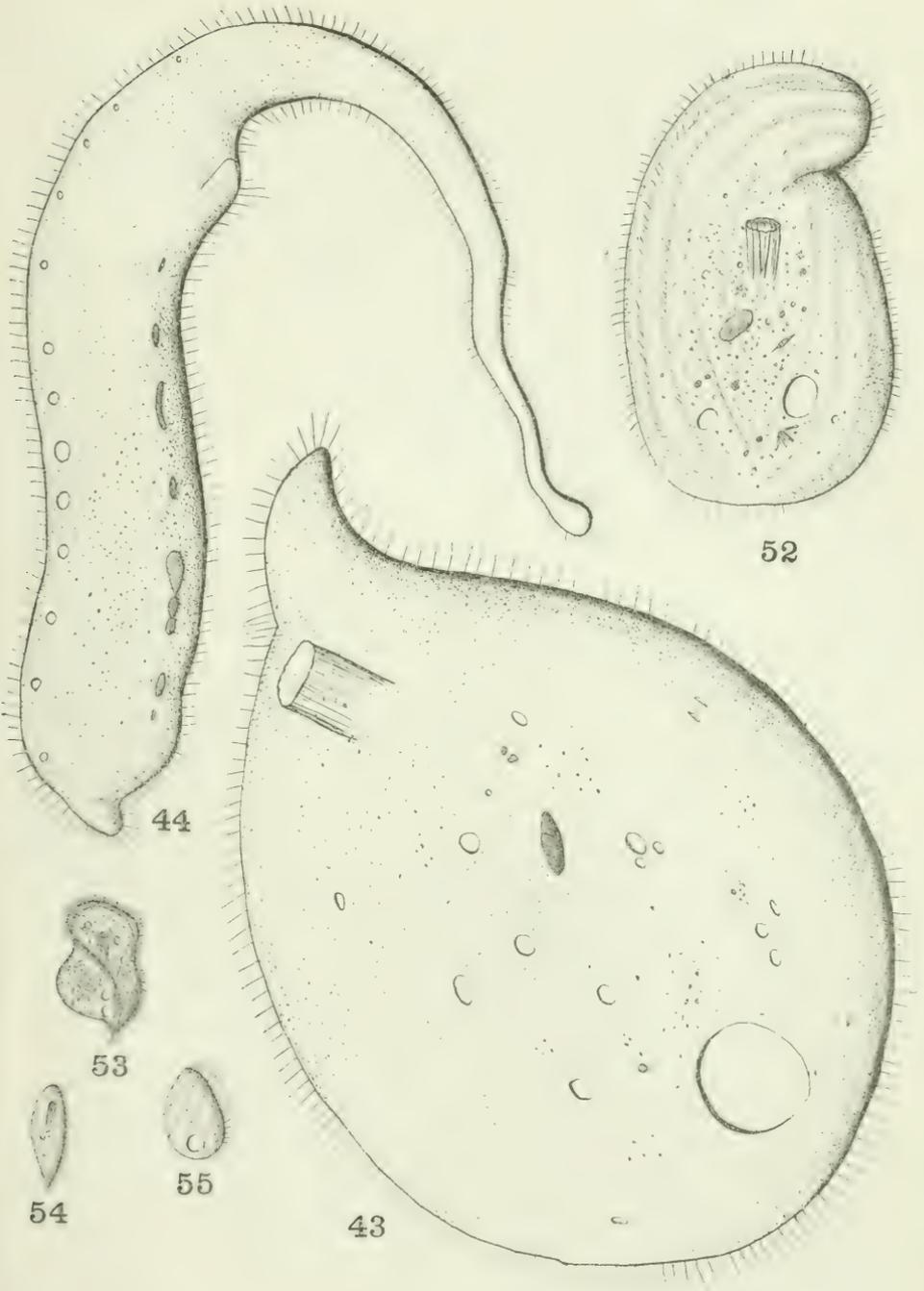
## EXPLANATION OF PLATE

- Fig. 31. *Tillina magna*, 195-225 $\mu$ .  
Fig. 39. *Loxophyllum setigerum*, 100-125 $\mu$   
Fig. 40. *Loxophyllum rostratum*, 125-150 $\mu$   
Fig. 41. *Trachelophyllum tachyblastum*,  
120-150 $\mu$   
Fig. 42. *Flexiphyllum elongatum*, 200-300 $\mu$   
Fig. 43. (See Plate IV)  
Fig. 44. (See Plate IV)  
Fig. 45. *Loxodes rostratum*, 250-350 $\mu$   
Fig. 46. *Nassula ornata*, 200-400 $\mu$   
Fig. 47. *Nassula* sp. 1, 30-35 $\mu$   
Fig. 48. *Nassula microstoma*, 50-60 $\mu$   
Fig. 49. *Chilodon megalotrocha*, 40-50 $\mu$   
Fig. 50. *Chilodon vorax*, 50-70 $\mu$   
Fig. 51. *Chilodon caudatus*, 35-50 $\mu$



## EXPLANATION OF PLATE

- Fig. 43. *Trachelius ovum*, 280-350 $\mu$   
Fig. 44. *Dileptus gigas*, 450-800 $\mu$   
Fig. 52. *Chilodon cucullulus*, 125-225 $\mu$   
Fig. 53. *Phascolodon vorticella*, 40-70 $\mu$   
Fig. 54. *Scaphiodon* sp. 1, 25-35 $\mu$   
Fig. 55. *Trochilia sigmoides*, 30-40 $\mu$



# COPPER: ITS OCCURRENCE AND RÔLE IN INSECTS AND OTHER ANIMALS<sup>1</sup>

By

RICHARD A. MUTTKOWSKI, PH.D.  
University of Idaho, Moscow, Idaho

1. General.
2. Respiratory Proteins in Insects; the Copper Nucleus.
3. Copper in Other Animals.
4. The Sources of Copper: Soil, Water, Plants.
5. Discussion.
6. Summary.
7. Bibliography.

## I. GENERAL

The present paper arose from certain experiments on the respiration of insects, particularly on the gases of the blood, and its rôle as a respiratory factor.

As at present understood, respiration in insects proceeds by the tracheal method: Atmospheric air is led directly to the cells by the tracheae, while the blood acts primarily in the transportation of food and metabolic products. This is modified in aquatic stages, in some of which the oxygen in solution in the water is absorbed by means of tracheal gills. In certain *secondarily* aquatic insects,—that is, insects originally aquatic which became terrestrial in habit, but which in some stages again have sought the water,—there are found structures which fundamentally are of the gill type. Here a thin membrane separates the blood from the water; the blood takes up oxygen through this membrane and distributes it directly to the tissues, or indirectly by yielding it to the gaseous supply in the tracheae. Such structures are the gill filaments and gill pouches of Trichoptera larvae and aquatic caterpillars, the caudal gill pouches of Chironomid larvae, Culicid larvae, Simulium larvae, etc.

Considered from both the physiological and morphological standpoint, these structures are meaningless unless a respiratory protein is postulated in the insect blood to fix the transfusing oxygen. Without such a protein, there could exist only an oxygen balance between the fluids divided by the animal membrane,—between the oxygen in solution in the blood serum and that in solution in the water. But since the available dissolved oxygen decreases with the rising temperature of the water, and the temperature of the insect rises with that of its environment, the oxygen supply of the water becomes impoverished, and with it the amount in solution in the blood,—that is, if the blood lacked a respiratory protein.

<sup>1</sup> (Contribution from the Zoological Laboratory of the University of Idaho, Moscow, Idaho.)

Furthermore, many insects are found in places where the available oxygen is nearly entirely used up in the decomposition and oxidation of organic waste. Indeed, a number of species are known which live, grow, and transform under anaerobic conditions (Juday 1909, Muttkowski, 1918).

Now, a respiratory protein is known for a few insect species, among them some of the anaerobes just referred to, especially the so-called "blood-worms" or Chironomid larvae. This pigment has been identified as hemoglobin, dissolved in the blood plasma, and not included in the corpuscles. As far as known to the writer, it is restricted among insects to the "blood-worm" type of Chironomid larvae. The important point in connection with the hemoglobin of these larvae is this: It is confined to a few species, but not all of these species live under anaerobic conditions; nor do all anaerobic Chironomids contain hemoglobin. One is forced to the conclusion that among these latter the hemoglobin is replaced by some other respiratory pigment. Hemocyanin has been suggested by the writer (1920), altho it had not been demonstrated for a single insect species.

It is known from the study of vertebrate blood that hemoglobin forms oxyhemoglobin with oxygen and carbohemoglobin with carbon dioxide. From its identity with vertebrate hemoglobin it can be supposed that the activity of the hemoglobin as found in the Chironomid "blood-worms" is similarly two-fold,—that it transports both oxygen and carbon dioxide. For the rest of insects it was shown by the writer that both oxygen and carbon dioxide are present in the blood (account published elsewhere). Hence it is logical to assume that, similar to Chironomid "blood-worms," there is a definite carrier which fixes both oxygen and carbon dioxide.

The following recounts a series of experiments undertaken in an attempt to prove or disprove the foregoing assumption. The experiments were performed during the spring, summer and fall of 1920, altho some earlier observations made in the course of the past ten years are included.

## II. RESPIRATORY PROTEINS IN INSECTS; THE COPPER NUCLEUS

In its development the problem presented several distinct phases: (1) Aside from the few insects possessing hemoglobin, is a respiratory protein available at least in those aquatic insects provided with blood gills? (2) If such a protein can be demonstrated, what is its nature? (3) If available, is it confined to aquatic stages or is its distribution universal among insects?

The presence of hemoglobin in Chironomid larvae (blood-worm type) is easily verified. For the blood responds to the various oxidation (Guaiac, O-tolidin, and Benzidine reactions) and crystallization tests (Hemin) that have been elaborated for the recognition and study of hemoglobin in vertebrate blood. Except for the hemin test, none of these is conclusive

as far as general differentiation between Invertebrate and Vertebrate blood is concerned. The hemin test alone indicates positively the presence of hemoglobin *as such* in Chironomid "blood-worms," or any other animal. Yet in a number of tests made for hemoglobin in the colorless blood of species like *Anax*, *Aeshna*, *Dytiscus*, and others, isolated crystals other than Sodium chloride were found which resembled the familiar prisms of hemin.

The oxidation tests are conclusive only in so far as they reveal the presence of blood, specifically the respiratory protein. They do not indicate the identity of this pigment. For it is noteworthy that the blood of crayfish as well as that of all insects reacts with Guaiac, and Benzidine, and produces color changes identical with those produced by vertebrate blood (See table I). Note that these oxidations are produced by blood which shows little or no trace of hemoglobin. Furthermore, as with vertebrate blood, boiling the test substance does not stop the reaction.

TABLE I. REACTIONS OF INSECT BLOOD TO HEMOGLOBIN TESTS

Name of Species	Guaiac Test (Oxidation)		Benzidine Test (Oxidation)		Hemin Test-Crystals (Nippe's Reagent)	
	Number	Result	Number	Result	Number	Result
<i>Aeshna</i> larva.....	8	pos.	3	pos.	5	4 neg., 1 trace
<i>Anax</i> , yg. larvae....	2	"	1	"		
<i>Enallagma</i> larvae...	2	"	1	"		
Mayfly nymphs....	1	"				
<i>Belostoma</i> .....	3	"	1	"	7	5 neg., 2 trace
<i>Chironomus</i> larvae	6	"	3	"	12	12 pos.
<i>Dytiscus</i> larvae....	2	"			6	5 neg., 1 trace
<i>Dytiscus</i> adults....	2	"	1	"	7	5 neg., 2 trace
Controls						
<i>Cambarus</i> blood...	6	"	3	"	5	5 neg.
Blank, with FeCl <sub>2</sub> ..	5	"	3	"		
Blank, with CuSO <sub>4</sub>	5	"	3	"		

In hemoglobin the iron is the active agent in the oxidation, in the hemocyanin of the crayfish it is the copper. That such is really the case was readily shown by the introduction of a crystal of ferric chloride or copper sulphate into some of the blank control tests. Such "salted" controls reacted positively, before and after boiling.

The tests described proved two things: (1) The oxidation tests for hemoglobin do not serve to differentiate between this and other respiratory proteins, or between the blood of Vertebrates and Invertebrates. (2) They proved the presence of a respiratory protein in insects.

Two possibilities at once presented themselves,—that the carrier in question could be either hemoglobin, or hemocyanin; or both, as in some

mollusks. It was definitely ascertained, however, that in only a very few insects could hemoglobin be the respiratory protein. In most insects, if present at all, it was found only in infinitesimal quantities, and therefore negligible as a respiratory factor. This left the alternative of hemocyanin. This respiratory protein has been reported for a number of higher Crustacea and some Arachnida (Scorpion, *Limulus*). It is also widely known among the Mollusca, and there is no valid reason to assume that it might not be present in other groups of animals, including the insects.

To ascertain if this is the case, both direct and indirect methods were resorted to in this study. Unfortunately, no direct method for the recognition of hemocyanin is known such as the hemin test for hemoglobin. A large number of experiments were attempted to find such a reagent, but all were unsuccessful. Hence an indirect method was adopted.

In hemocyanin copper forms the nucleus of the respiratory compound. If the presence of copper could be shown in insect blood in amounts comparable to the copper content of equal quantities of crayfish blood,—then it would be logical to assume that this copper forms the nucleus of a respiratory protein similar to that of crayfish. Since, as already related, various tests indicated the presence of extremely minute quantities of hemoglobin, and since hemocyanin responded positively to the various hemoglobin tests, the latter were useless for differentiation between an iron and a copper compound. It therefore became necessary to separate the two, and to test separately for copper. This, of course, could be accomplished only after incineration of the tissues.

Among larger insects the blood and entire specimens, in small insects only whole specimens, were incinerated in the course of this study. The incinerations were begun in June 1920 and continued thru the summer and fall, as material became available. The usual analytical methods were followed: the ash was dissolved in hot dilute hydrochloric acid, a portion tested for iron, while the remainder was treated with excess of ammonia, precipitating the iron and dissolving the copper. The solution was then filtered, the filtrate concentrated by slow heating, acidulated with acetic acid, and tested for copper. Where the amount of ash was very small, the residue was redissolved and reprecipitated several times in order to obtain all the copper present.

As expected, iron showed heavily in all the incinerations, as it is universally present in animal cells. The thiocyanates were the chief reagents used in testing for this substance. Only qualitative tests were performed on copper, with notation of the approximate intensity of the reaction as compared with the control substance, namely crayfish blood. No exact quantitative estimates were possible, as the amounts dealt with

were microscopic. The reagents employed were Potassium ferrocyanide, Ammonium mercuric sulphocyanate, especially after the test drop had been inoculated with zinc salts (acetate or sulphate) or Caesium and Rubidium chloride; and finally, the Lead acetate—Potassium nitrite method for the formation of the triple nitrite Lead-Copper-Potassium. The ammonia so generally employed as a test for copper was not sufficiently delicate. It is sensitive only to about 1:2000, and the copper obtained in the few milligrams of ash was insufficient to react with it. The other reagents mentioned are sensitive to copper in dilutions up to 1:50000 and over, sufficiently so to give definite reactions.

The incinerations covered practically every order of insects (see Table II). The material incinerated was collected by the writer from Paradise Creek, two or three ponds, and the fields in the immediate vicinity of the university at Moscow, Idaho; except no. 45, Sialis larvae, which were obtained from Lake Mendota, Madison, Wis., and kindly sent me by Prof. Chancey Juday, of the Wisconsin Geological and Natural History Survey.

TABLE II. COPPER IN INSECTS

Name	Stage	Tissue	No. of Incinerations	Result Cu	Remarks
Coleoptera					
1. Dytiscus	larva	blood	2	pos.	Equal to Cambarus
2. "	"	whole	2	pos.	slightly less
3. "	adult	blood	5	pos.	nearly equal
4. "	"	whole	4	pos.	slightly less
5. Gyrinus	adult	whole	1	pos.	less
6. Harpalus	adult	whole	1	pos.	less
7. Leptinotarsa	"	"	1	pos.	less than C.
Hymenoptera					
8. Apis mellifica	adult	whole	2	pos.	less than C.
9. Bombus sp.	"	"	1	pos.	less
10. Polistes	"	"	2	pos.	nearly equal
11. Formica	"	"	1	pos.	less
Lepidoptera					
12. Pieris rapae	larva	blood	1	pos.	about equal
13. " "	"	whole	1	pos.	about equal
14. " "	adult	whole	2	pos.	less
15. Noctuid moths	adult	whole	2	pos.	less
Diptera					
16. Musca domestica	larva	whole	2	pos.	slightly more
17. " "	adult	whole	2	pos.	about equal
18. Stomoxys	adults	"	2	pos.	less
19. Tachinid flies	"	"	1	pos.	less

TABLE II. COPPER IN INSECTS (Continued)

Name	Stage	Tissue	No. of Incinerations	Result Cu.	Remarks
<b>Hemiptera</b>					
20. <i>Belostoma</i>	yg. nymphs	blood	3	pos.	slightly less
21. "	yg. nymphs	whole	3	pos.	slightly less
22. "	adults	blood	6	pos.	about equal
23. "	"	whole	5	pos.	slightly less
24. <i>Ranatra</i>	"	"	1	pos.	" "
25. <i>Gerris</i> sp.	"	"	1	pos.	" "
26. <i>Notonecta</i> sp.	"	"	3	pos.	" "
27. <i>Corixa</i> sp.	"	"	2	pos.	" "
28. <i>Aphis</i> sp.	mixed	"	1	pos.	" "
<b>Odonata</b>					
29. <i>Anax</i> & <i>Aeshna</i>	yg. larvae	blood	2	pos.	less
30. <i>Anax</i> & <i>Aeshna</i>	yg. larvae	whole	2	pos.	less
31. <i>Anax</i>	larvae	blood	1	pos.	nearly equal
32. "	"	whole	1	pos.	less
33. <i>Aeshna</i>	"	blood	8	pos.	nearly equal
34. "	"	whole	7	pos.	" "
35. <i>Sympetrum</i>	"	blood	2	pos.	less
36. "	"	whole	2	pos.	"
37. <i>Libellula</i>	yg. larvae	whole	1	pos.	"
38. "	old larvae	whole	3	pos.	slightly less
39. <i>Enallagma</i>	yg. larvae	whole	1	pos.	less
40. "	late larva	"	3	pos.	about equal
41. "	adult	whole	2	pos.	less
<b>Ephemeroidea</b>					
42. Several spp.	nymphs	whole	1	pos.	slightly less
<b>Trichoptera</b>					
43. Several spp.	larvae	whole	1	pos.	nearly equal
<b>Neuroptera</b>					
44. <i>Myrmeleon</i>	adults	whole	3	pos.	equal or stronger
<b>Megaloptera</b>					
45. <i>Sialis infumata</i>	larvae	whole	1	pos.	nearly equal
<b>Isoptera</b>					
46. <i>Termes</i> sp.	mixed	whole	1	pos.	nearly equal
<b>Orthoptera</b>					
47. <i>Gryllus</i>	adult	whole	1	pos.	nearly equal
48. <i>Ceuthophilus</i>	"	"	1	pos.	less
49. <i>Locusta</i>	"	"	1	pos.	nearly equal
50. <i>Melanoplus</i>					
<i>bivittatus</i>	adult	"	2	pos.	nearly equal
51. <i>Dissosteira</i>	"	"	1	pos.	less

Total—34 species, 108 incinerations.

A glance at the results in Table II shows positive reactions for copper in all insects incinerated, no matter what the stages chosen for ashing.

This universal presence of copper among the Insecta, not only in aquatic forms, but also in terrestrial species, indicates that it has an important function which hitherto has been overlooked. Its universal distribution is certainly not adventitious. Such a contingency might be explained for aquatic insects on the basis of the food supply (a large percentage of Crustacea), but would hardly apply to terrestrial insects, especially those among the latter which feed on plants only, or whose food is even more restricted, as in the case of honey bees. Furthermore, the copper present in the blood of many insects exists in practically the same proportions as in the blood of Crustacea. In *Belostoma*, for instance, five cc. of incinerated blood reacted to ammonia, showing a slightly fainter shade of blue than an equivalent amount of incinerated *Cambarus* blood. Indeed, copper was present to such extent; that an incineration of one cc. showed decisive reactions with all the reagents listed except ammonia. Other examples might be adduced, such as wasps and ant-lions. Here the ash of a single individual gave positive response to tests for copper.

Based on the foregoing results, the writer offers the hypothesis that the rôle of copper in insects is to form the nucleus of a blood protein,—namely a hemocyanin, similar in constitution to the known hemocyanins of Crustacea and mollusks; and that it serves a similar purpose, that of a respiratory pigment.

Based on experiments, recorded elsewhere, on the presence of oxygen and carbon dioxide in the blood of insects, the writer advances the further suggestion, that the function of this hemocyanin is parallel to that of hemoglobin,—namely, that the hemocyanin carries both oxygen and carbon dioxide, that compounds are formed in the respiratory cycle similar to the oxy- and carbohemoglobins. This second hypothesis has not been proved directly, but it is logical to assume that analogy of function in a respiratory protein, as hemocyanin is analogous to hemoglobin, should result in analogous compounds during the respiratory cycle. In short, it is reasonable to assume the formation of oxyhemocyanin with oxygen, and of carbohemocyanin with carbon dioxide.

From this standpoint, the various respiratory structures of advanced aquatic insects, such as the gill filaments and gill pouches of Trichoptera larvae and aquatic caterpillars, and the others referred to in the opening paragraph of this paper, acquire a real significance. If considered as of the category of true gills, to which type they undoubtedly belong, it is easy to understand how effective they would be with a respiratory protein. Without such a pigment to fix the gases they would seem purposeless as structures, and inefficient physiologically.

## III. COPPER IN OTHER ANIMALS

As already stated, hemocyanin has been reported for mollusks, several species of higher Crustacea, scorpions and *Limulus* among Arachnida, and more recently for Coelenterates and fish. To ascertain whether or not it is found in the other classes of Arthropoda, the writer incinerated several species of plankton Crustacea, spiders, daddy-long-legs, centipeds, and millipeds (see Table III). In all of these copper was discovered in quantities equal to or exceeding the amount present in *Cambarus* blood.

As seen from this same table, examples of other phyla were also incinerated, including snails and slugs among mollusks, *Lumbricus* among Annulata, *Ascaris* among Nematelminthes, *Volvox* among Protozoa, and human blood and snake blood for Chordata.

This material also was collected locally, except nos. 13-16 inclusive, which were collected from Wisconsin lakes, and kindly sent me by Prof. Chancey Juday, of the Wisconsin Geological and Natural History Survey.

The determination of copper in the blood of *Cambarus* has been noted repeatedly in this paper. The blood of this Crustacean was used constantly as a control for other incinerations. Only a small number of these controls are listed in the table. A second pigment has been reported for marine Decapoda, called Tetroneurhythm, which is found also in our fresh water crayfish. The function of this pigment is unknown, altho it has been stated definitely that it is not a respiratory pigment. It is probable, however, that the pigment is used in the coloration and markings of the exoskeleton, and that it is carried passively in the bloodstream, similar to the pigments found in insect blood, and elaborated during the ecdysis. It is readily perceived in crayfish blood, from which it may be crystallized in orange-red crystals. Ordinarily it is not very abundant, but previous to moulting it is present in quantities sufficient to give a distinct pink or reddish color to the blood. Indeed, in larger crayfish, exceeding four inches in length, I have found the blood a bright red or scarlet, so that it resembled the diluted and aerated blood of a vertebrate. This blood clots in dark red masses, also resembling the clots of vertebrate blood. In "soft," freshly moulted crabs the blood appears transparent and contains little or none of this pigment.

Among species other than Arthropod *Volvox* furnished perhaps the greatest surprise by its show of copper, not merely as a trace, but in appreciable quantity. About 15 cc. of filtered *Volvox* were used in this incineration. That the reaction could not have been due to residual water is indicated by the fact that 100 cc. of water from the same pond showed not the slightest trace of this element. Its function in *Volvox* is problematical.

*Ascaris* furnished an additional surprise. Surely no one would suspect copper in an internal parasite. However, as barely a trace was found,

TABLE III. COPPER IN ANIMALS OTHER THAN INSECTS

Name	Stage	Tissue	No. of incinerations	Result	Remarks
Crustacea					
1. Cambarus sp.	1 in. long	blood	3	pos.	
2. "	1 in. long	whole	4	pos.	
3. "	2 in. long	blood	4	pos.	More Cu. than in No. 1.
4. "	2 in. long	whole	3	pos.	" " " " No. 3
5. "	3 in. long	blood	6	pos.	" " " " "
6. "	3 in. long	whole	3	pos.	" " " " No. 5
7. "	4 in. long	blood	5	pos.	" " " " "
8. "	4 in. long	whole	3	pos.	" " " " "
9. "	5 in. long	blood	3	pos.	" " " " No. 7
10. "	5 in. long	whole	2	pos.	" " " " "
11. Hyalella	adults	whole	2	pos.	less than No. 2
12. Cladocera & Copepods	adult	whole	1	pos.	" " "
13. Daphnia pulex	adults	whole	1	pos.	" " "
14. Microcystis	adults	whole	1	pos.	" " "
15. Copepods chiefly Limnocalanus)	adults	whole	1	pos.	" " "
16. Daphnia pulex	adults	whole	1	pos.	" " "
Arachnida					
17. Argiope sp.	adult	whole	1	pos.	Equal to No. 9
18. Phalaena sp.	adults	whole	1	pos.	More than No. 9
19. Several spiders	adults	whole	1	pos.	Equal to No. 9
Myriapoda					
20. Millipeds spp.	adults	whole	1	pos.	More than No. 9
21. Centipeds spp.	adults	whole	1	pos.	More than No. 9
Annulata					
22. Lumbricus		whole	1	pos.	trace
Mollusca					
23. Physa sp.	mixed	whole	5	pos.	Equal to No. 3
24. Slugs	adult	whole	2	pos.	Equal to No. 4
Nemathelminthes					
25. Ascaris	♂ & ♀	whole	1	pos.	trace
Protozoa					
26. Volvox	mixed	whole	1	pos.	Equal to No. 2
Chordata					
27. Thamnophis <del>viridis</del>	adult	blood	1	pos.	trace in 2.8 gr.
28. Homo sapiens	adult	blood	1	neg.	about 1.6 gr. used.

and this due probably to mechanical storage, it would hardly be justifiable to attribute any physiological rôle to copper in this parasite. Its source is most probably the plant food taken in by the host.

In snails and slugs the copper undoubtedly occupies the same rôle that it has in squids, clams, and other mollusks. *Lumbricus*, like *Ascaris*, showed only a trace. Aside from leeches, this is the first time copper has been noted for an Annelid.

The fact that an abundance of copper was found in Myriapoda and in several representatives of the Arthropoda, in some even more than in the control substance, lends definite support to the assumption that for all Arthropoda copper is an essential element, and functions in the rôle of a respiratory protein in all members of this largest of phyla.

The discovery of copper in snake blood was due to pure chance. Two and eight tenths grams of snake blood besides a small quantity of human blood had been incinerated for another purpose. While waiting to utilize the ash at some later date it occurred to the writer to test for copper. (At the time I did not know of Rose and Bodansky's discovery of copper in marine fish.) The various tests were negative except two in which the test drop had been placed under alcohol vapor for several hours. No reaction showed in the first fifteen minutes, but after that indications of a positive reaction were noticeable. Later, when examined after an interval of several hours, the reactions showed definitely positive.

The ashed human blood referred to in the foregoing paragraph was also tested, but gave negative results. However, since the quantity was even less (1.83 gr.) than the snake blood used, the experiment is inconclusive.

#### IV. THE SOURCES OF COPPER

For aquatic animals the source of copper is the slight amount in solution in the water. It is thus that mollusks and Crustacea obtain the copper necessary to their respiration. The soluble copper originates from the soil. Since the distribution of mollusks and Crustacea is universal, copper must likewise be available universally.

For terrestrial animals such as bees, wasps, caterpillars, moths, spiders, centipeds, etc., the soil cannot be considered as a direct source of copper. Their food consists largely of plants and animals, and perhaps minute droplets of water from wayside pools. It is evident that their copper must eventually come to them by way of their plant food. To determine this positively, a number of plants were incinerated and tested for copper (Table IV).

All plants reacted positively, but only to the more sensitive reagents, as the copper is present only in traces, not at all in amounts comparable to that of Arthropoda. All parts of the plant showed the presence of copper, with this difference: the fruit generally contained a less amount than the stem, leaves, or root. Because of the minimal amounts, its rôle in the plant is

probably not an active one, and its presence due to mechanical storage. As far as the relation of copper and plants is concerned, the copper ion is known to be highly toxic to plants, especially to the lower forms of plant life.

TABLE IV. COPPER IN PLANTS

Name	Part	No. of Incinerations	Result for Copper
1. Watermelon	Rind	2	Pos. trace
2. Pear	Leaves	2	pos. trace
3. Pear	fruit	2	pos. less than No. 2
4. Tomato	leaves and stem	2	pos. trace
5. Tomato	fruit	2	pos. less than No. 5
6. Potato	leaves	1	pos.
7. Lettuce	leaves	2	pos.
8. Red Beet	leaves	2	pos.
9. White Beet	leaves	2	pos.
10. Apple	leaves	2	pos.
11. Apple	fruit	2	pos. less than No. 11
12. Currant	leaves	2	pos.
13. Celery	stem	2	pos.
14. Clover	heads	1	pos.
15. Clover	leaves	2	pos. more than No. 14

Total—11 species, 30 incinerations.

Here in Moscow the copper content of the soil is very low. The surface soil is of aeolian origin and what copper it contains is brought by dust-storms from mountains 100 to 200 miles to the west in Washington and Oregon. In a quantitative estimate of copper in the soil 50 grams yielded only sufficient copper to permit a qualitative test.

Samples of water, each 3500 cc., taken from Paradise Creek during the summer and concentrated to 5 cc., likewise showed little more than traces of copper. A sample of water taken more recently (November) from under the ice showed a copper content of 0.0187 gr. by the sulphocyanide method or approximately 0.00534 gr. per liter. The mud of Paradise Creek shows somewhat larger amounts, some of which may be due to organic matter.

## V. DISCUSSION

In a recent paper on the occurrence of copper in marine organisms, Rose and Bodansky (1920) note the previous demonstration of copper in the following groups:

1. Echinodermata—starfish, urchins, sea-squirts.
2. Annulata—leech.
3. Crustacea—various Decapoda.
4. Arachnida—Scorpion, *Limulus*.

5. Mollusca—clams, oysters, snails, cuttle-fish, octopus.
6. Tunicata—Ciona.
7. Pisces—shark, 2 Teleosts.

To this list Rose and Bodansky add:

1. Coelenterata—jellyfish, Portuguese Man-of-War.
2. Mollusca—oysters, clams.
3. Crustacea—shrimps and crabs.
4. Pisces—Torpedo and sting ray, 12 Teleosts.

In all they add some 35 species, demonstrating for the first time that the copper in fish is not due to pathological causes. A survey of the groups studied in the present paper shows some interesting additions to the foregoing lists:

1. Protozoa—Volvox.
2. Nematelminthes—Ascaris.
3. Mollusca—snails, slugs.
4. Annulata—Lumbricus.
5. Arthropoda.
  - a. Crustacea—plankton, Cambarus, Hyalella.
  - b. Arachnida—Phalaena, spiders.
  - c. Myriapoda—centipeds, millipeds.
  - d. Insecta—13 chief orders, over 35 species.
6. Chordata—snake.

To these must be added the occurrence of copper in higher plants.

Such a wide distribution of an element in a variety of living organisms, representing eight of twelve phyla, must have some significance. Its occurrence cannot be wholly adventitious, especially since it may be present in considerable quantities in the organism. Where present only in traces, it may well be ignored. In a number of forms its physiological rôle has been known for some time, altho physiologists believed that it was restricted to a few scattered species, and really was more or less an abnormality or rarity. Schulz, for instance (in Abderhalden 1910), states, "Hemocyanin occurs in the blood of higher Crustacea. It is present *only in a few members* (italics mine) of this class (Homarus, Maja, Portucuco, etc.)" Yet it is evident from the work of Rose and Bodansky and from the experiments herein noted that copper is not at all restricted to a few Decapoda among Crustacea, but that even the simplest and smallest Crustacea contain it.

Indeed, the writer, once he found positive indications of copper in a few species of insects, set out to test representatives of as many different groups as he could obtain locally. These were taken wholly at random, representing a variety of living conditions, from aquatic to parasitic, and entirely without regard to possible favorable results. (As a matter of fact,

in the end I purposely selected some of the least likely animals, such as *Volvox*, *Ascaris*, *Lumbricus*, and snakes.) This same attitude held for the work on lower Crustacea, spiders, and so on. The results, I believe, more than justified such a procedure, by indicating copper in the most unexpected places.

Yet this very fact of random selection is all the more convincing in a general application of the phenomena discovered. It signifies that these random selections are representative of whole groups and that what pertains to the few pertains also to the many. Since in these representative species copper has been found, and since in at least some of these few the physiological rôle of the copper has been definitely ascertained, it can be concluded that all or most remaining members of these groups also possess copper and that its physiological rôle is also similar. In other words, in at least the *Mollusca* and *Arthropoda* all species contain copper in appreciable quantities, and this copper functions as the nucleus of a respiratory protein, hemocyanin.

In thus extending particulate findings to entire groups of organisms I do not think I am overstepping the bounds of proper scientific conservatism. For copper was found in many groups, while in certain groups where a greater variety of material was available every species studied showed positive results. The uniformity of the results in these groups, unexpected as they were, is convincing and, I believe, warrants the above generalization.

## VI. SUMMARY

1. Both oxygen and carbon dioxide are present in insect blood in appreciable quantities. Insect blood aids in the transportation of gases, and the respiratory function is not confined to the tracheae.

2. Over 100 incinerations were made of insect blood, or whole specimens, and the ash tested for copper. Copper was found in all cases. The 35 species studied represent 13 of the chief orders of insects, in both larval and adult stages.

3. Copper is found in insect blood in quantities comparable to that of crayfish blood. Its rôle is therefore interpreted as being identical,—namely that it serves as the nucleus of a respiratory protein,—hemocyanin. Insects, therefore, have two sources of oxygen,—atmospheric air led directly to the tissues by way of the tracheae, and fixed oxygen carried by the respiratory protein of the blood.

4. Incinerations of plankton Crustacea, spiders, and centipeds gave positive results for copper, showing that copper is distributed among all classes of *Arthropoda*. It may therefore be regarded as an element essential to the physiological activity of *Arthropods*, its rôle being to act in a respiratory pigment for all members of this phylum.

5. As representatives of other phyla Volvox, Ascaris, snails and slugs, Lumbricus, human and snake blood were incinerated. All of these, except human blood, showed varying amounts of copper.

6. As sources of copper the water, soil, and plants were tested. All plant ash showed traces of copper. The water samples of this region showed only small quantities, while the soil showed varying amounts.

## VII. BIBLIOGRAPHY

- GUÉRITHAULT, B. Sur la présence du cuivre dans les plantes et particulièrement dans les matières alimentaires d'origine végétale. C. R. Acad. Sci., Paris, 171, pp. 196-198 (no. 3), 1920.
- JUDAY, C. Some Aquatic Invertebrates that Live Under Anaerobic Conditions. Trans. Wis. Acad. Sci., 16, part I, pp. 10-16, 1909.
- MUTKOWSKI, R. A. The Fauna of Lake Mendota. Trans. Wis. Acad. Sci., 19, part I, pp. 374-482, 1918.
- MUTKOWSKI, R. A. The Respiration of Aquatic Insects: A Collective Review. Bull. Brooklyn Ent. Soc., 15, pp. 89-96, 131-140, 1920. Bibliography.
- MUTKOWSKI, R. A. Copper in Animals and Plants. Science, N.S., 53, No. 1376, pp. 453-454, May 13, 1921.
- MUTKOWSKI, R. A. Studies on the Respiration of Insects. I. The Gases and Respiratory Proteins of Insect Blood. Ann. Ent. Soc. Am., 14, pp. 150-156, 1921.
- ROSE, W. C., and BODANSKY, M. Biochemical Studies on Marine Organisms. I. Copper in Marine Organisms. JN. Biol. Chem., 44, pp. 99-112, 1920. Bibliography.
- SCHULZ, F. N., in Aberhalden, H.B. d. Biochem. Arbeitsmethoden, Vol. II, pp. 335-346, 1910.

## DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

---

### MICROSCOPE ILLUMINATION WITH REFERENCE TO BROWNIAN MOVEMENT AND COMBINATION LIGHTING<sup>1</sup>

By

ALEXANDER SILVERMAN

**BROWNIAN MOVEMENT.**—For the study of this phenomenon china clay was mixed with distilled water and passed through a double quantitative filter paper. The opalescent filtrate was run into the cavity of a hollow-ground slide, and this in turn placed over dull black paper on the stage of the microscope. A 4mm. apochromat objective surrounded by a ring-lamp<sup>2</sup> was lowered into the solution and the instrument focused.



FIG. 1

<sup>1</sup> Published by courtesy of the American Chemical Society. Read before the Division of Industrial and Engineering Chemistry at the Rochester, N. Y. meeting of the American Chemical Society, April 25-29, 1921.

<sup>2</sup> J. Ind. Eng. Chem., 9(1917), 971; 10(1918), 1013; 12(1920), 1200.—J. Soc. Chem. Ind., 38(1919), 126.—J. Royal Mic. Soc., No. 253 (1920), 98.

A 10x compensating ocular gave a magnification of 440 diameters. Not only was Brownian movement clearly evident, but the dot-like, rod and lenticular shapes of particles were shown with great definition in white against a black background.

COMBINATION LIGHTING.—A comparative study was made of (1) the effect of transmitted light, (2) of direct super-stage illumination from a ring-lamp surrounding the objective, and (3) of combination lighting from above by the same lamp, a part of whose light passed through a glass slide and was reflected back by a sub-stage mirror placed parallel to the stage.

For this study a fossil insect in amber was employed. The length of the insect, 0.9 mm., will enable the reader to judge the magnification in the illustrations. A combination of 32 mm. objective and 10x ocular together with the length of bellows used in photographing gave a magnification of 60 diameters in the original photographic prints.

Figure 1 shows the effect of transmitted light from below, placing the mirror at an angle and using a 25 watt frosted spherical lamp. While sufficient contrast is obtained details are lacking because of the varying thickness of the insect.

Figure 2 shows the effect of direct light from the ring-lamp above the object with dull black paper placed under the slide. Some details are visible, but there is no contrast. Beautiful detail may be obtained by substituting white paper for black.



FIG. 2

Figure 3 is the result of a combination of light from the ring-lamp above, with its own light reflected upwards by the sub-stage mirror placed parallel to the microscope stage. A black paper was held over the sub-stage mirror after 15 seconds. This



FIG. 5

Department of Chemistry,  
University of Pittsburgh.

affords contrast and detail, and the method may prove desirable for photographing an object which has transparent, translucent and opaque parts.

Seeds Graflex plates, exposed 60 seconds, with a Davies shutter closed to the smallest stop, gave the results obtained in the insect photographs.

**Conclusions.**—This paper emphasizes the importance of a study of background colors and of combination lighting in microscopy and cites specific examples.

TRANSACTIONS  
OF THE  
American  
Microscopical Society

ORGANIZED 1878

INCORPORATED 1891

---

PUBLISHED QUARTERLY

BY THE SOCIETY

---

EDITED BY THE SECRETARY

PAUL S. WELCH

ANN ARBOR, MICHIGAN

---

VOLUME XL

NUMBER FOUR

---

Entered as Second-class Matter August 13, 1918, at the Post-office at Menasha Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in Section 1103, of the Act of October 3, 1917, authorized Oct. 21, 1918

---

The Collegiate Press  
GEORGE BANTA PUBLISHING COMPANY  
MENASHA, WISCONSIN  
1921

## TABLE OF CONTENTS

For Volume XL, Number 4, October, 1921

On the Effect of Some Fixatives upon Myxosporidian Spores, with four figures, by R. Kudo . . . . .	161
New Species and Collections of Arrhenuri: 1921, with three plates by Ruth Marshall . . .	168
Some Work on Marine Phytoplankton in 1919, by W. E. Allen . . . . .	177
The Accessory Chromosome of <i>Anasa tristis</i> again, by A. M. Chickering . . . . .	182
DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES	
The Literature of Diatoms, by Fred B. Taylor . . . . .	187
A Compendium of the Hosts of Animal Parasites contained in Ward and Whipple's Fresh-water Biology, compiled by H. J. Van Cleave . . . . .	195
The Endocrines, by S. W. Bandler, reviewed by T. W. Galloway . . . . .	200
List of members . . . . .	206
Index . . . . .	207

TRANSACTIONS  
OF  
American Microscopical Society

(Published in Quarterly Instalments)

Vol. XL

OCTOBER, 1921

No. 4

ON THE EFFECT OF SOME FIXATIVES UPON MYXOSPORIDIAN  
SPORES<sup>1</sup>

BY  
R. KUDO

Although it has generally been recognized that when myxosporidian spores are fixed, stained and mounted as either smears or as section preparations, they appear smaller than in the fresh state, it was Cépède who first called attention to the matter. He recognized differences between fresh and stained spores of the species he studied, and concluded as follows (Cépède, 1906:63): "en présence de telles différences de taille et de l'importance donnée actuellement aux dimensions des spores des Myxosporidies dans la systématique comme caractère distinctif des espèces, je crois utile de faire remarquer qu'il serait bon d'indiquer si les mensurations des spores ont été faites in vivo ou sur des préparations fixées et colorées, et montées au baume."

My observations upon the species which I have studied up to date agree with Cépède's results and have suggested that the dimensions of spores of a species should be accompanied by the statement of conditions under which the measurements were made (Kudo, 1920:49).

Most authors agree that at the present state of our knowledge regarding this group of Protozoa, a satisfactory classification of genera and species of Myxosporidia, must have as its basis the study of the spore (Kudo, 1920:52-59). The size, dimensions and structure of spores show a certain amount of variation even in one species, yet they are far more typical of the species than are the vegetative forms. In every case, the identification of a species has been successfully done only when the spores were present.

Since the characters of the spore vary to a more or less recognizable extent, according to the difference of conditions under which the spores are observed, it naturally follows that the characters of spores of two different species can only be correctly compared when they are observed

<sup>1</sup> Contributions from the Zoological Laboratory of the University of Illinois, No. 191.

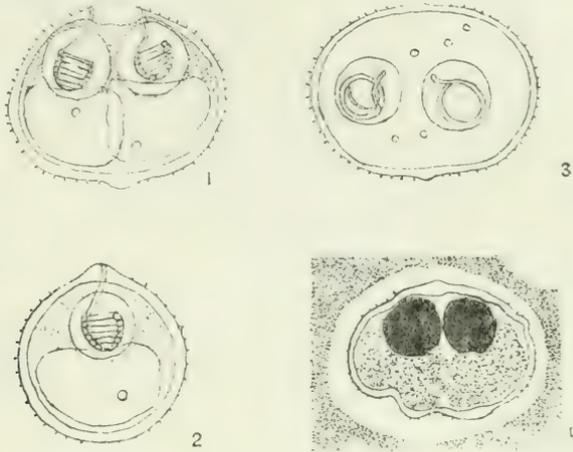
under exactly the same conditions. In other words, if the spores were originally observed and measured in the fresh state, those of the species to be compared with the former, should also be studied in the same condition. This kind of comparison, however, has happened only in a few cases, and can not always be carried out even in future. Many species of Myxosporidia have accidentally been found and described from stained smears or section preparations only by several authors, who were engaged with studies on other topics. Furthermore, even if one deals exclusively with Myxosporidia, one is frequently compelled to omit the study of fresh spores and to confine oneself to that of stained preparations under various circumstances.

The characters of spores observed only in section preparations can, of course, not only be compared properly with those obtained from fresh spores, but also should not be used for the data of establishing a new species. Special precaution must be exercised in cases where two forms are similar in habitat and locality and whose vegetative stages are not known.

Recently Schuurmans Stekhoven (1920) described three new species of Myxosporidia, *Sphaerospora gasterostei*, *Myxidium rhomboideum* and *Henneguya renicola*, found in the-uriniferous tubules of the kidney of *Gasterosteus pungitius* (misprinted as *pungiticus*) from Holland. The author studied section preparations of the host kidneys which were fixed with 60 per cent. alcohol and stained with Delafield's hematoxylin, and compared the characters of the spores observed therein with those of already known three species, *Sphaerospora elegans*, *Henneguya media* and *Henneguya brevis* (Kudo, 1920:30). Thélohan (1895) who described these latter forms, seems to have studied them in both fresh and fixed conditions, although his description is unfortunately brief. When the forms are so similar in every respect except the size of the spores, one finds it extremely difficult to decide whether the newly observed species studied only in sections, are identical with the former or not. If, however, we can calculate the dimensions of spores in the fresh state from those obtained from the stained ones, we can undertake a more satisfactory comparison between the species observed under diverse conditions.

In order to see exactly how the fixation, staining and mounting would affect the shape, dimensions and structure of Myxosporidia, a few experiments were performed on the spores of *Leptotheca ohlmacheri*. This Myxosporidian was first found by Ohlmacher and Whinery (1893) in the kidney of *Bufo lentiginosus*. I have recently observed it in the kidneys of *Rana clamitans* and *Rana pipiens*. Although I formerly placed it provisionally in the genus *Wardia* (Kudo, 1920:83-84), my recent study on its morphology and life history which will be published elsewhere, has shown that it should be placed in the genus *Leptotheca* as Labbé suggested.

The fresh spore of *Leptotheca ohlmarcheri* (Figs. 1 to 3) is oblong with its longest diameter at right angles to the sutural plane. The anterior end is slightly attenuated due to the thickening of the spore membrane at that point, while the posterior end is rounded. In profile, it is nearly circular with the slightly attenuated anterior extremity. In the anterior end view, it is regularly oblong. The shell is moderately thick. The sutural



Spores of *Leptotheca ohlmarcheri* (Gurley) Labbé. Figs. 1, 2 and 3. Three optical sections in front, side and anterior end views respectively of fresh spores which were kept in a hanging drop preparation with physiological solution and which are typical of the species in form, structure and dimensions. Fig. 4. A spore from a smear fixed with absolute alcohol, stained with Giemsa stain and mounted in cedar oil, showing the shrinkage of the entire body. All about X 2100.

ridge is well marked and protrudes conspicuously at the ends. The spore membrane is somewhat irregularly striated. Three to seven striae run parallel to the sutural line on each valve, the remaining ones make somewhat similar angles with the former. The striae in lateral view are mostly placed horizontally. The number of striae on each valve varies from 25 to 35. Two spherical polar capsules usually equal in size in one spore, occupy the anterior portion of the spore. The polar filament, coiled 4 to 6 times, is distinctly visible. Two independent sporoplasms occupy the extracapsular cavity of the spore. They are extremely homogeneous and each to be the karyosome of the nucleus). The size of the spore varies to some extent. There are some larger and some smaller than the average spores as is the case with every species found, doubtlessly due to the malformation. The average dimensions are as follows:

Sutural diameter	9.5 to 12 $\mu$ ; average	10.8 $\mu$
Breadth	13 to 14.5 $\mu$ ; average	13.75 $\mu$
Thickness	9.5 to 12 $\mu$ ; average	10.8 $\mu$
Diameter of polar capsule	3.5 to 4.5 $\mu$ ; average	4.0 $\mu$
Length of polar filament (KOH)	42 to 62 $\mu$ ; average	52.0 $\mu$

A drop of the emulsion of fresh spores in physiological salt solution was smeared on a slide. The amount of the emulsion and the area over which it was smeared were made approximately the same in every smear so as to obtain the similarity in the number of spores and the conditions under which the spores existed until they were fixed. Soon after the smear was made, it was fixed in one of the following fixatives before it dried up one smear being purposely made to dry up: 50 per cent., 70 per cent. and absolute alcohols, 4 per cent. formol, Schaudinn's (warmed), Bouin's and Flemming's (weak) mixtures. The fixatives were allowed to act upon the smears for 16 hours, after which they were removed from the latter by proper washings. At the same time, small pieces of the infected host organ were fixed in Schaudinn's fluid and 4 per cent. formol respectively, sectioned in paraffin and stained with Heidenhain's iron hematoxylin. The fixed smears were stained with Heidenhain's iron hematoxylin, Delafield's hematoxylin or Giemsa stain, and mounted in Canada balsam or cedar oil (for Giemsa stained smears only).

From each of the preparations, one hundred mature spores which could be distinguished distinctly from those that are in the course of development, were drawn at a scale of 2,100 magnification, and measured. The results are as follows:

	Sutural Diameter			Breadth			Diameter of Polar Capsules			
	Range in $\mu$	Average in $\mu$	Loss in $\mu$	Range in $\mu$	Average in $\mu$	Loss in $\mu$	Range in $\mu$	Average in $\mu$	Loss in $\mu$	
SMears	Fresh spores (control) . . . . .	9.5-12.0	10.8	0	13-14.5	13.75	0	3.5-4.5	4.0	0
	Air-dried, unstained, alcohols and balsam . . . . .	7.6-9.5	8.6	2.2	10.5-11.4	10.7	3.05	2.6-3.2	2.9	1.1
	50% alcohol; Giemsa . . . . .	9.2-10.0	9.6	1.2	10.5-10.9	11.0	2.75	3.0-4.0	3.5	0.5
	70% alcohol; Delafield . . . . .	9.0-9.8	9.4	1.4	9.8-10.9	10.4	3.35	2.8-3.9	3.4	0.6
	Absolute alcohol; Giemsa . . . . .	9.0-9.5	9.25	1.55	9.5-10.9	10.2	3.55	2.8-4.0	3.4	0.6
	4% formol; Giemsa . . . . .	8.9-9.3	9.1	1.7	9.5-11.4	10.5	3.25	2.9-3.8	3.4	0.6
	Schaudinn; Giemsa or Heidenhain . . . . .	9.2-9.8	9.6	1.2	10.9-11.9	11.4	2.35	3.0-3.8	3.4	0.6
	Bouin; Heidenhain . . . . .	8.5-9.5	9.0	1.8	9.5-10.9	10.2	3.55	2.6-4.0	3.3	0.7
	Flemming; Heidenhain . . . . .	8.8-9.6	9.2	1.6	10.0-11.2	10.6	3.15	2.4-3.2	3.3	0.7
	Average . . . . .		9.2	1.5		10.6	3.09		3.4	0.6
Per cent. of loss, calculated from the dimensions of spores from smears . . . . .			16.0%			29.0%			17.0%	
SECTIONS	Schaudinn; Heidenhain . . . . .	8.6-10.0	9.3	1.5	10.6-11.8	11.1	2.65	3.0-3.6	3.3	0.7
	4% formol; Heidenhain . . . . .	8.4-9.9	9.15	1.65	9.5-11.3	10.4	3.35	2.8-3.8	3.3	0.7
	Average . . . . .		9.23	1.58		10.75	3.00		3.3	0.7
	Per cent. of loss, calculated from the dimensions of spores in sections . . . . .			16.0%			28.0%			21.0%

From the above table, the following may be noted:

1) The amount of loss in sutural diameter of the spore of *Leptotheca ohlmacheri* is greatest when the spore is airdried and smallest when it is fixed with either Schaudinn's fluid or 50 per cent. alcohol. The average loss amounts to about 14 per cent. of the sutural diameter of the fresh spores.

2) The amount of loss in breadth of the spore is greatest when the spore is fixed with Bouin's fluid or absolute alcohol and smallest when it is fixed with Schaudinn's fluid. The average loss amounts to about 22 per cent. of the breadth of the fresh spores.

3) The amount of loss in the diameter of polar capsule is greatest when the spore is air-dried and smallest when it is fixed with 50 per cent. alcohol. The average loss amounts to about 15 per cent. of the diameter of polar capsules in fresh spores.

4) The losses in smear and section preparations are almost similar.

In the case of *Myxobolus cycloides*, Cépède gave the following dimensions: Fresh spores: sutural diameter, 13.5-16 $\mu$ , breadth 11-13 $\mu$ . Schaudinn-Heidenhain's spores: sutural diameter 10.5-12 $\mu$ , breadth 7.5-8 $\mu$ . Thus in this case, the fixation caused loss of 3.5 $\mu$  and 4.25 $\mu$  respectively in sutural diameter and breadth of the spore. These losses amount to 25 and 35 per cent. compared with the fresh spores.

The loss in the latter species is greater than the former species. It, however, shows distinctly from these two different types of Myxosporidia, that the sutural diameter undergoes a smaller amount of shrinkage than the breadth.

Concerning the change in the dimensions of spores by fixation, Cépède states that the reason why the spores appear smaller in fixed and stained conditions than in fresh condition, is simply because the refractive power of Canada balsam makes the unstained spore membrane invisible, and not because the shrinkage of the body caused by fixation takes place.

In the case of *Leptotheca ohlmacheri*, this is not the case. Very frequently spores such as shown in Fig. 4, are seen in the smears. The spore undoubtedly occupied the entire area which appear as blank zone before it was fixed. When fixed, the contents underwent a strong shrinkage, thus leaving a clear unstained zone between it and the smear. In such a spore, one can distinctly see the spore membrane in an irregular outline. I have noticed similar change of spores in smears of many species from various genera suggesting shrinkage as the main cause for the loss in dimensions. I, therefore, consider the decrease in the dimensions of myxosporidian spores, in general, is caused by the shrinkage of the entire spore body under the influence of the fixative and subsequent treatments.

The amount of shrinkage caused by fixation will apparently be different in different genera and species. Unless a large number of measurements

on different species be made, we do not know exactly the data on which the dimensions of fresh spores of the species observed by Schuurmans Stekhoven may correctly be calculated. Assuming that the spores of *Sphaerospora gasterostei* and *Heneguya renicola* underwent shrinkage similar to that of *Leptotheca ohlmacheri*, we obtain the following comparison:

	<i>Sphaerospora gasterostei</i>		<i>Sphaerospora elegans</i>
	Schuurmans Stekhoven	Calculated	Thélohan
Length	6.7 $\mu$	7.8 $\mu$	10 $\mu$
Breadth	7.0 $\mu$	9.0 $\mu$	11 $\mu$
Length of polar caps.	3.5 $\mu$	4.1 $\mu$	---

If the calculation is correct, it seems probable that *Sphaerospora gasterostei* is independent from *S. elegans*.

	<i>Heneguya renicola</i>		<i>Heneguya media</i>	<i>Heneguya brevis</i>
	Schuurmans Stekhoven	Calculated	Thélohan	Thélohan
Length of spore	8 $\mu$	9.28 $\mu$	20-24 $\mu$ (?)	10 $\mu$
Breadth	3.5 $\mu$	4.5 $\mu$	5-6 $\mu$	5-6 $\mu$
Polar capsule (length)	4.5 $\mu$	5.4 $\mu$	4-5 $\mu$	4.5 $\mu$
Tail	15 $\mu$	17.4 $\mu$	---	14-15 $\mu$

The calculated value of *Heneguya renicola* resembles closely the dimensions given by Thélohan for *Heneguya brevis*. The form of spore becomes so highly modified in section preparations that it is hard to make out the form in the fresh state. Therefore, it is highly doubtful whether Schuurmans Stekhoven saw a new species or not.

The form of spores changes to a variable extent according to the difference of the fixatives used. The more shrinkage the spore undergoes, the more irregular outlines it assumes. Careful fixation in Schaudinn's fluid often preserves the form of spores very nicely.

When a spore is fixed with any one of the fixatives, the coiled polar filament becomes entirely invisible. This is probably caused by the coagulation of the wall of polar capsule which becomes opaque by the

fixation. The distinction between the two sporoplasms is harder to determine in fixed and stained spores than in fresh spores. The sporoplasms become coarsely reticulated, losing the homogeneous condition seen in the fresh state.

In conclusion, I may again suggest that a new species should be described after studying the spores in fresh as well as fixed and stained conditions and if possible the fixed vegetative forms.

#### SUMMARY

1) The ordinary fixation causes about 14 and 22 per cent. decrease respectively of the sutural diameter and breadth of fresh mature spores of *Teptotheca ohlmacheri*.

2) The possibility of calculating the dimensions of fresh spores from those of fixed and stained spores is discussed.

3) The decrease in the dimensions of spores is due to the shrinkage of the entire spore body.

4) Fixation makes the coiled polar filament invisible.

#### WORKS CITED

- KUDO, R., 1920. Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. Ill. Biol. Monogr., 5:245-503, 25 pl. and 2 textfig.
- SCHUURMANS STEKHOVEN, JR., J. H., 1920. Ueber einige Myxosporidien des Stichlings. Arch. Protist., 41:321-329, 1 pl.

## NEW SPECIES AND COLLECTIONS OF ARRHENURI: 1921.

By

RUTH MARSHALL  
Rockford College

The genus *Arrhenurus*, the largest group of the water mites, continues to yield new material from collections in lake regions. The new species described in this paper came from regions as far apart as Canada and China; while additional notes on already described species are based on material secured in several states of northeastern United States, some of them from new localities. Through the kindness of Professor N. Gist Gee, of Soochow University, material was secured for the description of new species from China. Professor Frank Smith and Dr. H. R. VanCleave, of the University of Illinois, were good enough to contribute some material from Michigan, New York and Massachusetts. Through the interest of Dr. R. A. Muttkowski an opportunity was given for the examination of some collections of the Biological Station of the United States Bureau of Fisheries at Fairport, Iowa; and more recently the author was privileged to see some collections of Dr. F. A. Stromsten, of the University of Iowa. The author's own collections from the Muskoka Lake region of Ontario, together with other material from various sources, form the basis of a preliminary account of the genus *Arrhenurus* as it has been found in Canada. This topic will be discussed first.

Practically the only account of the water mites of Canada so far is that contained in a paper by Dr. F. Koenike, "Nordamerikanische Hydrachniden," and a revision of this paper, "A Revision of my 'Nordamerikanische Hydrachniden.'" The descriptions were based upon material sent to him by Dr. J. B. Tyrell, of Toronto, and were collected in Alberta and British Columbia, near the international line. Of the thirty species listed by Dr. Koenike, four were *Arrhenuri* and new species. These were *A. laulus*, *A. interpositus* (a young male), *A. setiger* and *A. krameri*. The last named species has since been found by the author and further notes are given in this paper. In addition to this, one new species is now added for Canada (*A. uniformis* nov. spec.), and four more are recorded for the first time, as follows. *A. americanus* Mar. and *A. manubriator* Mar. were found by the author at Parry Sound and *A. americanus* var. *major* Mar. in a small lake near Bala, Ontario. *A. marshalli* Piers., the most widely distributed species, had previously been found in material from Long Point, Canada, in some collections of the United States Fish Commission.

In the descriptions of species which follow the Canadian material will be discussed first.

*Arrhenurus uniformis* nov. spec.

Pl. IX, fig. 1-3.

This species resembles *A. scutiformis* Mar. and belongs in the group of "long-tailed" Arrhenuri in which the very long and rather simple appendix is decidedly narrower at the end than it is at the base. The outline of the body is approximately circular; the enclosed dorsal area and the appendix are moderately high and rounded. Details of structure given in the figures show this to be a new species. The last joint of the fourth leg is long and slim; the spur on the fourth joint is moderately developed.

The single male on which this description is based is 1.33 mm. long and 0.73 mm. wide. The color is dull olive green. It was found in a small lake near Long Lake, at Bala, Ontario, August 25, 1920.

*Arrhenurus krameri* Koenike.

Pl. IX, fig. 7-9.

The author has already recorded (1908) the finding of a mite from Oregon which appeared to be *A. krameri*. Drawings of this specimen are now given for the first time and its identity with the single male from British Columbia on which Dr. Koenike's description was based seems to be established. It is slightly smaller, however, measuring only 1.29 mm. A dorsal view is shown, which did not appear in the original paper, together with the lateral view and a drawing of the palpus.

*Arrhenurus simulans* nov. spec.

Pl. X, fig. 17-21.

Material sent to the author by Dr. H. J. Van Cleave from collections in Dump Lake, Woods Hole, Massachusetts, contained sixty individuals of this species. It was at first thought to be *A. krameri*, altho a larger form, the length being 1.45 mm. and the extreme width, 0.83 mm. The males bear some resemblance also to *A. rectangularis* Mar., especially when a comparison is made of the side views of the long appendix, the end of which in the three named species shows a double scallop, one part above the other. The body of the new species is conspicuously elevated where it joins the appendix. The wing-shaped genital areas are rather small and the ends of the line enclosing the dorsal area are far behind them on the appendix. Twenty-seven males were present. The color in the preservation is dull brown green.

Over half of the individuals in the collection were females; the examination of the palpi shows that they belong to this species. The body of *A. simulans* fem. is broadly ovate. The epimeral plates are relatively small; the third and fourth have about the same width throughout, the two posterior groups being well separated from each other and from the genital area. The fourth epimera are narrow, scarcely wider than the third. The genital plates are of nearly uniform width and extend straight out from the aperture, which is large. The body is 1.32 mm. in length.

*Arrhenurus pseudosetiger* nov. spec.

Pl. IX, fig. 4-6.

In a former paper (1910) the author identified as *A. setiger* Koen. an individual from Madison, Wisconsin. But a more careful study of this specimen shows that it belongs to another, though closely related species, which will be designated as *A. pseudosetiger*. The body proper is stouter than it is shown in Dr. Koenike's figures of the Canadian species, being nearly circular in outline, not oblong, and the appendix is smaller. The dorsal enclosed area runs over on the appendix and is depressed. The entire length of the body is 0.8 mm., the greatest width, in the region of the fourth leg, 0.7 mm. The color is deep brick red.

*Arrhenurus trifoliatius* Marshall

Pl. IX, fig. 10-12.

It is not often that the collector succeeds in securing a large number of Arrhenuri at any one time; it is still more unusual to find any one species in numbers large enough to make possible a thorough examination of all structures and to identify with certainty the females of the species. Collections made by the author in the marshy sloughs at Burlington, Wisconsin, July 5, 1919, consisted largely of individuals of the rather uncommon species, *A. trifoliatius*, twenty-five males and fifteen females being secured. It is now seen that the earlier description of the species (1908) did not show completely the details of the appendix of the male. As seen in Fig. 10, a young male, a delicate bladder-like structure, A, is attached to either side of the stout petiole, a structure which is easily injured in preservation.

The female, which is now described for the first time, is broadly oval in form. The posterior groups of epimera are close together and the genital area lies immediately behind them. The genital wing-shaped areas are unusual in form, the outer ends curving strongly upward. The length of the body is 1.15 mm., the extreme width, 1.05 mm.

*A. major* was represented in this Burlington collection by one male. The Lake Beulah region which was visited at the same time yielded four species of Arrhenuri. *A. marshalli*, *A. megalurus*, *A. americanus* and *A. reflexus*, the latter having the unusual color of orange red.

*Arrhenurus compactilis* Marshall

Pl. X, fig. 13-16.

This somewhat rare species was found in collections from Fairport, Iowa, and again in the collections of Professor Frank Smith from Douglas Lake, Michigan. This new material adds two states to the range of the species and makes possible a more complete study of its structure. Drawings of the palpus and the last leg are now given. The latter is seen to be very characteristic of this group of stout bodied petiolated Arrhenuri in having a long fourth joint with a conspicuous spur ending in a tuft of curved hairs, while the fifth and sixth segments of this appendage are short.

The female of the species is now known from the study of the palpi which agree in all details with those of the male except that they are somewhat larger, as usual. The body is stout and oval and measures 1.3 mm. in length. Details of the dorsal and ventral surfaces are shown in the figures.

The collections of water mites from the Biological Station at Fairport, summer of 1917, have already been referred to. They were secured from small lakes in the vicinity of the Station and were rich in Arrhenuri. This material is especially interesting since there have been no previous records of Arrhenuri from Iowa that the author is aware of. Nine species were found, as follows:

<i>A. marshalli</i> Piers.	<i>A. fissicornis</i> Mar.
<i>A. americanus</i> Mar.	<i>A. compactilis</i> Mar.
<i>A. americanus</i> var. <i>major</i> Mar.	<i>A. laticaudatus</i> Mar.
<i>A. apetirolatus</i> Piers.	<i>A. dentipectiolatus</i> Mar.
<i>A. birgei</i> Mar.	

The last named species is rare, only two other specimens, from Colorado, having been recorded.

The collections of Dr. F. A. Stromsten, mentioned in the introduction, add *A. lyriger* Mar. to the list.

The following descriptions of two new species of Arrhenuri from China continue the study of the water mites of the region of Soochow which was begun in a former paper (1919), and are made possible through the continued interest of Professor N. Gist Gee who furnished the material.

*Arrhenurus soochowensis* nov. spec.

Pl. XI, fig. 22-25

The new species belongs to a type of Arrhenuri which is seen in *A. kraepelini* Koen. described from Java, a type apparently common in the Asiatic members of the genus. This is the type of *A. forpicatus* Neum. of Europe, represented in America by *A. lyriger* Mar. It is characterized by a deep incision in the end of the appendix, the hyaline appendix lying on the dorsal side of this.

The single individual upon which this description is based has these characteristics well marked. The appendix is relatively long; the median incision runs into a round opening over which lies the large and highly developed hyaline structures, closely resembling the same parts in *A. limbatus* Koen. of Madagascar. The dorsal enclosed area of the body is rather small and the line which encloses it is not quite closed. The fourth joint of the fourth leg has a short spur. The fourth joint of the palpus is unusually broad at the distal end. This is a small mite, 0.8 mm. long and 0.5 mm. wide. The color is dull green. The specific name refers to the locality where it was found.

*Arrhenurus geei* nov. spec.

Pl. XI, fig. 26-29

This species resembles *A. madaraszii* Daday found in Ceylon and belongs to the *forpicatorum* group mentioned under the last species. The appendix is small and narrow but well developed; its median incision is large at the upper end. The hyaline appendix is an oblong structure; on either side of it is developed a delicate claw-like piece. The body is broad; the enclosed dorsal area is large, the line enclosing it not quite closed posteriorly. The fourth leg lacks the spur on the fourth joint. The palpus, as in the related species, has a broad fourth joint. The single male on which this description is based is 0.73 mm. long and 0.56 mm. broad. The species is dedicated to Professor Gee.

## LITERATURE CITED

KOEENIKE, F.

1895. Nordamerikanische Hydrachniden. Abh. natur. Vereins Bremen, 13:167-226.  
 1912. A Revision of My "Nordamerikanische Hydrachniden." Trans. Canadian Institute, Toronto, p. 281-296

MARSHALL, R.

1908. The Arrhenuri of the United States. Trans. Am. Mic. Soc. 28: 85-134.  
 1910. New Studies of the Arrhenuri. Trans. Am. Mic. Soc., 29:97-110.  
 1919. New Species of Water Mites of the Genus Arrhenurus. Trans. Am. Mic. Soc., 38:225-281.

## EXPLANATION OF THE PLATES

## Plate IX

1. *Arrhenurus uniformis*, dorsal view.
2. *Arrhenurus uniformis*, genital area.
3. *Arrhenurus uniformis*, lateral view.
4. *Arrhenurus pseudosetiger*, dorsal view.
5. *Arrhenurus pseudosetiger*, lateral view.
6. *Arrhenurus pseudosetiger*, left palpus
7. *Arrhenurus krameri*, dorsal view.
8. *Arrhenurus krameri*, lateral view.
9. *Arrhenurus krameri*, left palpus.
10. *Arrhenurus trifoliatus*, appendix of young male.
11. *Arrhenurus trifoliatus*, female, epimera.
12. *Arrhenurus trifoliatus*, right palpus.

## Plate X.

13. *Arrhenurus compactilis*, female, dorsal view.
14. *Arrhenurus compactilis*, female, ventral view.
15. *Arrhenurus compactilis*, male, part of left fourth leg, the last three joints rotated.
16. *Arrhenurus compactilis*, right palpus.
17. *Arrhenurus simulans*, female, epimera.
18. *Arrhenurus simulans*, male, dorsal view.
19. *Arrhenurus simulans*, appendix, ventral view.

20. *Arrhenurus simulans*, lateral view.
21. *Arrhenurus simulans*, left palpus.

## Plate XI.

22. *Arrhenurus soochowensis*, dorsal view.
23. *Arrhenurus soochowensis*, lateral view.
24. *Arrhenurus soochowensis*, ventral view.
25. *Arrhenurus soochowensis*, left palpus.
26. *Arrhenurus geei*, lateral view.
27. *Arrhenurus geei*, dorsal view.
28. *Arrhenurus geei*, appendix, ventral view.
29. *Arrhenurus geei*, left palpus.

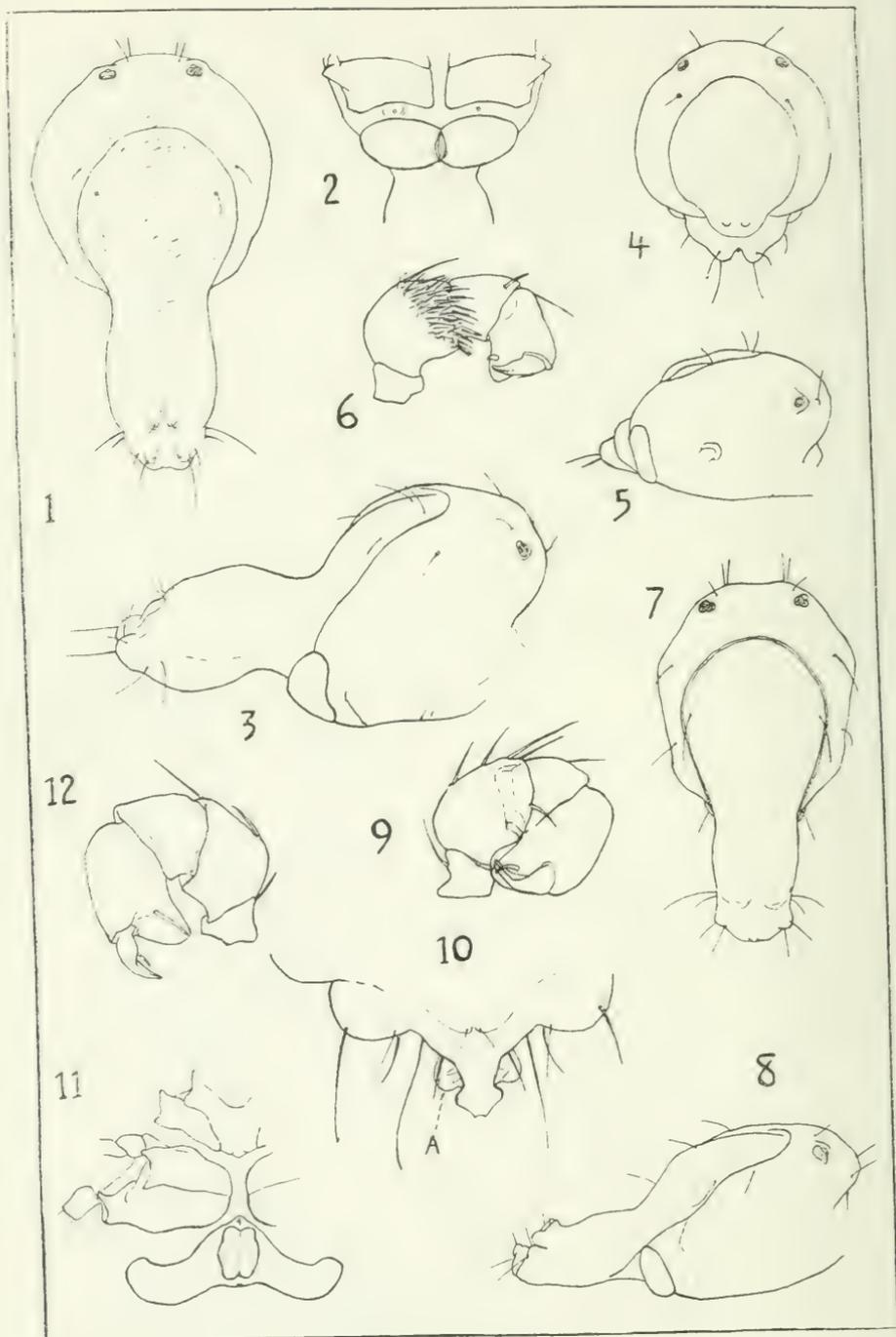


PLATE IX

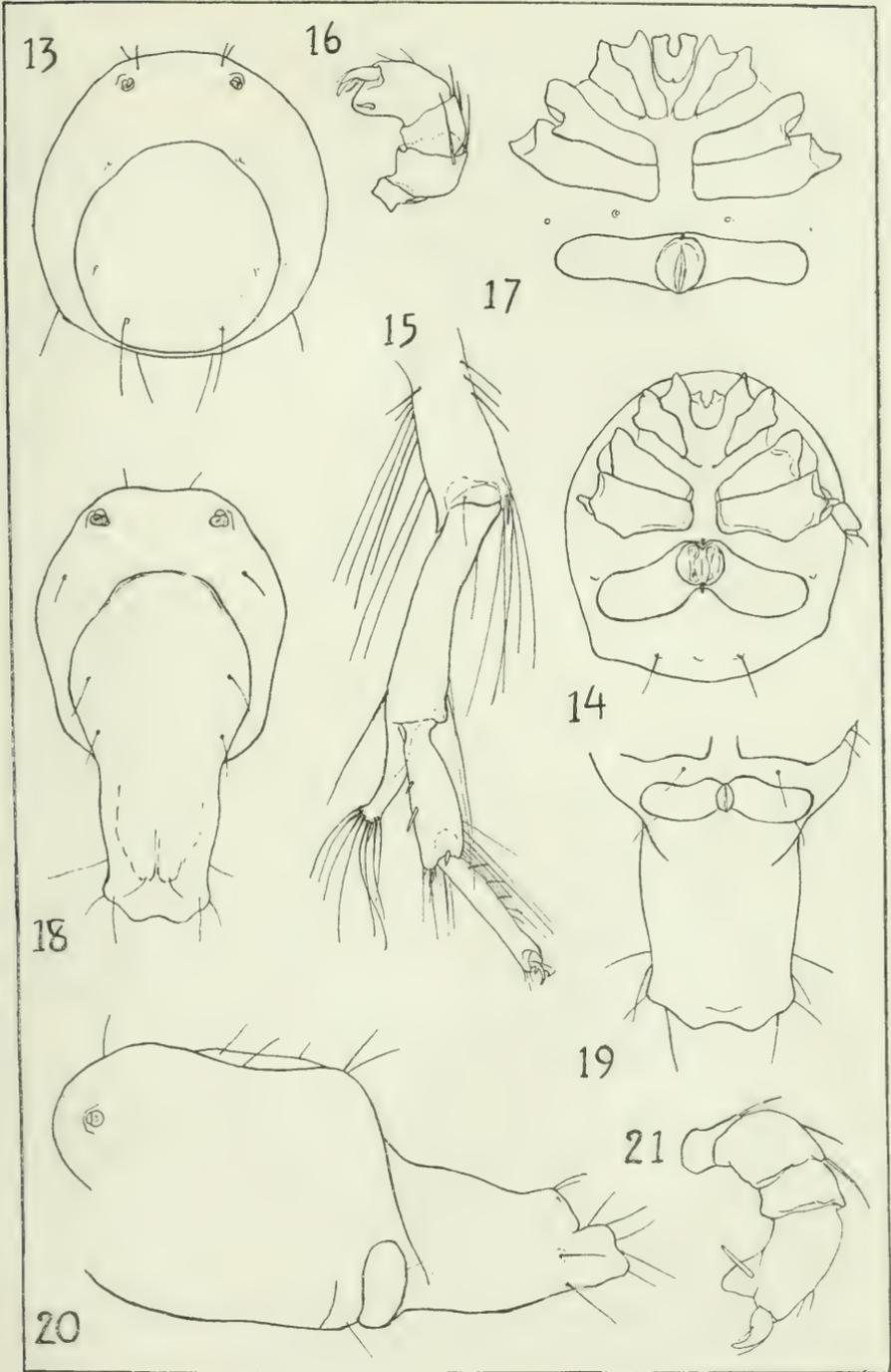


PLATE X

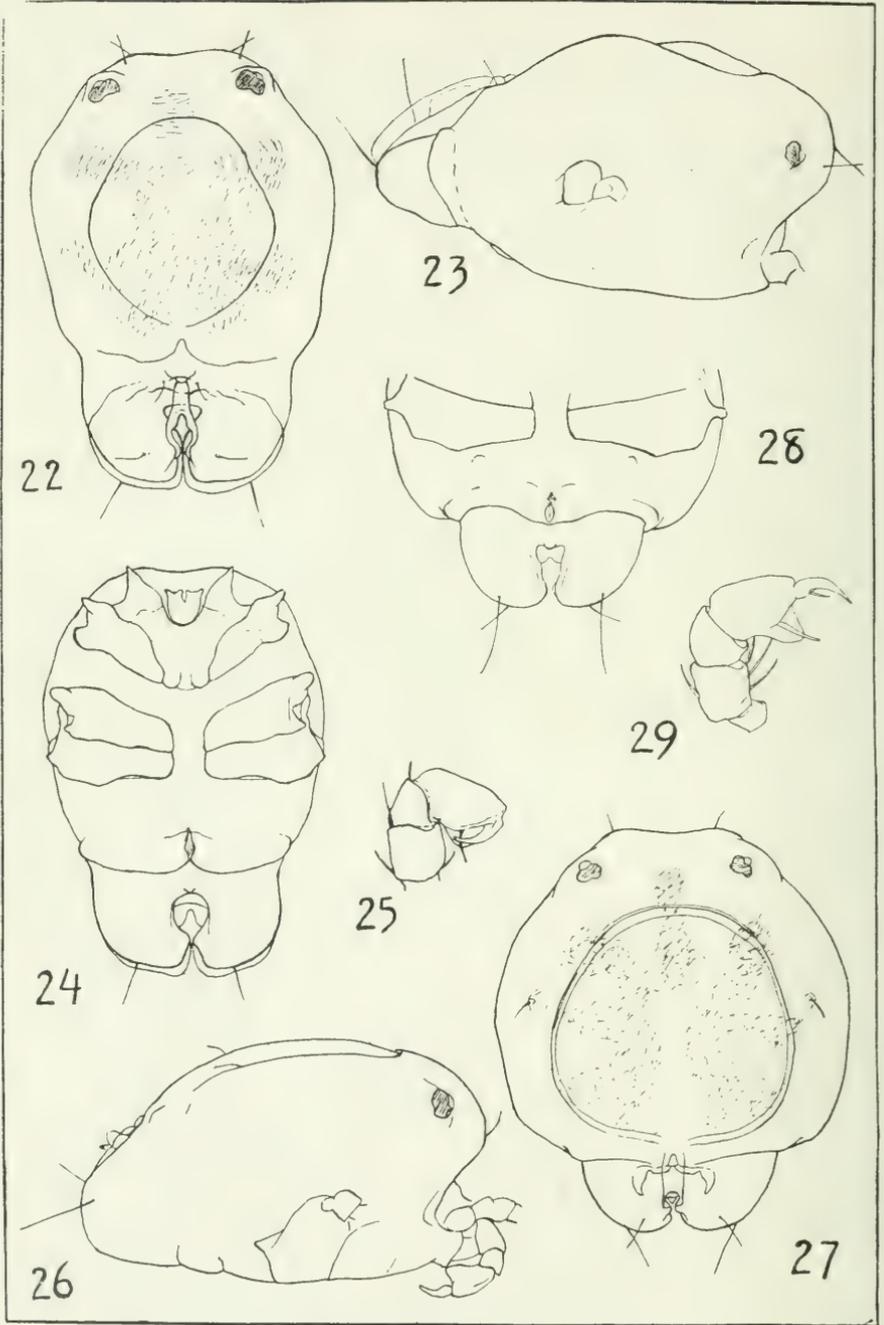


PLATE XI

## SOME WORK ON MARINE PHYTOPLANKTON IN 1919

By

W. E. ALLEN

Scripps Institution for Biological Research of the University of California.

On September 1, 1919, efforts to use tow nets for quantitative work with phytoplankton in the La Jolla area were abandoned and the resources of the Scripps Institution available for such work were temporarily concentrated upon collection and study of a series of catches made from our pier by the measured water method at intervals of twelve hours. These catches were taken by the simple procedure of dipping water from the surface of the sea at a point about one thousand feet from shore and immediately pouring it through a filtering net made of number 25 bolting silk. This net was made in the form of a funnel into the small end of which a bottle or other receptacle could be tied. After filtration catches were preserved in formalin for quantitative study at convenience.

Quantitative studies consisted in roughly approximate identifications of the species present and in an enumeration of representatives of each found in a certain fractional part of a catch mounted in a Sedgwick-Rafter counting cell. Necessary aids to this work were a Whipple eyepiece micrometer and a mechanical stage. Records were later assembled in the form of tables which were studied with reference to occurrence and prominence of species in the locality at the time and through the period of observation.

It is interesting to note that the locality in which this work was done was much farther south than points at which extensive studies of phytoplankton have been made in Atlantic waters, i. e., it is in about the latitude of Northern Egypt. Furthermore the fact that catches were made regularly and continuously for months at intervals of twelve hours (8 a. m. and 8 p. m.) marks the series as somewhat different from most other groups of catches of marine plankton material.

Surface water temperatures taken at the time of making the catches showed a range from 23° C. in August to 13° C. in December, but the range within the limits of discussion of this paper was from 20.8° C. in September to 13° C. in December.

Although there are other plants which take some part in synthetic activities in the open sea and although dinoflagellates of many kinds are generally analytic rather than synthetic in character, it has appeared from preliminary studies of marine plankton in the Southern California region that the groups most promising for quantitative study as synthesizing organisms are the diatoms and dinoflagellates, (or at least the armored dinoflagellates). There seems to be no reasonable doubt that these two

groups of organisms are on account of their small size, large numbers, rapid growth, facile reproduction, wide distribution and cosmopolitan character peculiarly favorable objects for quantitative study especially since they seem to be the most easily and continuously accessible of all marine organisms.

Both groups show rhythms and pulses of production which are more or less evident in each month of the year. Such rhythms and pulses are, however, characterized by changes in prominence of particular species according to season and according to certain other variable conditions.

For convenience in the present discussion a pulse may be defined as a marked increase in numbers of organisms which extends over a period of three or more days before decreasing to or near the numbers found at its beginning. In this four month period there were five such pulses of diatoms and four of dinoflagellates. In both groups they were unevenly distributed in the period. The records show that out of four times possible for coincidence of pulses of the two groups there were two close approximations to coincidence. This, of course, indicates that both groups of organisms are sometimes favored by the same stimuli to production.

But it is true that some other evidence indicates different possibilities, e. g., there were fourteen cases in which a catch of diatoms was more than three times as large as either the catch preceding or the one succeeding it and there were fifteen such cases of dinoflagellate catches. Out of fourteen chances for coincidence of such catches in the two groups only four occurred, a fact which leads one to think they may be to some extent mutually deterrent. This view gains support from the fact that catches distinctly low in numbers as contrasted with those catches nearest them show no coincidence in five chances although we might expect that there would be coincident absence in both groups if both were similarly responsive to changes in local conditions. In view of such considerations one seems to be driven to the provisional assumption that plants in the open sea like those on land may sometimes find such generally favorable conditions that widely different types may live and thrive together without prejudice but that usually some factor has given one form a better opportunity than another which may be used to the detriment or to the complete exclusion of that other.

The above mentioned exhibits of presence and absence are still more suggestive in regard to the perennial assumption that marine organisms are uniformly distributed through considerable areas of marine waters. A catch markedly larger than both of those at twelve hours from it or a catch markedly smaller than both surely indicates that distribution is not uniform in the given area.

Since catches were taken rather early in the day and early in the night the records were examined for evidence of greater productivity in light

or darkness. In October and November, two months out of the four, about four-fifths of the larger catches were made in the morning and in the other two months there was not much difference. So far as this limited evidence goes it favors the view that growth and reproduction occurs most vigorously at night, as might be expected from our general knowledge of distribution of plant activities in the twenty-four hours.

Although many species of diatoms and dinoflagellates may be found in the Southern California region there are not many which are ever very prominent or numerically important and there are very few which are frequently and continuously thus important. Since most of these can be identified fairly well under ordinary conditions of examination, statistical study is not seriously hampered by the requirements of taxonomy.

For most purposes it is best to study the distribution of diatoms and dinoflagellates as separate groups. Thus considered the following points may be noted concerning diatoms: Some representatives of the group were to be found throughout the period although distribution was very irregular. Large numbers appeared in the last three months of September thus producing an autumnal maximum similar to those noted in European waters.

In connection with this maximum I was interested in noting that for two or three days previous to its inception there had been rather strong and constant currents from the north. I also noticed that large numbers of mackerel came to the vicinity of our pier in the latter part of August and left about the time that the great increase in diatom production began. Whether these points were mere coincidences or whether they had significant relationship to increased diatom production, I have no means of knowing.

Forty six species of diatoms were recorded in the four months but only twelve of these were readily identified although fourteen were usually approximated, i. e., confusion limited to only one or two other forms. These included most of those of numerical importance. Eleven forms were found to have been represented in the most abundant five in one or more months. Five of these belonged to the genus *Chaetoceras*. They were mostly rather small species and difficult to identify.

Detailed study of the records has clearly shown the important fact that when there is increased production of the most prominent forms there is also increased production of the less prominent forms and an increase in the number of different forms. Such facts naturally lead to the assumption that conditions favorable to high productivity of diatoms in the sea affect a large number of forms in the same way. They also lead to the inference that determination of the species which shall lead in production is largely due to biological factors such as rapid multiplication and vigorous development.

As to the dinoflagellates I may say that they are usually much fewer in numbers than are the diatoms. Otherwise the general features of their distribution are not greatly different except in the periods of maximum production. In the last four months of 1919 the greatest numbers were produced in November but there had also been some heavy production in August several weeks before the maximum production of diatoms.

Thirty-seven forms of dinoflagellates were recorded eight of which were fairly easy to identify. Usually satisfactory identification of twelve forms could be made and these included most of those showing numerical importance. Six easily identified species were found amongst the five most numerous in one or more of the four months. Two of them belonged to the genus *Ceratium*.

Two of these most prominent species deserve special mention because of their connection with the phenomenon called "red water." *Gonyaulax polyedra* Stein has at various times been mentioned as responsible for extensive areas of "red water" in Southern California which have attracted especial attention because of the bad odor where it was washed upon the beaches and because of the large number of littoral animals killed by it and then stranded upon the beaches. The brownish or reddish color of the water is due merely to the vast numbers of these small organisms present in it. The destruction of littoral animals is usually said to be due to products of decay after death of such quantities of the microscopic organism. But it is possible that the living *Gonyaulax* is also poisonous to animals. In the last three or four years *Prorocentrum micans* Ehr. has been more often detected as a cause of "red water" than has *Gonyaulax* but no cases have been reported in which littoral animals died as a result of its presence. It is noticeable that in "red water" areas (some of which extend for miles in open water) very few other organisms, large or small, are found amongst those which cause the discoloration.

Several different kinds of dinoflagellates cause the appearance of water called "phosphorescence." More or less glow of this sort may be observed in waters of our section at almost any time of year although not continuously present. At times there are present in the water sufficient numbers of individuals of this sort to cause at night a glowing pathway where fishes stimulate them by swimming through.

A more detailed report of this work in 1919 is awaiting publication in another place. Its conclusions may be briefly stated as follows:

First, the measured water method seems to be by far the best to use for a *standard* method and the surface level to be the best for a *standard* level of collecting for quantitative study. Other methods and other levels should be regarded as special methods more or less supplementary to the standard.

Second, there is evidence that drift currents have pronounced influence on phytoplankton production at our pier.

Third, large numbers of phytoplankton organisms respond to conditions of production favorable for any one.

Fourth, it seems probable that some of the more prominent forms may be useful as indicators of certain conditions in the ecologic complex.

Lastly, it is evident that the problems of the ecologic complex of the sea are fascinating as well as intricate and baffling and that in many ways good returns are sooner or later to be expected as the results of time and energy expended in study upon them.

## THE ACCESSORY CHROMOSOME OF ANASA TRISTIS AGAIN

BY

A. M. CHICKERING

Albion College, Albion, Mich.

During the examination of literature in connection with cytological studies on other Hemiptera I became much interested in the case of *Anasa tristis*. I was astonished to find a very marked variation in the results of the cytologists who have studied the spermatogenesis of this form. Therefore, when in the summer of 1919 there occurred an excellent opportunity to procure an abundance of material, I decided to make some observations of an independent nature. It would seem not out of place occasionally to examine some of the commonly accepted cases and particularly where, as in this instance there has been a decided disagreement.

Altogether ten investigators have worked on the male germ-cells of *Anasa tristis*. At one time or another four of this number have been opposed to the now generally accepted view, first stated by E. B. Wilson. Two of these have corrected their former statements and now agree with the latter in his conclusions.

Paulmier ('99), who was the next after Henking ('91) to study the history of the accessory chromosome, decided that the spermatogonial number of chromosomes was twenty-two. He discovered the pair of m-chromosomes in the spermatogonia and described their behavior. He believed these united in synapsis to form a single condensed bivalent chromosome-nucleolus which persisted throughout the growth period and became the small central tetrad of the first maturation division. Furthermore he stated that this tetrad divided equally in the first division but that the products of this division passed undivided to but one of the poles of the second spindle giving ten and eleven chromosomes respectively to the spermatids. He therefore identified the chromosome-nucleolus of the growth period as the microchromosome bivalent and thought this to be identical with the accessory.

Montgomery ('01, '04) followed Paulmier in giving the spermatogonial number of chromosomes as twenty-two. He also regarded the accessory chromosome as being derived by a fusion of the m-chromosomes. A re-examination of his material after the publication of Wilson's second paper on chromosomes led to a change in his statements so that they were then in agreement.

After a very careful study of Paulmier's material as well as his own Wilson came to the following conclusions in a remarkable series of papers ('05, '06, '07, '11); (1) that there are twenty-one and not twenty-two

chromosomes in the spermatogonia; (2) that these chromosomes exist in pairs and this leaves one without a mate; (3) that this odd one is one of the three largest and is the so-called accessory chromosome; (4) that this chromosome exists as a chromosome-nucleolus throughout the growth period; (5) that the m-chromosomes previously identified by other observers as the accessory have an entirely independent history and divide in both of the maturation divisions; (6) but that the real accessory divides in the first and then passes undivided to one only of the two spermatids derived by division of the secondary spermatocytes, thus giving rise to two kinds of spermatozoa.

Foot and Strobell ('07), using smear methods to the entire exclusion of sections and illustrating only with photo-micrographs, took sharp issue with Wilson. These investigators asserted that Paulmier was right in his spermatogonial count; that the so-called chromosome-nucleolus of the growth period is but "morphologically the equivalent of a nucleolus" or in other words the plasmosome; that there is no odd or accessory chromosome; that what has been called such is but a lagging chromosome which divides in each division as do all the others; that therefore all spermatids receive eleven chromosomes.

This disagreement among cytologists, of course, became a serious matter. Many fundamental facts came into direct question and consequently several people were interested enough to make independent investigation of the conditions.

Closely following the papers of Foot and Strobell there appeared a brief treatment of the question by Lefevre and McGill ('08). Their observations confirmed those of Wilson.

In connection with work on the chromosomes of some of the coreid Hemiptera Morrill ('10) confirmed Wilson's spermatogonial count.

The climax of the researches on the accessory chromosome of *Anasa* came in 1910 when McClung and Pinney went over the whole matter with great care. Miss Pinney made an entirely independent study and in order to avoid bias or prejudice in the matter refrained from reading any of the accounts published by other investigators until her own conclusions had been reached. McClung studied the original material of Paulmier, Wilson and Lefevre and McGill. Both McClung and Pinney agreed that the spermatogonial number is twenty-one. They further agreed with Wilson that there are ten bivalent chromosome and one, the accessory, which is univalent in the metaphase of the first division. This univalent body exists as a short, heavy thread, a compact mass or finally as a straight longitudinally split rod all through the prophase stages of the first division. It divides as do the others in the first but does not divide in the second mitosis.

In making this brief and confirmatory study of *Anasa tristis*<sup>1</sup> I have used the ordinary cytological methods now somewhat standardized. As usual I have found Bouin's and Flemming's fluids very valuable. Perhaps the best preparations have been made with Bouin's. The iron-haematoxylin method of staining has again proven the best general stain although I have had difficulty with the domestic preparations.

My own conclusions are not enough different from those of Wilson to warrant a lengthy treatment. In fact as regards the important stages the matter might be dismissed by a statement that the facts as I see them are as stated by Wilson, Lefevre and McGill, and McClung and Pinney. In order, however, that my results may be on record and that the constancy of the chromosome relationship within the species may be further evidenced I will state the main facts as I see them.

There are without question twenty-one chromosomes in the normal spermatogonia. I have examined dozens of these cells in the metaphase when the chromosomes are well spread out but I have never found one which clearly showed more than the expected number. The three large bean-shaped chromosomes and two small m-chromosomes are always present together with sixteen others of about equal size.

In the late prophase stages of the first division at least nine typical tetrads can be seen accompanied by what I think are three dyad bodies. I am sure one of these is the accessory. Probably the others are the m-chromosomes but the study of these has not made me entirely sure of this. When the chromosomes become placed in the metaphase plate preparatory to division the accessory usually occupies a position outside of the ring formed by the position of the nine ordinary tetrads. The m-chromosomes, now unquestionably formed into a tetrad, occupy a central position within the ring. All the bodies divide equally in this division. As a result then all secondary spermatocytes possess eleven chromosomes each. When these become arranged in the flat metaphase plate again the ring-like arrangement is succeeded by an irregular placing. In spite of this the accessory can usually be identified. When the second division occurs this accessory does not divide like the rest but goes to one pole undivided thus giving rise to two kinds of spermatids. Lateral and polar views of these stages show without the shadow of doubt that half the spermatids receive ten while the other half get eleven chromosomes.

At this point I will mention a condition observed several times in *Anasa* and other Hemiptera. Follicle cells in division frequently show double the normal spermatogonial number of chromosomes. How this is brought about and what the fate of the cells involved is I am not able to state.

<sup>1</sup> For the identification of my material I am indebted to Dr. Paul S. Welch of the Univ. of Michigan, Ann Arbor, Mich.

It is amazing that there should have been so much disagreement in the results of investigations up to this point. As is often the case in the Hemiptera when the preparations are good most of the stages so far outlined stand out with diagrammatic clearness. There should be no further difference of opinion in regard to these matters. Anyone can demonstrate the truth of the statements by using reasonable care in preparation and observation.

When we come to the question of the behavior of the accessory chromosome from the time of the last spermatogonial division down to the late prophases of the first maturation division the facts are more difficult to determine. Those who have worked upon the question of spermatogenesis will agree that it is often difficult to discover just what is going on in the nucleus at this time. However, I believe the main contentions of Wilson can be proven true

In many cells before and during synezesis a compact body can be found just outside of the more or less tangled mass of chromatin threads. Because of its size and appearance I think it reasonable to conclude that it is the accessory chromosome. In every stage following this the same body can be identified and need not be confused with the plasmosome which occurs with it for a large part of the growth period. Thus the accessory maintains its individuality throughout the maturation process.

In conclusion I would say then that I believe the current explanation of the "case of *Anasa tristis*" as given by text-books of zoology and genetics based upon the work of Wilson is correct. This classical example may be regarded as a permanent addition to our stock of knowledge. Our interpretations may change, of course, but the facts will stand as Wilson stated them.

The different stages of the spermatogenesis of *Anasa* are on the whole so clear and beautiful that I can recommend them for class use where it is desired to demonstrate the main facts connected with the behavior of the sex chromosome.

#### LITERATURE CITED

FOOT, KATHERINE and STROBELL, E. C.

1907. The "Accessory Chromosome" of *Anasa tristis*. Biol. Bull., Vol. XII.

A Study of the Chromosomes in the Spermatogenesis of *Anasa tristis*. Amer. Jour. Anat., Vol. VII.

HENKING, H.

1891. Ueber Spermatogenese und deren Beziehung zur Entwicklung bei *Pyrrhochoris apterus*. Zeitschrift. wiss. Zool. Band LI.

LEFÈVRE, GEORGE and MCGILL, CAROLINE.

1908. Chromosomes of *Anasa tristis* and *Anax junius*. Amer. Jour. Anat. Vol. VII.

McCLUNG, C. E. and PINNEY, EDITH.

1910. An Examination of the Chromosomes of *Anasa tristis*. Kan. Univ. Sci. Bull., Vol. V.

**MONTGOMERY, T. H.**

1901. A Study of the Germ-cells of the Metazoa. Trans. Amer. Phil. Soc., Vol. XX.  
1904. Some Observations and Considerations upon the Maturation Phenomena of the Germ-cells. Biol. Bull., Vol. VI.  
1906. Chromosomes in the Spermatogenesis of the Hemiptera heteroptera. Trans. Amer. Phil. Soc., Vol. XXI.

**MORRIL, C. V.**

1910. The Chromosomes in the Oögenesis, Fertilization and Cleavage of the Coreid Hemiptera. Biol. Bull., Vol. XIX.

**PAULMIER, F. C.**

1899. The Spermatogenesis of *Anasa tristis*. Jour. Morph. Supplement, Vol. XV.

**WILSON, E. B.**

1905. Studies on Chromosomes, I. Jour. Exp. Zool., Vol. II.  
1905. Studies on Chromosomes, II. Jour. Exp. Zool., Vol. II.  
1906. Studies on Chromosomes, III. Jour. Exp. Zool., Vol. III.  
1907. The Case of *Anasa tristis*. Science, N. S., Vol. XXV.  
1911. Studies on Chromosomes, VII. Jour. of Morph., Vol. XXII.

## DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS, AND BRIEFER ARTICLES

---

### THE LITERATURE OF DIATOMS

BY

FRED B. TAYLOR  
Bournemouth, England

The literature on the subject of diatoms is both extensive and expensive; many of the books are long out of print; much is scattered through periodicals English and foreign; and there is no recent general hand-book at a moderate price. The nearest approach to such a volume is in German, it is the part on Peridinales and Bacillariales in Engler and Prantl's *Pflanzenfamilien*, published at Leipzig in 1896. It is written by F. Schuett, and gives an account of the morphology and biology of diatoms, with a scheme of the genera divided into Centricae and Pennatae, a description of each genus then established, and a drawing of one or more species to illustrate the genus.

The *Diatomaceae of the Hull District* by Mills and Philip contains several plates covering most of the species commonly found in England. There is also a most instructive paper by Philip in the *Transactions of the Hull Scientific and Field Naturalists' Club* vol. IV. part iv (1912) p. 205, on *Diatoms of the Humber*.

For fresh water diatoms *Die Süßwasser Flora Deutschlands, Oesterreichs, und der Schweiz*, part 10, by H. von Schönfeldt deals with diatoms, it is published by Fischer of Jena, and contains many figures of English diatoms.

The early history of our knowledge of the *Diatomaceae* is given by Ehrenberg in the introduction to the *Bacillaria* in his first great work, *Die Infusionsthierchen*, published in 1838. Kitton in *Science Gossip*, 1880, pp. 78 and 133, gives a résumé of this introduction, which describes the work of Ehrenberg himself and other diatomists. Kitton also gives an interesting account of the views of Corda (1835) in *Science Gossip*, 1882, pp. 6, 22. The imperfection of the instruments then available, and the then commonly received belief in the animal nature of diatoms often led to the enunciation of opinions, which now appear ridiculous.

Kuetzing's *Kieselschaligen Bacillarien*, (1844), also contains an historical preface. This was translated by Professor H. L. Smith of Geneva, U. S. A. in nos. 2 and 4 vol. II of *The Lens*. A résumé of this translation with comments of his own was given by Kitton in *Science Gossip*, 1874, pp. 2, 25, 149.

Ehrenberg may be regarded as the father of diatomology, he was able to command a vast quantity of material; and although many of his views are now known to be erroneous, and many of his figures are incorrect or insufficient owing to the imperfect objectives he used, and to the want of sufficient magnification, his labors will always be of great value to the diatomist. About one third of the genera now recognized were founded by him. After him Greville and Grunow are the most prolific creators of genera, accounting for about another third between them.

Diatom literature may be taken as starting with Agardh's *Systema Algarum* in 1824, and Ehrenberg's early papers on the Infusoria, in which he included diatoms, published in 1829-1832. Kuetzing's *Synopsis Diatomearum* followed in 1834, and in 1838 Ehrenberg brought out his great work on Infusorial Animalcules, *Die Infusionsthierchen*, nine of the 64 plates being devoted to diatoms.

Kuetzing's second work, *Die Kieselschaligen Bacillarien*, 30 pl. was published in 1844; the following year saw the first edition of Pritchard's *Infusoria*, further editions were issued in 1852, 1861, and 1864. The part on the Diatomaceae was written by Ralfs, and plates iv to xvii give figures of diatoms. This is, perhaps, the book on diatoms most readily accessible to English students, and though portions of it are out of date or incorrect, it forms a very useful introduction to the subject.

Rabenhorst's *Süsswasser Diatomaceen*, 10 pl., appeared in 1853. Owing to the imperfection of optical apparatus many of the figures in these earlier works are wanting in detail. It is therefore often difficult to determine the identity of the species named and described; yet it is wonderful how much was seen and accurately recorded with instruments that would now be despised and rejected.

The last half of the nineteenth century was a period of great activity in this branch of research. From the year 1853 to 1866 the *Transactions and Journal of the Microscopical Society*, and the *Transactions of the Royal Society of Edinburgh* contain a number of papers by Brightwell, Gregory, Greville, Lauder, Roper, Walker-Arnott, Wallich and others, many of which are beautifully illustrated by Tuffen West. William Smith's *Synopsis of British Diatoms*, containing 69 plates by the same artist was published in 1853-1856; Gregory's *Diatoms of the Clyde* appeared in 1857 (*Proc. Royal Soc. Edinburgh*, vol. xxi, p 473). The figures were drawn by Greville.

Ehrenberg's colossal *Mikrogeologie* appeared in 1856. Besides his two large books he wrote numerous papers on diatoms in the *Transactions of the Berlin Academy of Science*. Naturally in the course of the progress of knowledge, and with the improvement of instruments, and the increase of material, the early conceptions of many genera and species have been modified, and relations have been acknowledged between forms at first

sight widely differing. The *Micrographic Dictionary* first appeared in 1854, with enlarged editions in 1859, 1872, and 1882. It contains several plates of diatoms.

Greville's fine series of 20 papers on New and Rare Diatoms, mostly from Trinidad, Barbadoes, Moron, Monterey, and Ceylon, appeared in the *T. M. S.* and *Q. J. M. S.* from 1861 to 1866, with monographs on *Asterolampra*, *Campylodiscus*, and *Auliscus*. He also wrote on Diatoms from Hongkong (*A. M. N. H.* 1865), the South Pacific (*Edin. N. P. J.* 1863) and *The Tropics and Southern Hemisphere*, (*T. B. S. Edin.* and *Edin. N. P. J.* 1865, 1866). A collection of poor photographic reproductions of 81 plates mostly Greville's from *T. M. S.*, etc., compiled by Moebius was published in New York. It is sometimes on the market.

In America J. W. Bailey wrote on American Bacillaria, and published several papers between 1841 and 1860. L. W. Bailey also wrote on the subject, and numerous papers by Dr. A. M. Edwards appeared in various periodicals between 1859 and 1877; his sketch of the *Natural History of the Diatomacea* is dated 1874; (cf. *Bull. Torr. Bot. Club*, 1877 p. 34). Lewis wrote in 1861 on *New and Rare Diatoms*, and in 1865 on *White Mountain Diatoms*.

Cleve, a Swede, began to write in 1864, and published various papers and books, many of them in English, on Diatoms from Spitzbergen, the Sea of Java, West Indian Archipelago, Greenland and Argentina, and Finland; also on Arctic Diatoms, and *New and Little Known Diatoms*, and on the diatoms found by the *Vega Expedition*, and later, several papers on *Plankton Diatoms*. His great work is his *Synopsis of the Naviculoid Diatoms* published in 1904, 1905. In this last book he proposes a rearrangement of the *Naviculae* and the related genera setting up a number of new genera, which have not been universally accepted; although many of them are recognized as useful subdivisions of the older genera; some writers, however, accept his proposals en masse.

Grunow, an Austrian, wrote from 1860 to 1890; his papers on *New Diatoms*, and on *Austrian Diatoms* (1860, 1862, 1863, and 1882 and 1883) are important contributions to diatom classification; and much of his work is embodied in Van Heurck's *Synopsis of the Diatoms of Belgium*, and in Schmidt's *Atlas*. His account of the diatoms of the *Novara Expedition* is dated 1867; the same year saw his paper on the diatoms of the *Sargasso Sea of Honduras*, an abstract of this is given in *M. M. J.* 1877, p. 165. He also wrote on *Caspian diatoms*, and conjointly with Cleve a book on *Arctic diatoms*, followed by his *Diatoms of Franz Josef Land*.

Kitton wrote from 1868 to 1884 a number of articles, many of them in *Science Gossip*, a magazine which during the years 1867 to 1877 printed several useful papers on the subject of diatoms, including some by Kitton on *North American deposits*. Kitton translated some of Grunow's

papers for various English periodicals; among these were the Novara diatoms, which appeared in *Grevillea* in 1872.

Harting's Banda See, Janisch on Guano diatoms, Janisch and Rabenhorst on the Marine Diatoms of Honduras, Heiberg's Danish Diatoms, and Schumann's Prussian Diatoms belong to the early sixties.

In the seventies followed De Brébisson on Diatoms contained in Corsican Moss. He had previously written on diatoms from the Cherbourg littoral, and wrote other papers.

Donkin published only three parts, (12 plates), of his *Natural History of the British Diatomaceæ*. About the same time appeared O'Meara on Irish Diatomaceæ (poor plates) and on diatoms from Kerguelen's Land; and Petit on Table Bay, Campbell Island, Cape Horn, etc. In 1872 Pfitzer wrote on the structure and development of diatoms; an abstract of this important paper is given by O'Meara in *Q. J. M. S.* 1872, p. 240. The *Lens*, published in Chicago, only lived two years, 1872, 1873. It contains valuable articles by Professor H. L. Smith and other American diatomists; among these is the professor's Classification of diatoms into Rhaphidicæ, Pseudo-Rhaphidicæ, and Crypto-Rhaphidicæ, which was followed by Van Heurck and De Toni, and by most diatomists until the present division into Centricæ and Pennatæ, (a modification of it) appeared. Many French diatomists follow Pfitzer's division into Placochromaticæ and Coccochromaticæ, which is based upon the nature of the endochrome.

In 1874, Adolf Schmidt, a canon of Aschersleben, commenced his splendid work, *Atlas der Diatomaccenkunde*. This is published in parts of four plates issued at irregular intervals. An index to the first four series, 240 plates, has been published. Up to August 1914 parts 1 to 79, containing plates 1 to 316 had appeared: parts 80-83 have been published since the war. Adolf Schmidt died in 1901, but the *Atlas* has been continued by his son and by Fricke, Heiden, and Hustedt, with the assistance of Cleve, Grunow, and other diatomists.

J. Brun of Geneva entered the field in 1880 with his *Diatomées des Alpes et du Jura*; following in 1889 with *Diatomées Fossilies du Japon* written in collaboration with Tempère; and in 1891 with *Diatomées, Espèces Nouvelles*. The plates in the two last are excellent: the first commences with an important sketch of the natural history of diatoms.

In 1886 Count Castracane's report on the diatoms found by H. M. S. Challenger was issued by the British Government. This contains 30 plates and valuable notes. Peragallo's *Villefranche* appeared in 1888 and Wolle's *Diatomaceæ of North America* (112 plates) was published in 1890. The plates are mostly copied from other authors, and are far behind the originals in execution; but the book is useful for reference, and is generally procurable at a moderate price, considering its size.

Le Diatomiste edited by Brun, a periodical devoted to the study of diatoms, only lived from 1890 to 1896. It contains many notable contributions including monographs on Pleurosigma and Rhizosolenia by Peragallo, on Entogonia by Bergon, and on the miocene diatoms of Barbadoes by Brun and Barbo.

Le Micrographe Préparateur under the editorship of J. Tempère ran from 1893 to 1906. It contains valuable papers on the structure and reproduction of diatoms, on the movement of diatoms, and on mounting and cleaning. But the most important is Les Diatomées Marines de France, of which some 115 plates were published in this periodical; the complete work, 137 plates, was separately published in 1908.

Leuduger-Fortmorel between 1879 and 1898 published books on the diatoms of the north coast of France, Ceylon, Malaysia, and West Africa.

In 1886-1887 in the Journal of the Quekett Microscopical Club appeared the well known paper on the Oamāru deposit in New Zealand by Grove and Sturt; also in 1886 Pantocsek published the first volume of his great work on the fossil diatoms of Hungary. The second volume appeared in 1889, and the third in 1892. The whole contains 102 plates. In the third volume are many new forms from Kusnetzki in Russia, and from fossil deposits in Japan. A second edition was issued in 1903-1905. Pantocsek has also written on the diatoms of Lake Balaton (Platten See), and of Kertsch, and of Szliacs in Hungary, (1902, 3). About this time Walker and Chase, and Kain and Schultze wrote on diatoms in America, the latter pair bringing to notice the interesting deposit of Atlantic City.

Van Heurck's Synopsis des Diatomées de Belgique, (1880-1885) contains 141 plates, and is a work of the greatest value. His Treatise on Diatoms translated into English by Dr. Wynne Baxter, was published in 1896 before the original; it contains 35 plates illustrating all the species found in the North Sea and the neighboring countries; and in the text are descriptions with typical figures of all the genera known at the time of publication, some 193 in number. Otto Witt in 1885 gave an account of the diatoms from the marine deposits of Ananino and Archangelsk in the province of Simbirsk in the interior of Russia.

Rattray's monographs on Aulacodiscus (J. R. M. S. 1888); Auliscus (J. R. M. S. 1888); Actinocyclus (J. Q. M. C. 1890); and Coscinodiscus (Proc. Royal Soc. Edin. 1890) are standard authorities.

About the same time appeared Pelletan's Les Diatomées, he was assisted by Deby, Petit and Peragallo. The account of the natural history of diatoms is good, and there are numerous illustrations of the most common species and genera. Truan and Witt's book on the fossil deposit of Jérémie in Haiti is dated 1888. Another work of great utility, though it is not illustrated, is Les Diatomées du Monde Entier, issued by Tempère and Peragallo as a companion to the series of 625 slides from various locali-

ties distributed by them. It gives lists of the diatoms found by them on the slides, and is a valuable aid to identification. A second series of 1000 slides is accompanied by a volume bearing the same title, the second edition of this is dated 1915; it is of course more comprehensive.

Deby's monograph on *Campylodiscus* appeared in 1891; with three or four exceptions the figures are copied from other works. About the same time a portion of Janisch's report on the diatoms of the Gazelle Expedition was privately distributed to certain favoured diatomists without text or list of contents. This work has never been completed; out of 22 plates numbers 7, 8, 10, 12, 13, 14, 17, and 18 are wanting. Many of the specimens figured are reproduced in Schmidt's Atlas.

In 1891 to 1894 De Toni, a professor of the University of Padua, published his monumental work, *Sylloge Bacillaricarum* as part of his *Sylloge Algarum*. This book has no illustrations, but it contains in Latin descriptions of all the genera and species described or named at the date of publication, with their synonyms and varieties, and references to all published figures and drawings. Nearly 6000 species are admitted. The book also contains a bibliography to date of the literature of diatoms by Deby.

Frère Héribaud of the college of Clermont-Ferrand wrote in 1893 on the diatoms of Auvergne. The volume commences with a short, but very clear, instructive, and succinct account of the subject, forming an admirable introduction to the study of diatoms. Between 1902 and 1908 he published four memoirs on the fossil diatoms of Auvergne.

In 1896 Schuett wrote the part on diatoms in Engler and Prantl's *Pflanzenfamilien*, (lieferung 143-145). This book, with its one or more typical figures of each genus, and its account of the morphology and biology of diatoms, forms a most useful handbook at a moderate price. It gives a description of all known genera classified as *Centricæ* and *Pennatæ*, the division now generally adopted; the *Pennatæ* are since 1902 subdivided into *Mobiles* and *Immobiles*, the *Centricæ* are all *Immobiles*. This last distinction is due to Mereschkowsky, a Russian diatomist: (*Script. Bot. Hort. Imp. Petrop.* 1902) and *A. M. N. H.* 1902, p. 65).

Karsten's book, *Die Diatomeen der Kieler Bucht*, 1899, is praised by Cleve and Mereschkowsky as a *vade mecum* for students of living diatoms.

In the present century we have Dippel's book on the Rhine and Maine districts, 1905; and a most useful and instructive report by Mann on the diatoms found in the Pacific by the U. S. ship *Albatross*, published by the Smithsonian Institute in 1907. Von Schonfeldt's *Diatomacæ Germaniæ* was published in the same year.

Peragallo's magnificent work *Les Diatomées Marines de France*, (13 plates, 1897-1908), and Meister's *Kieselalgen der Schweiz*. (48 plates, 1912), contain splendid figures, and include most of the English forms.

*La Diatomologia Española* by Azpeitia, (1911, 12 plates), treats of various Spanish deposits, including Moron. This establishes two quite new genera, *Dossetia* and *Secallia*.

I must not omit mention of three important papers by Laubeg, on the Palæobotany of France, (Soc. Bot. de France. Mém. XV. Jan. 1910): the Study of Sedimentary Deposits of Diatoms (Bull. des Services de la Carte Géologique de France, Mém. 125, 1910): and on Diatoms, their Deposits and Uses, (Revue Générale des Sciences, 1911).

In Nuova Notarisia professor Achilli Forti has written several papers, with photographic illustrations; on Bergonzano and Marmorito deposits in 1908 and 1914; and a monograph on the genus *Pyxilla* in 1909. His paper on the classification of diatoms as *Mobiles* and *Immobiles* in 1912 was anticipated by Mereschkowsky in 1902, (Script. Bot. Hort. Imp. Petrop. Fasc. xviii. p. 96.), as I have already pointed out. Mereschkowsky has also written other papers on diatoms, and has suggested the formation of various new genera, some for new forms, and some for species already described.

West's *Algæ* (Camb. Univ. Press, 1916) devotes 43 pages to Diatoms, and compresses much information into that space. Boyer's *Diatomaceæ of Philadelphia*, 40 plates, bears date 1916: it contains a useful introduction and fine illustrations.

Considerable attention has been given during the last thirty years to the Plankton diatoms. Cleve in 1889 wrote on Pelagic diatoms from the Kattegat, and followed this by papers on diatoms from Baffin's Bay and Davis' Straits (1894), Phytoplankton of the North Atlantic (1897), Diatoms of the Jackson-Harmsworth Expedition (1898), North Sea and English Channel (1900), Swedish Expedition to Greenland (1900), and Plankton of the South Atlantic (1900). And Oestrup wrote on the Marine diatoms of East Greenland (1895, 6 plates).

Van Heurck in 1909 wrote on the diatoms found during the voyage of S. Y. *Belgica* in 1897-1899 in the Antarctic Ocean, (13 plates).

Karsten wrote the account of the diatoms found during the German Deep Sea Expedition in 1898, 1899. This is a costly and magnificent work in three parts, on the Antarctic, Atlantic, and Indian Phytoplankton, with splendid plates. Other writers on the subject are Ostfeldt, Aurivillius, Hensen, Jörgensen and Lemmermann.

The Belgian Museum of Natural History has published *Microplankton de la Mer Flamande*, by Meunier, (Tome VII. Fasc. 2, 3. 14 plates. 1913, 1915), *Nordisches Plankton, Botanischer Teil*, by Gran, 1905, contains about 180 figures of interesting and new forms. Gran also wrote several other papers on Arctic Diatoms and Plankton.

I must also note Mangin's paper on the Study of Plankton in *Annales des Sciences Naturelles*, 1908, pp. 177-219; and Bachmann on the Phyto-

plankton on Fresh Water, with special reference to the Lake of Lucerne, (1911, pub. Jena). The report of the Imperial Fisheries Institute of Japan for 1911 contains 6 plates of littoral diatoms of Japan.

It is impossible in the space of a few pages to note all the contributions to the knowledge of the structure and history of diatoms, even to name more than some of the best known writers on the subject such as Nelson, Morland, Lauby, Cox, Brightwell, Roper, Wallich, the two Müllers, Butcher, O'Donohoe, and Murray.

THURGARTON, BOURNEMOUTH.

# A COMPENDIUM OF THE HOSTS OF ANIMAL PARASITES CONTAINED IN WARD AND WHIPPLE'S FRESH-WATER BIOLOGY

COMPILED BY  
H. J. VAN CLEAVE  
University of Illinois

Ward and Whipple's *Fresh-water Biology* contains by far the most comprehensive treatment of the animal parasites of the North American fauna that has ever been published. The chapters dealing with the parasitic worms, represent contributions, on the part of Professor Henry B. Ward, not only in a compilation of results of almost innumerable researches of varying magnitude but also in the inclusion of extensive data based upon previously unpublished records. Much of this information of especial interest to parasitologists and to field zoologists is not available for ready reference because the names of hosts mentioned in the text are not included in the general index of the book.

For personal use the present writer prepared a compendium of the hosts mentioned in the *Fresh-water Biology*. This proved so valuable an aid and received such favorable comment from workers to whom the manuscript was shown that it was considered desirable to put it into a form in which it could be generally available.

The authors of the *Fresh-water Biology* have made no attempt to include complete check lists of the hosts in the chapters dealing with parasitic forms, yet in many instances references are inclusive enough to be of great value as a point of departure in determining the recorded parasitic fauna of any given host animal. In using this compendium it should be recalled that in many instances only one or a few typical species are listed for each genus and even the hosts of such species as are cited do not constitute full check lists. Doubtless there have been numerous erroneous determinations of hosts in the works from which the host lists have been assembled but host names have been quoted directly as they stand in the original citations without attempt at verification. As a result, some of the names of hosts current in the older literature appear along with the valid names of the same species in this compendium.

In many instances where there seems to be no fixed specificity of hosts, as well as in the discussion of families and genera of parasites, group names such as 'fish,' 'birds,' or 'water birds,' are used frequently.

In the chapters dealing with Protozoa the parasitic forms have not received the attention of the writers, consequently the great group of Sporozoa and all other parasitic protozoans have received no treatment in this compendium.

To facilitate locations of words on the page in referring back to the text, specific, generic, vernacular, or group names have been used in the compendium as they stand in the text reference. In a few instances cross references have been inserted between vernacular and scientific names, but in such instances the page references to the two names have not been assembled. This is due to the belief that the inconvenience of cross citation is less than the confusion resulting from the necessity of visualizing both vernacular and scientific names while scanning the printed page in search for a given reference.

A host name cited under a given page reference may appear more than once on that page.

A direct means of determining the groups of parasites listed for any given host without necessity of referring back to the text is afforded by reference to the following list of page inclusions for the various groups containing parasitic forms:

Trematoda	374-424
Cestoda	429-451
Nematoda	520-535
Gordiaceae	537-542
Acanthocephala	545-551
Rotatoria	553-620
Discodrilidæ	644
Hirudinea	646-660
Copepoda	782-788
Malacostraca	841-850
Hydracarina	851-874

#### MAMMALIA

'mammals' 390, 404, 440, 441, 442, 444, 447, 522, 549  
 beaver 386  
 cat 390, 393, 447  
 cattle 389, 409  
*Didelphis virginiana* 410  
 dog 390, 523  
 Lepus 409  
 man 389, 409, 432, 433, 434, 521, 522, 523, 534, 656  
 mink 522, 523, 530  
 muskrat 383, 386, 391, 404, 447, 451, 522, 534, 535  
 Mustelidæ 522  
 otter 522, 523  
 pig 390  
 seals 433, 523, 549  
 sheep 389, 408

skunk 522  
 wapati 389  
 weasel 522  
 whales 433  
 wolf 523

#### AVES

'bird' 402, 404, 409  
 'birds' 440, 441, 442, 443, 444, 446, 447, 448, 526, 549, 550  
*Anas platyrhynchos* 388  
 Anseriformes 449, 548  
*Ardea herodias* 410, 524, 527, 532  
*Ardea minor* 391  
 bittern 532  
*Bolaurus lentigenosus* 550, 551  
*Bolaurus minor* 526  
 canvas back 442  
 chicken 391, 402, 446  
 coot 443

cormorant 533  
 crane 444  
 crow ('fish') 446  
 duck 411, 441, 442, 447  
 egret (see *Herodias*)  
 eider duck 549  
*Gallinago wilsoni* 382  
*Gavia immer* 391 (see loon)  
 geese 442, 447  
 grebe 440, 448  
 grebe (horned) 448, 449  
 gull 440, 442, 446 (see *Larus*)  
*Herodias egretta* 549  
 'heron' 408, 440, 444, 445, 532  
 heron (green) 444  
 heron (little blue) 444  
 herring gull 408  
 'ibis' 448  
 kingfisher 525  
*Larus philadelphia* 391  
 loon 440 (see *Gavia*)  
 pelican (brown) 533  
 pelican (white) 433, 533  
 pintail-duck 442  
*Plotus anhinga* 524 (see water-turkey)  
*Porzana carolina* 549  
 scoter 442  
 scoter (American) 449  
 scoter (black) 391  
 shore birds 442, 444, 445  
 snipe (grey) 382  
*Somateria dresseri* 549  
 sparrow (English) 384  
 spoonbill 444  
 spoonbill (roseate) 445  
 stilt (blacknecked) 447, 448  
 waterbirds 401, 431, 432, 442, 443, 446, 447, 548  
 water turkey 523, 533 (see *Plotus*)  
 wood-ibis 531

## REPTILIA

alligator 382, 408  
*Alligator lucius* 410  
*Alligator mississippiensis* 391, 530, 531, 532  
*Amyda* 402  
*Ancistrodon piscivorus* 439  
*Aromochelys carinatus* 376  
*Aromochelys odoratus* 376, 377, 397  
*Aspidonectes* 402

*Bascanion constrictor* 405  
 Chameleon 400  
*Chelonura serpentina* 524  
*Chelydra serpentina* 376, 384, 387, 396, 529  
*Chrysemys marginata* 377, 385, 394  
*Chrysemys picta* 394  
*Cinosternum pennsylvanicum* 376  
*Cistudo carolina* 522, 524, 533  
 'Emys' 546  
*Emys guttata* 529  
*Emys scripta* 529  
*Emys serrata* 524, 533  
 garter snake 407  
*Heterodon platyrhinus* 405, 406, 407  
*Malacoclemmys geographicus* 546  
*Malacoclemmys lesueurii* 378, 380  
*Nanemys guttata* 528  
*Natrix rhombifer* 407, 439  
*Pseudemys* 387  
*Pseudemys elegans* 546  
*Pseudemys scripta* 377  
 snakes 405, 438  
 terrapin (common, food-) 376, 386, 531  
*Trionyx ferox* 378  
*Tropidonotus rhombifer* 406  
*Tropidonotus sipedon* 406, 526  
 turtles 385, 433, 526, 652

## AMPHIBIA

amphibians 398, 399, 404, 408, 434, 438, 449, 522, 547  
*Amblystoma* 399  
*Amblystoma mexicanum* 528  
*Amblystoma tigrinum* 438  
 Anura 400, 403  
 Bufo 399  
*Bufo americana* 522, 533  
*Bufo lentiginosus* 521  
*Cryptobranchius alleghaniensis* 525  
*Diemyctylus viridescens* 547  
 frog 382, 399, 400, 402, 404, 387, 408, 411, 652, 654  
*Necturus lateralis* 379  
*Necturus maculosus* 439  
*Rana catesbiana* 408  
*Rana halecina* 531  
*Rana pipiens* 410, 524, 530  
 salamander 382, 399  
*Salamandra rubra* 533  
*Siredon mexicanus* 528, 533

## PISCES

- fishes 382, 398, 401, 408, 411, 433, 434, 523, 524, 528, 529, 547, 551, 652, 653, 654, 655
- Acipenser (European) 392
- Acipenser oxyrinchus* 433
- Acipenser rubicundus* 378, 392, 396
- Acipenser sturio* 374
- Ambloplites rupestris* 401, 436, 548, 785
- Ameiurus nebulosus* 399
- Amia calva* 392, 401, 432, 435, 436, 526, 548, 787
- Anguilla vulgaris* 435
- Anguilla chrysoptera* 401, 435, 524, 546
- Aplidnotus grunniens* 381, 395
- black bass 375, 379, 392, 395, 408, 524, 534
- white bass 528, 529
- blue-gill 408, 411
- Boleosoma nigrum* 379
- bull-head 382, 408, 439
- carp (German) 529
- catfishes 786
- cat (channel) 439
- Catostomus commersonii* 787
- Catostomus leres* 375
- chub 431
- Coregonus nigripinnis* 437
- Coregonus prognathus* 437
- Coregonus artedii* 437
- crappie (black) 528, 529
- Cristivomer namaycush* 437
- Cyprinidae 408, 430
- dace 395
- dace (horned) 411
- darter 395
- Dorosoma cepedianum* 545, 547
- Eromyzon succetta oblongus* 787
- Esox lucius* 392, 399, 401, 437, 546
- Esox reticulatus* 392, 437, 524, 529 (see Lucius)
- Fundulus 655
- Fundulus ocellaris* 784
- Gasterosteus 432
- herring (lake) 527
- Ictalurus punctatus* 395, 401
- Lepomis pallidus* 785
- Lepisosteus osseus* 788
- Lepisosteus platostomus* 435, 436
- Lepisosteus tristocchus* 788
- Lota lota* 392
- Lucioperca 392
- Lucius masquinongy* 787
- Lucius reticulatus* 788 (see Esox)
- Micropterus dolomieu* 374, 392, 398, 401, 436, 548, 785
- Micropterus salmoides* 392, 400, 436, 546
- minnow 379, 395, 398, 408, 411
- minnow (red-finned) 395
- Moxostoma macrolepidotum* 394
- Perca flavescens* 401, 524
- perch 379, 395, 396, 399, 408, 411
- pickerel 788
- pike 408, 411, 521
- pike (wall-eyed) 530
- Ptychocheilus oregonensis* 424
- pumpkinseed 395, 411
- rays 434
- Roccus lineatus* 375, 548, 785
- rockbass 375, 379, 392, 395, 398, 408, 411
- Salmo sebago* 434, 437
- salmonid fish 527
- Salvelinus namaycush* 392
- shad 533
- sharks 434
- sheepshead 528
- Siluridae 439
- Stizostedion canadense* 788
- Stizostedion vitreum* 401
- sturgeon 395
- sturgeon (lake) 530
- sucker 395, 431, 787
- sunfish 375, 395, 411
- teleosts 431, 524
- trout 408, 431, 433, 550
- trout (lake) 374
- trout (Great Lake) 431, 527, 547, 548
- whitefish 432, 521, 527, 547

## INSECTA

- insect 534, 851
- insect larvæ 528
- Achaeta abbreviata* 539
- Acrididae 538, 540
- Blasturus cupidus* 395
- Blattidae 538
- Gryllus (see Achaeta)
- Hexagenia 395
- Locustidae 540
- mayfly 395
- Neobius fasciatus* 539

## CRUSTACEA

- crustacea 528, 534
- Apus 411

Copepoda 451, 523  
 crawfish 395, 401, 644  
 Cyclopidae 442  
 Decapoda 842  
 Diaptomus 442  
 Isopoda 547  
 Ostracoda 451, 523  
 Palaemonetes 842  
 Palaemon 842

## GASTROPODA

snails 409, 415, 451  
*Campeloma decisum* 416, 419, 420  
 Gasteropoda 417  
*Goniobasis virginiana* 416  
*Helix albolabris* 423  
*Helix alternata* 423, 531  
*Helix arborea* 411  
 Lymnaea 419  
*Lymnaea catascopium* 411  
*Limnaea humilis* 389  
*Lymnaea proxima* 412, 417, 418, 419  
*Lymnaea reflexa* 415, 422  
*Lymnaea stagnalis* 411  
*Physa analina* 417  
*Physa gyrina* 412, 417, 419, 420, 423, 424  
*Physa heterostropha* 411, 413  
*Planorbis parvus* 413, 415  
*Planorbis trivolvis* 413, 417, 420, 421

*Pleurocera-elevatum* 416  
 slugs 451  
 Succinea 423  
*Succinea ovalis* 409

## LAMELLIBRANCHIA

Anodonta 379, 380  
 Fresh-water mussels 423, 851, 872  
 Unionidae 421

## ANNELIDA

annelids 451, 521  
 earthworm 430  
 Lumbriculus 451  
 Tubificidae 430

## ROTATORIA

rotifers 554  
 Albertia 589  
 Pleurotrocha 589

## COELENTERATA

Hydra 287, 291

## PORIFERA

freshwater sponges 856

## PROTOZOA

colonial protozoa 856

THE ENDOCRINES, BY SAMUEL WYLLIS BANDLER, M. D., W. B. Saunders Co., Philadelphia and London, 1920. 486 pp.; price, cloth, \$7.00 net.

REVIEWED BY  
T. W. GALLOWAY

In the sudden enthusiasm generated by new discoveries that are clearly important, we human beings are reasonably sure to be swept into fads and over-emphasis. Both our generalizations in scientific philosophy and the more practical applications of them to the treatment of human ailments are full of illustrations of the momentum of credulity. The student of the history of science will recognize instances under the terms,—“natural selection,” “adaptation,” “eugenics,” “eye strain,” “uric acid,” and the like, in which truth has been over-pressed.

It would be a miracle under the circumstances if the thoroughly admitted omnipresence of endocrine secretions in the blood, produced by all sorts of normal and abnormal groups of cells, should not congest human traffic over the same course, and lead the willing student of the subject into statements which are not now established. For example, the present author asserts that they (the endocrines) are the underlying factors in heredity;” and, more specifically, “The differences between animals of various species (and among individuals of the same species) are due to the ductless glands,” and more of like tenor. While it may well be true that the hereditary structural elements in the germ cells are influenced by the internal secretions of the soma in which the germ cells lie, and both soma and germ cells are profoundly influenced in their later development by their mutual secretions, such over-sweeping statements of causation are not altogether impressive. It is a bit like saying that the environment is responsible for heredity! which is doubtless true. In spite of this over-enthusiasm, the body of the book contains a mass of most interesting statements supported by many cases,—with history, symptoms and experimental therapy given,—as well as by inference. The author is a gynecologist and naturally stresses the hormones linked most closely with the functions of sex development, reproduction, pregnancy, parturition, and with the emotions connected with these.

The author with commendable boldness even undertakes to redeem some territory from the deluge of Freudian interpretation, by way of endocrine action. He makes a strong case for believing that many of the complexes, hysterias, bad orientations, and phobias which the psychoanalysts accredit to mental conflicts are in reality due to a poor balance in the endocrine system.

Rather unfortunately, because the author does not seem to have a large first-hand knowledge of investigation on inheritance, the book is introduced by a somewhat rambling and inconsequential jumble of illustrations and exhortations which is rather euphemistically labeled "Environment and Heredity,"—presumably because these terms combined make room for about all that can be said on any subject. An analogous looseness and lack of system in arrangement mars the treatment at many points and leads to much unnecessary repetition, and to some seeming conflicts. This is particularly illustrated in comparing chapter 3, The Introduction to the Story of the Endocrines; chapter 4, Internal Secretions, and chapter 18, The Balance between the Endocrines. The total is something like what one might use in a series of lectures on the subject to a class in which inattention or lack of preparation would make much restatement seem necessary, rather than what one expects in a scientific book.

Aside from those mentioned, the following are the principal chapter headings: Environment and Heredity; The Endocrines in Gynecology; Hypergenitalism and Hypogenitalism; Skin Affections and Internal Secretions; Puberty and Climacterium; The "Higher up" Theory of Sterility in Women; Pregnancy, Labor and Placental Gland; Constitutional Dysmenorrhea; Instincts and Emotions; Mental and Nervous Defects; Mental Deficiency and Criminality; Neuroses and Psychoses; Phobias; The Autonomic Nervous System; Therapeutic Suggestions concerning Endocrines; and several chapters dealing with histories, symptoms, clinics and cases.

Possibly an illustration will aid in making vivid the author's repeatedly emphasized point of the interdependence of the internal secretions. In respect to lactation after labor, the following facts are significant:—The mammary glands are developed at puberty by the interaction of secretions from the ripening ovaries, the posterior hypophysis, the thyroid and the adrenals. A secretion from the ovaries or from the endometrium causes them to swell at menstruation. Placental secretions during pregnancy produce hypertrophy and differentiation of tissue at that time, and seem to inhibit the action of the above mentioned secretions. Milk itself will not form however until this placental secretion is removed or inhibited. After birth the ovaries, posterior hypophysis and other endocrines resume sway and stimulate degenerative process by which milk is formed.

Assuming that feminine metabolism and emotional states are more variable and instable than the masculine, the explanation by the endocrinologist would include such facts as these:—men and women alike possess these numerous glands and with all degrees of original potency; all these glands are normally pouring their secretions into the blood constantly; the secretion of each gland modifies those of certain, if not all the other glands, as well as general metabolic conditions thruout the body; the glands, directly

or indirectly are also modified in their action by the metabolic conditions and by sudden changes of state (as of the emotions) which may be initiated by the environment; gradually under normal conditions and in average individuals these various forces come into an adjustment that represents a person's constitutional norm; in women 13 times a year there is a cyclical interruption of this balance by the introduction of new factors which influence all the endocrines involved and thus upset the balance; in the case of pregnancy, new secretions (and consequent modification of the endocrine balance and of the mental states dependent upon this) are introduced about each of these points: pregnancy, fetal development, parturition, lactation, and the cessation of lactation.

Probably it will be of most service to the readers of this review, in trying to give an idea of the comprehensiveness of the book, to outline the sources, effects and interrelations of the various internal secretions as the author conceives them, especially the more intimate interrelations between the thymus, thyroid, pituitary and the sex glands. The experiments and deductions pointing to, and partially explaining, the cyclical character of these sex-linked endocrine activities seem to the reviewer the most effective and valuable part of the book. The treatment of the sexual and reproductive secretions of the male is not nearly so adequate, in spite of their greater simplicity, as is that of the female.

TABLE OF ENDOCRINES AND THEIR ACTIONS  
(Doubling the signs + and - in column 3 indicates excessive action.)

SOURCE OF THE ENDOCRINE	RELATION TO OTHER GLANDS	Hyper, + or Hypo, -	SYSTEMIC AND GENERAL EFFECTS	FUNCTIONAL EFFECTS	REMARKS
<b>HYPOPHYSIS:</b>					
Anterior Lobe.....	Associated with thyroid; sex; adrenals.	-	Influences physical, mental and sex development. Leads to dwarfing; sexual infantilism, etc.		Has two somewhat antagonistic hormones. Broadly antagonistic to ovary.
Posterior Lobe.....	Associated particularly with ovaries, uterus, etc.  Associated with thyroid insufficiency.	+ + +  + + -  + +	Rapid growth at puberty. Normal giants; after puberty, acromegaly. Inhibition of bone development; feminine quality in males. Protective to pregnant uterus; stimulates embryo.  Affects metabolism, especially carbohydrate. Trophic control, especially over uterus and genitals. Contributes to libido. Adiposity. Modifies menstruation and accentuates labor. Before puberty, sexual infantilism and inhibition of menstruation; after puberty genital atrophy. Dysmenorrhea; sex feelings more pronounced; nausea, etc., of pregnancy.		Specially <i>male</i> in its influence. With testis, adrenal cortex and interstitial thyroid produces male peculiarities.  Specially <i>female</i> in its influence. Couple   with ovary (follicular), adrenal medulla and glandular thyroid, results in peculiarities of the female.
<b>THYROID</b> .....	Associated with sex, particularly in female. Mutual hypertrophy of thyroid and parathyroid on removal of either. Excites thymus.	+  -  + +	Influences calcium metabolism, and activates general growth and function. Retards growth; produces sluggishness and laziness. Myxodema, cretinism. Excitability, irritability, tremor.		Active at puberty; regresses in old age.
<b>PARATHYROIDS</b> .....	Antagonistic to thyroids.	+  + +	Influences calcium metabolism; moderates activity of central nerve cells; and increases sensibility of sympathetic system. Uterine or general tetany in pregnancy. Muscular weakness.		Important in pregnancy. Pre-pregnancy increases their activity.
<b>ADRENALS:</b>	Associated with thyroid and hypophysis in sex organs; and development.	-	Antidote for muscular fatigue; protects against toxemia of pregnancy. Aids growth and sex development.		Secretion increased by emotions.
Cortex.....	Enlarged at pregnancy.	+ +	More masculine qualities, as courage, anger, muscular energy.		Connected with anger.
Medulla		+ +	Over stimulation of medulla, nervousness, anxiety, fear, blushing.		Medulla responds especially to fear.
<b>THYMUS</b>	Associated with thyroid, parathyroid, pineal, and sex (especially in male). Depressed by ovaries and adrenals.	+ +  -	Inhibits development of gonads. Thin, short, fragile bones. Hastens sex and other maturity.		Influences calcium metabolism; regresses at puberty, and stops inhibiting effect.
<b>PINEAL</b> .....	With thymus; antagonistic to hypophysis.	+ -	Inhibits development of sex glands. Physical and mental precocity.		Develops from 1-7th year.

SEX GLANDS:— SOURCE OF THE ENDOCRINE	RELATION TO OTHER GLANDS	Hyper. + or Hypo.	SYSTEMIC AND GENERAL FUNCTIONAL EFFECTS	REMARKS
Ovaries (general) . . . . .	Inhibits (slightly) growth and function of anterior lobe of hypophysis.	+	Tends to control height by effect on epiphyses: late ripening (or castration) increases stature; early ripening interrupts.	
" (special)	Stimulates adrenals and anterior hypophysis.	+	Trophic control of mammary glands. Castration before puberty prevents libido. Responsible for menstruation. May produce original or regressive infantilism. Protects uterus from too great invasion from placenta.	
Interstitial cells. . . . . Stromal	Antagonistic to corpus luteum. Stimulates adrenal cortex and anterior hypophysis.	-	Produce, in connection with other secretions, the secondary female characters at puberty. Control cyclical changes in uterine membrane. Related to formation of blood; diminishes the coagulability of blood. Rheixis; menstruation.	
Follicles (ripening and matured); . . . . .	Influenced greatly by posterior hypophysis, adrenals, and thyroid. Stimulates adrenal medulla.	+	Trophic to uterus, particularly uterine lining.	These increase old or add new secretions to ovary (?) Influenced by and influences menstruation.
Corpus luteum (false; of menstruation); . . . . .	Reacts on thyroid and posterior hypophysis.	+	Stimulates thyroid in anticipation of pregnancy; inhibits posterior hypophysis to check uterine contractions.	
Corpus luteum (of pregnancy);—	Stimulates glandular thyroid, and inhibits contractile effect of post. hypophysis on uterus.	+	Inhibits maturation of follicles and ovulation during pregnancy; and sometimes afterward (?) Stimulates growth of decidual cells of lining, and aids imbedding of fertilized ovum. General nutritional effect on uterus in early pregnancy. Protects wall of uterus from too great invasion by trophoblast cells.	Is stimulated by placental secretion of embryo.
Uterine decidua (endometrium) . . . . .	Mutual stimulation of endometrium and ovary, involving the ovulation-menstruation cycle.	-	Sensitizes the ovary. Atrophy of uterus, through inhibition of activity of ovary (follicles).	
Mammary glands (Nursing)	Hormone antagonizes ovarian activity.	+	Contracts uterus; inhibits menstruation. Atrophy of uterus, and inhibition of evaluation.	Especially where underfunctioning of hypophysis.
Testes— Interstitial cells (Other products?)	Operates on, and with, anterior hypophysis, adrenal cortex, pineal, and thyroid.	+	To produce general male characteristics of body and mind; heavier and longer bones, shape, hair, changes in pharynx, impulses, etc. Derangement of associated glands, and inhibits sexual growth and differentiation.	Maybe overcome by ovarian extract and thyroid.

SOURCE OF THE EXUDICINE	RELATION TO OTHER GLANDS	Hyper, + or Hypo, -	SYSTEMIC AND GENERAL FUNCTIONAL EFFECTS	REMARKS
FETAL (Embryonic):— Fertilized egg and Trophoblast:  and Later placenta:  Fetus:	Inhibits joint work of interstitial secretion and posterior hypophysis. Cooperates with corpus luteum.  Increases the growth (permanently) and function of anterior hypophysis.  Related to placental glands.	+  +  + +  +	Gives off elements which inhibit menstruation, by checking rupture of blood vessels and escape of corpuscles, even during congestion. Inhibits normal regression of corpus luteum; i. e., stimulates its growth and activity.  Stimulates growth of uterus. Protection and trophic effect on both mother and embryo. Irritation of mucosa, gastric and other; and nausea.  Plays a rôle in developing function of mammary glands (?)	Settles in endometrium, and develops attachments therein.    Counteracted by corpus luteum (?)

## LIST OF MEMBERS

In order to reduce the cost of printing, the complete list of members will be omitted.

### MEMBERS ADMITTED SINCE THE LAST PUBLISHED LIST

Bicknell, Anna, B. S. . . . .	4411 39th St., Washington, D. C.
Bierbaum, C. H. . . . .	Mutual Life Bldg., Buffalo, N. Y.
Boyden, A. A. . . . .	1421 Oakridge Ave., Madison, Wis.
Cleveland, L. R., B. S. . . . .	220 W. Monument St., Baltimore, Md.
Danheim, Bertha L., B. S. . . . .	Blue Rapids, Kansas.
Dayton, Edna B., M. D. . . . .	1512 N. Gratz St., Philadelphia, Pa.
Depew, Ganson . . . . .	167 Summer St., Buffalo, N. Y.
Diago, Joaquin, M. D. . . . .	Aguila 72, Havana, Cuba.
Fellows, Harriette L. . . . .	220 S. Prairie Ave., Sioux Falls, South Dakota.
Gunns, Cecil A. . . . .	Dept. Zoology, Kansas State Agricultural College, Manhattan, Kansas.
Hallinen, J. E., B. S. . . . .	Cooperton, Oklahoma.
Herrick, Chester A., B. S. . . . .	Kansas State Agricultural College, Manhattan, Kansas.
Kamal, Mohammed, B. S. . . . .	Kansas State Agricultural College, Manhattan, Kansas.
Lofton, Robert E., A. . . . .	Bureau of Standards, Washington, D. C.
MacKay, Alexander H., A. B., B. Sc. LL.D., F. R. S. Canada . . . . .	61 Queen St., Dartmouth, Nova Scotia, Canada.
Mannhardt, L. A., Ph. B. . . . .	New York University, New York, N. Y.
Payne, Nellie M., B. S. . . . .	1400 Poyntz Ave., Manhattan, Kansas.
Putz, Alfred . . . . .	5117 Locust St., Philadelphia, Pa.
Root, Francis M., Ph.D. . . . .	310 W. Monument St., Baltimore, Md.
Sister Monica, M. S. . . . .	Notre Dame Training College, Glasgow, Scotland.
Sperry, Arthur., B. S. . . . .	Kansas State Agricultural College, Manhattan, Kansas.

## INDEX

### A

- Abstracts, 14; 89; 158; 190.  
Acanthocephala from the Eel, 1.  
Ackert, J. E., and Wadley, F. M., Observations on the Distribution and Life History of *Cephalobium microbivorum* Cobb and of its Host *Gryllus assimilis* Fabricius, 97.  
Allen, W. E., Some Work on Marine Phytoplankton, 177.  
Allen, W. E., A Brief Study of the Range of Error in Micro-enumeration, 14.  
American Mulletts, Life History and Scale Characters, 26.  
Animals, Occurrence and Rôle of Copper in, 144.  
Animal Parasites, Compendium of Hosts of, 195.  
Annual Report of Treasurer, 48.  
Arrhenuri, New Species and Collections, 168.

### B

- Baker, F. C., Preparing Collections of the Mollusca for Exhibition and Study, 31.  
Bandler, S. W., The Endocrines, a Review, 200.  
Briefer Articles, 14; 89; 158; 187.  
Brownian Movement, Microscopical Illumination with Reference to, 158.  
Bullard, Chas., A Method for Orienting and Mounting Microscopical Objects in Glycerine, 89.

### C

- Cambarus agrillicola* Faxon, Spring Migration in, 28.  
*Cephalobium microbivorum* Cobb, Distribution and Life History, 97.  
Cnidosporidian Spores, Structures Characteristic of, 59.  
Combination Lighting, Microscope Illumination with Reference to, 158.  
Common Field Cricket, A Sarcophagid Parasite of, 116.  
Compendium of Hosts of Animal Parasites, 195.  
Copper, Its Occurrence and Rôle in Insects and Other Animals, 144.

- Crayfish, Spring Migration in, 28.  
Cummins, H., Spring Migration in the Crayfish, *Cambarus agrillicola* Faxon, 28.  
Custodian's Report for the Year 1920, 47.

### D

- Desmid, Method of Demonstrating Sheath Structure, 94.  
Diatoms, Literature of, 187.  
Distribution and Life History of *Cephalobium microbivorum* Cobb, 97.

### E

- Eel, *Acanthocephala* from, 1.  
Endocrines, Review, 200.

### F

- Faust, E. C., Larval Flukes from Georgia, 49.  
Faust, E. C., Recent Advances in Parasitology, 75.  
Fixatives, Effect upon Myxosporidian Spores, 117.  
Fresh-water Biology, Ward and Whipple, Compendium of Hosts of Animal Parasites contained in, 195.  
Fresh-water and Marine Gymnostominan Infusoria, 118.

### G

- Galloway, T. W., Review of Endocrines, 200.  
Georgia, Larval Flukes from, 49.  
Glycerine, Mounting Microscopical Objects in, 89.  
*Gryllus assimilis* Fabricius, Distribution and Life History of, 97.

### H

- Hausman, L. A., Fresh-water and Marine Gymnostominan Infusoria, 118.  
Henderson, W. F., Treasurer, Annual Report, 48.  
Herrick, C. A., A Sarcophagid Parasite of the Common Field Cricket, 116.  
Hosts of Animal Parasites, A Compendium, 195.  
Hubbs, C. L., Remarks on the Life History and Scale Characters of American Mulletts, 26.

## I

- Infusoria, Gymnostominan, 118.  
Insects, Occurrence and Rôle of Copper in, 144.

## K

- Kudo, R., On the Effect of Some Fixatives upon Myxosporidian Spores, 161.  
Kudo, R., On the Nature of Structures Characteristic of Cnidosporidian Spores, 59.

## L

- Larval Flukes from Georgia, 49.  
Life History and Distribution of *Cephalobium microbivorum* Cobb, 97.  
Literature of the Diatoms, 187.

## M

- Marine and Fresh-water Gymnostominan Infusoria, 118.  
Marine Phytoplankton, 177.  
Marshall, Ruth, New Species and Collections of Arrhenuri, 168.  
Methods, Department of, 14; 89; 158; 187.  
Method of Demonstrating Sheath Structure of a Desmid, 94.  
Method for Orienting and Mounting Microscopical Objects in Glycerine, 89.  
Micro-enumeration, Range of Error in, 14.  
Minutes of Chicago Meeting, 47.  
Microscope Illumination, 158.  
Mollusca, Preparing Collections for Exhibition and Study, 31.  
Mounting in Glycerine, 89.  
Muttkowski, R. A., Copper; Its Occurrence and Rôle in Insects and Other Animals, 144.  
Myxosporidian Spores, Effect of Fixatives upon, 161.

## O

- Orienting and Mounting in Glycerine, 89.

## P

- Pflaum, M., Custodian, Report for the year 1920, 47.  
Phytoplankton, Marine, 177.  
Preparing Collections of Mollusca, 31.  
Proceedings of the Society, 47.

## R

- Range of Error in Micro-enumeration, 14.  
Recent Advances in Parasitology, 75.  
Reports of Auditing Committee on Treasurer's and Custodian's Reports, 48.  
Reviews, 14; 89;

## S

- Sarcophagid Parasite, 116.  
Scale Characters in Mulletts, 25.  
Sheath Structure of Desmid, 94.  
Silverman, A., Microscope Illumination with reference to Brownian Movement and Combination Lighting, 158.  
Spencer-Tolles Fund, Report on, 47.  
Summaries, Department of, 75.

## T

- Taylor, F. B., The Literature of the Diatoms, 187.  
Taylor, W. R., A Method of Demonstrating the Sheath Structure of a Desmid, 94.  
Treasurer, Annual Report, of, 48.

## V

- Van Cleave, H. J., Acanthocephala from the Eel, 1.  
Van Cleave, H. J., A Compendium of the Hosts of Animal Parasites contained in Ward and Whipple's Fresh-water Biology, 195.

## W

- Wadley, F. M., and Ackert, J. E., Distribution and Life History of *Cephalobium microbivorum*, 97.  
Ward and Whipple, Compendium of Hosts of Animal Parasites, 195.  
Welch, P. S., Secretary, Minutes of Chicago Meeting, 47.

# CLASSIFIED ADS

## LIVING AMOEBA

You have never attained ultimate satisfaction with your laboratory classes unless you have tried our

GIANT BULLFROGS  
ALLIGATORS

and other southern specialties.

All Material Guaranteed

**SOUTHERN BIOLOGICAL  
SUPPLY COMPANY, Inc.**

Natural History Building  
New Orleans, La.

Established 1883 Without Intermission 1916

## A. A. SPHUNG

*The Frog and  
Turtle Man*

Live Frogs, Turtles, Crayfish,  
Clams, Earthworms. Also pre-  
served materials shipped to all  
parts of the world.

*Write for Price List  
Lists Free*

NORTH JUDSON, INDIANA

## Powers & Powers HIGH GRADE Microscopic Slides AND Living Micro-Organisms

*Living Amaba Proteus and Living Fresh Water Hydra.*—We were the first in America to develop the rearing and shipping of these organisms to all parts of the continent. We ship to every State in the Union and even as far as Nova Scotia. Living delivery always guaranteed within the months of the school year. We have a private plant of our own especially adapted to these and other micro-organisms.

*Microscopic Slides.*—We have a growing list of high grade preparations for the microscope, chiefly in zoology. We endeavor to produce slides of unusual excellence. Many are quite unprocurable elsewhere.

*Circulars and Prices Upon Application*

**POWERS & POWERS**  
Station A LINCOLN, NEBR.

## Marine Biological Laboratory

Woods Hole, Massachusetts

## Biological Material

1. ZOOLOGY. Preserved material of all types of animals for class work and for the museum.
2. EMBRYOLOGY. Stages of some invertebrates, fishes (including Acanthias, Amia and Lepidosteus), Amphibia, and some mammals.
3. BOTANY. Preserved material of Algae, Fungi, Liverworts, and Mosses.

Price lists furnished on application to  
GEORGE M. GRAY, Curator  
Woods Hole, Mass.

Correspondence solicited on special orders, e.g., giving marine or aquatic material.

# MICROSCOPE SLIDES

Largest Stock in America. Get List No. 4

## LANTERN SLIDES

Large catalog and two supplements.

We make slides from your negatives or photographs.

## LIVE AND PRESERVED MATERIAL

(two catalogs)

We are the Western Representatives of the Supply Dept. of the Marine Biological Laboratory of Woods Hole, Mass.

## BIOLOGICAL APPARATUS AND SUPPLIES

Glassware	Aquaria	Instruments	Microscopes
Lanterns	Museum Cases	Breeding Cages	Life Histories
Skeletons, Etc.			(Catalogs are ready)

## GENERAL BIOLOGICAL SUPPLY HOUSE

1177 E. 55th St., CHICAGO

# LIVING BULLFROGS

(*Rana catesbiana*)

Extra large, selected specimens, 18", each.....	\$ 1.25
Large, head and body 6-7", total length 15-18", per doz.....	8.00
Same, per hundred.....	60.00
Medium, head and body 4-6", total length 10-15", per doz.....	5.00
Same, per hundred.....	35.00

Prices for preserved frogs are the same.

### Living Crayfish (*Camabrus clarkii* Girarg)

Large, from 3 to 3½", per dozen.....	.90
Same, per hundred.....	6.00
Same, preserved, per dozen.....	.75
Same, preserved, per hundred.....	6.00
Extra large, selected specimens, 3½" minimum, per doz.....	1.10
Same, per hundred.....	7.50
Same, preserved, per dozen.....	1.00

Prompt shipments, high quality and low price materials, are some of the factors that constitute my service to you.

## H. EDW. HUBERT

3615 Melpomene St.

NEW ORLEANS, LA.

WARD'S NATURAL  
SCIENCE  
ESTABLISHMENT

84-102 COLLEGE AVE.,  
ROCHESTER, N. Y.

Have you seen the slides prepared for the series described as Dr. Sigmund's Histology of Man and the Mammalian Animals? Each slide has been prepared with the greatest care to show some particular structure, and the results exceed belief. There is also a descriptive text which is in itself a treatise on histology. Write for circular M-127 which describes the set.

We also carry over 2000 different microscope slides covering Botany, Zoology, Parasitology and Histology. Complete catalogue free upon request.

ANCO BIOLOGICAL  
SUPPLIES

Imported and Domestic  
MICROSCOPIC SLIDES

For use in

ANATOMY  
BOTANY  
EMBRYOLOGY  
HISTOLOGY  
NEUROLOGY  
PATHOLOGY  
ZOOLOGY

Our Loose-leaf Catalogue  
No. 21 Sent Upon Request.

Quality  
First

Prompt  
Delivery

THE ANGLERS CO.

913 W. Randolph St.  
CHICAGO, ILL.

The Collegiate Press

Our specialization in college and university printing has equipped us to give our customers unexcelled service combined with best quality work at moderate prices.

We shall be glad to have members of the Society write at any time for quotations on material they wish to publish in book or pamphlet form, or for rates on accurate drawings or engravings of microscopic subjects.

Careful attention will likewise be given to requests for stationery and blank forms.

George Banta Publishing Company

Manufacturing Publishers

Menasha, Wis.



Comstock Publishing Company

Ithaca, N. Y.

THE MICROSCOPE—12th Edition, 1917.  
Contains the old and also the new things in microscopy. S. H. Gage. Postpaid \$3.00.

OPTIC PROJECTION—Especially full on the projection microscope and drawing. S. H. and H. P. Gage. Postpaid \$5.00.



**High Grade Microscopical and Dissecting  
Instruments, Glassware and Preparations.**

**Biological and General Laboratory Supplies**

**Anatomical Models, Osteological Preparations,  
Museum and Naturalists' Supplies.**

*Write for Catalogues and Prices*

**The Kny-Scheerer Corporation of America**

*Department of Natural Science*  
G. LAGAI, PH. D.

56-58 W. 23rd Street  
NEW YORK, N. Y.

**BIOLOGICAL SUPPLIES**

**Michigan Biological Supply Co.**

Manufacturers of

**HIGH GRADE MICROSCOPIC SLIDES**

for

**BOTANY, ZOOLOGY, PHYSIOLOGY, HISTOLOGY, and  
AGRICULTURE**

We make a specialty of supplying slides to accompany Shull's  
"Laboratory Directions in Principles of Animal Biology."

Cultures of Ameba proteus and other Protoczoa

Dealers in

Preserved and Museum Material, Glassware, Lantern Slides, and  
Microtechnical Reagents

All our supplies are guaranteed to be entirely satisfactory.

Catalog sent on request.

Nickels Arcade

Ann Arbor, Mich.

## New Stereoscopic Eyepiece



PRICE, with Pair of Matched Eyepieces, Eyepiece Diaphragms and Adapter for FFS Microscope, \$50.00.

A most significant addition to our microscopical line, for both laboratory and research workers; has been enthusiastically received by those who have already seen it demonstrated. Presents following advantages:

1. *Makes available the benefits of binocular vision at moderate cost;*
2. *Can be adapted to almost any monocular microscope;*
3. *Gives stereoscopic effect;*
4. *Parallel position of eyepiece tubes, adjustable for interpupillary distances, allows full relaxation of ocular muscles, with consequent relief from eye fatigue.*

Allowing the natural use of both eyes, this apparatus will be particularly appreciated by all those who are obliged to do frequent or extended work with the microscope.

Write for illustrated, descriptive circular

## Bausch & Lomb Optical Co.

552 St. Paul St., Rochester, N. Y.

New York Chicago Washington San Francisco London

Makers of Photographic Lenses, Microscopes, Projection Apparatus (Balopticons), Ophthalmic Lenses and Instruments, Photomicrographic Apparatus, Range Finders and Gun Sights for Army and Navy, Searchlight Reflectors, Stereo-Prism Binoculars, Magnifiers and Other High-Grade Optical Products.

Leitz Microscopes are the Standard of the World

# LEITZ "MON-OBJECTIVE BINOCULAR" MICROSCOPE

*The Modern Research' Type*

This Binocular Microscope can be used with any of the standard objectives from lowest to highest power.

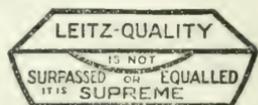
This model originates with Leitz and was successfully introduced in 1913. Other firms have copied this model but the individual design, superior workmanship and efficiency of the Leitz pattern will fully protect the prestige for the original type.



## Points of Merit

1. Binocular vision.
2. Perfect accommodation to any interpupillary distance.
3. Adjustment for any difference in refraction between the eyes.
4. Complete elimination of eye strain.
5. Improved quality of image.
6. Parallel eyepieces.
7. The possibility of using any favored objective from the lowest power to the highest oil immersion.
8. Reduction in numerical aperture.

Write for Pamphlet No. 1003



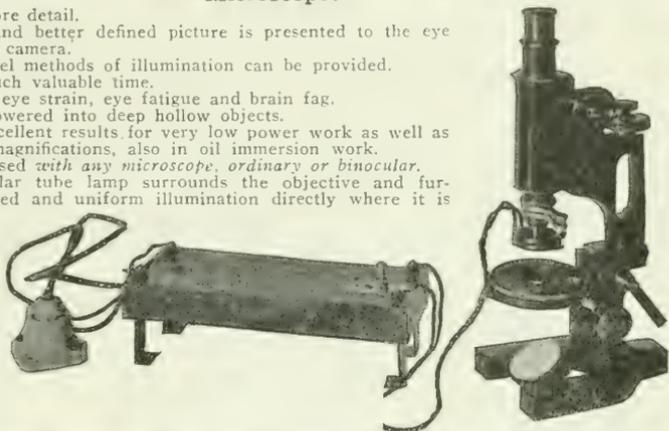
60 EAST 10<sup>TH</sup> ST.

Members and Friends Will Find Our Advertisers Reliable

## THE SILVERMAN ILLUMINATOR

offers important advantages for practically every application of the Microscope:

- a—It shows more detail.
  - b—A clearer and better defined picture is presented to the eye and the camera.
  - c—Several novel methods of illumination can be provided.
  - d—It saves much valuable time.
  - e—It prevents eye strain, eye fatigue and brain fag.
  - f—It can be lowered into deep hollow objects.
  - g—It gives excellent results for very low power work as well as higher magnifications, also in oil immersion work.
  - h—It can be used with any microscope, ordinary or binocular.
- A small circular tube lamp surrounds the objective and furnishes a diffused and uniform illumination directly where it is needed.



The Silverman Illuminator marks A GREAT ADVANCE in Microscope Illumination

WRITE FOR BULLETIN 45-C

LUDWIG HOMMEL & CO.

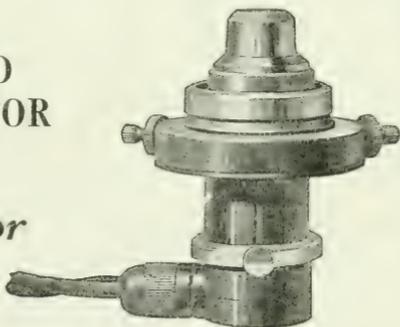
530-534 Fernando St.

Pittsburgh, Pa.

## NEW ELECTRIC DARK FIELD ILLUMINATOR

(U. S. Army Medical School Type)

*A Combined  
Dark Field Illuminator  
and Microscope  
Lamp*



It Fits the Substage Ring of All Standard Makes of Microscopes  
It Is New, Original, Unique, Compact, More Efficient

ANOTHER FORWARD STEP IN MICROSCOPE CONSTRUCTION. ANOTHER SPENCER TRIUMPH

Send for Booklet

SPENCER LENS COMPANY

Manufacturers

MICROSCOPES, MICROTOMES, DELINEASCOPES

BUFFALO, N. Y.

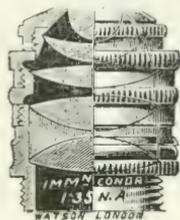


Members and Friends Will Find Our Advertisers Reliable

# Watson's Apparatus for Microscopes

Watson's manufacture a series of Achromatic Objectives of high and low aperture, and of varying powers, enabling the maximum effect to be obtained with every description of Object Glass.

They correspond in correction and workmanship with Objectives of similar powers and apertures, and the high power Condensers can be made suitable for lower power Objectives by the removal of the top lens.



**THE HOLOSOPIC OIL IMMERSION CONDENSER.** Power .22", numerical aperture 1.35, the whole of which is aplanatic if used on the thickness of slip for which it is corrected. The finest Condenser obtainable for high power work.

Optical part only, to fit the Royal Microscopical Society's Screw Thread, £9/12/6.

Particulars of the other Condensers of the Series are given below:

Condenser	Full Aperture	Aplanatic Aperture		Equivalent Focus		Diameter of Back Lens
		Complete	Top Lens Removed	Complete	Top Lens Removed	
Macro. Illuminator	—	—	—	Inch .2	Inch .	1.25
Aplanatic Low Power	.50	.48	—	.66	—	6
The Universal	1.0	.95	.40	.4	1.0	.77
Parachromatic	1.0	.90	.40	.29	.4	.62

**WATSON'S HOLOS. IMMERSION PARABOLOID** for the examination of Spirochetæ, and the exhibition of ultra-microscopic particles, is the best high power Dark Ground Illuminator.

Optical Part only. Price £3/11/6.

Particulars of all the above, and of Watson's Microscopes and Accessories of every description, are contained in their catalogue of Microscopes, which is published in 4 parts, as follows:

Part 1: Students' Instruments.

Part 2: Research and Other Microscopes, and appliances of every description.

Part 4: Instruments for Metallurgy, Petrology and Mineralogy.

Part 5: Photo-Micrographic Apparatus and Accessories. Sent post free on request to:

## W. Watson & Sons, Limited

Established 1837

313, High Holborn, LONDON, ENGLAND





QH                    American Microscopical  
201                  Society  
A3                    Transactions  
v. 39-40  
cop. 2  
Biological  
& Medical  
Serials

PLEASE DO NOT REMOVE  
CARDS OR SLIPS FROM THIS POCKET

---

UNIVERSITY OF TORONTO LIBRARY

---

**STORAGE**

