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REACTIONS OF VARIOUS PLANKTON
ANIMALS WITH REFERENCE TO THEIR
DIURNAL MIGRATIONS

BY

CALVIN O. ESTERLY



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INTRODUCTION

This paper reports the results of a year of study on the behavior of some marine plankton organisms. The chief aim of the work was to ascertain the factors that determine the diurnal migrations of such forms. It was outside my purpose to attempt to learn how sensitive the animals under experiment might be toward changes in surrounding factors such as light or temperature, for example. It was not in the plan, furthermore, to consider questions connected with the interpretation of behavior as applied to such matters as the mechanism of orientation. The end desired was to learn how the direction of movement is affected by various external conditions. The actual experimental facts were sought rather than the laws or principles underlying them. Since the experimental work was not concerned with the physiology of the movements controversial matters connected with that phase of the study of behavior are not discussed.

It is important that it be understood that this investigation of responses was undertaken with the definite end in view of obtaining a basis for explaining habits in nature. The natural conditions experienced by the animals in the course of the vertical movement were constantly considered, and the mode of procedure was based on that consideration. In general, it may be said that during descent the temperatures encountered are progressively lower, and during ascent

the animals pass into warmer and warmer water. From the surface to a depth of about thirty meters there is a slight decrease in salinity, but from thirty meters down the salinity gradually increases. The light intensity during the day grows less as the distance beneath the surface increases; this is probably true for the night also; but for that time the difference in degree of illumination at the surface and at lower levels is so much less than during the day that it is practically negligible, unless phosphorescence makes light that is more intense below the surface. Since plankton animals are commonly found in deeper water by day and nearer the surface by night, they pass through a range of temperatures and densities as they move up and down. Furthermore, during the day they are in water of higher salt concentration and lower temperature and in light of relatively low intensity, compared with the surface. While the illumination at the surface is low during the night also the temperature is higher and the salinity lower than the animals encounter by day, although forms that do not descend during the day farther than about thirty meters will be in water of less salinity than at the surface.

Now if natural surroundings are to be simulated in experiments conducted for the purpose already mentioned, it is a comparatively simple matter so to arrange conditions that if the animals move downward they meet with lower temperature, higher salinity, and decreased light intensity, or with the reverse of these three conditions if they ascend. It is needless to point out that there are some oceanic conditions that can not be reproduced in the laboratory, such as great depth of water, tidal currents, or distance from shore. But it is easy to provide a temperature gradient with cold water at the bottom of a column. If a light is placed at the top of the column the intensity will be greatest at the surface and progressively less below the surface. Or it may be arranged that cold water of higher salinity shall be found at the lower part of a cylinder where the light intensity is measurably less than toward the top. On the other hand, experimental conditions can be secured in the laboratory which would not be met by the organisms in the sea. An example of this is lighting a vertical container from below. It is permissible, of course, to introduce unnatural conditions but only for the purposes of interpreting activities observed under the more natural conditions. On the whole, however, the experimental conditions were patterned after the general oceanic environment as regards light, temperature, and salinity.

This experimental study of diurnal migration was undertaken only after a number of years of field investigation by the Scripps Institu-

tion. Such work has shown, we believe, that the vertical movement is wide spread among plankton organisms found in the San Diego region, and that the conditions and factors involved make a problem of great complexity. Studies of the field records indicate that it is possible to ascertain without recourse to experiment some of the relations existing between external conditions and the movements of the animals. Such studies, as carried on at the Institution, involve a large amount of statistical and mathematical work (Michael, 1916, p. xix). It is difficult to carry on the field and experimental work at the same rate, so that experimental results will be considered for the most part at this time. Some papers have already appeared dealing with the field results (Michael, 1911, 1913, Esterly, 1911, 1912) and others are in course of preparation. The published papers are based on statistical methods that as a rule give trustworthy and significant results. In some cases, however, the field data are inconclusive, and the experiments were not completed on some of the forms. It has seemed desirable, nevertheless, to point out in this paper the possible relation between the experimental results and the behavior in the sea as we understand it from our field data. Statements about this relation are more or less speculative, however, since both the field and experimental data are lacking in certain respects.

METHODS AND MATERIAL

The laboratory procedure and apparatus were purposely as simple as possible. Most of the work was done in a room with black walls. Daylight entered the room through a north window 50 by 40 cm., and this area could be reduced by means of a curtain and shutters of different sizes. Artificial light was obtained from Mazda lamps. The lamp in use was enclosed in a housing of black sheet iron. The rays from the bulb passed out through a filter of Corning "Daylite" glass, which according to Gage (1915) gives the electric light a quality closely approaching that of daylight. The lamps were 15-watt and 100-watt, the latter gas-filled and with a concentrated C-shaped filament; the opening in the housing of the former was 38 mm., in diameter, of the latter 46 mm. The relative intensities of the different light were determined roughly by means of a method suggested by Dr. G. F. McEwen. Two blocks of paraffine of the same thickness were clamped together with opaque cardboard between. The blocks were placed between the lights to be compared and by suitably varying the distance of the light from the paraffine it was possible to obtain approximately

equal illumination on the two sides of the cardboard screen. The light is diffused by the paraffine to such an extent that the edges of the blocks show when both are equally lighted. The 15-watt lamp was used as a standard of intensity. The intensity of the 100-watt is about 6, if that of the other lamp is called unity. The 50 by 40 centimeter window gives an intensity about 3800 times that of the 15-watt, and 400 times that of the 100-watt. These figures are only approximations, of course, but they serve for present purposes.

Temperatures lower than those ever found at the surface of the sea in the region about La Jolla were produced by putting a dish or tube of sea water into ice. With a vertical container the whole column or only the lower portion could be cooled, the latter case giving a gradient with a difference of 8°C or 10°C between the top and bottom.

Water of a salinity greatly above that of ordinary sea water was obtained by allowing evaporation to continue for days in a warm room. This highly concentrated water was diluted with normal ocean water until a desired degree of salinity was reached. The actual salinity was computed by means of Knudsen's tables (1901) from the specific gravity as given by a hydrometer. It should be noted that this method of increasing the salinity does not involve changing the relative proportions of the salts in sea water as does the addition of one salt, such as NaCl, to ordinary sea water. I did not attempt in any case to ascertain whether a decrease in the salinity has an effect on reactions.

In many experiments a single animal was enclosed in a glass tube 500 by 30 mm., which was filled with water and closed with a rubber stopper so that no air space remained. The tube could be clamped to a ring support and held in position so that light might enter from above or from below; or the tube could be kept horizontal. It is possible, furthermore, to arrange the test so that the animal must start at the top or at the bottom of the column of water, by swinging the tube through 180°. There was no apparent effect on the animals from the mechanical stimulus caused by turning the tube; if there was it could not have been as great as that caused by picking up the specimen in a pipette.

All the animals were collected by means of a conical plankton net. A wide-mouth bottle or jar was tied into the peak of the net, so that the organisms were never out of water and they were not handled at all until in the laboratory. It was found that the specimens were in better condition and lived longer if they passed from the net into a large amount of water, and accordingly, for the greater part of the collecting a two-quart jar was tied into the net.

The animals were kept in a large glass dish in the laboratory; and as a rule individuals were picked out of this stock as needed, and thereafter each one was kept in a finger bowl. Some species could be kept alive for a week, but most of the forms did not withstand laboratory conditions well. While I thought at the beginning that it would be desirable to test each one of a given set of individuals for several days, if possible, that plan was soon abandoned, and many, if not most of the experiments were performed with animals collected during the morning of the day on which they were used, or during the preceding night. But if specimens appeared to be in good condition on the second day I did not hesitate to use them. The precautions taken to have fresh material are not necessary, in my experience, to guard against alterations in the form of the responses. I believe that there is something connected with laboratory conditions that does affect the reactions of some species so that there is a change after the animals are removed from the ocean. But if the change occurs it appears shortly or within a few hours after the animals are brought in. Results are uniform after that, so far as sign of reaction is concerned, as long as there is any response.

The water in which the animals were kept after collection was either that from the laboratory system or it was brought into the laboratory from the end of the pier (1000 feet from shore) in glass jars. So far as I could determine from a large number of trials with different species, the behavior is practically the same in water from the two sources; though I believe that the animals remain in better condition and live longer if they are not put into water that has passed through the pipes of the pumping system.

The experiments were carried on with adults of the following forms: the copepods *Acartia tonsa* and *A. clausi*, *Calanus finmarchicus*, *Eucalanus clongatus*, *Labidocera trispinosum*, *Mctridia luccens*, and the chaetognath *Sagitta bipunctata*. It was originally intended to carry out the same series of tests with specimens of each species, but this proved impossible. The chief reason is that some species were not obtained in sufficient numbers or long enough through the year. *Acartia tonsa*, *Calanus*, and *Sagitta* could be counted on at almost any time, and were obtained in fair abundance, but the other species were more or less erratic in occurrence. There were times, indeed, when collecting was uniformly unsuccessful, even *Acartia* being absent from the surface at night though ordinarily very abundant then. The collections varied from time to time, also, so that it was necessary to use different species on different days as they happened to come.

LITERATURE

The first investigators to study diurnal migration from the experimental side were Groom and Loeb (1890). They worked with the larvae of a barnacle, *Balanus perforatus*. Their general conclusion (p. 173) is that the animals move down because they are negative toward the intense light of day, while they ascend at night because they are at least not negative to light of the intensity that prevails then. The authors consider that their experiments show “. . . dass die ganze Erscheinung der periodischen täglichen Tiefenwanderung der Tiere eben nur dadurch möglich ist, dass dieselben erstens heliotropisch sind, das heisst, dass sie durch den Lichtstrahl gerichtet werden; und zweitens, dass der Heliotropismus. . . Abends (im schwachen Licht) positiv, am Morgen (bei starkem Licht) negativ ist.” They state, however (p. 176) that the matter is different with certain copepods; they could not establish that there is a change in heliotropism in these forms.

A few years later Loeb (1893) studied several different kinds of smaller pelagic animals. *Polygordius* larvae are made positively heliotropic by cooling, and negative by warming (p. 90). Increase of concentration (by adding NaCl to sea water) has the same effect as cooling, while decreasing the concentration by adding fresh water to sea water causes the negative response (p. 94). Copepods, “probably *Temora longicornis*” are affected the same way as the worm larvae (p. 96). Loeb states (p. 104) that he has worked with copepods; larval crustacea, worms and mollusks, and has not found a pelagic animal of these sorts that would not become permanently or temporarily positive to light under certain conditions. Geotropism, however, as well as light has some effect in determining the vertical distribution since positive heliotropism and negative geotropism and negative heliotropism and positive geotropism work in the same direction (p. 105). A number of years later (Loeb, 1906) it was found that copepods are made positively heliotropic by CO₂ or any dilute acid (p. 565), as is *Daphnia* (p. 568). Fresh water *Gammarus* also is made strongly positive, but this condition endures only a few seconds (p. 570). The larvae of *Balanus* become positive in water to which carbon dioxide is added (p. 575).

A general statement of the bearing of such experimental results on the migration was given by Loeb in 1908. He regards light as

perhaps the most important factor (p. 732). "Diese periodischen Tiefenwanderungen der pelagischen Organismen führten wir darauf zurück, dass äussere Umstände einen periodischen Wechsel im Sinne des Heliotropismus derselben bestimmt. . . ." Acids and temperature are among the external conditions that affect the sign of heliotropism, and the application of such facts to diurnal movement is as follows (p. 733): As the animals descend they must reach colder water, and finally they encounter temperatures in which they become positively heliotropic; this checks the descent and brings about an upward movement. Since the temperature of the water nearer the surface decreases toward evening, the animals must reach higher and higher levels and ultimately the surface in consequence of the continuance of the positive heliotropism. As long as the temperature at the surface is sufficiently low the animals do not descend; but as morning advances the surface water becomes warmed, the positive heliotropism disappears, the active upward swimming ceases, and the animals sink or swim down. Then, as before, the descent is checked by low temperature at some level and the upward movement begins again. The fact that acids, especially carbon dioxide, cause positive heliotropism is also applicable to diurnal migration, particularly in fresh water animals. The carbon dioxide content of the water must diminish during the morning since the aquatic plants are consuming it in the manufacture of starch; and this decrease in carbon dioxide causes positive heliotropism to lessen in intensity. The animals, consequently, descend either by sinking or swimming, since they no longer make efforts to swim toward the light. As the light begins to wane in the latter half of the day the carbon dioxide gradually increases in amount up to a point where positive heliotropism is induced. Furthermore, light itself alters the sign of heliotropism (p. 734). Very intense light has a tendency to make certain forms negative, and the violet and ultra-violet rays have this effect also. This must mean that pelagic animals will descend by day and ascend by night. In addition, geotropism and viscosity of the water are factors that enter into depth migrations.

This paper of Loeb's (1908) has something of a controversial tone as regards certain statements by Bauer (1908), chiefly as to whether reactions to vertical and horizontal rays are similar. Without going into the question, it is sufficient to note here that Loeb (1908, p. 735) states that he has worked on thousands of positively heliotropic forms and has always found them to be positive to vertical as well as to horizontal light.

As recently as 1913 Loeb reiterated the same general views as to the factors that are concerned in the migration. He mentions the effects of temperature and carbon dioxide, and in addition suggests (p. 480) that "die durch das Light bestimmten chemischen Prozesse auf die Dauer ebenfalls den Sinn des Heliotropismus beeinflussen in der Weise, dass längerer starke Belichtung die Tendenz zur Indifferenz oder zu negativem Heliotropismus erhöht während länger andauernde schwache Beleuchtung die Tendenz zu positivem Heliotropismus erhöht." A fourth factor, possibly, is found in periodic variations in the internal chemical processes. "Wenn diese Schwankungen des chemischen Prozesses dem Wechsel von Tag und Nacht entsprechen (man denke an die nyktitropen Bewegungen der Pflanzen), so könnten sie auch periodische Tiefenwanderungen induzieren."

I think it is a fair statement of Loeb's views that change in heliotropism because of change in external conditions is the principal factor in the periodical depth migration. It would be well to note, also, that this seems to be regarded by Loeb as of general application, though he does not say so. His aim, however, evidently is to find a simple and general explanation for the habit.

The views of Loeb regarding diurnal migration do not meet with the unqualified approval of Bauer (1908). The disagreement between these writers has been impartially discussed by Burekhardt (1910). Bauer studied the movements of various Mysidae, and he insists that these animals do not react to horizontal light as they do to vertical rays. He also has this to say (p. 368) with regard to the periodic depth migration: "Die Erklärung der Tiefenwanderung planktonischer Organismen durch positive oder negative Phototaxis (geprüft mit der üblichen Anordnung für Phototaxisversuche) isht daher ein methodische Fehler." Later, in replying to Loeb (1908), Bauer (1909, p. 79) states that his only criticism was directed chiefly against the practice of applying to the migration problem the results obtained in experiments with horizontal light without further evidence as to the behavior in vertical lighting, since the light comes from above in the natural habitat of the animals.

The well known work of Parker (1902) on the copepod *Labidocera aestiva* is an example in which experimental results clearly reveal possible reasons for the diurnal movement. Parker believes that the behavior observed by him in the laboratory "undoubtedly imitated in miniature the natural daily mirations of female Labidocerae in the sea" (p. 119). The migrations of the animals are explained as follows

(p. 123): "Females rise to the surface with the setting of the sun, because they are positively phototropic to faint light and negatively geotropic; they descend into deep water with the rising of the sun, because they are negatively phototropic to strong light (their negative geotropism being overcome by their negative phototropism); the males follow the females in migration, because they are probably positively chemotropic toward the females." Parker notes (p. 122) that such an explanation need not apply to other plankton organisms, although their depth migrations may be made at the same time as those of *Labidocera*.

The important paper by Dice (1914) deals with the reactions of *Daphnia* and their bearing on the vertical movements. These careful and thorough experiments led Dice to conclude (p. 263) that "the diurnal movements of *Daphnia pulex* are caused chiefly by variations in geotaxis induced by changes in light intensity." Increase of intensity leads toward positive geotropism, decrease toward negative geotropism. Temperature also has an effect, since high temperatures tend to make the animals positively geotactic, while low temperatures have the opposite tendency. Dice gives (p. 262) the results of the experiments of others that may be taken to show that reversal of geotropism by changes in light and temperature occurs in other forms.

Another point of view, as regards the causes of the diurnal movement, is that of Ewald (1910, 1912). His laboratory observations on the behavior of Cladocera and of *Balanus* larvae led him to the conclusion that while light is an important factor it is not necessary to hold that there is continually recurring alternation between positive and negative heliotropism. The forms that Ewald used show what he called locomotion periods; these are, objectively, alternate ascents and descents. If the light is too intense, the animals cease their locomotion movements and therefore sink, and when they reach a region of lower intensity the swimming begins again. There are, therefore, successive periods of inhibition and stimulation (Ewald, 1912, p. 609) which have the effect of maintaining the animals in regions of about equal illumination all day long; they ascend in the evening and descend in the morning without giving positive and negative heliotropic reactions.

There is still another possible factor that may determine the diurnal movement. This is the effect of a physiological or metabolic rhythm. Such a factor has not received general consideration, but Menke (1911) makes much of it from his point of view. His experi-

mental work was done with the arthropod *Idothca*. He found that there is a periodical change in the chromatophores corresponding to day and night; the animals are darker by day than by night (p. 43). Menke formulates the proposition (p. 57) that variation in light intensity acts as a regulating mechanism. If external conditions are kept constant the chromatophores contract and expand corresponding to the usual periodicity of light and darkness. He was even able to reverse the rhythm, so that the old rhythm was set aside, by lighting the animals at night and keeping them in darkness by day. After nine days of this they were kept in constant darkness, and for about a week the pigment cells contracted by day and expanded at night. Menke cites other facts that show that there is a periodicity of metabolism: the animals are livelier at night and the heart beats twice as fast as by day (pp. 64-66), and, furthermore, agents such as temperature and acids affect the movements of the chromatophores.

Such facts are applied by Menke (pp. 79-80) to the vertical migrations of plankton animals. He notes that the heliotropism of such forms has been shown to be reversed by temperature changes, which in turn affect the chemical processes, and he says (p. 80): "In weiterer Verfolgung dieses Gedankenganges wird man zu den Schluss gedrängt, dass die primäre Ursache Vertikalwanderung mancher planktonischer Organismen in Stoffwechselländerungen liegt, und dass erst sekundär mit diesen chemischen Prozessen ein heliotropischer Stimmungswechsel verkettet ist." The periodical migrations and the periodicity of chromatophore movements are occurrences of essentially like nature, conditioned by periodicity in metabolism. The same agents that affect pigment cells alter the heliotropism and in corresponding ways. Raising the temperature causes negative heliotropism in many animals and it also brings about contraction of the pigment cells. Lowering the temperature causes expansion of chromatophores and reverses the heliotropism to positive (p. 81). An interesting suggestion is made (p. 82) as to the regulatory character of the migration. The osmotic pressure of the body fluids is altered as the metabolic changes take place, and the animals move into regions of corresponding pressure to compensate for this.

In brief, the conclusion reached by Menke, as given on page 88, is that the movements of the chromatophores and those shown in the diurnal migration are "autonomous," and that they parallel a periodicity in metabolism which is probably characteristic of all living matter. The fact that the metabolic rhythm follows the daily changes

in light intensity is explained on the ground that specific chemical processes are set up under the influence of light or darkness, and that these processes "in ihrer Art eben die Periode einer Erregung des Stoffwechsels resp. dessen Ruhe verursachen."

I believe that Menke's work has brought to light a factor that must be considered in the matter of the depth migrations. I have pointed out (Esterly, 1917*b*) that two species of *Acartia* exhibit a periodicity of behavior that is not connected at the time with recurring external stimuli; the same is true, though to a less extent, of *Calanus*. It remains to be determined by experiment how general this factor is.

The foregoing account of the literature has covered the papers that deal with depth migration more extensively from the experimental side. There are, in addition, some other references to this habit that may be mentioned. Harper (1907) showed that the insect larva, *Corethra*, is positively geotropic in strong light from above as well as in that from below, and that it is negatively geotropic in dim light. Harper suggests (p. 454) that the animals move down in the daytime because of positive geotropism in strong light, and that they ascend when the intensity decreases.

My own studies of the reactions of *Cyclops* (Esterly, 1907) were directed toward an understanding of diurnal movement. The females of *Cyclops albidus* were shown to be negatively geotropic in the dark and positive in the light, and it was suggested that phototropic responses are not even the main factors in causing the diurnal migrations. The effect of light on the geotropism, however, offers a possible explanation.

There are, of course, very many papers dealing with responses that might be applicable to the diurnal movement if the facts about natural distribution were known for those particular forms. But it does not seem desirable to include such accounts of behavior here. Similarly, the extended literature dealing with results derived from field investigations may be omitted since the purpose of this report is to give an account of a set of experiments on certain forms and of their possible bearing on the diurnal migrations of the same species.

In discussing the literature it would not be well to omit mention of the theories of Ostwald with regard to the diurnal movement. As is well known, the chief point in his theory is that the movement is largely due to alterations in the viscosity of the water, the viscosity in turn being dependent on the temperature. The migration of the animals is not so much due to their own activities as it is to the physical condition of the medium in which they live. Ostwald states,

however (for example, 1902, p. 627), that alterations of viscosity are not the only causes of vertical movements. Tropisms play a part but a subordinate one.

This is hardly the place to go more deeply into Ostwald's explanation, especially since my own experiments were along very different lines than those suggested by his work. It does not seem to me, however, that plankton organisms can be regarded as passive bodies that are carried up and down through physical agencies. Those forms that I have watched *swim* up when they ascend. Although they may drop down passively, this does not occur as a result of change of temperature so far as I can see; temperature has an effect but not in the way set forth by Ostwald.

In concluding the review of the literature, it seems necessary to note the somewhat unusual views expressed by Franz in a number of papers (1911, 1912, 1913). He holds that vertical migrations do not occur; that the reason for the larger number at the surface at night, as shown by collections, is that the animals can not see the nets then so as to avoid them. According to his view, the animals are really as abundant at the surface during the day as during the night but they escape the nets when it is light enough for them to see (1911, p. 10). I believe that the views Franz expresses are untenable so far as they relate to carefully conducted field investigations, but he has done well to point out that the results of laboratory experiment alone do not show what goes on in nature. For example (1913, p. 265) he states that in his opinion the observations concerning the phototaxis of *Balanus* larvae and similar forms do not offer any ground for assuming that plankton animals perform periodical vertical migrations. Phototactic phenomena in the laboratory give only a broken picture of behavior in the open. When he proceeds, however, to argue that because experimental results do not show what takes place in nature, the diurnal movement does not take place, he goes too far. He gives a good many reasons why laboratory phototaxis can not be identified with migration movements in the open and says (1912, p. 499): "Diese wohl einschneidende Kritik der Lehre der Phototaxis bei Tieren entzieht zugleich der Annahme von regelmässigen vertikalen Wanderungen der Planktontiere eine ihrer wichtigsten Stützen." The phototaxis (in the laboratory) is at most a "flight movement" (fluchtbe-*wegung*) which can only be of short duration in the open. It will be noted that Franz passes from the statement that a supposed habit can not be explained in a particular way to the argument that, for such a reason, the existence of the habit is questionable.

CONDITIONS OF LIFE IN THE SEA

It is advisable to point out something of what is known about the physical conditions of the sea in the region where the work of the Scripps Institution has been carried on, since the experiments here considered relate especially to the behavior of animals found under those conditions. The recent paper of McEwen (1916) summarizes the knowledge of the conditions in the San Diego region, and the statements that follow are based on that paper. The points that I desire especially to emphasize are the *variations* in temperature, salinity, and light intensity.

It is stated (McEwen, 1916, p. 267) that the annual range of surface temperatures is between 6° and 7°C, the upper and lower limits varying somewhat in different areas. The lower limit is from 14° to 15° and the upper from 20° to 22°; the coldest months are February, March, and April; the warmest season is the month of August. The water contains less salt in the spring than during the summer. The salinity varies from 33.50 ‰ in February to 33.75 ‰ in July or August in one area; in another section the minimum is 33.55 ‰ in January, the maximum 33.85 ‰ in July or August. The annual range of temperature "decreases from 6.2 C at the surface to 1.0 C at two hundred meters, while it can scarcely be detected below four hundred meters" (p. 268). Different months differ as to the monthly average of temperature and salinity, as do different days of the same month; and "each year is distinct from the others" (p. 269). Furthermore, there are significant differences in temperature at positions as little as sixty feet apart (p. 269). The bearing of such facts on the field study of plankton organisms is obvious and is noted by McEwen on page 270.

The diurnal variation in environmental elements is of more importance than the seasonal so far as the daily vertical migration is concerned. It is stated (p. 271) that the diurnal range in surface temperature in a region well offshore is about 1.0 C; the lower limit of the range occurs between 1 A.M. and 2 A.M., and the upper between 5 P.M. and 7 P.M. This slight diurnal variation decreases as the depth increases, and it does not appear at depths exceeding 25 meters.

The diurnal variation of salinity at the surface is given as about 0.05 ‰ (p. 271).

It will probably be granted without argument that daily variations in surface temperature and salinity are too small to have a marked

effect in causing diurnal migration. But temperature and salinity vary with the depth, as is well known, so that the migration of the animals carries them through a greater range of temperature and salinity. McEwen has given in plates 34 and 35 the relations of the mean annual temperature and salinity to depth.

The maximum of the mean annual temperature (pl. 34) is at the surface of course, and is 17° C. At 25 meters it is between 14° and 15° ; at 50 meters between 12° and 13° ; at 100 meters between 10° and 11° ; at 150 meters it is 10° C; while at 200 meters it is about 9.5° C. From that level it gradually decreases to 7.5° C at 400 meters. The temperature curve is much steeper between the surface and 100 meters than anywhere else.

The salinity increases with the depth (pl. 35), except that from the surface to about 25 meters there is a slight decrease. The mean annual salinity at the surface is 33.65 ‰; at 25 meters it is between 33.50 ‰ and 33.55 ‰; at 50 meters it is about 33.57 ‰; at 75 meters about 33.68 ‰; and at 100 meters it is 33.80 ‰. From that level to 200 meters the increase is at about the same rate for each 25 meters, the value of the salinity at the 200-meter level being 34.15 ‰. The increase is less rapid from there down, and at 400 meters the percentage is a little in excess of 34.39 ‰.

The light intensity at different depths has not been measured directly in the San Diego region, but McEwen has given approximations of the average distribution of radiant energy with respect to depth and time of day (pl. 38). The diagram is based on a formula that expresses the relation between intensity of radiation and depth and an "empirical one representing approximately the results of observations on the diurnal variation in the intensity of solar radiation on a horizontal surface at sea-level, between 20° N and 40° N" (p. 279). While McEwen's diagram gives theoretical results he states that the values agree well with photographic measurements made in the Atlantic.

According to the figures given in plate 38, if the intensity at a depth of one meter at noon is called 1000, it is relatively about 600 at nine meters, 300 at 20 meters, 200 at 25 meters, 100 at 35 meters, 50 at about 48 meters, 25 at 58 meters, 10 at a little over 70 meters, and 5 at 82 meters. It is doubtless understood that the light intensity at any depth increases until noon and decreases after noon, so that *time of day* is a rough expression of relative intensity.

EXPERIMENTAL RESULTS

REACTIONS OF THE COPEPODS *Acartia tonsa* AND *A. clausi*

These little copepods are usually abundant at the surface at night. *A. tonsa* is found throughout the year, while *A. clausi* occurs chiefly or only during the winter and spring. If it is desired to obtain animals during the day it is almost always necessary to do the collecting in deeper water than is found along the pier at La Jolla. We were successful, as a rule, at depths of 60 to 100 feet. There have been sunny days, however, when both species were abundant on the surface. Sometimes they were not obtained at the surface at night. The relations between these unusual modes of distribution at the surface and external conditions have not been worked out, but it is expected that something will be ascertained about such matters from a study of the large number of collections and the mass of data already on hand.

PHOTOTROPISM

Evidence has been given in another place (Esterly, 1917a) that *Acartia tonsa* if taken from the surface is always positive to horizontal light of any intensity if the temperature of the water is over 15° C. There is a tendency for the negative reaction to appear in colder water. Animals that have come from twenty or thirty meters are negative to light if tested soon, but if they are kept in the laboratory such specimens give only positive responses after a time. *A. clausi* reacts as does *tonsa* in most respects: it is positive to light at temperatures above 15° C, but shows a larger proportion of negative responses in colder water (Esterly, 1917a). Most surface specimens of *clausi* when first taken are negative to horizontal light if they are tested in water below 15° C, but after some hours most animals have become positive. Both species give negative responses to horizontal light if the animals are obtained below the surface. It seems important to note, first, that if cooling has any effect on the reactions of these animals to light, it causes negative phototropism; second, that the behavior is in some way related to or dependent on the habitat from which the experimental specimens come; third, something connected with retention in the laboratory modifies the character of the responses to light.

GEOTROPISM

It has been shown (Esterly, 1917*b*) that *Acartia tonsa* and *A. clausi* have a marked tendency to remain at or near the top of a column of water when in the diffuse light of a room. And specimens of both forms are to be found largely toward the bottom when kept in the dark during the day. But from 6 P.M. to 8 P.M. they both show a noteworthy increase in the upper levels even if they have been for hours continually in darkness except for occasional observations. This rhythm is less marked in *tonsa* at temperatures below 16° C, but it is clearly shown in *clausi* at those temperatures. In view of the tables published in the paper cited it is unnecessary to repeat the figures here.

Acartia tonsa is positively geotropic if the animals are obtained in deep water and tested within a short time; nothing could be more striking by contrast with the surface animals than the way in which the others swim down in diffuse light, or from the 15-watt lamp at the top of a tube, or in darkness. Some details of experiments may be useful at this point, although my material dealing with the geotropism of deep-water *Acartia* is not sufficient to warrant tabulation.

On November 1, 1917, a haul from about 100 feet was brought into the laboratory at 11:30 A.M. Fifteen animals (eleven females and four males) were tested one by one and all were negative to light from the window of the dark room. Ten of these animals (the four males and six of the females) were put, one by one, into a vertical tube 50 by 3 cm., beginning at 2:30 P.M. They all swam to the bottom in the light of the north windows in the main room and all were at or close to the bottom when observations were taken at 2:40, 3:00, 3:30, and 4:20 P.M. The next observation was at 6:20 P.M.; at that time there were three animals at the top while at 7:20 there were five at the top.

Another lot of animals was obtained from about 120 feet on December 1, 1917, and was brought into the laboratory at 10:15 A.M. Eight animals were tested in the light of the large window in the dark room, by releasing each individual at the center of a shallow dish and noting the direction of travel. I have records for seventeen such trips, and only two were positive. The two were made by an animal that also made one negative trip. At 11:15 A.M. the animals were put into a vertical tube (50 by 3 cm.), one by one. The tube was left standing in the dark room one meter from the window. The length of the tube was marked off into five equal divisions, and in giving the distribution of the animals the number in the top section is set down at the left, and

so on, to the number in the bottom section, at the right. At 11:20 A.M. the distribution was 1, 1, 0, 0, 6; at 12:45 P.M. and 12:55 P.M. it was 0, 0, 0, 0, 8. Then the tube was turned horizontal, so that what was the lower section became the one next the window. The animals all swam away from the light, and when they were in the section opposite the window, the tube was turned to the vertical position, at 1:33 P.M. This brought all the specimens into the top section. The distribution at intervals follows: 1:35 P.M., 1, 1, 1, 2, 3; 1:37 P.M., 1, 0, 1, 1, 5; 1:38 P.M., 0, 1, 0, 1, 6; 1:39 P.M., 0, 1, 0, 0, 7; 1:40 P.M., 0, 0, 1, 0, 7; 1:41 P.M., 0, 0, 0, 1, 7; 1:42 P.M., 0, 0, 0, 0, 8. At this point the room was made dark and the 15-watt lamp was placed ten centimeters from the bottom of the tube. None of the animals left the bottom in seven minutes of continuous observation. The tube was inverted then (so that all the animals were in the top section) and the 15-watt at the top of the tube was turned on at 1:56 P.M. At 2:00 P.M. the distribution was 2, 1, 1, 0, 4; at 2:03 P.M., 2, 0, 2, 1, 3; at 2:07 P.M., 1, 0, 1, 1, 5 at 2:10 P.M., 1, 0, 0, 0, 7. Here the light was cut off, and at the end of ten minutes in darkness the distribution was still 1, 0, 0, 0, 7. Evidently the animals did not ascend in darkness. Then at 2:21 P.M. the tube was inverted again, putting seven animals in the top and one in the bottom section and darkness was continued. At 2:25 P.M. the distribution was 0, 0, 2, 3, 3; at 2:28 P.M., 0, 0, 0, 0, 7; at 2:43 P.M., 0, 0, 0, 0, 7; at 3:00 P.M., 1, 0, 0, 1, 6; at 3:20 P.M., 2, 1, 2, 0, 3; at 3:45 P.M., 2, 0, 1, 0, 5; at 4:00 P.M., 2, 0, 0, 0, 6; at 5:25 P.M., 5, ?, ?, ?, 2; at 8:00 P.M., 6, ?, ?, ?, 1.

While these details are intended chiefly to show the positive geotropism of deep water animals, they incidentally reveal negative phototropism, and the onset of negative geotropism about 6:00 P.M. This latter point has been dealt with in another place (Esterly, 1917*b*, p. 396, table 3). There is no reason to doubt that the behavior shown by the animals from deep water is characteristic of the species, but the data might well be more extensive.

In contrast to the behavior of the deep-water specimens animals from the surface are negatively geotropic in the diffuse light of a room and positive in darkness. Since one could not depend upon getting surface animals except at night it was necessary to leave the experimental specimens in the laboratory for a few hours at least. A few preliminary tests indicated that individuals from the surface react at once as they do after six or eight hours, and I have assumed, therefore, that retention in the laboratory does not affect the behavior of animals

that were obtained at the surface during the night. Table 1 (p. 20) is a summary of the distribution of four different sets of animals (A-D) in a column of water. The tube containing the animals was marked off into five equal divisions and exposed to light for a short time and the distribution noted one or more times. Then it was covered so as to exclude all light, and after an interval the cover was removed and the distribution noted. The alternation of light and darkness was repeated several times, and all the records for a given set of animals are put together in the four lines of each half of the table.

The form of tabulation used in table 1 is found in others in this paper, and the following explanations of terms will apply to all tables of this sort. The "whole number of animals observed" is the sum of all records of distribution in all experiments. Each living animal was counted as many times as there were observations made: if there were five individuals in a tube and their distribution was recorded ten times, the "whole number observed" would be fifty. If it is necessary to count quickly it sometimes happens that all the individuals known to be in the container can not be located; in such cases the "whole number" does not equal the product of "number of individuals" by "number of observations." "Percentage distribution in the sections" is the proportion of the total number of animals observed *in each section*, to the whole number observed. "Number of observations" means the number of times the distribution was recorded. The "center of distribution" is the average position (as between the ends of the container) of the whole number of animals observed. It is obtained by multiplying the total number of animals recorded in each section by the number of the section, and dividing the sum of the products by the "whole number of animals observed." The centers are set down in units and tenths and the unit is considered as the middle point of the section having the corresponding roman numeral. If one center is compared with another the difference between them, if any, is the magnitude of the shift of the whole population. This useful method of expressing average position was first used, so far as I know, by Yerkes (1899).

The ascent is accomplished by means of swimming while in descending the animals drop passively, with the head uppermost and the anterior antennae spread to their full extent. The passive descent continues after the animals are lighted, following a short time in darkness, but in a few seconds they begin to swim up in characteristic jerky fashion.

TABLE 1

SUMMARY OF THE VERTICAL DISTRIBUTION OF DIFFERENT SETS OF *Acartia tonsa* FROM THE SURFACE FOR ALTERNATING PERIODS IN DIFFUSE LIGHT AND IN DARKNESS; ALL RECORDS FOR EACH CONDITION AND FOR EACH SET ARE COMBINED. TEMPERATURE ABOVE 17° C; SALINITY NOT OVER 34.0/00.

Set	Whole number of animals observed	Percentage distribution in the sections					Number of observations			Center of distribution
		top V	IV	III	II	bottom I	Total	per cent showing more animals in		
							upper %	lower %		
IN THE LIGHT										
A	68	53	33	8	4	2	4	100	0	4.1
B	221	55	21	13	4	7	11	100	0	4.1
C	184	51	24	7	2	16	9	100	0	3.9
D	99	57	18	9	8	8	5	100	0	4.1
IN THE DARK										
A	46	13	19	9	9	50	3	33	66	2.4
B	227	15	12	23	4	46	10	40	60	2.4
C	180	12	11	7	5	65	9	0	100	1.9
D	94	23	9	3	7	58	5	40	60	2.3

The evidence given in the table shows clearly the marked negative geotropism in diffuse light and the positive geotropism in darkness. It may be pointed out that most of the animals were in the upper two-fifths every time the distribution was recorded when the animals were in the light. The percentage distribution and the centers of distribution also show that there is a well-marked ascent in the light and descent in the dark.

In connection with the geotropism of surface animals it is suggestive to note that on the only occasion when I tested animals that were obtained at the surface during the day they were positively geotropic in diffuse light. The phototropism was not ascertained. These animals were obtained at 7 A.M., but were not used until 1 P.M. The majority swam to the bottom and that general distribution was not altered up to 4 P.M. when observation ended. Such observations are not of much value unless followed up, but they seem to fit in with those regarding the geotropism of subsurface animals. It almost seems that the physiological condition that leads to the descent was present in those particular surface animals even though they were obtained at the surface, and that the same state persisted for several hours. The difficulty about extending such observations is in obtaining animals from the surface during daylight.

I have made a few observations on the behavior of single animals from the surface when the 15-watt lamp is directly above or below the

tube. The animals are very hard to see under these conditions, but I believe that the small amount of data that I have to report really shows the behavior of the species. The account that follows is a summary of the results obtained with eight individuals. I recorded the position of each animal at intervals, noting whether the movement was up or down, and a record was kept also of the distance covered in each direction. It is possible, therefore, to state, first, what proportion of the animals moved up or down; second, the relative proportion of recorded changes of position in either direction; and third, the percentage borne by the distance traversed either up or down to the entire distance covered. There is more or less movement in both directions under either condition of lighting; but, as the figures will show, the direction of movement depends chiefly on the position of the light. *With the light at the top of the tube*, 80% of the records show movement toward the top and 20% show the reverse; and in a total of 380 centimeters traveled, 93% of the distance was toward the light (that is, negative geotropism) and 7% was away from the light (that is, positive geotropism). *With the light at the bottom of the tube*, 19% of the records show movement toward the top and 81% show the reverse; and in a total of 491 centimeters traveled, 91% of the distance was toward the light (that is, positive geotropism) and 9% was away from the light (that is, negative geotropism). It need not be said that the animals *swim* up, if they move in that direction at all, but they also swim down when the light is at the bottom. The downward movement is actively brought about instead of being a passive descent.

SUMMARY

The following facts about the behavior of *Acartia tonsa* and *A. clausi* seem to be adequately established: (1) Surface animals are positive to light of any intensity in water above 16° C. (2) At lower temperatures there is a tendency to negative phototropism, especially in *clausi* (Esterly, 1917a). (3) Animals from deep water show a strongly marked negative phototropism which disappears if they are kept in the laboratory (Esterly, 1917a). (4) Surface animals are negatively geotropic in diffuse light, and in darkness they are positive. (5) Specimens from deep water are positive in geotropism both in diffuse light and in darkness if tested soon after they are removed from the ocean. (6) Both species show a rhythm in geotropism which does not appear to be connected with external changes acting at the time (Esterly, 1917b).

POSSIBLE BEARING OF THE EXPERIMENTS ON DIURNAL MIGRATION

If only specimens from the surface were at hand the experiments would lead us to conclude that the animals are more abundant at the surface during the day and less abundant at night. While figures based on field data are not yet available it is certain that this sort of distribution is unusual. Specimens of *Acartia tonsa* from deep water react as one would expect of animals that forsake the surface during the day: they are positively geotropic in the light and negatively phototropic; but they are also positively geotropic in darkness until late afternoon or early evening, when there is an upward scattering. This latter fact, coupled with the rhythmic behavior of surface animals that are kept in darkness for hours, very strongly suggests that the ascent will take place in the absence of external stimuli. It is possible that the animals descend when the physiological state that led to the upward movement wears off. At any rate the experiments do not show why animals obtained at the surface should not be found there during the day; indeed the laboratory results show that the species "ought" to be at the surface during the day. The experiments furthermore suggest that during the colder months of the year more animals will be found at the surface than during the summer, since in colder water there is an increase in the number of animals in the upper parts of a column of water (Esterly, 1917*b*, p. 395, table 2).

REACTIONS OF THE COPEPOD *Calanus finmarchicus*

More attention was given to the reactions of this form than of any other because it was readily obtained and because it is the most abundant copepod in the field collections that have been studied. *Calanus* is from three to four millimeters in length and the body is somewhat opaque so that it is not as hard to see as some of the copepods. The animals are hardy and withstand laboratory conditions well, but for reasons already given I made it a general practice to use only fresh specimens. All the animals that were used in experiments were obtained at sixty to one hundred meters by lowering the net and allowing the boat to drift. None were obtained from the pier either by day or night. Several unsuccessful attempts were made to get animals from the surface at night by towing from a boat well offshore.

PHOTOTROPISM

When specimens are brought from such depths to the surface in a short time there is an abrupt change in environment of considerable extent. It is hard to say what effect this has on the responses observed in the laboratory. It is suggestive, however, that when the catch is first brought in and put into a dish before the window most of the animals are positive to the light. Individuals, also, when tested alone swim toward the light. In the stock dish the animals dart around continually; some even swim across the dish to the room side but immediately return to the side next the window. At any time, however, shortly after the animals are received there is a marked assemblage as near the window as possible. But this condition gradually disappears. More and more animals are found on the room side of the dish until all or nearly all are gathered there. It is not unusual to find one or two animals that are persistently positive, while others may swim from the negative to the positive side and back again. Such trips in either direction are straight across the dish; the animals do not wander about.

An example of the behavior of six animals in a dish 21 cm. in diameter will serve to illustrate what has just been said. They were brought to the surface from sixty meters at 9:15 A.M. and observations were begun at 9:40 A.M. The dish was marked off into five divisions parallel to the plane of the window and of equal width along the diameter perpendicular to the window. The number of animals in each division was noted at brief intervals, and the results are summarized in table 2 (p. 24).

Inspection of the table shows that there is a gradual increase in the number of animals in sections IV and V and a gradual decrease in numbers in I and II, while the center of distribution shifts from the window to the room side of the dish. The length of time varies a good deal within which the different sets of animals become predominantly negative. But when the animals are tested after they have been in the laboratory for some time they are very strongly negative to light.

If several animals are put into a dish which is turned end for end toward the window they swim from the end nearer the window to the one opposite, time after time, as the ends are reversed. In table 3 (p. 24) there are summarized the results of testing two different sets of animals in this way. Some records which enter into

the table were obtained on the fourth day after the animals were brought in, so that the table is not intended to show more than the strong negative reaction of the copepods. The animals were released in the center of the dish one by one, and in a few minutes the vessel was turned through 180° and the distribution noted at short intervals. The turning was repeated several times, and table 3 contains the summary of the results.

TABLE 2

THE CHANGING DISTRIBUTION OF SIX SPECIMENS OF *Calanus* IN A DISH OF WATER STANDING BEFORE THE WINDOW. THE ANIMALS WERE BROUGHT IN AT 9:15 A.M. AND WERE KEPT IN THE WATER THAT WAS IN THE COLLECTING JAR, AT TEMPERATURE OF ABOUT 16°C.

Time A.M.	Whole Number of animals observed	Percentage distribution in the sections					Number of observations per cent showing more animals in			Center of dis- tribution
		Negative V	IV	III	II	Positive I	Total	positive %	negative %	
9:40- 9:44	18	39	5	56	3	33	33	2.7
9:50- 9:55	18	11	33	17	39	3	33	33	2.2
9:57- 5:59	18	39	5	56	3	66	0	2.7
10:00-10:04	30	43	3	7	7	40	5	40	40	3.0
10:05-10:09	30	47	7	3	13	30	5	0	0	3.3
10:10-10:14	30	63	3	3	7	24	5	0	100	3.8
10:15-10:19	30	56	20	7	7	10	5	0	100	3.8
10:20-10:24	30	66	7	7	20	5	0	100	3.5
10:25-10:29	30	63	0	7	7	23	5	0	100	3.7
10:30-11:00	108	77	1	4	5	13	18	0	100	4.2
11:25 A.M.- 1:50 P.M.	30	93	0	0	3.5	3.5	5	0	100	4.8

TABLE 3

DISTRIBUTION OF SPECIMENS OF *Calanus* IN A DISH 20 BY 8 CM., DIVIDED INTO FIVE EQUAL SECTIONS. THE DISH WAS TURNED AT INTERVALS SO THAT THE ENDS CAME ALTERNATELY NEXT THE WINDOW. TEMPERATURES ABOVE 17° C; SALINITY NOT OVER 34 ‰.

Whole Number of animals observed	Percentage distribution in the sections					Number of observations per cent showing more animals in			Center of dis- tribution
	Negative V	IV	III	II	Positive I	Total	positive %	negative %	
946	59	9	10	5	17	138	5	95	4.

Since the intervals between observations were short some animals were recorded as in sections nearer the window when, as a matter of fact, they were really moving away from the light. Some, indeed, did not move out of a given end section at all, whether it was next the window or not. But on the whole the table shows the strong negative phototropism of *Calanus*.

Much of the testing was done with individual specimens. I followed the plan of making frequent records of the distance and direction traveled by each animal under observation. Each time that the position was noted, the distance and direction of any movement and the time elapsed since the last record was set down. While the rate of movement is relatively unimportant for our purposes, distance and direction indicate precisely the kind of response. Response means *position* at a given moment as compared with that at the previous time of observation. If it is decided to note the position every fifteen seconds, for example, each record that shows change of position surely denotes a response as compared with a preceding record. Of course an animal may be watched until it has traveled as far as the limits of its confinement will allow and that may be recorded as one reaction. But noting often will indicate whether an animal moves back and forth or not; whether its progress is continually away from the light, for example. Some records of position that do *not* show change of position on the part of the animals indicate, nevertheless, that there is a response and the nature of it. My plan has been to regard each record of position set down in my notes as showing the kind of reaction. The terms "observation," "response," "reaction" and "record of position" are practically the same.

In explanation of table 4 and others like it that follow, it needs to be said that the columns headed "records of position" show the number of times that the location was set down. If the specimen moved during the interval between two records (up or down, or toward the light, or away from it) the change of position is in the nature of a reaction. But it often happens that there has been no change in position from one observation to the next; this is also noted under "records of position." It may at once be asked, What is the use of entering a record that does not show movement on the part of the animals? One reason is that it seems desirable to present all the data in order to show as fully as possible just what the behavior is. A second reason is that "no movement" or "no change of position" may mean just as much in the way of reaction as actual locomotion. For instance, when an animal ascends in a vertical tube as far as it can and constantly maintains that position, successive records would show that there has been no upward or downward movement. But the fact that the specimen remains at the top is as good evidence that it is negatively geotropic as the fact that it swims to the top in the first place. Similarly, an individual may descend to the bottom

and remain there. The "records of position" obtained during the descent are entered as those that show movement. But if after the bottom is reached the animal remains there it is good additional evidence that it is positively geotropic. It is possible, of course, that an animal which remains at the bottom may be too fatigued to ascend although it is really negatively geotropic. I believe, however, that sufficient precautions were taken in these experiments to guard against such occurrences. The plus and minus signs at the top of the "no movement" columns refer to the *direction of the last recorded movement*. If the animal is in a vertical tube the plus sign in the "no movement" column means that after a descent of greater or less extent there had been no movement upward at the time the next record was put down. Likewise, the minus sign indicates that following a movement upward there was no descent. There is nothing in the summary in the tables to show at what level the animals remained stationary; but, as a matter of fact, most records that show no vertical movement are for animals on the bottom of the container or at the top of the column of water. Some records of position that show no movement were obtained because of what appears to be a thigmotactic reaction against the walls of the aquarium; at such times the animal is stationary. But these records are not numerous enough to change the general results.

As regards the "per cent of animals moving" (table 4, for example) it doubtless will be perplexing to the reader to note that the sum of the two percentages under the heading may exceed 100. In explanation it may be said if an animal moved at all toward the negative end that fact was noted, and if there was any movement toward the positive end that also was set down. It does not matter how many *times* or *how far* an individual is recorded as moving in one direction or the other; if the animal changed position once or a hundred times the tally would show 1 in either case, so far as "per cent of animals moving" is concerned. For example, it is possible that one individual in a set of five may be recorded as having changed position once toward the positive end and twenty times toward the negative end; but that particular specimen would count as one animal in the negative column and one in the positive column. The other four may all have moved toward the negative end only, and in the summary of the behavior of the set the figures would be: positive 20%, negative 100%. This example is not an actual one, but it ought to show why it is that such results appear as those in the fifth and sixth columns of table 4. The same explanation holds for other tables of the form of table 4.

It is worth noting that in the tables dealing with the summary of individual performances there are three items that indicate the kind of response and the relative degree to which it is manifested: (1) The relative number (expressed as percentages of the whole number of animals) of individuals that move in the positive and negative direction. These numbers may be about equal, but either or both of the following items will show more adequately the actual extent of positiveness or negativeness. (2) The proportion between the records that show positive and those that show negative changes in position. For instance, if an individual is recorded as moving toward the bottom three times out of four and toward the top once it is good evidence that it is a positively geotropic animal. (3) The distance traveled in either direction is a very satisfactory index of the degree to which one kind of reaction exceeds the other. If the figures giving the number of animals moving and those giving number of records should be about the same in the positive and negative columns, while the figures that show distance covered are very unequal (as between positive and negative), I should be inclined to hold that the latter reveal the kind of response given under the particular conditions of the experiment.

The differences between the figures in the positive and negative columns for each of the three items should always be compared when considering the tabulated results. When the differences point in the same direction in all three cases, the evidence is a good deal stronger than if one difference indicates one thing and a second shows something else.

It is desirable to note, in further explanation of the use of terms, that "number of experiments" means the total number of times it was attempted to ascertain the behavior of individuals under a given set of conditions. The average number of trials given each animal appears from the ratio between number of experiments and number of animals tested.

The table that follows (p. 29) contains the summary of the records of individuals. The behavior under all intensities of light is included in the summary, since the phototropism does not change with the intensity as it does with the temperature. The extreme ranges of intensity employed (as compared with the 15-watt lamp) were from 0.025 to 15,000 or more, with such intermediate intensities as 8, 196, 3800.

The figures in the first line show that *Calanus* is strongly negative to light. The range in temperature is that of the room at different seasons of the year, and the salinity is that of sea water as it came

from the pipes or from the ocean directly. It is possible to account for some of the positive responses. When an animal is enclosed in a tube that is horizontal with one end toward a light it may swim to the negative end and from there make short dashes toward the light and back again to the end. The method of recording that I used makes it possible to take note of such positive movements. I do not believe that such behavior is due to positive phototropism. It is rather due to the incessant activity, and after an animal has reached the end of a tube it can only swim toward the light. Some cases, however, are due to what seems to be a positive response, as such. If the animals are tested soon enough after they come into the laboratory they are, as already stated, usually positive, and some records of such animals enter into the table. It has occasionally happened that an animal after having gone from the light several times suddenly reversed and traveled toward the light a few times and then returned to the former kind of response. These records also are part of the first line in table 4. But in general laboratory animals are strongly negative to all intensities at ordinary temperatures and concentrations of the water.

If the results in the third line are compared with those appearing in the first it will be seen that the differences in the percentages are in favor of the positive instead of the negative side. That is, the animals become positively phototropic in water of low temperature. It was outside my purpose to try to ascertain the point at which there appears a well defined positive reaction when the temperature is gradually decreasing, or a negative reaction when the temperature increases slowly. But table 5 (p. 30) will give the results of observing the behavior of six animals in a dish while the temperature fell gradually and then slowly rose. As usual, the dish was divided into five sections, section I next to the light and section V opposite. In water as cold as 6° C the animals swim around as freely and actively as at 15° C.

The table does not need further explanation. It shows the gradual change from negative to positive as the temperature slowly decreases and the reverse when it increases from the low points. Individuals are continually swimming back and forth, and this accounts for most of the animals noted in sections other than the one next the light, or it is turned through 180° while the position of the light is not changed. Or they will swim back and forth across the vessel when a 15-watt lamp on one side is alternated with a 100-watt on the other, the movement being toward the illuminated side of the aquarium.

THE PHOTOTROPISM OF *Calanus* UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND SALINITY.
SUMMARY OF INDIVIDUAL RECORDS

Temperature	Salinity ‰/‰	Number of experi- ments	Number of animals tested	Per cent of animals moving		Total	Records of position				Total (cms.)	Distance	
				+	-		per cent of total showing		+	-		+	-
14°-20°C	33-34	386	65	52	88	1,167	movement		no movement		7,006	Per cent	
							20	56	10	14		21	79
14°-20°C	37-40	116	50	62	90	543	32	50	9	9	2,979	25	75
6°-9°C	33-34	83	17	74	24	100	82	18	0	0	728	80	20
6°-9°C	37-40	37	16	75	81	255	40	17	33	10	548	60	40

On the whole, the fact that the animals become positive in water of low temperature is clear and unmistakable.

If the salinity is increased while the temperature remains that of the room (second line of table 4) there is no significant change in the behavior as compared with that at ordinary temperature and salinity (line 1). The animals are clearly negative to light under both conditions, and it does not appear that there is positive phototropism to an increased extent when the concentration of the water increases. As a matter of fact it is shown in line 4 that at high salinities and low

TABLE 5
THE CHANGING DISTRIBUTION OF SIX SPECIMENS OF *Calanus* IN A DISH DIVIDED INTO FIVE SECTIONS, AS THE TEMPERATURE DECREASED SLOWLY AND THEN INCREASED SALINITY 33 TO 34 ‰.

Temperature (°C)	Time p.m.	Number of animals in the sections				positive I	Number of observa- tions	Center of dis- tribution
		negative V	IV	III	II			
16	1:50	6	0	0	0	0	1	5
13%	2:10	1	0	0	2	3	1	2
11%	2:20	2	1	0	0	3	1	2.8
10	2:25	0	1	1	0	4	1	1.8
8½	2:35	1	0	0	1	4	4	1.8
6-7	3:20-4:00	4	0	1	0	19	4	1.7
5½-8%	4:14-5:21	10	2	12	4	138	27	1.4
8%	5:35-5:37	0	0	0	0	12	2	1
9%-9½	5:40-5:43	1	1	0	1	15	3	1.4
10½-10%	5:45-5:47	2	2	1	0	13	3	1.9
10½-11%	5:50-5:55	10	1	1	0	13	5	2.3
13½-14	6:30-6:34	19	0	2	0	9	5	3.7
14-14%	6:35-6:40	18	0	0	1	17	6	3.7

NOTE.—From 1:50 to 2:35 the animals were exposed to the light from three north windows; for the rest of the time to the light from the 100-watt lamp without the "daylite" filter through an opening 10 cm. square, the center of the coil being 14 cm. from the wall of the aquarium; this intensity is about 200 times that of the 15-watt lamp.

temperatures there is less positive and more negative phototropism than when the temperature is low and the salinity normal. It is difficult to say, however, how much significance there is in the results given in the last line of table 4, but it would seem that the high salinity counteracts to some extent the effect of the low temperatures. Loeb (1893, p. 98) states that increase of concentration increases the positivity of copepods and also of *Polygordius larvae* (p. 94). Ewald obtained similar results with *Balanus larvae* (1912, p. 606). It is certain that *Calanus* does not become increasingly positive in water of high salinity.

Conclusions. *Calanus* is negative to light of all intensities except when the water is cooled. The phototropism becomes noticeably positive at about 13° C, and is pronouncedly so at temperatures below 10° C. There is no marked change in reaction when the salinity is increased if the temperature is not lowered; the phototropism is negative. But there is some indication that the positivity decreases, relatively, in water of low temperature and high salinity.

GEOTROPISM

In sea water of ordinary temperature and salinity *Calanus* is always positively geotropic except when lighted from below. The animals descend to the bottom and remain there except for what may be called accidental dashes upward. If put into a column of water the specimens do not swim down, under ordinary conditions, but they drop down passively. The descent is sometimes interrupted by swimming to the side of the tube where the animal may cling for a short time or bump against the glass, but it soon begins to drop again. An animal may even make a short ascent, then drop, then ascend, and so on, each upward movement being of less extent than one in the opposite direction so that the individual gets lower and lower in the tube. When dropping passively the long axis of the body is not quite perpendicular but the anterior end of the animal is always uppermost. The axis is inclined toward the dorsal side about 25° from the perpendicular, and the anterior antennae are always extended at right angles to the body. Under certain conditions *Calanus* will swim down, head foremost, with the axis of the body at an angle of 45° to 90°. The dorsal surface is uppermost in swimming down unless the body is perpendicular.

It is necessary to state that in the experiments on geotropism a temperature or salinity gradient was used as well as uniform conditions of both these factors. The lower two-fifths of a tube containing ordinary sea water was set into ice water, or this was done when the water in the tube was of high salinity, or the entire container was chilled when it held water of normal or of high salinity. And in some cases the tube held water of high salinity in the lower part and normal sea water in the upper part, with the lower two-fifths of the tube surrounded by ice. There is no evidence from my results that the behavior in a gradient differs from that shown when the water in the tube is of the same temperature or salinity in all parts. In tabulating

the results, therefore, no distinction is made between gradients and uniform conditions.

In diffuse light (table 6). When the lighting conditions are those of a room, or when the light comes from one side only, as in the dark room with the window open or with a lamp at one side, the geotropism of *Calanus* is almost uniformly positive. The figures given in table 6 offer abundant evidence of this. So far as the experiments go, the geotropism does not change with alterations in temperature or salinity (Cf. lines 1 and 3, and 1 and 2). The animals descend and remain at the bottom under all conditions. If the water in the tube offers a salinity and temperature gradient the animals drop into and down through the cold and highly concentrated strata without any change in behavior and remain at the bottom.

The general results in table 6 are so consistent that further comment is unnecessary.

In darkness (table 7). The prevailing positive geotropism in darkness is shown by the figures in the table. The animals descend to the bottom and remain there. The behavior in darkness was ascertained by momentarily lighting the aquarium to discover whether there had been a change in the position of the animal. When the light is flashed on, the animals are passive for a moment, then dash wildly about for two or three seconds and then suddenly become quiet; if they happen to have darted upwards they drop to the bottom as can be seen if the light remains on. There is no doubt, however, that if started at the top the animals descend and when once at the bottom they do not move up. If the time of illumination for the observation after a period in darkness is short enough the animal continues to drop without any other movement, and the presumption is that the same mode of progress is in operation in the dark. There are no significant changes of behavior when the temperature is lowered or when the salinity is increased. It may be well to explain, however, that in the third line of the table the large percentage of "no movement" records in the positive column is due to the fact that the time was prolonged after the bottom was reached in order to see if the animals would ascend in the cooled water. The result is that more records than usual were entered in my notes while the animals were on the bottom.

In vertical light from above (table 8, p. 35). All the experiments were performed with the 15-watt lamp directly above and ten or twenty centimeters from the surface of the water in a tall museum jar,

TABLE 6

THE GEOTROPISM OF *Calanus* IN DIFFUSE LIGHT UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND SALINITY.

SUMMARY OF INDIVIDUAL RECORDS. TUBE 50 CM. LONG, 3 CM. WIDE.

Temperature	Salinity ‰	Number of experi- ments	Number of animals tested	Per cent of animals moving		Total	per cent of total showing				Total (cms.)	Distance	
				+	-		movement	no movement	+	-		+	-
14°-20°C	33-34	201	32	72	12	580	70	2	26	2	6,498	99+	.06
14°-20°C	37-40	34	20	100	0	192	85	0	15	0	1,131	100	0
8°-11°C	33-34	5	5	60	0	36	47	0	53	0	135	100	0
8°-11°C	37-40	0	0

TABLE 7

THE GEOTROPISM OF *Calanus* IN DARKNESS UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND SALINITY.

SUMMARY OF INDIVIDUAL RECORDS. TUBE 50 CM. LONG, 3 CM. WIDE.

Temperature	Salinity ‰	Number of experi- ments	Number of animals tested	Per cent of animals moving		Total	per cent of total showing				Total (cms.)	Distance	
				+	-		movement	no movement	+	-		+	-
14°-20°C	33-34	109	42	95	7	385	78	4	17	1	4,302	98	2
14°-20°C	37-40	43	27	100	0	153	92	0	8	0	1,680	100	0
8°-11°C	33-34	21	15	66	20	103	42	6	41	11	470	93	7
8°-11°C	37-40	18	9	60	12	74	89	4	4	3	557	99	1

or more often a tube 50 by 3 centimeters. The animals are very strongly positive at room temperatures (lines 1 and 2), and this behavior is not altered by changing the salinity. In a good many instances the specimens swim down for at least a portion of the distance and swimming often alternates with dropping.

A comparison of the figures indicating movement in the first two lines with the last two shows that at the lower temperatures there is a considerable decrease in the percentages in the positive column and an increase in the negative column. In other words, the animals move toward the light more often when the temperature is low even if it involves overcoming gravitation. Such a change in behavior is not shown in tables 6 and 7, so it is to be presumed that the upward movement shown in table 8 is a reaction to light rather than to gravity. That is, the animals become positive to a vertical light as well as to a horizontal one, providing the water is of a sufficiently low temperature. It is interesting to note that when the lower two-fifths of the column is in ice water in some cases the animals do not ascend farther than the level of the cold water on the outside of the glass. On the other hand, some individuals, under similar conditions, will swim upward through the remaining three-fifths of the column (although that part is at room temperature) and will remain at the top for a short time. But even when the entire container is immersed in ice water the animals do not remain long at the upper levels, but gradually get farther and farther away from the surface. A number of short ascents may be made, but after each ascent the animal drops back a greater distance than it swims upward, so that finally it reaches the bottom.

It is evident, on the whole, that under the lighting conditions given in table 8 there is a certain amount of reversal of geotropism from positive to negative at the low temperatures. When the water is cooled and is also of high salinity (line 4), the behavior is substantially the same as in cooled water of the usual degree of salinity (line 3).

In vertical light from below (table 9). This experimental setting does not apply to natural conditions but it is helpful for interpreting behavior. In these tests the 15-watt lamp was used, and it was always about fifteen centimeters from the center of the bulb to the bottom of the aquarium. The containers were either museum cylinders, 35 by 6 centimeters, or tubes, 50 by 3 centimeters.

As is shown in the first line of table 9 there is more movement up than down at the usual temperatures and salinity. This is especially evident if line 1 is compared with the first lines of tables 6, 7

TABLE 8
THE CHROMISM OF *Calanus* IN VERTICAL LIGHT FROM ABOVE AND UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND SALINITY.
SUMMARY OF INDIVIDUAL RECORDS

Temperature °C	Salinity ‰	Number of experi- ments	Number of animals tested	Percent of animals moving		Records of position				Distance		
				+	-	per cent of total showing movement		no movement		Total (cms.)	Percent	
14°-20°C	33-34	81	40	97	1	88	11	1	0	3,689	99	1
14°-20°C	37-40	38	27	100	4	87	3	10	0	1,552	99	1
8°-11°C	33-34	22	16	56	75	22	22	47	9	626	53	47
8°-11°C	37-40	26	16	69	25	17	15	66	2	452	61	39

TABLE 9
THE CHROMISM OF *Calanus* IN VERTICAL LIGHT FROM BELOW AND UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND SALINITY.
SUMMARY OF INDIVIDUAL RECORDS

Temperature °C	Salinity ‰	Number of experi- ments	Number of animals tested	Percent of animals moving		Records of position				Distance		
				+	-	per cent of total showing movement		no movement		Total (cms.)	Percent	
14°-20°C	33-34	120	49	70	61	19	27	40	14	4,574	44	56
14°-20°C	37-40	48	27	81	81	25	47	22	4	2,022	14	86
8°-11°C	33-34	26	15	66	33	17	13	63	7	855	57	43
8°-11°C	37-40	30	16	81	31	27	14	59	0	654	74	26

and 8. When lighted from beneath many animals swim rapidly away from the light, but there is a good deal of difference in individuals. Some, apparently those that have been too long in the laboratory, do not leave the bottom at all, or, if they do ascend, move only a few centimeters. But animals that have been freshly brought in are very consistent in their behavior; they all leave the bottom and remain at or near the top for hours.

In order to show how pronounced the negative geotropism is when the light enters the tube from below an experiment may be cited in which six animals were put into a jar 35 by 6 centimeters (marked off into five sections) an hour after they were brought to the surface in the net. The light at the bottom was turned on at 10:44 A.M. and at 10:47 all were in the top section. From that time until 4:05 P.M., 98 observations were made; not an individual was observed in the bottom section; two were seen in the second from the bottom, six in the middle section, twenty in the second from the top, while 560 (about 94 per cent) were recorded in the upper section. Some animals are always dropping down from the top; they descend in that way for a longer or shorter distance and then swim back to the top. I have not looked closely into the reason for the persistence with which some animals, as shown in table 9, stay at the bottom, but it appears to be connected with some effect due to laboratory conditions.

When the temperature at the bottom of the column of water is low, there is an increase in positive geotropism; the animals stay more persistently at the bottom. This is probably a positive response to the light, but the whole effect is that of positive geotropism. If the whole column of water is chilled it can be noted that there is a tendency for animals that are started at the top to swim down. If, however, the upper part of the water in the jar is at room temperature while the lower portion is surrounded by ice the animals descend more slowly or even keep ascending time after time, but sooner or later they reach the cold water near the bottom. Then the tendency to ascend largely disappears. The most striking feature of the behavior when the aquarium is lighted from below and the lower part of it is in ice water is the failure of the animals to leave the bottom; but it is also evident that downward movement is increased and upward decreased when the animals encounter colder water. When the salinity is increased (line 2) while the temperature is that of the room, the negative geotropism is increased. But the positive geotropism is increased if the salinity is raised and the temperature lowered (line 4),

so that the behavior in cooled water of high salinity is about the same as in water of low temperature and normal salinity.

Physiological rhythm in geotropism. Will *Calanus* ascend in a column of water if there is no apparent change in external conditions that can direct the movement? It has been shown that the animals descend in diffuse light as well as in darkness under all conditions of temperature and salinity in the laboratory. They do not ascend during the day from 8 A.M. to 4 or 5 P.M. under experimental conditions except when driven up by light beneath or except for the continual and incidental movement already described. But I have found that under certain circumstances there is more or less of a general upward movement even when directive stimuli are absent.

On one occasion fifteen animals that had just come in were put into a cylinder 35 by 6 centimeters marked off into five equal divisions. The lower two-fifths of the cylinder were immersed in ice water and kept at 8°–10° C throughout the experiment, while the water in the remainder of the cylinder was from 16°–17° C. During the day the experiment was carried on in the dark room in daylight of an intensity amounting to about 1.2 as compared with the 15-watt Mazda. When night came on observations were taken in artificial light that was used for as few seconds as possible and then extinguished, the animals being in darkness for the rest of the time. The upper half of table 10 shows how the animals were distributed during the day and the early part of the night.

TABLE 10

THE DISTRIBUTION OF *Calanus* IN A COLUMN OF WATER, THE LOWER PART OF WHICH WAS 5°–10° C THE TOP 16°–18° C. TWO DIFFERENT EXPERIMENTS IN THE TWO HALVES OF THE TABLE. SALINITY 33 TO 34 ‰.

Time	Whole number of animals observed	Number of observations	Percentage distribution in the sections					Center of distribution
			top V	IV	III	II	bottom I	
1:00–7:00 P.M.	195	13	0	0	0	0	100	1.0
7:20–10:00 P.M.	195	13	10	3	4	11	72	1.5
10:30 A.M.–5:20 P.M.	610	61	0	0.1	2	7	91–	1.1
6:45 P.M.–10:30 P.M.	90	9	21	9	16	21	33	2.6

A second experiment of this kind was conducted with fresh material. Ten animals were put into a cylinder 35 by 6 cm. and the lower two-fifths of it was surrounded by ice; the temperature in those sections did not rise above 8½° C and it was as low as 5° C, while the temperature in the three upper sections was from 17°–18° C. The

distribution of the animals is summarized in the lower half of table 10. During the day they were in very dim diffuse light in the dark room; after dark the observations were made in the light of the 15-watt lamp. During this second experiment another cylinder was set up, identical with the other one and containing control animals from the same collection. But the temperature was about 18° C throughout the length of the control cylinder; otherwise the conditions were the same in both. The distribution in the control may be dismissed by saying that from 10:30 A.M. to 8:50 P.M. fifty-five observations were recorded. On two occasions one animal was noted in the second section from the bottom, but all the others were in the lower section throughout the experiment.

The results set forth in table 10 are more striking in the case of the second experiment, especially when the behavior of the control animals is considered. I believe that these experiments, in spite of the somewhat meager results, indicate the existence of a factor that must be taken account of in connection with diurnal migration. The fact that the upward movement in the laboratory does not take place except when the animals have been in cold water is significant in view of the temperature distribution in the sea. Table 10 shows that but a small part of the animals moved upward, but I believe that this can be accounted for in part at least. When the light is turned for observation after nightfall, the animals do not move at once; the quiescent period is long enough for one to note the distribution accurately. But even when the observation is made quickly there is more or less dashing around. Some dart down and others up, so that the distribution is upset every time an observation is taken. These unavoidable complications probably make the upward movement appear less extensive than it really is.

There is evidence, therefore, of a physiological rhythm in *Calanus* as in *Acartia*. The animals ascend at a particular time of the twenty-four hours but not during the period of daylight.

SUMMARY

(1) The phototropism of *Calanus* is predominantly negative at ordinary temperatures, but it becomes markedly positive in water of 10° C and less. The reactions to light do not change when the salinity is increased. (2) The geotropism is very strongly positive in diffuse light and in darkness, and the behavior does not change with changes in temperature and salinity. (3) When the light falls vertically from

above the vertical movements of the animals are practically all downward except in water of low temperatures. There is then a comparatively marked tendency to ascend. There is no marked change in behavior with change in salinity. (4) When the animals are lighted from below there is marked movement upward at ordinary temperatures, and this increases in water of high salinity. The amount of movement away from the bottom decreases at low temperatures, especially as regards the distance covered. The animals remain at the bottom more persistently when the water in the lower part of the column is chilled than when it is at room temperature. (5) Animals in weak, diffuse daylight do not show any tendency to ascend. But after nightfall a noticeable upward migration begins to appear in a column of water whose lower portion has been cooled to 8° C or 9° C, although no change that might affect the direction of movement has taken place in the surroundings. Some of the animals swim into water eight or nine degrees warmer than that in which they started. This behavior is not shown in a cylinder where the temperature throughout is 16°–18° C.

POSSIBLE BEARING OF THE EXPERIMENTS ON DIURNAL MIGRATION

The general facts of the distribution of *Calanus* in the ocean are well established by our field work. The animals are less abundant at the surface by day and more abundant in deeper water; at night they are less abundant in deeper water and more abundant at the surface. It is assumed that upward migration leads to the increase in numbers at the surface, and that downward migration leads to the greater numbers in deeper water. How can the results of experiments be applied to explain this general condition? Since the copepods are strongly negative to light at temperatures of 14°–20° C, that fact would explain their absence from the surface of the sea during the day, assuming that the reactions to vertical and horizontal light is the same. Furthermore, since the phototropism becomes positive at lower temperatures we might expect that the animals would begin to move toward the surface of the sea if, in the course of their descent, they reach water of a sufficiently low temperature while the surface is still lighted. There is experimental evidence showing that there is movement toward a light at the top of a column of water when its lower strata are cold enough. But the laboratory results indicate that there is practically no upward movement in darkness or in diffuse light if the observations are made during the hours of daylight. In view

of these various results, it appears that the upward migration requires water of relatively low temperature and light at the surface.

But, according to the published results of the field work (Esterly, 1912, pp. 282-285), the accumulation at the surface is greatest from 8 P.M. to 12 P.M. It is reasonable to suppose that some of the upward journey is accomplished in very dim light or in darkness, and it is difficult to account for such behavior on an experimental basis. If we grant that the organisms in descending reach water of a temperature so low that they *begin* to ascend, I do not see how the continuation of the ascent is to be accounted for after the animals reach the warmer water at higher levels. The initial stages of the upward migration may take place in the colder water of the deeper strata, but some other factor is apparently necessary in order to account for the completion of the journey to the surface. This factor, as shown by the experiments, may be the physiological rhythm. It is possible that the ascent may be accomplished in the absence of *directive* stimuli.

If the descent takes place as day begins to dawn it is readily accounted for, on the basis of these experiments, by negative phototropism at the temperatures that prevail at the surface and by positive geotropism in light from above. Our field data show, however, that these copepods forsake the surface several hours before dawn (Esterly, 1912, p. 284), though our observations are admittedly inconclusive. If more complete information should establish this point the positive geotropism in darkness might explain the descent and there would be no need to attribute anything to a response to light. When the "physiological state" that prevails during the time that the animals show negative geotropism wears off or is changed the descent will begin.

It is certain that under natural conditions large numbers of these animals arrive at the surface in the dark and continue to be obtained there during several hours of darkness. Their ascent begins in water of lower temperature than that at the surface, and the water becomes continually warmer at higher levels until temperatures are encountered in which there should be negative phototropism, as shown by the laboratory results. The problem is to explain the ascending into warm water in darkness. I believe that the facts brought out in connection with the physiological or metabolic rhythm offer the best basis for the desired explanation. The cause of the rhythm is, in turn, a separate question.

An explanation of the diurnal migration of *Calanus* based on geo-

tropic and phototropic reactions is beset by too many difficulties to be readily acceptable in view of the results of field work. The matter of the rhythm affords a simpler way of accounting for the habit, but it ought to be supported by more evidence than that brought forward here.

In discussing the results of field work (Esterly, 1911, p. 148, 1912, p. 292) it was stated, as a matter of opinion, that light appears to be the chief factor in the diurnal movement. There was no evident correlation to be found between abundance of organisms and temperature and salinity (Esterly, 1912, pp. 292, 294). It must be said that on the basis of the laboratory results the effects of variation in intensity of light (apart from direction) are negligible so far as vertical movements are concerned. There is some change in behavior when the temperature is lowered and the source of light is at the top or at the bottom of the column of water. The reactions do not change significantly when the salinity is increased. It is evident, therefore, that the causal interpretations based on field results do not conform to the laboratory results. Likewise, it is difficult to arrive at an understanding of what *Calanus* does in nature if we use the results of these experiments as a basis for judgment.

It is not improbable that a reëxamination of the field data, which are now more extensive than when the papers referred to were written, will show correlations between abundance of animals and external conditions that come into line with the results of experiment. It is also not improbable that continued experimentation will show that certain of the responses are due to the artificial conditions of the laboratory, and will reveal others that conform to the facts shown by the field data.

REACTIONS OF THE COPEPOD *Eucalanus elongatus*

This is an unusually large copepod, the length often being from six to eight millimeters. The body is of glass-like transparency, but in many animals there is a reddish or yellowish oil drop outside the digestive tube in the posterior part of the cephalothorax. The animals of this species are unique, so far as my experience goes, in that they do not have the incessant jerky movement so characteristic of copepods. A specimen of *Eucalanus* rests quietly for several minutes at a time, then suddenly darts away at such speed that it can hardly be followed with the eye. While resting the long anterior antennae are extended at right angles to the body, but when the animal swims they are folded

back against the body. There is another sort of movement, however, that is so slow it is barely perceptible. It is brought about by very gentle motion of the feet, some of the head appendages, and the abdomen. If the word "wiggle" may be used it serves to describe the action of the appendages. One is aware that there is a slow change of position, but it gives the impression of "creeping" or "drifting" as I have called it for myself. There are times, also, when none of the appendages are in motion, and then the body assumes almost any posture in the water; the head may be down or up, or the body tilted toward one side, so that a line passed through the extended antennae is neither horizontal nor vertical. It has seemed to me that the drop of oil in the body has something to do with the position taken; there is a tendency for the posterior end to gradually rise higher than the anterior end, and the oil drop is always nearer the posterior end. I believe that the "creeping" movement is due to the action of the appendages; I have not been able to determine that there is any change of position unless the appendages are moved. When an animal is in a vertical tube the "creeping" produces either ascent or descent.

If a specimen is in a shallow dish it can be gently moved about with a capillary rod so that the axis of the body takes any desired direction as regards the light. Under such circumstances one can easily see how the creeping will alter the position of the animal. The appearance is as if the body were drifting in a very gentle current of air over the surface. The animal moves in almost any direction, even sideways, or it swings around through part of a circle.

It does not seem to me that the so-called creeping movement is in the nature of a response. The direction of such movements is too irregular and aimless. They may lead to ascent or descent, or movement toward or away from the light, or in a line parallel to the window.

PHOTOTROPISM

Although the drifting is without definite direction, the other sort of locomotion is very definitely negative phototropism. The swooping dash is always away from the light. It takes place without any preliminary signs. If the head of the animal is directed toward the light one movement of extraordinary speed turns the body and carries the animal toward the side of the dish opposite the light. This behavior can be best observed in a shallow dish, but in a tube also the actual swimming is away from the light. Table 11 summarizes the results of the experiments; both the rapid and the slow movements are in-

cluded. All the tests were made in the light by the 50 by 40 cm. window of the darkroom.

TABLE 11

THE PHOTOTROPISM OF *Eucalanus*. SUMMARY OF INDIVIDUAL RECORDS IN ALL INTENSITIES. TEMPERATURE 16°-20°C; SALINITY 33 TO 34 ‰.

Number of experiments	Number of animals tested	Per cent of animals moving		Records of position							
				per cent of total showing				Distance			
				movement		no movement		Total (cms.)	Per cent		
+	-	+	-	+	-						
73	9	45	100	Total	11	69	20	0	1,432	3	97

It may be said that all the movements that show positive phototropism are due to the drifting, and if these records were omitted there would be only negative phototropism. But as the table stands the responses are strongly on the negative side.

GEOTROPISM

The movements of *Eucalanus* in a column of water are of the two general sorts. The animals "drift" up or down and also swoop in either direction.

Table 12 indicates the nature of the behavior in a vertical tube. The table summarizes records that were made at intervals of varying lengths, and the figures merely show the direction of movement without regard to the method by which it is brought about. Records that show no change of position are so few that they are omitted from the table.

TABLE 12

THE GEOTROPISM OF *Eucalanus* UNDER DIFFERENT CONDITIONS OF LIGHTING AND IN DARKNESS. SUMMARY OF INDIVIDUAL RECORDS. TEMPERATURE 16°-20°C; SALINITY 33 TO 34 ‰.

Number of experiments	Number of animals tested	Per cent of animals moving		Records of position				Distance			
				Total	per cent of total showing movement		Total (cms.)	Per cent			
					+	-		+	-		
IN DIFFUSE LIGHT											
35	5	100	100	223	58	42	1,637	67	33		
IN DARKNESS											
7	2	100	100	24	71	29	164	79	21		
IN VERTICAL LIGHT FROM ABOVE											
6	4	75	75	24	75	25	301	89	11		
IN VERTICAL LIGHT FROM BELOW											
8	5	100	100	27	22	78	203	8	92		

The table shows, in brief, that *Eucalanus* is positively geotropic except when exposed to light from below. It is then strongly negative, and we may suppose that the negative phototropism is stronger than the tendency to move downward.

The responses were not investigated in water of unusually low temperature or high salinity, so that it is not known whether the phototropism and geotropism change as these factors are altered.

The experiments have shown that *Eucalanus* is strongly negative to light of low and of high intensity, and positive to gravity in diffuse light, in darkness, and in light from above. It is negatively geotropic in light from beneath. Since experiments are lacking to show whether these reactions change with decreased temperature or when the salinity is increased, it seems hardly worth while to discuss the bearing of the experimental results on diurnal migration. It may not be out of place to state that the reason for the few experiments with *Eucalanus* is that the animals were obtained in such small numbers. Practically all the individuals brought in were used.

The results of an examination of the field records for *Eucalanus* are available in two papers (Esterly, 1911a, 1912, pp. 295-300). The data show that there are larger numbers of animals at the surface at night, but it does not appear that there is a well marked diurnal migration although there is an extensive movement below the surface.

REACTIONS OF THE COPEPOD *Labidocera trispinosum*

These animals have been extraordinarily abundant at times about La Jolla, but during the academic year 1916-17 they were scarce. The animals do not endure confinement in the laboratory very well, and they do not usually remain in suitable condition even over night. But if they were active on the second day after collection I did not hesitate to use them. I have not found any reason to suspect that the responses change after the animals are brought into the laboratory.

Labidocera differs from the other copepods used in having well developed eyes with prominent lenses. There are two on the dorsal and one on the ventral surface of the head.

The animals are rather slow swimmers as a rule, but if disturbed they dart about with great rapidity. The movement is steady and lacks the jerkiness of *Calanus* or *Acartia*. When swimming in a shallow dish the course is wavering and uncertain but the animals keep moving steadily. In the narrower confines of a tube the course is prac-

tically straight. The posterior antennae are in constant motion whether the animal is ascending or descending in a vertical tube; in the latter case the appearance is something like that of a bird hovering. I have seen no instances in which an animal sank immobile after the fashion of *Calanus*. It is not uncommon for individuals to circle in a vertical plane, and sometimes this behavior is kept up for a good many minutes.

PHOTOTROPISM

Labidocera is negative to light. I have not tested them in a wide range of intensities but the reactions are predominantly negative in the dim light of a 15-watt lamp as well in that of the 50 by 40 cm. window. Table 13 is a summary of the behavior of several animals in a rectangular aquarium 20 by 8 cm. The vessel was marked off into five sections, as usual, and turned end for end several times in the course of an experiment.

TABLE 13

THE PHOTOTROPISM OF *Labidocera*. SUMMARY OF RECORDS OF SEVERAL SETS OF ANIMALS IN LIGHT OF NORTH WINDOWS; TEMPERATURES 15°-20°C, SALINITY 33 TO 34 ‰.

Whole number of animals observed	Percentage distribution in the sections					Number of observations			Center of distribution
	negative V	IV	III	II	positive I	Total	per cent showing more animals in positive %	negative %	
304	85	5	4	1	5	24	0	100	4.6

Table 14 is of the usual form that deals with the responses of different individuals. The results of using different intensities of light are put together in the table since most responses are negative.

TABLE 14

THE PHOTOTROPISM OF *Labidocera*. SUMMARY OF RECORDS OF INDIVIDUALS IN LIGHT OF LOW AND HIGH INTENSITY. TEMPERATURES 15°-20°C; SALINITY 33 TO 34 ‰.

Number of experiments	Number of animals tested	Number of animals moving		Records of position				Distance			
		+	-	per cent of total showing				Total (cms.)	Per cent		
		Total		movement		no movement					
110	23	17	100	284	10	70	4	16	3,821	5	95

The results that appear in tables 13 and 14 are so evident that comment is unnecessary.

GEOTROPISM

In darkness and diffuse light. It is difficult to know what to say about the geotropism of *Labidocera*. There is a good deal of difference in the behavior of different sets of animals. I used two sets of individuals. Lot A was obtained at 8 A.M. at the surface and had been in the laboratory six hours before the experiment began. Lot B was obtained in a vertical haul from about 60 meters at 9 A.M. and had been in the laboratory about two hours. Except for these differences the treatment and surroundings of the two sets were similar. Table 15 summarizes the observations on the distribution of the two sets in darkness and in diffuse light.

TABLE 15

THE GEOTROPISM OF TWO SETS OF *Labidocera* (A AND B) IN DIFFUSE LIGHT AND IN DARKNESS. TEMPERATURES 15°-18°C, SALINITY 33 TO 34 ‰.

Whole number of animals observed	Percentage distribution in the sections					Number of observations			Center of distribution	
	top V	IV	III	II	bottom I	Total	per cent showing more animals in			
							upper %	lower %		
IN DARKNESS										
A	47	19	8	11	11	51	7	14	86	2.3
B	115	67	3	5	7	18	29	69	21	3.9
IN DIFFUSE LIGHT										
A	56	0	0	2	5	93	8	0	100	1.1
B	71	34	14	14	7	31	17	47	35	3.1

The table shows that in darkness the animals of lot A were strongly positive in geotropism while those of lot B were even more strongly negative. In diffuse light lot A was very strongly positive and lot B predominantly but not markedly negative. Yet it is shown that with each set there is an upward movement in the dark. The totals of the percentages in sections IV and V are larger for both A and B in the first two lines of the table than in the last two lines, and those in sections I and II are smaller in the first two than last two lines of the table. The centers of distribution, likewise, show that there is ascent in darkness. Yet the two sets of animals are significantly different in behavior if the distribution in darkness or in diffuse light is considered alone. It is upon comparison of the distribution under one condition with that under the other that the movement is indicated.

It does not seem that retention in the laboratory will account for the difference in the two sets of animals. Lot A was in the laboratory

for eight hours altogether and lot B for about eleven hours. But the latter animals were in darkness from the sixth to the eighth hour and were consistently and strongly negative and during that time twelve observations of the distribution was made. If something connected with the laboratory conditions affects the animals, it plainly does not affect all in the same way. In spite of the differences it appears, nevertheless, that there is a tendency for *Labidocera* to ascend in darkness, and to descend in the light.

Table 16 (p. 49) deals with the behavior as shown by the results with individual animals, the table being a summary. The possible effects of longer and shorter periods of confinement in the laboratory are considered by separating the recorded results into two groups, for both darkness and diffuse light.

It must be said that the results appearing in table 16 are confusing. Negative geotropism prevailed slightly in diffuse light among animals that had been a shorter time in the laboratory (C), at least so far as the actual swimming is concerned. The reverse is indicated for animals that had been longer in the laboratory (D); all the percentages are in favor of positive geotropism. The results of tests in darkness, so far as they go, show that there is prevailing positive geotropism in animals that were in the laboratory from 1-3 hours. This is also the case in the second line of the table except as regards the distance covered, where the difference is strongly in the negative column. It is well to note that the distance percentages show that there is an upward movement in the dark of animals kept for the longer time in the laboratory (compare lines B and D), but the records of position do not show that. If lines A and C are compared it is seen that the distance percentage shows upward movement in the light, and this is also indicated by the differences in the percentage of records that show change of position.

In view of the discordant results in table 16, especially when they are compared with table 15, it is difficult to know what to conclude as to the geotropism of *Labidocera* in the absence of directive light.

In directive light. The tendency of *Labidocera* to move away from a source of light is shown, I believe, by its behavior in a vertical tube when a lamp is at the top or bottom. In the former case the responses are negative to light and positive to gravity; in the latter they are negative to both. The facts are shown in table 17 (p. 49). Length of time in the laboratory does not make any difference in the behavior apparently, so all records are entered in the table. The source of light

was the 15-watt lamp. It was placed about ten centimeters from the top or bottom of the container, which was in most cases a tube 500 by 30 mm.

So far as the observations that are summarized in table 17 are concerned it is evident that *Labidocera* ascends or descends depending on the location of the light.

SUMMARY

The experiments show: (1) That *Labidocera* is negatively phototropic at ordinary temperatures. The responses are negative to light of low as well as of high intensity. (2) The results concerning geotropism are confusing. Table 15 shows that there is a tendency to ascend in darkness and to descend in diffuse light on the part of specimens that had been in the laboratory for ten hours at the most. In table 16 it appears that there is a tendency toward negative geotropism in diffuse light when the specimens had been in the laboratory from one to six and a half hours; but those not tested for twelve to twenty-seven hours after removal from the sea show positive geotropism. Two individuals that were tested in darkness within one to three hours after they were taken from the ocean showed positive geotropism; others, not used until twelve to twenty-six hours after capture, gave uncertain results. (3) In directive light from above the geotropism is strongly positive; in directive light from below it is strongly negative (table 17).

POSSIBLE BEARING OF THE EXPERIMENTS ON DIURNAL MIGRATION

Since there is nothing to show whether the behavior changes when the temperature is lowered or the salinity increased, a full discussion of the relation between the results in the laboratory and the natural habit is not possible.

So far as the experiments are concerned, it is not to be expected that *Labidocera* will occur at the surface during the day since it is negatively phototropic and positively geotropic when the light is from above; also the field data show that this copepod is very much more abundant at the surface from 6-8 P.M., and that it is practically absent at other times (Esterly, 1912, pp. 307-309). The upward movement of some sets of experimental animals when in the dark

TABLE 16
THE GEOTROPISM OF *Labidocera*. SUMMARY OF INDIVIDUAL RECORDS IN DIFFUSE LIGHT AND IN DARKNESS.
TEMPERATURES 16°-20°C; SALINITY 33 TO 34 0/1000

Hours in laboratory	Number of experiments	Number of animals tested	Per cent of animals moving	Records of position				Total (cms.)	Per cent				
				per cent of total showing		Distance							
				movement	no movement	+	-	+	-				
A	1-3	7	2	100	100	68	32	22	19	457	62	38	
B	12-26	5	4	50	25	37	16	11	62	68	37	63	
				IN DARKNESS									
				Total	+	-	+	-	Total (cms.)	+	-		
				IN DIFFUSE LIGHT									
C	1-6 1/2	27	9	56	89	107	24	33	29	14	610	43	57
D	12-27	19	7	86	86	142	53	18	24	5	695	60	30

NOTE.—The individuals in A, C, and D were all different; the four in B were also used in C but not in A or D.

TABLE 17

THE GEOTROPISM OF *Labidocera* IN DIRECTIVE LIGHT. SUMMARY OF INDIVIDUAL RECORDS.
TEMPERATURES 16°-20°C; SALINITY 33 TO 34 0/1000

Number of experiments	Number of animals tested	Per cent of animals moving	Records of position				Total (cms.)	Distance	Per cent		
			Per cent of total showing		Distance						
			movement	no movement	+	-	+	-			
16	5	100	0	0	34	80	0	20	745	100	0
			VERTICAL LIGHT FROM ABOVE								
			Total	+	-	+	-	Total (cms.)	+	-	
			VERTICAL LIGHT FROM BELOW								
17	7	57	100	74	20	53	4	23	805	7	91

(table 15) is in line with the distribution observed in the sea, and the negative reactions to light account for the small numbers at the surface during the day. But the inconstant results obtained in experiments on the geotropism make difficult their application to the behavior of the species under natural conditions.

REACTIONS OF THE COPEPOD *Metridia lucens*

The occurrence of this copepod as material for experiment was erratic, but the animals were obtained in fairly large numbers and at opportune times so that it was possible to work with them to advantage. The locomotion of these organisms is slow unless they are suddenly disturbed; there is nothing of the jerky character in the swimming; it is what may appropriately be called "sailing." This sort of movement is continual, but if the vessel is jarred the animals dart about with great rapidity. The sailing of *Metridia* is different from the "fluttering" or "hovering" of *Labidocera*, but the difference is hard to express in words. When specimens are in diffuse light in a vertical tube it is especially common when they are descending to see them sail in small circles that together make up a close spiral carrying them lower and lower. They also travel in vertical circles, something like "looping-the-loop" in an aeroplane, so that they gradually descend. Very rarely a specimen will drop down like *Calanus*, but practically always the animals are sailing about in circles. If a light is placed at the top of the tube it is not uncommon for the descent to be accomplished by swimming down head foremost.

Metridia lives very well under laboratory conditions, and I have not observed any difference in the behavior soon after they have been taken from the sea as compared with that several hours later.

PHOTOTROPISM

The reactions have been observed in the light of the window 50 by 40 cm., in the 15-watt, at distances of ten, fifty, and one hundred centimeters from the end of the tube or side of a dish next the lamp, and in the light of the 100-watt lamp about 200 times the intensity of the 15-watt. *Metridia* is negative to each of these intensities at room temperatures and in water of usual salinity. Positive responses predominate, however, if the water is cooled to 10°C. Table 18 shows a summary of the results when several animals are under observation at a time.

TABLE 18

THE PHOTOTROPISM OF *Metridia* AT ROOM TEMPERATURE AND AT 10°C OR LESS

Whole number of animals observed	Percentage distribution in the sections					Number of observations			Center of distribution
	negative V	IV	III		positive I	Total	per cent showing more animals in		
			positive	negative			positive %	negative %	
	TEMPERATURES 15°-20°C								
391	47	8	9	10	26	56	25	70	3.9
	TEMPERATURE 10°C OR LESS								
78	1	0	6	3	90	13	100	0	1.2

So far as my observations go, the animals will remain positive indefinitely providing the water is kept cooled. There is more or less swimming back and forth under any circumstances; but most of the animals are on the side toward the light in cold water, and on the side away from the light in the warmer water. The same animals are positive, in cold water, to the light of a 15-watt lamp and also to that of a 100-watt that is 200 times as intense. The movements away from the light when its intensity is low (the 15-watt at 50 or 100 cm.) do not begin as promptly as when the animals are exposed to the light from the window, and progress is slower at the lesser intensities.

When individual animals are used the results (table 19, p. 53) are similar to those set forth for the higher temperatures in table 18.

The table shows that the negative greatly outnumber the positive responses, while the distance covered is practically all in the negative direction.

GEOTROPISM

Table 20 (p. 53) gives the summary of results obtained when several animals were under observation at once. It shows the difference in behavior in water of low and high temperature. In all the experiments with cold water the entire tube or cylinder was in ice water, at temperatures ranging from 7°C to 9°C.

In diffuse light there is strong tendency for the animals to descend or to stay at lower levels when the temperature is that of the room. It is suggested by the results that negative geotropism is somewhat increased in the cold water.

In darkness the geotropism changes as the temperature changes. It is strongly positive at the higher temperatures, and strongly negative in cold water. If the animals are at the bottom in cold water when the light is extinguished they ascend and remain at or near the top;

their negative geotropism involves both locomotion and maintenance of position at high levels. If they are in section V when subjected to darkness they do not descend, providing the temperature is low enough.

When exposed to light from above in water of room temperature the animals descend and remain at the bottom. But in the cold water they ascend toward the light and remain at the top or near it. In light from below, however, there is a marked movement upward at room temperatures; at low temperatures, on the other hand, the animals will descend toward the light and remain in the bottom sections.

Inspection of the figures in table 20 showing the centers of distribution reveals the difference in distribution of the animals as the temperature is higher or lower.

The results obtained with individual animals are confirmative of those with sets of animals, although no tests at low temperature were made in the former case. Table 21 (p. 55) deals with such records and it shows that the dominant response is positive geotropism in all conditions except when the light is below.

SUMMARY

The experiments show: (1) That *Metridia lucens* is negative to light at ordinary temperatures, but positive if the water is cooled to 9°C. (2) The animals are positively geotropic at ordinary temperatures in darkness and under any condition of illumination except light from below, and these responses are reversed in cold water.

POSSIBLE BEARING OF THE EXPERIMENTS ON DIURNAL MIGRATION

It is safe to say that so far as these experiments go they offer a ready explanation of some phases of the vertical migration. *Metridia* is negative to light and is positively geotropic (except when lighted from below) at temperatures ranging from 16°C to 19°C. Both the geotropism and phototropism are reversed at 9°C.

Now if the animals in the sea descend to sufficient depths they will encounter temperatures in which the direction of movement is reversed and it may be expected that they will begin to ascend. The field data show that these copepods are found in largest numbers dur-

TABLE 19
THE PHOTOTROPISM OF *Metridia*. SUMMARY OF INDIVIDUAL RECORDS. TEMPERATURES 16°-20° C.;
SALINITY 33 TO 34 ‰/60°

Number of expert-ments	Number of animals tested	Percent of animals moving	Records of position				Distance			
			Total	movement	no movement	Total (cms.)	+	-		
33	9	52	83	2	94	4	0	798	1	99

TABLE 20

THE GEOTROPISM OF *Metridia* UNDER DIFFERENT CONDITIONS OF LIGHTING AND IN DARKNESS, AND AT LOWER AND HIGHER TEMPERATURES

Temperature	Whole number of animals observed	Percentage distribution in the sections						Number of observations		Center of distribution
		top V	IV	III	II	bottom I	Total	Percent showing upper 25%	Percent showing lower 25%	
16°-19°	354	15	6	7	9	63	59	20	76	2
7°-9°	32	44	0	0	6	50	4	0	50	2.8
16°-19°	570	8	2	7	10	73	92	2	98	1.6
7°-9°	102	63	12	6	9	10	13	85	8	4.1
16°-19°	186	2	11	11	10	66	28	14	82	1.7
7°-9°	112	77	11	4	3	5	15	93	0	4.5
16°-19°	370	42	8	6	5	39	52	54	42	3.1
7°-9°	141	13	8	6	8	65	18	8	92	1.7

ing the day at about 270 meters (Esterly, 1912, pp. 304-305), and according to McEwen (1916, pl. 34) the mean annual temperature at that depth is between 8° C and 9° C; at such temperatures in the laboratory the phototropism changes from negative to positive and the geotropism from positive to negative. If it is assumed that when the animals in the ocean reach water of 10° C they begin to ascend, the ascent into warmer water remains to be accounted for; this point is not covered by the experiments. We know, however, from the field records that *Metridia* is found in great numbers at the surface when the temperature is 19° C (Esterly, 1912, p. 306). If the ascent begins in a region of lower temperature, why does it continue into water of temperatures in which experimental animals are positively geotropic? It is possible that the answer to this question will be found in the occurrence of a physiological rhythm, but I have no evidence that the rhythm is present in *Metridia*.

REACTIONS OF THE CHAETOGNATH *Sagitta bipunctata*

A study of the reactions of this animal seemed especially desirable because of the striking results obtained from the field data, as shown by the study made by Michael (1911). He has shown that there is marked correlation between occurrence of this species and variations in certain external conditions, and the question at once arose whether similar behavior would be shown in the laboratory.

So far as I am aware, a chaetognath has never before been used in experiments on behavior. *Sagitta* is usually to be obtained at La Jolla in sufficient abundance if the collecting is done at depths of 75-100 meters. The animals come far enough toward shore to be taken from the pier, but occurrence there is exceptional.

A great deal of difficulty was experienced in obtaining specimens in the proper condition for experiment. On many occasions they show signs of morbidity even before they reach the laboratory. The body becomes slightly opaque or milky and bits of sand and débris adhere to the exterior. Moreover, the animals rest quietly on the bottom of the dish if they are in bad condition, instead of swimming around constantly. I soon learned that an animal that has sunk to the bottom will not react.

The reasons for the appearance of such unfavorable states are not clear. Our success was much better if a two-quart jar was tied into

TABLE 21
 THE GEOTROPISM OF *Metridia*. SUMMARY OF INDIVIDUAL RECORDS. TEMPERATURES 16°-20° C;
 SALINITY 33 TO 34 0/1000

Number of experiments	Number of animals tested	Per cent of animals moving		Records of position							
		+	-	Per cent of total showing movement		no movement		Total (cms.)	Distance		
				+	-	+	-		+	-	
11	6	100	50	Total							
				In DIFFUSE LIGHT							
				77	21	0	2	353	82	18	
				In DARKNESS							
4	2	50	50	18	50	22	28	0	117	85	15
				In VERTICAL LIGHT FROM ABOVE							
7	5	100	0	17	100	0	0	254	100	0	
				In VERTICAL LIGHT FROM BELOW							
10	5	80	80	57	28	53	10	9	275	31	69

the net so that the animals passed into a larger amount of water than when a bottle was used. But perfectly satisfactory material has been obtained in the latter case, and very poor in the former. Water from the laboratory supply, as contrasted with that carried in from the pier, is not always to be blamed. One animal lived for three days in sea water drawn from the pipes, without changing the water, and the specimen was apparently in as good condition on the third day as on the first. Its death was due to exposure to direct sunlight. If the animals are in good condition handling them does not have an unfavorable effect. It is necessary to pick them up with a pipette, and this must be done carefully of course, but it does not interfere with their activities. Pouring from one dish to another is not injurious.

On the whole, I do not know of anything that will insure one's having good material. It must be taken as it comes. Specimens can practically always be obtained but their condition is problematical. With identical treatment they may at one time be perfectly satisfactory and at another be the opposite.

Sagitta is not a particularly rapid swimmer, even when disturbed, as by the approach of the pipette. The animals are expert "dodgers," and this combined with the transparency of the body makes it difficult to catch them. Swimming is accomplished by making strokes with or by "flirting" the tail. At each stroke the animal moves forward a short distance, and this action is kept up continually. The animals can even swim backward by what is to all appearances the same method of propulsion as that used for locomotion in the opposite direction.

Another difficulty met with in using *Sagitta* is that of seeing the animals, particularly in a large dish of water. It is not hard to keep track of an individual nor to count a number of animals in a tube when one can look through the water toward the light. I found it impossible to do anything, however, by looking down into a dish of water from above.

I believe that there need be no hesitation in using animals that have been in the laboratory, so long as they are swimming about; that is the best test of condition I have found. I did not reject specimens even on the second day, and I think that their reactions then are as characteristic of the species as those of any animals in the laboratory.

TABLE 22
 THE PHOTOTROPISM OF *Sagitta*. SUMMARY OF INDIVIDUAL RECORDS. TEMPERATURES 16°-20° C.;
 SALINITY 33 TO 34.0/60°.

Number of experimental animals	Number of animals tested	Percent of animals moving		Records of position				Total (cms.)	Distance				
		+	-	Percent of total showing		movement			+	-			
38	14	100	29	Total	204	70	19	6	5	Total (cms.)	428	97	3

TABLE 23

THE GEOTROPISM OF *Sagitta* UNDER DIFFERENT CONDITIONS OF LIGHTING AND IN DARKNESS. SUMMARY OF INDIVIDUAL RECORDS. TEMPERATURES 16°-20° C.; SALINITY 33 TO 34.0/60°.

Number of experimental animals	Number of animals tested	Number of animals moving		Total	Records of position				Total (cms.)	Distance	
		+	-		Percent of total showing		movement			+	-
30	8	50	87	142	IN DIPYCNSE LIGHT				922	11	89
					IN DARKNESS						
16	6	16	66	60	2	23	0	75	671	1	99
					IN VERTICAL LIGHT FROM ABOVE						
22	9	66	78	105	28	41	1	30	902	30	70
					IN VERTICAL LIGHT FROM BELOW						
26	8	75	63	120	30	38	11	21	838	37	63

PHOTOTROPISM

The evidence that I can present regarding the reactions of *Sagitta* to light has all been obtained by observing individual specimens. So far as my records go the animals are strongly positive to light if they are in a horizontal tube that is parallel to the rays. In a stock dish before the window it seems to me that the specimens are swimming around indiscriminately, but the difficulty of observation under such circumstances is great. Table 22 (p 57) is a summary of the observations. All were made upon animals in the 50-centimeter tube. As soon as a specimen reached the end of the tube it was turned to the vertical position and the geotropism noted. The observations were made in intensities ranging from bright daylight, through the 50 by 40 centimeter window, to the 15-watt lamp at 10, 20, and 50 centimeters; the 100-watt lamp was not used.

The table clearly shows that the animals react positively to light. There is no reason to doubt that after moving as far as possible toward the light they would remain there. Some observations have shown that an animal may actually turn about and swim away from the light for a short distance, and then turn and swim toward the light. The behavior toward light was not tested in water of low temperature or high salinity.

GEOTROPISM

When the individual records are summarized it appears that there is a well defined negative geotropism at ordinary temperature and salinity. There is evidence that the geotropism changes in diffuse light of too great intensity, but in the dark room with the window unshaded or in the light of the 15-watt lamp, the animals ascend and remain at or near the top. The same is true of the behavior in darkness and in vertical light from below and from above. Table 23 (p. 57) gives the summary of results of observations on single specimens.

It seems to me that the evidence is conclusive that *Sagitta* is negatively geotropic in darkness as well as in light from any direction and of the intensities used in the experiments. When once at the top the animals keep bumping against the end of the tube, and if they stop swimming they drop down for a longer or shorter distance and then swim back to the top; this passive descent accounts for most of the records that show downward movement. When the tube is lighted

from below it is not unusual to see an animal swim down toward the light. When the tube is lighted from above I have seen animals swim from the bottom to the top, then drop from the top to the bottom, and after a few moments return to the top and stay here. In no case, however, has an animal descended by swimming except when the light was beneath.

When *Sagitta* is in a vertical tube that stands in a room with white walls and several high windows the negative geotropism changes to positive. The following case is a good illustration of this. A tube containing an animal that was positively phototropic to the light from the large window of the dark room, and negatively geotropic in darkness as well as in the diffuse light of the dark room, was carried out into the main laboratory. The light was much brighter there because of several large windows and white walls and reflection from the windows and walls of an adjacent building. In this light the specimen would swim down slowly and was also negatively phototropic. When the tube was shaded by the door of a closet, the animal would turn and ascend. If the tube was moved into the brighter light while the specimen was still swimming up, it turned and swam down, and if put into the subdued light while still descending the course was reversed. This was repeated several times with this particular animal, and I have no doubt that such behavior is characteristic of the species.

Confirmatory results have been obtained with several animals under observation at once. The specimens descend in the light of a room with white walls and three tall north windows, and they ascend in the dark. There are two different experiments to be reported on, and table 24 (p. 60) deals with the first of these. There were fourteen animals, obtained half an hour before the experiment began, in a cylinder 50 by 6 centimeters that stood 75 centimeters from the windows. A light proof box was set over the cylinder when it was desired to subject the animals to darkness; otherwise they were in the bright diffuse light of the room. The column was marked off into five divisions. Only those animals were counted in the lowest section that were actually off the bottom, so that all the records dealing with distribution relate to animals that were able to swim. In addition to giving a summary of the distribution, table 24 outlines very briefly the changes in external conditions.

TABLE 24

THE GEOTROPISM OF *Sagitta* IN BRIGHT DIFFUSE DAYLIGHT AND IN DARKNESS, AND UNDER VARYING CONDITIONS OF TEMPERATURE.

SALINITY 33 TO 34 ‰.

	Whole number of animals observed	Percentage distribution in the sections					Number of observations			Center of distribution
		top V	IV	III	II	bottom I	Total	Per cent showing more animals in		
							upper %	lower %		
1.	147	11:15 a.m.-3:15 p.m., darkness, temperature 16°-17°C					12	17	58	2.7
2.	18	3:15-3:20 p.m., diffuse light, temperature 16°-17°C					2	0	100	1.4
3.	80	3:21-8:45 p.m., darkness, temperature 17°-18°C					7	100	0	3.8
LEFT IN DARK OVERNIGHT; FOLLOWING RECORDS OBTAINED ON SECOND DAY OF CAPTIVITY										
4.	33	7:45-8:25 a.m., darkness, temperature 16°-17°C					3	100	0	4.0
5.	38	8:26-9:10 a.m., diffuse light, temperature 16°-17°C					5	0	80	1.6
6.	20	9:11-9:30 a.m., darkness, temperature 16°-17°C					2	100	0	3.8
DURING TIME OF LINES 7-10, THE LOWER ONE-FIFTH WAS AT TEMPERATURE OF 7°-8°C										
7.	44	9:42-10:20 a.m., darkness					5	40	60	2.9
8.	35	10:21-10:40 a.m., diffuse light					4	0	100	2.2
9.	17	10:41-10:52 a.m., darkness					2	100	0	4.1
10.	17	10:53-11:07 a.m., diffuse light					2	5	5	3.2
ICE JACKET REMOVED										
11.	18	11:08-11:20 a.m., diffuse light, temperature of lower one-fifth, 8%°-10%°C					2	0	100	3.6
12.	8	11:30 a.m., diffuse light, temperature of lower one-fifth is 12%°C					1	0	100	1.7
13.	25	11:30 a.m.-12:45 p.m., diffuse light, temperature of lower one-fifth goes up to 16°C					3	0	100	1.6
12:45 P.M., LOWER ONE-FIFTH IMMERSSED IN ICE WATER										
14.	23	12:55-1:40 p.m., diffuse light, temperature of lower one-fifth 7°-9°C					3	0	100	1.9
1:40-2:00 P.M., DIFFUSE LIGHT; 2:00-2:15 P.M., DARKNESS, ICE JACKET REMOVED										
15.	25	2:15-2:30 p.m., darkness, temperature of lower one-fifth 10°-11°C					3	100	3	4.2
2:30 P.M., LOWER THREE-FIFTHS IN ICE, DARKNESS										
16.	8	2:50 p.m., darkness since 2:30 p.m., temperature of lower three-fifths 8°-10°C					1	100	0	3.9
17.	32	2:50-3:25 p.m., diffuse light, temperature of lower three fifths 7°-9°C					4	75	0	3.2
3:25-8:25 P.M., DARKNESS, ICE JACKET REMOVED										
18.	26	5:30-8:20 p.m., temperature of whole column 15°-16°C					3	100	0	4.3

The first six lines of the table show clearly that the experimental animals are positively geotropic in the light and negatively geotropic in the dark. It is true that during the first four hours of the experiment (line 1) most of the animals while in darkness were in the lower two-fifths of the cylinder; but by comparison they were less strongly positive than they were in the light (line 2). It is possible that we have to deal here with effects that are due to removal from the ocean; or it may be that there is some indication of a physiological rhythm. Neither of these possibilities has been investigated, but the general facts as to the behavior in light and in darkness are clear.

The change in distribution that occurs when the temperature is decreased appears in lines 7-10. The lower section of the cylinder was immersed in ice water. The figures show that while the animals ascended and descended as before, they did not go down into the cold water in the light. The largest numbers were found in the second section from the bottom (line 8) and in the middle section (line 10), and this can only be due, apparently, to the cessation of the descent that takes place when the animals enter cold water. Such behavior in diffuse light is in sharp contrast to that shown in lines 2 and 5; the temperature throughout the cylinder was about 17° C during the time those records were obtained.

From 11:08 A.M. to 12:45 P.M. the temperature of the lower section gradually increased after the ice was removed (lines 11-13). The numbers of animals in the bottom section constantly increased and the center of distribution was situated at successively lower levels. At 12:45 P.M. the container was again set into ice water so as to cover the bottom section, the lighting being unchanged. There is some suggestion in line 14 that the animals moved up from the lowest section as the temperature decreased but this point needs more evidence. The same thing is suggested again, however, if line 15 is compared with line 16. In both cases the animals were in darkness, but they very evidently moved upward when the lower three-fifths of the column of sea water was chilled. At any rate that was the only external change that took place. It is probably shown in line 17 that some animals descend in diffuse light even through thirty centimeters of cold water; but it is noticeable that two-thirds of them remained in the two upper sections. Evidently the descent is checked by the low temperature. It should be stated that there is a possibility that the animals recorded in section I in line 17 were there previously but were not observed to be off the bottom; that is, there may not have been any downward move-

ment through the cold water leading to the distribution recorded in line 17 as compared with that in line 16.

Line 18 is of interest as showing that *Sagitta* may remain negatively geotropic in darkness for five hours; at least it may reasonably be assumed that there was no marked downward movement from 3:25-8.20 P.M.

Table 24 shows finally that specimens of this chaetognath may exhibit the same kind of behavior late on the day after their removal from the ocean that they did within the first hour.

In giving the results of similar experiments on a second set of twenty animals it is not necessary to go into so much detail as in table 24. The observations are summarized in table 25; there were alternating periods of diffuse light and darkness and all the records obtained under each condition are put together in the several divisions of the table. The experiments were carried on in a room with white walls and lighted by four tall north windows. The container was a cylinder 50 by 6 centimeters and the animals were put into it within half an hour of the time they were taken.

There is always more marked negative geotropism in darkness than in the light, regardless of the temperature. This is shown by the percentages and also by the centers of distribution. But it is evident that most of the animals do not descend in the light into the colder water. In sections *b* and *c* of the table most of the animals are shown to be in that fifth of the cylinder which is immediately above the upper limit of the cold water, during the time that the specimens were exposed to the light. In the first line of *b* in the table a larger proportion of animals is recorded in the middle section of the cylinder than in all the others in *c* most of the animals were in the upper section during the time they were in the light. When the temperature of the lower sections of the cylinder was higher more animals descended into those levels in the light.

SUMMARY

It has been shown by the experiments: (1) *Sagitta* is strongly positive to light ranging from that of a 15-watt lamp at 50 centimeters to daylight about 3800 times as intense. No tests were made with intensities beyond these limits. (2) The geotropism is predominantly negative in darkness and in light that is not too bright. In a room with white walls and well lighted from without the geotropism

THE GEOTROPISM OF *Sagitta* IN BRIGHT DIFFUSE DAYLIGHT AND IN DARKNESS UNDER VARIOUS CONDITIONS OF TEMPERATURE. SALINITY 33 TO 34 ‰/‰

TABLE 25

Whole num- ber of animals observed	Percentage distribution in the sections					Number of observations			Center of dis- tribution	Remarks
	top V	IV	III	II	bottom I	Total	Per cent showing more animals in			
							upper %	lower %		
97	27	12	11	6	44	5	20	80	2.7	Diffuse light
54	41	30	11	4	14	4	100	0	3.8	Darkness
41	17	2	59	5	17	4	25	25	2.7	Diffuse light
19	74	5	5	5	11	2	100	0	4.2	Darkness
94	60	12	5	8	15	11	100	0	4.0	Diffuse light
19	85	5	5	5	0	2	100	0	4.7	Darkness
48	23	4	4	8	61	6	0	100	2.2	Diffuse light
45	38	18	9	13	22	6	83	16	3.3	Darkness

a. 10:35 a.m.-12:45 p.m., whole column 17°-18° C

b. 1:10-1:35 p.m., lower two-fifths at 7°-9° C, upper three-fifths at 17°-18° C

c. 1:55-2:55 p.m., lower four-fifths at 7°-9° C

d. 3:05-4:25 p.m., lower four-fifths rises to 14° C

is strongly positive. (3) The animals do not descend into water of 10° C except in small numbers; if the lower part of a cylinder of sea water is chilled most of the animals remain in regions above the level of the cold water although they are in the same intensity of light in which they descend if the water is warmer. The upward movement in darkness is not restricted, however, by such low temperatures.

POSSIBLE BEARING OF THE EXPERIMENTS ON DIURNAL
MIGRATION

If these experiments suggest to any extent what the animals do in nature and reveal why they do it it is to be expected that the chaetognaths will desert the surface of the sea as the light grows brighter with the advancing day. It also seems clear that the descent will be checked as the animals reach colder water, and in the more subdued light they may begin to ascend. It is to be noted, in this connection, that the geotropism does not change as the temperature is altered; on the contrary, the direction of vertical movement is reversed as the light varies. The most that can be said with regard to temperature is that the animals do not descend into cold water; the descent is checked but the animals do not turn and ascend unless the intensity of the light is reduced. I think it is evident that the experimental results offer a basis for explaining the diurnal migration in its general outlines. Light intensity is apparently the determining factor; and it may be that this will explain some of the irregularities in the occurrence of the species at the surface mentioned by Michael (1916, p. xiii), where it is stated that large numbers have been obtained at the surface at all times of day and night and even at noon. The suggestion from the experiments is that on the occasions when there are many animals at the surface at noon the light is of comparatively low intensity, as on a cloudy day.

The distribution of *Sagitta* as regards different conditions in the sea has been very thoroughly studied by Michael (1911). The summary of facts derived from the study of field data (p. 157) shows that this species of chaetognath is found in unusually large numbers at the surface twice during the twenty-four hours, within an hour after sunrise and also within an hour after sunset. Below 25 fathoms it is most abundant between 10 A.M. and 2 P.M. The species is most abundant between the surface and 20 fathoms. Michael states (1911, p. 128, for example) that the facts of marked decrease about sunrise

and of marked increase soon after sunset "strikingly confirm our former inference that *S. bipunctata* migrates toward the surface during subdued light and away during intense light and darkness." The experiments confirm this statement in all respects except that the animals in the laboratory are negatively geotropic in darkness.

The distribution of *S. bipunctata* with regard to temperature shows (Michael, 1911, p. 133) that it is found in larger numbers at the surface when the temperature is low (15.9 C-17.5 C). "It is further suggested that low surface temperatures *retard downward* and *aid upward* migrations, and that high surface temperatures *retard upward* and *aid downward* migrations." It has been shown by the experiments that downward migration is retarded by low temperature, and there is some evidence in table 24 that there is more of an upward movement in darkness as the temperature is decreased than when it remains at 16° C or 17° C. This is an instance where experiment has verified a suggestion gained from a study of the field records. But the upward movement of animals in subdued light in an experiment is not held back by temperatures as high as 18° C; it is possible, however, that at 20° C the ascent would be checked, as suggested by Michael.

Since the field records and the experiments both show that in light of too great intensity there is downward movement, the question arises, How far will the animals descend under natural conditions? Michael (1911, p. 117) concludes that this chaetognath is rarely found below 75 fathoms and he states (p. 128) that it is most abundant between 40 and 75 fathoms from 10 A.M. to noon. For this discussion we may assume that 75 fathoms is the lower limit of the descent. The mean annual temperature at that depth (McEwen, 1916, pl. 34) is about 10° C, and this checks well with the observation in the laboratory that the animals do not descend into water that is cooled to 9° or 10° C. It cannot be stated at this time whether the downward movement stops at 75 fathoms because of the low temperature or because of the decrease in light intensity. The latter factor may be the determining one. Michael (1911, p. 158) has concluded that the region where the largest number of optimum conditions are found is from 15-20 fathoms. At that level the mean annual temperature is between 13° C and 14° C, and it is doubtful if the descent would be retarded at that temperature.

I believe that the results of these experiments and of the field work check each other in essential matters, in showing the conditions under

which *Sagitta bipunctata* ascends and descends, and why the downward migration does not extend deeper than 75 fathoms.

Since there are no experiments dealing with reactions in water of high or low salinity there does not seem to be any value in discussing the distribution of *Sagitta* in the sea as regards variations in salinity.

GENERAL DISCUSSION

THE EFFECTS OF LABORATORY CONDITIONS IN GENERAL ON EXPERIMENTAL ANIMALS

In the preceding accounts of responses it was pointed out that there is a noticeable change in the *kind* of reaction to light in the copepod *Calanus finmarchicus* and to both light and gravity in *Acartia tonsa* after the specimens have been kept in the laboratory for a time. To my mind this change is due to something connected with the artificial conditions under which the animals must be maintained, combined with the effect of removal from the ocean. Similar changes have been recorded by other observers in the case of other kinds of organisms. Harper (1905, p. 445) states that specimens of *Corethra* will collect on the lighted side of a dish when they are first brought in, and that this positive reaction gradually changes to negative. According to Jennings (1904, p. 83) certain rotifers show a like change; and there is a similar statement with regard to the copepod *Temora longicornis* by Loeb (1893, p. 94). Phipps (1915, p. 215) found that there is something associated with captivity that affects the phototropism of amphipods.

Such observations as have just been mentioned ought to make one question how far reactions that take place under laboratory conditions are likely to indicate the reasons for natural habit. If one is interested in what the specimens at hand are capable of doing under experiment it is desirable to allow them to "become accustomed" to laboratory surroundings. But if experimental results are sought for the information they may give concerning what the animals actually do *in nature*, it is necessary that the change in behavior be considered. What shall be said, for instance, about such a case as that of *Calanus*? The animals are positive to light at first, then they become permanently negative at room temperatures. The meaning of such a reversal ought to be considered. It may indicate that the state of the animals has returned to the condition that prevailed at the time of capture, and

which was upset by removal from deep water to the surface. If this is true there is no reason why the reactions observed in the laboratory should not be looked upon as affording a basis for ascertaining the factors that determine the migration. On the other hand, it may well be that the physiological state has *not* changed so long as the animals react positively to light, and that the change from positive to negative phototropism is the outward evidence of a condition that is the opposite of that prevailing at the time of capture. If this is really the case it seems plain to me that interpretations based on reactions will be erroneous.

Although it is not known just what the reversal in phototropism means, the fact that the change takes place should not be neglected. The purpose of observing the animals under laboratory conditions was to discover if possible why they are found during the day at 100 meters, for example, instead of at the surface. How can we expect to learn from the responses of a few representatives why the species as a whole is in deep water during the day unless the responses are evoked from animals that are in the same physiological state at the time of experiment as when they were swept into the net in deep water?

It must be admitted that the prevailing negative phototropism of *Calanus* appears to explain the absence of the animals from the surface during the day: they descend because they move away from the light. But is this true for animals in the sea? Specimens that are put under observation as soon as possible after they are brought to the surface are *positive* to light. Does that kind of response reveal the physiological condition of the specimens as it was at the level where they were obtained? If it does, we are prohibited from saying that the animals leave the surface because they are negative to light. As I look at this matter, we should consider whether or not the animals when brought to the surface have the same form of response that would be observed if it were possible to perform the experiments at a depth of 100 meters, for example. My own opinion is that the removal of the animals from deep water to the surface temporarily upsets the physiological state, and the brief period of positive phototropism is the sign of the disturbance. I believe that the animals when at 100 meters are really negatively phototropic. A good deal could be learned about the matter by comparing the behavior of animals from the surface with that of specimens from deep water. Experimental material of *Calanus* has not yet been obtained from the surface. But it may

reasonably be expected that the reactions of surface animals will be different from those of specimens from deep collections.

The behavior *Acartia tonsa* shows clearly how different the behavior of surface and deep water animals can be. This matter has been dealt with before (Esterly, 1917a, and also in this paper), but it is worth emphasizing again. This copepod is positive to light and negative to gravity in the light and positively geotropic in the dark, providing the specimens come from the surface. But animals that are obtained in deeper water (10-20 fathoms) are negatively phototropic and positively geotropic soon after capture both in diffuse light and in darkness. In this case, furthermore, there is a marked change in the behavior of specimens from deep water after they have been in the laboratory, for they come to react in all respects as do the animals from the surface.

I have not had the opportunity to compare the reactions of specimens from the surface and low levels of other forms than *Acartia*. It seems to me, however, that the results with that species are suggestive of possibilities in other forms. They show that the physiological state really is different, depending on the habitat from which the experimental specimens are obtained. What is true of one species is not necessarily true of another, but it is difficult to avoid the conclusion that the effect of laboratory conditions must be considered. At any rate an observer should look for the evidences of change in physiological state that may be connected merely with confinement in the laboratory before he uses experimental results to explain habits in nature.

SPECIFICITY IN BEHAVIOR

Diurnal migration is a complex natural phenomenon in which many different kinds of organisms take part. The cause of the migration in each species is a particular problem, and there is need to realize that the facts of behavior of one form may not apply to others. A general explanation of the movement is desirable, of course, and the matter may be approached from the experimental side with a working hypothesis in mind. But a 'blanket explanation' will lead to controversy unless it is based upon experimental results obtained from a wide range of organisms. It is necessary to work first on *specific* problems since the phenomena recorded and the animals studied are so varied. An explanation based on the results of experiments with one sort of copepod can not be assumed to be applicable to another or

to a chaetognath. It may not be out of place to state that the experiments that have been reported here were conducted without preconceived ideas as to the reasons for the migration. It was my belief, and still is, that it is possible to learn from laboratory experiments why a species shows rhythmic vertical movements in nature. But it has become more and more apparent that specific differences in reactions must be taken into account. I am not yet ready to state that I believe a general explanation of diurnal migration is possible. It seems evident to me, as Parker (1902, p. 122) has already said, that the simultaneous depth-migration of different organisms may really be the results of quite different causes. It is probably understood that reference is made here to the sum total of the responses rather than to the behavior with respect to any one external factor.

In order to show the differences between different animals the following brief summary of results is given. It is based entirely upon these particular experiments and no reference is made to the behavior in water of high salinity since that was tested only with *Calanus*.

PHOTOTROPISM

Acartia: very strongly positive at room temperature with surface animals; deep water specimens are strongly negative; surface animals become negative in cold water, *A. clausi* to a greater degree than *A. tonsa*. *Calanus*: strongly negative to all intensities used at ordinary temperatures, becomes strongly positive at 10° C and less. *Eucalanus*: very strongly negative; effects of cooling not known. *Labidocera*: markedly negative; effects of cooling not known. *Metridia*: strongly negative at ordinary temperatures; becomes positive when water is cooled to 10° C. *Sagitta*: very strongly positive, behavior in cold water not known.

GEOTROPISM

Acartia: both species, if the animals are obtained from the surface, are markedly negative in the diffuse light of a room, but positive in darkness, and there is an increase in negative geotropism in darkness if the water is cooled to points below 15° C; specimens from deep water are very strongly positive in diffuse light and in darkness; there is a well marked physiological rhythm in geotropism if the animals are kept in darkness. *Calanus*: at ordinary temperatures the animals are very strongly positive in darkness and under all conditions of lighting, except that when lighted from below the positive geotropism is de-

creased; there is some reversal in cold water (10° C), especially when the light is above; there is some evidence of a rhythm in the reactions of specimens kept in very dim light, but only in case the bottom water, at least, is chilled. *Eucalanus*: strongly positive at ordinary temperatures except when lighted from below, then strongly negative; behavior at low temperature not known; possibility of rhythm not investigated. *Labidocera*: it is safe to say that there is a tendency to negative form of response in darkness and to positive in diffuse light; strongly negative in light from below, strongly positive in light from above; behavior at low temperatures unknown, and existence of rhythm not investigated. *Metridia*: at ordinary temperatures it is strongly positive in darkness and in all conditions of lighting except from below, then strongly negative; the behavior shows reversal at low temperatures; existence of rhythm not investigated. *Sagitta*: if light is not too bright there is marked negative geotropism under all conditions and in darkness; in bright diffuse daylight the response is markedly positive; the animals do not descend in cold water, but the ascent occurs in darkness out of cold water as well as out of warmer.

There are of course similarities in the behavior of different forms, and this is to be expected. For instance, *Calanus*, *Eucalanus*, *Labidocera* and *Metridia* are negative to light at room temperatures; *Acartia* and *Sagitta* are positive; *Calanus* and *Metridia* become positive on cooling, *Acartia* becomes negative. It is recognized that the experimental work is incomplete, particularly in that the behavior of *Eucalanus* and *Labidocera* in cold water is not known. But even so, if the behavior as a whole, or the sum of the responses, is considered in the cases of *Acartia*, *Calanus*, *Metridia*, and *Sagitta* it is very clear that each species has its own mode of action. It is possible, of course, that as more is known about the responses of the different forms the differences will tend to disappear; and it is likewise possible that when more is known still other differences will appear. But as the results stand it is plain that a general explanation based on laboratory responses can not be given that will cover the diurnal migration for the different species. Such an explanation is desirable, but it is necessary to recognize that when many different kinds of organisms perform depth migrations, it is likely that the end results are due to quite different causes.

The matter of specificity in behavior has been referred to in publications from the Scripps Institution that deal with the result of field investigations. Michael (1913, pp. 32, 36) has shown that manner of distribution is as much a specific character as structure; and he refers

(1916, pp. viii, ix, xi) to specificities in other respects than structure. My study of the distribution of some of the Copepoda led to similar views (Esterly, 1912, p. 328) and this matter has been more recently discussed in papers on the experimental side (Esterly, 1917*a*, 1917*c*).

Other writers have had this point of view as regards the behavior of different organisms in the laboratory and also in nature. Walter (1907) was aware of specific differences in *Planaria*. The same attitude is apparent in the papers of Shelford (1911, 1912, 1914, 1915, and especially 1916) and of Allee (1912, 1913) though those investigators were primarily interested in other phases of behavior. It seems to me, as has recently been pointed out by Patten (1917, p. 267), that the attempt to arrive at blanket explanations is the main reason for the controversy over the nature of the processes involved in orientation. Such problems are primarily of physiological interest, and are therefore of a somewhat different nature than the problem of depth migration. But the general attitude of many investigators has been similar in the two cases, especially as regards the failure to recognize that we must deal with specific differences in behavior before a general law can be determined, if such a thing is at all possible. We cannot tell from studies conducted on a given kind of animal how another sort is going to act. Jennings (1909, p. 315) writes: "The man who, from a knowledge of how the starfish and sea anemone behave in the orientation to light, should attempt to predict how the infusoria behave under the same conditions, would fail completely. More striking still, he who from a knowledge of how *Stentor* becomes oriented in galvanotropism, should predict how *Stentor* becomes oriented in phototropism, would likewise fail completely. *Different organisms respond in the same tropisms by different methods of action. . .*" And again: (p. 324) "Different organisms differ in their behavior and reactions just as they do in their structure." If such statements are true as regards certain matters in the physiology of behavior, they are doubtless equally true as regards their relations between behavior in the laboratory and in nature.

If it is granted that a uniform and relatively simple explanation of the depth migration of the plankton is possible, the first preliminary step is to find out what each species does in nature. The facts about migration can not be explained until they have been ascertained. Then the study of reactions under controlled conditions should follow, and this study should be carried on with different kinds of plankton organisms. It is probable that sooner or later there will be duplications in behavior of different species, so that similar explanations will apply.

THE RELATIONS BETWEEN GEOTROPISM AND DIURNAL MIGRATION

When an animal in an experiment ascends it overcomes the influence of gravity that is always acting in the same direction with the same force; when the specimen descends it moves in the direction of the action of gravity. It is difficult to see how differences in distribution can be attributed to reactions to an unvarying agent, and I use the term geotropism to designate movement in a vertical direction or maintenance of position in an upright container. Whether upward and downward movements in a tube are reactions to gravity or not, it is certain that their *direction* is changed as variable factors in the environment are altered. In this sense it is permissible to speak of modification of the geotropism; the intention is to express the fact that direction of vertical movement changes under certain conditions, or that the position maintained in a column of water may vary as surrounding conditions change.

Variations in light intensity are constantly affecting organisms of the plankton. Such alterations are due both to increase or decrease in the amount of light entering the water and to movements that bring the organisms into regions of greater or less intensity, depending upon the direction of their locomotion. Since the source of the light that may affect the animals in the sea is always above, it seems probable that light will act as a directive stimulus. It has been suggested, however, by Franz (1913, p. 264) that organisms in the sea may be stimulated from all sides, even from beneath, by the diffusion of the light, except when they are close to the surface or to the bottom. It seems to me that this is a point worth considering. Is a copepod, for example, when at a depth of one hundred feet, aware, so to speak, that the light comes from one direction? It is possible that the light is so diffused that the external condition may be described as "more light" or "less light" instead of as "light from above." We ourselves have the experience, in a dense fog, of not being able to determine the position of the sun; the intensity of the light is nearly the same all about one, particularly toward the sky.

If, however, an animal at a moderate depth in the sea is stimulated by light from a given direction, it is necessary that a distinction be made between phototropism, as such, and modifications of geotropism that are related to the changes in light intensity apart from changes in direction. Are we dealing with "vertical phototropism" or with changes in the geotropism attributable to variations in the light intensity?

DOES THE GEOTROPISM CHANGE AS THE INTENSITY OF
LIGHT VARIES?

It was attempted, in the experiments that have been described, to ascertain whether the direction of movement in a vertical container is different in diffuse light and in darkness. In the latter case it is certain that responses coming under the head of phototropism are excluded, and it seems entirely probable that such reactions are done away with in the former case, as the experiments were conducted.

Let us note, in summary, the results of observing behavior in a column of water in darkness as compared with diffuse light.

In *Acartia tonsa* surface specimens go up in the light and down in darkness; animals from deep water are positively geotropic under both conditions. *Calanus* and *Eucalanus* are always positive in darkness as well as in diffuse light. In *Labidocera* some specimens are negatively geotropic in darkness and positively geotropic in diffuse light; but this sort of behavior was not apparent in all sets of animals. *Metridia* shows very strong positive geotropism in diffuse light and in darkness. *Sagitta* is positively geotropic in brighter diffuse light, but in more subdued light and in darkness it is negative.

So far as these experiments are concerned *Calanus*, *Eucalanus*, and *Metridia* do not show alterations in geotropism as the light intensity changes. A change is very clearly shown, however, in the behavior of *Sagitta* and of some specimens of *Acartia*, though in the latter case the habitat has some relation to the behavior. It is evident, therefore, that change in geotropism because of change in light intensity does not account for the vertical migration in most cases, on the basis of these experiments.

DOES THE GEOTROPISM CHANGE AS THE DIRECTION OF
LIGHT VARIES?

Sagitta and surface specimens of *Acartia* move toward a source of light above or below. All the others show a tendency to move away from the light whatever its position, when direction of rays is the only factor that is altered. This tendency to move from the light is more marked in some forms than in others. That is, two out of six species move toward the source of light while four move from it; or, the vertical phototropism is positive in two cases, and negative in four cases. It is not allowable, of course, to apply the results of experiments with the light beneath the column to the migration as it occurs

in nature. We come to the conclusion, then, on the basis of the experiments, that the geotropism is usually positive when the light is vertically above the column of water.

DOES THE GEOTROPISM IN DIFFUSE LIGHT AND IN DARKNESS
CHANGE AS THE TEMPERATURE AND SALINITY CHANGE?

Plankton animals that perform the depth migrations encounter variations in temperature and salinity as well as in light intensity. It is possible, therefore, that the direction of the vertical movement may be altered as the condition of the water varies. What do the experiments suggest? The tests with *Acartia* are not complete, but it has been shown (Esterly, 1917b, table 2 and p. 396) that negative geotropism increases in darkness if the water is 16° C or less, as compared with the distribution in warmer water. There is no change in the geotropism of *Calanus* in diffuse light or in darkness coincident with changes in the condition of the water. In *Metridia* there is some evidence that when the temperature is decreased the geotropism in diffuse light changes from positive to negative; the geotropism in darkness is strongly positive in warmer water, and strongly negative in colder. In *Sagitta* there is no reversal of geotropism in cold water, but the animals do not descend into strata of low temperature even when in light of such high intensity that they would otherwise be very strongly positive.

Four species have been tested as regards geotropism in cold as compared with warmer water, and in only one, *Metridia*, is it clear that the geotropism is reversed when the water is cooled.

DOES THE DIRECTION OF VERTICAL MOVEMENT IN DIRECTIVE
LIGHT CHANGE AS THE TEMPERATURE CHANGES?

The results of experiments are available for only two species, *Calanus* and *Metridia*, but the former shows some change in behavior while in the latter there is a marked reversal of the geotropism. In water at room temperature specimens of both forms move away from the light, but if in colder water they move toward the light and remain in the part of the container next the lamp. This sort of behavior is much more marked in *Metridia* than in *Calanus*.

Since but two species were used in this sort of experiment the interpretation cannot be very general. The results are suggestive, however, since they show that there is an alteration in the direction of vertical

movement in directive light as the temperature is decreased. It is important to note in this connection that the upward migration in nature does not take place, in general, in the full intensity of daylight; at any rate the accumulation at the surface occurs after dark in most cases. If, therefore, it is found in experiments that there is relatively more upward movement toward a light when the temperature of the water is lower than when it is higher, we ought not to apply this result to natural conditions unless we know that the results of field work are similar to those of the laboratory. Since we find that the upward movement in nature occurs in light of low intensity or in what is practically darkness, we should look to the results of experiments on geotropism in dim light or in darkness if we are to arrive at an explanation of the diurnal habit from laboratory tests.

TO WHAT EXTENT WILL BEHAVIOR UNDER RECURRENT EXTERNAL CONDITIONS EXPLAIN THE DIURNAL MIGRATION?

The general facts about the diurnal distribution of plankton organisms are these: at night there is greater abundance of a given species at higher levels, less abundance at lower levels; by day the abundance is less at higher levels, and greater at lower levels. One form may have a vertical range from the 100 fathom to the 200 fathom level and another may range between the surface and 100 fathoms. In each case, however, the general statement made above will apply, though it makes the matter of diurnal migration seem much simpler than it really is.

What responses are involved in such varying relations between the distribution of organisms and the environment? Since the vertical movement is rhythmic and corresponds to the change between day and night it is natural to expect that in the laboratory diurnal migration will be simulated as the light intensity changes. The upward and downward phases should take place under artificial conditions that are similar in general, if not in detail, to the natural conditions under which the animals ascend and descend if the laboratory results are to be applicable to the behavior in nature. For instance, the ascent in a vertical tube should take place in darkness if it is known that the species under experiment is most abundant at the surface of the sea during the night. Or, if the animals in an experiment remain at the top of a column of water at 10° C but not at 17° C it would be

unreasonable to hold that low temperature accounts for the negative geotropism under natural conditions, because the mean annual temperature of the surface of the sea is about 17° C. If a species is negatively geotropic in a cylinder of sea water that has been cooled to 10° C it does not necessarily follow that such animals will be found at the surface of the sea when the temperature is 17° C even though they descend to levels where the temperature is 10° C.

In the experiments dealt with here six species of plankton animals were tested. Aside from the metabolic rhythm of *Acartia* and *Calanus* there is but one form, *Sagitta*, which in experiment shows negative geotropism in darkness when the temperature of the water is about that of the surface of the sea during the summer months. Each species was tested in this regard. Even when the geotropism is reversed from positive to negative upon cooling the lower levels of the column of water, it remains to be shown that the animals will ascend and remain at the upper levels if the temperature there is 16° C or 18° C. The copepod *Metridia* is a good example of an animal that has very marked negative geotropism in water of low temperature. It would undoubtedly begin to ascend if the lower ten centimeters of a tube 50 centimeters long were in ice water; it has not been established whether it will continue to move upward under the initial impulse until the top is reached. It is just this point, however, that it is essential to settle so far as the correlation between experiment and habit is concerned. Again, the experiments on *Calanus* lead us to think that these animals will not be found at the surface in large numbers at night, yet we know that they are.

In addition to movements coincident with external changes the physiological rhythm should be considered. Rhythmic behavior in the absence of recurrent changes in light intensity was noted in two species of *Acartia* (Esterly, 1917b) and to some extent in *Calanus*. It has not been established whether the same is true of the other species. When there is an inherent tendency to move upward at a certain time of the day although the animals have been in darkness or dim light for hours, it seems evident that there is a possibility of doing away with the difficulties involved in explanations based on reactions evoked by changes in external factors. I believe that the matter of the physiological rhythm is well worth further study. In the case of *Acartia* the rhythm offers an adequate and satisfactory explanation of the ascent, at least, if the results of experiments are applied unquestioningly. But in *Calanus* so few animals in an experiment actually ascend

that the factor of the rhythm seems not to have much importance, though it probably plays some part in the depth migrations.

It is probably not well to pursue further the discussion of the matters in this section because the experiments are not complete. But omission of these points would be unwise because a most essential consideration is involved, namely:

THE RELATION BETWEEN EXPERIMENTAL AND FIELD WORK

After what has been said on this topic in other publications from the Scripps Institute (Michael, 1916, Esterly 1917*a*, 1917*c*) it is needless to say much here. It seems to me more apparent than ever that a knowledge of what organisms do in nature is indispensable for an intelligent evaluation of experimental work that is undertaken to obtain a basis for the explanation of behavior under natural conditions. An observer cannot learn what is to be explained except from knowledge of the organisms of his interest in nature. Experiment without field work will lead to error. For example, if it were said, on the basis of experiment, that *Acartia tonsa* would be most abundant at the surface by day and least abundant by night, it would be a perfectly fair statement so far as experiments with surface animals go. Yet we know that the reverse is true in nature. Again, the experimental results with *Calanus* would justify one in saying that this animal shows practically no diurnal movement. But our field results have led to a different conclusion, for if any plankton animal has the habit of diurnal migration this copepod has. If experiments point in one direction and field observations in another I believe that the latter are more trustworthy. As has been said by Michael (1916, p. xii), "Experiments . . . reveal only what transpires in a laboratory and are necessarily incapable of revealing what occurs in nature."

Both field and laboratory work are indispensable and the two should go hand in hand if it is desired to know the natural history of a species. It is practically impossible, however, in many cases to investigate behavior under experimental conditions on account of the size of the animals, trouble in maintaining specimens in captivity, and difficulty in even approaching the conditions of the natural habitat. The last consideration is one of more than passing importance. If it were possible to duplicate nature in the laboratory and then secure the element of experimental control there would be no doubt that behavior in the laboratory would reveal that in nature, in so far as individual behavior can be taken to represent that of a species.

While some conditions can be duplicated in a laboratory it is impossible to reproduce the entire environmental complex, unless it be in the case of a culture of protozoa or organisms like rotifers. This whole matter has been discussed by Hargitt (1912, pp. 51, 52) and one can hardly do better than quote him.

What right has one to assume that the reactions of an animal taken rudely from its natural habitat and as rudely imprisoned in some improvised cage are in any scientific sense an expression of its normal behavior either physical or psychical? Is it within the range of the calculus of probability that conclusions drawn from observations made upon an animal in the shallow confines of a finger-bowl, but whose habitat has been the sea, are trustworthy? . . . laboratory appliances are indispensable. But at the same time it must be recognised that they are at best but artificial makeshifts whose values, unless checked by constant appeals to nature, must be taken at something of a discount. This must be especially the case with higher organisms. It seems to the writer that until one has been able to place his specimens under conditions approximating the natural, or has at least brought them to a state of semi-domestication . . . he has small right to dogmatize as to conclusions or to make such conclusions the basis of so-called *laws of behavior*.

Burckhardt (1910, p. 200) writes as follows with regard to the artificial conditions that apply in experiments in the diurnal movement:

Kann eine Glühlampe das Tageslicht ohne weiteres ersetzen? Haben die stark divergenten Strahlen einer so nahen Lampe dieselbe Wirkung, wie die parallelen der Sonne? . . . Darf man vom plötzlichen Andrehen einer Lampe dieselbe Wirkung erwarten, wie vom allmählichen Aufgehen und Aufsteigen des Tagesstirns? . . . Mir persönlich scheint einstweilen immer noch, ausichtsvoller als die Sisyphusarbeit, im Laboratorium natürliche Bedingungen herzustellen, sei die Beobachtung der mannigfaltigen Experimente, die die Natur in den natürlichen Wasserbecken ausstellt. Haben wir das erschöpfend getan, werden uns unsere Kenntnisse auch die Sisyphusarbeit leichter machen.

The statements of Longley (1917, p. 550) may appropriately be referred to in this connection, where he mentions the color changes shown by fishes in tanks as compared with animals under natural surroundings.

There is no doubt that the questions asked by Hargitt and Burckhardt are pertinent. The attitude revealed by such questions will probably lead to another: Of what use, then, is the method of laboratory experiment? The answer will depend largely upon the end that the experimenter has in view. If he is desirous of finding, for instance, what organisms can do, or how sensitive they are to variations in outer factors, the laboratory method is the only one to use. But when the laboratory results are to be used as explanatory of habits in nature it must be said that the experimental method is of little use if it is followed without regard for its obvious limitations.

GENERAL CONCLUSIONS

The results of the experiments have led to the following conclusions of general application:

1. Owing to specific differences in behavior no general explanation of diurnal migration can be given at present. It is suggested by the experiments that each kind of organism will have its own way, so to speak, of performing the vertical movement, as each has its own peculiar responses in the laboratory.

2. There is evidence that the physiological state changes when the animals are removed from the ocean and kept in the laboratory. While this has not been noted for each species, it is necessary to guard against errors in interpretation that may arise through that cause. We should inquire whether reactions under controlled conditions in a glass vessel are similar in kind to those that would be observed if it were possible to have the element of control and at the same time retain the animals under otherwise natural surroundings.

3. It is suggested by some of the experiments that behavior is in some way related to the habitat from which the experimental specimens are obtained. In general it seems highly desirable to use both animals that are obtained in deep water and at the surface, and compare the reactions in the two cases. If this is not done the possibility remains that what is learned about habit from reactions in the laboratory is not characteristic of the species as a whole, but only of the particular specimens tested.

4. Field studies and laboratory investigation are both necessary and supplementary to each other. While studies on reaction possibilities do not require that the natural habits of the animals be known, this knowledge is a necessity when responses are studied in the expectation that they will reveal the reasons for a habit in nature.

5. So far as these experiments go (they are incomplete in many respects) there is apparently no adequate explanation of diurnal migration that can be based on responses to external stimuli except in the case of *Sagitta*. Additional information may make possible the explanations desired for the other species. It appears, however, that change in geotropism with change of light intensity or in temperature is not general enough to be considered as of wide significance.

6. The action of what may be called a physiological rhythm is clearly shown under certain conditions by the two species of *Acartia*.

There is some similar evidence in *Calanus*; but under the experimental conditions it accounts for the vertical movement of only about 20 per cent of all individuals. Whether this factor will appear in other species remains to be ascertained; but it may be of general occurrence and importance in connection with the diurnal movement.

7. While special attention was not given to the study of individual differences in behavior, the work with single animals has suggested that sooner or later experimental zoology may well be definitely concerned with the peculiarities that appear in different individuals of a species, even when all specimens are kept under the same conditions. Individuality manifested itself everywhere, and I believe that there is a fruitful field for study along that line.

In conclusion it is a pleasure to speak of the suggestions and assistance received from the resident staff at La Jolla. The seminars afforded opportunity for discussion, and there were besides many stimulating conversations. Mr. E. L. Michael was unsparing of his time in talking over matters connected with presenting the experimental data, especially as to arrangement in tabular form that will permit of testing probability by statistical methods. Professor Ritter was always ready to listen and advise, and Mr. W. C. Crandall most efficiently managed affairs connected with the business office. Capt. James Ross did the collecting offshore, and his interest and willingness are very much appreciated.

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THE PTEROPOD *DESMOPTERUS*
PACIFICUS SP. NOV.

BY
CHRISTINE ESSENBERG



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BY

CHRISTINE ESSENBERG



Nearly thirty years ago a new pteropod was discovered and described by Chun. This beautiful animal differed so greatly from any other pteropod that it did not fit in any of the larger subdivisions of that group and consequently was an object of dispute among the authors as to the place it should take in the classification. Chun (1889) named the mollusc *Desmopterus papilio*, placing it with the Gymnosomea on account of the absence of a shell. Pelseneer (1889) classified *Desmopterus* with the Thecosoma, putting it as a genus in the family Cymbuliidae. Meisenheimer (1904) in his revision of the Pteropoda separated the new genus from the Cymbuliidae and elevated it to the rank of a separate family, the Desmopteridae.

Meisenheimer (1904) gave a distribution of the group as it was then known. Reference is made to only one species, *Desmopterus papilio*. According to that report the genus is limited in its distribution to the tropical and sub-tropical zones, occurring on the entire African coast between 34° N and 37° 5' S lat., in the Mediterranean Sea, and in the Indian Ocean. In this ocean the distribution is strictly limited to the tropical zone (between 11° S and 13° N lat.). It is of interest to note that no Desmopteridae have been reported from American waters. Hence the species described on the following pages is the first representative of the group to be made known either from the Pacific or the Atlantic waters of America. The description of the present species is based on twenty-seven individuals. Two of these were taken in the surface plankton nets at the Scripps Institution for Biological Research of the University of California, 32° 53' N lat., 117° 15' 7" W long. on two successive mornings, December 7 and 8, 1917. Other specimens were taken by the United States Bureau of Fisheries steamer Albatross

on July 28, 1916, at $32^{\circ} 36' 5''$ N lat. and $118^{\circ} 7'$ W long. at stations 6684, 6686, 6687, and 6689 in the self-closing nets at the depth of fifty meters.

DESCRIPTION OF THE SPECIES

Desmopterus pacificus is one to two millimeters long, and four to five millimeters wide, measured from tip to tip of the spread wings. The body (fig. 2) is somewhat barrel-shaped. The anterior or the head end is bent ventrad. The shell is absent, but the body is covered completely by a transparent integument. The strongly developed wings, the epipodia, are attached to the anteroventral portion of the body and

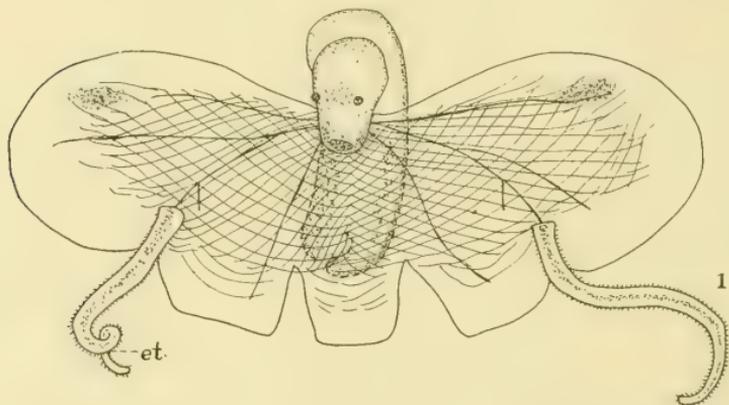


Fig. 1. Ventral view of *Desmopterus papilio* after Chun, greatly enlarged; *c.t.*, epipodial tentacles. Note the sharply bent head and the long wings.

are fused in the middle. Their posterior margin extends beyond the posterior extremity of the body. It is deeply indented, the entire margin being divided into five distinct lobes (fig. 2), one median, and two lateral on each side. The anterior margin of each wing is only slightly curved and is without indentations or lobes. Between the two lobes of the posterior margin of each wing are inserted the epipodial tentacles (fig. 2, *c.t.*). These are short, their length being only one and one-half times their width. Each tentacle is supplied with a strong nerve and is covered with cilia. Judging by the rich nerve supply these tentacles are sensory, probably tactile organs. The wings are supplied with well developed nerves. One strong nerve is sent to each lobe, subdividing and spreading there into several smaller branches, and one large nerve goes to each epipodial tentacle.

COMPARISON

Desmopterus pacificus greatly resembles in general appearance *Desmopterus papilio*, but differs sharply from the latter in some characteristics. The most striking difference is in the size and the shape of the epipodial tentacles (fig. 1). In *Desmopterus papilio* the tentacles are long, far exceeding the length of the wings and extending behind as two whip-like organs, sometimes coiled. They are broader at the base and decrease slightly in width toward the distal extremities. They are covered with cilia. In *Desmopterus pacificus* the epipodial tentacles are spade-shaped and short, their length being less than that of the

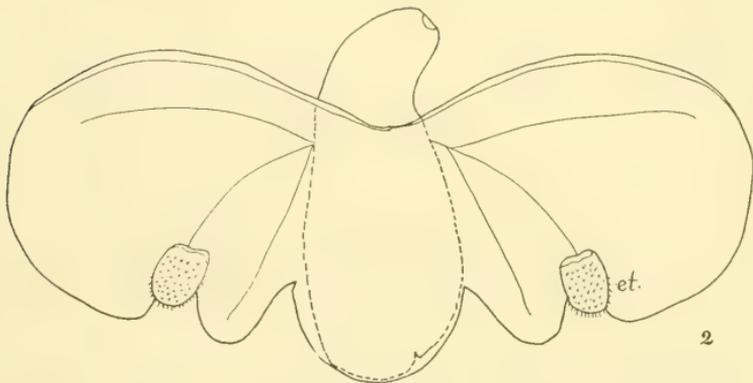


Fig. 2. Ventral view of *Desmopterus pacificus*. $\times 100$. Drawn from life.

protruding lobes of the wings. They are covered with cilia and rough elevations (fig. 2). The head of *D. papilio* is bent more abruptly than that of *D. pacificus*. The wings of the former are also proportionally longer than are those of the latter (figs. 1 and 2).

BEHAVIOR.

A few observations on the behavior of *Desmopterus pacificus* have been possible. The animal is graceful in its locomotion, and with its large wings and bright red color spots scattered in patches over the body and wings, it resembles more a butterfly than a mollusc. In locomotion *D. pacificus* uses its wings very much in the same manner as any broad-winged animal flying in the air. With a sudden, jerky movement of the wings the pteropod sets the body in motion and then

sails on through the water with great swiftness in either the horizontal or the vertical direction. From time to time when the motion becomes slower a new jerky flapping of the wings occurs, increasing thus the speed of locomotion to the original strength. Occasionally it rests motionless at the surface of the water with its wings widely spread, but the least disturbance of the water arouses the animal to sudden, new activity. Judging from the laboratory observations the pteropod seems to be in the habit of attaching itself to objects by means of the epipodial tentacles, standing then in an erect position and flapping its wings. When placed in a shallow dish, the mollusc, after some floating and swimming, usually attaches itself by its tentacles to the bottom of the dish, in the meantime gracefully moving its large wings. The latter operation is of some interest, considering the fact that the home of the genus *Desmopterus* is predominantly the high sea, where objects of attachment, except for a few floating weeds or some swimming animals, are not usually common. The fact that the animal balances its body so well while standing in an erect position for some time, suggests the possibility that this practice is not new to it but that it is an established adaptation. The observations are not sufficiently numerous to justify any definite conclusions. An abundance of living material would make possible some interesting observational studies.

On account of the transparency of the cuticle all the internal organs can be readily seen at work in the living animal. As soon as the animal dies the body turns opaque and only the solid, external form of the body and the large nerves of the wings are visible.

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STUDIES ON *GIARDIA MICROTI*

BY

WILLIAM C. BOECK



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THE CYCLE OF ENCYSTMENT IN *GIARDIA MICROTI*

INTRODUCTION

Most of the Protozoa which have been studied intensively for a long period of time have been shown to possess rhythms or cycles of what might be termed general vitality. These rhythms may be seen in the sensitiveness to environmental conditions of the individual organism or races of organisms, as in the case of *Paramoecium aurelia* (Woodruff), but more often the rhythms or cycles are evident in the reproductive activity of the protozoan. Cycles in the fission rate of *Paramoecium* were found by Calkins (1904) and by Woodruff (1905). Gregory (1909) showed that there were cycles of high and low vitality in *Stylonychia mytilus* and *Tillina magna*. The cycles were found to be fairly regular and the last work of Woodruff on *Paramoecium aurelia* (1917) has shown that even changes in the culture media and in temperature fail to modify the cycles of endomitosis which are characteristic for the species. There may be a slight initial influence on the cycle of the *Paramoecium* when the ciliates are suddenly placed in a changed environment, but after a short period, during which a readjustment of the organisms takes place, the endomitotic interval regains its normal length.

Cycles in the life history of *Haematozoa* are also known, the most classical examples of which are those of the various species of *Plasmodium*. In these protozoans not only is the reproductive process rhythmic in nature, but also that of sporulation.

In the flagellates the presence of cycles of vitality expressed in reproductive activities is also known. The haemoflagellates in their life history present striking examples of cyclic development, and one has only to watch the various free living flagellates in an ordinary aquarium to notice that a rhythm or cycle must exist for them in view of the fact that their numbers are seen to fluctuate from day to day and week to week.

For some time a cycle of encystment was suspected in *Giardia* found in the intestine of rodents, but it was during an investigation of mitosis in *Giardia microti* (Boeck, 1917) that evidence of such a cycle was found. A table in the paper referred to above showed that cysts were found upon examination in only five out of nineteen cases of infection. This fact raised the suspicion of the presence of a cycle of encystment, for if encystment occurs continuously in the life history

of each flagellate, then cysts should be present in every one of the hosts in which the examination of the large intestine had been made. (The large intestine is the region of the digestive tract in which cysts occur in greatest numbers.) Again, if encystment occurs at all times then cysts should always be found in faeces of rats infected with *Giardia*, and the number of cysts in the faecal sample of a rat should be approximately the same for each day. The process of encystment would then be an even, regular process from day to day and would not show evidence of a sudden rise and fall in the number of cysts found in the faeces.

The enumerations of the cysts of *G. intestinalis* in human dysenteric faeces made by Porter (1916) gave for the first time critical evidence of a cycle of encystment in this flagellate, which is a species allied to *G. microti*.

The importance of knowing whether or not there is a cycle of encystment in these flagellates cannot be overestimated. It would be of great significance in the therapy of dysentery caused by these organisms as well as a considerable factor to reckon with in the diagnosis of dysenteric patients by the daily examination of their stools, if a more or less regular cycle should be found to occur. It would be necessary to make a longer series of examinations in order to determine whether or not a patient were infected if a cycle of encystment is present in the life history of the flagellates causing the dysentery than it would if no such cycle were present. Accordingly daily examinations of the faeces of fifteen rats were begun and carried on throughout a period of twenty-eight days for the purpose of securing if possible sufficient additional evidence to definitely determine the facts.

MATERIALS AND METHODS

The faecal pellets of the rats vary in size from the average, about twelve millimeters in length and six millimeters in diameter, to about four millimeters in length and two millimeters in diameter. Constipation was present when the pellets of the smallest size were defaecated. The small size of the pellets could not be correlated with the absence or presence of cysts within them. At no time were the faeces liquid in consistency. There is no evidence of diarrhoea in the rats infected with *Giardia* like that caused by *G. intestinalis* in man and mice. The color of the faeces was usually a dark brown. Variations in the color from darker shades of brown to very light yellow were of no signi-

ficance as a diagnostic factor in the detection of cysts. The amount of stools defaecated each day varied and on no day did the rats fail to pass any stools.

In the examination of the stools for the first four days a modification (Boeck, 1917) of the method of faecal examination described by Cropper and Row (1917) was used. The method in its modified form is as follows: To at least one gram of faeces add thirty cubic centimeters of normal salt solution and stir with a Hamilton-Beach "cyclone" mixer for ten minutes. Then add five cubic centimeters of ether and stir for two minutes longer. The suspension is then placed in a separatory funnel and allowed to stand for five to seven minutes, during which time the two liquids will separate, the ether carrying most of the débris to the top while the cysts remain in the normal salt solution below. The normal salt solution is then drawn off into a centrifuge tube of a capacity of fifteen cubic centimeters and centrifuged for three minutes. The cysts are concentrated at the bottom of the tube and the supernatant fluid is drawn off with a pipette. A drop of neutral red solution, one part to ten thousand parts of distilled water, is added to a drop of the residue from the bottom of the tube and transferred to a slide for microscopic examination. The cysts are readily detected with a one-inch eye-piece and a four millimeter objective. The cysts measure about fourteen microns in length and six to seven microns in diameter.

The other examinations were made by making a suspension of the stools in distilled water, and stirring them until the mixture appeared uniform in density. Again a drop of neutral red solution of the same dilution as in the previous method, was added to a sample of the faecal suspension, which was then examined under the microscope for cysts. The neutral red is of great service in that it differentiates the cyst from the yeasts and débris, which in most cases are partially or totally stained while the cysts are very seldom affected by the stain and so stand out in the preparation as clear, transparent, ovoid bodies. The cysts also reveal in many instances their two or more nuclei, also the remains of the axostyle, intracytoplasmic flagella, and the parabasal bodies.

There is no doubt that the concentration of the cysts by the ether-centrifuge method is superior in accuracy to the simple microscopical examination described in the preceding paragraph, but because of the number of rats under observation this shorter method was used in this work. A count of the cysts was also undertaken at the time examinations were made. At first a haemocytometer was used, but it was

found that the cysts were never in sufficient numbers to make the use of this instrument practicable. The number of cysts were then counted in any twenty fields of the microscope, using a one-inch ocular and four-millimeter objective. The following table (1) shows the occurrence of the cysts in the faeces and the count that was made. The examinations commenced October 25, 1917, and were concluded November 21, 1917.

This table is a record of the daily examination of the faeces of the different rats, with the number of cysts counted in any twenty fields of the microscope. A negative sign signified that no cysts were found in the faeces for that day.

From a study of table 1 it will be seen that rat number 7 was negative for all the examinations. It received five treatments of magnesium sulphate in a twenty-five per cent solution and no cysts or living *Giardia* were seen in the semi-fluid stools. It was concluded that this rat was not infected with *Giardia*. Cysts were found in the faeces of rat 3 for three successive days, after which there was no recurrence. In rat 15 cysts appeared November 8 and not again until November 20 and 21, when the examinations for all the rats were concluded.

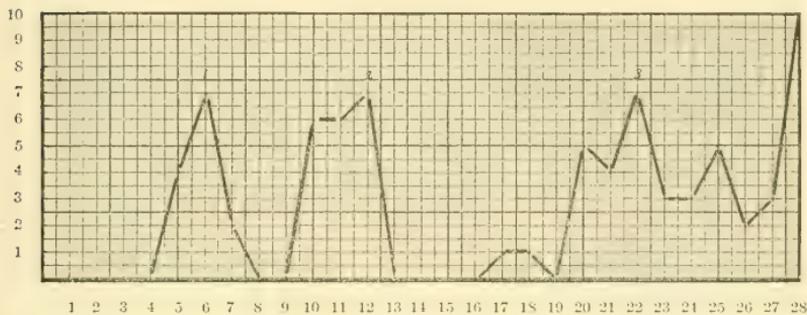
In the study of the rats in table 1, to determine whether or not there was evidence for a cycle of encystment, the data for rats 3 and 15 were not considered since neither showed two complete periods when cysts were ejected and consequently no interval could be determined between successive appearances of cysts. The data for rats 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, 13, and 14 are the only data, then, that were used in this study of periodicity in the appearance of the cysts.

The length of the period during which the cysts were defaecated with the faeces varied from one to fourteen days, and the interval during which no cysts were found in the faeces varied from one to eleven days. There is a common feature seen in the records of most of the rats in that the ejection of cysts occurred at three or four periods during the twenty-eight days of daily examinations. The highest count of cysts was recorded on November 21 in the examination of the faeces of rat 14. There were eighteen cysts in twenty fields of the high power objective.

The data for each one of the rats was plotted so that each graph resulting would represent more clearly the evidence for the presence of a cycle of encystment in *Giardia microti*. The points on the abscissa represent the days when the examinations were made, while the points on the ordinate represent the number of cysts counted in twenty

fields of the high power objective. Each one of these graphs will now be discussed.

Rat 1. This graph is plotted from the data of rat 1. Its modes are very sharply defined since the periods of depression are characterized by an absence of cysts. The first period when cysts were found in the faeces lasted from October 29 to November 1. The mode of this part of the curve was reached on October 30. A depression period of two days when no cysts were found in the faeces ensued. This period endured for three days. The mode recording the maximal number of cysts occurred on November 5. A period when no cysts were found



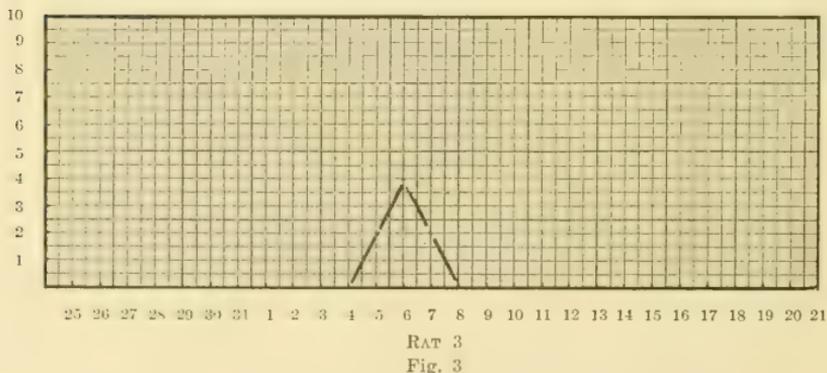
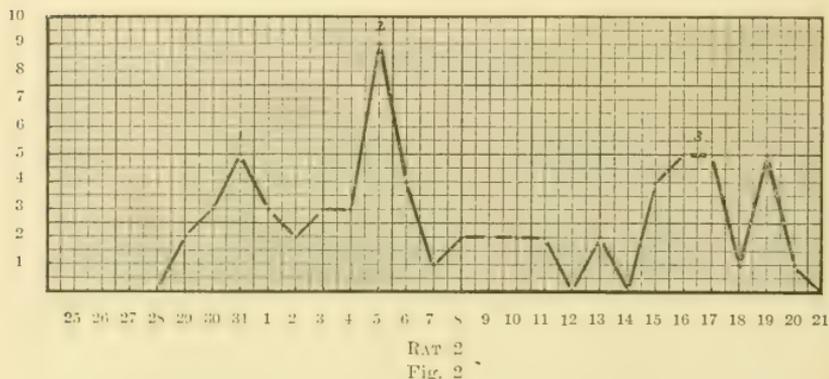
RAT 1

Fig. 1

in the faeces followed for four days, then there were two days when one cyst was detected, followed again by an examination in which no cysts were detected. Another period of cysts in the faeces took place from November 12 up to November 21, when the last examination was made. The mode of this period came on November 15, while another mode was in the process of formation because the number of cysts was increasing when the last examination was made. The interval between mode one and two is six days, and the second and third modes are separated by an interval of ten days.

Rat 2. This graph, made from the data of rat 2, is very similar to the graph in figure 1, there being three modes in the curve. The first mode occurred on October 31 when four cysts were counted in twenty fields and the period of depression followed, during which the number of cysts decreased to two on November 2. The cysts still continued to be found in the faeces and their number began to increase until another mode was reached on November 5. A sharp falling off

in the number of cysts then took place, but the cysts were not absent from the stools until November 12. Two cysts were found on the following day, but none occurred on November 14. The third period of cysts in the faeces began November 15, and although there was a decrease in the number of cysts on November 18, to rise again on the following day, this period may best be regarded as continuing from

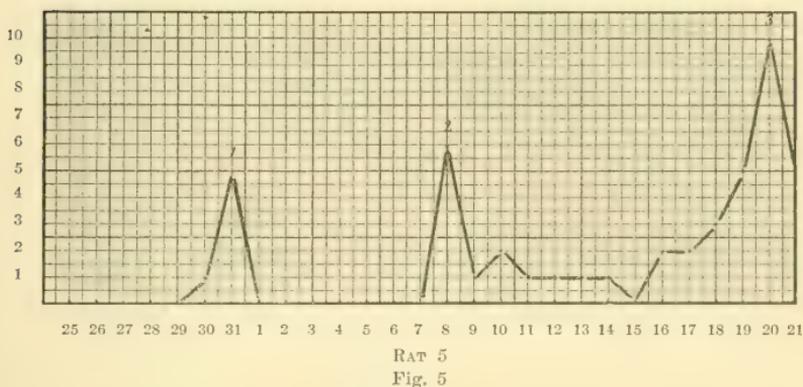
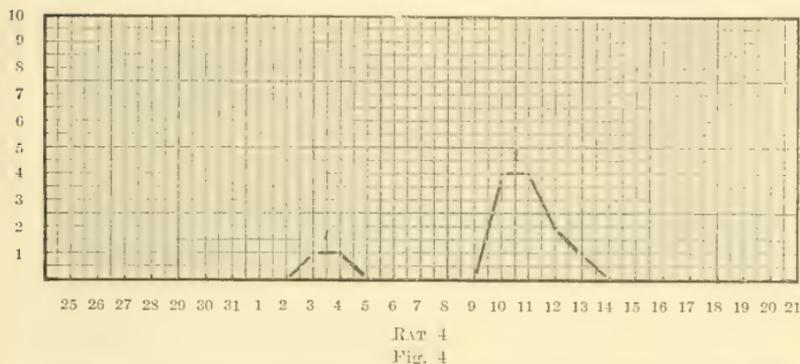


November 15 to 21, when no cysts were found in the last faecal examination. The intervals between modes one and two, two and three, are five and eleven days, respectively.

Rat 3. There was only one period when cysts were ejected, November 5 to 7, and because they never recurred again the data from this rat could not be used to contribute any evidence on our problem. The rat was found free from infection at autopsy on January 9, 1918; the organs were normal, and none of the lesions of the intestine were present which characterize an infection by *Giardia*. The fact that

this rat was infected and then became free, showed that it was capable of throwing off the infection. There was no reinfection as can be seen from the records, which run negative for thirteen days.

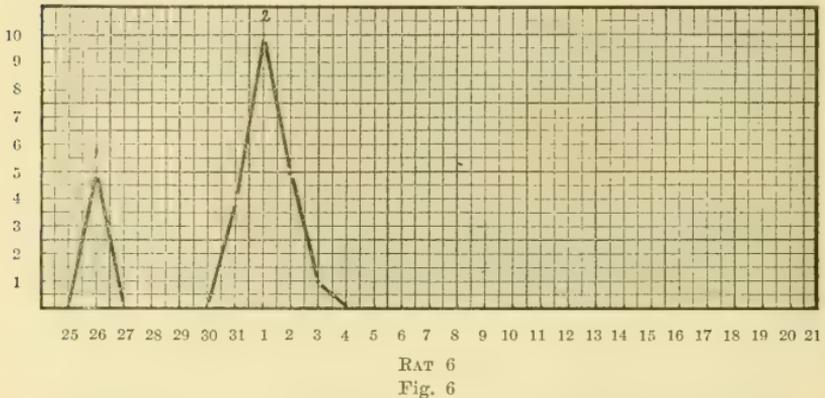
Rat 4. The degree of infection was very light in this animal for the greatest number of cysts found in the faeces was four on November 10 and 11. There were two periods when cysts were found in the



faeces. The first period was two days in length and only one cyst was found on each day. Then came an interim of depression of six days in which the examinations revealed no cysts. The mode of the second period of cysts in the faeces came on November 10, and the period ended November 19. The interval between the two modes is seven days.

Rat 5. The first period of cysts in the faeces, October 30 to November 1, results in a portion of the graph sharply set aside from the

remainder, for after this period there follows an interval of depression when eight negative examinations occurred. The next period of cysts in the faeces came on November 5, when the number of cysts increased very suddenly to six, then fell to one on the next day, and remained at two or one for the five following days. The mode of this period came on the first day, when six cysts in twenty fields were counted. No cysts were counted on November 15, but on the next day the number of cysts in the faeces began steadily to increase until the mode of this period was reached on November 20, when ten cysts were counted. The number began to decrease when the last examination was made. The intervals between modes one and two, two and three, are eight and twelve days, respectively.

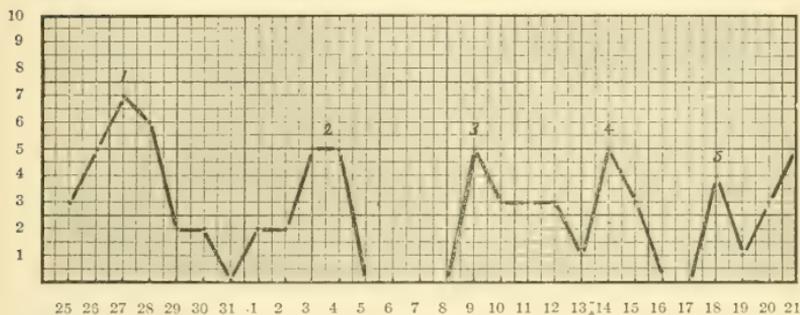


Rat 6. This graph shows that two periods of cysts in the faeces occurred. These two periods were recorded in the first eleven examinations; the remaining examinations did not reveal any cysts. The first period lasted only one day, while the second period lasted four days. The interval between the modes of these two periods was six days.

This rat at autopsy on January 9 was still infected, even though it had eighteen consecutive negative examinations. The organs were normal except that there was a small amount of gas in the jejunum. In other respects this autopsy was identical with that of rat 3. The fact that this rat was still infected after having had eighteen consecutive negative examinations showed that reinfection may have taken place between the day when the last examination was made and the day the autopsy was made. Reinfection was possible for the cages

were not cleaned of their faeces each day after the last faecal examination had been made. The cysts which might have caused this reinfection may have entered the cage by dropping through the wire netting with faecal pellets. On two instances an infected rat became free from its cage and ran over tops of the others and it is very likely that a few of its pellets could have dropped into the cages of the other rats.

Rat 8. There are three periods and an incomplete fourth when the cysts were found in the faeces. The mode of the first period occurred on October 27. The period of depression between this mode and the mode of the next period when cysts were in the faeces is very well defined in that the curve decreases abruptly from mode one until

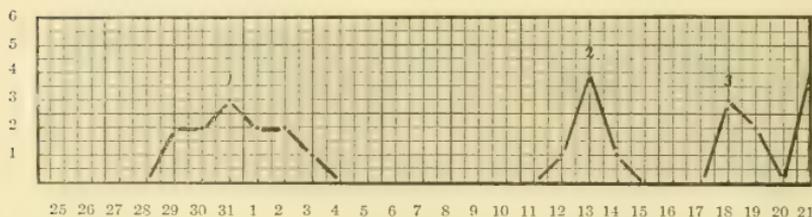


RAT 8

Fig. 7

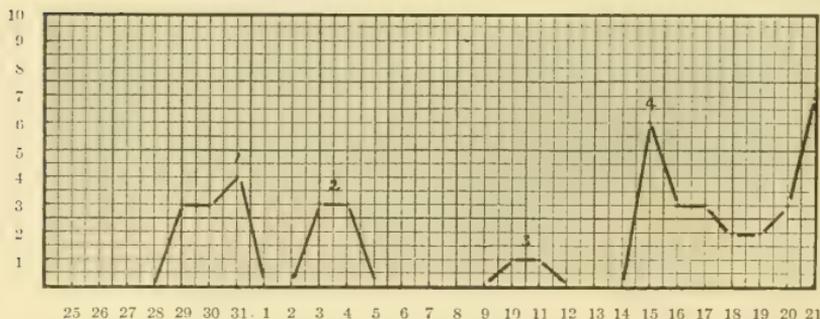
on October 31 no cysts were found in the faeces, then the curve rises again until the second mode is reached on November 3. The period of depression between the second and third mode is defined by four negative examinations. The third period of cysts in the faeces commences on November 4 and continues until November 16. Two maxima appear in the portion of the graph, and both of them have been termed modes since there is a distinct period of depression on November 12, but it is possible that we have in reality a single mode in this period. The next period starts on November 18 and was continuing when the last examination was made; but because of this last mode five may not be the true mode of this period; instead the last one may be only a stepping-stone to another mode which would have been reached if the examinations had continued a greater length of time. The intervals between the modes are $7\frac{1}{2}$, $5\frac{1}{2}$, 5, and 4 days, respectively.

Rat 9. There are three distinct periods when cysts were found in the faeces in this rat. This condition, we have seen, has been common to many of the curves already discussed. The first period of cysts in the faeces extended from October 29 to November 4. The number of cysts reached three in twenty fields at the mode on October 31. A period of depression then took place, from November 4 to 12, during which no cysts occurred in the faeces. From November 12 to 15 is



RAT 9

Fig. 8



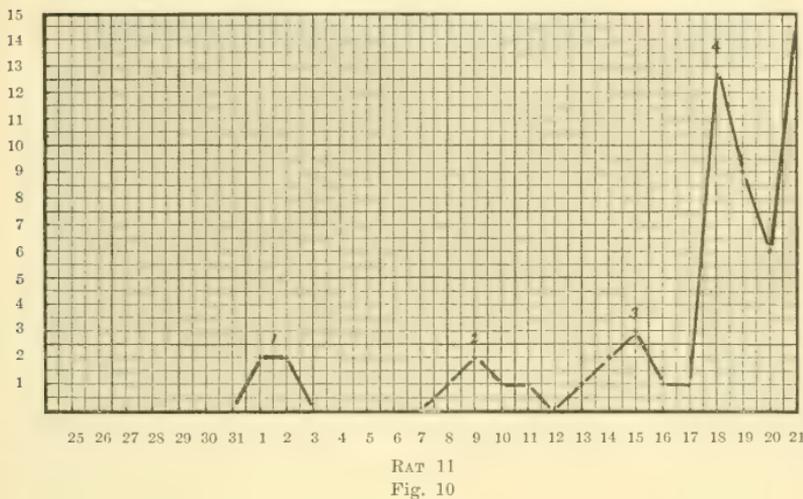
RAT 10

Fig. 9

the next period of cysts in the faeces, with the mode on November 13. A period of depression of three days followed; no cysts were counted when the examinations were made. The cysts then appeared again on November 18, on which day a third mode is reached. But as in mode five, shown in figure 7, mode three in this figure may have proved to be a part of the rising curve following it, had the examinations been continued for a few days longer. The intervals between modes one and two and two and three are thirteen and five days, respectively.

Rat 10. There are four modes representing the days when the highest number of cysts were counted in four distinct periods. The first period, October 29 to November 1, was three days in length and

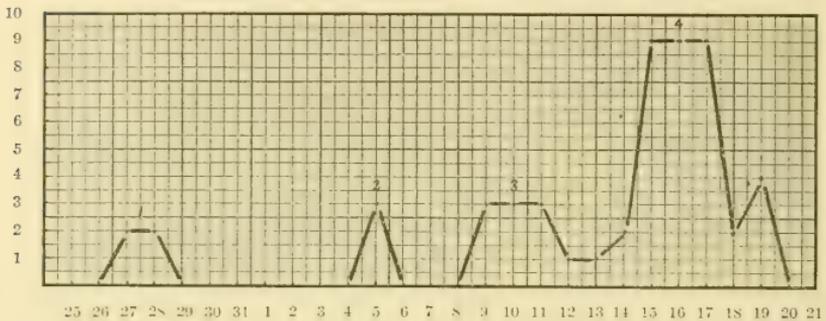
the mode came on October 31. The depression period between the first two cycles of encystment was two days in length during which no cysts were found in the faeces. The next period of cysts in the faeces was only two days in length, November 3 to 5, and four cysts were counted on each of these two days. No cysts were then found for five successive days when on November 10 and 11 one cyst only was found. A depression period of two days with no cysts precedes the last cycle of encystment, which was continuing when the last examination was made, to form another mode. The intervals between modes one, two, three, and four are 3, 2, 7, and 4 days, respectively.



The interval between the last mode and incomplete mode on November 21 is six days.

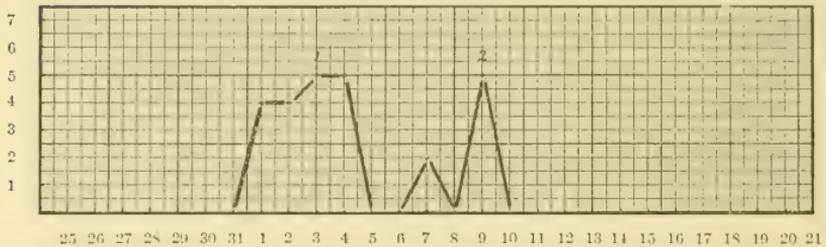
Rat 11. The first two periods or cycles of encystment were of short duration and the number of cysts was never high. The first cycle took place from November 1 to 3 and two cysts were counted on each day. A depression period of five days followed this cycle and no cysts were found in the faeces. The next cycle, from November 8 to 12, although longer than the first cycle, contained only a small number of cysts. The mode of this cycle appeared on November 9. Another mode is designated on November 15, which may be a true mode since a period of depression follows it for two days, or this portion of the cycle may be a part of the period of cysts in the faeces

which follows the period of depression on November 18, when a pronounced mode is seen in the curve. After November 18 a short period of depression is characterized by a falling off in the number of cysts found, but their number begins to increase again, and had reached fifteen cysts when the last examination was made. The intervals between the modes are $7\frac{1}{2}$, 6, and 3 days, respectively.



RAT 12

Fig. 11



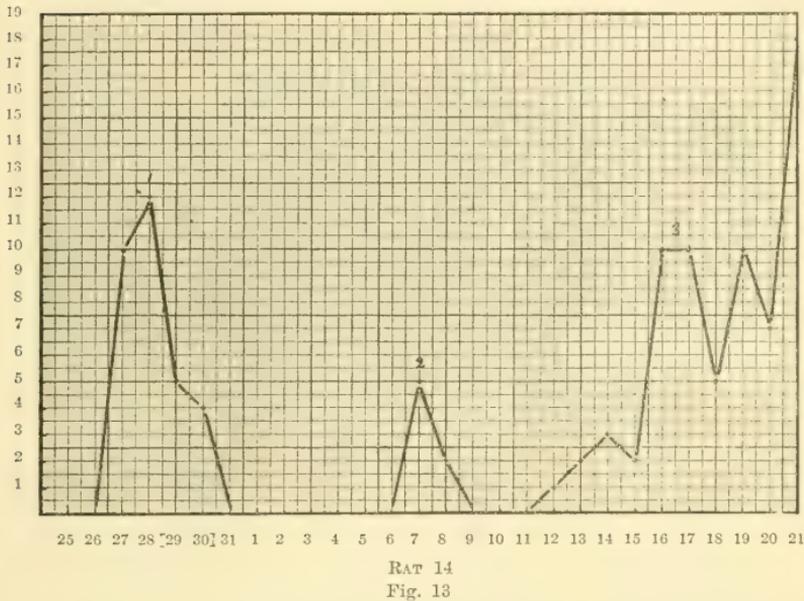
RAT 13

Fig. 12

Rat 12. There are four cycles of encystment represented in the graph which was made from the data of rat 12. The cysts were found during two successive days in the first cycle October 27 to 29. A period of depression then continued for seven days when no cysts were found in the faeces. The cycle which followed was only one day in length and only three cysts were counted in the examination of the faeces. The third cycle is preceded by two days when no cysts were found. The mode of the third cycle was reached on November 10 when three cysts were found in twenty fields. A couple of days when the number of cysts decreased to one in twenty fields then ensued, to

be followed by a cycle when the number of cysts reached nine for three successive days. The cysts had disappeared from the faeces when the last examination was made. The intervals between the modes one, two, three, and four are $8\frac{1}{2}$, 5, and 6 days, respectively.

Rat 13. The infection of the rat was light, for the number of cysts was small and there were only two cycles of encystment during the twenty-eight days. The first period when the cysts were in the faeces extended from November 1 to 5, with the highest number of

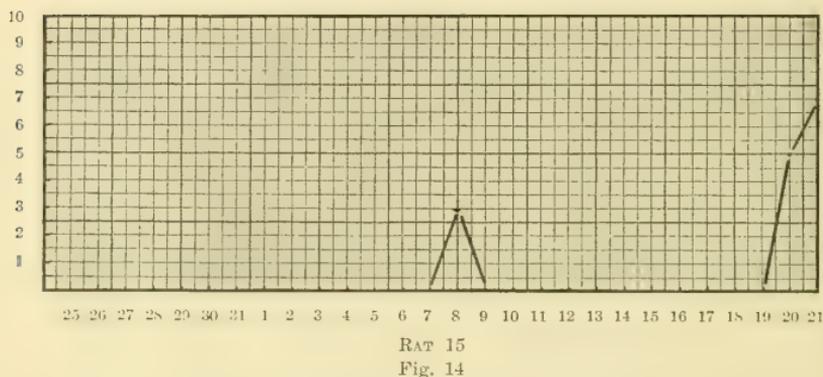


RAT 14
Fig. 13

cysts coming on November 3 and 4; then for a day no cysts were found. The next period of cysts in the faeces was from November 7 to 10. In this period there was one day, November 8, when no cysts were found; but since the number of cysts found in the other days was very small it is probable cysts were present on November 8 and escaped detection because of their small numbers. The mode of this second period was reached on November 9 when five cysts were counted in twenty fields. The interval between the two modes is six days in each case.

Rat 14. The infection in this rat was the heaviest for all the rats examined. The maximum numbers of cysts detected ran higher than

the numbers of the other rats. There were three distinct periods when the cysts were found in the faeces. The first period lasted four days, October 27 to 31. The mode of this period came on October 28, when 12 cysts were counted. A period of depression of eight days when no cysts were found in the faeces preceded the next period when the cysts recurred in the faeces. This period was short, lasting only two days, with the mode on November 7. Two days followed during which the cysts disappeared from the faeces. Then the third period when cysts recurred again in the faeces began November 12. This period extended to November 18, when a period of depression marked by a fall in the number of cysts took place. The mode was reached on



November 16, when 10 cysts were recorded for twenty fields. After the single day when the number of cysts decreased to five the number immediately increased and when the last examination was made the number had reached eighteen in twenty fields. This was the largest number of cysts found in twenty fields for all the rats examined. The intervals between the modes one and two and two and three, are 10 and $9\frac{1}{2}$ days, respectively.

Rat 15. The infection in this rat was very light, for during the twenty-eight days cysts occurred only three times and in small numbers, though the number of cysts was increasing when the last two examinations were made. It will be seen that there are not two complete periods when cysts were found in the faeces, and because of this fact these data of rat 15 could not be used to determine the cycle of encystment in *Giardia* of the rat.

The series of graphs just presented may be divided into two groups. In the first group may be placed those curves which show positive

examinations comprising less than one-half the total number of examinations. The graphs of figures 3, 4, 6, 12, and 14 fall into this class. In the second group may be placed all the other groups; in these more than one-half the total number of examinations were positive.

What caused this great difference in the degree of the infection in the rats is not known at the present time for there is no evidence at hand with which to attack this problem; but it is very significant that, even when the positive examinations comprised less than half the number of all the examinations which were made, there are still distinct periods into which the positive examinations fall.

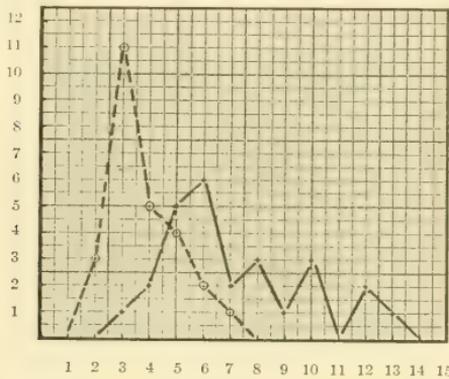


Fig. 15

Frequency curve of modal intervals.

Curve (solid) plotted by single-day units (on abscissa) of the day intervals between the modes of all the figures 1, 2, 4, etc.

Curve (broken) plotted by two-day units, of the day intervals between modes of all the curves. It is a more typical frequency curve.

The interval (average) between modes is shown to be seven days. Seven days is the interval then, between the maxima number of cysts in the faeces.

This graph represents the combined plot of the intervals between the modes of all the curves described in the preceding pages. The units on the abscissa stand for the length, in days, of each interval; the units on the ordinate stand for the frequency with which any given interval occurred.

The solid line is a frequency curve whose descent is characteristically lytic in nature; the latter feature is eradicated if only the outside points are represented in the curve. The broken line curve was made from the same data as the solid line curve except that the units on the abscissa are two days in length instead of a single day. This

doubling of the units on the abscissa results in the curve being one-half as long on the abscissa, while the height of the mode is far more increased; furthermore, it serves to straighten out the descent of the curve, giving a more typical frequency graph.

This graph presents striking proof of the existence of a cycle of encystment in *Giardia* in the rat, for we see that the average interval between the modes of the cycles of encystment is about seven days. Most of the intervals were about six days in length. In other words, the maximum number of cysts were found in the faeces about every seven days. This curve indicates that encystment falls into regular periods, the climax of each recurring period being reached about every seventh day.

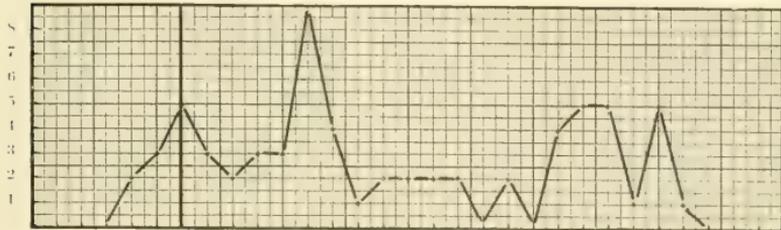
If a series of curves be so placed one above the other that the first mode of each curve lies on the same ordinate line and the rest of the curve be allowed to fall as it will, if there is a similarity or a close identity of the interval between the other modes of each curve, the modes of all the curves should, in the majority of the cases, fall along as many common ordinate lines as there are modes. Since most of the curves have three or four modes we should be able by such a handling of the graphs as that given above to detect four common ordinate lines upon which the majority of all the modes of the curves will be located. The distance between these lines would be the true interval between the crest of each mode, or the interval between the days when the maximum numbers of cysts were found in the faeces.

Such a treatment of the curves is the most conclusive proof of the presence of similar intervals common for all the curves. Obviously a high degree of exactness is impossible with the curves made from a study of the cycle of encystment in rats, since the incidence of infection varies in the case of each rat and there is also a margin of error in the detection of the infection. However, there is, as we have seen, evidence for an interval between the ejection of the maximum numbers of cysts, and this interval can be seen by superimposing the curves one above the other with their first modes coinciding, in order to determine whether or not the other modes in the curves will in the majority of cases also coincide on other common ordinates.

In figure 16 the curves have been placed one above the other so that their first modes lie on the ordinate line at point 6 on the abscissa. In this figure of superimposed curves it will be seen that the second mode for the majority of the curves is on an ordinate line, at a place between points 11 and 12 or 13 on the abscissa; the third



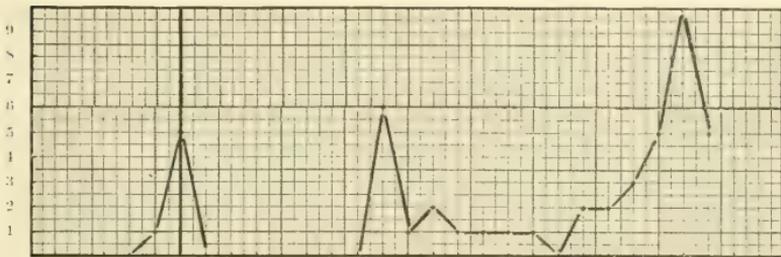
RAT 1
Fig. 1



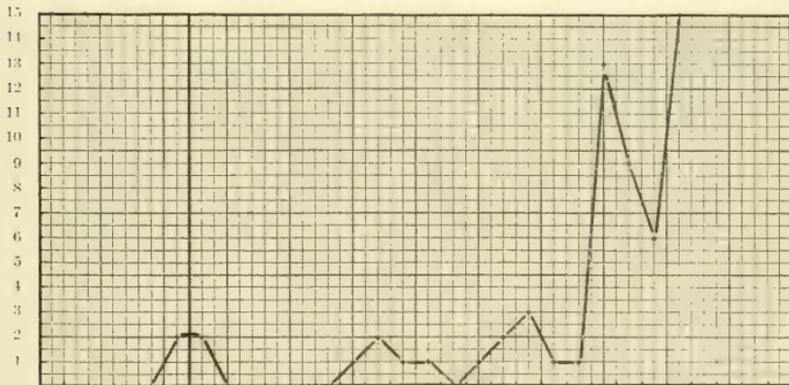
RAT 2
Fig. 2



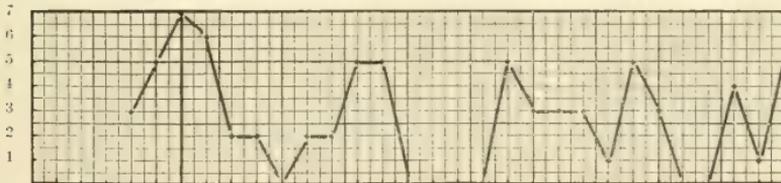
RAT 4
Fig. 4



RAT 5
Fig. 5



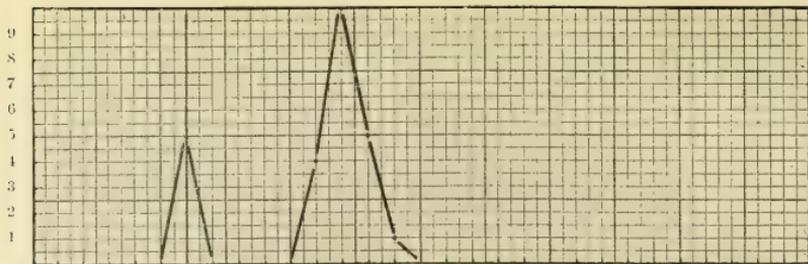
RAT 11
Fig. 10



RAT 8
Fig. 7

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

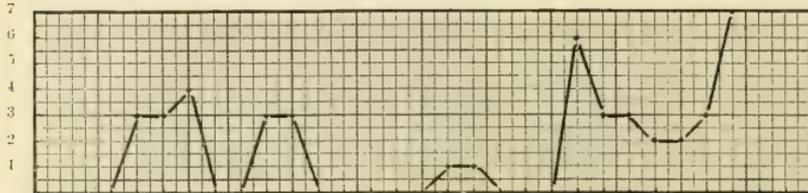
Fig. 16



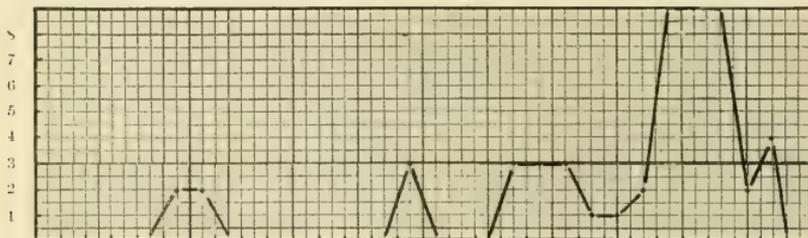
RAT 6
Fig. 6



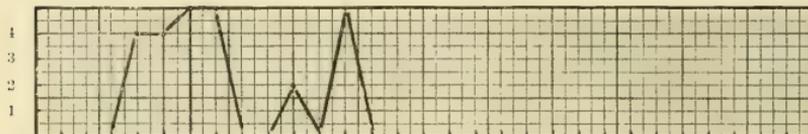
RAT 9
Fig. 8



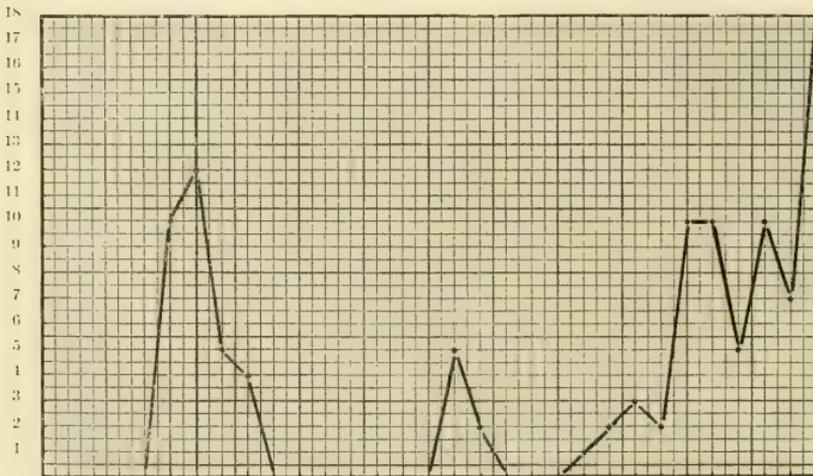
RAT 10
Fig. 9



RAT 12
Fig. 11



RAT 13
Fig. 12



RAT 14
Fig. 13

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

Fig. 17

mode of those curves having three or more modes lies on the ordinate line in the region of points 19 and 20 on the abscissa, while the fourth mode of the curves which have four modes is seen to lie on the ordinate line at points 25 or 26 on the abscissa.

In all cases these ordinate lines determined above were selected because the majority of the modes of all the curves fell on these four lines. Thus ten curves had at least two modes, and of these ten six fell on the ordinate line in the region of points 11, 12, or 13 on the abscissa. Five curves showed a distinct mode in the region of points 19 and 20 on the abscissa, and eight curves showed definite modes on the ordinate line in the region of points 25 and 26 on the abscissa. From this evidence it is justifiable to conclude that there is a common interval between the modes of all the curves when they are placed one above the other so that their first modes lie upon a common ordinate line. This interval is approximately equal between all the ordinate lines. The intervals between the ordinate lines are 6, 7, and 7 days; their average interval is $6\frac{2}{3}$ days.

The average interval of $6\frac{2}{3}$ days as obtained from the superimposition of the curves, is almost identical with the average interval of 7 days obtained by plotting the frequency of all the modes (see fig. 15). Thus by two different methods approximately the same interval has been obtained, setting the interval between the maximum numbers of cysts ejected in the faeces at about every seven days. In other words, the cycle of encystment of *Giardia* occurred about every seven days in the rats under observation during a period of nearly one month.

In the superimposition of the curves and in the consequent study of the data to determine the common ordinate lines no consideration was taken of the terminal point of all the curves, because the curve was in the process of forming another mode in most cases when our observations were suspended. Therefore, because of its incompleteness this portion of the curve was useless in the determination of the cycle of encystment.

In order to substantiate the evidence for these common ordinate lines which mark the days when the maximum number of cysts occurred in the faeces of most of the rats examined I plotted the occurrence of the average number of cysts for each day when the curves were thus superimposed. This is the average number of cysts that occurred on each ordinate line in figure 17. For example, on ordinate line 4 in figure 16 two cysts were found twice, three cysts

twice, and four cysts once. The average number of cysts on this ordinate was three cysts, and this was the number plotted in figure 18 on ordinate line 5 as representing the average number of cysts for that day. In like manner the average number of cysts was determined for the other days and plotted in figure 17.

The modes 1, 2, and 4 are at once very evident and it will be found that they lie on the same ordinate lines that were determined in figure 16. The third mode is not so conspicuous, which I interpreted to mean that the incidence of encystment was small and disturbed, possibly by some environmental factor for all the rats whose modes go to make up this *common mode*. But it will be seen, as was pointed out in connection with figure 16, that 6 rats showed a distinct mode at this area in their curve. Of the other six rats one was showing a small number of cysts at this time (fig. 5), three were negative, and no cysts were found in the following examinations (figs. 4, 6, 12), so these curves could not be considered; the other two rats were negative during these days. Therefore, *out of eight possible cases six* of them showed a distinct mode at point 19 (fig. 17), which made it justifiable to pick out this line as representing a *common mode* for most of the curves. It was the day when a maximum number of cysts was found.

The mode at 26 (fig. 17) was chosen rather than the mode at 28 because more individual curves showed a distinct mode at this day, and so represented more truly the day when the maximal number of cysts was found for the majority of all the rats.

The average interval between the modes 1, 2, 3, and 4 (fig. 17) is found to be $6\frac{2}{3}$ days, which corresponds to the interval between the ordinate lines determined in figure 16.

DISCUSSION

From the data just presented the conclusion is derived that there is a cycle of encystment in *Giardia* in the rat, that this cycle is regularly periodic, and that the interval between successive maximum numbers of cysts is about seven days.

It is fully realized that the possibility of error is fairly large in the study that has been made in determining this cycle. In the first place because of the time required for examination only one examination was made to determine whether or not a rat was negative for any given day. To eliminate this error at least three ordinary examinations should be made to determine whether a rat is negative. Had it

been possible to continue the examinations by means of the ether-concentration method, adopted later, then three ordinary examinations would not have been necessary, one examination by this method would have sufficed.

Again, in all the studies of this nature the more cases one works with the safer are his conclusions; therefore, if a greater number of rats could have been handled the conclusions would have a greater degree of certainty. But even with this relatively small number of cases, when the results are to a great extent uniform, the conclusions are at least highly significant and to a considerable degree may be relied on as presenting the truth of the situation. The data from

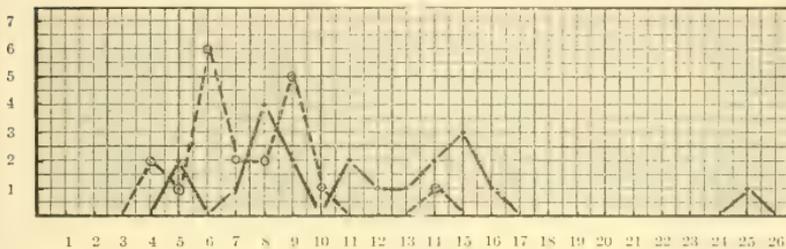


Fig. 18

Frequency curve of mode intervals; data taken from charts submitted by Porter (1916).

Solid line, interval plotted by single-day units, modes at 8, 15, 24.

Broken line, interval plotted by two-day units, modes at 6, 9, 14.

The broken line curve, a typical bi-modal polygon.

The interval between the maximal number of cysts is about 7-8 days. Fourteen days represents two intervals, and the small mode at 24 represents three intervals.

These results from studies of seven cases of *Giardia intestinalis* are very similar to those given by me for *G. microti*.

the fourteen rats presented by several methods yield in every case the same fairly uniform and equivalent cycle of encystment.

Another source of evidence is available for comparison to demonstrate the value of these results in this small number of cases. In determining the cycle of encystment for *Giardia intestinalis* from man and mice Porter (1916) noticed that there was a period of about a fortnight between the maximum number of cysts recurring in the faeces. She did not, however, call attention to the fact that there was also a period of about seven or eight days when another maximum number of cysts was to be found in the faeces. This she would have found apparently if the day-interval between the modes in all her figures had been plotted.

It is significant that a plot made of analogous data in my own study reveals only one mode (fig. 15) and sets the period at seven days as the interval between maximum numbers of cysts in the faeces. The curve is a typical frequency curve with a single mode.

In plotting the interval between the modes in the curves submitted by Porter (1916) there are two distinct modes present (fig. 18). It is a bimodal frequency curve. The solid line in figure 18 represents the interval plotted by single-day units on the abscissa, just as was done in figure 15 of my own study. Two distinct modes are seen at points 8 and 15 and a small mode at point 24. There was only one instance of an interval of 24 days (see chart 1, Porter, 1916). When these same data were plotted by two-day units on the abscissa, the

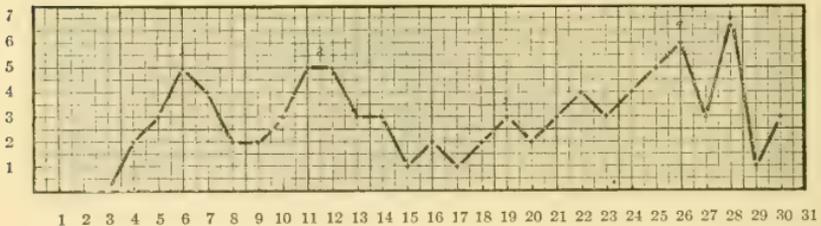


Fig. 19

A plot of the average number of cysts for each day for rats 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, leaving out of consideration the number of cysts which occurred on the last examination in each rat.

Modes 1, 2, and 4 are very definite; mode 3, although not so pronounced as the other modes, was selected since in six rats this point represented the days of a maximal number of cysts in the faeces. Mode 4 at point 27 on the abscissa was selected instead of the mode at point 29 because it was more representative of all the curves; five curves had a mode at or near point 27, while only two curves had a mode at point 29.

The average day-intervals between modes 1, 2, 3, 4, is 6½ days are almost identical with the intervals found in figure 15.

broken curve results, and again we find that there are two modes present. This curve thus plotted represents two, if not three (point 24), distinct periods at which time maximum numbers of cysts were found in the faeces for *Giardia intestinalis*.

From this study of Porter's charts and the plottings of the modes of her graphs it is evident that the cycle of encystment in *G. intestinalis* has an interval of about seven to eight days between the maximum number of cysts in the faeces. The mode at point 24 (fig. 18) is the locus of a third occurrence in one instance only of a maximum number of cysts, which suggests that there was a tendency at least for the cycle to continue at the second interval of fourteen days on the interval

of the first two maxima. The results are very striking and uniform in this series of only seven cases, and taken together with the data derived from my study of the cycle of encystment in *Giardia* of the rat give ground for concluding that there is a regular periodicity in the appearance of the cysts of *Giardia* in the faeces of about six to seven days.

It is quite possible that the species of *Giardia* found in some of the rodents, especially in *G. microti*, is one and the same species as that found in man, namely, *G. intestinalis*, because of the similarity in structure. Another reason for believing that these species may be identical is revealed by the study of periodicity made by myself on *G. microti* and by Porter (1916) on *G. intestinalis*, and also by the manipulation of her data in the plotting of the curve representing the frequency of the interval between modes, or the days when the maximum number of cysts was detected. This study shows that both of these species have almost identical cycles of encystment. This feature may be common for all the species of *Giardia*, therefore a characteristic of the genus and not a peculiarity of a single species only; but in either case it is significant as evidence toward the solution of the true etiology of giardiasis (lamblia) of man.

SUMMARY

1. There is a cycle of encystment in *Giardia* in the rat; the recurring interval between the maximum numbers of cysts in the faeces is about seven days.
2. The cycle of encystment in *G. intestinalis* is about seven or eight days, as seen in the charts submitted by Porter (1916), and not a fortnight, as she has concluded. This in reality, if not fully plotted, represents two cycles of encystment.
3. Because the cycles of encystment of *Giardia* in the rat and of *G. intestinalis* are almost identical there is another reason to infer that these two species may be one and the same. This fact may lend some aid in the solution of the true etiology and prevention of giardiasis (lamblia) of man.

DEVELOPMENT OF *GIARDIA MICROTI* WITHIN THE CYSTS

INTRODUCTION

The author (1917) has pointed out the presence of three distinct types of cysts for *Giardia microti*. These types were placed in three categories because their morphology warranted such a classification. The first of these types is the "single individual" cyst (pl. 1, fig. 1). This cyst harbors only one individual which has the usual two nuclei. The second type is the "binary" cyst (pl. 1, figs. 5-8), containing four nuclei. The cytoplasmic body may not have undergone plasmotomy, (pl. 1, figs. 4, 5) or plasmotomy may be completed with two daughter flagellates formed (pl. 1, figs. 7, 8). The third type is the "multinucleate" cysts, so called because it always contains more than four nuclei and never more than sixteen nuclei (pl. 1, figs. 12-16).

A study of these cysts and their location in the intestine of the mouse or rat at once suggest definite progressive development of the flagellate within the cyst. To give an account of this development with a view to supplying more evidence toward completing all the stages in the life history of this parasite, the following data are submitted.

MATERIALS AND DATA

The first part of these data were gathered from a study of the material from five different meadow mice, *Microtus californicus californicus* (Peale). Other preparations were made but only five series revealed the presence of cysts. The following table shows the types of cysts found in the different regions of the digestive tract of the meadow mice.

Cysts were not always found in all the regions of the digestive tract (series 28, A4) and this was explained by virtue of a cycle of encystment. In series 28 the process of encystment had been going on for some time and the cysts had already progressed as far as the colon and rectum, while in series A4, the process of encystment had just begun and so the cysts were found only in the small intestine. In series 29 and 33 preparations made from all the regions of the intestine showed the presence of cysts. Encystment was in the midst of its cycle. Series 29 and 33 present two parallel lines of data, identical in all respects except that in series 29 only single and binary cysts were

TABLE 2

Series	Sex	Date	TYPES OF CYSTS OF <i>Giardia microti</i> IN INTESTINE OF <i>Microtus</i>				
			Small Intestine	Caecum	Colon	Rectum	
28	♀	March 2, 1916	single	single	single binary	single binary	
29	♀	March 4, 1916	single	single	mostly binary	mostly binary	
33	♀	March 8, 1916	single multinucleate	single	single mostly binary	single mostly binary	single mostly binary
36	♀	February 8, 1917	single multinucleate	no preparation made			
A4	♂	September 27, 1917	single				multinucleate

found, while in series 33 not only were the single and binary cysts present but also multinucleate cysts. This latter type of cyst was found chiefly in the small intestine.

The probable reason why the cysts were found in so few of the infected mice at autopsy was the fact that a negative period in the cycle of encystment was then in progress.

In *Giardia microti* there are two types of reproduction, binary and multiple fission. In taking up the evidence for the development of this flagellate within the cysts a review will be made first of the data that are related to the reproductive method by binary fission and then the data concerned with the method of multiple fission.

Binary fission may occur in the free state of the flagellate, as has been shown by the work of Kofoid and Christiansen (1915) and by the author (1917), and also within the cyst; the latter fact has been known for a long time. Previous to the work mentioned above, describing binary fission taking place in the free state, it had been held that this method of fission took place only within the cyst. Schaudinn (1903) had noticed two individuals within a single cyst wall and had called such a cyst a "copulation cyst" because he thought the two flagellates were in syngamous union. It had been previously shown, however (Boeck, 1917), that these cysts were only binary cysts and that there is no evidence of sex in *Giardia* as Schaudinn had inferred.

BINARY FISSION WITHIN THE CYST

When encystment takes place it involves the formation of a wall around a single flagellate. This process begins in the ileum and caecum of the meadow mouse. The greatest number of single cysts are consequently found in the ileum or caecum of the digestive tract. From table 2 it will be seen that these single cysts may also be found in the colon and rectum, but their number in these regions is very small compared with the number found in the small intestine. The ratio in series 29 in one preparation was fifty-five binary cysts to five single individual cysts in the colon and rectum.

The fact that there were great numbers of single cysts in the ileum and only a few in the large intestine, great numbers of binary cysts being present in their stead, coupled with the presence of nuclear changes within the cysts, is conclusive evidence of progressive multiplication within the cysts.

The single cyst (pl. 1, fig. 1) is strongly indicative of recent encystment because one can easily detect within it all the organelles of the

flagellate characteristic of its free state, the only exception being the absence of the extracytoplasmic parts of the anterolateral, posterolateral, and caudal flagella. The intracytoplasmic portions of these last named structures are still present within the cyst.

In the metamorphosis of a single individual cyst into a binary cyst the nuclei undergo division. Distinct anaphase spindles were found in many cases (pl. 1, fig. 3), but no late prophase, metaphase, or telophase stage was found. The mitotic activity during these stages is probably very rapid while the anaphase is of longer duration. The telophase was detected in cysts of *Giardia* found in the rats (pl. 1, fig. 12). In no instance was the chromatin of either nucleus seen to have resolved itself into chromosomes. Centrosomes were distinguishable in some of the cysts (pl. 1, figs. 2, 4), but in no case were they found to be divided with one daughter centrosome located at each pole of each nucleus.

In the cyst shown in plate 1, figure 1, the nuclei of the organism appear to be in the resting stage, for the karyosome is a single ovoid mass of chromatin. This stage in the development within the cyst is very typical of the nuclear constitution of all the single-individual cysts found in the ileum and the caecum.

In the caecum single-individual cysts were also found, which, however, present the first stage of definite progressive development beyond that described in the preceding paragraph. These cysts (pl. 1, figs. 2-4) show the chromatin of the nuclei dividing or already divided into two separate masses upon a spindle, with evidence of their migration to the poles of each nucleus. This stage corresponds to the anaphase in mitosis. Passing along the digestive tract from the caecum to the colon we find a few of the single-individual cysts are still to be found, even as far down as the rectum, but their number is very small.

In the colon the next stages of development within the cysts were found. The nuclei have divided to form four daughter nuclei (pl. 1, fig. 5), which are very close to each other and in the resting stage. The cytoplasmic body within the cyst is still in the condition of a single encysted individual, no plasmotomy having as yet taken place. The axostyle has partially divided, but only two distinct parabasal bodies are to be seen.

The next stage in the progress of binary fission within the cyst is also to be found in the cysts in the colon. In this stage (pl. 1, fig. 6) two of the nuclei have migrated to a position which was earlier the

posterior pole of the body of the parent, but no plasmotomy has as yet taken place. During this stage another set of intracytoplasmic organelles, namely axostyle, anterior peristomal fibrils, and posterolateral flagella have been formed. The method of forming the axostyle is one of splitting of the parent structure (pl. 1, fig. 3), but there is no evidence at hand to determine the exact method of formation of the other organelles.

In the colon and in the rectum occur the binary cysts (pl. 1, figs. 7, 8) which represent the completion of binary fission. The method of plasmotomy appears to be a longitudinal division of the parent on a plane parallel to the major axis of the body and at first horizontal, at least in the matter of nuclear separation, for even before plasmotomy has begun (pl. 1, fig. 6) two of the nuclei and the axostyle are seen to be upon a different optical plane from that of their sister structures. The axostyle, however, appears to split in the sagittal plane. When plasmotomy has been completed a side view of the two flagellates within the cyst shows one individual above the other. The line of separation of the two flagellates may have no reference to any plane of plasmotomy. Kofoid and Swezy (1916) have shown that movement in trichomonads is very active during plasmotomy in the free state. It is quite possible that this also occurs within the cyst in *Giardia*. An oblique view (pl. 1, fig. 8) shows the two flagellates occupying such a position that their anterior ends are at opposite ends of the cyst; this is the position which they might assume if plasmotomy occurred by a longitudinal cleavage of the body in a plane horizontal to the major axis of the body. However, in typical binary cysts the two zooids lie in an end to end, back to back position.

It is noteworthy that in all the cysts of the binary type the individuals resulting from binary fission of the parent flagellate lack their respective parabasal bodies. This absence has been shown by the author (1917) to be correlated with the depletion of the reserve material in the form of these bodies during encystment, and with excessive mitotic and motor activity of the flagellate while in the free state. These bodies at first hypertrophy during encystment, often they are scattered like a cloud of chromotoidal substance in the cytoplasm (Kofoid and Christiansen, 1915), but are always absent in the free somatella stage or when plasmotomy during binary fission within the cyst has been completed.

Infection is known to occur by the ingestion of the cysts; no intermediate host is necessary. The cyst wall in all probability is digested

off when the cysts reach the small intestine. Those cysts which contain two individuals, products of binary fission, would discharge two flagellates into the intestine when the cyst wall was digested away. It is also possible that cysts might be ingested which had not completed all the stages of binary fission, because such cysts were found in the rectum, and if this happened and the cyst wall was digested away then a somatella would be liberated which could continue its development in a free stage.

A word may be said here regarding these somatella stages described many times by Kofoid and Christiansen (1915) for *G. muris* and by the same investigators and myself for *G. microti*. Most of these somatella stages represent plasmodial bodies resultant from previous mitotic activity of individuals which had not encysted. Obviously the large number of individuals present in the intestine can be explainable on the ground of binary and multiple fission in the free stage of the flagellate, but another interpretation may be proposed for the somatella stages.

These stages may be interpreted to be somatellas liberated from cysts while the flagellate body within was in the course of binary fission. Especially does this seem plausible when we compare the free somatella described by Kofoid and Christiansen (pl. 8, fig. 55; 1915) and the encysted somatella described in this paper (pl. 1, fig. 6). Furthermore, these authors figure cysts with part of the cyst wall apparently digested away and the somatella in a process of binary fission (pl. 7, fig. 38; Kofoid and Christiansen, 1915). The interpretation that these somatellas may have originated from encysted individuals which have escaped from the cyst seems justifiable in view of the facts just presented. But most of these somatella stages no doubt originate from an individual undergoing binary fission in the free state.

MULTIPLE FISSION WITHIN THE CYSTS

During the process of multiple fission within the cysts the two nuclei of the encysted flagellate go through three successive divisions to form a total of sixteen daughter nuclei. The method of division is that of mitosis, for anaphase spindles were found in many cases (pl. 1, fig. 10), but the other phases of mitosis were much obscured and were therefore detected with great difficulty. The chromatin of each nucleus was never seen to be divided into chromosomes. A somatella containing sixteen nuclei is found in every multinucleate cyst which has completed all its nuclear divisions (pl. 1, figs. 10, 11).

From table 2 we see that the greater number of these cysts were found in the small intestine, and in one series they were found in the rectum. This was interpreted to mean that encystment commenced in the small intestine (duodenum) and the cysts were in the rectum because they had been carried there by the peristaltic movement of the bowels.

Encystment, it appears, begins with the formation of a wall around a single individual, and the immediately subsequent stages are similar to those in binary fission preceding plasmotomy of the two-zoöid somatella. Many of the stages following the first division of the nuclei were not found. Some of the cysts showed twelve nuclei (pl. 1, fig. 9) due to the fact that some of the nuclei have not divided the third time. In some cysts (pl. 1, fig. 10) two of the nuclei were seen to be dividing for their last time (third division). No plasmotomy was seen to occur within the cyst, but from the evidence given by Kofoid and Christiansen (1915) it is more probable that plasmotomy takes place outside the cysts after the digestion of the wall of the cysts in the small intestine.

This method of reproduction within the cysts does not seem to be the common method employed by this flagellate, for the cysts were detected in only two cases in my preparations of *G. microti* from the meadow mouse and were never detected in some two hundred examinations of cysts of a species of *Giardia* found in the rat. It is a method which results in a greater proliferation of the individuals when compared with binary fission. In the rat the binary cysts were found in the faeces at regular intervals, showing that encystment was cyclic and not continuous or sporadic in nature. But since the multiple cysts were not found with such regularity it appears that this method of reproduction does not follow at regular intervals unless its interval of recurrence is much longer than that found for binary fission. It may be, then, that disturbance of the normal environment causes an increased fecundity on the part of the flagellates which results in the method of multiple fission for its expression. The possibility of multiple fission following the formation of a zygote by syngamy is not excluded.

These cysts when ingested would have their cyst walls digested away as in the case of the binary cysts. Since each one of the multinucleate cysts contained a somatella of sixteen nuclei an eight-zoöid somatella would be liberated to continue its plasmotomy in a free state. Cysts showing part of the cyst wall absent were figured by Kofoid and Christiansen (1915, pl. 7, figs. 42, 53) and these cysts

contained somatellas in various stages of multiple fission. The absence of part of the cyst wall is interpreted to be due to digestion and a continuance of the process would result in the liberation of the somatella. Somatella comprising as many as four zoöids were found by the investigators just mentioned, but in no case were eight-zoöid somatellas found. The case of the four-zoöid somatella appears to have resulted from the multiple fission of a flagellate which had never encysted, instead of representing a possible half of the eight-zoöid individual which might have come from a cyst.

In all the multinucleate cysts the axostyle had split to form two daughter axostyles. This is similar to the activity of the parent axostyle during binary fission, but the other organelles were not duplicated which is unlike the condition in binary fission. It is probable that the formation of the other organelles is deferred until the process of plasmotomy begins, that being the time that they appear in binary fission.

It is evident from all the data at hand that the processes of binary and multiple fission are very similar if not identical in their early stages. Both methods begin with the encystment of a single individual, and the subsequent stages up to the formation of a two-zoöid somatella are alike. The process of binary fission ceases when plasmotomy of the two-zoöid somatella results in the production of two daughter flagellates; but with the formation of the two-zoöid somatella the process of multiple fission still goes on. The nuclei have gone through one division to form four nuclei, the four nuclei go through another division and eight nuclei are formed, and these eight nuclei, when they divide, form sixteen nuclei. There are three successive divisions of the nuclei in multiple fission but only one division in binary fission. The two processes differ in that multiple fission does not appear to occur so often as binary fission since the cycle of encystment during which binary fission takes place is shorter than the cycle of multiple fission, if a cycle of multiple fission exists. It is probable also, as has been stated, that multiple fission is initiated by some environmental disturbances and, therefore, may not be cyclic but sporadic in nature.

The data for binary and multiple fission within the cysts of *Giardia microti* were taken from preparations made from the meadow mouse. A further collection of data will now be presented to corroborate the evidence for binary and multiple fission within the cyst. These further data were taken from a study of the cysts of a species

of *Giardia* found in culture rats. This species of *Giardia* resembles *G. microti* as far as the general form of the body and the organelles are concerned. The species is longer than *G. microti*, measuring 13 to 17 microns in length and 6 to 9 microns in width. The cysts, however, are approximately the same size as those of *G. microti*; they measure 11 to 13 microns in length and 5 to 7 microns in width (compare pl. 1, figs. 5, 13).

The locus of infection in the rat differs from that of the meadow mouse. In the meadow mouse the flagellates were found in all the regions of the small intestine, but in the rat the jejunum is the area that is most heavily infected. No flagellates occurred in the duodenum and only a few in the ileum. Again, it was found that the intestine of the meadow mouse was not discolored by an infection with *Giardia* (Boeck, 1917), but in the rat the jejunum is orange colored and filled with gas when *Giardia* are present. This latter condition is very similar to the condition found in culture mice and *Peromyscus gambeli* when infected with *G. muris*.

A study was made of the cysts found in the different regions of the small and large intestines in order to see if the method of development for this species of *Giardia* during encystment was similar to that of *G. microti* during its encystment.

The following table gives the types of cysts found in the different regions of the digestive tract of the mouse.

TABLE 3
TYPES OF CYSTS OF *Giardia* IN INTESTINE OF RAT

Series	Sex	Date	Small intestine	Colon	Rectum
A5	♂	Oct. 12, 1917	single binary	binary	binary
A6	♂	Oct. 21, 1917		binary	binary
200		Feb. 8, 1918	binary	binary	binary
304		Feb. 8, 1918		binary	binary
307		Feb. 8, 1918	single binary	binary	binary
313		Feb. 8, 1918	binary	binary	binary

It will be seen at once that the only types of cysts encountered in the preparations were the single-individual and the binary cysts; no multinucleate cysts were ever found. In the meadow mouse the binary cysts did not occur until the cysts had reached the colon on their transit through the intestine to the outside of the mouse, but in the rat the binary cysts occurred in the jejunum along with the flagellates in the free state. The single-individual cysts were few in number compared with the binary cysts, and so it would seem that the

division of the nuclei to form four daughter nuclei follows soon after the encystment of the flagellate. The cysts are defaecated as binary cysts, for over two hundred preparations from the faeces were examined and showed the cysts at a stage of development in binary fission the same as that found in the small intestine.

In the rat the binary cysts occurred throughout the large intestine and were found in the faeces in a stage of development which was not in advance of the stage that was found in the jejunum and ileum (pl. , figs. 14, 16). A few of the single-individual cysts were also found in the large intestine, but the number was almost insignificant compared with the number of binary cysts. The single-individual cysts of *Giardia* found in the rat resembled very closely those of *G. microti* which were found in the meadow mouse (cf. pl. 1, figs. 1, 12).

Mitosis, with its phases somewhat modified, is the method of nuclear division. At no time in the cysts examined could chromosomes be detected during the process of mitosis. Anaphase spindles (pl. 1, fig. 12) were very common. When the new nuclei are formed two remain in the anterior region of the cyst; but the other pair appear to migrate to a more posterior position, and they lie upon a different optical plane from that occupied by the other pair (pl. 1, figs. 13-16). This difference in optical planes of the two pairs of nuclei is due to the direction of the major axis of the spindle during the division of the parent nuclei. The major axis of the spindle is directed dorsoventrally, but in many cases the spindle may be tilted obliquely in an anteroposterior direction (pl. 1, fig. 12).

The fact that cysts found in the faeces are in the same stage of development as those found in the small intestine makes it appear that further development of these cysts in the intestine is arrested. The two-zoöid somatella present in the binary cysts was never found to have undergone plasmotomy while in the cyst, as was the case in *G. microti* found in the meadow mouse. It is very probable that further development is dependent on the cysts being ingested by another rat. The action of the enzymes of the stomach it is believed serves to prepare the cyst wall for digestion when the cyst reaches the small intestine; otherwise, we should expect those cysts found in the small intestine before the defaecation to lose their walls, without making ejection of the cysts from the rat and subsequent ingestion necessary. The reason why the development of this species of *Giardia* found in the rat should be arrested during encystment is a matter of

conjecture at this time. It is very probable that this species is different from *G. microti* although their structure is very similar. If it is a new species then it is very probable that there is simply a difference in the rate of development between *G. microti* and this new species. The first stages of development are similar in all respects and there is reason to believe that the following stages are also similar. The greatest difference is in the location of the different stages in the various regions of the intestine. The fact that binary cysts were found in the small intestine leads one to believe that the early stages of development, including the formation of the two-zooid somatella in this new species, were more rapid than the corresponding rate in the formation of these stages in *G. microti*, but that after the cysts had gone through these stages of binary fission the rest of the development was slower or arrested when compared with *G. microti*, which continued to pass through all the other stages of binary fission even to the completion of plasmotomy within the cyst.

On the other hand, the presence of cysts in the faeces in an early stage of binary fission might be accounted for by a more rapid rate of peristalsis in the rat compared with the rate of peristalsis in the meadow mouse. In order to test this hypothesis the cysts which were found in the faeces were incubated at 31° C for several days continuously and examined at intervals of one, four, and five days. The temperature of 31° C was chosen because it was lower than the optimum of most of the bacteria found in the faeces. This would tend to prevent excessive proliferation on the part of the bacteria which would hasten the death of the encysted flagellates. This temperature is considerably lower than the body temperature of the rat, but it has been used for the cultivation of Protozoa with some degree of success.

Upon the examination of the cysts at the intervals stated above no noticeable changes in the development within the cysts were found. The condition of the body within the cyst was identical with that when the cysts were defaecated. The cysts decreased markedly in their number, which may be due to their death through the agency of the bacteria. The incubation failed to cause any further development but it cannot be said that this experiment disproves the supposition of the effect of peristalsis. Incubation with higher temperatures and with cultures of cysts free from bacteria should be tried before condemning the hypothesis.

SUMMARY

From the data which has been reviewed in the foregoing pages the following facts regarding the life cycle of *G. microti* are established.

There are two distinct phases in the life history of the flagellate: the one a free vegetative phase; the other encystment.

During the vegetative phase the animal may pass through the following stages:

As an adult organism it may undergo mitosis in which case there is:

Subsequent formation of a two-zoöid somatella and later plasmotomy to form two daughter flagellates; or,

The formation of an eight-zoöid somatella, followed by plasmotomy to form eight daughter flagellates; or,

A single flagellate may encyst.

During encystment the animal may pass through the following stages:

By binary fission two daughter flagellates may form within the cyst which will be liberated when the cysts are ingested by another host.

By multiple fission an eight-zoöid somatella may be formed which will be liberated to complete plasmotomy upon the ingestion of the cyst by another host and the digestion of the cyst wall.

In case cysts should be ingested before they have completed either binary or multiple fission, a somatella would be liberated upon the digestion of the cyst wall which would continue its development as a somatella in a free state.

THE PARABASAL BODIES OF *GIARDIA MICROTI*

INTRODUCTION

In many of the parasitic Protozoa the process of nutrition does not always result in a growth of the cytoplasmic body, but instead there may result the formation of bodies in the cytoplasm, which act as reserve food materials. These bodies may be intimately connected with the motor activity of the organism, especially with the metabolic activity, in which case they will be used later during rapid growth, during reproductive periods, or during encystment, when the original source of nutrition is cut off.

These bodies have been called *metaplastic* bodies because they result from the metabolic activity of the organisms and are deposited in the cytoplasm. Among these bodies may be cited the paramylum grains of flagellates, the paraglycogen grains of gregarines and ciliates, the plastinoid granules of coccidia, and the *parabasal* bodies of flagellates.

FUNCTION OF THE PARABASAL BODIES

In a previous paper by the author (1917) a short account was given of the behavior of the parabasal bodies of *Giardia microti*. It was pointed out then that these organs were metabolic reserve centers of food materials, which acted as "conveniencies on the part of the flagellate for coping with the intestinal medium in which it lives. They appeared to be more intimately connected with the metabolic activity of the parasite rather than its motor activity."

This hypothesis was based only upon morphological aspects of these bodies during encystment and during the life of the parasite in the free state. For the sake of completeness the activity of these parabasal bodies during encystment and in the free state of the flagellate may be reviewed.

In the free-living adult (full-sized) flagellate there are two parabasal bodies located in the posterior third of the body and lying dorsal and usually across the axostyle. The bodies are usually elongate fusiform in shape. In the cyst the bodies may be two or more in number and are greatly hypertrophied at the beginning of encystment. In the cyst the bodies are located in various regions but always in the posterior part of the body. They often spread out like the tail of a

comet (pl. 1, figs. 1, 4, 5, 13, 15, 16), or they may appear as conglomerations of a material, cloud-like in character through the cytoplasm. Such was the condition of the parabasal bodies during multiple fission in *G. muris* (Kofoed and Christiansen, 1915).

During mitosis in the free state of *G. microti* the parabasal bodies are always present in the stages of the prophase and in the metaphase, but they were often lacking in the anaphase and telophase and almost without exception they are missing in the stages of plasmotomy. The only exception was an instance described in which a parabasal body was seen in one of the daughter flagellates in the process of plasmotomy of a two-zoöid somatella in the free state (Boeck, 1917). In the investigations upon *G. muris* during mitosis the parabasal bodies were absent in the late phases of mitosis and always absent in the two-zoöid somatellas resulting from binary fission (Kofoed and Christiansen, 1915). Likewise in the four-zoöid somatella resulting from multiple fission of a flagellate in the free state, the parabasal bodies were lacking.

With the encystment of *G. microti* the parabasal bodies hypertrophy to a great extent (pl. 1, fig. 1) and as the process of binary fission within the cyst continues the bodies disappear (pl. 1, figs. 6-8). In multiple fission within the cyst of *G. microti*, there also occurs an increase in size of the parabasal bodies (pl. 1, fig. 9), and when the sixteen nuclei have been formed in many cases the parabasal bodies are absent or they appear faint (pl. 1, fig. 11). The somatella stages found in a free state may have resulted from their liberation from a cyst by the digesting away of the cyst wall, and in these stages, it has been pointed out, the parabasal bodies have disappeared.

It was on these morphological aspects of the parabasal bodies during mitosis and during encystment that the conclusion was reached that these bodies were reserve food centers to be utilized during periods of reproduction and during encystment, when the original source of food supply had been cut off. At such periods as these there is an extra drain on the food supply of the flagellate, since the rate of metabolism during reproductive activity is great, and since during encystment not only are reproductive processes carried on, necessitating a drain on the food supply, but also encystment itself may extend over a long period of time, which necessitates an extra food supply. This food reserve is depleted at the end of encystment, when, for the most part, the reproductive processes within have also been completed.

BIOCHEMICAL AND STAINING QUALITIES OF THE PARABASAL BODIES

More evidence, not morphological but biochemical in nature, is required to determine the function of the parabasal bodies.

When either the free forms or cysts of *G. microti* are stained with Heidenhain's iron haemotoxylin the parabasal bodies appear very dark, and for this reason it was previously thought that these bodies were chromatoidal in nature and of probable chromatinic origin. In *Giardia microti*, however, there has been no evidence of chromidia within the nuclei or escaping from the nuclei. Again, increase in size of the parabasal bodies is not accompanied by a decrease in size of the karyosome of the nuclei, so this evidence would militate against the origin of the parabasal bodies from the nuclei.

Since iron haemotoxylin is a selective stain for both chromatin and cytoplasmic structures, which in most cases are of cytoplasmic origin, it was thought more prudent to use other stains substantively, in order to fix the chromatophilly of the parabasal bodies. When the preparations of flagellates and cysts were stained with acid fuchsin and methyl green the parabasal bodies appeared red and the chromatin of the nuclei appeared green. From this reaction the parabasal bodies were thought to be acidophillic, and so differed from the chromatin of the nuclei which was basophillic. In about half of the flagellates the parabasal bodies appeared to be situated upon or in a definite area of the cytoplasm, limited by a membrane-like structure. In other cases the two parabasal bodies appeared as if stained by iron haemotoxylin, i. e., just two fusiform bodies. But when the area enclosed by a membrane-like structure appeared, that portion of the area not occupied directly by the parabasal bodies was lighter in staining reaction than the parabasal bodies or the cytoplasm surrounding this parabasal area. There seemed to be evidence of another substance along with the parabasal bodies, the two together constituting the parabasal complex, so to speak. This other substance might well be the ground substance, or parabasal-plasm within which the parabasal bodies are situated.

To substantiate the acidophyllie nature of the parabasal bodies preparations were treated with basic fuchsin. In these preparations no flagellate was seen in which the parabasal bodies or parabasal-plasm was stained, but in all cases the parabasal-plasmic region was identified as a more refractive area than the surrounding cytoplasm. It was certain, then, that the constitution of the parabasal bodies

differed from that of the chromatin of the nuclei, that their constitution was acidophyllic. Some of the very preparations which when stained with basic fuchsin revealed no parabasals were subsequently treated with iron haematoxylin and the parabasals reappeared; they had been present but the basic fuchsin did not stain them.

Alexeieff's (1917) work with flagellates, especially *Trichomonas augusta*, has shown that the parabasal rod is of mitochondrial constitution. Instead of the rod, in times of division there may be a row of mitochondrial granules which he believes form the parabasal rod by forming chondriomites. He has also shown that the mitochondria are structures which secrete glycogen, which is utilized by the motor and metabolic activity of the organism.

When preparations of free flagellates killed in Schaudinn's fluid and fixed in ninety-five per cent alcohol were treated with Lugol's solution to test for glycogen in the parabasal bodies two distinct conditions of the parabasal-plasm were noted. The parabasal bodies themselves did not appear but only the glycogen contained in the parabasal-plasm. In the first place the glycogen was seen as a rectangular, mahogany colored mass filling all the parabasal-plasm for no membrane-like structure could be detected; or the glycogen assumed a thin rod-shaped mass, or was in two smaller masses. In the latter two cases, however, these glycogen masses were lying the parabasal-plasm, which was easily identified by the more darkly stained, ovoid, membrane-like structure which surrounded them. In the second place, other individuals did not show any glycogen present in the parabasal-plasm, but the area itself was clearly seen as a refractive, rectangular body lying in a position identical with that occupied by the parabasal bodies. The cases which revealed no glycogen in the parabasal-plasm were about the same in number as those that showed glycogen present. Out of fifty flagellates, twenty-three showed glycogen present, twenty-four showed the absence of the glycogen, and three flagellates showed no glycogen or parabasal-plasm present. These three cases were interpreted to mean the complete absence of the parabasal bodies and parabasal-plasm.

It was thought at the time that perhaps those flagellates which showed only the parabasal-plasm present actually lacked parabasal bodies. Accordingly some of the preparations treated with Lugol's solution were stained with iron haematoxylin, and after examining fifty flagellates forty-seven were found to have parabasal bodies and three did not. This was identical with the ratio counted in the preparation

treated with only Lugol's solution. But out of the forty-seven flagellates showing parabasal bodies present twenty of them showed the presence of the parabasal-plasm lying between the parabasal bodies. This was attributed to previous treatment with iodine since preparations not treated with iodine previous to staining with iron haematoxylin, fail to show as distinctly the presence of the parabasal-plasm.

Some glycogen is lost through processes of killing and fixation of the flagellates, so that the glycogen which remains is only a portion of the original amount present in the parabasal-plasm. In view of this fact, in the case of those flagellates which showed only parabasal-plasm without glycogen, the lack of glycogen may have been due to the loss of the comparatively small amount present in the parabasal-plasm previous to the killing and fixation of the preparations.

ORIGIN OF PARABASAL BODIES

The origin of the mitochondrial granules found in *Trichomonas augusta* around the nucleus, along the parabasal rod, and in the axostyle may be of nuclear origin since previous to mitotic activity a distinct chromidial cloud is seen about the nucleus. These chromidia may have been extruded from the nucleus since there is also an intranuclear chromidial cloud present at the same time. Alexeieff (1917) also finds that the mitochondrial granules are azurophyllic when stained with Giemsa. This reaction being characteristic of the chromatin of the nucleus Alexeieff believes that the reaction is further evidence of the probable nuclear origin of the granules. It seems safe to infer from this paper that if the mitochondrial granules form the parabasal rod indirectly this rod is also of nuclear origin.

It has been mentioned previously, however, that in *G. microti* there is no evidence of chromidial extrusion or chromidial clouds, and because the parabasal bodies are markedly acidophyllic there seems little evidence for attributing nuclear origin to these organs. Again, glacial acetic acid in the killing fluid failed to dissolve the parabasal bodies, which would have been expected if they were mitochondrial in constitution. From the evidence at hand it appears best to designate these parabasal bodies along with the parabasal-plasm as structures derived directly from the cytoplasm. They are metaplastic in nature, since they are formed from the anabolic processes of metabolism and tend to disappear when the katabolic activity exceeds anabolism. This is especially marked during the final stages of mitosis, plasmotomy

and encystment with its incident processes of reproduction. When the parabasal bodies have disappeared they are reformed from substances out of the cytoplasm, when metabolism is again normal.

Alexeieff (1917) has termed the parabasal bodies of flagellates the "kinetoplaste;" but it does not seem that this name is appropriate for the parabasal organs of *Giardia* since as we have seen that there are actually two substances which make up the parabasal complex. In cognizance of the presence of the parabasal bodies themselves lying in or upon another substance it seems to the author best to refer to the bodies as parabasal bodies which secrete glycogen. The glycogen is stored up in the other substance, the ground work or *parabasal-plasm*. The word kinetoplaste connotes the motor but not the metabolic significance of the parabasal bodies and because, as we have seen, the parabasal bodies are more intimately correlated with the metabolic activity of *Giardia* it is best to discard the word and to still refer to the complex as parabasal bodies and parabasal-plasm.

SUMMARY

From the foregoing data the parabasal bodies were found to be:

Acidophyllic in constitution.

They are of cytoplasm origin.

Their function is to secrete glycogen which is retained for subsequent use in the parabasal-plasm. The glycogen constitutes a reserve food supply which is utilized during the reproductive period and during encystment.

THE THERAPEUTIC VALUE OF BISMUTH SUBNITRATE
AND BISMUTH SALICYLATE IN THE TREATMENT
OF GIARDIASIS (LAMBLIASIS) OF RATS

INTRODUCTION

The use of these two salts in the chemotherapy of giardiasis of man was attended with some success in England and consequently encouraged further treatments with these same chemical compounds in order to ascertain their true therapeutic value.

The account of the bismuth treatments of giardiasis of man was given by Porter (1916), who made an enumerative study of the cysts of *Giardia intestinalis* occurring in the stools of dysenteric patients.

PROCEDURE

Finding a large number of rats infected with *G. microti*, a species similar to *G. intestinalis* (the history of which was followed for a period of one month by daily faecal examinations) six were selected which showed the heaviest infection. The degree of infection was determined by the number of positive examinations of the faeces made daily during the month. Three of the rats were treated with bismuth sub-nitrate and the other three with bismuth salicylate.

It was found that the best way to administer the dose is to spread the salt (powder) on water-soaked bread each day. The rats then ate the bread at the same time, receiving approximately a full dose of the salt. The rats did not object to the treatment, although at different intervals they appeared very nervous, slow in movement, often sluggish, to some degree ferocious, and their coats manifested a certain degree of roughness. Periods of constipation also occurred, and in the case of one rat no faeces were defaecated on one day. Otherwise constipation was indicated by the defaecation of very small pellets.

As has been said previously, a history of the six rats was known throughout a period of about a month. Table 4 shows the degree of infection of each rat for each day during a period of twenty-eight days when daily examinations had been made of the faeces. The number in each square represents the number of cysts counted for that day in any twenty fields of the microscope by the use of a one-inch ocular and four-millimeter objective. A negative sign in the square means that no cysts were detected in the stools for that day.

TABLE 4

DAILY NUMBER OF CYSTS IN THE FECES OF THE SIX RATS TO RECEIVE BISMUTH TREATMENT

	October											November															
	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Rat 1	4	7	2	6	6	7	1	1	5	4	7	3	2	5	2	2	10
Rat 4	1	1	4	4	2	1
Rat 8	3	5	7	6	2	2	2	2	5	5	5	3	3	3	1	5	3	4	1	2	5
Rat 9	2	2	3	2	2	1	1	4	1	3	2	4
Rat 10	3	3	4	3	3	1	1	6	3	2	2	2	2	3	7
Rat 12	2	2	3	3	3	2	1	1	2	9	9	9	2	4

The treatments began on December 18, 1917, and continued for three weeks, until January 8, 1918. The dose for man was a teaspoonful of either of the bismuth salts three times a day, and usually after five to ten days of treatments the infection appeared to be cured (Porter, 1916). This dose for man amounted to about 3.6 to 4 grams of the chemicals each day.

To arrive at dosage for the rats a dose was proportioned according to the ratio of the body weight of man (about 146 pounds) and the average weight of the rats (about 300 grams). This dose was found to be about 20 milligrams each day. This was the first dose tried with the rats, but was considerably increased as the treatments progressed (table 5).

In making the daily faecal examinations of these six rats faecal emulsions of the stools were prepared in distilled water each day during the course of treatment. A drop of this emulsion was transferred to a slide, adding to it a drop of neutral red solution $\frac{N}{10,000}$ (Boeck, 1917), and then the preparation was examined for the cysts of *Giardia*.

When the cysts were found their number was counted in any twenty fields of the microscope, using a one-inch ocular and a four-millimeter objective. A plus sign was placed in the square of the table (table 5) for that day and the number of cysts counted was placed above the sign. If no cysts were detected in the first coverslip preparation from the emulsion, after stirring the emulsion a second preparation was made and by the same procedure a third preparation if the second one turned out to be negative. At the end of three preparations no cysts were detected, then the stools were designated negative for that day, and a minus sign was written in the square of the table for that day. If, however, cysts were found in either the second or third preparation their number was counted and a plus sign written in the square for that day, placing the number of cysts counted above the sign and the number of the preparation below the sign. It took three negative examinations to make a rat negative for that day on which the stools were collected.

The dose of twenty milligrams was first administered to the rats. The dose was the same for the bismuth subnitrate and bismuth salicylate. After seven treatments with a dose of twenty milligrams the daily examinations still showed rats 1, 4, 9, and 12 to be infected, but rats 8 and 10 showed no evidence of infection, there being no cysts in

TABLE 5
 TABLE SHOWING THE DOSAGE OF THE BISMUTH SALTS GIVEN TO THE RATS. IT ALSO SHOWS THE RESULT OF THE FAECAL EXAMINATION FOR EACH

Dose in milligrams	December												January										
	18	19	20	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	
1. Bismuth salicylate	0	3	11	8	...	4	4	4	4	4	6	2	3	3	3	2	5	5
8. Bismuth salicylate	0	1	2
12. Bismuth salicylate	0	...	2	2	3	10	18	5	5	5	11	6	2	1	2	5	4	4	4
4. Bismuth subnitrate	0	2	2	1	1	1	2	2	2	2	1	3
9. Bismuth subnitrate	0	...	2	2	4	5	7	1	*	...	2	2	2	2	2	2	2	2	2	2	2	2	2
10. Bismuth subnitrate	0	1	1

* No faeces.

the faeces. On December 25 the dose was increased to thirty milligrams for two days and then to fifty milligrams for three days, for the fact that four rats remained infected led one to believe that the dose previously used in the treatments was insufficient to effect a cure.

After increasing the dose to fifty milligrams the crystals of the bismuth subnitrate were seen in the faeces of rats 4, 9, and 10. The dose certainly must have been adequate since the faeces contained a great amount of the crystals, and the chemicals must have come in contact with the flagellates.

The rats appeared quite ill as a result of the large dose and the treatment was dispensed with for two days. On January 1 a dose of thirty milligrams was again administered and this dose was increased to sixty milligrams on the following day. A dose of thirty milligrams followed by fifty milligrams was given the rats on the next two successive days. Treatment was suspended on January 5 and 6, and sixty milligrams were given on the last day.

At the end of this treatment with these two bismuth salts rats 1, 4, 9, and 12 were still infected. Cysts were found in the faeces of all these rats, except rat 4 on the last day. But rat 4 showed cysts in the faeces two days before the treatments were concluded. Only rat 8, treated with bismuth salicylate, and rat 10, treated with bismuth subnitrate, appeared to be cured.

DISCUSSION

From table 4 we see that these two rats, 8 and 10, were heavily infected during the twenty-eight days that their faeces were examined. But during the treatment cysts were found in the faeces of rat 8 on only two different and successive days, while cysts were found in the faeces of rat 10 only on one day. Apparently the drug had cured these rats.

The rats were then posted and at autopsy no sign of pathological disturbance of the organs was seen. The jejunum, the seat of infection by *Giardia* in rats, was not discolored nor did it contain any gas. In cases of infection the jejunum is usually orange colored and contains gas. The upper and lower duodenum, jejunum, and ileum were examined for free forms and cysts of *Giardia* and none were found, nor were there any cysts in the colon or rectum. *Octomitus muris* was very abundant in the jejunum and ileum. The absence of *Giardia*

in both these rats at autopsy, showed them to be free from infection by this flagellate.

It would seem that these two rats, 8 and 10, were cured by the action of the chemicals, but such a conclusion is not altogether warranted when we consider that the other four rats remained infected. Another interpretation of these two cases is justifiable, viz.: In the study of the cycle of encystment in *G. microti* rat 3 showed only one period when the cysts were found in the faeces. At autopsy this rat proved to be free from an infection by *Giardia* and the conclusion reached was that the rat had been capable of ridding itself of the infection, one way or another.

It will be noticed that in the cases of rats 8 and 10, which appeared cured by the chemicals, treatment with these salts of bismuth did not take place until December 18. This date was twenty-six days, approximately four weeks, after the day when their period of infection of twenty-eight days, November 21, had been concluded. There was an interim, then, of nearly four weeks during which these two rats could have thrown off the infection.

If rats 8 and 10 threw off the infection previous to the treatment with the salts of bismuth then all the days subsequent should have showed no cysts in the faeces. This, however, was not the case for rat 8 showed cysts in the faeces on two successive days, December 27 and 28, and rat 10 showed cysts in the faeces on December 22. A possible explanation of the presence of these cysts may be that they are perhaps the result of a short period of reinfection.

Reinfection might have occurred by the transfer of pellets from rat 9 into the cages of rats 8 and 10. Rat 9 escaped from its cage one night and was found the next day running along the cages of the other rats. If reinfection took place then, according to the data both rats 8 and 10 must have thrown off the infection for the second time for no cysts were in the faeces after the single period of infection in either of the rats and they were not present at autopsy.

Even though reinfection might have taken place it is altogether probable that the salts of bismuth did cure these two rats; but the persistence of the infection in the other four rats certainly militates against the practicability of these chemicals as a specific cure for giardiasis.

Porter (1916) reports the cases of three men infected with *G. intestinalis* and treated with bismuth salts in which an apparent cure was affected. It is significant to note that daily faecal examinations of

these men were not made after the time the cysts disappeared from the stools, following treatment with the chemicals. These men were then discharged from the hospital as cured from giardiasis. Porter recognized the presence of a cycle of encystment in *G. intestinalis*, but this factor was not taken into account when the men were determined free from infection. It is very probable if these men who appeared cured could have been watched for several days longer and their stools examined daily, that the cysts might have recurred. The fact that the cysts were absent from the stools after several treatments with the salts of bismuth was not conclusive proof that the men were free from the infection of *Giardia*; this could only have been ascertained by a series of daily examinations for the purpose of determining whether or not the cysts recurred in the faeces at the proper time in the cycle.

SUMMARY

From the experimental work conducted with the salts of bismuth on rats infected with *Giardia* and the results obtained with the treatment of giardiasis in man with the same chemicals it is certain that the therapeutic value of bismuth subnitrate and bismuth salicylate is negative in the treatment of giardiasis. The conclusion is supported by W. L. Yakimoff, W. J. Wassilevski, and N. A. Zwietkoff (1918), who state the inefficacy of these chemicals in the chemotherapy of this disease.

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EXPLANATION OF PLATE

All figures are of cysts of *Giardia microti* from the meadow mouse and of the cysts of *Giardia* found in the culture rats, drawn with camera lucida from smear preparations. Magnification, $\times 2750$.

PLATE 1

Fig. 1. Ventral view, single-individual cysts found in small intestine and caecum; nuclei in resting stage. Two parabasals dividing.

Fig. 2. Dorsal view, single-individual cyst found in caecum; nuclei dividing, parabasals in division.

Fig. 3. Dorsal view, single-individual cyst found in caecum of meadow mouse; nuclei in anaphase, small parabasals, axostyle completely split.

Fig. 4. Dorsal or ventral view, single-individual cyst, chromatin in one nucleus divided into two masses. Parabasals dividing. Cyst from caecum of meadow mouse.

Fig. 5. Dorsoventral view, binary cyst from colon of meadow mouse; nuclei divided, two parabasals.

Fig. 6. Dorsoventral view, binary cyst from colon of meadow mouse; four nuclei, new organelles, no parabasals.

Fig. 7. Side view, binary cyst from colon of meadow mouse; shows plan of cleavage of the parent body to form two daughter flagellates. No parabasals.

Fig. 8. Side view, binary cyst from colon and rectum of meadow mouse. Two complete individuals with their organelles, no parabasals.

Fig. 9. Dorsoventral view, multinucleate cyst, two axostyles, large parabasals, twelve nuclei. Found in small intestine of meadow mouse.

Fig. 10. Dorsoventral view, multinucleate cyst, two axostyles, twelve nuclei, parabasals. Found in small intestine of meadow mouse.

Fig. 11. Dorsoventral view, multinucleate cyst from small intestine of meadow mouse; two axostyles, no parabasals, sixteen nuclei.

Fig. 12. Dorsal view, single-individual cyst from jejunum of rat; nuclei in anaphase, large parabasals, axostyle partially split.

Fig. 13. Dorsoventral view, binary cyst from jejunum of rat; four nuclei, large parabasals.

Fig. 14. Dorsoventral view, binary cyst from colon of rat; four nuclei and three large parabasals.

Fig. 15. Ventral view, binary cyst from jejunum of rat; four nuclei in resting stage, parabasals.

Fig. 16. Side view, binary cyst from jejunum of rat; four nuclei two exostyles, large parabasals.

Many of the cysts in the preparations show a shrinkage away from the cyst wall; this is attributed to plasmolysis at the time of fixation.



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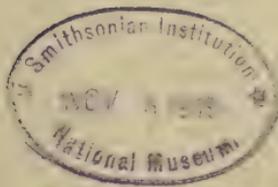
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October 4, 1919

A COMPARISON OF THE LIFE CYCLE OF
CRITHIDIA WITH THAT OF *TRYPANOSOMA*
IN THE INVERTEBRATE HOST

BY

IRENE McCULLOCH



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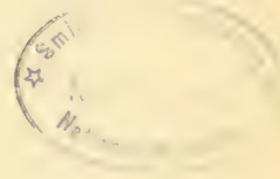
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A COMPARISON OF THE LIFE CYCLE OF
CRITHIDIA WITH THAT OF *TRYPANO-*
SOMA IN THE INVERTEBRATE
 HOST

BY

IRENE McCULLOCH



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INTRODUCTION

In my work on the flagellate parasites of hemipteran insects during the past five years the evidence indicating a close relationship between *Crithidia* and the crithidial stages of *Trypanosoma* has been continuously accumulating and has become more and more convincing. The relationship is shown both in their morphology and in the stages of their life cycles. This paper presents a comparison of the morphology and the life cycle of the genus *Crithidia* with that of *Trypanosoma* in its crithidial stages.

Before taking up this comparison a brief discussion of the phylogenetic relations of *Leptomonas*, *Herpetomonas*, *Crithidia*, and *Trypanosoma* will be of value in clarifying the subject. In addition a short discussion concerning the hosts, their food, and their methods of infection will give a fundamental conception of the problem in hand.

The phylogenetic relation of these intestinal flagellates, *Leptomonas*, *Herpetomonas*, and *Crithidia*, to the haemoflagellates, or *Trypanosoma*, has received the attention of many investigators and consequently has been the subject of much controversy. Only a brief statement of the historical side of this controversy need be given here. Minchin (1908) discusses the possible sources from which the trypanosomes may have been evolved, namely, from the herpetomonad-like and the trypanoplasma-like ancestors. At that time as well as now the evidence pointed to the crithidial (herpetomonad-like) forms as the true recapitulative, developmental, primitive stage in the life cycle of *Trypanosoma*. Minchin (1912) stated:

The types denoted by the generic names, *Leptomonas*, *Crithidia* and *Trypanosoma* form a perfect evolutionary series with monogenetic parasites of invertebrates culminating in digenetic blood parasites. It must be emphasized, however, that any such conclusions are of a tentative nature and can have no finality but are liable to modification with every increase of knowledge concerning these organisms.

Wenyon (1913) also discusses the whole question of the phylogenetic relationship of *Leptomonas*, *Herpetomonas*, *Crithidia* and *Trypanosoma*. If the trypanosome be regarded as the highest stage of development then his conclusion is that the phylogenetic order of these flagellates would be: *Leptomonas*, *Crithidia*, *Herpetomonas*, and *Trypanosoma*. He makes a distinction between the genera *Lepto-*

monas and *Herpetomonas*. *Leptomonas* is a flagellate having the non-flagellated and herpetomonad form in its life cycle; *Herpetomonas* has these two stages, together with a crithidial and a trypaniform stage. But such a distinction has not been generally accepted. His chief reason for placing *Herpetomonas* next to *Trypanosoma* is the presence of a trypaniform phase in the life cycle of a few herpetomonad flagellates, as, for example, those found in *Musca domestica* and *Drosophila confusa* (Chatton and Leger, 1911).

From the viewpoint of the present study of these flagellates the hemipteran insect hosts have been frequently divided, for convenience, into the plant-feeding and the blood-sucking types. According to Patton and Cragg (1913) some species of the three families of plant-feeding Hemiptera, the Pentatomidae, the Lygaeidae, and the Coreidae have been found to serve as hosts for either crithidial or herpetomonad flagellates. One more family should now be added to this list, namely, the Pyrrhocoridae, to which the "lupine bug" (*Euryophthalmus convivus*) belongs. Among the blood-sucking Hemiptera some species of the family Reduviidae and the family Cimicidae are parasitized by haemoflagellates having a cycle in a vertebrate host and in some cases by other flagellates having only the invertebrate or insect host. Among the hosts discovered in the family Reduviidae we find *Triatoma* (= *Conorhinus*) *megista* Burm., the invertebrate host of *Schizotrypanum cruzi* (Chagas, 1909) and *Triatoma protracta* Uhler, the invertebrate host for *Trypanosoma triatomae* (Kofoid and McCulloch, 1916).

In addition to the hemipteran insects the Diptera (flies, sheep ticks, Siphonaptera (fleas), and Anapleura (lice) also serve as hosts for some of the herpetomonad, crithidial, and trypaniform flagellates. the rat-flea (*Ceratophyllus fasciatus*) is one of the invertebrate hosts of *Trypanosoma lewisi*. Frequent reference will be made to this flagellate in the comparison of *Crithidia* with *Trypanosoma*.

The methods of infection of the above hosts have been described as casual, "cross," and hereditary. Of these three methods Patton (1908) proved that nymphs of *Lygacus militaris* became infected by ingesting with their food the feces of infected adults. The feces contained encysted or spore forms. Porter (1910) described two additional methods for the infection of sheep ticks, the "cross," and the hereditary. The former occurs among insects with cannibalistic habits, the contents and parasites of the digestive tract of one host individual being eaten by another. The hereditary infection has been

described for *Melophagus ovinus* (Porter, 1910). The erithidial flagellates penetrate the wall of the digestive tract, make their way into the ova, and thus infect, the next generation. Infection among the blood-sucking insects is usually due to the taking in of infected blood from the vertebrate host.

Closely associated with the habits of these hosts in securing their food are the variations in the structure and life cycles of the infecting flagellates. In general it may be stated that the haemoflagellates, i.e., trypanosomes, have two hosts in their life-cycle, an invertebrate and a vertebrate, while "natural," or leptomonad, herpetomonad, and erithidial flagellates are thought to have normally only the invertebrate host. Blood-sucking hosts can be infected with both types of flagellates, the so-called natural flagellates and the haemoflagellates, or trypanosomes. Plant-feeding hosts, so far as known, are infected with the natural flagellates only. Patton (1909) came to the conclusion that all the erithidial stages found in the insect host are natural parasites and that the blood parasites, trypanosomes, undergo no developmental process in the invertebrate host. On the contrary, the work of Minchin and Thomson (1915) on *Trypanosoma lewisi*, which is the most conclusive that has been done, clearly proves that this haemoflagellate at least does pass through a definite life-cycle in the digestive tract of the flea.

Another interesting feature of the natural flagellates is brought out in the recent work of Fantham and Porter (1915a, b, c). They conclude that intestinal flagellates of insects such as *Herpetomonas pattoni*, a natural flagellate of the intestine of the flea, can adapt themselves to life in the blood of mice. These investigators have also proved that other intestinal flagellates from insects, such as *Herpetomonas jaculum*, *H. stratiomyiae*, *H. pediculi*, and *Crithidia gerridis* become pathogenic to mammals when the latter have been fed on, or inoculated subcutaneously or intraperitoneally with them. *Herpetomonas jaculum* and *Crithidia gerridis* have also been successfully fed or inoculated into cold-blooded hosts, namely, fishes, frogs, toads, lizards, and grass snakes, according to the above investigators.

My work with flagellates of the hemipteran insects has been confined to a study of the erithidial flagellates of two supposedly plant-feeding insects, the box elder bug, *Leptocoris trivittatus* Hahn, parasitized by the flagellate *Crithidia leptocoridis* (McCulloch, 1915), and the lupine bug, *Euryophthalmus convivus* Stal, containing *Crithidia euryophthalmi* (McCulloch, 1917), and flagellates of one of the blood-

sucking bugs, *Triatoma protracta* Uhler, infected with *Trypanosoma triatomae* (Kofoid and McCulloch, 1916).

It is the purpose of the present paper to compare the structure and life cycle in the *Crithidia* of a plant-feeding bug with the crithidial stages of *Trypanosoma* in the invertebrate host. In this comparison I shall use as a basis *Crithidia euryophthalmi*, parasitic in *Euryophthalmus convivus*, and the crithidial stages of *Trypanosoma lewisi* found in *Ceratophyllus fasciatus*, the account of which as given by Minchin and Thomson (1915) is the most complete of its kind. It is desired to emphasize at this point that our knowledge of the whole of the life cycle of *Crithidia* in any one insect host is meager as compared with the knowledge gained from the vast amount of time and energy which has been devoted to the study of the invertebrate cycle of *Trypanosoma lewisi* in the rat-flea. However, *C. euryophthalmi* for some stages has proved to be the most valuable material for study, and has added materially to our knowledge of the life cycle of these parasites; for instance, numerous stages of an endogenous budding process have been found in preparations of this flagellate.

In addition to considering the comparative morphology and life history of these two flagellates, comparison will be made with *C. leptocoridis* (McCulloch, 1915) and with *Crithidia* as studied by previous investigators. *Schizotrypanum cruzi* (Chagas, 1909) and *Trypanosoma triatomae* (Kofoid and McCulloch, 1916) will also be discussed.

This work has been carried on under the direction of Dr. C. A. Kofoid, to whom I am indebted not only for his personal interest in the progress of the work but also for advice and helpful criticism.

HISTORICAL SUMMARY

In 1915 Minchin and Thompson published the results of their long and careful investigation on the life history of one of these flagellates, the rat-trypanosome, *Trypanosoma lewisi*, in its relation to the rat-flea, *Ceratophyllus fasciatus*. These authors stated that their purpose in the investigation was to work out the life history and mode of transmission of a trypanosome (in its relation to an invertebrate host) as fully as possible, in order to give us a standard with which the life histories of other species of trypanosomes might be compared as they become known. They selected the rat-trypanosome, *Trypanosoma lewisi*, a haemoflagellate unable to live in human blood and non-

pathogenic to its vertebrate host, the rat. The invertebrate host of this haemoflagellate is normally the rat-flea, which transmits the flagellate from rat to rat. Both the vertebrate and the invertebrate host of *T. lewisi* are widespread, abundant, easily procurable, and adaptable to laboratory conditions. To make certain that none of the stages of the life history of the so-called natural flagellates should be confused with stages of the life history of *T. lewisi* a stock of uninfected fleas was procured for the breeding cages. The rat-fleas have frequently been found to be infected with *Leptomonas pattoni*.

At the time of the publication of the Minchin and Thomson paper I was working on the morphology and life history of *Crithidia caryophthalmi* (McCulloch, 1917), a form which occurs in the alimentary tract of *Euryophthalmus convivus*. This material was of great interest; the intracellular process of multiple fission was found, and the initial infective spores were relatively abundant. Previously some time had been spent in studying the morphology and life history of *Crithidia leptocoridis* (McCulloch, 1915), which infects the digestive tract of the box elder bug, *Leptocoris trivittatus*. It had also been pointed out in a superficial way that the individuals of various forms, shapes, and structures found in a typical infection of this crithidia, *C. leptocoridis*, were apparently analogous to many of the corresponding figures of *Schizotrypanum cruzi* (Chagas, 1909) in the invertebrate host. As the investigation of *C. caryophthalmi* and *C. leptocoridis* proceeded it became increasingly easy to link the life history of *Crithidia* with the life history of *Trypanosoma* in the invertebrate host. This was especially true of the life history of *T. lewisi*. In order to demonstrate clearly the essential facts of the life cycle of one of these important flagellates a brief outline of the life history of *T. lewisi* will be given, based upon the work of Minchin and Thomson.

The developmental cycle of *T. lewisi* in the flea is divided into two phases, characteristic of the parts of the digestive tract in which the trypanosomes are found, viz., the stomach and the rectal phase. The cycle in the rat-flea requires a minimum of five days for its complete course. The trypanosomes enter the stomach of the flea with the blood of an infected rat. They show the characteristic structure of a trypanosome, the "kinetonucleus," or parabasal body, being posterior to the nucleus and the undulating membrane well developed. The change in the medium is probably responsible for the physiological changes which result in the bodies becoming more cylindrical. The trypanosomes then penetrate the epithelial cells of the stomach and

undergo the process of multiple fission, a process which characterizes the stomach phase. The initial steps in this process after the trypanosome has entered an epithelial cell of the stomach, is the formation of a "tailed" sphere. This is a spherical structure with the flagellum and some cytoplasm protruding at one point. A little later the the "tailed" sphere becomes "tailless," and shows internally the formation of a variable number of daughter individuals. In the formation of these daughter individuals, the nucleus, parabasal body, and blepharoplast each divide an equal number of times, forming merozoites of a crithidiomorphic type, i.e., a long, free type of trypanosome whose external movements are crithidial but whose structure still shows the parabasal body posterior to the nucleus. The crithidiomorphic flagellates may do one of two things: enter other epithelial cells and undergo the process of multiple fission, or collect at the pyloric opening of the stomach, to be carried down through the intestine into the rectum with the food. The intestine ordinarily serves as a passageway for the parasites from the stomach to the rectum, but under certain conditions rectal forms may migrate forward and attach themselves to the wall of the intestine in the postpyloric region.

The rectal phase is established by the entrance of the crithidiomorphic forms into this region of the digestive tract, and this region becomes the permanent source of infection throughout the life of the flea. During the migration from the stomach to the rectum structural changes take place in the crithidiomorphic trypanosomes. The posterior end of the body becomes club-shaped, and this shifting of the cytoplasm assists in the forward movement of the parabasal body to a position anterior to the nucleus. Following this condition, binary fission brings about the production of the smaller, minute crithidias which characterize this phase of the life history. The established rectal phase is described as consisting of: (1) the attached or haptomonad form, which is the multiplicative stage of the rectal development; (2) the free or nectomonad form; and (3) the final trypanosome form which is instrumental in infecting another host.

The above outline of the life history deals only with the developmental series; but in both the stomach and rectum there are present individuals which show degeneration and belong consequently to the degenerative series. I have found that the life history of *Crithidia caryophthalmi* can be correlated advantageously with the life history of *T. lewisi* in the invertebrate host, the flea. The life history of *C. caryophthalmi* is not the life history of a haemoflagellate but

apparently that of a more primitive flagellate (pls. 2-6) found living in the medium of the fluid contents of the alimentary tract of a plant-feeding insect. As such, *C. euryophthalmi* (fig. A, 2) lacks in the stomach phase the trypaniform characteristics, namely, the blepharoplast (*bl.*), the parabasal (*pb.*) (or kinetonucleus) located posterior to the nucleus (*n.*), and the well developed undulating membrane. In this connection it is interesting to note that, regardless of the contrast in the initial stages of the stomach phase in each life cycle, the process of multiple fission characterizes the stomach phase for both the haemoflagellate and the more primitive crithidial flagellate. In the stomach phase of the crithidial parasite there are also found the "rounding up" forms (pl. 3, figs. 24, 25), the spheres (pl. 3; figs. 26-32), and the final stages of multiple fission wherein the resulting merozoites are about to escape from the epithelial cell of the host (pl. 3, fig. 39). In addition to this I found numerous stages of an endogenous process of multiple fission (pl. 2, figs. 11-23).

Correlation of the life histories of these forms (*C. euryophthalmi* and *T. lewisi*) became more difficult in the stage following the process of multiple fission, on account of the structure of the digestive tract of the host, *Euryophthalmus convivus*. The digestive tract of the flea (cf. Minchin and Thomson, 1915, text-fig. 1) shows the stomach as a prominent enlargement of the tube, followed by a comparatively long, slender intestine, at the posterior end of which is the rectal enlargement. The digestive tract of *Euryophthalmus convivus*, or lupine bug, on the other hand, is quite unlike that of the flea (cf. McCulloch, 1917, text-fig. 1). The first enlargement of the mid-gut proper is followed by a second and a third enlargement, a narrow constriction of the digestive tract separating the three expansions. Posterior to these there is a relatively long intestine which passes through the center of a ruffled, ribbon-like gland. The intestine opens into the slight enlargement near the entrance of the malpighian tubules, which in turn opens into the rectum. Since the several parts of the digestive tract of the hemipteran and other insects have not been satisfactorily homologized as yet and the nomenclature used in describing the divisions is confused, it is exceedingly difficult to ascertain the homology of these several parts of the digestive tract. However, as indicated in my preliminary paper (McCulloch, 1917), all three enlargements anterior to the intestine were considered as parts of the stomach proper and were accordingly designated as the "crop," mid-stomach, and pyloric expansion. With this disposal of the three enlargements of the digestive tract, the

question immediately arose as to whether or not all the erithidial infections of these regions are a part of the stomach phase of the life history of *C. euryophthalmi*. A careful study of the erithidial infection of each of these portions of the digestive tract together with many examinations of the contents of the intestine, the gland, and the rectum, convinced me that food conditions do not permit the parasites to establish a permanent rectal phase in the rectum but that they are forced to remain anterior to the intestine, in the pyloric expansion. Preparations of the gland and intestine have as yet shown no infection posteriorly, and the rectum has contained an occasional infection of spore forms only.

To add to the difficulty in determining the extent of the stomach phase and the beginning of the rectal phase no structural changes of a striking nature occur in the stomach phase of *C. euryophthalmi* as is the case in *T. lewisi*; nevertheless, the behavior of the erithidias is of some assistance and the study of the serial sections and stained smears of the several parts have added materially to our knowledge of the phases. Taking into consideration the evidence from these sources the erithidial infection of the "crop" is interpreted as the stomach phase. The mid-stomach serves as a temporary location for the slowly migrating forms of the stomach phase, and the pyloric expansion becomes the region of the permanent location of the "rectal" phase during the life of the lupine bug. The established "rectal" phase of the life history of *C. euryophthalmi* in the pyloric expansion has three general types of individuals, the attached, or haptomonad, the free, or nectomonad, and the final spore forms, which probably serve to infect another insect. Only the last type of parasite has been found in the normal contents of the rectum.

With these brief explanatory outlines of the life histories of *T. lewisi* and of *C. euryophthalmi* a detailed discussion of the more important points concerning the comparative morphology of the erithidial stages of *Trypanosoma* and of *Crithidia* will now be given. The comparison of the life cycles which follows is based upon the work of Minchin and Thomson on the life cycle of *T. lewisi*, and upon the life history of *C. euryophthalmi* with special reference to the accompanying plates (pls. 2-6). For further details of the life history of *T. lewisi* the reader is referred to Minchin and Thomson's paper (1915).

THE COMPARATIVE MORPHOLOGY OF *CRITHIDIA* AND
THE CRITHIDIAL STAGES OF *TRYPANOSOMA*

The morphology of *Crithidia* and *Trypanosoma* has been the subject of careful investigation for a number of years, and our conception of the structure of these simple organisms has been modified from time to time by additional discoveries. This is especially true of the extranuclear organelles of these flagellates, owing to the recent investigations carried on in the Zoological Laboratory of the University of California by Dr. C. A. Kofoid (1916) and Dr. Olive Swezy (1916), the latter studying particularly the binuclear theory of Hartmann (1911). The work of these investigators has centered attention upon the extranuclear organelles of these flagellates, consisting of the blepharoplast (fig. A, *bl.*) at the base of the flagellum, the parabasal body (*pb.*), or kintonucleus, the rhizoplast (*rh.*), the parabasal rhizoplast (*pb. rh.*), the flagellum (*fl.*), and the undulating membrane (*und. m.*). They have homologized the kintonucleus of the Protomonadina (*Herpetomonas*, *Crithidia*, and *Trypanosoma*) with the parabasal body of the Polymastigina and the Hypermastigina. Bearing this in mind, it at once becomes clear that this extranuclear complex of organelles is the neuromotor apparatus of *Crithidia* and *Trypanosoma* (Kofoid, 1916). In a previous paper (Kofoid and McCulloch, 1916) the term *parabasal body* was used in place of *kintonucleus* throughout and I shall employ this nomenclature in the present paper.

The position of this extranuclear complex of organelles determines largely whether the flagellate is a trypanosome or a crithidia. The trypanosome is characterized by the presence of the parabasal body and the blepharoplast posterior to the nucleus, and by a well developed, undulating membrane which passes forward laterally along the edge of the ribbon-like body. These characteristics are common to the flagellates found in the blood, and modifications of this structure take place as soon as the medium is changed, as in the transfer to the stomach of the flea. The transition stages between a trypanosome and a crithidia have been designated as crithidiomorphic trypanosomes by Minchin and Thomson, as previously noted. In the transition forms the parabasal body and the blepharoplast are still posterior to the nucleus at a greater or less distance, but the movement and shape of the body of the flagellate are distinctly like those of a crithidia. In

the crithidial forms the extranuclear complex of organelles is anterior to the nucleus (fig. A, 1, 2) and the body has a tendency to be cylindrical in cross-section and to show only a slight undulating membrane (*und. m.*, fig. A). These crithidial forms are common to the life cycle of both *Crithidia* and *Trypanosoma*. The next step in our

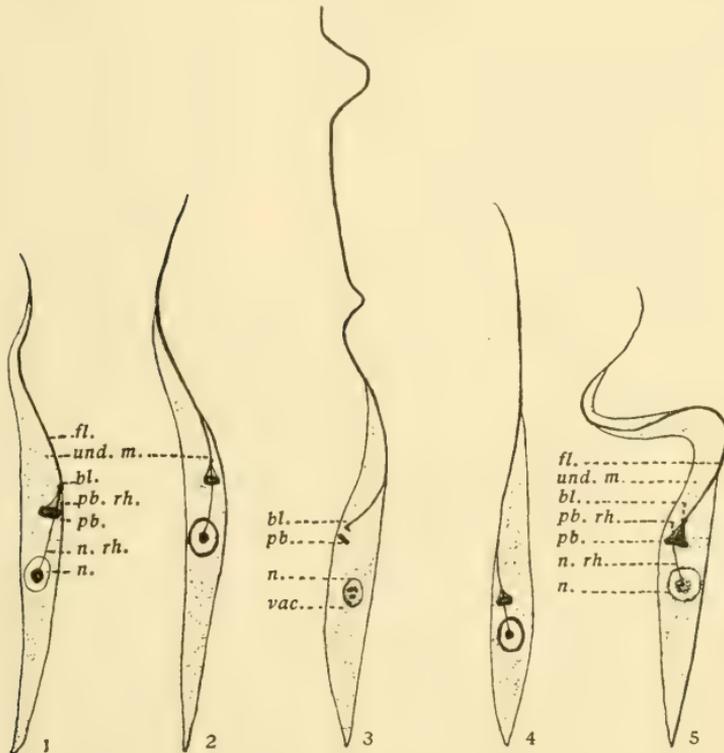


Fig. A. Typical crithidial forms of: (1) *Crithidia leptocoridis*; (2) *C. euryophthalmi*; (3) *Trypanosoma lewisi* (after Minchin and Thomson, 1915, pl. 37, fig. 66); (4) *T. triatomae*; (5) *Schizotrypanum cruzi* (after Chagas, 1909, pl. 13, fig. 16); showing the similarity of the several flagellates in size, form and structure. $\times 3500$. *bl.*, blepharoplast; *fl.*, flagellum; *n.*, nucleus; *pb.*, parabasal body; *pb. rh.*, parabasal rhizoplast; *n. rh.*, nuclear rhizoplast; *und. m.*, undulating membrane; *vac.*, vacuole.

discussion will be directed to the demonstration of the close resemblance of the crithidial forms of the genus *Crithidia* to those of *Trypanosoma* in the invertebrate host.

A series of typical crithidial flagellates (fig. A) has been selected from the stomach phase of the life histories of both *Crithidia* and

Trypanosoma to show in detail the comparative morphology in this particular stage. *C. leptocoridis* (fig. A, 1) and *C. euryophthalmi* (fig. A, 2) have been selected to represent the structure of *Crithidia* and *Trypanosoma lewisi* (fig. A, 3, after Minchin and Thomson, 1915, pl. 37, fig. 66), *T. triatomae* (fig. A, 4), and *Schizotrypanum cruzi* (fig. A, 5, after Chagas, 1909, pl. 13, fig. 16) that of *Trypanosoma*.

Each erithidial flagellate of this series has an elongate body, cylindrical in outline but slightly flattened at the anterior portion, which forms the undulating membrane. At the edge of this membrane there is a sharply defined flagellum of variable length (fig. A, 1, 3). The length of the flagellum has no particular significance since considerable variation exists within each species. Posteriorly, however, there is in the stomach phase a consistent difference between the crithidial form of *Crithidia* and the erithidial form of *Trypanosoma*. The posterior ends of the bodies of the true crithidias are more attenuate (fig. A, 1, 2) in the stomach phase, and do not show the slight tendency to become club-shaped until the rectal phase is reached. In figure A, 4, *T. triatomae* is quite blunt at the posterior end, and the shifting of the cytoplasm into this region is increasing the width of the body at the expense of the length. Another difference almost as consistent as the one just noted is the variation in the nucleus (*n.*). In *Crithidia* (fig. A, 1, 2) it is usually anterior to the center, and in the erithidial forms of *Trypanosoma* it is posterior to the center (fig. A, 3, 4, 5).

Internally the similarity of the structure of the organelles and their relationship in general is quite marked. The nucleus (*n.*), the blepharoplast (*bl.*), and the parabasal body (*pb.*) are common to each of these flagellates. The nuclear rhizoplast (*rh.*) and the parabasal rhizoplast (*pb. rh.*) are found in all the above flagellates with the exception of *T. lewisi* (fig. A, 3). The absence of these organelles in the erithidial form of *T. lewisi* is questionable since they are present in all the others. They may have been overlooked because of the delicacy of their structure and the faintness with which they stain.

The nucleus and the extranuclear organelles will now be taken up in detail.

Nucleus.—This organelle in each of the above flagellates may be described as round or slightly oval in shape and of the vesicular type. It varies somewhat in size from 1μ in figure A, 3 to 1.7μ in figure A, 5, but it is usually about two-thirds of the width of the body in diameter. In *Crithidia leptocoridis* the nucleus shows clearly the ves-

icular type of structure, having a relatively large karyosome, a light area outside of this, and a clearly defined nuclear membrane. In figure A, 3 a slightly different condition exists; the nucleus of *C. euryophthalmi* is of the vesicular type, but the nuclear membrane is encrusted with considerable chromatin, and the central karyosome is somewhat reduced as compared with that of *C. leptocoridis* in figure A, 1. An examination of plate 5 indicates that the nucleus of this flagellate (*C. euryophthalmi*) is characterized by the heavily encrusted nuclear membrane, the small central karyosome, and the broad, clear area between the membrane and karyosome.

In the trypanosomes (fig. A, 3, 4, 5) the nucleus is slightly posterior to the center of the body, which has a tendency to be shorter and more club-shaped at the posterior end than is that of the crithidias (fig. A, 1, 2). The nucleus of *Trypanosoma lewisi* (fig. A, 3) shows a peculiar structure. In this particular form it is difficult to demonstrate the nuclear membrane, since the light area encircling the more deeply stained portion could be interpreted as either intranuclear or extranuclear. However, taking into consideration other figures of *T. lewisi* (cf. Minchin and Thomson, 1915, pls. 37, 42) this light area has been regarded as being extranuclear and the diameter is 1μ instead of 1.4μ . Within this relatively deep-staining nucleus the chromatin is divided unequally into a large, irregularly shaped granule and a smaller one. The nucleus of *T. triatomae* (fig. A, 4) resembles that of *C. euryophthalmi*. It is also characterized by the chromatin-encrusted nuclear membrane, the wide clear area, and the small central karyosome, as in *C. euryophthalmi*. A nucleus of a somewhat more complex type is found in *Schizotrypanum cruzi* (fig. A, 5). A faint nuclear membrane limits the clear nuclear space which surrounds the central karyosome containing a centriole at the base of the rhizoplast. This nucleus is 1.7μ in diameter and somewhat larger than that of any other flagellate in the series.

Blepharoplast.—This structure is of great interest since it is the center of the extranuclear organelles, or neuromotor apparatus. In the crithidias this structure is not a sharply defined basal granule at the base of the flagellum. For instance, in *Crithidia leptocoridis* a slight enlargement at the base of the flagellum is noted (fig. A, 1). Since this slight enlargement stains with almost the same intensity as does the flagellum it is exceedingly difficult to get a satisfactory conception of its structure. By careful focusing of the binocular microscope with Watson's no. 20 holoscopic oculars, however, the small enlarge-

ment of slightly greater staining intensity can be observed at the junction of the parabasal rhizoplast with the nuclear rhizoplast. In *C. euryophthalmi* (fig. A, 2) even greater difficulty is experienced in endeavoring to find the blepharoplast. In this form there is no enlargement of the base of the flagellum, but the area at the junction of the rhizoplast is darker in appearance, and frequently a small granule at one side can be seen by careful focusing.

Among the trypanosomes the blepharoplast is apparently a more clearly defined structure. Our investigations of *T. triatomae* (fig. A, 4) have yielded more tangible results. We found in this trypanosome a slight enlargement of the base of the flagellum but no differentiation in the staining capacity of the flagellum and basal granule. In *T. lewisi* (fig. A, 3) and in *Schizotrypanum cruzi* (fig. A, 5) the figures of these trypanosomes show what is evidently a well defined basal granule, or blepharoplast. This is particularly true of *T. lewisi* (fig. A, 3). In both the crithidias and the trypanosomes the blepharoplast frequently divides independently of the nucleus. In the binary fission of *C. euryophthalmi* the blepharoplast and parabasal body may divide before the nucleus divides (pl. 3, fig. 4), or the nucleus may divide first (pl. 3, fig. 33). In figure 25, plate 3, the nucleus is dividing but there are no indications of the division of blepharoplast and of the parabasal body. A similar condition exists among the trypanosomes, indicating that the blepharoplast is the kinetic center of the extranuclear organelles, or neuromotor apparatus. But this is not the only kinetic center of these simple organisms. The nucleus also contains a kinetic center which initiates division in the form of a centrosome, at the base of the nuclear rhizoplast in those flagellates containing this organelle. In the nucleus of *S. cruzi* (fig. A, 5) this centriole, or centrosome, at the base of the nuclear rhizoplast is clearly defined within the central karyosome of the nucleus. The origin of the blepharoplast is still the subject of investigation and as yet no conclusive evidence is at hand to establish beyond doubt the way in which this structure originates. In the endogenous buds, which are exceedingly small and difficult to interpret, the blepharoplast seemingly originates from the single nuclear structure as an outgrowth rather than by a mitotic process. The outgrowth forms a second deep-staining mass anterior to the nucleus, and at this stage the nonflagellated forms have the appearance of "binucleated" spores. As development proceeds the kinetic center of the blepharoplast is probably established as the center of the neuromotor apparatus, and

from this blepharoplast the flagellum grows anteriorly and the parabasal body to one side. Connecting the parabasal body with the centrosome, or blepharoplast, is the parabasal rhizoplast (*pb. rh.*). Parallel cases indicating such an origin of the blepharoplast can be found among other flagellates.

Parabasal body and rhizoplasts.—In all of these flagellates the parabasal body (*pb.*) presents the same general appearance with respect to its location, size and shape. In *C. leptocoridis* (fig. A, 1) the parabasal body is a bar-shaped structure made up of two deeply stained granules lying in close proximity. Immediately surrounding this deep-staining bar is a clear area, limited by a sacklike covering of dense cytoplasm, evidently continuous with the contractile cytoplasmic sheath of the flagellum. Between the parabasal body and the blepharoplast region the cytoplasmic sheath takes on the appearance of a cone-shaped mass of contractile fibers (*pb. rh.*), the apex of which is found at the base of the flagellum and continuous with the outer contractile cytoplasm of the flagellum. The axial or central part of this parabasal rhizoplast is presumably of blepharoplastic origin and connects the blepharoplast and parabasal body.

The parabasal body of *C. euryophthalmi* is similar to that of *C. leptocoridis*. It is not so large comparatively (fig. A, 2) and it seldom has the bilobed appearance. In *C. euryophthalmi* the most interesting and valuable evidence has been found concerning the relation of the parabasal body to the blepharoplast. In text figure B, 1–5 a series of flagellates has been drawn from good iron-haematoxylin preparations to show clearly that the position of the organelle is lateral and not axial. In figure B, 1 the end of the bar-shaped parabasal body, which lies lateral to the nuclear rhizoplast, extends outward to the periplast. From another angle the entire length of this organelle is visible (fig. B, 2) peripheral to the nuclear rhizoplast, which passes behind and underneath the parabasal rhizoplast. Still another aspect of the possible position of this structure is indicated in figures B, 3, 4. In each of these figures it is medial to the nuclear rhizoplast and behind or beneath it as it passes from the centrosome of the nucleus to the centrosome, or blepharoplast, at the base of the flagellum. In such a figure as B, 5 the relationship of this organelle to the others of the neuromotor apparatus can be seen most clearly. The cytoplasm has become exceedingly clear and does not conceal the other structure. The parabasal body in the flagellate stains very deeply and has the appearance of a chromotoidal mass suspended in

a sacklike structure from the region of the blepharoplast. Unlike the suspensory apparatus of *C. leptocoridis*, *C. euryophthalmi* does not show a fan-shaped mass of contractile cytoplasmic fibrils covering this axial portion, which is of blepharoplastic origin (figs. A, 1, 2; figs. B, 1-5). The outline of this cytoplasmic envelope in *C. euryophthalmi* is definite, slightly opaque, and continuous with the cytoplasmic sheath of the flagellum.

The parabasal body of the trypanosomes (figs. A, 3, 4, 5) is similarly a bar-shaped structure, which stains deeply, located to one side of the nuclear rhizoplast and blepharoplast. In *T. lewisi* (fig. A, 3) the parabasal body is relatively small while the blepharoplast is correspondingly large. As previously noted, no parabasal rhizoplast has been figured by Minchin and Thomson in *T. Lewisii*. Here and there suggestions of a connection might be pointed out in their figures. While it is possible that *T. lewisi* is exceptional in this respect, yet, since the structure is found in other flagellates of this group, we may infer that a critical study of the preparations with a binocular microscope will reveal the presence of such a connection in the crithidial stages of this flagellate.

Trypanosoma lewisi (fig. A, 3) is likewise the only flagellate here figured in the crithidial stages without a nuclear rhizoplast. Here again *T. lewisi* is either exceptional or the structure has perhaps been overlooked, since it is usually discerned with difficulty. Chagas (1909) has figured a nuclear rhizoplast in *Schizotrypanum cruzi* and we have found it also in *T. triatomae*. Therefore we may expect that it will be found in *T. lewisi*.

The parabasal body in *Trypanosoma triatomae* (fig. A) is a relatively large structure, its width approximately equal to one-half of its length. It is located a short distance anterior to the nucleus, and is suspended from the blepharoplast by a fan-shaped parabasal rhizoplast like that of *Crithidia leptocoridis*, i.e., a suspensory apparatus with a fibrous appearance. In the crithidial stage of *T. triatomae* a nuclear rhizoplast was observed connecting the blepharoplast with the centriole of the karyosome, but such a connection was not found in the trypaniform individuals. If a nuclear rhizoplast be present in the trypaniform stage, in which we did not find it, it is possible that we may have been prevented from observing it because of the density of the cytoplasm in this stage and a tendency in this delicate thread in the trypanosomes to stain lightly. In the crithidial stages of this trypanosome, on the other hand, the cytoplasm is more or less vacuolated in appearance thus making the nuclear rhizoplast more evident. When a try-

panosome rounds up for the process of multiple fission no nuclear rhizoplast is in evidence, but after the complete transition into the crithidial form the nuclear rhizoplast can be readily demonstrated with the high power binocular microscope and Watson's holoscopic eyepiece. It is also possible that further work will show that the centrosomic structure of the trypaniform flagellate differs from that of the crithidial form, and consequently that the rhizoplasts are absent in the former.

In *Schizotrypanum cruzi* (fig. A, 5) the parabasal body is slightly bilobed in appearance, and is suspended from the blepharoplast by a clearly defined, fibrous rhizoplast. The suspensory apparatus of the parabasal body is here relatively larger and apparently more highly developed than in any of the other flagellates. A few of the trypaniform stages also show a similar parabasal rhizoplast, according to the figures of Chagas (1909). In the crithidial stages of this trypanosome, as shown in figure A, 5, there is a sharply defined nuclear rhizoplast passing from the centriole of the nucleus to the blepharoplast. The position of the parabasal body in *S. cruzi* is similar to that of *Crithidia euryophthalmi* and is situated apparently to one side of the nuclear rhizoplast.

One of the most important discoveries in connection with the study of these organelles is the fact that the parabasal body, or the so-called kinetonucleus, is not axial in position in *Crithidia euryophthalmi* (figs. B, 1-4) and apparently not in the other flagellates (figs. A, 1, 3-5). With the evidence at hand to show definitely that this body is not axial but a lateral appendage of the blepharoplast in *C. euryophthalmi* it is necessary to look upon this organelle as something other than a second nucleus or a kinetonucleus. Reference has already been made to the work of Dr. Kofoid (1916) concerning the homology of this organelle with the parabasal body of other flagellates. The observations concerning the origin of this structure, the location and relation of the parabasal body to the other organelles as found in the study of *C. euryophthalmi* offer some of the best evidence against the binuclear conception of these flagellates (Hartmann, 1911).

Flagellum and undulating membrane.—Owing to the relationship which exists between the flagellum (*fl.*) and undulating membrane (*und. m.*) it is convenient to describe these two organelles together. The flagellum consists of an outgrowth from the blepharoplast and is surrounded by a cytoplasmic sheath which is continuous with the sack-like sheath covering the parabasal body. In the ordinary preparations no distinction can be made between this central portion originating

from the blepharoplast and the cytoplasmic sheath surrounding it. The entire flagellum stains as a single heavy line, almost chromatoidal in appearance. It does not stain so deeply as does the nucleus, but much deeper than the cytoplasm of the body and of the undulating membrane. As the flagellum forms and lengthens, there is an accompanying elongation of the protoplasm which forms the undulating membrane (*und. m.*). Both endoplasm and ectoplasm enter into the formation of this organelle. Usually there is a thin, narrow band of clear ectoplasm lying parallel to the flagellum (fig. A, 5). The length of the membrane, and consequently of the intracellular part of the flagellum, is greater among the crithidias of the stomach phase than of the rectal phase owing to the shifting of the cytoplasm of the body in the transition. This is usually true also of the crithidias as compared with the crithidial stages of the trypanosomes (fig. A, 1, 2, 3, 4). As previously noted, the length of the free flagellum (fig. A, 3) has no significance from the viewpoint of comparative morphology, since there is a wide variation in the length of this organelle for each species of *Crithidia* and of *Trypanosoma*.

THE LIFE CYCLE OF *CRITHIDIA EURYOPHTHALMI*

The life cycle of *C. euryophthalmi* in *Euryophthalmus convivus* begins, so far as known, with the casual ingestion with food of spores from the fecal matter of infected insects. *E. convivus* commonly feeds upon the sap from the growing tips of the lupine, which show many indications of excreta. In these same regions of the lupine galls and other abnormal growths occur in great abundance. The possibility that these insects were getting their infection of *C. euryophthalmi* from the sap of the lupine was suggested by the work of França (1914). Examinations of the sap of the lupine were accordingly made. Nematodes, numerous yeast-like spores, and bacteria were found. No organisms were discovered, however, of any description which could be linked to the known stages of *C. euryophthalmi* in the digestive tract of the host.

The large number of parasites in the life cycle of *C. euryophthalmi* can be grouped readily into two series: the developmental, or infective, and the degenerative, which are comparable to the developmental and degenerative series of *T. lewisi* in the flea, as described by Minchin and Thomson. That the correlation between these two life cycles is marked will become clear in following the discussion of the life cycle

of *C. curyophthalmi*, notwithstanding the fact that the initial stages of the two life cycles are entirely different in the insects. The initial stages of the crithidial life cycle are small, oval spores, which develop into elongate crithidial flagellates, whereas the initial stages of the trypanosome are trypanosomes from the blood of a rat, which indirectly by a process of multiple fission, produce very similar elongate crithidial flagellates. Subsequently all of the rectal stages in each life cycle are very similar.

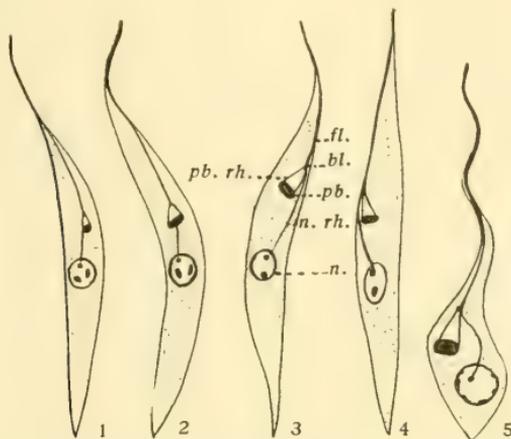


Fig. B. Five figures of *Crithidia curyophthalmi* to show the relation of the parabasal body to the flagellum, blepharoplast, rhizoplast, and nucleus. $\times 3500$. Abbreviations same as in figure A.

The developmental or infective series of *Crithidia curyophthalmi* consists of the stomach phase or the crithidias of the "crop," mid-stomach and upper part of the pyloric expansion. The degenerative series includes the late rectal phase or the attached crithidias of the pyloric expansion.

THE DEVELOPMENTAL SERIES

STOMACH PHASE

The stomach phase of *Crithidia curyophthalmi* is apparently initiated when the small, oval spores (pl. 2, figs. 1, 2) casually ingested with food begin to develop in the "crop." Such initial infective spores develop rapidly into a swarm of multiplying crithidias (pl. 2, figs. 2-7). Later as mature flagellates (pl. 2, figs. 9, 10) the crithidias

may be carried immediately to the pyloric expansion through the mid-stomach by the current of food, or they may undergo a process of multiple fission extracellularly or intracellularly in the "crop." The process of multiple fission may be similar to that of the spheres of *Trypanosoma lewisi* (Minchin and Thomson, 1915), which have certain characteristics in common with the somatella in the multiple fission of some of the Polymastigina (trichomonad flagellates) described by Kofoid and Swezy (1915). In addition to this type of multiple fission there is a second and entirely different process of multiple fission, the internal or endogenous budding (pl. 2, figs. 11-23). These two processes of multiple fission have been described briefly in a preliminary communication (McCulloch, 1917). Endogenous budding has been emphasized in this paper and described in detail because of its interest and importance.

EXTRACELULAR CRITHIDIAS

Oval spores.—The initial infective spores (pl. 2, fig. 1) are ovoid and stain deeply. They are found in small numbers in the "crop," and present several distinguishing marks which serve as a means of identification. They average 1.7μ in width and about 3.4μ in length. The nucleus stains diffusely and forms a solid mass of chromatin at the extreme posterior end of the body. The parabasal body lies within the anterior half of the spore, about equally distant from the nucleus and forward end. One end of this bar-shaped structure, or parabasal body, lies close to the thick periplast. On all the other sides of this organelle there is the characteristic light area, which quickly destains in iron-haematoxylin preparations. Careful focusing has revealed a faint nuclear rhizoplast passing from the nucleus toward the region of the parabasal body.

Developing crithidias.—When the initial infective spores begin to develop in the "crop" a change occurs in their shape and staining capacity (pl. 2, fig. 9). The forward outgrowth of the flagellum assists in the elongation of the anterior end and the formation of the undulating membrane (pl. 2, figs. 6-9). The posterior region elongates less rapidly, but in time it frequently attains an even greater length than the anterior end (pl. 2, fig. 9). The length of the free flagellated crithidias which result from the developing forms varies greatly at all times regardless of their location in the digestive tract. In figure 9, plate 2, the crithidial flagellate has reached the extreme

length, comparatively, of 32μ . At the other extreme, a short crithidia of 7μ (pl. 2, fig. 12) is evidently mature, since it is undergoing a process of multiple fission of the endogenous-budding type. Between these extremes is a series of intergrading forms. The sizes and shapes of a swarm of free crithidial flagellates from the life cycle of anyone of these species of flagellates under discussion show great diversity, and *Crithidia euryophthalmi* is not an exception, as indicated in plates 2 and 4. The change in the appearance of the nucleus as development proceeds is noticeable. The deeply staining mass of chromatin (pl. 2, figs. 1, 2) becomes a nucleus containing a prominent central karyosome and a chromatin-encrusted nuclear membrane (pl. 2, figs. 3-10). Between the karyosome and membrane is a clear area, which destains very readily after iron-haematoxylin.

MULTIPLE FISSION

Endogenous budding.—In looking over a large number of preparations of the digestive tract of *Euryophthalmus convivus*, in the early part of 1916, I chanced upon a splendid preparation of a "crop" from a nymph which contained an exceedingly heavy infection of crithidias of all shapes and sizes. The smear was well fixed and well stained.

Among other things the nuclear structure was studied in detail to determine whether the nucleus of these crithidias divided by a mitotic process or by a more primitive method of mitosis. This search led to the discovery of a flagellate which apparently contained two nuclei (pl. 2, fig. 12), in a linear arrangement with respect to the long axis of the body. Shortly after another crithidia (pl. 2, fig. 13) was found in the same preparation, containing apparently three similar nuclei, which were arranged in a like linear series. In the latter (pl. 2, fig. 13) careful focusing revealed the outline of a fourth partially concealed beneath the most anterior nuclear structure. In each of these flagellates (pl. 2, figs. 12, 13) no indications of any ordinary process of binary fission were detected. The blepharoplast, parabasal body, and the rhizoplasts in each were still intact in so far as could be determined. The alternative hypothesis that these nucleus-like structures, which are relatively small, are internal parasites of a bacterial or protozoan nature naturally was given full consideration. Drawings of these flagellates were made with the camera lucida and the readings were taken for future reference. Owing to the abun-

dance of the parasites in this preparation of the "crop" ample material was at hand for an extensive study of the morphology of this phase of the life cycle of *Crithidia euryophthalmi*. From time to time more flagellates were observed, apparently multinucleated, but little additional light was thrown upon their peculiar nuclear structures until a large flagellate, such as shown in figure 22, plate 1, was observed. This crithidia was relatively large and contained approximately twelve "binucleated" spore-like forms. The structure of the sporelike forms within the periplast of the large flagellate was similar to that of the numerous small, oval spores in the immediately surrounding field, which were, beyond doubt, stages of the life cycle of *C. euryophthalmi*. Another large flagellate containing six nuclear structures within the periplast (pl. 2, fig. 21) was also drawn. The flagellum and parabasal body were clearly outlined, as in the former, multinucleated flagellates. Some of the enclosed nuclear-like structures were deeply stained, owing to the thickness of the preparation, but the majority presented the same appearance as did the nuclear-like structures in figures 12 and 13.

Investigation of the smear revealed more and more evidence of a possible endogenous, or internal budding, in the life cycle of *Crithidia euryophthalmi*. Past experience indicated that the preparations of the "crops" of young nymphs furnished the best smears for the study of the initial infections, of which the endogenous budding forms were evidently a part. An effort was accordingly made during the next breeding season of *Euryophthalmus convivus* to collect as many young nymphs as possible in order to obtain additional preparations of the digestive tract, with reference to the "crop" in particular.

Out of a large number of preparations of the "crops" prepared during the following season, only two contained additional stages of the process of endogenous budding. The percentage of infection of the "crops" of young nymphs was found to be approximately twenty per cent as compared with two per cent among adult insects.

In the two preparations there were numerous small, "binucleated," spore-like forms, grouped near discarded flagella, with or without blepharoplasts, and with parabasal bodies still attached (pl. 2, fig. 23). It was easy to conceive of the degeneration of the cytoplasm surrounding the spore-like forms, leaving a field covered with the internal spores, or zooids, and the extranuclear organelles of the parent cell. In the earlier stages of degeneration presumably the parabasal bodies were still attached by the parabasal rhizoplasts to the blepharoplasts

at the bases of the flagella. Later stages showed only flagella among the zooids. This additional evidence from the two smears at once suggested the work of Moore and Breinl (1907), in which "latent bodies" were described in the life cycle of a haemoflagellate, *Trypanosoma gambiense*.

According to these investigators *T. gambiense* in the blood of a vertebrate host formed latent bodies in the nuclear region, under certain conditions. These latent bodies are pictured and described as being small nucleated structures having a nuclear membrane and a single mass of chromatin in the center. The exact method of the formation of the bodies in the nucleus or from the nucleus is not clear, since none of the early stages in the formation of the latent bodies are figured or described in detail. Apparently only the results of an endogenous process in the life cycle of *T. gambiense* were observed by these investigators. The final stages of the process in the life cycle of *T. gambiense* are the presence of large, degenerating trypanosomes with minute, nucleated latent bodies in the nuclear area. The flagellum, kinetonucleus, or parabasal body and cytoplasm are in various stages of degeneration. Their figures of the latent bodies (Moore and Breinl, 1907) should be compared with figure 23 of this paper.

The latent bodies in the life cycle of *Trypanosoma gambiense* and the endogenous zooids of the life cycle of *Crithidia euryophthalmi* are not the only instances of an endogenous budding in the life cycle of the Protomonadina (*Trypanosoma*, *Crithidia*, *Herpetomonas*, *Leptomonas*). Minchin and Thomson (1915) searched for evidence of an endogenous budding in the life cycle of *T. lewisi* but failed to find any indications of the process. However, in the life cycle of *Leptomonas pattoni*, one of the so-called natural flagellates frequently found in the digestive tract of the flea, they found several crithidial-like flagellates containing a nucleus and an endogenous bud. These authors suggested that the endogenous buds found therein were doubtless comparable to the latent bodies of Moore and Breinl.

The early stages of the endogenous, or internal budding of *Crithidia euryophthalmi* are shown in figures 11 and 12, plate 2. In figure 11 there is a relatively short flagellate undergoing multiple fission in this way. The nucleus has budded off two circular, nucleus-like buds, each showing a chromatin-encrusted nuclear membrane. The parabasal body and the flagellum of this flagellate are still intact and have no indications of binary fission. Somewhat similar to this flagellate

is the one shown in figure 12, plate 2. The anterior end of the body is slightly shortened but the nuclear structure has the same general appearance. Only one bud has been given off, and this lies directly posterior to the nucleus proper. As in the former case, the chromatin is collected on the inner surface of the nuclear membrane of both the nucleus and endogenous bud. In figure 13, plate 2, a more complex organization was observed. The nucleus and two clearly defined nuclear buds are arranged in a linear series, the two buds being posterior to the nucleus proper. Partially concealed by the nucleus is a third bud, which is being constricted off from the nucleus. This particular view of the process is in all probability an end view and only a portion of the bud is visible. If the observations and interpretation of this structure be correct, the nucleus, with all of its chromatin collected on the inner surface of the membrane, repeatedly constricts or buds off a portion, forming a series of nuclear buds (pl. 2, figs. 13, 20, 21). There is at hand at present no evidence of a central karyosome being present in the nucleus when endogenous buds are formed. The nuclear buds, therefore, in so far as our observations have gone, are due neither to a clearly defined amitosis nor to a primitive form of mitosis of the chromatin material which normally occurs in a central karyosome.

Although evidence of a mitotic process in this nuclear division is wanting, nevertheless some evidence of a promitosis has been found in the division of the nucleus in an early stage of the formation of a somatella (pl. 3, fig. 25). The formation of the somatella involving the "rounding up" of an elongate flagellate into a sphere will be described shortly; it will suffice for our purpose here to point out salient features of the internal structure of the "rounding up" flagellate (pl. 3, fig. 25). The blepharoplast, parabasal body, and the rhizoplast of this flagellate have not yet begun to divide but the nucleus is beginning to form two daughter-nuclei. The central karyosome which is normally present in *C. euryophthalmi* has formed two unequal masses of chromatin connected by a centrodesmose. No critical evidence was found showing that there is present at each end of the centrodesmose a centriole or centrosome differentiated from the chromatin material in this minute form. Peripherally there is the nuclear membrane still present and intact, but it is constricting in the center to form two nuclei. If a similar division of the chromatin material occurs in the nuclear budding it has thus far escaped observation. The number of early stages of nuclear budding studied has been small, owing to the rarity of

preparations showing the process. In every instance of nuclear budding so far observed the chromatin has been peripheral, with a more or less unequal distribution on the nuclear membrane. Frequently there is one heavy mass with a uniform amount elsewhere on the membrane (pl. 2, fig. 15).

A somewhat larger, elongate flagellate, with sharply defined endogenous buds, is shown in figure 14, plate 2. Unlike the endogenous forms just described the buds here are not posterior to the nucleus. In figure 14, opposite the parabasal body, there is a bud decidedly anterior and lateral in position. A second endogenous bud is also anterior between the nucleus and parabasal body. Within all of these nuclear structures the chromatin is massed irregularly upon the nuclear membrane.

In addition to these elongate flagellates undergoing multiple fission of this type there are also pear-shaped crithidias (pl. 2, figs. 15-18) containing several or numerous endogenous buds. Possibly it is the greater thickness of these flagellates, with consequent decrease in destaining capacity, which makes the buds within stand out so distinctly. Another marked characteristic of the pear-shaped flagellates is the lack of differentiation between the nucleus and the buds. There is no evidence of a parent nucleus which has given rise to the buds. The distribution of the chromatin within these buds presents some interesting variations. In figure 15 the buds contain a clearly defined chromatin encrusted nuclear membrane together with one distinct mass or chromatin granule. In figure 17 the massing of the chromatin may mean a more advanced stage since it is no longer on the entire nuclear membrane but has been segregated into two masses, which give the buds a bipartite appearance. Proceeding to figure 18 in this pear-shaped individual more steps in advance are portrayed, namely the unused portions of the flagellate are beginning to degenerate around the endogenous buds. The parabasal body has already disappeared, and only a portion of the discarded flagellum remains near by. The structure of the endogenous buds of this spherical crithidia is remarkably uniform. In each bud the chromatin granule is adherent to the nuclear membrane.

Other elongate flagellates undergoing multiple fission are observed in figures 19, 20, and 21. Some interesting variations can be pointed out in these crithidias. In figure 19 there is a nuclear rhizoplast, which can be traced from the edge of the nuclear membrane to the blepharoplast. Of greater interest are the variations shown in figures

20 and 21. In figure 20 the binucleated appearance of the nucleus and buds is very prominent. The chromatin granules have a paired effect which is difficult to interpret. In figure 21 a more complex structure is represented. Anterior to the nucleus there is a group of five endogenous buds. Two of these, owing to their position, are not destained sufficiently to make clear their nuclear structure. This large flagellate has a sharply defined parabasal body and flagellum, similar to those in figures 19 and 20. The large size of such forms has aided materially in the interpretation of the endogenous buds, which, upon developing, form the so-called zooids.

The most mature stage of endogenous budding yet found, wherein the resulting zooids are still within the periplast, is pictured in figure 22. There are approximately twelve clearly defined zooids massed near the central part of this large flagellate. The structure of the zooids could be studied readily. The nucleus and parabasal body in each, because of their deep stain, helped to distinguish the several zooids. The other organelles were not visible. Another interesting feature concerning these zooids is the difference in the stages of their development. Some are larger and more mature and were doubtless budded off from the nucleus first. Probably the zooids of the flagellate have been retained within the periplast of the parent for a longer period than usual. If the size of these zooids be compared with that of the free zooids shown in figure 23 the former, on the whole, are larger and more fully developed. There are, moreover, no marked signs of degeneration of the parabasal body, of the flagellum, or of the cytoplasm. The parabasal body is almost hidden by the zooids in that region.

The final step in the endogenous-budding process comes with the degeneration of the body-plasm of the parent flagellate, leaving a mass of zooids, together with the flagellum and parabasal body of the parent. Portions of smears have been observed to be literally covered with zooids and discarded flagella. As degeneration proceeds the parabasal bodies next disappear, and finally the flagella, leaving only numerous zooids of various sizes and structure. The last phase is the one most frequently observed in preparations of *Crithidia euryophthalmi*. I had been working with *C. euryophthalmi* more or less for a period of almost two years before the clue as to the origin of these small zooids was found. It had been most puzzling to find so many of these small, non-flagellated, binucleated forms (pl. 4, fig. 40), which were obviously unlike the initial infective spores (pl. 2, figs. 1, 2). They

were smaller in size, their periplast was thinner, and they destined much more rapidly after iron-haemotoxilin. Serial sections of the mid-stomach showed them grouped in pockets between the epithelial cells. Possibly the endogenous process occurred there or they were lodged there as the current of food carried the others down into the pyloric expansion. They could be arranged in a series, beginning with those averaging about 1.7μ in length (pl. 4, fig. 40) and gradating to the size of the spores which were regarded as the initial infective spores (pl. 2, fig. 1; pl. 4, figs. 40-54).

The development of the endogenous buds into binucleated zooids is not easy to interpret. In figure 23, plate 2, the numerous zooids present variations in both size and structure. Unlike the latent bodies of *Trypanosoma gambiense* of Moore and Breinl (1907) and the endogenous buds of *Leptomonas pattoni* of Minchin and Thomson (1915), these endogenous buds show no central chromatin granule within the nuclear membrane. Their chromatin is distributed at the periphery of the nucleus usually in one of three ways. In the elongate flagellates (pl. 2, figs. 11, 14, 19) the buds have their chromatin material massed irregularly on the nuclear membrane. A second form of peripheral chromatin in the nucleus is a noticeable mass or granule at one point. This is characteristic of the buds within the pear-shaped flagellates (pl. 2, figs. 15-18). The third type of nuclear structure in the buds is possibly only a slight modification of the others. In figure 20, plate 2, the internal or endogenous buds appear to be binucleated because of the peculiar segregation of the chromatin material on the nuclear membrane. At present these several modifications of the nuclear structure are not regarded as having any special sequence or significance. In some of the smallest zooids of figure 23 there are still other modifications wherein a single, deeply staining mass is frequently observed. Owing to the size of these zooids it is extremely difficult, even with a binocular microscope, to form any adequate conception of their structure.

A diligent search has been made among these small forms to find a series of developing zooids which would clearly show the whole process of the formation of the several organelles. While *Crithidia euryophthalmi* undoubtedly furnishes the necessary material for such a study, the size of the endogenous buds makes the interpretation exceedingly difficult. It is therefore disappointing that a complete series has not yet been accumulated showing the steps which are thought to take place in the development of a bud into a typical

erithidia. The results of the study now indicate that the extranuclear organelles are apparently formed as outgrowths from the nucleus. It is conceived that the centrosome of the nucleus divides, giving rise to the extranuclear centrosome, or blepharoplast. From the blepharoplast, which is the dynamic center of the nuclear outgrowth, two other organelles are formed. The flagellum grows forward anteriorly and the parabasal body to the side. The connection which persists between the blepharoplast and the nucleus is the nuclear rhizoplast. Between the blepharoplast and the parabasal body is the parabasal rhizoplast. Careful observation of some of the larger forms reveals a cytoplasmic sheath around the flagellum, which is continuous with a similar sheath around the parabasal body. The latter sheath is like a sack, in which the deeply staining, bar-shaped parabasal body is suspended. The variation in size of the parabasal body may explain the light area which is frequently observed to surround this organelle. The fan-shaped appearance of the parabasal rhizoplast is due in all probability to this sacklike sheath. Any general conclusions concerning the origin of these organelles would, at this time however, be premature, although the observations of the zooids under the binocular microscope tend to give this conception of their origin. It is necessary to keep in mind constantly the fact that the material with which we are dealing is the complex life cycle of a flagellate and that several stages of the life cycle have not yet been followed, step by step, in the living material. The possibility of confusing two life cycles is always present, and the fact that all of the work is done near the limits of microscopical magnification adds further possibility of misconception.

Aside from these difficulties and doubtful points, however, the discovery of all of these stages of what has been interpreted as a process of endogenous budding, opens up further problems for investigation in the life cycles of these flagellates. The origin of the parabasal body in the endogenous bud is a big problem in itself. In addition, the light thrown upon the probable origin of the numerous binucleated spores or *Leishmania*-like bodies, which occur so abundantly in the life cycles of such flagellates as *Schizotrypanum cruzi* (Chagas, 1910), *Crithidia melophagia* (Porter, 1910), and *C. leptocoridis* (McCulloch, 1915), is very suggestive.

Somatella.—Previous to the discovery of the multinucleated flagellates which were undergoing a process of internal or endogenous budding, another type of multiple fission had been studied in prepara-

tions of the "crop" of *Euryophthalmus convivus*, namely, that which leads to the formation of the somatella (pl. 3, figs. 28-32). These spherical crithidias have certain characteristics in common with the tailed and tailless spheres described in the life cycle of *Trypanosoma lewisi* by Minchin and Thomson (1915). In a general way they resemble also the somatellas in the life cycle of some of the Polymastigina described by Kofoid and Swezy (1915). Beginning with the earliest stages of this type of multiple fission a series of flagellates can be arranged which parallels the successive stages of multiple fission of *T. lewisi* (cf. Minchin and Thomson, 1915, pl. 37). At the beginning of this series such flagellates as shown in figures 24 and 25 of plate 3 are to be found. In figure 24 the elongate flagellate is beginning to round up. The attenuate ends are being drawn up to the central part of the body and the flagellum has become intracellular throughout its length. A somewhat different formation of the sphere is shown in figure 25, plate 3. The long, attenuate ends are being wrapped about the body and the flagellum is likewise entirely intracellular in this flagellate. Other important features to be observed in connection with this flagellate are the first indications of nuclear division in the spherical formation. The centriole or centrosome within the nucleus of the rounding-up flagellate is initiating the division of the organelles. It has divided into two daughter-centrosomes, which are still connected by a centrodesmose, and with each daughter-centrosome there is present a varying amount of chromatin material. No chromosomes are present at this stage of the division, and their presence at any period throughout the process in *Crithidia euryophthalmi* has not been established. Indications of chromosomes in the ordinary binary fission have been observed, but no definite number has been determined. The nuclear membrane has begun to constrict on either side of the centrodesmose. The entire division of the chromatin material takes place within the nuclear membrane, and the process is apparently a primitive type of promitosis. The centrosome within the karyosome is not always the first to divide; on the contrary, in many instances the first indication of the division of the organelles is shown by the blepharoplast or extranuclear centrosome. When the blepharoplast divides the division of the parabasal body follows immediately. A repetition of the division of the several organelles occurs and the spheres finally break up into a number of merozoites, or daughter individuals.

The spherical formation is completed in figure 26, plate 3. A flagellum is protruding beyond the surface of the sphere. Internally

division of the blepharoplast, the parabasal body, and the nucleus has occurred. A second flagellum growing out from the daughter-blepharoplast cannot be observed. Another more advanced stage of multiple fission is shown in the somatella in figure 27. The number of nuclei and of the parabasal bodies is the same as in figure 26, but the outgrowths of the daughter-flagella are clearly shown in this sphere. Spheres without protruding flagella are to be found in figures 28 and 29, plate 3. The formation of the merozoites within the spheres, as presented in figure 29, has advanced to the point where they are clearly defined. In figure 28 another important observation can be made, namely, that the divisions of the blepharoplast and the nucleus do not occur simultaneously. In this particular sphere there are three parabasal bodies present but only two nuclei. In figure 29 there are four merozoites visible, each of which shows the outgrowth of a flagellum, and a similar spherical formation is shown in figure 30, wherein the four merozoites are somewhat larger and more developed. In the latter somatella, however, the thickness of the sphere prevented the usual amount of destaining necessary to show the nuclear structure. In each of these merozoites the body is elongating and the anterior end is becoming attenuate.

Another thick sphere is found in figure 31, and all the nuclei therein have the appearance of being a solid mass of chromatin. In this somatella the number of merozoites which could be counted is twelve. The irregular outline of the sphere indicates that the breaking up or plasmotomy of the sphere is about to occur. Possibly some of the merozoites have already escaped. In the investigation thus far the number of merozoites in a somatella has been exceedingly variable. In some of the Polymastigina, Kofoid and Swezy (1915) found the number of merozoites to be eight, which is apparently constant for the somatellas of these flagellates. Minchin and Thomson (1915) report a variable number of merozoites in the spheres of *Trypanosoma lewisi* but they found the average number to be approximately ten. In the spherical mass of flagellates shown in figure 31, plate 3, the number is twelve, but in a still larger sphere (pl. 3, fig. 32) the number is probably double that, or twenty-four. The denseness of the latter may have obscured some of the parabasal bodies and nuclei. Protruding from the surface of this sphere are numerous flagella which are outgrowths from the daughter-blepharoplasts. In the somatellas of the Polymastigina the nucleus and the extranuclear organelles may divide simultaneously, but in the spheres of *T. lewisi*

and in the somatella of *Crithidia caryophthalmi* either the extra-nuclear or the nuclear organelles may divide first. The ultimate number of nuclei is equal to the number of parabasal bodies and of the blepharoplasts.

The stage of development of the merozoites when plasmotomy occurs, is variable. Usually the rupture of the somatella occurs when the merozoites are just beginning to elongate, as in figure 31. All the organelles of these merozoites are definitely outlined, and the flagella are free for a certain distance of their length. In a few instances extremely elongate flagellates have been observed wriggling about within the spherical structures. There is no evidence of a residual mass of cytoplasm.

A comparison of these two methods of multiple fission in the life cycle of *Crithidia caryophthalmi* reveals the fact that they are fundamentally different. In the first place, the former method, endogenous budding, involves only one organelle of the crithidia, namely, the nucleus. In the latter method a somatella results from a repeated division of the nucleus, blepharoplast, parabasal body, and, in all probability, the nuclear and parabasal rhizoplasts. The flagella, however, in each case are new outgrowths from the newly formed blepharoplasts in endogenous buds or from the daughter-blepharoplasts in the somatella. Secondly, the flagellates undergoing endogenous budding retain their normal shape as elongate or pear-shaped crithidias. In some instances their size increases as the buds develop. The flagellates forming somatellas round up into spheres. The comparative efficiency of the two processes from the standpoint of the multiplicative phase in the host cannot be estimated. The length of time necessary to complete each process and the conditions under which each occurs are at present unknown. On the whole, the number of individuals resulting from an equal number of endogenous flagellates or of somatellas is approximately the same. Our conclusion, however, concerning the two methods is that the endogenous budding is of greater importance in the life cycle of *C. caryophthalmi* since in the preparations there are relatively many more evidences of the endogenous budding than of the somatella.

BINARY FISSION

Another method of multiplication of *Crithidia caryophthalmi* in the "crop" is binary fission. This process has also been observed in the pyloric expansion. While the number of crithidias found dividing

in this way is relatively small, on the whole, yet crithidias of almost any stage of development apparently may thus increase their number. Some conception of the prevalence of the process throughout the life cycle may be gained by studying figures 33 to 37. In addition to these smaller crithidias in various stages of development many instances of binary fission have been observed among the elongate or mature flagellates.

The blepharoplast or the centrosome of the nucleus may initiate the division of the several organelles. In figure 33, plate 3, the centrosome of the nucleus is in the process of binary fission and there are present two daughter-nuclei. In each of the nuclei the chromatin is peripheral on the nuclear membrane. The blepharoplast and the parabasal body have not yet begun to divide. The general appearance of this binary fission form is very similar to that of the smaller somatella. In the unflagellated crithidia shown in figure 34, plate 3, the blepharoplast and parabasal body have divided but the nucleus is still intact. A more advanced stage of binary fission is found in figure 35. In this small, unflagellated crithidia the blepharoplast, parabasal body, and the nucleus have divided, and a light, thin area, which is preliminary to the cleft in the cytoplasm, extends longitudinally between the two sets of organelles. The flagella can also be observed. The longer, more clearly defined flagellum is evidently that of the parent since the second is shorter and less distinct. The last stages of binary fission are shown in figures 36 and 37. In figure 36 the cleft in the cytoplasm can be traced from the anterior to the posterior end, and the daughter flagellum has also attained a greater length. The two flagella in figure 37 are the same length; the separation of the two daughter individuals is more marked and almost complete in figure 38. The posterior ends are the last to remain attached and the lashing about of the anterior ends assists efficiently in the final tearing apart.

One of the most interesting problems in connection with this method of reproduction has always been the origin of the daughter-flagellum. Is it due to the division of the flagellum of the parent or to a new outgrowth from the daughter-blepharoplast? A review of the work already done on this genus indicates that with the exception of Porter (1909, 1910) all authors regard the daughter-flagellum as a new outgrowth and consider that the parent-flagellum does not split to form two daughter flagella. My work on *Crithidia leptocoridis* and *C. euryophthalmi*, unlike that of Porter, is an agreement with

the results of other investigators. For some time the evidence of the new outgrowth was difficult to obtain (McCulloch, 1915), but very clear evidence of such an outgrowth from the blepharoplast has finally been discovered. Text-figure C gives a very clear picture of the origin of the daughter-flagellum in *C. leptocoridis*. The larger size and the greater abundance of erithidias undergoing this process make *C. leptocoridis* the most desirable material to illustrate this point.

The importance of binary fission in increasing the number of parasites in the multiplicative phase in the "crop" is not great. At the present time no evidence of the process has been found in the mid-stomach, which serves chiefly as a passage way for the crithidias mi-

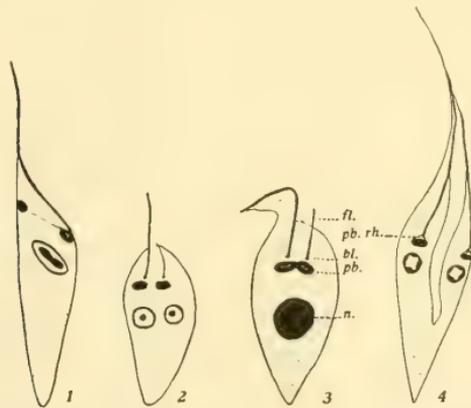


Fig. C. Flagellate stage of *Crithidia leptocoridis* to show the outgrowth of the new flagellum in binary fission. $\times 3500$. Abbreviations the same as in figure A.

grating from the "crop" to the pyloric expansion. In the pyloric portion of the digestive tract, however, binary fission is of great importance in increasing the number of crithidias, which attach themselves to the epithelial lining of the pyloric expansion.

In connection with the process of binary fission in *Crithidia euryophthalmi* another problem of interest has occurred, namely, the nature of the difference, if there be any, between the division of the several organelles in binary fission and the division of these same organelles in the somatella. Minchin and Thomson (1915) have regarded the increase in the number of nuclei and kinetoculi in the spheres of *Try-*

panosoma lewisi as being due to a repeated binary fission wherein the resulting individuals failed to separate immediately. Kofoid and Swezy (1915) have described in detail the process of binary fission and of multiple fission for the trichomonad flagellates, and these authors found the increase of organelles in multiple fission to be due to thrice repeated mitosis. The larger size of those flagellates together with the correspondingly increased size of the organelles presents better material for the study of binary and multiple fission than do the trypanosomes or the crithidias. In *C. euryophthalmi* the minute size of the flagellates undergoing either process have made accurate interpretation thus far impossible. For this reason the relation of binary fission to multiple fission in a somatella must remain an open question for the present.

INTRACELLULAR CRITHIDIAS

Another salient similarity between the life cycle of *Crithidia euryophthalmi* and the life cycle of *Trypanosoma lewisi* in the invertebrate host is the appearance in each life cycle of a stage of intracellular multiple fission. In *C. euryophthalmi* there is figured for the first time an epithelial cell in the life cycle of a crithidia taken from a "crop" containing numerous crithidial parasites. Careful examination of this infected cell shows that there are three distinct groups (pl. 3, fig. 39, *a, b, c*) of parasites and a number of scattered crithidias. Altogether there are approximately seventy parasites in this one epithelial cell. In figure 39*a* the crithidias are of two sizes: long, slender flagellates, and short, non-flagellated forms. Since multiple fission of either the endogenous or somatella type produces zooids or merozoites of approximately the same size, it appears that several infections have occurred in this cell. It is conceivable that all the elongate flagellates are due to one infection while the short, non-flagellated crithidias are due to a second infection. In figure 39*b* there are ten small oval forms. In structure they show a diffuse nucleus, as do the other parasites within this host-cell, which is probably due in part to the thickness of the cell. A nuclear rhizoplast can be observed passing from the nucleus to the blepharoplast, and a short intracellular flagellum extends forward to the anterior end of the body. The parabasal body of each is a relatively small and deeply staining structure. At *c* and *e* of figure 39 are more of the small oval forms. In the former region the cytoplasm of the host-cell has been

completely destroyed within a certain radius of the parasites. In the latter the small forms are scattered in the cytoplasm of the host-cell. At *d* there is still another group of elongate flagellates, which are probably the results of another process of multiple fission. They are approximately the same size and shape, and are in the same stage of development. Whether they are merozoites from a somatella or zooids from endogenous buds is not clear, but they have broken apart somewhat and are making their way to the periphery of the host-cell. At *f* a flagellate is protruding through the cell wall and the posterior end is directed forward to penetrate the tissue. In the study of the living material of both *C. leptocoridis* and *C. euryophthalmi* crithidias have been observed to direct their posterior ends forward and to use the flagellated ends as propellers in penetrating tissues.

The early stages of the intracellular multiple fission have not yet been found within the epithelial cells of the crop and the more advanced stages, such as shown in figure 39, do not indicate definitely the method of multiple fission. These intracellular crithidias had been described as the results of multiple fission within somatellas before the discovery of the stages of the endogenous process of multiple fission in this life cycle. The evidence is not absolutely convincing either way, but there is at the present state of the investigation a preponderance of evidence in favor of their probable origin by the plasmotomy of a somatella. The circular outlines of the vacuoles in which the groups of parasites are found in figure 39 suggest the formation of the spheroidal somatella. Further there are no evidences of discarded flagella within these cells which suggests the possibility that endogenous budding might have occurred with the formation of circular cavities in the cytoplasm of the host-cell. On the other hand, if the numerous small oval spores are due to a process of multiple fission within a somatella the subsequent plasmotomy has taken place very early. Usually the breaking apart of the merozoites does not occur until they have become elongate flagellates. Moreover, the exact method of the process of intracellular multiple fission is not so important as the fact that under certain conditions crithidias become intracellular and destroy the epithelial lining of the digestive tract which they penetrate. Each destruction of a host-cell thus also means a tremendous increase apparently in the number of the parasites. The intracellular crithidias are not found frequently in the preparations. We have no proof that it is an obligatory phase, though it might well be so. Nor have we evidence that it follows the forma-

tion of a zygote, as does the somatella of *Plasmodium* in the wall of the digestive tract of a mosquito. There is no evidence that the zygote precedes the somatella in the Polymastigina. In the life cycle of *Trypanosoma lewisi* the process of intracellular multiple fission is apparently obligatory. The trypanosomes, or haemoflagellates, penetrate epithelial cells of the "crop," undergo multiple fission, and crithidiomorphic merozoites are produced. This phase brings about the early stages of transition from a trypanosome to a crithidia. A similar need for such a phase is not present in the life cycle of *Crithidia curyophthalmi*. At present our knowledge of intracellular multiple fission in the life cycle of *C. euryophthalmi* is too meager to permit of extended correlation between the life cycle of this more primitive flagellate with that of the more highly developed haemoflagellate *T. lewisi*. It is conceivable that in the evolution of trypanosomes from the crithidial-like flagellates the intracellular multiple fission was carried over and became more specialized and more important in the life cycle of the haemoflagellate.

RECTAL PHASE

The stomach phase of *Crithidia curyophthalmi*, beginning with the initial infective spores and ending with the great swarm of parasites resulting from binary, extracellular, and intracellular multiple fission in the "crop," is followed by the established rectal phase of the life cycle in the pyloric expansion.

Owing to the structure of the digestive tract of *Euryophthalmus convivus*, with its three divisions separated only by a narrow constriction, which allows a possible intermingling under normal conditions of the crithidias in the "crop" with those of the mid-stomach and pyloric expansion, it is extremely difficult to say where the stomach phase ends and the rectal phase begins. In *Trypanosoma lewisi* the transition between the stomach and rectal phase, as has already been pointed out, is marked by definite structural changes. The trypanosomes, brought into the stomach of the flea with blood from a rat, enter epithelial cells and undergo multiple fission, producing merozoites of a crithidiomorphic type. The merozoites thus produced may do one of two things, either enter other epithelial cells of the stomach or collect at the pyloric opening and be carried down the intestine to the rectum with food. As they pass through the intestine the structural

changes which convert erithidiomorphic forms into crithidias are taking place.

The frequency with which we find preparations of the "crop" free from infection with *C. curyophthalmi*, the less frequency of infection of the mid-stomach, together with the almost invariably heavy infection in the pyloric expansion, lead us to the conclusion that the crithidias of *Euryophthalmus convivus* have the same tendency to migrate posteriorly as does *T. lewisi* in the flea. In *C. curyophthalmi*, however, the movement is less marked than in *T. lewisi*. This tendency of slow progression posteriorly doubtless depends upon the movement of food contents from the "crop" into the mid-stomach and pyloric expansion.

The migrating mass of crithidias from the "crop" soon establishes three distinct types of parasites in the pyloric expansion: the nectomonads or free flagellates, the haptomonads or attached flagellates, and the infective spores which serve for transmitting *C. curyophthalmi* to another host. These types or classes of parasites are comparable in almost every way to the nectomonads, or free flagellates, the haptomonads, or attached flagellates, and the final trypaniform stage of the rectal phase of *T. lewisi*. The nectomonads and haptomonads of each life-cycle are almost identical in structure and behavior.

NECTOMONADS

Smear preparations of the mid-stomach and of the pyloric expansion show little difference in the morphological structure of their crithidial infection. The serial sections of these two parts, however, show a sharp distinction in the structure of haptomonad forms and the nature of the epithelial lining to which they are attached, and a slight distinction in the structure of the nectomonads in the mid-stomach and in the pyloric expansion. The nectomonads of the former are usually of the elongate, slender type (pl. 5, figs. 73-80). The zooids, the result of the processes of multiple fission (pl. 4, figs. 40-54) are abundant in the smear preparations of both regions. The series sections thus far have shown numerous zooids (pl. 4, figs. 40-50) in the anterior portion of the mid-stomach. These zooids (pl. 4, fig. 40) are frequently grouped together in the grooves between epithelial cells. For this reason it is possible that the current of food in passing down the digestive tract failed to carry them on into the pyloric expansion. The zooids are small forms,

(pl. 4, figs. 40-47) from 1μ (pl. 4, fig. 40) to 1.5μ (pl. 4, fig. 47) in length and from 0.7 to 1μ in diameter. In comparing them with the initial infective oval spores (pl. 2, figs. 1, 2) it is noticed that they are smaller, stain less densely, and do not show a heavy periplast. The nuclear structure also is unlike that of the initial spore forms, and the location of the nucleus within the zooid adds another distinguishing character. These zooids have always been found in the anterior portion of the mid-stomach in the serial sections while the sections immediately posterior contain developing crithidias (pl. 4, figs. 59-64). Groups of elongating forms are frequently found (pl. 4, fig. 59). With the elongation of the body of the zooids, especially of the anterior end, the nucleus instead of being filled with chromatin is now vesicular and contains a distinct karyosome. This group (pl. 4, fig. 59) shows the parabasal bodies in close proximity to the nuclear membranes. Judging from the conditions found within the great majority of forms, figure 59 is probably an exceptional case in this respect. The parabasal body normally moves anteriorly in the early development of the zooid (pl. 6, fig. 40) before the flagellum and the anterior end grow out (pl. 4, figs. 41-53). In the figures just noted the flagellum is not yet visible. The nuclear rhizoplast, extending from the nucleus forward to the region of the blepharoplast and parabasal body, is found by focusing carefully. In figure 54 the flagellum is growing out from the blepharoplast but there is no noticeable lengthening of the anterior end of the body. In figures 55 to 58 the flagellum and anterior end of each are lengthening simultaneously. In these same figures the nuclei are like the nuclei of the zooids, being completely filled with chromatin. Farther on posteriorly are found crithidias such as are shown in figures 63 to 72. Beginning with figure 65 there is also an elongation of the posterior end, which is equal to that of the anterior.

The majority of these developing crithidias have the vesicular type of nucleus. Figures 65, 67, and 68 are exceptions, but no significance can be attached to them since the position, relative thickness of the body, or the technique, could explain these exceptions in this region of the digestive tract. The study of the serial sections leads us to think that the time necessary for the stomach crithidias to reach the rectum is approximately the amount of time required for the zooids to develop into mature flagellates. Not all of the developing zooids become mature in the mid-stomach. Under certain conditions the food current probably carries many of the non-flagelated stages or zooids back into the pyloric expansion before they have

scarcely begun to develop. As the developing forms and the fully matured forms enter the pyloric expansion, they become part of the permanent crithidial infection therein. The content of the crithidial infection of this region varies from time to time. Nectomonads may predominate or all nectomonads may become haptomonads, or attached forms. Usually the nectomonads and haptomonads are both present in large numbers. Mature nectomonads in the mid-stomach are usually the elongate, slender crithidias (pl. 4, figs. 73-79). In figure 74 the body size is approximately 25μ in length and 1.7μ in width. Figures 75 to 78 are yet narrower, averaging about 1μ in width. The nectomonads of the pyloric expansion are frequently like figures 73 to 79, long, slender forms together with numerous shorter and stouter crithidias (pl. 4, figs. 80-90). It is conceivable that the elongate slender forms give rise to the shorter, stout forms. The length of the body is decreased and the width is increased. Both the anterior and the posterior ends of the body become less attenuate.

The structure of the nucleus of the elongate forms varies considerably. In figures 73 and 80 there are distinct central karyosomes with chromatin-encrusted membranes. In figure 74 the chromatin is in five granules scattered within the nuclear membrane. The chromatin is broken up into granules in figures 76 and 79. The breaking up of the chromatin material in these nectomonad forms doubtless means the beginning of a degeneration, which will be discussed shortly. The nuclear structures in figures 77 and 78 are unique. In figure 77 the nuclear membrane is rather densely encrusted with chromatin and is elongate and irregular in outline. In figure 78 the nuclear membrane is oval, chromatin-encrusted, with two masses of chromatin at the anterior and posterior regions of the nucleus. In the series of figures 81, 83 to 86 a karyosome of a variable size is found in each and its location is not always central. In figure 82 a distinct chromidial fragmentation of the chromatin has taken place, which is another indication of degeneration. The short, stout forms (pl. 4, figs. 87-90) are transition forms from the nectomonads to haptomonads. While these flagellates are still free forms they resemble the haptomonads of the pyloric expansion, which are attached in the mid-region of this division. In the anterior part of the pyloric expansion the haptomonads are relatively long, slender flagellates while posterior to the middle portion they are still short and more pear-shaped. The nuclear structure of these transition forms indicates no degeneration as yet. They all have a central karyosome and a more or less encrusted nuclear membrane.

HAPTOMONADS

One of the most characteristic features of the life cycle of a crithidial flagellate is the great mass of attached forms which line definite parts of the digestive tract of the host. Minchin and Thomson found three regions of the digestive tract of the rat-flea where the crithidias of *Trypanosoma lewisi* might attach themselves, namely, the prepyloric, post-pyloric, and the rectal regions. In the lupine bug there are likewise three regions where the haptomonads may attach themselves: (1) in the posterior part of the "crop," where they are possibly comparable to the prepyloric crithidias in the posterior part of the stomach of the flea; (2) in the posterior half of the mid-stomach, where they are probably comparable to the post-pyloric crithidias of the rat-flea, attached to the anterior part of the intestine; and (3) in the pyloric expansion, where they are comparable to the haptomonads of the rectum of the rat-flea. In *Trypanosoma lewisi* in the flea these investigators regard the prepyloric haptomonads as being due to a forward migration from the rectum, possibly as a result of the food conditions. The prepyloric haptomonads are not found frequently in the flea, and in only one preparation of the "crop" of the lupine bug were haptomonads found. In the lupine bug haptomonads were found in a number of preparations of the mid-stomach, but they were commonly present in preparations of the pyloric expansion. The haptomonads observed in the single preparation of the "crop" were of the rectal type, small oval forms similar to those from the pyloric expansion shown in plate 6, figures 107 and 117. The serial sections of the digestive tract, however, showed no haptomonads in the "crop."

From the serial sections of the mid-stomach abundant material was obtained for the study of the haptomonad of this region. The attached forms here (pl. 6, figs. 93-96) are relatively small, slender flagellates. They are uniform in size and shape, on the whole, and form a definite fringe on the inner surface of the epithelial lining of the sections of the mid-stomach. These flagellates attach themselves to the epithelial cells by means of the flagella. They frequently almost surround the elongate, columnar epithelial cells, which project into the lumen of the digestive tract. The grooves between masses of epithelial cells evidently afford a particularly good place for the haptomonads, since they are found in compact layers in such places. Haptomonad crithidias of this type are the only ones found in the mid-stomach and they continue to line the digestive tract posteriorly

into the pyloric expansion. Considering the attached crithidias the most anterior sections of the pyloric expansion are almost identical with the sections from the posterior part of the mid-stomach. The lumen of the narrow constriction separating the mid-stomach from the pyloric expansion is frequently almost blocked with the bodies of the flagellates extending out into the passageway between these two enlargements of the digestive tract.

The structure of these haptomonads (pl. 6, figs. 93-96) is also relatively uniform. The majority of the crithidias show the vesicular type of nucleus, with a central karyosome and a nuclear membrane containing an inner lining of chromatin material. In figure 94 the nuclear membrane is slightly distorted in appearance and the karyosome is excentric, being posterior. In figure 96 the nuclear membrane is not distinct and the enlarged mass of chromatin material is in the form of two granules. These haptomonads are usually characterized by their attenuate anterior and posterior ends. The posterior ends of these crithidias more than of any others show relatively extreme attenuation.

Beginning in a region just posterior to the mid-stomach end of the pyloric expansion a series of transition haptomonads is found (pl. 6, figs. 97-106), lining the middle part of this division of the digestive tract, these transition haptomonads, which vary considerably in size. Figure 97 shows a broad, stout form. Such forms undergo binary fission, producing two smaller, more slender individuals. Possibly figures 100 and 101 are the products of such a division. The binary fission of the broad, stout forms rapidly increases the number of haptomonads which make up the dense and compact layer of attached crithidias. The nuclear structure of these forms shows comparatively little variation. They have the vesicular nucleus with the small central karyosome and chromatin-encrusted nuclear membrane.

At the posterior portion of the pyloric expansion are found the normal rectal forms (pl. 6, figs. 107-124) which are common to the life cycles of so many of these flagellates. One of the interesting things observed in connection with these forms is that the wall of the pyloric expansion becomes exceedingly thin. There are few indications left of the epithelial cells lining this part of the mid-gut. Sections of this portion of the tract previous to any infection by the flagellate are not at hand, unfortunately, and consequently it is difficult to estimate the total amount of destruction incurred. The pyloric expansion of an infected adult bug, however, is extremely weak and

becomes torn very readily; it has every appearance of having been almost entirely destroyed by the erithidias. Among these small haptomonads numerous variations of size and shape are noted. The length of the free flagellum (pl. 6, figs. 109, 110) is exceedingly variable. In other erithidias whatever flagella are present are intracellular throughout their length. The changes brought about in the flagella are probably due to their absorption. In every case the flagellum attaches the erithidia to the wall of the digestive tract. The nuclear structure of the haptomonads in this region, with the exception of the forms in figure 91, shows no indication of degeneration. The round haptomonads finally become free forms. They can be seen to drop off in the living preparations and to roll up into round or oval forms (pl. 6, figs. 118, 119, 122). The cytoplasm of these forms is vacuolate and stains lightly. These round erithidias then degenerate along with the nectomonads, which are constantly degenerating. The degeneration of the haptomonads and nectomonads will be described under the degenerative series.

FINAL SPORE FORMS

The structure of the digestive tract of *Euryophthalmus convivus*, including three portions of the stomach proper and the intestine with its gland, differentiates very clearly between the degenerative series of erithidias and the final spore forms which can be transmitted to another host. In the anterior part of the digestive tract is found the developmental series which becomes the degenerative series together with the final spore forms. Posterior to the gland only the final spore forms have been observed. The preparations of the rectum show only these final spore forms (pl. 6, figs. 125-131). These are oval, non-flagellated forms containing a thick periplast, within which are the nucleus and parabasal body. In figure 126 the characteristics of these final spore forms may be noted. They are approximately 2.8μ in length and 1.4μ in width. The nucleus lies in the extreme posterior end of the body and stains deeply. The parabasal body is sharply outlined within a vacuolate area. A faint nuclear rhizoplast may be visible, extending from the nucleus toward the parabasal body. As previously indicated in a preliminary paper (McCulloch, 1917) it was some time before the true rectum was discovered, and consequently the significance of these final spore forms was not entirely clear in the beginning of the investigation. However, with the discovery of the true rectum and the fact that it contained only these

final spores the characteristics of these rectal forms became clear and it was relatively easy to find them in both the mid-stomach and pyloric expansion. In the "crop" of the lupine bug the multiple fission gives rise to numerous small zooids. Some few of these zooids, on reaching a certain stage of development, become the well protected final spore forms. Their development into mature flagellates is arrested for an indefinite time, perhaps in response to some unfavorable internal or external chemical condition. The periplast becomes thickened and these zooids stain deeply and retain the stain much better than the unprotected zooids. Since only a few of the zooids become thus encysted the change cannot be regarded as a general change in response to some general stimulus. In the infections of the mid-stomach and pyloric expansion some of the final spore forms can be found at almost any period in the life history of the flagellates. Considering that these are the only stage of the life cycle of the flagellate which have been found in the rectum they have been regarded as the final spore forms, which upon being ingested with food by another insect host become the initial infective spores. Our observations upon these cannot be regarded as conclusive as yet, owing to the fact that experimentally we have not produced infection with these spores.

THE DEGENERATIVE SERIES

The degenerative series includes all individuals of the life cycle other than the final spore forms and their antecedents which have just been discussed. It has already been pointed out that the rectal phase of *C. euryophthalmi* in the pyloric expansion of the lupine bug is comparable to the rectal phase of *T. lewisi* in the rectum of the flea. In the lupine bug the degenerating forms abound in the posterior portion of the pyloric expansion and their number is apparently not decreased by constant elimination under normal conditions of such parasites from the intestinal contents, which pass through a relatively long intestine before reaching the colon and rectum. If any flagellates succeed in passing out with the intestinal matter into this long intestine they are evidently destroyed by the new chemical medium before they reach the rectum. In the flea the degenerating forms are in the rectum and it is possible that their number is repeatedly being decreased with each discharge of the feces.

There is little danger of confusing the developmental and degenerative series in the life-cycle of *C. euryophthalmi* since the differentia-

tion of the parasites into the final spore forms and the ordinary propagative forms takes place apparently in the stomach phase in the "crop." The majority, in fact, nearly all of the parasites become ordinary propagative forms which develop and increase their numbers in the digestive tract of the lupine bug. After passing through the various processes of the life cycle they degenerate in the late rectal phase in the pyloric expansion. The permanent rectal phase persists through the life of the lupine bug after the first infection; the degenerative series is soon formed, and likewise persists throughout the life of the host.

The individuals of the degenerative series are distinguished among the mass of living crithidias by a sticky periplast, to which bacteria frequently adhere by virtue of the tendency of crithidias to adhere to each other, by slow sluggish movements, and by odd sizes and shapes. The degenerating forms are detected in stained preparations by nuclei with diffused chromatin or by a vesicular nucleus breaking up into a number of chromatin granules, and by the vacuolated cytoplasm. The size, shape, and location of the crithidias also assists in distinguishing between the developmental and degenerative crithidias.

CONCLUSIONS

1. The crithidial flagellates of the life cycle of *Trypanosoma* are structurally like the crithidial flagellates of the life cycle of *Crithidia*. The extranuclear organelles, the blepharoplast, parabasal body, parabasal rhizoplast, nuclear rhizoplast, and the flagellum are all common to the crithidial flagellates of both *Trypanosoma* and *Crithidia*.

2. From the viewpoint of comparative morphology the differences existing between the crithidial forms of *C. euryophthalmi* and the crithidial forms of *T. lewisi* are less marked than are the differences between similar stages of *T. lewisi* and *Schizotrypanum cruzi*.

3. Using the life cycle of *T. lewisi* as a standard for comparison of the life cycle of a haemoflagellate or a trypanosome and the life cycle of *C. euryophthalmi* as the standard of the life cycle of a more primitive crithidial flagellate, there are more parallel stages and phases in these two life cycles than exist between the life cycle of any trypanosome and the life cycle of any herpetomonad or of any leptomonad now known. Furthermore the close correlation between these two life cycles of *T. lewisi* and of *C. euryophthalmi* affords new evidence that

the evolution of a trypanosome has probably taken place from a crithidial flagellate rather than from a herpetomonad or leptomonad flagellate.

4. The process of multiple fission in the somatella of *C. euryophthalmi* is fundamentally like the multiple fission (sphere formation) of *T. lewisi* and also like the multiple fission (somatella) of the trichomonad flagellates. In each of these flagellates the nucleus, parabasal body, blepharoplast, and flagellum, or flagella, are concerned in the process. In each after multiple fission plasmatomy occurs.

5. The process of multiple fission by endogenous budding in the life-cycle of *C. euryophthalmi* tends not only to establish another link in common between the life cycles of *Trypanosoma* (e.g., *T. gambiense*) and the life cycle of *Crithidia* but also to link the life cycle of *Crithidia* more closely to the lower protozoan forms which contain numerous *Leishmania*-like bodies in their life cycles.

6. The endogenous buds in the life of *C. euryophthalmi* are comparable to the latent bodies in the life-cycle of *T. gambiense*.

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EXPLANATION OF PLATES

All figures were outlined with a camera lucida using a $\frac{1}{12}$ objective on the binocular microscope and a Watson no. 20 holoscopic eye-piece. The magnification is in all cases approximately 3500. Unless otherwise stated all figures were made from iron-haematoxylin preparations.

PLATE 2

Crithidia euryophthalmi from the "crop" of *Euryophthalmus convivus*.

Fig. 1. Small, oval infective spore which has been casually ingested with food. This spore shows the structure common to these phases, thick periplast, deeply staining nucleus in extreme posterior end, heavily stained parabasal body. Faint nuclear rhizoplast.

Figs. 2-8. A series of developing crithidias showing successive stages of development. Both anterior and posterior ends are becoming attenuate. The nucleus changes from a solid mass of chromatin to a vesicular nucleus, chromatin-encrusted membrane and central karyosome. Flagellum grows forward and carries anterior end of body along with it, which forms undulating membrane.

Figs. 9-10. Two mature flagellates illustrating extremes in length, width, and shape, common to *C. euryophthalmi*. A whole series of intergrading forms occur between these two extremes.

Figs. 11-23. Multiple fission; endogenous budding. (Fig. 11). Flagellate with a nucleus, two endogenous buds posterior to nucleus. Chromatin of both nucleus and buds massed on nuclear membrane. Blepharoplast and parabasal body intact, no indications of division.

Fig. 12. A short flagellate; one endogenous bud; chromatin peripheral in each nuclear structure.

Fig. 13. Flagellate with three clearly defined nuclear structures and the beginning of a third bud from the most anterior, the nucleus proper. Chromatin peripheral in each of these nuclear structures.

Fig. 14. Elongate flagellate nucleus; two endogenous buds anterior to the nucleus; chromatin peripheral.

Figs. 15-17. Pear-shaped crithidias with endogenous buds. If nucleus be present in each there are no differences in structure between nucleus and bud. Buds in these flagellates are always sharply defined. Chromatin material on membrane but most of it is in definite granules.

Fig. 18. Pear-shaped form with numerous endogenous buds but no definite nucleus. First stages of degeneration present, parabasal body has disappeared and only a fragment of discarded flagellum is near. Chromatin peripheral but also in form of one granule.

Fig. 19. Elongate flagellate; two endogenous buds posterior to nucleus. Nuclear rhizoplast still present. Chromatin distributed irregularly on the nuclear membranes.

Fig. 20. Elongate flagellates with nucleus and two endogenous buds; chromatin massed in the anterior and posterior portion of each nuclear structure. Parabasal body has taken no part in this multiple fission.

Fig. 21. Large endogenous flagellate with five buds, all anterior to nucleus proper. Two of the buds are not destined sufficiently to see their structure. The organelles other than nucleus have taken no part in the process of endogenous budding. Flagellum, blepharoplast, parabasal rhizoplast and parabasal body are still intact and clearly shown.

Fig. 22. Late stage in endogenous budding. Buds have formed zooids, each of which has a nucleus and a second, deeply-staining structure anterior to nucleus. Flagellum, parabasal body, parabasal rhizoplast, and the blepharoplast are still intact. No signs of degeneration are to be observed in this flagellate.

Fig. 23. Drawing made from a field literally covered by discarded flagella and small zooids. The blepharoplast and parabasal body are still attached to some of the flagella. Zooids show various stages of nuclear structure. Some have a single mass of chromatin, others two granules within a chromatin-encrusted membrane. Some few of the zooids contain a nucleus, nuclear rhizoplast, and a second mass of chromatin anterior to these.

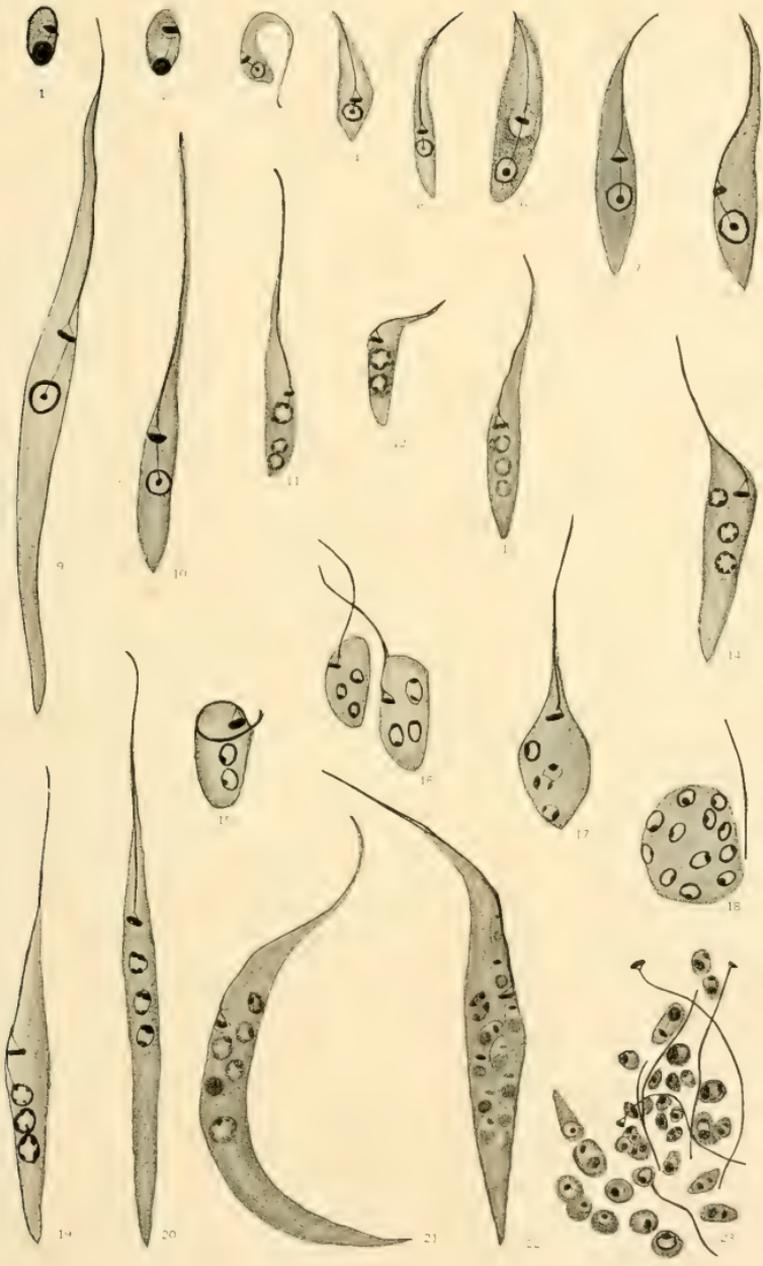


PLATE 3

Figs. 24-32. Multiple fission; somatellas. (Fig. 24). An early stage in the formation of a somatella. The flagellate is rounding up, and the flagellum is entirely intracellular. No indications of division of the nucleus, parabasal body, or the blepharoplast are present.

Fig. 25. A somewhat different type of rounding up. The attenuate anterior and posterior ends are being wrapped about the body. The nucleus has begun to constrict or to divide by a process of primitive promitosis. The blepharoplast and parabasal have not yet begun to divide.

Fig. 26. A more advanced stage in the formation of the somatella. The flagellum of the rounded up flagellate is protruding and both the nucleus and blepharoplast, together with the parabasal body, have divided. The new daughter-flagellum is not yet visible.

Fig. 27. A sphere, or somatella, still more advanced in its development. Not only have the blepharoplast, parabasal body, and the nucleus divided but the new outgrowth of the second flagellum from the daughter-blepharoplast is clearly visible.

Fig. 28. A spherical crithidia showing a repeated process of division on the part of the nuclei and parabasal bodies. Three parabasal bodies and two nuclei are present. There are possibly indications in one of the nuclei wherein the chromatin has been divided that another division was about to occur.

Fig. 29. A sphere, or somatella, without protruding flagella, containing at least four definitely outlined merozoites. The nuclei, parabasal bodies, nuclear rhizoplasts, and the outgrowths of the flagella are clearly visible.

Fig. 30. A densely stained, small somatella containing four relatively large merozoites which are beginning to elongate.

Fig. 31. A sphere, or somatella, breaking up and the merozoites about to escape. The destruction of the sphere has occurred later than usual and the merozoites have become almost mature flagellates. All nuclear structures are deeply stained, owing to the thickness of the sphere.

Fig. 32. An exceedingly large sphere, comparatively, showing many protruding flagella. Here again the nuclear structures are deeply stained because of the thickness of the sphere. The exact number of merozoites cannot be determined, but approximately twenty-four nuclei and parabasal bodies can be counted.

Figs. 33-38. Binary fission. (Fig. 33.) A small spherical crithidia undergoing binary fission. The nucleus has divided but the blepharoplast and parabasal body show no indications of fission.

Fig. 34. Binary fission, in which the blepharoplast and parabasal body have divided but the nucleus has not yet divided. A flagellum from the daughter-blepharoplast has already grown forward.

Fig. 35. Binary fission taking place in a developing crithidia. Both the nucleus and parabasal body have divided, and a new flagellum can be observed growing from the daughter-blepharoplast.

Fig. 36. Simple binary fission; blepharoplast, parabasal body, and nucleus have divided. The chromatin in the nuclei is peripheral, about the membrane.

Figs. 37, 38. A more advanced stage of binary fission, showing in addition to the division of the blepharoplasts, parabasal bodies, and nuclei a cleavage in the cytoplasm to form two crithidias in each case.

Fig. 39. Intracellular multiple fission: one of the many infected cells from the "crop" of *Euryophthalmus convivus*. This cell is in a degenerating condition. The nucleus stains a blue-gray color in iron-haematoxylin. There are at least three and possibly five intracellular infections by *C. euryophthalmi* in this cell. There are approximately seventy parasites within this cell: (a) A group of parasites of two sizes, small oval forms, non-flagellated and elongated *Crithidia*. Nuclei of all crithidias are diffuse, possibly due to thickness of smear. Nucleus and parabasal body readily observed but the other organelles are not always clear. (b) Another group evidently the result of a process of intracellular multiple fission. (c) Similar to b. Circular cavity about these non-flagellated crithidias may indicate the outline of a former somatella wherein plasmotomy has occurred early. (d) Elongate merozoites probably resulting from another intracellular somatella. Plasmotomy has occurred and the merozoites are about to make their way out of the host cell. (e) A scattered group of oval merozoites. Considerable variation in size is noted. Parabasal body, nucleus, and intracellular portions of flagellum clearly shown. (f) A mature merozoite making it way out of the host cell. The non-flagellated, or posterior, end directed first.

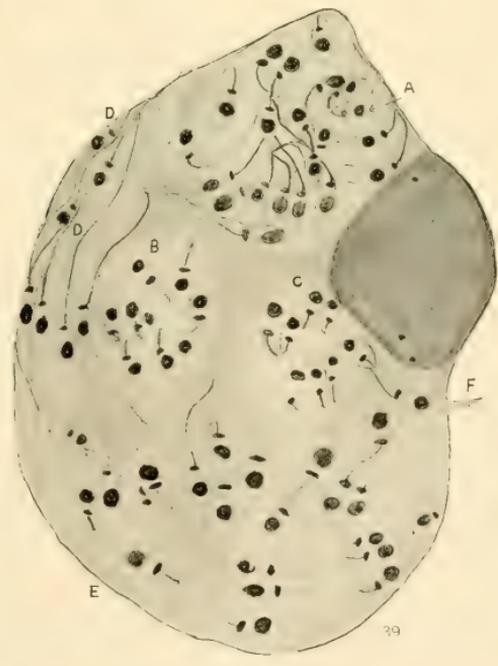
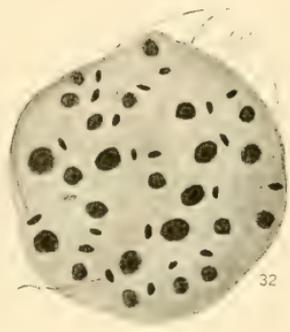


PLATE 4

Crithidia euryophthalmi from mid-stomach of *Euryophthalmus convivus*.

Figs. 40-92. Nectomonads (Figs. 40, 41). Small oval forms at the beginning of the developmental series of merozoites or zooids in the early rectal phase, showing nucleus, parabasal body, nuclear rhizoplast, and light area around the parabasal body. Nucleus at posterior end of the body. Anterior end pointed or blunt.

Figs. 42-47. More advanced stages in the developmental series; width of body and distance between nucleus and parabasal are increasing.

Figs. 48-54. Size of the oval forms increasing; nucleus still diffuse and located at extreme posterior end of body. Nuclear rhizoplast present, but no indication of the flagellum except in figure 54.

Figs. 55-58. Developing forms. Anterior end elongating and extending out along the flagellum. Nucleus still diffuse. In figure 58 the posterior end of the body is slightly elongated.

Fig. 59. A group in upper part of mid-stomach. Note vesicular type of nucleus, with the centriole-like karyosome. Both ends of the body are more or less pointed. The position of the parabasal body near the nucleus is unusual.

Figs. 60-62. Both anterior and posterior ends are elongating. Vesicular nucleus, nuclear rhizoplast, parabasal body, parabasal rhizoplast, and flagellum very distinct.

Fig. 63. Posterior end blunt; vesicular type of nucleus. Parabasal body near the nucleus. Anterior end of the body well developed; a slight undulating membrane present.

Fig. 64. Form a little more developed. Posterior end of the body is blunt or round, and nucleus is diffuse.

Fig. 65. Almost mature flagellate; nucleus diffuse; posterior end elongated and quite broad.

Fig. 66. Free form, showing vesicular nucleus and a distinct undulating membrane.

Fig. 67. Nectomonad developing into elongate flagellate; nucleus diffuse.

Figs. 68-72. Nectomonads of mid-stomach, long, slender, flagellates showing the common variations in the nuclear structures. The diffuse type in figure 68; vesicular in figures 69 and 71; chromatin-encrusted nuclear membrane in figures 70 and 72.

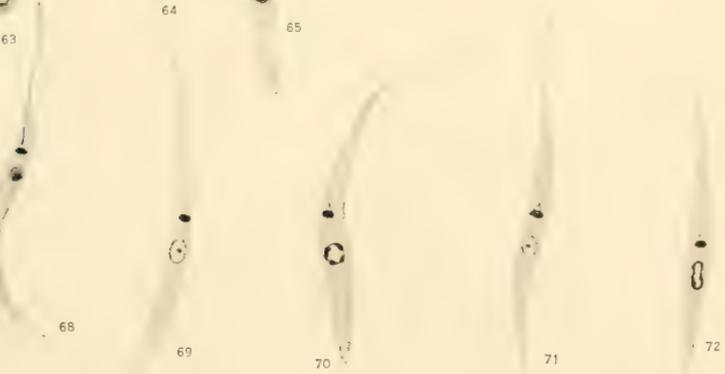
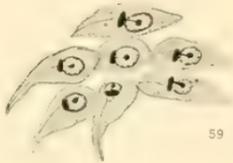


PLATE 5

Figs. 73-92. Nectomonads from pyloric expansion (Figs. 73-80). A series of elongate, slender flagellates from crithidial infection of the pyloric expansion. Vesicular types of nuclear structure in figures 73, 75, 80. Chromatin broken up into granules in figures 74, 76, and 79. Chromatin of nucleus on the membrane in figures 77, 78.

Figs. 81-86. Another group of nectomonads from pyloric expansion, illustrating the changes which take place in shape of body, length decreases, width increases. These are shorter, broad types of crithidias, and probably come from the longer, more slender forms. Nuclear structure usually shows the central karyosome. Figure 82 shows the chromatin broken up, a condition which may indicate an early stage of degeneration of the nectomonads.

Figs. 87-90. Transition flagellates from nectomonads to haptomonads. By a process of binary fission these transition forms become reduced in size. Certain nectomonads become attached to wall of the pyloric expansion or they form haptomonads. Others degenerate before reaching this stage.

Figs. 91-92. Free flagellates. Their size is doubtless due to a process of binary fission and they resemble the attached haptomonads of the pyloric expansion. They are either nectomonads which are about to become haptomonads or *vice versa*. If the latter, the haptomonads upon becoming free again usually degenerate at once.

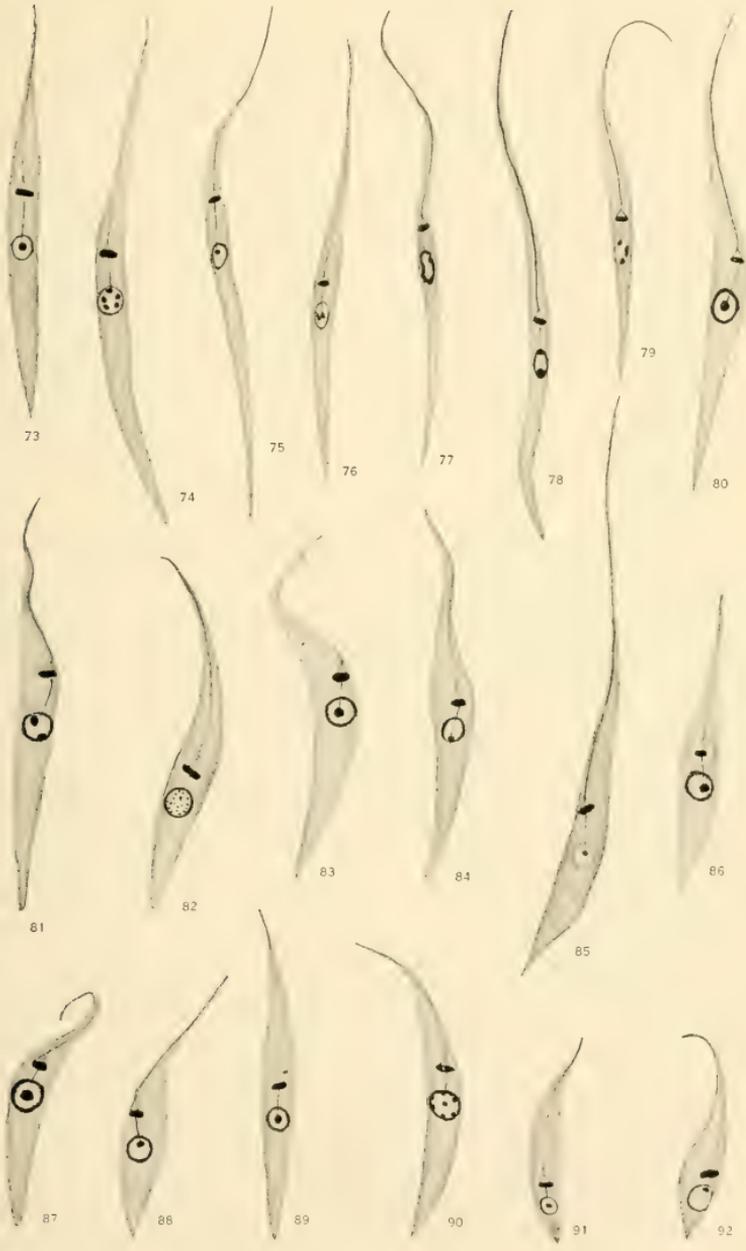


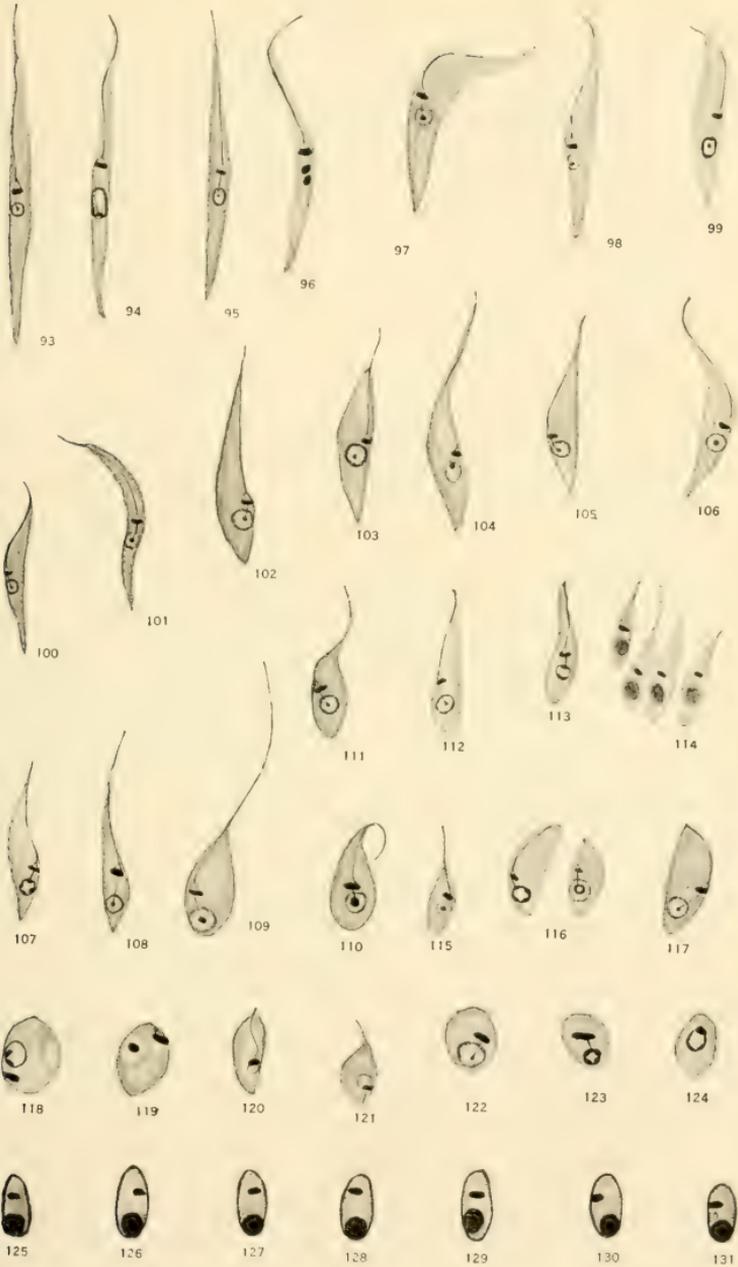
PLATE 6

Figs. 93-96. Haptomonads from mid-stomach. Haptomonads of this type may be found attached to wall of the "crop," posterior part of mid-stomach or anterior part of pyloric expansion. Small, slender flagellates usually with a vesicular type of nucleus are shown in figures 93-95. Figure 94. Nuclear membrane distorted, karyosome excentric. Figure 96 may show degeneration since there is no nuclear membrane and the chromatin is breaking up.

Figs. 97-124. Haptomonads from pyloric expansion (Figs. 97-106). Transition haptomonads lining mid-portion of the pyloric expansion. Bodies becoming pear-shaped; flagella largely absorbed and intracellular throughout length. Nucleus usually vesicular. Binary fission is common among these forms. Their size is reduced in this way.

Figs. 107-124. Small haptomonads, found in the extreme posterior portions of the pyloric expansion. They form a dense compact layer of parasites attached to epithelial lining. Reduction in size shown, beginning with figure 107. Degeneration is shown in the nuclear structure, as in figure 114, and in the cytoplasm of figures 117, 118. Figures 118-124 are all haptomonads which show advanced stages of degeneration; the cytoplasm is vacuolate and the nuclear structure is no longer normal. Most of these have become detached from the wall of the pyloric expansion.

Figs. 125-131. A series of final spore forms from rectum, showing thick periplast, deeply stained cytoplasm, and two chromatin bodies. Nuclear rhizoplast not visible in these deeply stained spores.



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A MUSCID LARVA OF THE SAN FRANCISCO
BAY REGION WHICH SUCKS THE BLOOD
OF NESTLING BIRDS

BY
O. E. PLATH



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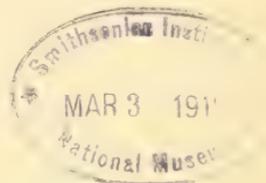
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A MUSCID LARVA OF THE SAN FRANCISCO
BAY REGION WHICH SUCKS THE BLOOD
OF NESTLING BIRDS¹

BY

O. E. PLATH



Up to the middle of the nineteenth century it was generally believed by zoologists that all muscid fly larvae were scavengers living in and depending upon decomposing animal or vegetable matter. The first one to point out that this belief is erroneous was the French scientist Leon Dufour (1845). In the spring of 1844 while examining a brood of young swallows this French scientist accidentally came across some fly larvae and pupae, the adults of which were later identified as Calliphorinae belonging to the species *Lucilia dispar*. He noticed that the larvae were "gorged with blood," and concluded that they were external blood-sucking parasites and not scavengers, as most fly larvae are. Since then a number of similar cases of parasitism by the larvae of *Protocalliphora azurea* (Fallen) and *Protocalliphora chrysorrhoea* (Meigen) have been recorded by other scientists (see Coutant, 1915, pp. 138-150); and within the last decade or two it has been discovered (Austen, 1907, and Roubaud, 1913) that the larvae of several African flies belonging to the Calliphorinae habitually suck the blood of mammals, such as the aard-vark and wart-hog, and even man, and that they are absolutely dependent upon vertebrate blood in order to mature.

In the summer of 1913 I accidentally discovered some sixty or seventy fly larvae and pupae in two nests of goldfinches, one belonging to the species *Astragalinus psaltria hesperophilus* Oberholser and the other to *Astragalinus tristis salicamans* (Grinnell). The two nests

¹ A popular and more comprehensive paper on this subject is to appear in the January-February number of *The Condor* for 1919.

contained nine young birds, four of which died shortly before they were full fledged. The fly larvae were creamy white in color, about 1.5 centimeters in length, and 0.5 centimeters in width. A number of them contained a bright red substance which looked like blood. From this I concluded that the larvae were blood-sucking parasites, and that the young birds had died from loss of blood. Both larvae and pupae were destroyed, as I supposed that this form of parasitism was no doubt generally known to zoologists.

Later in the summer I met Dr. C. A. Kofoid, head of the Department of Zoology of the University of California, and told him about the larvae and pupae. He regretted that they had been destroyed and suggested that I look into this matter more closely the following summer. It was impossible for me to do this, however, until the summer of 1917.

The period of investigation which is now about to be discussed extended from the latter part of June, 1917, until about the middle of September. The work was carried out under the supervision of Professor W. W. Cort of the Department of Zoology of the University of California, to whom the writer is indebted for a number of important suggestions.

The first nest examined, that of a Nuttall sparrow (*Zonotrichia leucophrys nuttalli* Ridgway), contained thirty-six, full grown fly larvae, and thereafter the larvae were found at various stages of development, ranging from 0.3 centimeter in length and 0.1 centimeter in width to 1.5 centimeters in length and 0.5 centimeter in width, in about two out of every three nests (the exact figures are shown in tables 1 and 2 of this paper). Nearly all of the small and half grown larvae showed a bright red substance in the anterior part of their intestine, while, on the other hand, this was the case with only a very few of the full grown larvae. Smears made from this red substance and examined under a high power microscope showed that it was vertebrate blood, which the maggots could have obtained only from the birds inhabiting the nests. To prove this beyond a doubt a number of experiments were carried out. Several dozen fly larvae were placed in a number of bird's nests containing young and the latter observed from day to day. Some of these nestlings died and the others seemed to be retarded in their growth; but otherwise these experiments furnished no absolute proof that the vertebrate blood found in the larvae was avian blood, for I had never seen any of the fly larvae attached to the young birds.

As I had found it rather difficult to observe nestlings of wild birds closely in the open, I decided to use a brood of tame canaries for further experiments. I succeeded in securing a female bird with two nestlings about a week old, but both young died within a few days, probably because they had taken cold while being transferred to my room. For these I substituted four, nearly full fledged, green-backed goldfinches (*Astragalinus psaltria hesperophilus* Oberholser).

A few days later forty of the most active larvae were selected from some two hundred. None contained any fresh blood and many of them were only half grown. These forty larvae were placed on the young birds in the early part of the afternoon and developments observed from time to time until about 8:30 P.M. At first the nestlings showed some uneasiness by shaking themselves, but this only lasted for a minute or two, after which they again came to complete rest. There was nothing further unusual in their behavior during the next six or seven hours.

At 3 A.M. the next morning one of the nestlings was found at the bottom of the cage and the others, including the mother bird, were sitting on the rim of the nest and not huddled closely together in the center. Two of the fly larvae were crawling about at the bottom of the cage and had evidently been dragged down by the nestling, because it would have been impossible for them to get out of the nest-box in any other way. They were only half grown and were gorged with fresh blood. Thereupon the four remaining birds were carefully examined. About four or five of the larvae were clinging to the feet and lower parts of the body of each one of the nestlings. None were found on the mother bird. Most of the larvae released their hold while the birds were being examined, but some of them had to be forcibly detached. These, like those found at the bottom of the cage, were not full grown and were filled with fresh blood. No marks, visible to the naked eye, could be seen where the larvae had pierced the skin of the birds, nor were any traces of blood noticeable on the nestlings. The four nestlings were then transferred to another nest and their own carefully examined. From the latter sixty-eight larvae were taken, showing that it had contained twenty-eight before the forty were added. More than half of the sixty-eight larvae showed fresh traces of blood, the smaller ones being most gorged. The soft cotton lining of the nest-box was then removed and examined, but no additional larvae were found. Thereupon the box was refilled with cotton and the nestlings put back. The latter now appeared completely

at ease. They again huddled closely together as formerly, the mother bird sitting on them during the night.

During the next few days a number of other experiments were carried out with this brood of goldfinches. While one of them was being held in the hand, a few vigorous, half grown larvae were placed among the nestling's feathers. After crawling about among them for a brief period, the larvae would invariably drop from the bird. One evening several dozen, half grown, hungry larvae were put in a pasteboard box and covered with a layer of soft cotton about an inch thick. Two of the nestlings were then placed on the cotton and the box closed. In less than ten minutes eight of the larvae were found firmly attached to the two nestlings, ingesting blood, only a few releasing their hold while the birds were being examined. This last experiment was repeated the following afternoon, but none of the larvae were found attached to the birds. When this experiment, however, was repeated in the evening, and on subsequent evenings, some of the larvae were found attached to the nestlings. This indicates that the larvae are mainly active at night and that they probably rest in the lower parts of the nest during the day. It may be of interest to note that both of the nestlings that were used for these experiments died several days later, apparently from loss of blood.

During the eleven weeks over which this investigation extended, a careful study was made of the activity of the larvae and the resulting flies; these have been since identified as *Protocalliphora azurea* (Fallen) by Mr. C. W. Johnson, curator of the Boston Society of Natural History. The larvae were kept in pasteboard boxes, in some of which soft cotton or bird-nest material was placed. They usually crawled about until they had found a secluded, dark spot, the crevices between the layers of paper of the boxes being their favorite resting place.

In these pasteboard boxes various organic substances were placed, such as fruit, bread, meat, and boiled potato. Although the fly larvae crawled through some of these substances, they fed upon none of them. One day I secured a large bone containing many blood cavities. Among these blood cavities I bored several holes and placed the bone in a box containing several dozen fly larvae. The latter had been starved for about a week so that there was not the slightest trace of food left in their intestines. The next morning I found two or three of them with fresh traces of blood in their alimentary canal, conclusive evidence that they had been feeding upon the ox blood contained in

the bone. Several other larvae were placed near a drop of human blood, but they invariably crawled in the opposite direction as soon as they came in contact with it. Similar experiments with ox blood brought the same results. Larvae which had not reached their full growth when taken from nests invariably died, unless they could feed upon blood. On the other hand, full grown larvae always pupated, even if they had no access to food. This shows that the larvae of *Protocalliphora azurea* (Fallen) are dependent upon blood in order to mature.

The blood ingested by the larvae is stored in a kind of reservoir, the diverticulum, which branches off from the esophagus close behind the pharynx, and there retains its red color for three or four days, gradually passing into the posterior end of the intestine as a dark brown substance, which appears as a longitudinal streak in the middle of the animal's body. If the larvae are not fed, this streak is gradually discharged as feces, so that after five to seven days more the larvae become creamy white in color throughout. From this we may infer that it is not necessary for the larvae to replenish their supply of food at frequent intervals to attain full growth.

When the larvae are about to pupate they crawl, anterior end downward, into the feces which they deposit at the bottom of the nest and there sometimes form a kind of disk, the lower side of which is made up of the anterior ends of the larvae, and the upper side of the posterior ends. About two weeks after pupation, the young flies emerge from the lower side of the disk. In captivity the fly larvae, before they pupate, usually attach themselves to objects located in dark places by means of viscous fluid which solidifies a few moments after it has been discharged.

The power of resistance of the larvae was surprising. Several of them, after having been immersed in a 70 per cent alcohol solution for twenty-four hours, were still wriggling vigorously. Several others which were being prepared for dissection were placed in a fixing fluid for six hours, then washed in a 50 per cent alcohol solution and placed in one of 90 per cent. They were still alive two days later and it was necessary to resort to a stronger fixing fluid, such as Gilson's, to prepare them for dissection. Other larvae were placed in a very strong insect powder, but they remained alive in it for two or three days.

As soon as the larvae pupated the pupae were placed beneath inverted tumblers. After the flies emerged from the pupae they were

kept in a large, narrow-mesh cage and their habits carefully studied. It may be of interest to state that the flies, about 1500, practically without exception emerged from the pupae between 7 A.M. and 2 P.M. Upon emerging they were of a slightly lighter hue than the adult of our common housefly, *Musca domestica* (Linnaeus), their wings being shriveled up, but in the course of about an hour or so these straightened out and the young flies assumed the dark blue, metallic luster of adult *Protocalliphora azurea* (Fallen).

Various kinds of food were placed before these flies, such as milk, crushed fruit, cheese, and meat in various forms. The flies readily ate the milk and fruit, especially if the latter was placed on the cage wire instead of the cage floor, but they were rather indifferent to the meat and cheese. Although some of the flies were kept in the cage for six or seven weeks, none of them, to my knowledge, deposited eggs or maggots.

It will now perhaps be of interest to zoologists, as well as to bird lovers, to state how frequently the larvae of *Protocalliphora azurea* (Fallen) were encountered in bird's nests and what effect their blood-sucking habit has on the nestlings. During the eleven weeks in which these experiments were carried on no less than sixty-three nests, representing six species of birds, were examined. Of these, thirty-nine, or nearly two-thirds, were infested by blood-sucking larvae. The accompanying tables will help to illustrate.

From the first table it will be noticed that birds which build a rather compact nest, as the goldfinches and the linnets, show a considerably larger proportion of infection than those which construct nests of a looser texture, as for instance the California brown towhee (*Pipilo crissalis crissalis* [Vigors]). This may possibly be due to the fact that it is difficult for the fly larvae to keep from falling out of loosely constructed nests.

All of the 1844 larvae in table 2, excepting the seventy-one indicated by the footnote, were those of *Protocalliphora azurea* (Fallen). These seventy-one larvae were taken from a linnet nest which contained the skeletons of three young which had been overtaken by death just before they were full fledged. Death had probably been caused by the larvae. When the latter were discovered they were in their pupal stage and were considerably smaller than the larvae of *Protocalliphora azurea* (Fallen). All of them had hatched except about a dozen. These unhatched pupae were guarded very carefully, but they all proved to be parasitized by the chalcid fly, *Nasonia brevicornis* (Girault).

The death of six other nestlings, including the four which had died in 1913, could be traced with more or less certainty to the muscid larvae, as in the case of the three linnets whose skeletons I found and the two goldfinches used for experimenting. One of these six nestlings, a nearly full fledged California linnet (*Carpodacus mexicanus frontalis* [Say]), was discovered when it had been dead only a short time. A number of fly larvae had actually penetrated into its body. The nest in which this dead bird was found contained another nestling of the same brood. Though apparently rather weak, this nestling took wing

TABLE 1

Species of bird	Nests examined	Infested nests	Uninfested nests	Infestation
Nuttall sparrow (<i>Zonotrichia leucophrys nuttalli</i> Ridgway)	4	4	0	100%
California purple finch (<i>Carpodacus purpureus californicus</i> Baird) and California linnet (<i>Carpodacus mexicanus frontalis</i> (Say)	31	21	10	67%
Green-backed goldfinch (<i>Astragalinus psaltria hesperophilus</i> Oberholser)	13	8	5	61%
Willow goldfinch (<i>Astragalinus tristis salicamans</i> (Grinnell)	7	4	3	57%
California brown towhee (<i>Pipilo crissalis crissalis</i> (Vigors)	8	2	6	25%
Totals	63	39	24	61%
				Average

TABLE 2

Species of bird	Larvae in each nest	Totals	Larvae per nest
	8, 10, 14, 15, 17		
California purple finch (<i>Carpodacus purpureus californicus</i> Baird), and California linnet (<i>Carpodacus mexicanus frontalis</i> (Say)	20, 23, 29, 50, 52 54, 60, 62, 71*		
	86, 94, 103, 103		
	106, 108, 155	1239	59
California brown towhee (<i>Pipilo crissalis crissalis</i> (Vigors)		44, 62	106
Willow goldfinch (<i>Astragalinus tristis salicamans</i> (Grinnell)		13, 24, 34, 81	152
Nuttall sparrow (<i>Zonotrichia leucophrys nuttalli</i> Ridgway)		24, 36, 36, 37	133
Green-backed goldfinch (<i>Astragalinus psaltria hesperophilus</i> Oberholser)		13, 18, 19, 21 28, 30, 35, 50	214
Total		1844	47
			Average

* A considerably smaller but apparently more deadly species than *Protocalliphora azurea* (Fallen).

when I approached the nest. Nearly all of the fly larvae found in this nest showed traces of fresh blood.

As far as I am able to ascertain, there are only two cases of blood-sucking fly larvae on record for the United States. In 1908 Henshaw recorded the infestation of two successive broods of bluebirds, *Sialia sialis* (Linnaeus), by the larvae of *Protocalliphora chryssorrhea* (Meigen), which had been reported to him by Mrs. Emma F. Everett, of Wellesley Hills, Massachusetts. These two cases of parasitism were decidedly fatal, seven out of the eight nestlings dying as a result. Henshaw closes with a note of warning about the danger of this insect pest to our native birds.

Seven years later Coutant (1915), while studying blood parasites of the common crow (*Corvus brachyrhynchos* Brehm) at the Biological Laboratory of Cornell University, came across some larvae of *Protocalliphora azurea* (Fallen). Most of his deductions, based upon the study of these larvae, agree with my observations. However, his conclusion (p. 139) that "the larvae prefer rather dry places to moist ones and are therefore not accustomed to living in decomposing or fecal material" and that (p. 143) "the larvae when ready to transform, apparently leave the more occupied parts of the nest in the vicinity of their food-supply and seek a dry . . . portion" of the nest, were not borne out by my observations and experiments. In all cases the larvae preferred the moist fecal material and pupated in it. This discrepancy between Coutant's results and mine is undoubtedly due to the fact that Coutant based his conclusions upon the study of a comparatively few larvae, and that even these few were not studied by him in their natural environment, the bird's nest.

Commenting upon the fact that *Protocalliphora azurea* (Fallen) are recorded by collectors and dipterologists as "rare" or "very rare" and that specimens of this fly are only to be found in the larger museums and collections, Coutant (1915, pp. 144, 145) correctly assumes "that they are not so rare as is generally supposed, but that the adults are peculiar in their habits, flight, etc., and for this reason are rarely taken." He then goes on to say (p. 145): "Few collectors, I imagine, have taken insects very often from the zone of air, from fifty to one hundred feet above the ground, in the woods; yet from the habits of the larvae, this is where we would naturally expect that the adults would occur." This may be partly correct, but the lower limit will have to be extended considerably. Most of the thirty-nine infested nests taken during the course of my experiments, as well as the two

encountered in 1913, were found far distant from forests, and all of them came from a height three to fifteen feet above the ground.

Turning now to the effect which these blood-sucking larvae of *Protocalliphora azurea* (Fallen) have on nestling birds, my observations seem to warrant the following conclusions: (1) from 5 to 10 per cent of the parasitized nestlings die from loss of blood; (2) some of the parasitized nestlings which do become full fledged are so weakened by the loss of blood that they fall an easy prey to rapacious animals.

Much remains to be done along this line of investigation in order to determine how large and universal the damage is which is wrought on our continent by this insect pest. Although the adults of both *Protocalliphora azurea* (Fallen) and *Protocalliphora chrysorrhoea* (Meigen) are very rarely taken by collectors (see Henshaw, 1908, p. 88, and Coutant, 1915, pp. 144, 145), my investigations show that the former is not so rare, at least not in certain parts of the country. So far, however, only forty-four bird's nests, infested by the larvae of one or the other of these flies, have been recorded. All forty-four of these infested nests were found at three places, one near Ithaca, New York (Coutant, 1915), two at Wellesley Hills, Massachusetts (Henshaw, 1908), and the remaining forty-one in the San Francisco Bay region. It would be highly interesting, and perhaps beneficial to our wild birds, if bird students in other parts of North America as well as South America, would thoroughly investigate this matter in their home districts.

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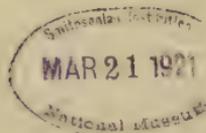
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BINARY FISSION IN COLLODICTYON
TRICILIATUM CARTER

BY
ROBERT CLINTON RHODES



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INTRODUCTORY AND HISTORICAL

In February, 1916, I found in an aquarium containing goldfish a dominant and persistent, free living flagellate which seemed to warrant further investigation. Being large and transparent, except when filled with green and opaque inclusions, and occurring in quantities, it presented an opportunity for continuous and repeated



observations, especially upon its metabolic character and nuclear changes. The form has been identified as *Collodictyon triciliatum* Carter (= *Tetramitus sulcatus* Stein).

This genus was first described by Carter (1865, p. 289) as follows:

Collodictyon, nov. gen. *C. triciliatum*, nov. sp.

Pyriiform, straight, or slightly bent upon itself, bifid at the small extremity, presenting at the larger one an indentation, from which spring three cilia. Structure transparent, cancellated, composed of globular cells, with a strongly marked, greenish granule here and there in the triangular spaces between them. Locomotive, swimming by means of the cilia; subpolymorphic, flexible, yielding, capable of assuming a globular form . . . or one more or less modified by the body it may incept . . . ; enclosing crude material for nourishment in stomachal spaces, and ejecting the refuse, like *Amoeba*. Provided with a nucleus and contracting vesicles.

He gave its habitat (p. 289) as "fresh water, chiefly among *Euglena* and Infusoria of that kind." Its length was $1/771$ in. (30μ) and its location the Island of Bombay. Among his observations he added (p. 289) the following: "The plastic nature of this Infusorium, and its mode of incepting food being like that of *Amoeba* (for it does not appear to possess any oral aperture), induce me to think that it should be placed among the Rhizopoda. Still it seems to have some analogies to *Bodo* Ehr." "Its generic name has been derived from its plasticity and delicate cellular structure, which gives it a reticular or cancellated appearance; and its specific designation from the presence of three cilia."

The above description is satisfactory for identification, though not detailed. My own observations coincide with it with these exceptions: there are four instead of three flagella; it may or may not be bifid at the posterior end; there is at the anterior end of the body a blepharoplast from which the flagella arise, these not springing from, but near the indentation, which is a continuation of the median groove, or sulcus, which functions in food ingestion; I have found no contracting vacuole, though Carter stated that he had observed "contracting vesicles" having no fixed position, but he figures none.

In 1878 Stein figured a similar form, showing, however, the four flagella, a median sulcus and a contractile vacuole, naming it *Tetramitus sulcatus*. Kent (1880-1882, p. 314) accepted these organisms as described by Carter and Stein as belonging to separate genera of the family Trimastigidae, but that there is little cause for such a distinction can be seen from his characterization of Stein's genus as follows:

Body obtusely pyriform or subcordate, widest and rounded anteriorly, tapering towards and bluntly pointed at the posterior extremity, about one and a half times as long as broad; a deep groove traversing the entire length of the centre of the ventral side and imparting to the posterior extremity, as seen from beneath, a bilobate contour; flagella four in number, of equal length, inserted close together in the centre of the anterior border; endoplast and contractile vesicle located side by side near the same anterior margin; parenchyma granular, soft and plastic. Length 1/700. Hab., fresh water.

Bütschli (1887, p. 841) recognized these two forms as the same and *Tetramitus sulcatus* Stein as a synonym of *Collodictyon triciliatum* Carter, characterizing the genus as follows under the family Tetramitina:

Mässig gross (L. bis 0,035 Mm.), estalt vorn etwas verbreitert und quer abgestutzt, nach hinten wenig verschmälert und abgerundet. Wahrscheinlich etwas abgeplattet; über die eine Fläche zieht eine breite Längsfurche hinab. Vorderende mit vier gleich langen aus einem Punkt entspringenden Geisseln (Carter gibt nur drei an). Nucleus und contractile vacuole im Vorderende. Nahrungsaufnahme sicher. Vermehrung durch Längstheilung. Süsswasser. Europa und Ostindien.

In 1893 Klebs investigated a form which he designated as *Tetramitus sulcatus* Stein. He found the four flagella of unequal length and a contractile vacuole in the posterior end of the body, the longitudinal furrow a spiral, the size being 17 by 15 microns. These differences lead me to conclude, after careful consideration of his description and figures, that he is mistaken in his verification and the form he described is not *Collodictyon triciliatum*, but probably some species of *Tetramitus*.

Francé (1899) studied Carter's organism thoroughly; his description is accurate and detailed, his figures typical and true to life. My own observations coincide with his in practically all details. He concluded that Klebs' (1893) description was not of *Collodictyon*. He dealt fully with the morphological features and metabolic changes, to which I have little to add. In only one important point do our observations fail to agree. I can find no contracting vacuole. He left untouched, however, the method of mitosis, merely stating that reproduction was by longitudinal division, which was also noted by Carter (1865); it is to this especially that I shall address myself.

I am indebted to Dr. Olive Swezy for suggesting the desirability of working out the mitosis of this form, the correction of the bibliography, sketching figures 75, 78, and 83 of plate 8, in my absence, and for repeated criticism and help. I also wish to thank Professor

C. A. Kofoid for his suggestive interpretations and criticisms, both constructive and destructive, and for the determination of the extra-nuclear division center.

MATERIAL AND TECHNIQUE

Plentiful material was found in an aquarium where goldfish were kept. These were obtained from the Yorizuya Company, who represent the Nippon Gold Fish Company and import direct from Japan. In no other cultures have I found *Collodictyon*. Since it has only been noted from India and central Europe, it is possible that it is not native to California, and may have been introduced with the importation of goldfish from Asia or Hawaii. On the voyage from Japan, however, the barrels in which the fish are contained are emptied and fresh water added at Hawaii and also at the wharf in San Francisco. It would be easy for the flagellates to be brought through notwithstanding this change of water, either by being transferred with the fish, with water plants which are brought in the same aquaria, or by clinging to the moist sides of the containers (barrels). Thus, though there is a possibility that *Collodictyon* has been introduced into California, the cosmopolitan distribution of Protozoa makes this highly improbable, and this genus may be regarded as indigenous.

These forms have persisted and usually have been dominant in an aquarium $26.5 \times 60 \times 20$ cm., the bottom of which is covered with sand to a depth of about an inch, in which *Ulothrix* has grown in quantities and to which I have added *Lemna*, *Ranunculus*, and *Myriophyllum*. At least one goldfish has been present all the time, at times two and four. These fish have fed freely on the plant life of the aquarium, making it necessary to replenish all higher plants and on more than one occasion, the algae, although the aquarium has been sufficiently well balanced for one fish to survive since January, 1916. The aquarium has been placed outside a window with southern exposure, partly protected by glass plate and wooden cover. The variations of temperature for the year have been from about 28° F to 92° F. Considerable variation of temperature from the heat of the direct rays of the sun at mid-day to the cool nights failed to destroy the culture. Since the cooler weather of last December *Collodictyon* has been supplanted at intervals by a dinoflagellate, *Peridinium penardii* Lemm., as the dominant organism, the latter seeming to be favored by the cooler weather. On one occasion the aquarium froze over during the

night, the ice being one-eighth to one-quarter of an inch in thickness, forming at a temperature of 28° F. Under the ice and throughout the following day *Collodictyon* seemed even more numerous than ever and many dividing forms were found. The following night, temperature 32° F, abundant mitotic stages of *Collodictyon* and *Peridinium* were found. Many of the dinoflagellates escaped from their theca by ecdysis and many division stages were observed. Greeley (1903) noted the effect of reduction of temperature upon the artificial production of spore formation and multiple fission in *Monas*. I obtained nothing resembling resting spores or somatellas. At 4° C Greeley (1903) found that *Monas* rounded up into a resting spore in about six hours, and at 1° C multiple fission, resulting in a resting somatella, was observed within five days. It is interesting to note in comparison that binary fission was simply accelerated in both *Collodictyon* and *Peridinium* at a temperature of 32° and 28° F.

Collodictyon seemed most abundant on the surface, naturally tending to accumulate in the corners and around the edges of the aquarium. But during the day and night at all temperatures above freezing, I have found them present throughout the aquarium, from the bottom to the top, under the protected area as well as the open end. I placed slides and covers, suspended at various depths, as well as covers suspended in cylinders to eliminate currents, and found abundant organisms in all parts of the aquarium. In these experiments, attempted primarily to determine the time and conditions of division and probable multiple fission, I was led to conclude that division was determined more by chemical than physical conditions, that it was no more abundant at night than during the day, that individuals undergoing binary fission remained at the surface for the most part, but could be found at all depths, in all degrees of light and temperature, though more abundant at 32° F. At no time have I found a clear case of multiple fission. One instance (pl. 7, fig. 62) of a somatella was observed, but on careful comparison of the staining reactions, I was led to conclude that this was a cyst of *Amoeba radiosa*, vegetative stages of which were abundant in the aquarium. The life cycle of *Collodictyon*, as far as traced, is thus simple, reproduction being by binary fission only. When the organisms wholly disappear from the cultures, I have been unable, by varying conditions, to start the culture up again. It seems, therefore, that there may be no cysts or resting stages for this flagellate, at least under the conditions observed. The extreme variations in size (pl. 2, figs.

5-6) would naturally lead one to suspect a somatella stage, though this variation may be accomplished by reduction through successive binary fissions.

In culture experiments I have tried malted milk (one-sixteenth of one per cent solution and varying strengths), crushed *Myriophyllum*, boiled mushroom solution, amoeba agar, sterilized soil with tap water boiled thirty-five minutes, beef extracts, and quince-seed jelly as suggested by Turner (1917) for *Euglena*. Most of these were partially successful, but only temporarily so, *Collodictyon* soon disappearing from the culture.

Among associated forms in the aquarium, I have found: *Pandorina*, *Peridinium*, *Euglena*, *Amoeba* of the *limax* group and others, *Platydorina*, *Gonium*, *Actinophrys*, *Bodo*, *Chlamydomonas*, *Chilomonas*, *Coleps*, *Stylonychia*, *Euplotes patella* and *E. charon*, *Microthorax*, and *Colpidium*; rotifers (*Branchionus*, *Philodina*, and *Chaetonotus*); *Ulothrix*, *Oscillatoria*, *Chlorella*, *Tetraspora*, *Spongomonas*, *Lagerheimia*, *Scenedesmus*, *Pediastrum*, *Selenastrum*, *Coleochaete*, *Navicula*, *Closterium*, *Cosmarium*, and several undetermined desmids and diatoms.

I have considered the possibility that the life history of *Collodictyon* may in some way be related to its association with goldfish. Aside from the balancing of the plant and animal life of the aquarium, I have looked for some symbiotic or parasitic relationship, but have found none, other than the fact that I have been unable to keep a permanent culture in other than a goldfish aquarium. I tried a fairly well balanced stickleback aquarium without success. In aquaria where there were abundant *Ulothrix* and other algae, *Collodictyon* did not persist. The voracious habits of this animal led me to believe that it is not symbiotically dependent upon goldfish, nor has it any other method of food-taking involving absorption; but its habits of engulfing free living protozoa and algae may wed it to a well balanced condition such as would be found in a satisfactory goldfish aquarium. There is a bare possibility that the life-history is dependent upon the presence of living fish, though Carter and Francé's observations are opposed to such an interpretation. In examination of stomach and intestinal contents I found no evidence of symbiosis or parasitism relating the two. Examination of the gills and body for ectoparasites never failed to yield some of these forms. This was probably due, however, to their abundance in the water.

It is rather a prevalent custom to use cover-glass preparations in

protozoan technique. I refer here not to Romanowsky's dry film, which has no general use or approval, but to a modification of Schaudinn's moist film method, which involves the use of some fixative, pipetting the organisms on to the cover smeared with this and then the evaporation of the water until the animals will adhere to the cover when dropped or floated directly on the surface of the killing fluid. I found on attempting this method that conditions accompanying the evaporation process ruptured the body, and normal killing or fixation could not be obtained with *Collodictyon*, which is evidently more susceptible than many other free living forms.

Collodictyon when exposed to excessive evaporation may rupture instantaneously or more frequently pass through moribund phases as shown in text figure C. All foreign bodies are ejected, the vacuoles become larger, gradually fuse into still larger pathological vacuoles, which finally rupture. The organism becomes much distorted and disintegration usually takes place along the sulcus.

Wherever the death rate is above the normal or even at equilibrium, the number of abnormal moribund forms is undoubtedly great. Many life cycles could be and possibly have been built up on such fallacious interpretations. Protozoa may be potentially immortal but the vast majority do not survive. The real problem, therefore, is to find some way to determine the normal from the moribund, either physiologically or pathologically abnormal.

I resorted to killing *en masse*, either with or without centrifuging. I found that in lightly centrifuged material there was no nuclear displacement or other variations which could be detected from the non-centrifuged, so I adopted the centrifuge as the best and quickest method of running up the material. After a normal killing in a beaker and running up through the alcohols in centrifuge tubes, some of the organisms were then fixed to covers, where it was essential for the quick handling of material, as in Mallory's stain. I also found it convenient and satisfactory in dehydrating rapidly after certain stains, as in phosphotungstic haematoxylin, to pass from an aqueous stain by adding 3 to 5 c.c. of 50 per cent and then 40 to 50 c.c. of absolute alcohol, immediately centrifuging and adding carboxylol.

At times *Collodictyon* was found to be rapidly increasing in numbers in the aquarium and from this it was judged that there was little or no death taking place, but that conditions were favorable for a maximum growth and normal reproduction. Material collected at such times as this was regarded as normal, at least for the phase of

purely vegetative existence. By collecting water from the aquarium and putting in petri dishes I was at times able to get a determinate increase in numbers and low mortality. By killing at such times, material normal as it was possible to get was obtained. Various methods of introducing the material to the killing fluid was used: by pipette, by pouring, or by pouring the killing fluid on concentrated masses of the organisms. The proportion of the killing fluid was never allowed to be less than ten times the amount of the water containing the flagellates.

The most satisfactory material was obtained by killing either in hot Schaudinn's fluid or strong Flemming, and staining in Heidenhain's aqueous iron haematoxylin. By this method the nuclear differentiation becomes evident as figured in accompanying plates. Counterstaining in eosin, either just after destaining or from 50 per cent alcohol, brings out the flagella.

Other killing fluids used in addition to Schaudinn's and strong Flemming were: picro-mercuric, Gilson's, Flemming's weak, Carnoy's, Zenker's, Bouin's, and osmic acid. None of these was perfectly satisfactory, possibly through lack of proper proportions or some undetected flaw in technique.

Heidenhain's aqueous iron alum haematoxylin I regard as by far the safest, most differential and permanent stain. Alcoholic solutions were about as satisfactory, but required from one to two hours for the mordant and eight to twenty-four for the stain, thus not being much quicker than the aqueous solutions. Acid fuchsin or eosin were excellent counterstains with either of the above. Delafield's haematoxylin yielded beautiful but not so critical a differentiation. The granular organization of the chromatin, even of the karyosome, was emphasized. Phosphotungstic acid haematoxylin was of assistance in determining the nuclear membrane, but did not show up the spindle fibers as was hoped. Mallory's connective tissue stain as modified for Protozoa yielded only fairly satisfactory results with the fixatives tried. By its use the two basal granules embedded in the blepharoplast could be distinguished. For some reason *Collodictyon* seems able to withstand chemicals, especially the usually quick stains, far more than other free living Protozoa with which I have dealt, the time required for all stains being longer than that ordinarily scheduled. Flemming's safranin, gentian-violet, orange G was tried, for the purpose of differentiating the macrokaryosomes and microkaryosomes, but no variations of color diagnostic of chemical differences were obtained. The

cytoplasmic differentiation, however, was good. The blepharoplast and evidence of a rhizoplast were emphasized by preceding Heidenhain's iron haematoxylin with weak Bordeaux red for twenty-four to thirty-six hours, and destaining until the chromatin was almost colorless, as directed by Heidenhain. Paracarmine with aluminum chloride as mordant did not yield satisfactory results. *Collodictyon* proved susceptible to neutral red and methylen blue of the intra-vitam stains. It was unaffected by Bismarck brown. Neutral red after a considerable time differentiated the food vacuoles and acted somewhat upon the plasma. It never disclosed a pulsating vacuole. Methylen blue largely reacted upon the plasma and was not differential, possibly showing up the protoplasmic vacuoles with some intensity.

In order to section I resorted to the capsule method of embedding. The material was taken from the xylol-paraffine, saturated solution, in the centrifuge tube, and placed in a capsule. Melted paraffine was dropped in and this process was repeated successively until the material was satisfactorily embedded. Sections were cut 3, 5, and 7 μ thick.

GENERAL MORPHOLOGY

Collodictyon is variable both in size and shape. Its length is 15 to 60 μ ; width, 10 to 40 μ ; thickness, 8 to 36 μ . These figures show that many of the individuals I have found are smaller than those recognized by previous observers. The typical shape of the body (pl. 7, figs. 1-4) is ovoid, cordate or bifurcated posteriorly. A longitudinal furrow, or sulcus, is always present, though at times showing only a slight indentation, but usually evident as a deep groove on one of the narrower sides. Four equal flagella about as long as the organism arise from the anterior ovoid end. Anteriorly the body is ovoid, at times cordate, the sulcus extending around as an indentation. The general shape is rounded or compressed in the plane running through the sulcus, the nucleus and the blepharoplast. The posterior end may be truncated, oval, acuminate, bifid, with the cusps pointing posteriorly, curved spirally or diverging at an angle up to seventy-five degrees, or with three, four, or five cusps (pl. 7, figs. 5-8) caused by secondary sulci which run parallel to the primary longitudinal sulcus. Changes of form are gradual except when altered by engulfed food or the extrusion of undigested products. The peculiarities of the posterior cusps are held by a single individual indefinitely. Few individuals, if any, are exactly alike in form.

Seldom does the sulcus extend far enough forward to modify the regularity of the anterior end, which is fairly constant in form, much more so than the posterior end. This sulcus is a furrow or depression which cleaves the body on one side, which side may consistently be called sulcal or adsulcal, analogous to ventral, as opposed to absulcal, which is analogous to dorsal. The secondary sulci usually branch from the chief longitudinal sulcus, the resulting cusps being variable in size, shape and permanence. At times the general form becomes spherical and globular, the posterior end truncated, or ovate and conical, with acute posterior end, the sulcus being faint in both cases.

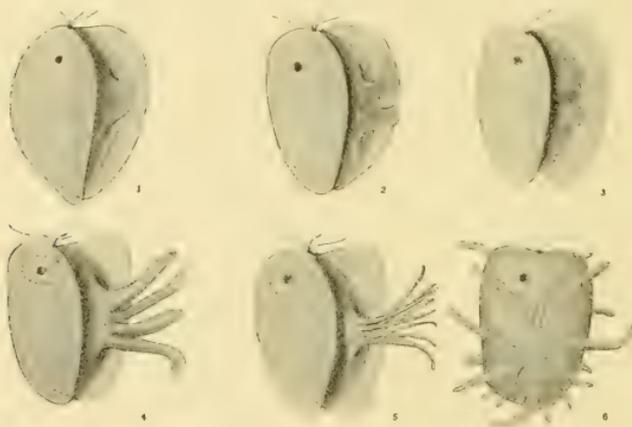


Fig. A. Pseudopodia of *Collodietyon*. Diagrammatic. $\times 1000$. 1-2. Lobose. 3. Undulate. 4. Digitate. 5. Filose. 1-5. From the sulcus. 6. From all parts of the surface.

The four flagella are paired, each pair arising from a single basal granule, the two granules being embedded in the irregular chromatoidal blepharoplast which is surrounded by a granular, less darkly staining archoplasm or modified cytoplasm. The flagella are typically whip-like, in length averaging that of the major axis of the body, at times a little longer, measuring in one instance 68 to 70 μ . They taper toward the tip. Francé (1899) used zinc chloride to bring out their full length and further observed their base to be granular. Language is inadequate to describe the beauty of their elegant backward curves. They function both in pulling and propelling the body forward, in attachment to the substrate while the organism rotates on its major axis, as a tactile organ for directing locomotion, and actively in offense

and defense. In attacking a dinoflagellate they were observed to catch their prey with all four flagella; they then allowed themselves to be pulled about until the dinoflagellate was exhausted, when it was drawn back to the anterior end of the sulcus and engulfed. On another occasion when *Collodictyon* was being drawn toward the mouth of a rotifer, it spread its flagella and lodged upon the oral membrane; at other times it was enabled to guide itself to one side of the oral current. While not the most conspicuous, the flagella constitute the most useful organelles of this slightly differentiated unicellular organism.

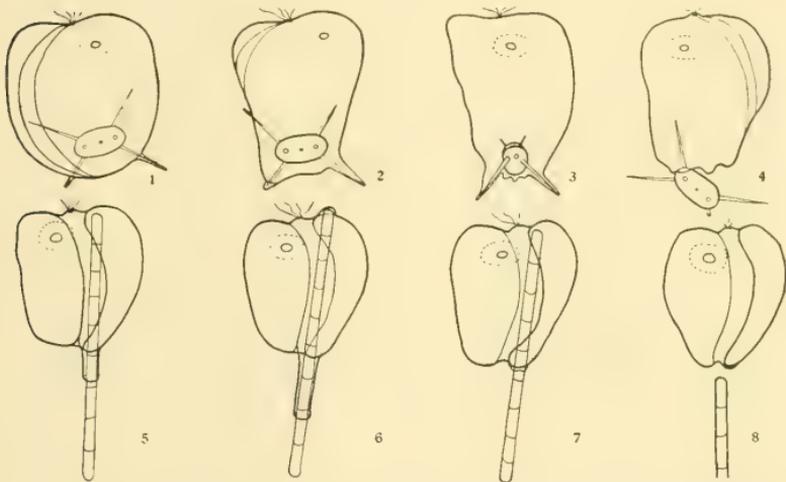


Fig. B. 1-4. Extrusion of *Lagerheimia* by *Collodictyon*, showing false pseudopodia. 5-8. Unsuccessful attempt of *Collodictyon* to engulf long *Ulothrix* filament. Diagrammatic. $\times 750$. 5. Extension of protoplasmic sheath along filament. 6. Contraction of protoplasmic sheath forcing the filament anteriorly, pushing out the surface. This was repeated several times. 7. Retraction of protoplasmic sheath. 8. Extrusion of filament.

The sulcus is usually a smooth depression, but when *Collodictyon* is actively searching for food, pseudopodia are extended from any or all portions of the sulcal region. These may be of various types, hatchet-shaped, lobose, finger-like, or filose (text fig. A, 1-5). Finger-like pseudopodia from over the entire surface of the body were observed (text fig. A, 6), but such are very rare. The entire sulcus at times may become serrated and undulating, exceedingly amoeboid and metabolic. The posterior end may put forth lobose pseudopodia or flow around objects being engulfed (text fig. B, 1-6). The posterior cusps

are themselves slightly amoeboid and may slowly change position and form, though this is unusual.

On many occasions I have noticed a change of form of a different character and at first thought true pseudopodia were being extended from the whole body surface, but by continued observations it was discovered that some undigested plant, as *Scenedesmus* or *Lagerheimia*, was escaping or being extruded (text fig. B, 1-4). The pellicle and cytoplasm extended out around the rays or processes and undoubtedly part of the cytoplasm was lost when the object was set free. The cytoplasm immediately contracted, the ruptured edges or margins drew closer together until they were rounded up and fused (text fig. C, 1-7). This seemed not so much a healing process as simply protoplasmic contraction and rounding up. I have noticed on two occasions, however, that the ruptured edges met and fused, enclosing a water vacuole in the healing or coalescing process (text fig. C, 5). These phenomena make it evident that there is no cuticle and that the pellicle has developed little beyond the state of a firm protoplasmic gel. Changes in shape in the sulcal region are amoeboid, most other changes involving the whole body are metabolic or euglenoid. There is no differentiation of ectoplasm and endoplasm. The surface is exceptionally smooth and well rounded, in spite of the fact that the cytoplasm is highly vacuolated.

The function of the sulcal region is not hard to determine, for undoubtedly it is through this region almost altogether that food is ingested; but to decide its true status and homology is a more difficult problem. It is a restricted area of the body, which has either retained or evolved an amoeboid and miscible surface. It is always a unit, a persistent though variable character, and of constant function. Amoeboid pseudopodia are almost entirely restricted to this area. Many flagellates, such as *Euglena* and *Astasia*, are exceedingly metabolic, constantly varying in shape, but they have no such differentiated surface area as this found in *Collodictyon*. *Mastigamoeba* is classified as a polarized rhizopod, the entire surface of which is amoeboid. The exceeding voraciousness of *Collodictyon* is indicative of a fairly advanced organism and in view of this fact it seems best to regard the whole sulcal region as a modified cytostome. It must be considered more homologous to the amoeboid surface of *Mastigamoeba*, however, than to the more restricted gullet of *Euglena*, which is probably homologous with only the anterior end of the sulcus. This structure indicates a possible origin of the more specialized cytostomes as found

in *Trichomonas*, *Costia*, and *Giardia*. *Collodictyon* must in any case be looked upon as a form of simpler organization and probably of a more primitive type than these parasitic forms.

The anterior extremity of the sulcus, just beside the base of the flagella, may be modified into a depression (pl. 9, fig. 19) which has

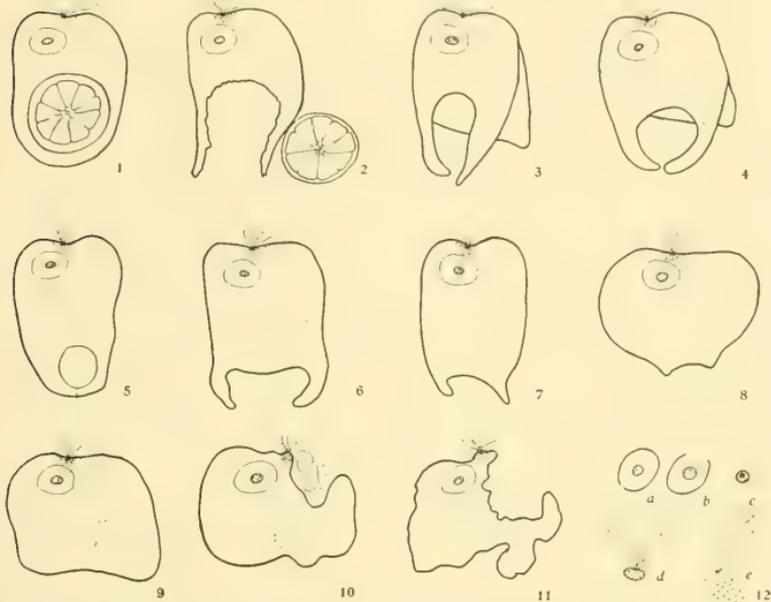


Fig. C. Escape of *Pandorina* and subsequent dissolution of *Collodictyon* due to drying. Diagrammatic. $\times 750$. 1. *Pandorina* within food vacuole. 2. *Pandorina* swimming away; ruptured surface of *Collodictyon*. 3, 4. Apparent healing of torn surface. 5. Fusion of ends of protoplasmic processes, enclosing water vacuole; of frequent occurrence. 6-8. Resorption of protoplasmic processes. 8, 9. Flattening of body; formation of pathological vacuoles indicative of dissolution. 10. Bursting of vacuole at anterior region of sulcus. 11. Further dissolution, rupturing posteriad along the sulcus. 12. Nucleus and blepharoplast freed by dissolution. *a*. Nucleus; nuclear membrane persisted for two minutes. *b*. Rupture of nuclear membrane. *c*. Karyosome; persisted for thirty minutes; finally broke up into small granules. *d*. Blepharoplast; basal granules surrounded by archoplasm, flagella still moving. *e*. Rupturing of archoplasmic mass; flagella cease beating.

a probable function of a more specialized cytostome. It takes the form of a permanent depression in an amoeboid surface. It does not usually function in food getting, for this is accomplished by the amoeboid surface of the entire sulcal region. I have, however, observed unicellular algae and small dinoflagellates engulfed through or near this

depression. Here then is a structure which by location and analogy may be correlated, or at least compared, with the highly specialized gullet of forms like *Euglena*.

There is a tendency at times for *Collodictyon* to become exceedingly eccentric in its form (pl. 8, figs. 9-18). Its irregularity is for the most part accompanied by a flattening and warping, the sulcus cleaving one of the narrow margins, making a secondary if not, indeed, a fundamental bilateral symmetry. On occasions when such irregularities were prevalent, I have tested the culture, trying to determine if possible a cause for such variation. The water of the aquarium was neutral or only slightly alkaline, by litmus paper and litmus solution tests. Alkalinity tended to produce rounded, globular, conical, or pear-shaped forms, the sulcus itself being reduced to a minimum. Concentration tests were not accurate, but in my judgment I could detect no variation upon this. Death always resulted when the density was such as might be judged sufficient to rupture such a fragile organism. That oxygen content plays an important part in the variation in shape there can be no doubt. Sufficient or excessive oxygen supply tends to produce well rounded forms, a deficient supply, flattened, eccentric forms. This test was easily made by having a substrate of filamentous and unicellular algae, which in the sunshine kept the aquarium filled with bubbles of oxygen. The alternative interpretation that light and heat caused the rounding up, was tested by placing the aquarium without any algae in the sunlight. The forms then retained their original shapes. The chemical content so far as organic salts in solution is concerned was probably not variable enough to produce the variations, tests having been made for sodium, calcium, and magnesium salts with negative results. By adding carbon dioxide slowly in small quantities similar eccentricities of shape resulted as from deficient oxygen. From these tests I concluded that variation of shape was largely a question of respiration, irregularities being either degenerative stages or adaptations to meet deficient oxygen supply. Carbon dioxide in excessive amounts would be immediately converted into carbonic acid gas and thus make the culture slightly acid. This is in accord with the acidity tests.

In observing moribund forms disintegrate (text fig. C, 1-12), all food vacuoles were seen to be extruded, the body flattened, and pathological vacuoles, largely water, became apparent in the sulcal axis. These ruptured leaving the body very irregular in shape. Successive formation of these vacuoles finally caused complete disintegration of

the body. Even then the nucleus persisted as a unit for some two minutes, when the nuclear membrane ruptured and the karyosome alone remained as a unit, retaining its form for over half an hour. The blepharoplast persisted as an irregular granular mass surrounding the two basal granules. As long as this mass remained as a unit, the flagella could be seen to wave back and forth, but ceased moving as soon as the mass disintegrated.

It is especially noticeable that the body may be distended, elongated, or distorted by newly engulfed food (pl. 9, figs. 19-27). It may elongate to twice its normal length by inclusions of filamentous algae, desmids, or diatoms. Such elongation is usually, though not always, anteroposteriorly. The modifications due to inclusions of such organisms as *Scenedesmus* or *Lagerheimia* I have already mentioned. *Chlorella* and *Protococcus* when engulfed were arranged peripherally within the vacuolated cytoplasm, just underneath the surface reticulum. At times this made the animal appear perfectly green. These frequently popped out through the pellicle and when first observed made me think of zooids from flagellated forms in multiple fission. Such a condition was but temporary, however, the algae either being digested for food, early showing the surrounding vacuole, or else, I am led to believe, at times assuming the state of transient symbiosis. For three months such a congested condition was both typical and dominant. Seldom was *Chlorella* digested and I am inclined strongly to the idea of transient, or facultative symbiosis.

As seen in the living state, the cytoplasm consists of large, hyaline vacuoles, in the interstices of which are smaller vacuoles, and the spaces between these filled with granules or plasmosomes in a fluid matrix (pl. 8, fig. 5). The periphery of each vacuole seems to consist of a definite membrane, more the result of a turgid surface tension, while the interior is filled with a hyaline fluid.

The surface of the cytoplasm consists of smaller vacuoles with a greater number of granules. The arrangement of these gives the appearance of a surface reticulum, with the larger, deeper vacuoles lying against or within it. When disintegration takes place, the pellicle ruptures, the cytoplasm goes to pieces rapidly, the hyaline fluid diffusing into the water, and the granules, which do not appear nearly so numerous as the mass of the organism might indicate, are scattered by diffusion currents.

The nucleus possesses no vacuoles, but seems to consist, much as the blepharoplast and the immediately surrounding cytoplasm, of granules

in a fixed matrix, denser and more refractive than the cytoplasmic hyaloplasm. In the resting state these are arranged peripherally just within the membrane. The nucleus is usually disc-shaped, being flattened anteroposteriorly. It is, when seen from the front or rear, round, oval, or irregularly elongated. In the living state, and when intravital stains, such as neutral red and Bismarck brown, are used, there is at the center a karyosome which seems at times to consist of closely compacted granules surrounded by a light hyaline area of nuclear sap in which there are no granules, the periphery of which seems usually to be bounded by a membrane. When the nucleus disintegrates this granular karyosome persists for from fifteen to thirty minutes. When stained with iron-alum haematoxylin, Delafield's haematoxylin, phosphotungstic haematoxylin and others, this karyosome appears homogeneous and this whole mass to be surrounded by the hyaline area, thus giving a perfect vesicular nucleus.

As to the reticular nature of its protoplasm, I am inclined to regard the surface of the vacuoles as modified, perhaps by stress or strain, into thickenings or longitudinal strings of plasmosomes, the interstices of the larger vacuoles being filled with still larger granules of various kinds, food, mitochondria, plastids, metaplastic granules, and foreign organic and inorganic bodies. There is little or no circulation of vacuoles or protoplasm visible in *Collodictyon*. That the nuclear protoplasm has the power to create and absorb or eliminate a membrane, metabolic in character, around the microkaryosome, will be described as a prophase phenomenon; even so, a cytoplasmic vacuole may be bounded by a definite membrane, kinetic in character, which may be modified so as to appear reticular when stained, and on which are accumulated small granules, either scattered or in long, bead-like strings. All of this is in addition to the other granules which may be held in the interstices of both large and small vacuoles.

The physical nature of the protoplasm of *Collodictyon*, therefore, appears to be a fluid, which may be modified by metabolic, kinetic and other life processes into granular or reticular variations, these however, being subject to reabsorption into a hyaline fluid, or becoming by-products, such as plastids, never again functioning in metabolism or life processes, though still retained in the cytoplasm.

Besides the protoplasmic vacuoles, other kinds may be present.

1. Food vacuoles, which may contain plants or animals just engulfed, or which may be very old and alkaline in reaction, simply water or digestive spaces in which little remains. These may flow together

when *Collodictyon* is in a moribund state, giving rise to what Francé calls water vacuoles. 2. Water vacuoles: I can not differentiate these from the old food vacuoles except that they are larger. They give similar reaction with Congo red. Undoubtedly they are pathological, in the sense that they do not appear except in moribund condition. The smaller food vacuoles either are entirely absorbed or their contents are extruded. 3. In the moribund state the simple protoplasmic vacuoles may rupture and flow together, thus creating large degeneration vacuoles, indicative of a quick collapse.

“Contractile vesicles” were seen and described by Carter (1865). He did not figure them, however, and merely indicated that their position was so variable that he evidently failed to locate them for his sketches. Stein (1878-83) described one pulsating vacuole in the anterior end and figured the same. Klebs (1893) indicated one in the posterior end of his organism, which neither Francé nor I believe to be a true *Collodictyon*. Francé (1899) found one at the anterior end, about 6μ in diameter, which pulsated two to three times a minute. He says that it is near the nucleus, but very hard to see on account of the numerous granulations. In all my observations, even with the compound binocular microscope, I have failed to find this vacuole or any other pulsating vacuole. The individuals were often free from inclusions, were studied when actively moving about, when stained intra-vitam with neutral red, Bismarck brown, and methylen blue, when retarded by nicotine, Congo red, anilin solution, litmus solution, weak hydrochloric acid and carbon dioxide. Several times in watching these forms until cytolysis occurred, I have seen the protoplasmic vacuoles flow together, resembling somewhat contractile vacuoles discharging. I am sure these were not pulsating vacuoles. However much I regret to differ from previous observers, especially Francé, who measured and observed the period of pulsation, I am constrained to believe that in the *Collodictyon* of the culture under discussion there is no contractile vacuole.

Francé (1899) sums up most satisfactorily his reasons for believing that there is no cuticle; I agree with him. At the same time, the characteristic form is such, and so constant for the individual, especially for the anterior end, that I am convinced there is a periplast or pellicle, thin and undifferentiated, of smaller vacuoles or homogeneous protoplasm. This must be a coagulation product. It at least is rather impermeable to quick action of many chemicals, especially anilin dyes.

There is no central digestive region, but food vacuoles are held

suspended within the body, at times rupturing or displacing many protoplasmic vacuoles. Small food granules, as *Chlorella*, are arranged peripherally just underneath the periplast, showing that the suspension capacity of the smaller peripheral vacuoles is greater than that of the larger and more centrally located ones. The only evidence of circulation of food vacuoles is that most of the undigested products are evacuated from the posterior portion of the body.

The sulcus has been described in discussing the various modifications of form but its chief features may be here summarized. It cleaves one side, may extend anteriorly so as to cause a cordate or irregular depression in the usual oval contour; posteriorly it may fade out, leaving the general shape conical, or it may divide the body into two cups, thus giving the bifurcated appearance; by secondary branches, which also tend to run longitudinally, there may be produced as many as five posterior cusps. The whole of the sulcal region is amoeboid and functions in food engulfing. Much of the irregularity of shape is due to variations in this region. At its anterior end there is a depression (pl. 9, fig. 19) which may function as a cytostome or esophagus. This seems to end blindly, having no connection with any vacuole. If it may at all be regarded as a cytostome, it is most primitive, more potential and functional than structural.

Collodictyon possesses a true vesicular nucleus, located anteriorly near the base of the flagella and may be either centrally located or displaced, usually away from the sulcus. It is surrounded by a distinct nuclear membrane, from which granular cytoplasm extends out into the body between the protoplasmic vacuoles. The large karyosome is located centrally with a surrounding hyaline area.

The blepharoplast is located anterior to the nucleus, at the base of the flagella and immediately beside the depression caused by the anterior extension of the sulcus. When killed and fixed in strong Flemming and stained in Bordeaux red and iron haematoxylin, the blepharoplast seems to consist of two basal granules surrounded by a more darkly staining granular archoplasm (pl. 9, figs. 23-27). It usually appears, especially when not sufficiently destained, as an irregular chromatic mass in which are embedded the two basal granules which protrude as tubercles, to each of which paired flagella are attached, each also surrounded by a granular archoplasm (pl. 12, figs. 19, 20). It is probable that the irregular chromatic mass is, in fact, simply a lateral view of an archoplasmic plate or cap bounding the granular area and in the center of which are the two basal

granules. There is evidence in vegetative stages for a faint rhizoplast, probably two strands, connecting the blepharoplast to the nucleus, and at times such strands can easily be observed (pl. 8, fig. 13). In division, thickened striations and fibers connecting the blepharoplast and nucleus are more evident than at other times (pl. 11, figs. 40, 44). These no doubt are rightly interpreted as dividing rhizoplasts.

HABITS AND ACTIVITIES

Normally *Collodictyon* is pelagic, floating near the surface of the water in the aquarium, but at all times of the day and at all temperatures tested and under all conditions of light and darkness some have been found scattered throughout the aquarium. In a free drop of water on a slide they tend to stratify near the substrate, but in a hanging drop they swim about throughout the drop, only occasionally accumulating near the slide. In the aquarium they rest both in the direct sunlight and also in shaded portions; but there is a marked tendency to gather nearest the source of light. When there are abundant algae floating on the surface of the aquarium, they can be found at or just beneath the surface and then there is a region of scant distribution below which they tend to accumulate in greatest abundance. This may be on account of a superabundance of oxygen or to too great heat at the surface, due to the absorption of heat by the algae and the surface reflection.

As to the association of *Collodictyon* with water pollution and pools in which decay has been or is progressing rapidly, I am less positive in my convictions than Francé (1899) seemed in his conclusions. My own observations have been that *Collodictyon* can not live where there is a great amount of decay. They are holozoic, however (with the possible exception of times when there is a symbiotic association with *Chlorella*), and live on Protozoa and algae which are associated with decay. Their own life seems far removed from saprozoic nutrition and I find little in the rate of multiplication that tends to confirm such a supposition or conclusion. As to the fact that they were found in pools where disintegration was rapidly increasing or at a maximum, I am not in a position to question except from cultural experiments, in which other factors might have played a determining part; but I urge this same factor, unknown as it is, in explanation of Francé's observation. Francé's argument that *Euglena* is its chief and only

source of food will not hold, since I find engulfed *Ulothrix*, dinoflagellates, *Pandorina*, *Gonium*, *Chlorella*, etc., more common in my aquarium than *Euglena*. I do find the species collectively a "lover of pure water," thriving in sunshine and in a balanced aquarium. I can therefore, at least conclude that increasing or maximum decay is not essential to the life of the organism, and that *Collodictyon* is not a determining factor in water pollution.

When free swimming, *Collodictyon* moves forward by beautiful lashings of the flagella in true tractellar style, the flagella undulating as the animal circles about. It may also move backward by the anterior adaxial action of the flagella, but only seems to do so in an avoiding reaction. It rotates on its longitudinal axis more frequently clockwise, but seemingly without cause or provocation may reverse and rotate counter-clockwise. The flagella may beat back on all sides of the body, closely appressed to the pellicle. It frequently, when near the substratum, attaches itself by its flagella and rotates clockwise about its longitudinal axis. As to the explanation of this I am in doubt. I am inclined to believe it simply a thigmotactic response, possibly combined with positive geotropism; but in swimming through the water when nearing an object it touches it with its flagella and usually passes to one side or jostles the object out of the way if small enough.

In its feeding habits, *Collodictyon* is most interesting. When hungry, it can be distinguished from moribund stages in which all food is extruded by pseudopodial projections from the lateral groove or sulcal region (text fig. A, 1-5). Francé emphasized the adhering engulfing process, speaking little of the pseudopodia. I wish to emphasize these pseudopodia, for I observe that they function actively whenever the organism is seeking food. At these times when coming in contact with Protozoa or algae which it may use for food, they are wafted to the sulcal region by the flagella, or else *Collodictyon* aligns itself alongside of its prey with the pseudopodia in contact. If an elongated filamentous alga is to be engulfed, the relationship between the two is nearly always with the alga lying in the groove longitudinally; but I have noticed with diatoms that they just as frequently are engulfed by the end. Both the flagella and the pseudopodia appear sensitive to food stimulus and usually there is coordination between the protoplasm of the sulcal region and the flagella, though there seems to be no mechanism for this other than the primitive characteristics of the protoplasm. The process of the organization of a food vacuole is a combination of circumvallation and

circumfluence (Minchin, 1912, p. 189). The food is frequently surrounded by engulfing protoplasm of the pseudopodium before it begins to sink into the vacuolated body, but the latter process always takes place and at such times there may be a considerable shifting of internal vacuoles. Once I noticed the rupture of several vacuoles on the engulfing of a very large *Pandorina morum*, undoubtedly explicable by the movements of the captured organism. *Collodictyon* may engulf much food, almost as much as its own size and still appear very little larger. Francé cites an instance where ten *Euglena minima* and *Chlamydomonas* filled up the interior of *Collodictyon*. Its normal cytoplasmic vacuoles must, therefore, not only be displaced, but also ruptured and the food vacuole take the place of one or more of these. There is always a slight water ring surrounding the new food vacuole, but as it grows older this seems to be supplanted by a protoplasmic film coming directly in contact with the substance being digested. Tests with Congo red and litmus bring out these differences intra-vitam. In the use of the former, the food vacuoles present, for some time appear red, thus indicating alkalinity, but the small vacuoles in final stage of digestion are blue, indicating acidity. Litmus did not yield such good results, though the water film was shown up very well. *Collodictyon* has been seen to be engulfed by a larger form of its own species. It is not only a cannibal, but is very voracious, and almost omnivorous. *Peridinium*, *Pandorina*, *Euglena*, *Amoeba*, *Chlamydomonas*, a ciliate (presumably *Colpidium*), *Pediastrum*, *Scenedesmus*, *Lagerheimia*, *Ulothrix*, *Chlorella*, *Navicula*, and *Gonium*, have been observed being ingested or in food vacuoles within the body (pl. 9, figs. 19-27).

MITOSIS

RESTING STAGE

In the normal nucleus of *Collodictyon* in the resting stage the following organelles appear to play important rôles. The nucleus is surrounded by a *nuclear membrane*, which stains very lightly with iron haematoxylin, but a dark red with acid fuchsin and safranin. The shape of the nucleus is variable but typically is an ovoid flattened on the posterior side or anteroposteriorly, the longitudinal axis lying perpendicular to the major axis of the cell. This nucleus is *vesicular*. The central *karyosome* measures 2 to 3 μ in diameter and appears homogeneous with all stains except Bordeaux red, iron haematoxylin, and neutral red used intra-vitam, with which it appears granular;

with safranin, the periphery appears to contain masses of granules of chromatin while the center is homogeneous and more or less translucent. Surrounding this karyosome is a *hyaline area*, measuring from 1 to 3μ in width, which is always transparent and takes none of the nucleus stains but is lightly colored with eosin and acid fuchsin about the same as the cytoplasm. Around this there is a *peripheral area*, varying in width and definiteness, in which irregular *chromatin masses*, small, variable in size, number and shape, occur. This area is from $\frac{1}{2}$ to 3μ in width. It is surrounded by the nuclear membrane.

The *chromatin* material is frequently encrusted upon the nuclear membrane in the resting stages of the nucleus. Much chromatin is accumulated in masses scattered peripherally between the hyaline area and the membrane, at times (presumably when anabolic processes greatly predominate over the katabolic) reducing the hyaline area to a minimum. But the largest amount of the chromatin is found in the karyosome which stains deeply with all nuclear stains. Thus the chromatin encrusted upon the membrane, that occurring in the peripheral zone, and that making up the karyosome, or the larger part of it, all has to be accounted for later in mitosis.

The *blepharoplast*, located at the anterior end, is irregular in size and shape. It consists of a mass of chromatoidal protoplasm which tends to become stellate in shape, much like a nerve cell, deeply staining strands extending out into the cytoplasm between the vacuoles (pl. 9, fig. 20). Embedded in this blepharoplast are two *basal granules*, which are distinctly red when stained with safranin, gentian-violet, orange G, and acid fuchsin. From each of these basal granules two equal flagella arise. From the blepharoplast, probably from each of the basal granules, arise the two *rhizoplasts*, which extend as strands from the chromatoidal mass, but instead of remaining upon the surface as the other strands do, run interiorly to the nucleus, enlarging at the nuclear membrane into a minute granule. In some instances the nuclear membrane is drawn up at the point of attachment of the extranuclear rhizoplast. In several instances (pl. 7, fig. 1), an intranuclear rhizoplast seems to penetrate the membrane and run to the central karyosome.

The cytoplasm immediately surrounding the nucleus is closely appressed to the membrane, making it difficult at times to distinguish the latter. It is denser and more granular, and extends out into the body in strands which lie between the protoplasmic vacuoles. It stains much as the peripheral nuclear area with iron haematoxylin,

but is not so evident with safranin, gentian-violet, orange G, or Mallory's modified connective tissue stain. This area of cytoplasm is not differential cytoplasm, for the interstitial material between or bounding the protoplasmic vacuoles throughout the cytoplasm stain thus deeply with all nuclear as well as plasma stains that I have tried. It seems, therefore, to be merely the cytoplasm in which the nucleus is suspended, being largely granular instead of vacuolar. This denser cytoplasmic area just surrounding the nucleus is a condition of the resting nucleus and not a mitotic phenomenon.

The state of the microkaryosome is of help in determining the progress of mitosis especially of the prophase; but the expanding of the kinetic membrane is the best criterion of this. A chromatin halo inside of the nucleus usually accompanies this.

When division begins, or very soon thereafter, all undigested food particles and other foreign bodies are extruded. There is no rounding up of the cell, the characteristic shape being retained throughout mitosis. I have never seen pseudopodia or amoeboid protrusions from the sulcal region in division stages.

UNEQUAL CONSTRICTION OF THE KARYOSOME

Preliminary to true mitosis, the karyosome usually elongates and constricts into a dumb-bell shape with the knobs of unequal size. These pull apart until connected only by a strand, which finally breaks, accomplishing an unequal or differential division of the karyosome (pl. 10, figs. 29, 30). The resulting large and small daughter karyosomes are not equivalents either in size or behavior, and by reason of their size I shall designate them macrokaryosomes and microkaryosomes respectively (pl. 10, figs. 31, 32).

During this preliminary unequal constriction of the karyosome, I have been unable to detect any change in the blepharoplast, which consists of two basal granules surrounded by granular archoplasm. I have not seen anything resembling a splitting of the rhizoplast at either end. The flagella are still four in number at this stage (pl. 10, figs. 28-31, 34, 36).

The macrokaryosome is homogeneous in appearance with all stains used and contains no residual body. It may be regarded as consisting of plastin impregnated with chromatin. Its behavior is passive. It loses its surrounding hyaline area, and may round up or form a crescentic mass 'around and outside' of the kinetic membrane. It is

then pushed by this expanding membrane out to the periphery of the nucleus, where it reposes in a niche of the nuclear membrane, but separated from the mitotic area within by the persistent kinetic membrane (pl. 11, figs. 38, 44).

It has been found on several occasions (pl. 11, fig. 43) to break up and form an intranuclear chromatin cloud. Even where it has been traced repeatedly to its position near the nuclear membrane, I have never located it or any similar chromatin mass outside the nucleus. In some instances it seems that the cytoplasm just around the nucleus is more deeply stained by a chromidial cloud as though the macrokaryosome or a part of it were extruded in fine granules. Such is undoubtedly its fate in many individuals. But much evidence points to the gradual absorption of the macrokaryosome *in situ* in some instances within the nuclear membrane, with the formation of an intranuclear, intrakinetic membrane chromatin cloud (pl. 10, figs. 35, 36, pl. 11, figs. 37, 38, 41, 42, 44, 45). There is some evidence (pl. 11, fig. 46, pl. 12, fig. 48) that it may in other instances persist and pass over to one of the daughter nuclei without complete dissolution. There is no evidence for its splitting or division on the equatorial plate. Its behavior seems dependent in some way upon the metabolic equilibrium of the nucleus and cytoplasm. Its significance, regarding possible relations with the parabasal body of parasitic flagellates, the macronucleus of ciliates, Hertwig's theory of trophochromatin and idiochromatin, and Hartmann's binuclear theory, in so far as flagellates are concerned, will be taken up in subsequent discussion.

Individuals in evident stages of the prophase have been observed in which there is a total absence of any evidence of a constriction of the karyosome. The kinetic karyosome (microkaryosome) entering into mitosis may likewise on rare occasions be larger than the other mass, the passive macrokaryosome.

MITOSIS

With the organization of the microkaryosome mitosis begins. Binary fission by longitudinal division seems to be initiated within this karyosome. This forms about itself the kinetic membrane, which continues to expand until it becomes commensurate with the nuclear membrane. The faint rhizoplasts extend from the kinetic membrane which surrounds the organizing microkaryosome, through the

peripheral granular area to the nuclear membrane; from here they pass through the intervening cytoplasm to the basal granules within the blepharoplast. The rhizoplasts are not sufficiently prominent to be evident to the uninitiated eye. They may easily be confused with the attenuated strands from the blepharoplasts radiating out into the surface cytoplasm.

Two small granules have been observed just at the point where the rhizoplasts enter the nuclear membrane on their way to the central karyosome (pl. 8, figs. 9, 13). In early and late prophase stages (pl. 14, fig. 75) these separate and the nuclear membrane appears heavier or thicker between these points in comparison with the rest of the membrane, as though an extranuclear paradesmose were forming. In a lately discovered metaphase (pl. 14, fig. 78) this paradesmose is well formed, connecting the polar ends of the spindle. In one particularly favorable anaphase (pl. 14, fig. 83) granules of considerable size are located at the polar ends of the daughter chromatin masses and these are connected by a heavy paradesmose upon the nuclear wall.

This evidence points definitely to the presence of an extranuclear division center or centrosome. Such is typical of parasitic polymastigotes (Kofoid and Christiansen, 1915, Kofoid and Swezy, 1915*a*, 1915*b*, Swezy, 1915, Boeck, 1917) and may be considered typical of polymastigotes in general. In this case, however, the blepharoplast and centrosome are separate, the latter adhering to the nuclear membrane.

The rhizoplasts split first at the end near the nuclear membrane, presumably with the division and separation of the centrosomes. The split extends anteriorly, giving the appearance of a V-shaped striated region (pl. 8, fig. 13). Finally with the separation and division of the basal granules of the blepharoplast (pl. 8, fig. 9) the rhizoplasts appear distinct and their points of contact with the nuclear membrane become farther apart.

In justice to truth, it must be said that the problem of the division center has been full of difficulties. The material in hand is of such a nature that the possibility of error must not be overlooked. The apparent points of contact of the rhizoplast with the nuclear membrane are exceedingly faint. The extranuclear cytoplasmic granules and vacuoles and the intranuclear chromatin encrusted in granules upon the nuclear membrane, which are connected in prophase by somewhat chromatic lines, are exceedingly confusing. This renders

the above interpretation still tentative. Much work is still needed upon *Collodictyon* and related forms to clear up fully the question of the division center.

The time at which the blepharoplast divides has not been definitely determined. There is evidence of its division as early as the middle of the prophase (pl. 11, figs. 40, 40a). It seems, from the figures just referred to, that the method is one of doubling of the basal granules, thus making two pairs, which gradually separate (pl. 11, fig. 46, pl. 12, figs. 48, 53, 54). No splitting of the flagella has been observed, but since only equal flagella have been found in these stages, it seems safe to conclude that the flagella split longitudinally or new ones grow out at about the same time as the doubling of the paired basal granules. At the metaphase the doubling is complete (pl. 12, fig. 48).

PROPHASE

With the unequal constriction of the karyosome, sometimes even before this differential division has been completed, the microkaryosome organizes about itself a membrane, which seems to have a kinetic or metabolic function and which I shall designate as the *kinetic membrane*. The macrokaryosome is passive in behavior and remains outside of this active membrane. The kinetic membrane does not simply bound the hyaline area, but is almost surrounded by such a zone (pl. 10, fig. 32). At first the space between the microkaryosome and the membrane is hyaline, but it is soon filled with a dense chromatin cloud (pl. 11, figs. 37, 38, 41, 42, 44). This kinetic membrane seems somewhat less chromatic than the nuclear membrane. Usually it is spherical or irregularly globular, but in two or three instances angular, almost polygonal (pl. 10, figs. 32, 33), which is probably an artifact. As it enlarges, however, it may elongate and its shape become modified by the organizing spindle.

When the organization of the microkaryosome is begun, the chromatin outside the kinetic membrane tends to accumulate in granules or masses which are linked together by slightly chromatic strands (pl. 10, figs. 30, 32, 36). Much of it is encrusted upon the nuclear membrane (pl. 10, fig. 32); much forms immediately around the hyaline area surrounding the kinetic membrane (pl. 10, fig. 30), frequently also about the macrokaryosome (pl. 11, fig. 40). The whole peripheral zone becomes thus involved and with the expansion of the active hyaline area is more and more encroached upon until

only the chromatin encrusted upon the nuclear membrane remains outside of it. As to the behavior of this peripheral chromatin, there is some doubt. It may pass into solution and become a part of the metaphase chromosomes, or spread out upon the nuclear membrane, making this appear heavier and darker, or it may be extruded into the cytoplasm. There is an indication in a few individuals of a peculiar splitting of peripheral encrusted chromatin bodies (pl. 11, fig. 46). I can not verify this as a regular occurrence nor can I regard it as typical. This splitting is prior to the metaphase and may be closely correlated with the precocious splitting of the segmented spireme (pl. 12, fig. 47).

The microkaryosome elongates and divides, the separation being characterized by connecting fibrils somewhat resembling spindle fibers, rather than a dumb-bell constriction as in differential division of the primary karyosome (pl. 10, figs. 34, 35, 36). A chromatin cloud forms immediately around this elongating microkaryosome and fills up the intervening space, almost obliterating the fibers (pl. 11, figs. 37, 38). This cloud expands until the whole area within the kinetic membrane becomes diffusely filled with fine granules.

This early microkaryosome organization is exceedingly complex for microscopic analysis, but it soon becomes evident that instead of having divided simply into two masses, the microkaryosome is undergoing a segmenting process (pl. 11, figs. 39, 40, 41, 42, 44, 45), preliminary to a final prophase spireme (pl. 12, fig. 47). The first division is followed by a second elongation of each mass, apparently passing through a tripod and ring stage, in time forming two crescentic masses or a segmented skein (pl. 11, figs. 40, 41, 42, 44). This retains terminal chromatin masses or knobs which probably form the basic elements of the future chromosomes. The next phase is a longitudinal splitting of each segment (pl. 11, fig. 45). If all terminal knobs divide at this time, eight chromatin masses would result and this would probably determine the correct count of chromosomes. It is possible that one of the four terminal knobs fails to divide, and this would give but seven chromatin masses—as many chromosomes as I have been able to count (pl. 12, fig. 50). Such a phenomenon is common in mitoses of higher animals (Wenrich, 1916; Carothers, 1917), but it must be admitted that the evidence here is not conclusive (pl. 11, fig. 45). The middle and final prophase stages are characterized by an active organization of chromatin upon the segmenting skein. That there is some chromatin in the original

microkaryosome there can be little doubt and this seems to persist as the terminal knobs to the skein segments.

That all the chromatin entering into the formation of the chromosomes can not possibly be of the original microkaryosome is obvious. Considering the average individuals, the average size of the microkaryosomes when separated from the macrokaryosomes may be estimated at about 1.5 to 2μ in diameter. The size of six of the chromosomes may relatively be estimated at $0.75 \times 1.5\mu$ each, the small one being only half as large (pl. 12, fig. 50). All other chromatin is outside the kinetic membrane and it does not seem probable that it could go through as granules or mass units, since the area immediately around the kinetic membrane is hyaline and in a state of solution, while the area within becomes filled with a dense chromatin cloud, and the only chromatin masses consist of the persisting and reorganizing elements of the microkaryosome. It appears, therefore, that there is a solvent action within the hyaline area around the membrane and that chromatin from the granules in the peripheral zone, possibly from the macrokaryosome, and probably from that enerusted upon the nuclear membrane, is dissolved and passed through the kinetic membrane by diffusion and enters into the composition of the chromosomes. This demands a higher pressure from without, which can easily be accounted for by the chromatin within the membrane being condensed and precipitated upon the achromatic linin fibers of the skein. When the kinetic membrane has expanded to the limits of the nuclear membrane, this early phase of a segmented spireme (pl. 12, fig. 49) finally organizes into a skein resembling a more or less continuous ribbon, with chromatin granules embedded upon it. This seems to be accomplished by a longitudinal separation of the segments, the terminal knobs especially showing this division. A final split involving the formation of sixteen chromatin masses (pl. 12, fig. 49) may be found and may be regarded as the precocious splitting of the definitive chromosomes. From these chromatin masses which are already arranged in an equatorial belt the seven or eight chromosomes of the equatorial plate are finally organized (pl. 12, fig. 50), probably re-fused by a telosynaptic process.

The interpretation of a segmenting spireme seems necessary because of the fact that its elements seem to be directly produced by the early division and organization of the microkaryosome, this being evident before the metabolic membrane has expanded to its final proportions. This spireme frequently has the appearance of a

tripod, ring, or double crescent (pl. 11, figs. 39, 40, 41, 42, 44). I found one difficulty of technique here hard to overcome. The chromatin cloud is usually so dense that whenever this is sufficiently destained to see the skein, this latter is rendered unfit for detailed interpretation.

In *Phrynotettix* (Wenrich, 1916, p. 112) plasmosomes may change into polar granules of chromosomes and vice versa.

One of the most puzzling problems that cytologists have to deal with is the behavior and function of the so-called "plasmosomes" or "nucleoli." They apparently exhibit such a variety of reactions to methods of technique, and exhibit such varying relationships to other structures in the cell, that it is almost hopeless even to attempt to classify them. That they play some important rôle in the physiology of the cell, there is not the slightest doubt, but what that rôle is, or what relation they bear to the question of chromosome-individuality, are problems that are far from a solution at the present time.

There is hardly a close analogy between *Phrynotettix* and *Collodictyon*, but here they offer a most interesting comparison. The terminal knobs of the segmenting skein of *Collodictyon* lend themselves to the interpretation that they are elements at least of the chromosomes. In *Phrynotettix* the individuality of the chromosomes is traced by similar chromatin masses which, however, are not all terminal and several of which may enter into the composition of a chromosome.

The blepharoplast divides during the middle or final prophase. The rhizoplasts thicken and evidently split soon after the kinetic membrane begins to expand (pl. 11, fig. 40). The spindle is not organized until after the spireme and skein are far advanced. The cytoplasm immediately surrounding the nucleus may become darker, due to the presence of a chromidial cloud from extruded chromatin. The nuclear membrane persists. The protoplasmic vacuoles may or may not be large. They are as normal as in the vegetative stages and their variations are largely the result of food vacuoles extruded at the beginning of division. If one so cares, the prophase may be regarded as beginning with the unequal constriction of the karyosome. I have separated these stages arbitrarily for advantages of analysis.

METAPHASE

The nucleus becomes filled with a perfect spindle, which lies at right angles to both the major axis of the cell and to the sulcal axis, usually on one side or the other of the sulcus. The chromosomes are now seven or eight in number (pl. 12, fig. 50). There is a general

uniformity of size and shape, being ovoid, from 1.5 to 2μ in length, 0.5 to 0.75μ wide; with one exception, namely that one chromosome is but half so long and hardly so wide. These are arranged equatorially with very slight tendency to V-shape or crescent-shape. Their elongated axes are meridional to the spindle.

In division (pl. 12, fig. 50) all the chromosomes but one in this spindle appear to have parted transversely. The spindle fibers are attached to the ends and not to the centers or sides. This parting may be fundamentally similar to the parting of the chromosomes as found by Tschenzoff (1916) in *Euglena*. The precocious splitting is evidently not so far removed from the metaphase, however, since in *Euglena* it apparently takes place in the preceding telophase of the parental individual, while in *Collodictyon* it takes place no farther back than the late prophase just preceding the metaphase.

In the most satisfactory metaphase I have found (pl. 12, fig. 50), the small chromosome has not as yet divided. No constriction can be found in it in this figure. It may best be interpreted as a lagging chromosome. In such a case it would probably divide later on in the metaphase or early anaphase, though there is no available material to determine this matter.

In one anaphase (pl. 12, fig. 51) there are obviously unequal chromatin masses. These are either so deeply stained as to prevent an accurate chromosome count or else the chromosomes were contracted and massed in the killing and fixation. It is barely possible that this inequality of mass is due to the failure of the lagging chromosome to divide, thus giving an unequal qualitative as well as quantitative division comparable to the sex chromosome; but we have as yet no evidence of gamete formation in this genus. Such inequality may also be explained by the passing over to one of the daughter nuclei of the remnants at least of the macrokaryosome (pl. 12, figs. 48, 56). Any inequality of mass which is evident in early anaphase is soon obscured by the growth of chromatin, which is proceeding rapidly. I regret that I have not found a sufficient number of metaphase stages to warrant a detailed study. Of the many thousand individuals studied I have seen but three or four equatorial plates.

As suggested above, the indication of a peculiar splitting of certain peripheral chromatin granules is a prophase, not a metaphase phenomenon, related rather to the precocious splitting of the segmented spireme. I have found no indication of a division of the

macrokaryosome at the time of the splitting of the peripheral granules, or when it persists and is found on or near the equatorial plate (pl. 11, fig. 46). As noted above, it may (pl. 12, fig. 48) pass undivided to one of the poles and thus to one of the daughter nuclei (pl. 12, fig. 56). This would explain the inequality of the anaphase chromatin masses. It may disintegrate (pl. 11, fig. 43), go into solution, and finally enter into the composition of the chromosomes. It may, which is very probable, pass from its niche in the nuclear membrane, out of the nucleus to form an extranuclear chromidial cloud or simply be dissipated or absorbed into the cytoplasm. It was found to divide into two unequal segments in several instances in the early prophase (pl. 10, fig. 33), but it is hardly probable that much significance attaches to this, since it is not coordinated with chromatin divisions elsewhere.

ANAPHASE AND TELOPHASE

After the transverse splitting and separation of the chromosomes, each daughter group passes toward its respective pole. When only slightly separated, the chromosomes fuse into a densely staining mass (pl. 12, figs. 52, 54, 55), but can hardly be said to lose their identity here, since knobs and masses resembling ends of chromosomes protrude irregularly from the mass. These chromatin masses become organized into a skein or spireme, a great number of small granules arranged on linear linin threads, before the nuclear membrane has divided (pl. 12, fig. 53). The spindle fibers still persist (pl. 12, fig. 52) after the daughter chromatin masses have drawn near their respective poles, but are only slightly visible at the former equatorial plate. The nuclear wall constricts and nuclear division is accomplished, with the chromatin still in compact masses.

As the daughter chromatin masses pass to their respective poles, they increase perceptibly in size until each equals or exceeds the size of the original karyosome (pl. 12, figs. 52, 55). This is evidently growth and not concretion or deposition, since the chromidial cloud practically disappears at the metaphase and then deepens again in the anaphase.

Toward the final anaphase the chromatin mass or skein breaks up into numerous chromatin masses scattered irregularly through the daughter nuclei. A cloud seems to fill the nucleus and spreads to the surrounding cytoplasm, indicating excessive metabolic activity.

The plane of division runs parallel to the major axis and the sulcus or chief longitudinal groove. The daughter blepharoplasts separate and move to either side of this plane. The basal granules divide earlier in the prophase, as do probably also each of the two flagella, thus producing for each daughter blepharoplast two basal granules and four flagella. I find no stages in which there are shorter or unequal flagella, indicating outgrowth of new flagella, and yet no evidence of splitting has been observed.

The major sulcus deepens and in this plane there is a complete peripheral cytoplasmic constriction (pl. 13, figs. 57-60). The cytoplasm rounds itself up and the daughter organisms are then held together by a thin, highly vacuolated, protoplasmic connection, containing one or more large vacuoles. Division is finally accomplished by the rupturing of these vacuoles. At the time of final separation, the chromatin masses are scattered irregularly through a clouded nucleus. Part of these mass together, round up into a karyosome on which is deposited the immediately surrounding cloud, thus leaving a hyaline area. A large part of the chromatin remains on the outside of this hyaline area and tends to disassociate, forming the peripheral granular area (pl. 13, fig. 59). There is great metabolic activity at this stage, for the whole cell, especially the anterior end, is darkened by a chromidial cloud. At completion of nuclear reorganization the rhizoplasts assume their small, almost invisible appearance and the typical vegetative organism results.

SUMMARY OF OBSERVATIONS

1. Verification of the work of Carter (1865), Stein (1878), and Francé (1899).
2. Failure to find a contracting vacuole, a point upon which previous observers are at variance.
3. Determination of a fundamental polarity and a superficial symmetry, with anterior and posterior, sulcal and absulcal areas.
4. There is evidence of a very primitive cytostome just at the base of the flagella. The sulcus itself may be regarded as an extension of the cytostome.
5. The blepharoplast consists of two basal granules surrounded by a granular archoplasm. When not sufficiently destained, it resembles a more or less branched and attenuated mass, from which

two tubercles protrude. From each granule two equal flagella arise. Two faint rhizoplasts join the blepharoplast to the nucleus and karyosome. At the points of contact of the rhizoplasts with the nuclear membrane, very small granules are found, which function as extranuclear centrosomes.

6. The typical vesicular nucleus undergoes a true mitosis of a type probably related to mesomitosis.

7. There is an unequal constriction and differential division of the initial karyosome, the resulting karyosomes being designated macrokaryosomes and microkaryosomes.

8. A kinetic membrane is organized around the microkaryosome and during the prophase expands until it apparently becomes commensurate with the nuclear membrane.

9. The macrokaryosome rounds up in a niche of the nuclear membrane, not being involved in mitosis. Its possible fate and significance will be discussed in the latter part of this thesis.

10. The nuclear membrane is persistent during mitosis.

11. There is evidence for an extranuclear centrosome, such as has been found in parasitic flagellates, but in this instance it is separated from the blepharoplast and connected with the same by rhizoplasts. Further work on this and other free living flagellates is much needed to more clearly demonstrate this and related problems.

12. There is an intranuclear chromatin cloud during the prophase. In the final prophase, the anaphase and telophase an extranuclear chromidial cloud is also formed.

13. The microkaryosome organizes within the kinetic membrane, apparently separates into two masses connected by fibers and may pass through a tripod and ring stage. This passes into a double segmented spireme stage, in which there are four terminal chromatin masses or knobs.

14. A separation of this spireme takes place, the resulting terminal masses, seven or eight, forming the chromosomes. There is a precocious splitting of the peripheral chromatin granules.

15. In the final prophase when the segmented skein is arranged about the equatorial plate, there is a precocious splitting of the chromatin masses, which may be indicative of the division and distribution of the chromosomes which is about to take place. In this way the transverse division of the chromosomes may be explained as a fundamental longitudinal division, as determined by this precocious splitting.

16. The number of chromosomes is seven or eight, which in metaphase are arranged on the equatorial plate of a perfect spindle.

17. The chromosomes part transversely. In the only satisfactory metaphase observed (pl. 12, fig. 50) one lags on the spindle.

18. The resulting chromatin masses in early anaphase are sometimes unequal in size, but this is soon concealed by a rapid growth of chromatin in the later anaphase and telophase.

19. The reorganization of a typical skein, which breaks up into chromatin masses, some of which go to form the karyosome, some the peripheral chromatin, and some may be extruded.

20. The basal granules separate, the flagella split longitudinally or grow out anew, the rhizoplasts split from the nuclear end, and the two resulting blepharoplasts contain two new basal granules from division of one of the old, inherited from the old blepharoplast, and are connected with four equal flagella and a rhizoplast.

21. A paradesmose typical of polymastigotes in general is present between the separating centrosomes, on the nuclear membrane.

22. Final separation of the cells takes place in the plane of the sulcus, parallel with the major axis of the cell, by the rupture of one or more of the vacuoles of the constricted protoplasmic connection.

DISCUSSION

CLASSIFICATION AND RELATIONSHIP

Prowazek (1903a) attempted to classify the nuclei of Flagellata, distinguishing four different types, which Dobell (1908) has summed up:

1. Simple nuclei, with an evenly distributed chromatic network, and no internal structures (karyosome, division centre, etc.), e.g., *Herpetomonas*.

2. Vesicular nuclei, with direct division; with central chromatin mass surrounded by a clear zone, across which a more or less distinct network extends outward to the nuclear membrane. Such a nucleus may be seen in some species of *Bodo*, and is well seen in *Copromonas*.

3. Centronuclei containing a "nucleolo-centrosome" (Keuten, 1895) and separate chromatin masses. This type of nucleus is characteristic of *Euglena* and its allies. (The centro-nucleus, as defined by Boveri, is a nucleus which contains a cyto-centre, either in a consolidated or diffuse form. In the case of *Euglena*, etc., the cyto-centre is the nucleolo-centrosome, i.e., is of the consolidated type.)

4. Vesicular nuclei with karyokinetic division: e.g., *Polytoma*, *Chlamydomonas*, etc.

To these Dobell added a fifth:

5. Nuclei in which the achromatic division-centre lies freely in the cell, whilst the chromatin is diffuse in the form of chromidia.

In such a classification *Collodictyon* finds no place. Recent work of Tschenzoff (1916) on *Euglena* and of Bělař (1916) on *Astasia* show related nuclear phenomena which furnish an interesting comparison with *Collodictyon*. These possess a "nucleolo-centrosome" (Keuten, 1895), the significance of which seems not to be well understood. In division it presents the appearance of a centrodesmose. *Collodictyon* possesses no such evident body or nucleolo-centrosome, but there is evidence of an extranuclear centrosome similar to that in other polymastigotes. There is a much more perfect spindle and all chromatic material of the nucleus in the metaphase is located upon the equatorial plate. It thus seems to have a more advanced type of mitosis than do the above-mentioned Euglenoidea. With the present advancement in the science of protozoology it would seem possible to review the various groups of flagellates already recorded, not only describing their typical vegetative, but their mitotic phenomena, and to establish complete life cycles for the majority. But such is not the case. All previous investigations of *Collodictyon*, to use it as an example, have omitted description and discussion of mitotic and related phenomena. It is hoped that this knowledge has been brought out in sufficient detail and accuracy to warrant future correlations and comparisons, though little more than a beginning is claimed to have been made.

Because of its plastic nature and its mode of incepting food, "for it does not appear to possess any oral aperture," Carter (1865, p. 289) classified *Collodictyon* as a rhizopod. He, however, acknowledged its similarities to *Bodo* Ehr., in its voracity especially, to *Polyselmis viridis* Duj., and to *Actinophrys eichornii* in its vacuolated cytoplasm, the "cellular spaces which pervade its body." These comparisons have little significance today.

Stein (1878), naming it *Tetramitus sulcatus* Stein, placed it in the flagellate group and in the first family, Monadina, together with the genera: *Cercomonas*, *Monas*, *Goniomonas*, *Bodo*, *Phyllomitus*, (*Tetramitus*), *Trepomonas*, *Trichomonas*, *Hexamitus*, *Lophomonas*, and *Platytheca*.

Kent (1880-81) interpreting Carter's and Stein's organisms as different, put both under Order IV, Flagellata-Pantostomata; *Collodictyon* in Family XIV, Trimastigidae with *Trichomonas*, *Dallingeria*

and *Trimastix*; *Tetramitus* in Family XV, Tetramitidae with *Tetraselmis* and *Chloraster*. He compared the vacuolated cytoplasm to that of *Noctiluca*, *Leptodiscus*, *Trachelius* and *Loxodes*.

Bütschli (1883-87), recognizing *Tetramitus sulcatus* as the synonym of *Collodictyon*, characterized it briefly under the Family Tetramitina of the Suborder Monadina Bütschli, with *Tetramitus* Perty, *Monocercomonas* Grassi, *Trichomonas*, and *Trichomastix*, and placed the Polymastigina as the succeeding family with the genera *Hexamitus*, *Megastoma*, and *Polymastix*.

Francé (1899, p. 19), after discoursing on the inadequacy of the classification of flagellates, says:

Man eröffnet einfach systematische Rumpelkammern, in die man der "Urväter Hausrath drein gestopft." Dort liegen sie, ein trübseliges Chaos von Bodoninen, Monadinen, Dendromonaden, mit dem man nichts anzufangen weiss. Von dort holte ich mir auch meine *Collodictyon*, deren Bau und Lebensgeschichte darzulegen, die Aufgabe der folgenden Zeilen ist.

In concluding his paper (p. 26) he wrote:

Ich begrüßte die generische Selbständigkeit mit umso mehr Freude, als ich der Ansicht bin, *Collodictyon* sie mit den viel höher organisierten Tetramitiden gar nicht näher verwandt. Es ist eine sehr primitive Zelle, welche nach Art der Monadinen gebaut ist, so lebt und sich sowie sie vermehrt. Höhere Differenzierungen besitzt es gar nicht, sondern nur lauter solche Charaktere, welche es erfordern diesen Organismus den Monadinen anzugliedern. Damit wäre aber mein anfangs gestecktes Ziel erreicht, diesem Wesen endlich seinen dauernden Platz im System anweisen zu können.

There is little agreement among taxonomists since 1900 as to the group with which *Collodictyon* is associated. G. Senn (1900), probably as consistent as any, placed it in the Order Protomastigineae (coördinate with I. Pantostomatineae, III. Distomatineae, IV. Chrysoomonadineae, V. Cryptomonadineae, VI. Chloromonadinae, VII. Eugleninae), in the ninth and last family, Tetramitaceae, with *Costiopsis*, *Tetramitus*, (*Collodictyon*), *Trichomastix*, *Trichomonas*, and *Polymastix*. Lemmermann (1910) follows Senn, accepting *Costia* instead of *Costiopsis*.

Klebs (1893) and Blochmann (1895) accept five orders: Protomonadina, Polymastigina, Euglenoidea, Chromomonadina, and Phytoomonadina, including *Collodictyon* in the Polymastigina.

Hartmann and Chagas (1910) put the Protomonadina and the Polymastigina into a single order, the Protomonadina, and add the

orders Rhizomastigina and Binucleata (Rhizomastigina, Protomonadina, Binucleata, Chromomonadina, Euglenoidea, Phytomonadina). This is the system adopted by Hartmann.

Calkins (1909) and Lankester (1909), as did Delage and Hérourard (1895), put *Collodictyon* in the tribe or subtribe Monostomatina, organisms with mouth opening at the base of the group of from four to six flagella, as contrasted with the Astomea, which have no special mouth openings. The sulcal region of *Collodictyon* may indeed be regarded as a cytostome. In this structure, however, we have an extension of the cytostome as a metabolic surface. Its pseudopodia mark it as a generalized rather than a specialized type. *Collodictyon* may thus be regarded as comparable with organisms like *Mastigamoeba* Schultze, *Cercomonas crassicauda* Dujardin, and *Tetramitus* or *Trichomonas*. Regarding the above classification, therefore, the validity of the tribes Astomea and Monostomatina is lessened or *Collodictyon* must be regarded as an intermediate type.

Neither Doflein in his *Lehrbuch* (1911) nor Minchin in his *Introduction to Protozoology* (1912) classify *Collodictyon*. Senn, Klebs, Doflein, and Minchin, however, all accept the Order Polymastigina, into which *Collodictyon* naturally falls. Klebs and Doflein contrast this with Protomonadina, Minchin with Pantastomina and Protomonadina, Senn with Pantastomatineae and Distomatineae. These distinctions by various authors are not so much opposed to one another as it at first seems. All accept the number of flagella as of determining value. It is very desirable to have no all-inclusive taxonomical groups such as the older Monadina. At the same time artificial distinctions do not tend to clarify the situation. Hartmann was evidently actuated by such a feeling when he combined the Polymastigina with the Protomonadina. *Collodictyon* emphasizes the difficulties arising in establishing the distinctness of the groups Pantostomatina, Protomonadina, Distomatineae and Polymastigina, especially when the nuclear phenomena are considered.

As to the location of the mouth, *Collodictyon* seems to have all of its body somewhat metabolic, the anterior end alone being comparatively constant in shape, but the function of ingesting food is localized in the sulcus, about one-fourth of the surface of the organism, assisted materially by the posterior part of the body. Thus, in this feature, *Collodictyon* seems to lie midway between the Pantostomatina and the Protomonadina or Polymastigina. If the primitive phylogenetic type be regarded as a polarized flagellate, with a surface entirely

amoeboid, then the sulcus of *Colloidietyon* may be regarded as a vestigial character, a restricted surface area retained from a *Rhizomastigina* type. *Colloidietyon* would thus be closely related to the rhizopods, not far removed from the ancestral type from which the latter diverged from the evolving flagellates. By such an interpretation it would naturally be considered an organism of a generalized or possibly a primitive type. If, however, the primitive phylogenetic type be regarded as a polarized flagellate with a non-amoeboid surface, then the sulcus of *Colloidietyon* must be regarded as a highly specialized cytostome.

Among the specific and generic characters of *Colloidietyon* which may be called diagnostic, I find:

1. The number of flagella is four. Carter erred in this.
2. I find no contracting vacuole (I am loath to say that there is none). Carter (1865) described but did not figure "contracting vesicles;" Kent (1880-82, p. 308), with seeming authority from Carter, denied that there were any; "such an open vacuolar character of the parenchyma would seem to obviate the necessity for a contractile vesicle, the presence of which structure Mr. Carter was unable to detect." Stein (1878-83) described and figured one in the anterior end near the nucleus; Francé (1899) with difficulty found one in the anterior end and timed its pulsation at about every forty seconds; Klebs (1893) found one in the posterior end in the *Tetramitus* he described as "*sulcatus*."
3. The flagella are equal in length, being as long or slightly longer than the body. This possibly was the basis of Kleb's mistaken identity.
4. The sulcal region is a modified surface area and may be regarded as a cytostome. The classification of Delage and Hérouard (1895), Lankester (1909), and Calkins (1909) involves this in their Tribe Monostomatina.
5. There is an extranuclear centrosome just at the point where the rhizoplasts enter the nuclear membrane. It is, therefore, outside of, but connected with, the blepharoplast. In mitosis a paradesmose is formed between the dividing centrosomes. These characters establish polymastigote affinities.
6. Blepharoplast (so defined as not to include the division center) consists of two basal granules embedded in a chromatoidal matrix or surrounded by a granular archoplasm. The basal granules are connected to the nucleus by faint rhizoplasts.

7. In mitosis the nuclear membrane is persistent.

8. There is formed a perfect spindle of achromatic mantle-fibers with at least seven, probably eight, chromosomes and an equatorial plate.

9. Life cycle so far discovered is simple, binary fission by longitudinal division being the only method of reproduction known.

Collodictyon is typically an animal of simple organization. Its phylogenetic stem grows out of the unknown past. It is very close to the stem from which the Rhizopoda and Mastigophora branch, having much in common with each. That *Collodictyon* is one of the simplest and most primitive of the Polymastigina, there can be no doubt. With the free living members of the genus *Tetramitus* (not accepting *T. chilomonas* as such) it finds its nearest relatives. *Costia*, *Tetramitus* (saprophytic and parasitic), *Trichomastix*, *Polymastix*, and *Trichomonas* are derivatives of either the *Collodictyon* type or free living *Tetramitus*. Thus knowing possibly the simplest Tetramitidae, I am prone to regard this group as not so complex in its entirety as Francé (1899) would have us believe when he wished *Collodictyon* related to the Monadaceae rather than the "complex Tetramitaceae." Much of this complexity may be interpreted as the result of morphological changes resulting directly or indirectly from parasitism.

In *Collodictyon* the two basal granules with very faint rhizoplasts connected with the microkaryosome are not necessarily so highly differentiated, when it may be regarded as simply a slight advance over *Cercomonas* (Wenyon, 1910, text fig. 18), due to the multiplication of flagella, or a doubling of the flagella of a biflagellate type. *Collodictyon* is a primitive polymastigote.

PARASITISM AND SYMBIOSIS

For some three months, January to March, 1916, the majority of all individuals of *Collodictyon* were filled with algae, which were identified as *Chlorella vulgaris*. Only at the period of longitudinal division and in moribund stages would they become free from these inclusions. The algae were arranged peripherally, just beneath the surface and it looked at times as though *Collodictyon* were hollow. Often when under observation these algae could be seen to form small nodules and pop out of the pellicle. These were seldom surrounded by a hyaline area indicating that they were being used for food, though at periods nearly all were so absorbed.

From the above similar and repeated observations I was forced to conclude that we have here a case of parasitism or possible symbiosis. Such a *Collodictyon* seldom engulfed food and seemed well nourished, as though by holophytic nutrition. For some months, in spite of daily observations, I never observed pseudopodia protruded from the sulcal region in such individuals, which condition is characteristic of the holozoic phase. If such be regarded as parasitism, *Collodictyon* must be regarded as the parasite, and *Chlorella* must provide the nourishment. Possibly it had best be regarded as a case of benign domestication, the by-products alone being used. To be truly a symbiotic relationship, *Chlorella* would have to be benefited, and I have not been able to determine that this was the case. I do know that it thrived fully as well, if not better, outside the organism.

The question of inclusions functioning in a parasitic or symbiotic relation, is not a new one. The "yellow cells" of Radiolaria is one of the most interesting as well as most disputed points, and one which is still far from being satisfactorily solved. They were first described by Huxley in *Thalassicolla*, and verified by Johannes Müller and Haeckel. Cienkowski (1871) strongly contended that they were parasitic algae. The discussion progressed with Richard Hertwig (1898, 1902), K. Brandt (1881, 1882, 1885), Entz (1882), and F. Keeble (1909), adhering with certain reservations or modifications to Cienkowski's interpretation. Müller had at first indicated the possibility that they were phases in the life cycle, but later gave up this conception. In 1909 Moroff and Stiasny contended that the yellow cells of *Acanthometron pellucidum* were part of the developmental cycle; in 1910 Stiasny extended this interpretation to Sphaerozoa and Radiolaria generally. Such a view was not accepted without reservation by Minchin (1912); so the question stands, awaiting further and decisive evidence.

In *Collodictyon*, there can be no doubt that the inclusions are algae. They correspond with the free *Chlorella* of the aquarium. I did at first mistake them in observations upon unstained material to be elements of multiple fission, but was forced to give this up upon critical examination. Neither can it be a situation similar to *Euglena gracilis* (Zumstein, 1899), in which the typical chloroplast is lost and an *Astasia*-like phase results; though such evidence furnishes a most interesting possible analogy. That an ultimate lichenoid condition might result is a possibility and from such a relation *Collodictyon* might be transformed from a holozoic to an independent holophytic state.

The possibility of *Collodictyon* becoming ectoparasitic upon the gills or endoparasitic in the intestine is a very fertile field for speculation and experiment. I have nearly always found *Collodictyon* when examining preparations brushed from the gills of goldfish of the aquarium. This may have been due to their normal abundance in the water. In attacking dinoflagellates, large *Euglena*, and other rapid, free-swimming organisms, *Collodictyon* may grasp the body of its prey in a death clasp with all four flagella. At times I have seen one flagellum free in such attacks. Normally, in attaching itself to the substrate all four flagella are spread out radially and its body may be drawn down close and tight or left suspended at a considerable height. In such a state it can revolve clockwise or counter-clockwise upon its major axis. We can hardly imagine *Collodictyon* modified directly into a *Costia*-like animal, though the differences are not so great. In *Costia* there are four flagella, two of which are long and used for fixation, two of which are short and used for wafting food to the cytostome. All four function in locomotion. In *Collodictyon*, the four flagella occur in two pairs with separate basal granules, but all are of equal length and undifferentiated in structure and function. When we consider its omnivorous propensities it seems possible that it could easily adapt itself to an ectoparasitic life, and possibly does so.

Its extreme delicacy of protoplasmic texture and tendency to rupture under slightly unfavorable conditions, makes it improbable that it may directly become an endoparasite, even in the rectal region of fishes. That it could not pass uninjured through the stomach and into the intestine, would be evident to all who could observe it closely. But its great variation in size with correlated reduction of cytoplasmic vacuoles, and the possible modification of the sulcal region into a cytostome similar to that in *Trichomonas*, or a cytostomal region of attachment, leads me to offer *Collodictyon* as a possible free-living ancestral type, or near relative of such at least, of these highly specialized genera.

MITOSIS

Since the discovery of a method of cell division by Remak, the evidence of mitotic phenomena has accumulated rapidly. The early discussion, and late as well, centers about the universality and then the very existence of amitosis as a method of cell or nuclear division. To this mooted question my observations can add but little of decisive value. It is evident that *Collodictyon* presents still another example,

among flagellates, of mitosis. There is not sufficient evidence yet to generalize, but there is a probability, previously expressed by many, that with a fuller knowledge of flagellates, amitosis as a normal method of cell division in Protozoa will be reduced to a minimum. All work on flagellates and rhizopods in which amitosis has been recorded, should be reworked carefully and patiently, for the metaphase spindle is not a structure of long duration, very few instances being met with in the vast number of vegetative and karyokinetic individuals, and the various stages and figures of the prophase are readily capable of erroneous interpretation.

There are several important problems related to the differential division of the karyosome. In the first place it in no way involves the question of *amitosis* as a type of reproduction.

That there is here any phenomenon of chromatin reduction, homologous to *maturation*, must be regarded with equal skepticism. The phenomena of sex have been established for the Phytomonadina for years (Dobell, 1908). The nuclear details of the sexual process in this group are still hard to explain. In other flagellates sex phenomena have received little confirmation. Maturation, such as is regarded as necessary in sexual reproduction among higher plants and animals, has had little corroborative evidence among flagellates. Dobell (1908) worked out the life cycle of *Copromonas subtilis* and figures nuclear extrusion of chromatin in the form of two polar bodies. These are not described as the products of mitosis, however. On critical examination Dobell's work is not convincing. His evidence is inadequate and thus far has not been verified. Goldschmidt (1907) described similar phenomena for *Mastigella vitrea*. His observations are less satisfactory than Dobell's. Schaudinn (1904) in error extended the sexual process to *Trypanosoma noctuae*.

Nuclear extrusion of chromatin is, however, a common phenomenon in flagellates and protozoa generally. Work on *Trichomonas* (Kofoid and Swezy, 1915) reveals chromatin extrusion in each of these forms. Among *Amoeba* of the *limax* group (Alexeieff, 1911*b*, 1912*a*, 1912*b*), a similar extrusion occurs. But all of these extrusions as described, with the exception of *Copromonas*, seem to have no relation to maturation phenomena.

Collodietyon (pl. 10, figs. 29-36) presents a clear case of separation of chromatin from that which organizes for mitosis in the differential division of the karyosome (pl. 10, fig. 32). In one instance the macrokaryosome is seen in a state of division (pl. 10, fig. 33) very

comparable to the division of a polar body, but there is no adequate basis for any maturation process. Another phase (pl. 13, fig. 60) was found in which there are two nuclei in a partially constricted individual. This may be a late telophase, but in each nucleus can be seen a small chromatic body near the central karyosome. Furthermore there is a food vacuole containing a recently engulfed *Pandorina*. This is contrary to the rule. At the beginning of mitosis all food particles are extruded and this is the only instance in which it seems food has been engulfed before karyokinesis is completed. It is interesting, therefore, to contemplate the possibilities. It may be interpreted as follows: 1. A telophase phenomenon, presenting the anomalies of the small chromatic mass near the karyosome of unknown function, but probably metabolic, and the engulfed *Pandorina*. 2. A somatella of two cells or a suspended telophase which has actively begun to engulf food. 3. Conjugating individuals in which polar bodies are being extruded. Since conjugation has never been observed in living material and this is the only instance capable of such an interpretation, it seems improbable. The first alternative leaves much to be desired, since it gives no explanation of the exceptional phenomenon. This leaves the interpretation of a suspended telophase or a two-celled somatella as the most probable explanation. It can not be considered of much critical value until further verified and elucidated. We may, however, at least exclude the probability of maturation phenomena. In *Collodictyon* is found a beautiful illustration of the separation of excessive chromatin from the mass undergoing mitosis, thus freeing that body for its generative function. It presents little probability of sexual phenomena. If I may be allowed to surmise, acknowledging how illogical such a surmise is, all such maturation phenomena, recorded in the Flagellata, may be nothing more than the amitotic division of the karyosome, as is evident in *Collodictyon*, a freeing of the nucleus of excessive or surplus chromatin.

The *surface-volume ratio hypothesis* is not supported by the cell division of *Collodictyon*, since all sizes of cells are found undergoing division (pl. 11, figs. 40, 45, pl. 12, figs. 47, 51, 54). There is an apparent indifference to size.

The *theory of nucleao-cytoplasmic ratio* is much more difficult to prove or disprove positively. *Collodictyon* presents a double line of evidence: *First*, the differential division of the karyosome may be interpreted as either (a) a method of chromatin extrusion, such as has repeatedly been recorded for many, the majority of the Plasmodro-

mata; (b) simply the freeing of the karyosome of sufficient chromatin, which may here be regarded as passive and inert, to permit of unretarded kinetic activity on the part of the generative microkaryosome, or (c) both. The *second* line of evidence points toward a separation of the chromatin preliminary to a reorganization and growth of the same. It is necessary for the peripheral chromatin as well as that in the macrokaryosome to pass through solution, usually through the stage of a chromidial cloud as well, before entering into the prophase skein and the metaphase chromosomes whose organization takes place within the metabolic membrane. The chromatin upon the equatorial plate is much greater in amount than the mass of the original microkaryosome. Growth and organization have, therefore, definitely taken place. Subsequently, growth proceeds much more rapidly in the anaphase, during which time each daughter nucleus comes to contain almost if not fully as much chromatin as the original nucleus (pl. 12, figs. 52-55). This would indicate that cell division was not initiated by an unbalanced ratio and that the chromatin-cytoplasmic equilibrium may be simply a metabolic phenomenon, not at all related to initiating cell division. It would point, however, to the necessity of freeing the microkaryosome to insure its better kinetic activity and that a dividing nucleus seeks to be unretarded by surplus chromatin when organizing for division. *Collodictyon* thus presents evidence which tends to contradict the nucleo-cytoplasmic ratio theory.

The problem of dual chromatin is especially inviting. There are two types of chromatin in *Collodictyon*, differing clearly and unmistakably in behavior. Repeated efforts to get a differential stain for the macrokaryosome and microkaryosome, however, have not succeeded, and we have no evidence whatever of any chemical or physical difference between the chromatin of these structures. Observations upon the microkaryosome lead me to interpret that body as consisting of the generative chromatin. In its function at least, this chromatin is different from that of the separated macrokaryosome, which is both physiologically and morphologically passive. It required little speculation to conceive, in fact it is perhaps probable, that with differentiation in functioning, the chemical nature of the chromatin might be sufficiently altered by purely metabolic changes to produce differential staining of the two bodies. That such a differential staining has not been achieved, points emphatically to the fundamental similarity and chemical nature of all the chromatin of *Collodictyon*. *Collodictyon* seems to present an example among flagellates of trophochromatin and

idiochromatin. Such a distinction in this instance is, however, more physiological than morphological.

Conceived in such a category, *Collodictyon* may stand closely related to the parasitic flagellates containing a parabasal body (kinetocore of Hartmann). This would emphasize the fact that Hartmann's Binucleata is based upon a fundamentally physiological rather than a true morphological character. The macrokaryosome, through the changing metabolic equilibrium, brought about by change from a free living to a parasitic mode of life, might be preserved and persist as a result of purely chemical reaction, thus becoming the parabasal body. This possible origin of the parabasal body is purely theoretical. *Rhynchomonas* (Bělár, 1916) and *Bodo caudatus* (Alexeieff, 1911a), as far as I know, are the only free living flagellates having any persistent character comparable to a parabasal body. The fact that the parabasal body is not present in all parasitic flagellates, nor constant in the life-history of some, would emphasize its dependence upon chemical nucleo-cytoplasmic equilibrium and its probable origin from a primitive condition such as is found in *Collodictyon*.

Werbitsky's work (1910), in which, by feeding host rats para-fuchsins, tryparosin or oxazine, the parabasal body was dissolved and a strain of parasites obtained in which this body did not appear through several successive generations, may be similarly interpreted. Anything tending to counterbalance or upset the metabolic equilibrium of the cell would be expected so to affect a simple passive mass of chromatin which no longer functions in its original rôle, but persists as a surplus or reserve. Since chromatin seems to be the substance in which metabolism centers, the parabasal body would thus probably function as a kinetic reservoir for the motor activities of the cell. Evidence from *Collodictyon* emphasizes this interpretation of Kofoid and Swezy (1915).

Mitosis in Metazoa is variable in its details, but as found in the Protozoa it is variable in its fundamental features, so much so that it may be classified into categories. Little distinction was made in the types of mitosis until Nägler (1909, p. 46) designated what had previously been called amitosis in *Amoeba* and many Protozoa as "promitosis" in contrast to the type of mitosis as found in Metazoa and Metaphyta. "Für die sogenannte Amitose der Protozoen führt man daher am besten eine neue Bezeichnung ein und definiert sie als eine Kernteilung, die weder ausgesprochene Mitose, noch Amitose ist und sich charakterisiert durch die Teilung eines Nucleocentrosoms, des Caryosoms. Ich schlage deshalb für diese Teilungsform den Namen

Promitose vor." Flemming (1882) suggested the terms, "amitosis" and "mitosis," and defined the former as a nuclear division without formation of chromosomes and a spindle, while in mitosis these are more or less evident. Nägler (1909, p. 46) thinks amitosis would be better characterized "durch die unregelmässige Durchschnürung des Kernes (Fragmentierung)." He concludes that an extreme instance of amitosis is not known in the Protozoa, instances so interpreted, with division of centriole within the karyosome and the apparent division of the whole chromatin mass, being more analogous to mitosis than amitosis.

Chatton (1910) distinguished three types of mitosis, which, as he characterized them, may be analyzed as follows:

Promitosis.—(Protokaryon type of nucleus, consisting of a fundamental mass of plastin, impregnated with chromatin, and containing a centriole).

1. Nuclear membrane is persistent and division is intranuclear.
2. Karyosome is the equivalent, morphologically and physiologically of the centrosome.
3. Equatorial plate (chromosomes?) organized from peripheral chromatin material.
4. Chromatin is not distributed equally by the nuclear mechanism.
5. Achromatic separation fibers are apparent when the karyosome divides.

Thus all essential elements and the primitive substances, except peripheral chromatin (which may be most important), are condensed within the karyosome. In higher forms of mitosis these tend to separate. "In proportion as the karyosome loses its plastin and chromatin elements, and becomes reduced to the centriole alone, so the primitive promitosis will approach more and more to the type of an ordinary mitosis" (Minchin, 1912, p. 110). This reduction of the karyosome may be either temporary, taking place only during the process of mitosis, or permanent, as is characteristic of higher types of mitosis.

Mesomitosis.—

1. Nuclear membrane persistent and division intranuclear.
2. Centriole, more or less separated from the karyosome, rests within the nucleus.
3. Chromosomes derived from the karyosome.
4. Chromosomes (equatorial plate) organized upon a spindle.
5. Plastin is reduced or disappears.

Metamitosis.—

1. Nuclear membrane disappears during process of mitosis, the mitotic figure resting in the cytoplasm.
2. Centriole, separate from the karyosome, may be intranuclear (as in *Pelomyxa*), but is generally extranuclear.
3. Evident chromosomes splitting longitudinally upon an equatorial plate.
4. With the zones of differentiated, surrounding cytoplasm the centriole forms the centrosome. Polar asters are usually present.

Chatton thus emphasized or summarized three categories:

1. Division center within the karyosome (nuclear membrane persistent, chromosomes organized from peripheral chromatin).
2. Division center outside the karyosome, but within the nucleus (nuclear membrane persistent, the karyosome organizing the equatorial plate).
3. Division center extranuclear (nuclear membrane disappears, chromosomes organized from nuclear chromatin).

Collodictyon falls into none of these categories. This system is based upon *Amoeba* and can not be extended without modification to include the typical polymastigote type of mitosis, in which the nuclear membrane is persistent and the division center extranuclear. Analysis of the mitotic phenomena of *Collodictyon* may be correlated and summarized here.

1. The unequal constriction or differential division of the karyosome into microkaryosomes and macrokaryosomes, which differ at least in behavior. The microkaryosome organizes directly into the segmenting skein. The macrokaryosome is: (a) extruded from the nucleus and absorbed into the cytoplasm, producing an extranuclear chromidial cloud; or (b) distributed as a unit to one of the daughter nuclei; or (c) is absorbed in the intranuclear cloud and probably is involved in chromosome organization. These two derivatives seem to offer good examples of so-called trophochromatin and idiochromatin.

2. The presence of an extranuclear centrosome. Intranuclear chromatin cloud.

3. The presence of a paradesmose.

4. Nuclear membrane persistent.

5. Successive separation of promitotic segmented skein. Indication of a precocious splitting of the peripheral chromatin granules of the nucleus. Evident precocious splitting of final segmented skein in prophase.

6. A well defined intranuclear spindle (of mantle-fibers). No extranuclear astral rays.

7. Definite chromosomes, organized upon equatorial plate, probably derived from chromatin of the microkaryosome and the intranuclear chromatin cloud formed by the going into solution of the peripheral chromatin granules and probably the macrokaryosome.

8. Apparent transverse splitting of the chromosome in metaphase.

That it is impossible to use Chatton's categories, unmodified, will inevitably be concluded from only a slight survey of protozoan mitosis. If emphasis be laid upon the division-center, accepting this as the

homologue of centriole, Binnenkörper and centrosome, and other characters be excluded, then the classification may be all-inclusive. But whenever other characters are correlated, exceptions or connecting links become as common or more so than the rule, and only arbitrary progress is made.

Alexeieff's "Systematization de la mitose dite 'primitive,' " (1913) into five types—promitose, haplomitose, mesomitose, paramitose, and panmitose—each subdivided into two subtypes, is the best possible illustration to what extremes one will be led who attempts to bring order out of the chaos of protozoan mitoses. With this elaborate schedule, there are many misfits and the exceptions become the rule or the logical connecting links. *Euglena* (Tschenzoff, 1916) fits into none of the categories. *Collodictyon* fails to conform to any of his categories, the centrosome being extranuclear and the nuclear membrane persistent. Furthermore, the chromosomes form partly from the chromatin of the microkarosome, partly it seems from the macrokaryosome and partly from peripheral chromatin granules, just as in panmitose. Thus it, too, is a conspicuous exception and emphasizes the arbitrariness of such attempts at elaborate classification.

Alexeieff (1913) attached little importance to the presence or absence of an equatorial plate, as a basis for classifying mitoses. His reasons assigned are very good and can be referred to by all interested. He emphasized the "centriole theory" and distinguished three types of mitotic figures as they possess (1) polar bodies, (2) centrioles, or (3) neither polar bodies nor centrioles. He suggested that polar bodies, reduced, might be homologous to centrioles, but then, with fine-spun distinctions, claimed that such were only present in mesomitosis and rheomitosis. What he regarded as the "pseudo-polar bodies" of haplomitosis, not being siderophile, could not be homologized with centrosomes by him; he, therefore, concluded that haplomitosis was very primitive and "particuliere." Hartmann (1911), Nägler (1909), and Chatton (1910) considered centrioles very general in Protozoan nuclei; Dangeard (1901), Alexeieff (1913), and Gläser (1912) considered them very rare.

Calkins (1903) suggested the constitution and rôle which chromatin plays in division as a basis for classification of the types of mitosis. Tschenzoff (1916) expressed the same suggestion, having failed to find a division center in *Euglena*, except the nucleolo-centrosome, the Binnenkörper not initiating cell division, but persisting much as a nucleolus of metozoa.

Chatton (1910) attached much importance to the absence, persistence, or disappearance of the nuclear membrane. Alexeieff (1913, p. 357) said, "Je ne puis pas partager l'opinion que la persistance ou la disparition de la membrane nucléaire a quelque importance." After citing *Centropyxis aculeata*, *Octomitus intestinalis*, where the nuclear membrane persists through all phases of mitosis, and *Hexamitus intestinalis* in which the nuclear membrane disappears, Alexeieff (1908) concluded: "En effet dans beaucoup de cas il est très malaisé de décider si la membrane nucléaire a disparu complètement, ou si elle est seulement amincie; souvent il n'y a qu'une séparation physique entre le cytoplasme et le suc nucléaire (comme entre deux liquides immiscibles) et l'image cytologique peut être dans ce cas difficile à interpréter."

Calkins in 1898 and in later works describes *Tetramitus chilomonas* as an example of a non-nucleated flagellate or rather of a distributed nucleus. He likewise (1899) considered *Chilomonas paramoccium* to have no nuclear membrane. Kepner and Edwards (1916) prove this latter to be incorrect. I came to the same conclusion from observations of material put up in 1916. I am, therefore, skeptical of the accuracy of Calkin's description of *Tetramitus chilomonas*. This form, so far as I can judge, is not a true *Tetramitus* but of a typical *Chilomonas* structure. The granules of the cell may or may not be chromidia. Distributed granules of some character (idiochromatin or paramylum) are characteristic of the genus *Chilomonas*. The division center would thus be considered either within the nucleus or the karyosome. It needs correction, verification, or elucidation.

The nature of the division center in flagellates is not well understood. Prowazek (1903) classifies flagellates with regard to this division center and his work was accepted with slight additions by Dobell (1908). Chatton (1910) classified primitive mitoses in *Amoeba*, basing his system upon the relationship of the division center to the nucleus and karyosome. Alexeieff (1913) gave a most elaborate system of the primitive mitoses, so elaborate, in fact, that it is of little service. Most references in recent years have reverted to the simpler system of Chatton (1910). In all of these the relationship of the division center to the nuclear chromatin has been accepted as the basis of differentiation. Calkins (1903) based his system upon the behavior of the chromatin. He (1899), however, based his conception of the evolution of metazoan mitoses upon the extranuclear division center of *Noctiluca*.

Hartmann (1910) in his new group Binucleata adopted the conception of an extranuclear division center located in the blepharoplast. This seems to hold good for many polymastigotes. There is always present a parademose, which is probably comparable to the centrodemose of rhizopods or the "Binnenkörper" of free-living flagellates.

KINETIC MEMBRANE

The phenomenon of a membrane organizing around the microkaryosome, commensurate with the inner boundary of the hyaline area, and expanding progressively during the prophase until it approaches and becomes identified, identical except for the part where the macrokaryosome rests, with the nuclear membrane, as is found in *Collodictyon*, so far as I know, has been recorded in no other instance. Achromatic radiations from the karyosome through the surrounding hyaline area are found in the nuclei of some amoebas (usually described as characteristic of protokaryon type of nucleus). These have been interpreted as being related more or less closely to chromosome formation. Sutton (1900) discovered what he designated "chromosome vesicles," surrounding the organizing chromosomes in an orthopteran insect. Carothers (1915) finds the same in the nuclei of *Culex* and modifies the term to "chromomere vesicle." Wenrich (1916) interprets these vesicles as expanded granules.

In *Collodictyon* the nuclear membrane is not one of the most evident features of the resting nucleus, but undoubtedly it is both present and persistent. With the separation of the microkaryosome and the beginning of organization, a second membrane, very faint but evident, is formed immediately around the microkaryosome, and progressively expands. Just around this membrane is a progressively expanding hyaline area, and inside is a more or less homogeneous clouded area filling up the entire intramembranous space, in which can also be distinguished the organizing microkaryosome, segmented skein and spireme. No such membrane surrounds the macrokaryosome and there is no evidence that any kinetic activity is present in or around this mass of inert chromatin.

R. S. Lillie (1902, p. 420) in discussing the oxidative processes of the cell-nucleus, concludes that "in many tissues the nucleus is the chief agency in the intracellular activation of oxygen . . . The active or atomic oxygen is in general most abundantly freed at the surface of contact between nucleus and cytoplasm." The nucleus in much recent literature is regarded as the kinetic or metabolic center of cell

activity. It is usually concluded, on account of this, that the chromatin is the substance upon which metabolism is dependent. *Collodictyon* presents evidence bearing on this point. The macrokaryosome, consisting of separated chromatin, appears wholly passive during the prophase. The microkaryosome, on the other hand, consisting of chromatin which organizes the skein, is the center of great activity. The radius of this activity is bounded by the metabolic membrane. If this activity be regarded as due to the entering into solution of peripheral chromatin and related nuclear substance, which must pass into the interior, the so-called membrane must be either merely a static atomic equilibrium zone, through which outer and inner activities are counter-balanced, or it must be a definite membrane organized by great pressure from within and without, through which, by the process of osmosis, the peripheral solution passes into the inner sphere of organization, where the pressure is relieved and less than on the outside, by the condensation and precipitation of chromatin on the organizing skein. The latter seems the more plausible interpretation. In this light the generative chromatin is the kinetic factor, at least in mitosis probably in protozoan metabolism. The free chromatin is non-active and non-kinetic, acting in a purely passive way in the prophase. Since no food is engulfed and all inclusions are extruded, nutritive processes seem suspended in *Collodictyon* during mitosis. Oxidative (katabolic) processes would naturally be at their height, and better subject to analysis since they are here separate from the anabolic. Since the chromatin of the macrokaryosome shows no kinetic phenomena, chromatin as such may be eliminated as the center of "activation of oxygen." But since this oxidative katabolism is a part of nuclear activity, it is possible that it may be performed by the generative chromatin in *Collodictyon*, possibly in other protozoans; while anabolic or constructive processes of intussusception may find their center in chromatin.

There seems little doubt that the metabolic membrane found in *Collodictyon* is related in some way to the chromosome vesicles of the Metazoa, especially the radiations from the karyosome in a protokaryon type of nucleus. By its unity and simplicity I judge it to be more primitive. The chromosome vesicles fuse as mitosis progresses in higher forms; in *Collodictyon* it begins as a unit and ends in becoming practically continuous with the nuclear membrane. It may not be too imaginative to regard the nuclear membrane itself, with its peculiar phenomena of persistence or disappearance in mitosis.

as related in some way to this kinetic membrane, either in origin or in underlying causes.

The substance of the microkaryosome is quantitatively smaller than the chromatin of the metaphase chromosomes (equatorial plate). These latter seem undoubtedly to be derived from both peripheral and karyosome chromatin. The most probable way in which peripheral chromatin can get upon the skein or into the metabolic membrane, is by entering into solution and being again deposited on the inner achromatic framework, within the membrane. This would eliminate any interpretation of the continuity of all of the substance of chromosomes in *Collodictyon*. Much has been written of late concerning the individuality of chromosomes and this is regarded by many as fundamental to any mechanism of Mendelian inheritance, especially as interpreted by Janssen and Morgan in their "chiasma-type theory" or "theory of crossing over." Wenrich (1916) presents probably the best morphological evidence of the continuity of the chromosomes, so far recorded. He, however, does not claim to present observable proof of such continuity, but finds in his observations and correlations, together with reasonable conclusions from hybridization and other experiments, that the evidence is greatly in favor of such continuity. Moenkhaus' (1904) conclusion from his hybridization experiments is typical: "If from such a nucleus, two kinds of chromosomes again emerge, it amounts almost to a demonstration that the chromatin substance of a given chromosome forms a unit and that unit persists."

In *Collodictyon* the chromatin cycle consists of an apparently homogeneous karyosome, separation and possible elimination of major part, an apparent solution phase, prophase segmented spireme with terminal knobs, metaphase chromosomes, anaphase synizesis and growth, followed by a typical spireme of small nodular granules, which later unite into irregular masses, from which the daughter karyosome is reorganized.

INHERITANCE IN BINARY FISSION

In sexual plants and animals the hereditary substance can be localized in the germ cells, though all reproduction is not by means of such germ plasm. In flagellates, reproducing alone by the method of simple binary fission, and in which sex is unknown, the problem of heredity becomes more complex, instead of simpler of analysis.

Were the problem solved for higher organisms, those conclusions

might be extended to the majority of the simpler Protista, but not necessarily to all. For in this group nature has her experimental laboratory, and we would expect to find discards. We may well believe that the mechanism found in sexual plants and animals is an extremely modified and specialized adaptation of a much simpler, more fundamental, but thoroughly satisfactory type, characteristic of most binary fission as found today. So much at least we may assume.

Parthenogenesis might be pointed to as a reversion to such a primitive type and such may be the case; but a study of this phenomenon points rather to its being a still further specialization and adaptation, based upon advanced sexual phenomena or their suspension. If such be the case, we need not look here for the simpler type mechanism of inheritance characteristic of binary fission. Some do regard it as such, however, and such an interpretation naturally leads to the conclusion that sex is a universal phenomenon. Minchin (1912, p. 130) makes the generalization that sex is "of universal occurrence in all truly cellular organisms." This attitude does not seem to accord, nor can it be satisfactorily harmonized with facts as understood today. Coulter (1914) would refute such a view, at least in algae. In the simpler flagellates a satisfactory example of syngamy has yet to be found. Dobell's (1908) life cycle of *Copromonas* has not been verified. What he figures as maturation phenomena may be well explained by comparing his figures with the differential division of the karyosome in *Collodictyon*. Still variation and evolution are characteristic of flagellates. In fact, it is back to the flagellates that the origin of higher plants and animals is traced by the large majority of biologists.

It is little that *Collodictyon* adds to this much discussed subject. There is a mitotic figure, a mechanism which may well be interpreted as a distributor of hereditary characters. Chromosomes are present. The actual number of these in *Collodictyon*, as in most Protozoa, is very difficult to determine. They seem composed for the most part, the most evident part, of chromatin, probably upon an achromatic center or skeletal structure. Such achromatic elements must not be confused with "interzonal or connecting fibers," exposed by the diverging chromosomes (pl. 12, figs. 51-53). The chromosomes in the metaphase split transversely. Such a transverse division is capable of interpretation as a longitudinal split in two ways. Either the chromosomes split longitudinally, separate at one end and finally pull apart at the other end, or, during the precocious splitting of the spireme and final prophase the number of chromosomes is doubled

(pl. 11, fig. 47), these being fused end to end into half the number on the equatorial plate. It is, then, these doubled chromosomes which separate transversely in the metaphase. A third alternative is that the chromosomes do split transversely, the inheritable characters being usually halved physiochemically but not necessarily according to the chiasma-type hypothesis.

In behavior, at least, there are two kinds of chromatin. That of the macrokaryosome is largely passive, that of the microkaryosome is either active or activated by a close association with the division center. The former may possibly be analogous to the macronucleus of ciliates and the parabasal body of parasitic flagellates; the latter to the micronucleus of ciliates and the typical nucleus of flagellates. The distinction of trophochromatin and idiochromatin might be applied here as well as in any of the typical usually cited instances. The chromatin may be all of like character, its behavior being determined in all cases by associated elements. Its close association with achromatic elements and its inclusion in all chromosomes is, therefore, essential.

GENERAL SUMMARY

1. The first evidence of mitosis is an unequal constriction and differential division of the primary karyosome of the vesicular nucleus into a macrokaryosome and a microkaryosome, the latter alone functioning directly in the formation of the prophase skein.

2. The skein originates by the successive segmentation of the microkaryosome, resulting in two crescents and four terminal knobs.

3. These crescents split longitudinally, producing presumably eight terminal knobs which are the elements at least of chromosomes. It is possible that one of the terminal knobs fails to split. In this case the number of chromosomes would be seven, which coincides with the best count so far made.

4. Coincident with the beginning of the segmenting skein, there is organized around the microkaryosome a kinetic membrane which expands until it becomes apparently commensurate with the nuclear membrane.

5. In the final prophase there is a precocious splitting of the segmented skein, in which the number of terminal chromatin masses is doubled, and all are organized in an equatorial belt. These are probably fused in telosynapsis.

6. The spindle is intranuclear. There are seven or eight chromosomes which part transversely at the metaphase.

7. Growth of chromatin is very rapid in the anaphase. As the chromatin passes to the poles of the spindle, a distinct granular spireme is organized.

8. *Collodietyon* conforms to no category in the classification of mitoses of either Chatton (1910) or Alexeieff (1913). Its nuclear membrane is persistent and its centrosome extranuclear. A typical parademose is present. The chromosomes are organized from both peripheral chromatin and the karyosome.

9. *Collodietyon* is one of the simplest of the Polymastigotes, both in morphology and mitotic phenomena.

10. The blepharoplast consists of two basal granules, surrounded by a granular archoplasm. In the middle of the prophase these granules separate and divide into four, thus producing a double blepharoplast for each daughter cell. The flagella either split or grow out anew. The rhizoplasts split longitudinally, being doubled about the time the basal granules separate.

11. Division finally takes place by a longitudinal constriction along the sulcus.

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EXPLANATION OF PLATES

Figures in plates 7 and 14 are diagrammatic, those of plate 7 being based upon observations of living and stained material, plate 14 being sketched from camera lucida drawings of accompanying plates. Figures in plates 8 to 13 are camera lucida sketches. All figures except plate 13, figure 62, are of *Collodictyon triciliatum* Carter, killed in hot Schaudinn's fluid and stained in Heidenhain's aqueous iron-alum haematoxylin, unless otherwise stated. $\times 1700$.

PLATE 7

Fig. 1. Laterosulcal view, showing vacuolated cytoplasm, sulcus, vesicular nucleus with karyosome and peripheral chromatin, blepharoplast of two basal granules surrounded by granular archoplasm, four flagella, and rhizoplast.

Fig. 2. Absulcal view, showing bifurcated posterior end, nucleus, blepharoplast, and flagella.

Fig. 3. Sulcal view, showing median sulcus, posterior bifurcation, nucleus, blepharoplast and flagella.

Fig. 4. Anterior view, showing sulcus, nucleus, blepharoplast, rhizoplast, and four flagella.

Fig. 5. Sulcal view of truncated posterior end; two cusps.

Fig. 6. Sulcal view of truncated posterior end; three cusps.

Fig. 7. Laterosulcal view of truncated posterior end; four cusps.

Fig. 8. Laterosulcal view of truncated posterior end; five cusps.

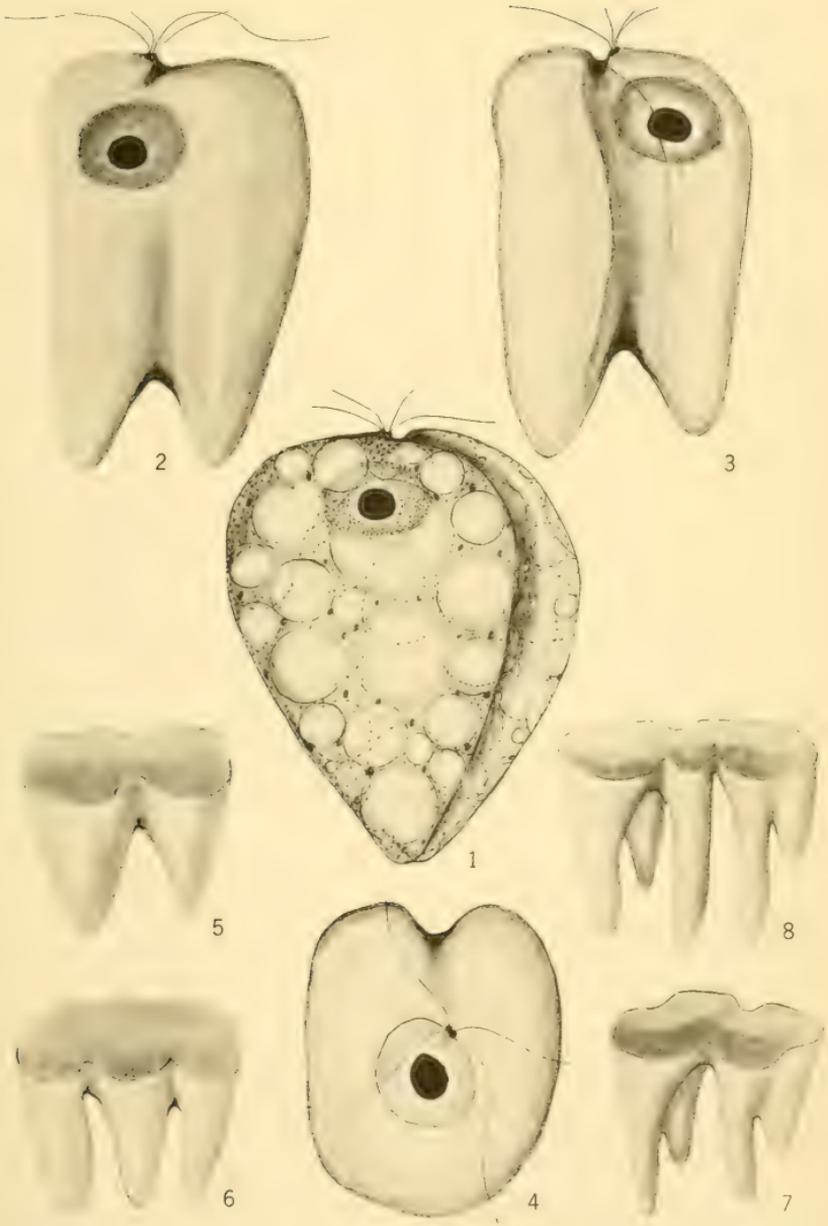


PLATE 8

Figures 9 to 18, showing variations in size and shape.

Figs. 9 and 16, from material killed in picro-mercuric, stained in Bordeaux R, aqueous iron haematoxylin.

Figs. 10, 13, and 18, from material killed in strong Flemming, stained in aqueous iron-alum haematoxylin.

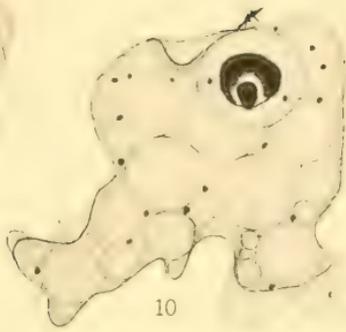
Figs. 11, 14, and 15, from material killed in hot Schaudinn's fluid, stained in alcoholic iron-alum haematoxylin.

Fig. 12, from material killed in picro-mercuric, stained in Mallory's connective tissue stain, modified.

Fig. 17, from material killed in picro-mercuric stained in phosphotungstic acid haematoxylin.



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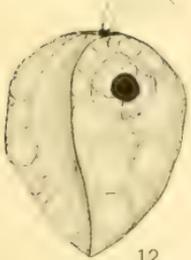
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PLATE 9

Figures 19 to 27, showing food inclusions, fig. 19 killed in hot Schaudinn's fluid, fig. 26 killed in strong Flemming's and stained in iron haematoxylin, and figs. 20-25 and 27 killed in strong Flemming's and stained in Bordeaux R iron haematoxylin.

Fig. 19. *Ulothrix*.

Fig. 20. Two dinoflagellates, presumably *Peridinium*.

Fig. 21. Gelatinous capsule of *Pandorina* and *Scenedesmus*.

Fig. 22. *Scenedesmus*.

Fig. 23. *Pediastrum*.

Fig. 24. A diatom, presumably *Navicula*.

Fig. 25. A ciliate, presumably *Colpidium*.

Fig. 26. A diatom, presumably *Navicula*.

Fig. 27. *Pandorina*.

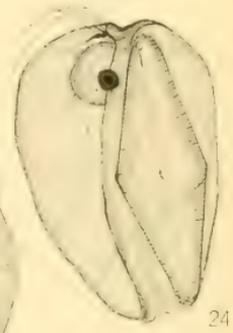
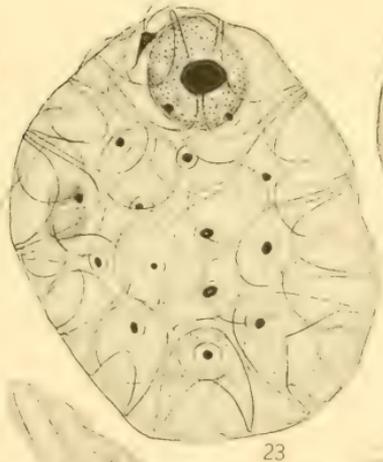
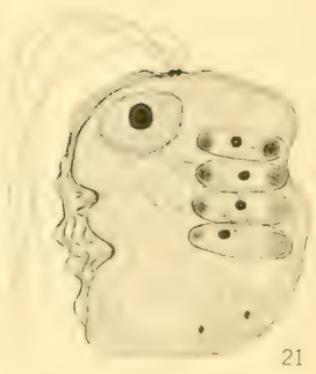
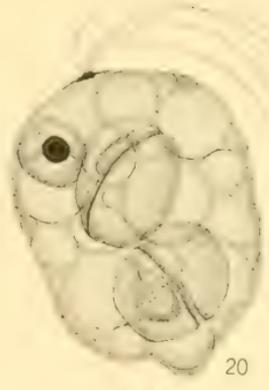


PLATE 10

Prophase.

Fig. 28. Typical vesicular nucleus. Chromidial granules forming around the hyaline area.

Fig. 29. Unequal constriction of primary karyosome.

Fig. 30. Unequal constriction of primary karyosome. Organization of peripheral chromatin into granules connected by a slightly chromatic continuous fiber. Partial separation of basal granules.

Fig. 31. Differential division of primary karyosome into a macrokaryosome and a microkaryosome. Kinetic membrane surrounding the microkaryosome.

Fig. 32. The same as figure 31 with peripheral chromatin enerusted upon the nuclear membrane. Extranuclear chromidial cloud.

Fig. 33. The unequal constriction of the macrokaryosome. Note the granular organization of the microkaryosome.

Fig. 34. First signs of segmentation of microkaryosome.

Fig. 35. Segmenting microkaryosome with fibers connecting the two polar chromidial masses. Intranuclear chromidial cloud.

Fig. 36. The same as figure 35; nucleus elongated; peripheral chromatin granules; separation of basal granules, only four flagella.

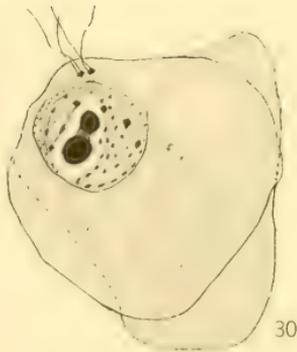


PLATE 11

Prophase.

Fig. 37. Chromidial masses with connecting fibers in the segmenting skein. Chromidial cloud within the kinetic membrane. Macrokaryosome being pushed aside by the expanding kinetic membrane. Peripheral chromatin gathered just within the nuclear membrane.

Fig. 38. Macrokaryosome in a niche of the nuclear membrane. The segmenting skein within a precocious spindle formation.

Fig. 39. Further organization of the skein into the tripod stage.

Fig. 40. Tripod stage of segmenting spireme. Peripheral chromatin in larger granules. Further separation and initial division of basal granules, producing four basal granules and eight flagella, splitting of rhizoplast.

Fig. 40a. Another view of figure 40, showing the four basal granules.

Fig. 41. Segmenting skein in the form of a double crescent with four terminal knobs of chromatin. Two small granules which may be the division center. Chromidial cloud within the kinetic membrane, macrokaryosome passive.

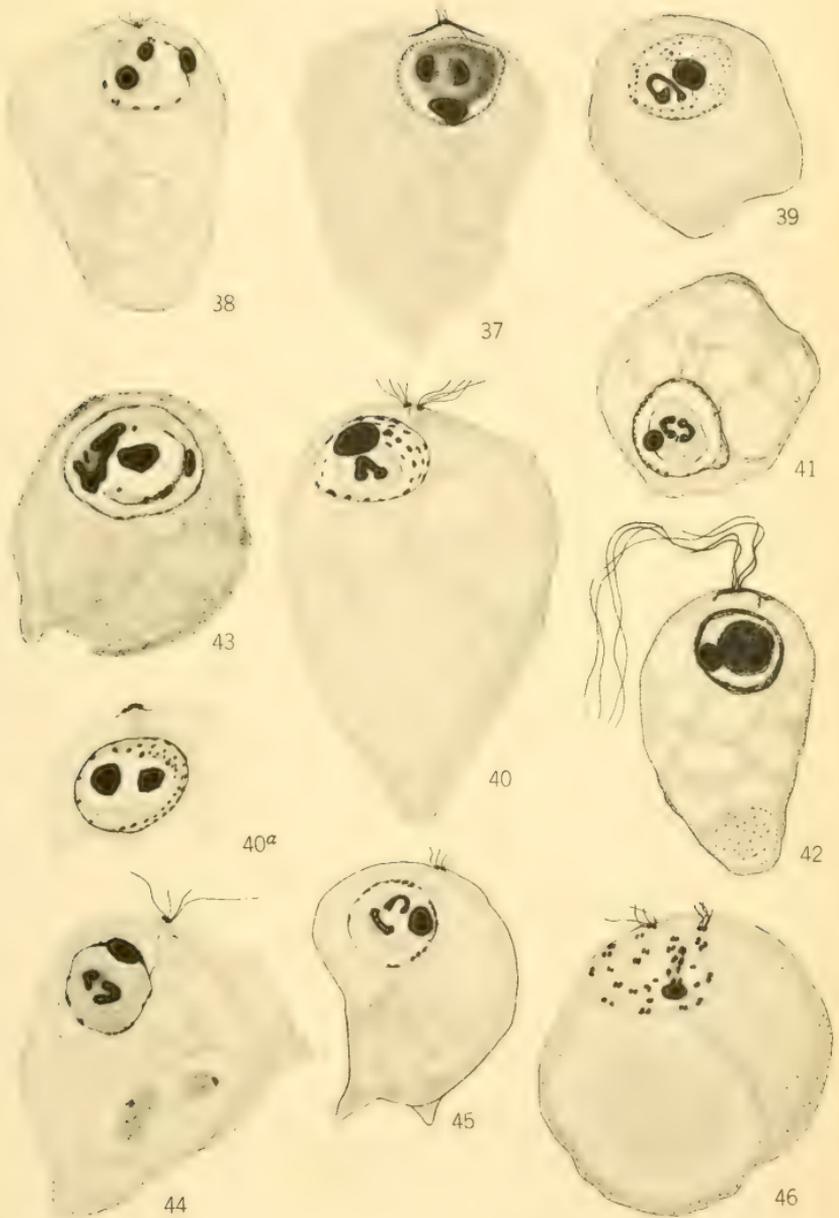
Fig. 42. Same as figure 41.

Fig. 43. Disintegration of macrokaryosome. A moribund stage.

Fig. 44. Expanding kinetic membrane commensurate with the nuclear membrane. Macrokaryosome in a niche of the nuclear membrane. Some chromatin still encrusted upon the membrane. Rhizoplast evident.

Fig. 45. Longitudinal splitting of segmenting skein, producing seven or eight terminal knobs, in all probability the elements of the future chromosomes.

Fig. 46. Blepharoplasts separated. Precocious splitting of peripheral chromatin. A precocious equatorial plate with macrokaryosome apparently upon it.



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PLATE 12

Metaphase, Anaphase and Telophase.

Fig. 47. Precocious splitting of the terminal knobs of the final segmentation skein, forming an equatorial belt of chromidial cloud in which are embedded eight paired chromatin masses. Final prophase.

Fig. 48. Metaphase equatorial plate. Macrokaryosome apparently a part of it. Two blepharoplasts of two basal granules each. Spindle oriented either in relation to the blepharoplasts or the major axis of the cell. Intranuclear chromidial cloud.

Fig. 49. Same as figure 48; chromatin granules lodged upon the spindle fibers. Clearing up of intranuclear chromidial cloud.

Fig. 50. Metaphase spindle. Seven chromosomes can be seen, one being only half so large as the others. Transverse splitting of all chromosomes except the small one. No chromatin within the nucleus except that upon the equatorial plate. No evidence of macrokaryosome, peripheral chromatin, or centrosome granules. From material killed in hot Schaudinn's fluid and stained in safranin, gentian-violet, orange G.

Fig. 51. Anaphase. Unequal amounts of chromatin passing to the daughter poles. The chromosomes are stuck together. Intranuclear chromidial cloud.

Fig. 51. Anaphase. Irregularly lobed chromatin masses collected at respective daughter poles. Separation fibers evident. Intranuclear chromidial cloud.

Fig. 53. Anaphase. Organization of daughter chromatin masses into linear skeins. Daughter rhizoplasts connecting the daughter blepharoplasts.

Fig. 54. Telophase. Daughter nuclei separated. Intra- and extranuclear chromidial cloud. Daughter blepharoplasts and related nuclei shifted to opposite sides of the sulcus.

Fig. 55. Telophase. Same as figure 54. Extranuclear chromidial cloud deepens.

Fig. 56. Chromatin has separated out into four large masses, an extra large mass being in one daughter cell. Daughter rhizoplasts heavy.



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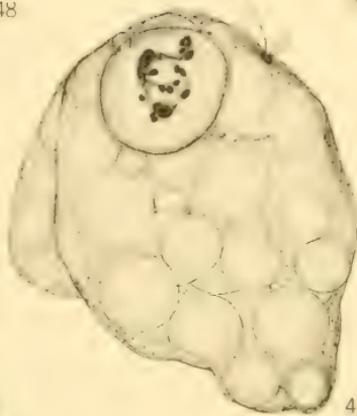
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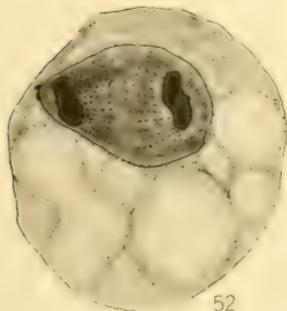
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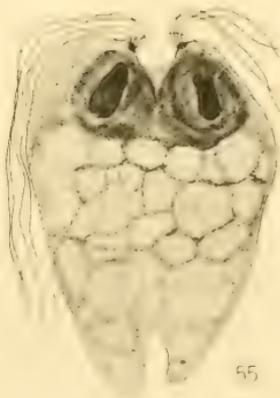
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PLATE 13

Fig. 57. Telophase. Cytoplasmic constriction along the sulcus. Blepharoplasts deeply stained. Rhizoplasts evident. Heavy extranuclear chromidial cloud. Chromatin further broken up into irregular masses.

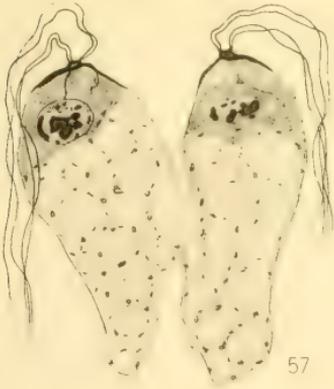
Fig. 58. Large vacuole in the cytoplasmic connective. Further dissociation of chromatin masses into granules which show the beginning of concentric organization.

Fig. 59. Reorganization of central karyosome and peripheral chromatin. From material killed in Flemming strong, and stained in Bordeaux R iron haematoxylin.

Fig. 60. Suspended telophase. Engulfed *Pandorina* in food vacuole. Karyosome and peripheral chromatin. Small chromatin mass outside the karyosome of unknown significance, possibly the division center. From material killed in Flemming strong, and stained in Bordeaux R iron haematoxylin.

Fig. 61. Individual just after fission is completed. Double rhizoplast extending into karyosome. Chromidial organization still evident in the karyosome.

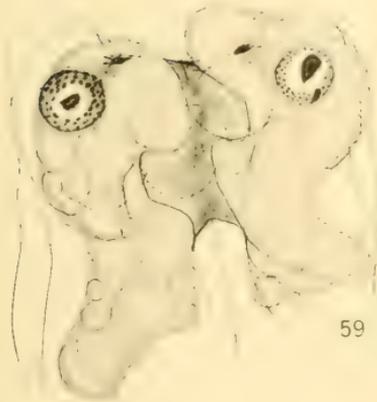
Fig. 62. Somatella, probably of sixteen cells, of *Amoeba radiosa*. At first considered a *Collodictyon* cyst. Such may possibly be the case though reaction to the stain does not warrant such a conclusion. $\times 1900$.



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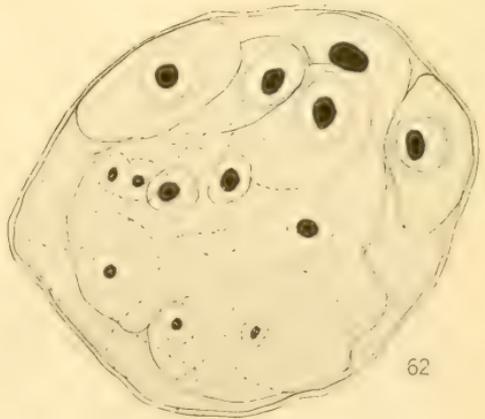
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PLATE 14

Nuclear changes in binary fission in *Collodictyon*. Any differences between these figures and those of associated plates must be referred to the original camera lucida sketches for critical interpretation.

Figs. 65-76. Prophase phenomena.

Figs. 63-65. Stages of the resting nucleus.

Figs. 77-79. Metaphase.

Figs. 80-83. Anaphase.

Figs. 84-87. Telophase.

Figs. 66-67. Unequal constriction of primary karyosome.

Figs. 68-69. Differential division of the primary karyosome into a macrokaryosome and a microkaryosome. Organization of a kinetic membrane around the microkaryosome.

Figs. 69-73. The segmenting skein, with associated expansion of the kinetic membrane.

Fig. 71. Precocious spindle formation. Macrokaryosome in a niche of the nuclear membrane.

Fig. 72. Double crescent stage of segmenting skein showing four terminal knobs and a possible intranuclear division center dividing.

Fig. 73. Longitudinal splitting of crescents, producing seven or eight terminal knobs of chromatin which are the elements of the chromosomes.

Fig. 74. Precocious splitting of peripheral chromatin granules. Precocious equatorial plate formation with macrokaryosome upon it.

Fig. 75. Final prophase. Division of blepharoplasts with apparent splitting of flagella; separating centrosomes upon the nuclear membrane connected by a paradesmose.

Fig. 76. Precocious splitting of final stage of segmenting skein. Sixteen chromatin masses in a chromatin cloud in the form of an equatorial belt.

Fig. 77. Metaphase spindle. Macrokaryosome apparently a part of it. Intranuclear chromatin cloud.

Fig. 78. Metaphase spindle; centrosomes at poles of spindle and connected by paradesmose.

Fig. 79. Metaphase spindle showing seven chromosomes. Transverse parting of all chromosomes except one which is only half so large as the rest.

Fig. 80. Anaphase. Apparent unequal distribution of chromatin.

Fig. 81. Anaphase. Daughter chromatin masses organized into linear spiremes.

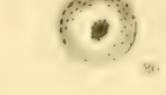
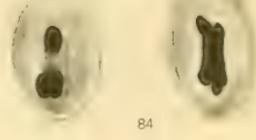
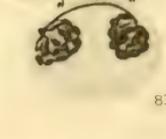
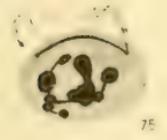
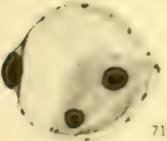
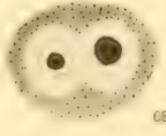
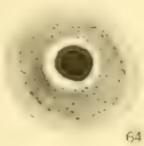
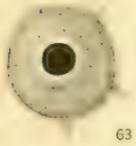
Fig. 82. Anaphase. Linear spireme of chromatin granules closely related to centrosomes, which are connected by paradesmose.

Fig. 83. Telophase. Complete separation of daughter nuclei. Extranuclear and intranuclear chromidial cloud.

Fig. 85. Distribution of chromatin as granules throughout nucleus.

Fig. 86. Reorganization of central karyosome with surrounding hyaline area, and peripheral chromatin.

Fig. 87. Suspended telophase. Vesicular nucleus with small chromatin mass of unknown significance near the periphery. Daughter blepharoplasts with double basal granules surrounded by granular archoplasm.



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OF A STYLET CERCARIA

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THE EXCRETORY SYSTEM OF A STYLET
CERCARIA¹

BY

WILLIAM W. CORT



In the summer of 1915 while working on the development of trematodes at the University of Michigan Biological Station, Douglas Lake, Michigan, I found *Cercaria polyadena* Cort in a small beach pool on the north shore of the lake. This species of cercaria was described from one specimen of *Lymnaea reflexa* Say from near Chicago, Illinois (Cort, 1914, p. 16). At Douglas Lake I found it in *Lymnaea exilis* Lea, *Lymnaea emarginata angulata* (Sowerby), *Lymnaea humilis modicella* (Say), *Lymnaea stagnalis appressa* (Say), and *Lymnaea stagnalis perampla* Walker. With an abundance of material available it was possible to work out the details of the excretory system of this cercaria by using the method described in a previous publication (Cort, 1918).

The excretory system of *Cercaria polyadena* is shown in figure 1. The capillaries and flame cells of the postacetabular region were very difficult to work out on account of the heavy cystogenous glands and the crowding of the tubules in this region. Only two flame cells were found in the posterior groups on each side, and the exact positions of the flame cells of the next to the last groups were doubtful. The excretory pore is located in the mid line within the cup-shaped depression into which the tail fits, and dorsad of that portion of the tail within the depression. No part of the excretory system extends into the tail. The muscular bladder (fig. 1, *b*) is Y-shaped or bicornuate, with the horns about the same length as the main stem. The common collecting tubes (*cct*) on each side which enter the tips of the horns of the bladder are complexly coiled and receive the anterior and posterior collecting tubes (*act*, *pct*) at the level of the acetabulum.

¹ A research study from the University of Michigan Biological Station.

Each of these collecting tubes receives three accessory collecting tubes (*acct*) making a total of twelve accessory collecting tubes. Each accessory collecting tube, except the posterior on each side, is joined by a group of three capillaries. The formula $2 \times 6 \times 3 = 36$ has been used by Looss (1894, p. 68) for this type of excretory system, the "2" representing the sides of the system, the "6" the number of accessory collecting tubes on each side, and the "3" the number of capillaries in each group, making a total of 36 flame cells. The arrangement of the capillaries of each group is significant. Each accessory collecting tube divides into two subdivisions, one of which forms directly a capillary ending in a flame cell while the other subdivides into two capillaries. The paired capillaries of each group go to one surface of the body and the single capillary to the other. The dorso-ventral arrangement of the capillaries of any particular group varied in different individuals and no regularity in this respect could be discovered. The positions of the flame cells of the various groups were modified greatly by the extension and contraction of the body of the cercaria. Whether the finding of only two flame cells in the posterior groups in *Cercaria polyadena* was due to failure to locate the other member of each group or whether only two are present cannot be determined at this time. It is possible that these two posterior groups had lagged behind the others in division. Looss in his figure of this same type of system in *Anchitrema sanguineum* (Sonsino) (Looss, 1896, p. 110, pl. 8, fig. 77) shows not only the posterior but the anterior groups on each side with two flame cells.

The method of division of the capillary groups of *Cercaria polyadena* supports the hypothesis that each capillary group is formed in the development of the system by longitudinal divisions of a single flame cell (Cort, 1918a). According to this hypothesis an excretory system of the type found in *Cercaria polyadena* passed through a stage in development when it was composed only of the bladder, the common collecting tubes and the anterior and posterior collecting tubes, each of which received three capillaries from three flame cells, making a total of twelve flame cells. The capillaries of these flame cells would correspond to the accessory collecting tubes of the fully developed system. In further development each of these flame cells with its capillary first divided into two flame cells and capillaries, one going to the dorsal, and one to the ventral side of the body. One of these two flame cells again divided making the groups of three found in the fully developed system.

That the excretory system of *Cercaria polyadena* has reached its full development in arrangement and number of flame cells is suggested by the fact that the capillary groups have been definitely established, and by the finding in the literature of the descriptions

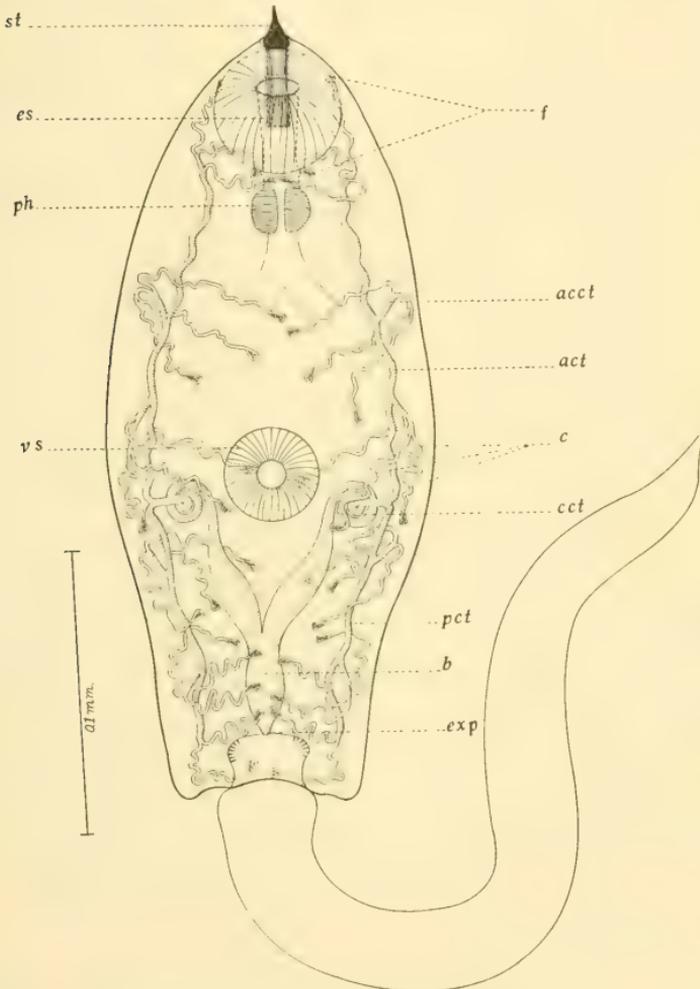


Fig. 1. The excretory system of *Cercaria polyadena*. Ventral view; body spines, stylet and cystogenous glands are not shown; *acct*, accessory collecting tube; *act*, anterior collecting tube; *b*, bladder; *c*, capillary; *cct*, common collecting tube; *exp*, excretory pore; *f*, flame cell; *os*, oral sucker; *pct*, posterior collecting tube; *ph*, pharynx; *st*, stylet; *vs*, ventral sucker.

of six excretory systems in adult distomes which correspond to this general type. Of the six adult distomes showing the " $2 \times 6 \times 3$ " formula for the excretory system, two, *Haplometra cylindracea* (Zeder) (Looss, 1894, p. 68, pl. 8, fig. 163) and *Opisthioglyphe ranae* (Frölich) (Looss, 1894, p. 87, pl. 8, fig. 157), are placed (Odhner, 1910, pp. 22 and 23) in the family Plagiorechiidae Lühe (= Lepodermatiidae Odhner). The other four, *Pleurogenes medians* (Olsson), *Pleurogenes claviger* (Rudolphi), *Prosotocus confusus* (Looss) (Looss, 1894, pp. 95, 103, 107) and *Anchitrema sanguineum* (Sonsino) (Looss, 1896, p. 110, pl. 8, fig. 77) are placed by Odhner (1910, pp. 76 and 177) in the family Lecithodendriidae Odhner. The first three of these which are closely related form the basis of the subfamily Pleurogenetinae Looss. The excretory system of these six adult distomes and that of *Cercaria polyadena* show fundamental homologies. They correspond in the arrangement of the principal collecting tubes, in the number and general position of the accessory collecting tubes, and, finally, except in the case of *Anchitrema sanguineum* and possibly in *Cercaria polyadena*, in the number and grouping of the flame cells in the capillary groups. Differences are to be found in the relative lengths of the main stem and subdivisions of the bladder, which vary from the condition of *Haplometra cylindracea* in which the divisions are very short to the condition of *Pleurogenes medians* in which the main stem has been almost completely reduced and the bladder has assumed a V shape. These differences in the bladder produce also differences in the relative positions of the capillary groups. I consider the correspondence in number of flame cells and capillary groups and in the number and arrangement of the collecting tubes in these six adults and one cercaria to represent a fundamental homology, while the differences in the shape of the bladder seem to me to be secondary modifications. It is possible that the primitive type of bladder in the " $2 \times 6 \times 3$ " type of excretory system is that of *Opisthioglyphe ranae* and *Cercaria polyadena*, in which the divisions of the Y almost equal the main stem and the common collecting tubes enter the horns of the bladder back of the middle of the body. The other types of bladder could easily be derived from this by a shortening or lengthening of the main stem or the horns.

The excretory system of *Cercaria polyadena* agrees most closely with that of *Opisthioglyphe ranae*. The cercariae of this species and *Opisthioglyphe rastellus* (Olsson) are stylet forms which Lühe (1909, p. 192) places in his group of *Cercariae armatae* and which also belong

to the narrower polyadenous group (Cort, 1915, p. 57), of which *Cercaria polyadena* is the type. Of the other adult distomes with the "2 × 6 × 3" type of excretory system only the cercaria of *Haplo-metra cylindracea* is known, which is also a stylet cercaria belonging to the *Cercariac armatac* group. The adult into which *Cercaria polyadena* develops is not known, but the homologies described above place it in the family Plagiorehiidae close to *Opisthioglyphe*.

So little is known of the excretory system in the stylet cercariae that it is not possible in the present state of our knowledge to draw any conclusions in regard to the prevalence and modifications of the "2 × 6 × 3" type of excretory system in the whole group. It seems very probable as suggested by Faust (1918, p. 104) that the stylet cercariae do not represent a closely related group of trematodes. The Y-shaped type of excretory bladder, however, is quite common among stylet cercariae (Looss, 1896; Cort, 1915; Faust, 1918 and 1918a). Faust (1918 and 1918a) has partially worked out this excretory system in a number of stylet cercariae. In most cases, however, it is difficult to determine from his drawings and descriptions the number of flame cells or the pattern of the finer vessels. His description of the system in *Cercaria isocotylea* Cort, also placed in the polyadenous group, is more complete (Faust, 1918, p. 103, fig. 20). In this form he describes a system strikingly different from that of *Cercaria polyadena*. He finds a total of twenty-two flame cells on a side, and shows capillary groups of both two and three.

In every stylet cercaria of which Faust describes the excretory system he shows branches extending into the tail. Magath (1917, pl. 1, fig. 3) shows a branch of the excretory system of the stylet cercaria of *Lissorhis fairporti* Magath in the tail. I have made examinations of the excretory systems of eight different stylet cercariae and have never seen any trace of excretory tubules in the tail. Unfortunately I have never had the opportunity of studying the excretory system of any of the stylet cercariae in which Faust shows this peculiarity. The excretory tubules which Faust figures in the tails of his stylet cercariae vary from a simple tube to a very much branched tube (cf. Faust, 1918a, figs. 18 and 20). He never shows flame cells in the tail nor does he show tubules opening to the outside. In my studies on the excretory system of cercariae I have seen only two parts of the excretory system invading the tail: either extensions of the bladder which form usually two larval excretory

pores, one on each side of the tail as in echinostome cercariae, amphistome cercariae or schistosome cercariae, or flame cells which are connected with accessory collecting tubes extending back into the tail from the body as in the various types of fork-tailed cercariae. In Faust's (1918a, pl. 2, fig. 18) figure of *Cercaria stilifera* he draws twenty-one subdivisions of the excretory-branch in the tail which appear to correspond to capillaries of the body. If this representation is correct there should be twenty-one flame cells in the tail of this form although Faust does not figure any.

Since the study of the tails of stylet cercariae is very difficult in the living condition, which is the only way in which the finer tubules of the excretory system can be seen, I feel that many more careful studies on this point will have to be made before final judgment can be passed on the differences noted above.

The finding of such fundamental homologies in the excretory systems in such a wide variety of forms is very suggestive and must in my opinion indicate some degree of relationship between the groups to which these species belong. I prefer, however, not to attempt to draw any far-reaching conclusions in regard to relationships since the excretory systems of so few species are known. Such a comparison, however, emphasizes the great importance of further studies on the excretory systems both of cercariae and adult trematodes for the determination of the relationship of the larger groups and the establishment of a natural classification.

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INTRODUCTION

The examination of thirty individuals of the California red-legged frog, *Rana aurora* Baird and Girard, obtained from a frog farm near San Francisco, showed a characteristic parasitic fauna differing in a number of respects from that described from frogs in other parts of the United States. One of the most interesting forms found was a new distome from the intestine. Since this species does not fit into any of the described genera of trematodes I shall establish the new genus *Margeana* for it and name it *Margeana californiensis*, nov. gen., nov. spec. A discussion of the classification of this species and the diagnosis of the new genus to which it belongs will follow the description of its structure.

DESCRIPTION OF SPECIES

Margeana californiensis was found in the intestines of fourteen of the thirty specimens of *Rana aurora* examined. The number of parasites in each host was comparatively small. Two hosts harbored one parasite, three two, three three, one four, one five, one six, one seven, one nine, and one ten. The flukes were attached to the wall of the intestine by the oral sucker but were easily loosened when the intestine was opened. They were most often found in the anterior third of the intestine and never in the posterior third.

Practically all the observations recorded in this paper were made from the study of living material. Whole mounts and sections, however, were made for comparison.



EXTERNAL ANATOMY

Margeana californiensis (fig. 1) is a small distome ranging in length when alive from 2.4 to 5 mm. The smallest sexually mature individual found had a length of 2.4 mm., and had large numbers of eggs in the coils of the uterus. Several immature specimens were also found, the smallest of which (fig. 5) had a length of 1.5 mm. Figure 1, which is a camera lucida drawing of a large-sized living specimen of *Margeana californiensis* slightly compressed, gives an idea of the size and shape. The body has considerable power of extension and contraction, so that the dimensions vary in both living and preserved specimens according to the degree of contraction. Measurements of width and thickness were made from a series of sections. The thickness of the body in the region in front of the testes was found in this series to be about two-thirds of its width. Posteriorly the body was somewhat flattened, having a thickness in the region back of the testes of a little less than half of its width. In the series of sections studied, in the region of the oesophagus the width was 0.56 mm. and the thickness 0.36 mm.; at the level of the ventral sucker the width was 0.64 mm. and the thickness 0.38 mm.; and half way between the testes and the posterior end the width was 0.68 mm. and the thickness 0.23 mm.

All the surface of the body except the posterior one-fifth is covered with tiny scale-like spines set very closely together in rows encircling the body. These spines are so small that they cannot be clearly discerned at the magnification of figure 1. Back of the testes the spines begin to thin out, and in the larger specimens the posterior one-fifth of the body is entirely smooth. In the smallest immature specimen studied, however, the body was completely covered with spines. This suggests that the thinning out of the spines in the posterior part of the body of this species is due to the very great proportional growth of the postacetabular region.

The oral sucker has a slightly greater width than length, and in a living specimen 5 mm. in length had a diameter of 0.4 mm. The ventral sucker in this same individual had a diameter of 0.25 mm. The measurements of the suckers of seven toto mounts of specimens killed by Looss' shaking method is shown in the following table.

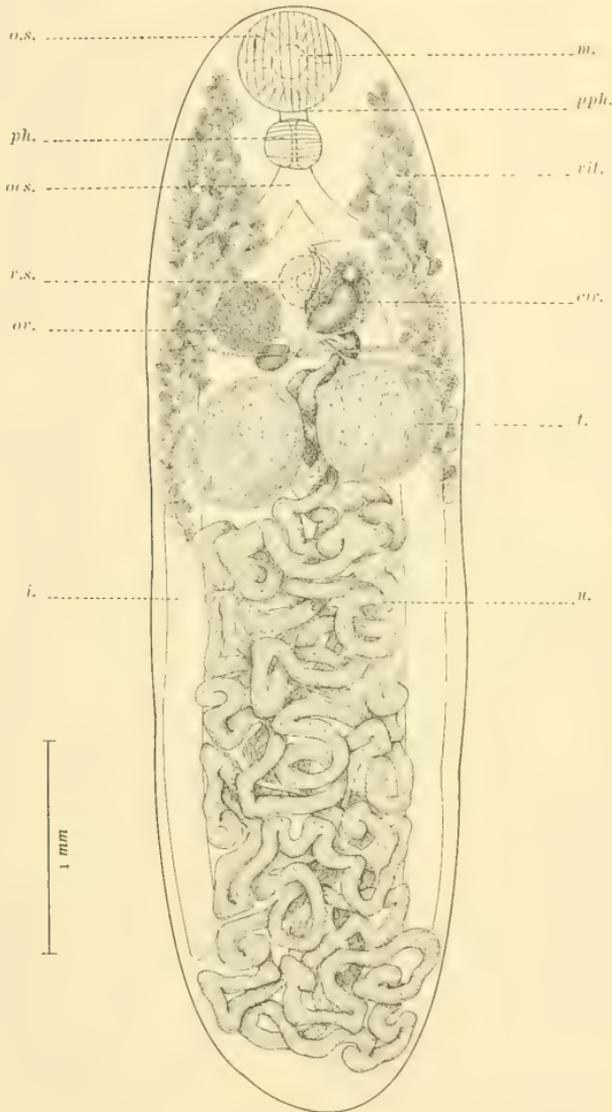


Fig. 1. Camera lucida drawing; dorsal view of a living specimen *Margeana californiensis*, slightly compressed; *cir.*, cirrus sac; *oes.*, oesophagus; *i.*, intestinal caecum; *m.*, mouth; *o.s.*, oral sucker; *ov.*, ovary; *ph.*, pharynx; *pph.*, prepharynx; *t.*, testis; *u.*, uterus; *vit.*, vitellaria; *v.s.*, ventral sucker.

DIMENSIONS OF *MARGEANA CALIFORNIENSIS*, IN MILLIMETERS

	1	2	3	4	5	6	7
Length of individual	2.41	3	2	2.9	1.9	1.6	1.8
Length of oral sucker	0.28	0.31	0.26	0.31	0.24	0.24	0.24
Width of oral sucker	0.32	0.41	0.29	0.36	0.26	0.25	0.25
Diameter of ventral sucker	0.17	0.19	0.17	0.20	0.14	0.16	0.15

From this table it is seen that the proportions of the ventral sucker to the oral sucker vary from 1:2 to 2:3. The measurements of this table are all taken from sexually mature individuals and give some idea of the amount of contraction of the body and suckers in preserved specimens.

The parts of the digestive system are shown in figure 1. The mouth (fig. 1, *m.*) is sub-terminal, and the oral sucker (*o.s.*) is separated from the pharynx (*ph.*) by a short prepharynx (*pph.*). The pharynx (*ph.*) is large, having a diameter of more than half that of the oral sucker. The oesophagus (*oes.*) is short, being very difficult to make out in toto mounts and the intestinal caeca (*i.*) extend almost to the posterior end of the body. The intestinal caeca always extend as far back as the posterior fifth of the body and sometimes into the last tenth of the body length, although in the sexually mature specimens they never extend clear to the posterior end. It is interesting to note that in immature specimens (fig. 5) the intestinal caeca extend practically to the posterior end.

All the reproductive organs of *Margeana californiensis* (fig. 1) except the coils of the uterus which extend to the posterior end are in the anterior half of the body. The genital pore (fig. 2, *g.p.*) is located slightly to the right of the mid-ventral line of the body close in front of the ventral sucker. The cirrus sac (fig. 1, *cir.*, and fig. 2) is large and has strong musculature. It is located to the right side and in front of the ventral sucker. It has a width about equal to the diameter of the ventral sucker and a length of about three times this diameter.

The size and proportions of the cirrus sac are shown in figure 2. The posterior half of the cirrus sac contains a large seminal vesicle (fig. 2, *s.v.*) full of sperms, which is constricted near its anterior end. This is followed by a short prostate region (*pr.*) into which empty large numbers of small unicellular prostate glands (*p.g.*), which fill the space between the seminal vesicle and the walls of the cirrus sac, but which do not extend further forward in the cirrus sac than the anterior limit of the prostate region of the male duct. These glands are densely

granular and in the living specimens are dark grey in color in contrast with the other structures of the cirrus sac. The cirrus is about as long as the seminal vesicle, is thick-walled and can be protruded. At its posterior extremity the cirrus sac receives the ducts from the testes (*v.e.*), which unite into a very short vas deferens (*v.d.*) just before

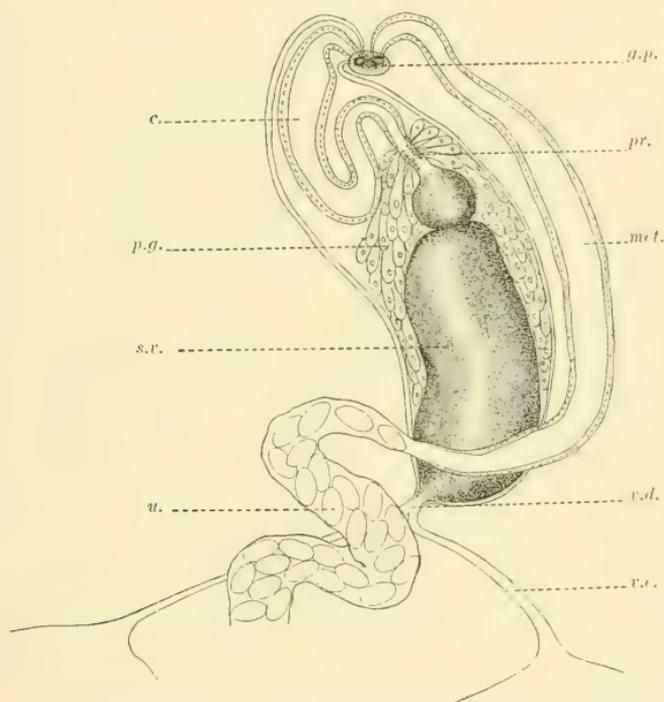


Fig. 2. End ducts of reproductive systems of *Margeana californiensis*; *c.*, cirrus; *g.p.*, genital pore; *met.*, metraterm; *p.g.*, prostate glands; *pr.*, prostate region; *s.v.*, seminal vesicle; *v.d.*, vas deferens; *v.e.*, vas efferens; *u.*, uterus.

enlarging into the seminal vesicle (*s.v.*). The testes (fig. 1, *t.*) are large and, in the living animal, spherical except for the slight projections anteriorly at the points where the vasa efferentia (fig. 2, *v.e.*) are given off. The testes are located at the same level in front of the middle of the body and just behind the ventral sucker. Their size varies with the size of the worm. In the largest individuals (fig. 1, *t.*) they fill almost four-fifths of the width of the body and have a

thickness but little less than the thickness of the body. In immature specimens (fig. 5, *t.*) they are much smaller and behind the middle of the body.

The ovary (figs. 1 and 3, *ov.*) is spherical and has about two-thirds the diameter of one of the testes. It is located to the left of the ventral sucker and may overlap this organ dorsally. The relations of the female ducts are shown in figure 3. The oviduct (fig. 3, *ovd.*) leaves the inner margin of the ovary near its posterior end. It runs but a short distance before receiving the duct from the small pear-shaped seminal receptacle (*s.r.*). Laurer's canal (*L.c.*) opens into the oviduct just beyond the duct of the seminal receptacle and passes dorsad and posteriad between the testes to open on the dorsal surface. The oviduct further receives the common vitelline duct from the small vitelline reservoir just before it enters Mehlis' gland (*M.g.*) to change into the ootype (*oot.*). The beginning of the uterus (*u.*) curves forward for a short distance and then turns back, passing between the testes dorsad. The coils of the uterus fill the whole posterior body region and it was impossible to follow their exact course. The terminal portion of the uterus (fig. 2, *u.*) immerses from the posterior coils near the ventral surface of the body and passes forward between the testes, changing into the metraterm (fig. 2, *mct.*) just ventrad of the posterior end of the cirrus sac. The metraterm is thick-walled and runs parallel to the cirrus sac to open at the common genital pore (fig. 2).

The vitellaria (fig. 1, *vit.*) are made up of small irregular follicles which are not arranged in definite groups. The vitellaria are located along each side of the body both dorsally and ventrally and extend from the region of the oral sucker back to the posterior margin of the testes. Dorsally or ventrally they may extend nearly to the mid line of the body. The vitelline ducts (figs. 1 and 3, *v.d.*) run transversely near the dorsal surface and join the small vitelline reservoir.

The eggs of *Margeana californiensis* are brownish in color and have shells of medium thickness. The operculum is well defined and each egg contains a fully developed miracidium. Measurements of twenty-five normal eggs from two different specimens showed a range of variation from $46\mu \times 19\mu$ to $56\mu \times 26\mu$. The size most frequently found which represents about the average was $51\mu \times 24\mu$.

The excretory system of *Margeana californiensis* (fig. 5) was worked out completely from living material. The excretory pore (fig. 5, *ex.p.*) is located on the ventral surface near the posterior end.

From it the muscular club-shaped bladder (*b.*) extends almost up to the testes. In the immature specimens the anterior end of the bladder extends to about the same position but the bladder is very much shorter (fig. 4, *b.*). The anterior end of the bladder is somewhat widened to receive the common collecting tubes (fig. 5, *c.c.t.*) on each side, but there is no trace of bifurcation. The filling and contraction of the bladder could be followed in the immature specimens, in which

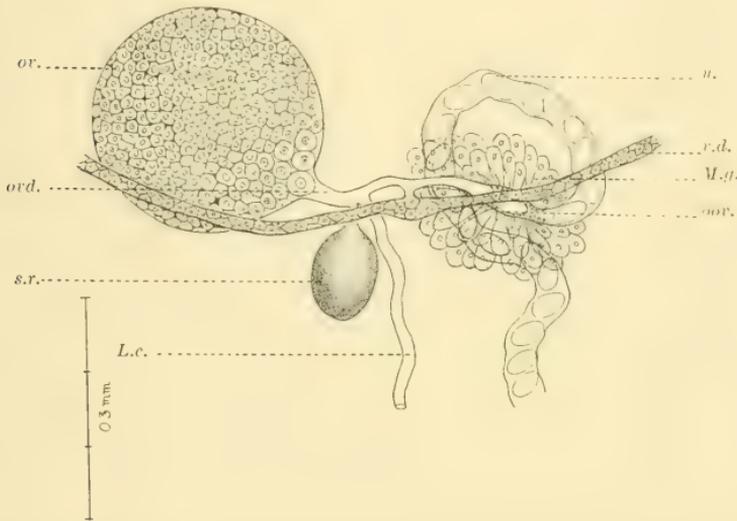


Fig. 3. Connections of the ducts of the female reproductive system of *Margreana californiensis*; *L.c.*, Laurer's canal; *m.*, Mehlis' gland; *o.o.t.*, ootype; *ov.*, ovary; *ovd.*, oviduct; *s.r.*, seminal receptacle; *u.*, uterus; *v.d.*, vitelline duct.

it was not obscured by the folds of the uterus. Starting with the bladder entirely contracted and empty for its whole length, there can first be noticed a dilation of the common collecting tubes near the points where they enter the anterior end of the bladder. The anterior end of the bladder then fills with liquid and finally becomes much distended. The muscular contraction of the anterior end then drives the liquid down into the posterior bulb, which becomes distended. At this time the whole bladder contains liquid. Then the anterior half of the bladder becomes entirely contracted, further distending the posterior bulb. With the contraction of the posterior bulb and the expulsion of the liquid contents the whole bladder becomes empty.

The common collecting tubes (*c.c.t.*) extend forward diagonally from the bladder, pass ventrad to the testes and receive the anterior and posterior collecting tubes (*a.c.t.*, *p.c.t.*) at the level of the ventral sucker. Each anterior and posterior collecting tube receives three accessory collecting tubes (*ac.c.t.*), each of which in turn receives a group of three capillaries (*c.*). The positions of the accessory collecting tubes and their capillary groups is shown in figure 5. In each capillary group the accessory collecting tube divides into two divisions, one of which forms directly a capillary which ends in a flame cell and the other further subdivides into two capillaries and their flame cells. In every group the paired capillaries go to one surface of the body and the single capillary to the other. The dorso-ventral arrangement of the capillaries of any particular group varied in different individuals and no regularity in this respect could be discovered. The significance of the two-one arrangement in the capillary groups in relation to the development of this type of system was discussed in my description of the excretory system of *Cercaria polyadena* (Cort, 1919). The formula for the excretory system of *Margeana californiensis* is the same as that for *Cercaria polyadena*, $2 \times 6 \times 3 = 36$ (Cort, 1919).

HOMOLOGIES OF THE EXCRETORY SYSTEM

Of the six adult distomes described by Looss with the "2-6-3" formula (Cort, 1919) for the excretory system, two, *Haplometra cylindracea* (Zeder) and *Opisthioglyphe ranae* (Frölich), are now placed (Odhner, 1910, 22 and 23) in the family Plagiorechiidae Lühe syn. Lepodermatiidae Odhner. The other four species, *Pleurogenes medians* (Olsson), *Pleurogenes claviger* (Rudolphi), *Prosotocus confusus* Looss, and *Anchitrcma sanguineum* (Sonsino), are placed by Odhner (1910, 76-77) in the family Lecithodendriidae Odhner. The first three of these species which are closely related form the basis of the subfamily Pleurogenetinae Looss. A comparison of the excretory systems of these six distomes with that of *Margeana californiensis* will show how fundamental are the homologies. The excretory system of *Haplometra cylindracea* is fully described and figured by Looss (1894, p. 68, pl. 8, fig. 163). The excretory system of this species corresponds more closely to that of *Margeana californiensis* than those of any of the other five species mentioned above. The most important

difference between the two systems is in the bladder, which in *Haplometra cylindracea* is shorter than in *Margeana californiensis* and is divided at its anterior end into two lobes. In *Haplometra cylindracea*

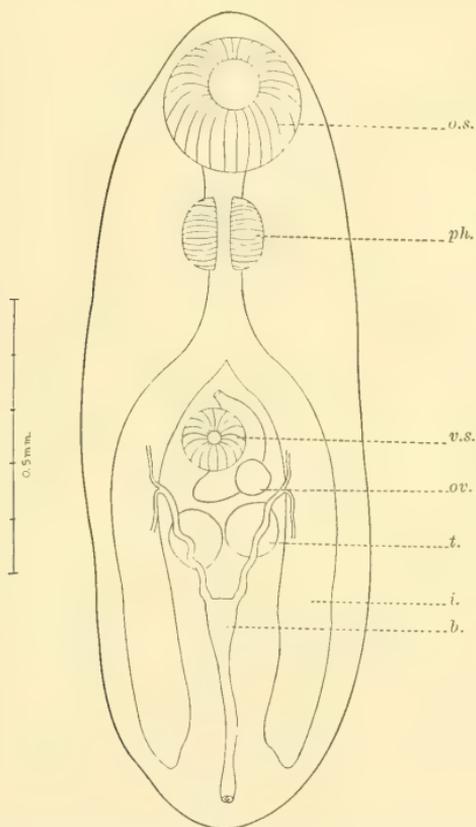


Fig. 4. Camera lucida drawing of immature specimen of *Margeana californiensis*, ventral view; *b.*, excretory bladder; *i.*, intestinal caecum; *o.s.*, oral sucker; *ov.*, ovary; *ph.*, pharynx; *t.*, testis; *v.s.*, ventral sucker.

the points on each side where the anterior and posterior collecting tubes meet the common collecting tubes from the division of the bladder are at about the mid line of the body, while in *Margeana californiensis* these points are at the posterior limit of the anterior third of the body. This difference produces variations in the positions

of the capillary groups and in the length of the accessory collecting tubes of the posterior collecting tubes. These differences are related to the differences in length of the post-acetabular region in the two forms. In other characters the excretory systems of these two species are exactly alike. They correspond in the arrangement of the common and anterior and posterior collecting tubes, in the number and arrangement of the accessory collecting tubes and the capillary groups, and finally in the number of flame cells in each group and in the grouping of the capillaries of each group into a 2-1 arrangement as explained in the description of the excretory system of *Margeana californiensis*. The agreement of the excretory system of *Opisthoglyphe ranae* (= *Distomum endolobum* Dujardin) (Looss, 1894, 87, pl. VIII, fig. 157) with the two just described is almost as striking. Here the "2-6-3" grouping is carried out exactly as before. The only difference is to be found in the more characteristic Y-shape of the bladder, the divided lobes being almost as long as the main stem. The point where the anterior and posterior collecting tubes meet the common collecting tube in this species is still further back, being at the posterior limit of the second third of the body. This of course makes a difference in the position of the accessory collecting tubes and the capillary groups.

Anchitrema sanguineum (Looss, 1896, p. 110, pl. 8, fig. 77) the third species with the "2-6-3" type of excretory system has a bladder in which the divisions are equal to the main stem and the points of bifurcation of the collecting tubes are at the level of the ventral sucker just in front of the middle of the body. There are also slight differences in the position and length of the accessory collecting tubes. The three grouping of the capillaries is carried out in this species except in the anterior and posterior groups on each side which have only two capillaries. This makes four capillary groups of two each and eight of the regular three. This difference is easy to understand when we consider that in each three group of capillaries the end of the accessory collecting tube is divided into two branches, one of which is directly connected with a flame cell and the other of which divides into two capillaries with flame cells. It is evident that the fundamental pattern of the excretory system of *Anchitrema sanguineum* is the same as that of the other forms just described, but that the anterior and posterior capillary groups in this form have lagged behind in division (Cort, 1919).

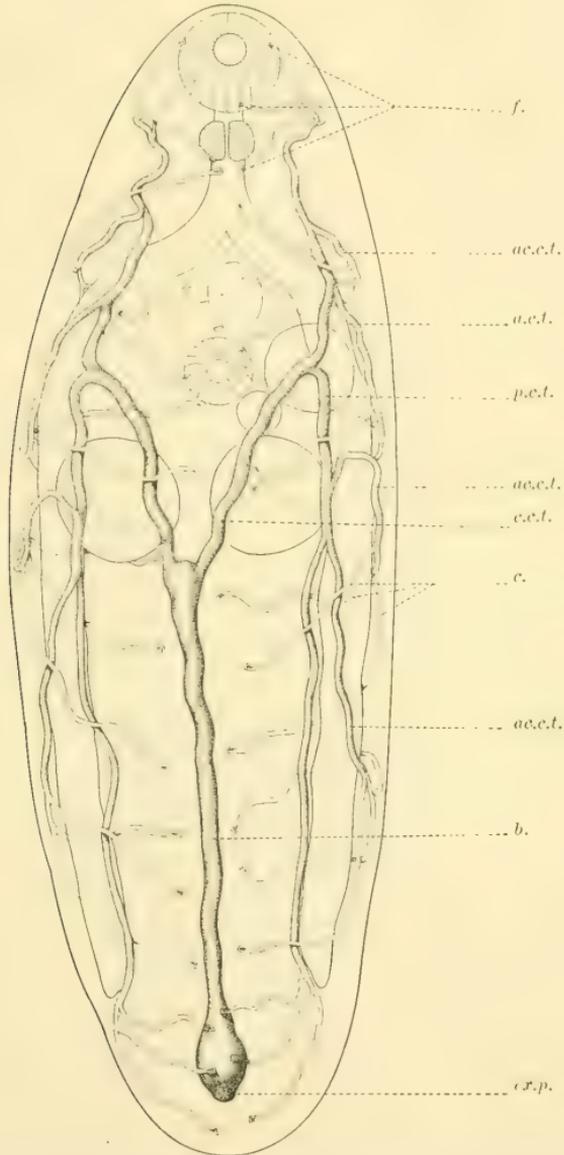


Fig. 5. Free hand drawing of the excretory system of *Margeana californiensis*; *ac.c.t.*, accessory collecting tube; *a.c.t.*, anterior collecting tube; *b.*, bladder; *c.*, capillaries; *c.c.t.*, common collecting tube; *ex.p.*, excretory pore; *f.*, flame cells; *p.c.t.*, posterior collecting tube.

In *Pleurogenes medians*, *Pleurogenes claviger*, and in *Prosotocus confusus*, Looss describes the same "2-6-3" arrangement in the flame cells capillaries and accessory collecting tubes (Looss, 1894, pp. 95, 103, 107). Since he does not figure the excretory systems in these three forms it is more difficult to make detailed comparisons. The chief variation from the species already described seems to be in the shortening of the main stem of the bladder, which in *Pleurogenes median* and *Prosotocus confusus* is so much reduced that the bladder becomes V-shaped instead of Y-shaped (Looss, 1894, pl. 2, figs. 33, 36).

I consider the correspondence in number of flame cells and capillary groups and in the number and arrangement of the collecting tubes of the seven species having the "2-6-3" type of excretory system to represent a fundamental homology. The differences in the shape of the bladder I consider to be secondary modifications. It seems probable that the primitive condition of the excretory bladder in the "2-6-3" type of excretory system is that found in *Opisthio-glyphe ranae* or in *Cercaria polyadena* (Cort, 1919), where the divisions of the Y are about equal to the main stem. From this type of bladder by lengthening the main stem and reducing the subdivisions would be derived that of *Haplometra cylindracea* and finally by the complete elimination of the divisions would be produced the club-shaped bladder of *Margeana californiensis*. An intermediate condition in bladder structure between *Haplometra cylindracea* and *Margeana californiensis* is described by Looss (1902, p. 851) for *Brachycoelium salamandrae* (Frölich) (= *Distomum crassicolle* Rudolphi), in which the divided lobes are reduced to little pockets at the anterior tip of the bladder. *Brachycoelium salamandrae*, as I will show later, is very closely related to *Margeana californiensis*, and although the details of its excretory system have never been worked out, I would expect on account of this close relationship to find that its excretory system is of the "2-6-3" type. On the other side the progressive reduction of the main stem of the bladder would finally produce the V-shaped condition found in *Pleurogenes medians*. The above considerations indicate that it is dangerous to lay as much stress as is at present done on differences in the shape of the excretory bladder, in the classification of trematode families and subfamilies when the whole pattern of the excretory systems is known in so few forms.

I consider the fundamental homology of the pattern of the excretory systems of those forms having the "2-6-3" type to indicate some degree of relationship. The excretory system is so conservative that

the "2-3-6" type and its derivatives may represent a character surmounting family limits, as is suggested by the present classification of the forms exhibiting this peculiarity. On the other hand an increase in the number of species for which the excretory system is known may make necessary fundamental rearrangements in the classification.

DEVELOPMENT

The character of the cercaria from which *Margeana californiensis* develops is not known from direct observations. However, some idea can be given of the type of cercaria of this species from what is known of the cercariae of related forms, from comparisons with the types of excretory systems found in cercariae, and from the structure of immature individuals. The structure of *Margeana californiensis*, especially as shown in immature specimens (fig. 5) agrees in the relative size and position of the suckers, the character of the digestive system and the position of the genital pore with the stylet cercariae. The finding of the "2-6-3" type of excretory system in *Cercaria polyadena* (Cort, 1919), a stylet cercaria of the polyadenous group, strengthens this comparison. Also the cercariae of two of the species with the "2-6-3" type of excretory, *Haplometra cylindracea* and *Opisthioglyphe ranae* are known to be of the stylet type. From these considerations I conclude that *Margeana californiensis* develops from a stylet cercaria.

A comparison of figure 4 and figure 1 gives a number of interesting points in regard to post-cercarial development in this species. In the most immature individual found (fig. 4) no eggs were as yet developed and the outlines of the vitellaria could not be discerned. The testes (*t.*) and ovary (*ov.*) are relatively much smaller and further back than in mature specimens. The excretory bladder (*b.*) is also much shorter than in mature individuals. In the change from the stage shown in figure 4 to that of figure 1 there is relatively a much greater growth of the post-acetabular region, undoubtedly correlated with the development of the coils of the uterus to hold the enormous numbers of eggs produced. The ratio of the pharynx to the oral sucker and of the ventral sucker to the oral sucker in the immature individual (fig. 4) is very different from the usual condition of the adult. Also the intestinal caeca of the immature specimen reach to the posterior end of the body, and the whole surface of the body is covered with spines.

CLASSIFICATION

I consider that the genus *Margeana* should be placed in the sub-family Brachycoeliinae Looss and give for it the following diagnosis:

Margeana nov. gen.

Characters of Brachycoeliinae; digestive system with prepharynx, short esophagus, and intestinal caeca extending into the posterior fifth of the body but not reaching the posterior end; excretory system of the "2-6-3" type with a club-shaped bladder; vitellaria extending from in front of the pharynx to the posterior limits of testes; cirrus sac large; testes large, filling most of the width of the body; only one species recorded, from intestine of red-legged frog, *Rana aurora* (Baird and Girard), from California.

Margeana californiensis shows close affinities to the genus *Brachycoelium*. Not only have I been able to make comparisons with the descriptions of the European species of this genus, *Brachycoelium salamandrae* (Frölich) (= *Distomum crassicolle* Rudolphi) (Lühe, 1909, p. 119) and (Odhner, 1911, pp. 91-94), but I have had opportunity for a first hand study both of living and preserved material of specimens of the American species *Brachycoelium hospitale* Stafford from the intestine of *Diemictylus viridescens* from Douglas Lake, Michigan. *Margeana californiensis* and *Brachycoelium hospitale* are alike in the size and shape of the body and in the position and relative size of the ventral sucker. They agree in the position of the genital organs which in both species lie in the anterior half of the body, the posterior half being filled with the coils of the uterus, which both in ascending and descending pass between the testes. The position and relations of ducts of the ovary and testes also agree closely. The cirrus sac in both species is at the side and partly in front of the ventral sucker and contains a good sized seminal vesicle, a short prostate region, and a protrusible cirrus. Both forms have a small seminal receptacle and Laurer's canal, and the vitellaria have practically the same distribution anterior to the testes. In fact the resemblances between *Brachycoelium hospitale* and *Margeana californiensis* are so fundamental that I would have no hesitation in including my new species in the genus *Brachycoelium* were it not for the striking differences in the character of the digestive system. The species of the genus *Brachycoelium* have a moderately long esophagus and intestinal caeca, not reaching beyond the ventral sucker, while *Mar-*

geana californiensis has a short esophagus and intestinal caeca extending at least four-fifths of the distance from the anterior to the posterior end, but not reaching into the posterior extremity of the body. The pattern of the excretory system has never been worked out for any species of the genus *Brachycoelium*. Looss (1902, p. 815) describes the excretory bladder of *Brachycoelium salamandrae* as club-shaped with the bifurcation reduced to mere pockets at the anterior end, which differs from the condition of the bladder in *Margeana californiensis*, in which the reduction of the bifurcation has been complete. The testes and cirrus sac are also larger in *Margeana californiensis* than in the species of *Brachycoelium*. It was for the reasons given above that I established the new genus *Margeana* for my new distome and included it in the subfamily Brachycoeliinae as amended by Odhner (1910, pp. 89, 90). This subfamily as amended by Odhner contained only the genus *Brachycoelium* and therefore its diagnosis coincided with the diagnosis of this genus. The inclusion of *Margeana* in the subfamily Brachycoeliinae requires its revision only in the characters of the digestive system and the excretory bladder. Johnston (1912, p. 336) places the genus *Mesocoelium* Odhner in the subfamily Brachycoeliinae. While the resemblances between these two groups are so great that they indicate close relationship, the position of the ovary behind the testes, and the difference in the position of the genital pore in *Mesocoelium* lead me to doubt the propriety of placing this genus in the subfamily Brachycoeliinae.

There has been considerable uncertainty in regard to the systematic position of the subfamily Brachycoeliinae. Odhner (1910, pp. 89, 90) after his revision of this subfamily, placed it in his new family Dicrocoeliidae, considering it to be closely related to the subfamily Dicrocoeliinae Looss. Differences in habitat and in structure of the members of Dicrocoeliinae and of Brachycoeliinae suggest that this grouping is unnatural. Ward (1918, p. 400) separates the subfamily Brachycoeliinae from the family Dicrocoeliidae. In my opinion the subfamily Brachycoeliinae should be placed in the family Plagiorchiidae Lühe, syn. Lepodermatidae Odhner. The departure of the excretory bladder of the subfamily Brachycoeliinae from the typical Y-shaped condition of the family Plagiorchiidae was shown above to be a secondary modification. In all other features this subfamily falls within the diagnosis of the family Plagiorchiidae as given by Odhner (1910, pp. 22, 23) and by Ward (1918, p. 402).

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December 6, 1919

THE OCCURRENCE OF A ROCK-BORING
ISOPOD ALONG THE SHORE OF
SAN FRANCISCO BAY,
CALIFORNIA

BY

ALBERT L. BARROWS



UNIVERSITY OF CALIFORNIA PRESS
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INTRODUCTION

Near the railway station of Rodeo, on the shore of San Pablo Bay, in the northern portion of San Francisco Bay, California, there are ledges of Pinole tuff and San Pablo sandstone into which marine isopods have bored in great numbers. These isopods have been identified as *Sphaeroma pentodon* Richardson by Dr. Harriett Richardson Searle of the Smithsonian Institution, to whom I am indebted for the courtesy of examining them. The other elements of the associated fauna of this locality are mainly small barnacles, bryozoans, small crabs and pelecypods of the species *Mya arenaria* Linnaeus and *Mytilus edulis* Linnaeus. Some of the latter species are found in the holes of the isopods.

The water of this part of San Francisco Bay is brackish, but the salinity varies greatly during the year, its degree being determined by the amount of water discharged into the bay by the Sacramento

and San Joaquin rivers. During the months of flood water, April and May, the water of the upper part of San Pablo Bay may be fresh, or nearly so, for considerable periods, especially after seasons of heavy precipitation. During the months when the amount of water discharged is least, September and October, the salinity may rise as high as 25 parts per 1000. The surface temperature of the water of San Pablo Bay ranges during the year from about 6° to 19° C.

OCCURRENCE AND HABITS OF THE SPHAEROMIDAE

Sphaeromidae form a prominent constituent of the isopod part of marine faunas. Fifteen or twenty genera are recognized and two or three scores of species have been described. Of these species at least five in the genus *Sphaeroma* are reported as boring into wood or stone and several of the genus *Limnoria* of the closely related family Limnoriidae are reported as habitual borers into wood. Richardson (1904, p. 28) regards the Sphaeromidae as extensively distributed within the temperate zones and composed "nearly all of cold water species, though not reaching into the Aretalian Realm." The members of this family are largely shallow water forms, but some marine species have been dredged from a depth of 75 fathoms (Gerstaecker and Ortmann, 1901, pp. 179, 244, and 268). "The list of fresh water Sphaeromidae is large for a marine family" (Richardson, 1904, p. 24), nine species being listed from brackish and subterranean waters and even from artesian wells and warm springs, as well as from rivers.

The free-living forms of the Sphaeromidae "frequent the rocky shores of the ocean . . . and are found under stones and along the muddy banks of estuaries. . . . Others adhere to marine plants floating on the surface of the sea. . . . They are able to run with considerable agility" (Bate and Westwood, vol. 2, p. 400). Some species are said to swim only on their backs, and the specimens of *Sphaeroma pentodon* from San Pablo Bay which were kept in the laboratory frequently darted about the aquarium in this way when taken out of their holes. They also moved about on the smooth glass floor of the aquarium by walking and by a combination method of walking with the legs and of propulsion with the swimmerets.

In feeding habits most of the isopods are carnivorous, living as scavengers, and a few are parasitic, but *Sphaeroma* is said to be a

vegetable feeder (Bate and Westwood, vol. 2, p. 404). The related wood-boring genus, *Limnoria*, is also a plant feeder (Zirwas, 1910, p. 43). It is yet to be demonstrated, however, that *Limnoria* makes direct use of wood fibers as food. Undoubtedly the boring species of *Sphaeroma* must live upon the microscopic organisms washed into their holes by the currents of water set up by the swimmerets.

Among the isopods the Sphaeromidae are especially noted for hiding away in crevices, under rocks, and in the abandoned holes of other animals. Certain species are found regularly in the empty shells of barnacles. The known occurrence of Sphaeromidae such as *Sphaeroma quadridentatum* Say "under raised bark and in deserted holes of *Teredo*, etc., of such dead trees as are periodically immersed" (Henger, 1878, p. 370) in several localities along the Atlantic coast is suggestive of the origin of the boring habit in the few species of this genus which have adopted this manner of life. In seeking refuge in all sorts of holes and crevices a few species of this genus, quite accidentally at first, may have brought their stout mandibles to play in enlarging or reshaping their borrowed domiciles. From this beginning the capacity for completely excavating their own holes in any soft material, either wood or stone, may have readily developed.

One cause initiating the instinct of hiding under stones and in crevices, which is common to this group, and which has perhaps developed later into the boring habit, may have been the effort on the part of these isopods to escape from their predaceous enemies, especially fish. It seems hardly probable that the isopods found on the shore of San Pablo Bay resorted to the boring habit to escape the force of the waves. On the open coast this motive seems to drive many animals to seek shelter in natural crevices and hollows or in holes of their own making. But large waves seldom occur on so small a body of water as San Pablo Bay.

The development of the boring habit in the genus *Sphaeroma* is the more remarkable in view of the very slight morphological modifications, if any at all, for such a habit. In fact, the specific differences within the group are apparently of minor consideration in the ecology of these animals, consisting of such characters as the number of tubercles on the abdomen, the number of setae on the exterior margin of the uropods, slight modifications of the appendages, etc. The stout mandibles with which the whole group of isopods has been supplied, and which seem to have been originally used in attacking gross food material, have in the plankton feeding isopod borers merely been

turned against the medium selected for a domicile, and as excavating implements have proven very efficient.

The conclusion upon comparing the large number of free-living species of the genus *Sphaeroma* with the five or six species for which the boring habit is reported is that this habit is probably of rather recent acquisition in this group, though in *Limnoria*, with its very extensive distribution for a comparatively small number of species, the wood boring habit may have had a longer history; it seems to have become so thoroughly established here that *Limnoria* is not known to live in a free condition. References to the occurrence of fossil rock-boring isopods are not known to the writer, though some search has been made for them.

OTHER BORING SPECIES OF SPHAEROMIDAE

The earliest report of the occurrence of *Sphaeroma* boring into wood or stone seems to be that of Fritz Müller from Brazil. In this case *Sphaeroma terebrans* Bate was "procured in timber which had been immersed in the sea" (Bate, 1866, p. 28).

In 1866 C. Spence Bate described *Sphaeroma vastator* from Madras, taken "from a piece of wood which had formed part of a railway bridge over one of the back waters on the west coast of the Indian peninsula. The wood was honeycombed with cylindrical holes, from about one-tenth to two-tenths of an inch in diameter, placed close together. In many of these holes the animal was rolled up like a ball" (Bate, 1866, p. 30).

The occurrence of *Sphaeroma destructor* Richardson (Richardson, 1897, p. 105) in wooden piers in the fresh-water portion of the St. John River near Palatka, Florida, 75 miles from the sea, where the water is fresh enough for the growth of the water hyacinth (Snow, 1908, p. 11), is said to be the first reported instance of isopods damaging submerged woodwork in America. Dr. Richardson in describing this says:

Sections of the wood show that the diameter had been reduced during a period of eight years from 16 inches to 7½ inches. The whole surface of the wood was bored with holes averaging in size about 5 mm. in diameter, and in end sections the holes were arranged in concentric rings between the rings of annual growth, showing the little animals' preference for soft pine. Very strong mandibles projecting beyond the labrum most conspicuously provide a perfect equipment for destructive work.

Charles Hedley (1901, p. 239), in a report read before the Australian Association for the Advancement of Science, described a species of *Sphaeroma* boring in a log from the fresh-water portion of the Rewa River, Fiji. He also reports *Sphaeroma quoyama* Milne-Edwards boring in wood in Sydney harbor, Australia, giving illustrations both of the borer and of its work. *Sphaeroma verrucauda* White is reported from Bay of Islands, New Zealand, "in rotten wood in cavities bored by the Teredo." The work of the *Sphaeroma* is said to be very destructive to marine timber consisting both of hard and soft wood in Sydney, but its operations are said to be limited to the region between low and high tide levels, though Dr. Teesdale (1914, p. 357) states in his discussion of all marine wood borers, including *Sphaeroma*, that their operations are carried on "from high tide to many feet below the surface."

Moll (1914) suggests that since isopod wood borers have frequently been brought to the surface from relatively great depths they cannot be regarded as limited to the shore or to the surface in their operations. Other reports suggest to the writer, however, that of the borers it is *Limnoria* which probably ranges more widely in depth than does *Sphaeroma*, but that, as Moll notes, the operations of all these borers may range from the bottom to the surface in any available wood, though their destructive work is the more rapid at the surface because of the added action of wave erosion, as well as because of the greater number of individuals congregating at the surface.

Finally, Mr. Hedley (1901, p. 240) adds that "this species [*Sphaeroma verrucauda*] has been observed by Mr. Whittlegge to bore holes in sandstone rock at Mosman's Bay," Sidney Harbor, Australia, and that "*Sphaeroma verrucauda* has been accredited with a similar habit." *Janira*, another isopod, is mentioned as always associated with *Sphaeroma verrucauda* when the latter occurs as a wood borer. Stebbing (1904, p. 21) in commenting upon the occurrence of *Sphaeroma verrucauda* White expressed the opinion, however, that it is "not likely to have produced the hollows in sandstone in which it is found." If *Sphaeroma verrucauda* actually bores at times into wood and at times into sandstone, this species would seem to represent a transition stage in the development of the boring habit, parallel to what may have been a similar transition stage in the independent development of the same habit in an American species, *Sphaeroma destructor*.

Stebbing (1904, p. 16) also describes borings of an isopod which he attributed to the species *Sphaeroma terebrans* Bate from cocconut

piles in Ceylon. Dr. Arthur Willey in transmitting these specimens to Mr. Stebbing says:

While collecting in a salt-water lake having both fluvial and marine connections I came across some piles of coconut driven into the water by fishermen, which harbored great numbers of small organisms, tubicolous amphipods, and boring isopods, and in amongst the tubes several errant species, isopods, amphipods, and what I took to be tanaids. . . .

The tubicolous amphipods were mostly on the inner surface of the bark stripped off the piles. The boring isopods were mostly above the water line. I cut off the top of the block and had it photographed and send copy [published by Bate, 1904, opp. p. 16]. . . . It looks like a bee or wasp nest, each cell containing an isopod when fresh and several with young.

Dr. Teesdale (1914, p. 356) speaks of *Sphaeroma destructor* as one of the principal borers in marine woodwork, entering even palmetto piles in Florida, which are said to be immune from attacks from *Teredo*, and as of general occurrence from Louisiana to South Carolina in both fresh and salt water. The bores of this species are described as "from one-eighth to three-sixteenths inch in diameter and one-half inch long," though the borer is said to be "larger than the holes it bores" [!]. The galleries are described as approximately uniform in diameter without a calcareous lining, the lack of which distinguishes them from the borings of *Teredo*, though the occurrence of this borer in the same timber with *Teredo* probably accounts for the fact that the separate origin of their holes was overlooked until the late discovery of the isopod as a borer in 1897. Woodwork in fresh water as well as in salt water in the region where these isopods are abundant is to be protected only by impregnation with creosote.

In personal correspondence with the present writer (dated May 16, 1914, and quoted by permission), Dr. W. de C. Ravenel of the United States National Museum says:

There are in the museum collections specimens of *Sphaeroma destructor* Richardson from Colleton River, South Carolina, found by the Fish Commission in 1911 in soft sandstone between tide marks, also specimens of *S. Seiboldii* Dollfus from Yokohama imbedded in holes in tufa. Other boring species are *Sphaeroma peruvianum* Rich. and *Limnoria japonicum* Rich.

Of *Sphaeroma peruvianum* Richardson, the so-called mangrove louse, "piojos de mangle," the describer (Richardson, 1910, p. 83) says:

A large number of specimens were collected in the oyster beds of Matapolo (near Capon), Peru. They were found in wood, in holes bored by them. The wood was completely honeycombed. . . .

The following notes were supplied by Dr. Coker [who collected the specimens]: "These small Crustacea are economically significant, since they enter

the green stems and roots of the mangroves, causing the wood to decay. The falling away of these destroyed branches and roots causes the loss of many oysters attached to them. As I rarely observed the *Teredo* in the green stems, it seems that these Crustacea are the most pernicious form and undoubtedly they prepare the way for the more rapidly destructive *Teredo*."

OCCURRENCE OF *SPHAEROMA PENTODON* RICHARDSON

This species, *Sphacroma pentodon* Richardson, was first described from collections of the Harriman Alaska Expedition of 1899 (Richardson, 1904, p. 214, fig. 96). The species has also been reported from "marshy ground" along the shore of San Francisco Bay (Holmes, 1904, p. 323) and from mudflats at Sausalito, near San Francisco (Richardson, 1905, p. 287). This isopod is extremely abundant at the locality described on San Pablo Bay and is probably common along the Pacific coast. The species may, therefore, follow a versatile life, boring into rocks when ledges of sufficiently soft or fine texture are accessible and also living in marshes and in mudflats.

The sandstone into which these isopods bore on the shore of San Pablo Bay may be described as "a fine grained, friable sandstone of open texture and not well cemented." The results of an acid test for calcium were negative. For an examination of a sample of this sandstone I am indebted to Professor A. C. Lawson, Department of Geology, University of California.

The tuff is even more friable than the sandstone and is "composed of a whitish or light yellowish pumice, partly in fragments, ranging in size from 1 to 50 millimeters, and partly in fine dust" (Lawson, 1914, p. 13). The tuff in the region where the boring isopods are found is largely composed of the finer materials with occasional beds almost of the fineness of fine clay.

In the tuff the isopods bore with little if any apparent localization on account of varying hardness or fineness of constituent beds of material. In the sandstone, however, certain beds, presumably the softer, have been selected in preference to others probably harder or more firmly cemented, though there is no apparent difference in hardness. The result both of the activity of the borers and of the friability of these layers of sandstone is that the sandstone beds in which the isopods bore are more rapidly eroded than the intervening beds. The tuff probably presents rock of such consistency as to be readily attacked by the borers, while the San Pablo sandstone is, on the average, somewhat too hard for the borers. The isopods bore, therefore, only into the softer of the sandstone beds. This indicates a

rather delicate choice on the part of the borers of those beds which are best suited to receive their excavations (see pl. 15, figs. 1 and 2).

The size of the holes made in these rocks varies from bores 6 mm. long and 2 mm. in diameter, containing small and presumably young isopods, to bores 35 mm. long and 9 mm. in diameter, containing large and presumably adult isopods. Each bore is of approximately uniform diameter throughout its length, indicating that the animals either make new bores to suit their increasing growth in size or that they ream out and lengthen their original bores. Though there is no direct evidence against the possibility of a progressive exchange of holes, such a procedure seems altogether unlikely, in view of the prodigious capacity of these animals for boring into any suitable ledge, the scarcity of empty isopod holes, especially small holes, the general accurate fitting of the isopods to their holes, and also in view of certain laboratory observations (see pl. 17, fig. 2).

The direction of the holes is in general downward, but not always vertical or even perpendicular to the surface of the rock. Most of the holes are roughly parallel in direction, but they may vary widely in direction (see pl. 17, fig. 1). Observations on these rock-boring isopods on San Pablo Bay have found them only between the tide levels, and it is not known how deep they may live in rocks of favorable texture beneath the surface of the bay. Regions of deposition of mud or sand, even if periodically swept by currents, would, of course, be unfavorable for the boring activity of *Sphaeroma*, and since the deposition of mud is probably a more or less continuous process in San Pablo Bay, except perhaps in the midchannel, it is not to be expected that these isopods in this locality bore into rock much below the level at which wave action prevents the deposition of silt.

THE PROCESS OF BORING OF *SPHAEROMA PENTODON*

RICHARDSON

The evidence for believing that these animals themselves bore the holes in which they are found is supported by the following observations.

The holes were evidently made by some active purposeful agency. They are not of accidental occurrence, and it is impossible to conceive of them as due to wave erosion. The surface of the bore, moreover, presents the appearance of freshly cut stone. These freshly cut holes

invariably contain an isopod of approximately the same size as the hole in which it is found. When contracted into a ball the isopod can sometimes be made to roll out of its hole much as a shot may roll out of a gun barrel.

There is no other animal in evidence which could have made these holes. If holes so numerous as these were freshly made by another animal than the occupants found in them, one would expect to find traces of the other animals alleged to have made the holes. There is no indication that the animal association of these reefs contains in large numbers other animals than those already mentioned in an introductory paragraph of this paper.

There are to be seen on the walls of the bores fine scratches such as would be made by the mandibles or hard parts of the exoskeleton of such animals as the isopod occupants in excavating the bore.

Still stronger evidence, however, that these isopods in nature bore their own holes comes from laboratory observations of their attacks upon chalk. Into a glass dish containing some twenty isopods and a little *Ulva* three cone-shaped pieces of carpenters' chalk were placed, two of the blocks flat side downward and the third apex downward. The isopods had previously crept out of sight among the folds of the *Ulva*. Between two subsequent observations about twenty-four hours apart one isopod, 4 or 5 mm. in diameter, bored for a distance a little more than its own length into the base of one of the chalk blocks which was flat side down. Other isopods showed an inclination to creep about the base of the chalk blocks and to huddle under the inverted block. Probably the instability of that block, resting on its apex and curved side, prevented their treating it as a permanent abiding place.

Within a few days the under surface of the two blocks which rested with their flat sides on the bottom of the aquarium dish were riddled with borings of the isopods (see pl. 17, fig. 2), some of the borings striking upward into the heart of the block, with two or even three isopods occasionally lodged in a single bore. The bottom of the dish became covered with the fine chalk particles from the borings. One bore approached very close to the side of the block so that its outer wall became broken, making a sort of window through which the isopod might be watched industriously at work.

This animal did not use its feet in boring except to grip the sides of the bore with the terminal claws so as to give a purchase for the effective application of the mandibles. The feet are, moreover, too feeble to be expected to accomplish any great effect against rock.

As the isopods crawled over the edges of their bore, however, scratches were seen to be made by the feet in the thin edge of chalk around the mouth of the bore, and certain longitudinal scratches found in the bores may have been made by the claws or by the uropods. The softening of the chalk block by its immersion in water may have facilitated the making of these adventitious scratches.

The effective boring is, however, done with the mandibles. Gripping the sides of the hole by striking the claws of its feet into the chalk, the isopod spreads its mandibles apart and drives them into the chalk near the head of the bore. At the height of this exertion the anterior end of the body of the animal is pressed down so tensely as to cause the surface of the back in the region between the head and the anterior thoracic segment to be indented. At the next moment the fragment of chalk is bitten off, handed back by the palpi and the feet until under the abdomen, and is then washed out of the bore by the current of water set up by the swimmerets. By continually turning around in the bore the shape of the hole is made cylindrical and its size is automatically determined by the size of the borer. So energetic and efficient was the work of this little borer that a quiescent isopod lying in the mouth of another hole closely adjacent to the hole under observation was covered with chalk dust, as if with snow, in the course of twenty or thirty minutes. Boring into the chalk is undoubtedly more easily and rapidly accomplished than boring into sandstone or tuff, but there is no reason to suppose that the boring process in nature is essentially different from that observed in the laboratory.

Disturbed by the opening of the window in its bore, the particular isopod observed during this process was seen to turn around in its bore occasionally and to work at the mouth of the bore or at the sides of the hole, evidently reaming out some narrow portion to the proper size. This confirms a former suggestion that in nature the growing animal probably occupies but one hole during its lifetime, enlarging this hole as may be necessary. In nature also the boring process is probably not a slow or tedious one, but is fairly rapid while in progress, though interrupted by many periods of quiescence after the original entry has been made into the rock.

In setting up the currents by which the hole is cleaned the water seems to enter the hole in a diffused stream and is drawn gently about the head by suction from the action of the swimmerets. The sides of the body fit down closely against the side of the hole so as to confine

and strengthen the outgoing current, which emerges as a pulsating stream below the posterior end of the body.

Moll (1914) states that it has been impossible to demonstrate the presence of secretions in the case of *Chelura*, a related amphipod borer, which might aid in the process of boring, but adds that ligneous chips have been found in their stomachs (i.e., of *Chelura*). It seems certain therefore that fragments of wood gnawed off by *Limnoria* or by wood-boring species of *Sphaeroma* will be found in the stomachs of these borers as well, though whether this wood is made to contribute to the nourishment of the borer is another matter. Chalk particles readily identified by color, consistency, and acid reaction have been found in the alimentary canal of specimens of *Sphaeroma pentodon* which have bored into chalk, indicating a certain amount of lack of selection or of accident in admitting material into the digestive tract. The greater amount of chalk debris resulting from the excavation is, however, washed directly out of the bore by the swimmerets.

It is not known how extensively these borers may leave their bores when covered by the tide. If exclusively plankton feeders, they probably rarely make excursions away from their holes. In the laboratory, specimens of rock containing numerous borings with isopods in the holes have been kept for weeks and only very rarely have the isopods been seen to leave the holes voluntarily to wander over the surface of the rock or to swim about the aquarium.

Both in the laboratory and in the natural condition in the rocks of the bay shore the isopods have been repeatedly seen resting in the mouth of the bores, probably because a current of water for good respiration and food supply can be the more easily set up there than at the head of the bore. The animal, however, invariably presents the posterior end outward. It is to be noted in this connection also that the uropods seem to be exceedingly sensitive, probably acting as efficient tactile organs in the place of antennae in perceiving danger. When alarmed the isopods retreat to the inner end of the hole, and if pursued roll up into a nearly spherical ball, so that the popular term of "pill-bugs," given to the group, seems very appropriately applied.

That the boring habit was thoroughly established in the race of the isopods brought into the laboratory appears from the activity with which the isopods attacked the chalk. If this race be morphologically the same as that collected from the mudflats and marshy ground by Ritter and Holmes, it seems to differ from those races physiologically

in being possessed of the characteristic habit of boring, resuming this habit in the first favorable material offered even in the quiet of a laboratory aquarium, unprovoked by such conditions of its natural environment as might be supposed to have caused the boring habit.

SUMMARY

An isopod, *Sphaeroma pentodon* Richardson, occurring abundantly in marshes along the margin of San Francisco Bay, California, is found in one locality near Rodeo, on the shore of this bay, boring into ledges of soft San Pablo sandstone and Pinole tuff. Specimens of this species, kept in laboratory aquaria, excavated characteristic holes in blocks of chalk and were observed to use their mandibles in biting away particles of the chalk, which were washed out of the bore by the action of the swimmerets. The habit of boring was probably developed in this genus and in the related wood-boring genus, *Limnoria*, from attempts to reach out crevices in which most of the genera of the family Sphaeromidae habitually seek protection, becoming a permanent characteristic in *Limnoria* and a less extensive habit in a few species of *Sphaeroma*.

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EXPLANATIONS OF PLATES

PLATE 15

Fig. 1. Ledge of San Pablo sandstone exposed between tide levels (viewed from the shore bluff), about one-half mile southwest of Rodeo station, showing the uneven erosion of the beds, the location of the boring isopods in those beds which have been eroded most rapidly, and the scarcity of the borers in the intervening beds.

Fig. 2. The same ledge as that shown above (viewed from the southern side), showing the erosive action of the isopod borers. A small island of Pinole tuff lies in the background.



Fig. 1



Fig. 2

PLATE 16

Fig. 1. Close view of a portion of the ledge of San Pablo sandstone shown in plate 15.

Fig. 2. Natural surface of a fragment of San Pablo sandstone, showing isopod borers and sessile surface fauna.



Fig. 1

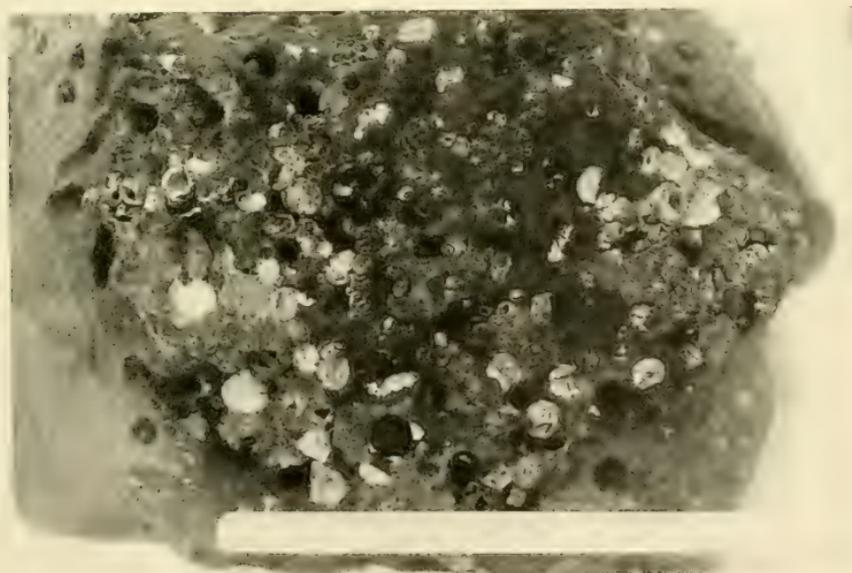


Fig. 2

PLATE 17

Fig. 1. Fragment of San Pablo sandstone, sectioned to show the natural depth of the borings.

Fig. 2. Under surface of a chalk block into which the isopods bored in a laboratory aquarium.



Fig. 1

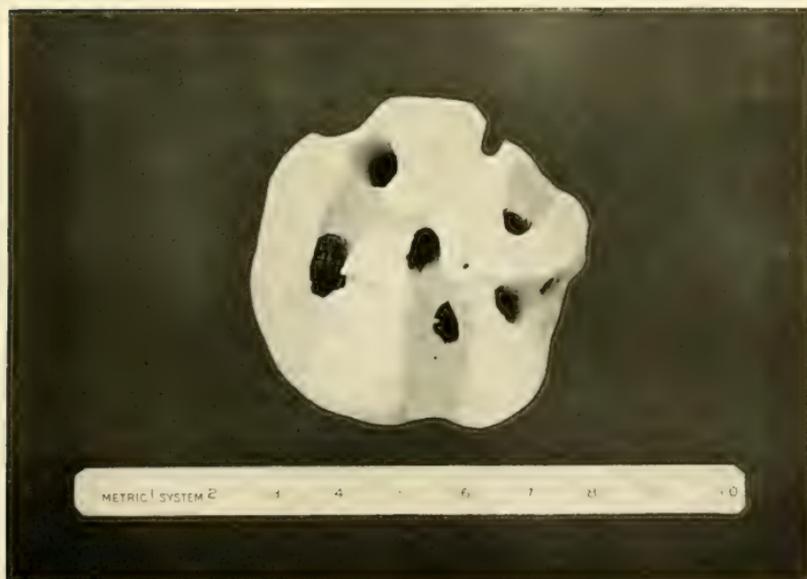


Fig. 2

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A NEW MORPHOLOGICAL INTERPRETATION
OF THE STRUCTURE OF *NOCTILUCA*, AND
ITS BEARING ON THE STATUS OF THE
CYSTOFLAGELLATA (HAECKEL)

BY
CHARLES A. KOFOID



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CHARLES A. KOFOID



The purpose of this paper is to set forth certain observations on the structure of *Noctiluca*, to call attention to the bearing of some recently discovered types of Gymnodiniidae upon the relationships of *Noctiluca*, and to present a new interpretation of the orientation and morphology of this well-known organism. This interpretation indicates that the allocation of *Noctiluca* as the generic type of a separate order of the Mastigophora, namely the Cystoflagellata Haeckel (1873), is no longer justifiable by the canons of comparative morphology, and harmonizes its structures with those of the tentaculate dinoflagellata.

In the recent fourth edition of his *Lehrbuch der Protozoenkunde*, Doflein (1916, p. 663) places the Cystoflagellata as the fifth and last, and presumably the highest order of the Zoömastigina, quite remote from Dinoflagellata, with this qualifying statement:

“Die systematische Stellung dieser Ordnung ist noch nicht klar erkannt. Man hat vielfach, auch wegen der äusserlichen Aehnlichkeit der Schwärmer von *Noctiluca* mit solchen an die Verwandtschaft mit Dinoflagellaten gedacht. Vorläufig liegen keine einleuchtenden Beweise für solchen Annahme vor. Doch müssen wir manche der Charaktere der Cystoflagellaten für Anpassungen an die planktonische Lebensweise halten und eine nähere Erörterung der Verwandtschaftsverhältnisse wird erst möglich sein, wenn Bau und Fortpflanzungsweise der hierher gerechneten Formen genauer erforscht sein wird.”

Data of a comparative and interpretative nature we here offer to elucidate this relationship of *Noctiluca* to the Dinoflagellata.

Although the essential elements in the structure of *Noctiluca*, in the typical large, unicellular phase commonly found in the neritic plankton, have long been known, and are figured more or less imperfectly by the earlier investigators of this unique animal, there are in the literature no comparative morphological analyses and correlations of these structures with those of any dinoflagellate. The grounds upon which Jollos (1910) proposed the inclusion of the Cystoflagellata (*Noctiluca*) in the Dinoflagellata are very general ones, including the resemblances in nuclear structure and mitosis, the presence of a tentacle, as in *Erythroopsis*, and the inflated sphere of *Pyrocystis* (*Gymnodinium*) *lunula* with its local aggregation of the cytoplasm. Most stress, however, is laid upon the swarm spores of *Noctiluca* as being indicative of dinoflagellate affinities. Beyond these elemental resemblances Jollos does not develop the homologies, and concludes by placing the Cystoflagellata in the Dinoflagellata as an order coördinate with the "Peridineen," within which he includes all those organisms previously allocated in the Dinoflagellata. In these allocations he is followed by Poche (1913) in his *System der Protozoen*, but Poche gives no elaboration of the basis of such allocation. In making this allocation these authors have adopted a view earlier proposed but not followed by Bütschli (1885).

Data brought to light by a monographic investigation, now in press, of the unarmored Dinoflagellata of the San Diego region, by the writer and Dr. Olive Swezy, make possible a reconsideration of the morphology of *Noctiluca* and a detailed homologizing of its essential structures with those of the *simple, typical, naked* dinoflagellate, and with those of some recently discovered, *more highly specialized, tentaculate* forms of the Dinoflagellata.

The mononucleate, vegetative, free, and active stage of *Noctiluca*, comparable with the usual unencysted free stage of dinoflagellates, has certain structural features noted in a fragmentary fashion by various earlier writers on the morphology of this animal. These I have recently verified on living and preserved *Noctiluca* from San Francisco Bay, and present herewith carefully oriented figures, and a new analysis and homology of these structures with the organs of the Dinoflagellata.

These features (pl. 18, figs. 1-6) are (1) a deep groove on the ventral face at one end of which is found (2) the transversely striate tentacle. This groove is continued at the opposite end in (3) a straight, narrow prolongation, which in small individuals consider-

ably extends the body along its axis. The groove just above the tentacle (4) sinks rather deeply into the body, forming a recess, the oral pouch. At one side of this at some distance from the tentacle arises (5) a threadlike flagellum which almost reaches the base of the tentacle with its free end. At one side, and slightly in advance of the origin of this flagellum there is (6) a membranous or somewhat prolonged and ribbonlike projection called by Huxley (1855) the tooth.

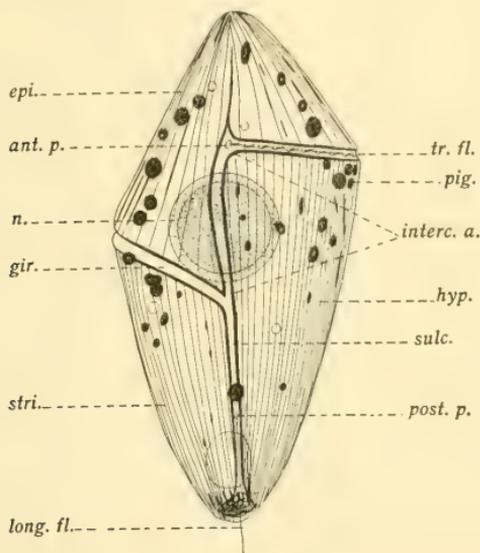


Fig. A. Typical dinoflagellate, *Gyrodinium corallinum*, gen. nov., sp. nov., Kofoid and Swezy MSS, showing sulcus, girdle, and two flagella. $\times 500$. *Ant. p.*, anterior pore; *epi.*, epicone; *gir.*, girdle; *hyp.*, hypcone; *interc. a.*, intercingular area; *long. fl.*, longitudinal flagellum; *n.*, nucleus; *pig.*, pigment; *post. p.*, posterior pore; *pus.*, pusule; *sulc.*, sulcus; *tr. fl.*, transverse flagellum.

Close to this and at one side of it there appears, especially in the smaller and presumably younger individuals, (7) a shallow trough, the girdle, hitherto unnoted, which branches off from the main groove in a short arching curve which speedily dies out on the rotund side of the body. Adjacent to the deep pit above the tentacle is gathered the denser, granular mass of the cytoplasm, enclosing the nucleus, and from it radiate the anastomosing strands of cytoplasm passing to the outer pellicle of the body.

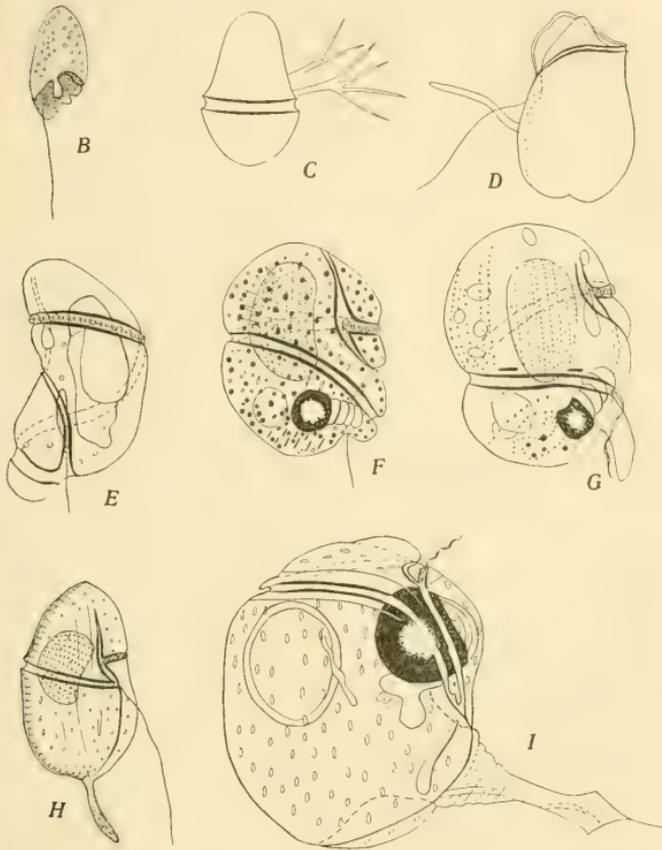
The fundamental organs upon which are based the structure of the Dinoflagellata are the girdle and the sulcus (fig. A, *gir.* and *sulc.*).

The former encircles the body transversely or in a descending left spiral of 0.5 turn in *Hemidinium* and *Oxyrrhis* (fig. B), for a full turn in *Gymnodinium* (figs. C, D), for not to exceed 1.5 turns in *Gyrodinium* (fig. A), and up to 3 or 4 turns in *Cochlodinium* (fig. E). Except in *Hemidinium* and *Oxyrrhis* its distal end joins the sulcus.

The girdle in dinoflagellates is occupied by the transverse flagellum (fig. A, *tr. fl.*), a broad ribbonlike flagellum, which passes from its origin at the junction of girdle and sulcus on the ventral face, distally in the girdle for the whole or a part of its length. It is free except for its attachment at its base, but in life normally lies in the girdle, where short waves of contraction traverse it distally. When fixed by preservatives it may be thrown out of the girdle in close-set undulations (fig. I).

The sulcus (fig. A, *sulc.*), on the other hand, is longitudinal and extends for a varying distance in the morphological midventral line between the apex and antapex of the body. As the body undergoes torsion in the higher genera, such as *Pouchetia* and *Cochlodinium*, the sulcus is involved in the torsion and makes one less turn than the girdle. The sulcus always contains the two flagellar pores. The anterior one, from which the transverse flagellum arises, is at or near its junction with the proximal end of the girdle, and the posterior pore, from which the posterior or longitudinal flagellum arises, is at its junction with the distal end of the girdle or below it.

The anterior end of the sulcus may extend far anterior to the girdle to the very apex, or beyond it, and, in species with torsion, this anterior extension of the sulcus may be continued in a spiral, apical loop about the apex. In like manner the posterior section of the sulcus below the distal end of the girdle may be carried around the antapex in an antapical loop continuing the torsion, though it usually runs directly posteriorly in the midventral line. The longitudinal or posterior flagellum extends distally in the sulcus and projects beyond the posterior end of the body. In contradistinction to the transverse flagellum it is a slender thread not thrown in close-set undulations. The intercingular part of the sulcus, between the two ends of the girdle, is increased in length by the posterior displacement of the distal end of the girdle, especially in the higher genera, such as *Gyrodinium* (fig. A) and *Cochlodinium* (fig. E), where there is much torsion. It is mainly through the sulcus, especially its intercingular section, that holozoic species of Gymnodinioidae engulf the organisms upon which they feed. It is thus the homologue of the cytostome of



Figs. B-I. Evolution of tentacle in the Gymnodinioidae.

Fig. B. *Oxyrrhis marina* Dujardin after Senn (1911, pl. 30, fig. 9), showing tentacular lobe adjacent to bases of the flagella in the generalized sulcal area.

Fig. C. *Gymnodinium zachariasii* Lemm. showing temporary pseudopodia emerging from the sulcus. After Zacharias (1899, fig. 4). $\times 500$.

Fig. D. *Gymnodinium pseudonoctiluca* (Pouchet) showing temporary(?) tentacle and smaller pseudopodia arising from margin of the sulcus. After Pouchet (1885, pl. 4, fig. 2, right).

Fig. E. *Cochlodinium cavatum* Kofoid and Swezy MSS, showing protuberant antapex adjacent to sulcus. $\times 500$.

Fig. F. *Pouchetia maculata* Kofoid and Swezy MSS, showing short mobile extension at the side of the sulcus. $\times 500$.

Fig. G. *Proterythropsis crassicaudata* gen. nov., sp. nov., Kofoid and Swezy MSS, showing protuberant, pendant and mobile tentacle with both flagella present. $\times 500$.

Fig. H. *Pavillardia tentaculifera* gen. nov., sp. nov., Kofoid and Swezy MSS, showing normal sulcus and girdle with the two flagella and a tentacle at the posterior end of the girdle as in *Noctiluca*.

Fig. I. *Erythroopsis extrudens* Kofoid and Swezy MSS, showing stout, very active tentacle or prod., protruding from the lower end of the sulcus, which is transformed into the tentacular chamber or recess. No longitudinal flagellum figured.

other flagellates. The sulcus is, therefore, not only an organ of great lability in the speciation of the Dinoflagellata but may also be very mobile in the individual.

It is likewise from the margins of this region that the pseudopodia and in higher genera the tentacles of the Dinoflagellata are developed; and it is this development of these outgrowths of the sulcal region in otherwise typical Dinoflagellata that affords one of the grounds for the dinoflagellate affinities of *Noctiluca*.

The lability of the cytostomal area in *Collodictyon triciliatum*, a polymastigote flagellate, has recently been demonstrated by Rhodes (1919), who figures the formation of temporary pseudopodia from the sulcus of this polymastigote flagellate, which are used in the capture of organisms for food.

Among the dinoflagellates there is but one instance (fig. C) of pseudopodia so designated on record, that of Zacharias (1899) who found such structures in *Gymnodinium palustre* (later called *G. zachariasii* by Lemmerman, 1900). The protoplasmic protrusions of this species take the form of simple hyaline, tenacle-like, or branching outgrowths from the sulcus or its immediate margin adjacent to the flagellar pore. No use of these processes in the capture of food was noted, but the conjecture was made by Zacharias that they were utilized in rapid saprophytic nutrition prior to encystment. It is quite possible that they are in reality degenerative phenomena. Extrusions of plasma from the flagellar pores of thecate forms as cytolysis approaches is rather a common phenomena in our experience.

There are four genera thus far known among Dinoflagellata in which a distinct tenacle is formed from the margin of the sulcus. These are *Gymnodinium pseudonoclituca* Pouchet (1885), *Pavillardia tentaculifera* Kofoid and Swezy MSS, *Proterothropsis crassicaudata* Kofoid and Swezy MSS, and *Erythroopsis agilis* Hertwig (1884). The evolution of tentaculate species among the Dinoflagellata appears to have taken place independently in different genera, and not in a single series of species within a single genus. The addition of *Noctiluca* to the series of tentaculate Dinoflagellata therefore accords with the conditions known to prevail in that subclass.

In *Gymnodinium pseudonoclituca* Pouchet (1885, 1892), we have a species with the typical morphology of *Gymnodinium* (fig. D), with girdle of one turn, straight sulcus, transverse flagellum in the girdle, and trailing posterior flagellum. In addition to these *two* flagella there is a "tentacle" originating in the posterior region of the sulcus

and extending posteroventrally. Its length is about 0.6 that of the body and its form is that of a rod of uniform caliber with rounded end. It is thrown into curves strikingly similar to those of the tentacle of *Noctiluca*, but it is not figured as striate as it is in *Noctiluca*.

The exact point of origin of this tentacle does not appear to be constant in Pouchet's (1885, 1892) figures. In certain ones (1885, pl. 4, fig. 37; 1892, pl. 11, figs. 3, 6, 7) the tentacle arises from the posterior region of the sulcus. In one instance (1892, pl. 11, fig. 2) it arises from the left margin of the sulcus and in another (1892, pl. 11, fig. 4) from the posterior end of the body. In the latter instance the point of origin may be determined by the very evident mobility of the various regions of the body, and may not be a morphological displacement. The point of origin indicated in the former instance represents either an oversight in drawing or a real shifting of the point of origin of the "tentacle" out of the sulcus to the left margin. There may be a single secondary amoeboid projection, or a bilobed one immediately anterior to the base of the tentacle, either of which would be suggestive of the labile condition of the region. Some individuals entirely lack the "tentacle," whether by autotomy or retraction is unknown. If the latter is the case the structure should be called a pseudopodium. The adjacent secondary process suggests the pseudopodial interpretation, but the form of the major process is clearly tentaculate.

The cytoplasm of the body is much vacuolated and is aggregated around the nucleus, as in *Noctiluca*. It is 175 to 200 μ in length, has yellow chromatophores, a complete girdle, and no extension of the sulcus upon the epicone. For these reasons it does not appear to be a developmental phase of *Noctiluca*, although the tentacle and vacuolation are suggestive of it. Its small zoöspores are typical *Gymnodinium* without tentacle, unlike those described for *Noctiluca* (pl. 18, figs. 4a, 4b), which have at this stage a large tentacle. If the "tentacle" is, in reality, only a pseudopodium the species may well remain in *Gymnodinium*, but if it is a permanent tentacle it should be removed to *Pavillardia* gen. nov. Kofoid and Swezy MSS.

The second genus of the tribe Gymnodinioidae with tentacle is *Pavillardia* nov. gen. Kofoid and Swezy MSS, with one species, *P. tentaculifera* Kofoid and Swezy MSS (fig. H). This has a simple *Gymnodinium*-like body with girdle, sulcus, and the two flagella. The longitudinal flagellum, in the somewhat limited material we have examined, is fully developed but in some instances was not found.

At the posterior end of the sulcus and of the body arising from the postmargin, is a short, stout, highly pigmented (dark orange), mobile, permanent tentacle. Its location is posterior to the origin of the longitudinal flagellum, where it is developed directly from the end of the sulcus, a position comparable to that of the tentacle in *Noctiluca*. Beyond this possession of a tentacle there are no other indications of special relationship to *Noctiluca*. There is no marked vacuolation and zoöspores are unknown.

The third genus of the tribe Gymnodinioïdae with a posterior tentacle is *Proterythropsis* nov. gen. Kofoid and Swezy MSS, with a single species, *P. crassicaudata* Kofoid and Swezy MSS. This is a highly specialized form (fig. G) with slightly more than one turn of the girdle, which forms a descending left spiral with a displacement of its distal end posteriorly for a distance of about 0.6 of the total length of the body. The sulcus runs from near the apex to near the antapex. Both transverse and longitudinal flagella are present in their normal locations. There is an ocellus with melanosome and lens, as in *Pouchetia* and *Erythropsis*. Arising from the margin of the antapical section of the sulcus is a stout, ventrally recurved, pendant tentacle, in length about 0.3 of the length of the body. This arises immediately behind the base of the posterior flagellum. It is foreshadowed in the genus *Pouchetia* by species (*P. maculata*, fig. G) which have a very mobile antapical region at one side of the sulcus. The position of this tentacle with relation to the sulcus and posterior flagellum is comparable to that of the tentacle of *Noctiluca*. Intermediate stages leading towards *Proterythropsis* are also seen in *Cochlodinium cavatum*, in which the area adjacent to the sulcus is protuberant and mobile.

The genus *Erythropsis* is represented in literature by Hertwig's (1884) original species *E. agilis*, and by two species described by Schütt as *Pouchetia cornuta* and *P. cochlea*, which have been transferred by Kofoid and Swezy (MSS) to *Erythropsis*. We have found the tentacle on the former; the latter probably has it. Both have the structure of *Erythropsis*. A fourth species has been described and figured by Pavillard (1905) and Fauré-Fremiet (1914) as *E. agilis* Hertwig, yet it is not Hertwig's species but a new one, which is designated as *E. pavillardi* Kofoid and Swezy MSS. A fifth species, *E. extrudens* Kofoid and Swezy MSS, appears in our text figure I.

Fauré-Fremiet (1914) has reversed the orientation of *Erythropsis*, placing the tentacle anterior, and Doflein (1916) has adopted this

erroneous orientation in his *Lehrbuch*. Comparative morphology and observations on the living animal establish the posterior location of the tentacle (see Kofoid and Swezy, 1917).

The species of *Erythroopsis* mentioned have the following structural features. There is a girdle with bordering paracingular lines sharply displaced posteriorly near its distal end, making one turn about the body. In it lies the transverse flagellum, thrown out of the girdle in our figure I. The sulcus is a highly modified structure extending anteriorly upon the epicone in a more or less spiral apical loop. Posteriorly it expands into a deep recess, the tentacular chamber, from which protrudes the cylindrical, sometimes capitate tentacle, or prod. Adjacent to the base of this prod in the upper part of the chamber is the insertion of the longitudinal flagellum, which sometimes can not be found, and is usually short and difficult to see. Here also the tentacle is an outgrowth of the posterior part of the sulcus, specialized as the tentacular chamber, posterior or adjacent to the longitudinal flagellum.

It is obvious, from this comparative analysis of tentacle-bearing dinoflagellates, that if *Noctiluca* is one of the Dinoflagellata it will not be exceptional in the possession of a tentacle, and also that this interpretation involves the presence in *Noctiluca* of the girdle, sulcus, and of the two flagella, as well as of the tentacle. We will now proceed to the demonstration that these organs are present in *Noctiluca*.

For the proper understanding of the morphology of *Noctiluca* it is essential to so orient its body that a consistent terminology of planes and axes, as well as of organs is both feasible and logical. This is accomplished by considering that the deep straight groove is the sulcus and on the midventral line. It follows then that the tentacle is in this line and we locate it at the posterior end of the sulcus as in other dinoflagellates, and not anterior as Bütschli (1885) located it. Granted these points, it follows perforce that the face opposite to the groove is the dorsal one and that the anterior pole is that towards which the "superficial ridge" of Allman (1872) is extended, this "ridge" being in fact the apical section of the sulcus. The girdle must then be looked for at right angles to and to the left of the sulcus (right of fig. 3, pl. 18). The transverse flagellum would be located in this girdle if normally developed and would take its origin at or near its junction with the girdle. The longitudinal flagellum would be found running lengthwise in the sulcus, with its origin in front of the tentacle and near or posterior to the junction of girdle and sulcus.

According to these criteria the single flagellum of *Noctiluca* is the longitudinal one and the transverse one is represented by the degenerate toothlike or ribbon-like process at the left of the sulcus, while the girdle itself is reduced to a mere trough which branches off to the left side of the sulcus or at a short distance anterior to the posterior flagellum, and dies out quickly on the distended left face (pl. 18, figs. 3, 5, 6).

This interpretation coincides with the results to be expected from the adaptations to pelagic life manifested by *Noctiluca*, as shown in (1) great distention by hydrostatic vacuoles, (2) consequent loss of locomotor capacity, and (3) degeneration of locomotor organs.

The cessation of rotation about the axis and the limitations on progression established by the inflation of the body, reduce the locomotor organs, and the two flagella, to mere rudiments, and the girdle to but a trace of its proximal end. The sulcus, however, is retained in highly developed form, with its cytostomal function in full operation because of the holozoic nutrition of the organism. In plate 18, figures 1, 2, and 3, are seen anterior, lateral, and ventral views of *Noctiluca*, so oriented and labelled as to bring out the interpretation here advanced.

It is not the purpose of this note to discuss the earlier literature on the morphology of *Noctiluca* or to elaborate this subject. A brief analysis only of the figures will be attempted. In figure 2, plate 18, we have a view of the right side of the body so oriented that the tentacle (*tent.*) is posterior, the sulcus midventral and longitudinal with a deeply excavated oral pouch (*o. p.*) containing the cytostome, directed posteroventrally. The sulcus consists of two main regions, the more or less deeply recessed oral pouch and cytostome extending from the base of the tentacle anteriorly to the median anterior tip of the recess, and the apical groove (*ap. gr.*) continued beyond the lip in the median line in the anterior direction. It is a shallow rectilinear groove slightly elevated above the general surface and extending from the anterior lip of the recess on the anteroventral surface to the neighborhood of the apex of the body.

This tract in its entirety corresponds to the sulcus of *Gymnodinium pseudonoctiluca*, of *Pavillardia tentaculifera* and of other dinoflagellates. The morphological limits of three regions, precingular or apical, intercingular, and postcingular, of the dinoflagellate sulcus are here recognizable. (1) The apical or precingular section from the proximal junction of the girdle to the anterior end is represented by the region

included in that part of the cytostome anterior to the girdle, plus the apical groove. (2) The intercingular part of the sulcus extends posteriorly from the girdle. Its posterior limit is undefined, owing to the failure of the girdle to make the circuit of the body. Because of the fact that the posterior flagellum, as a rule, originates at or near the junction of the distal end of the girdle with the sulcus, it may be assumed that the base of this flagellum in *Noctiluca* represents approximately the posterior limit of the intercingular sulcus, which normally is found between the junctions of the proximal and distal ends of the girdle with the sulcus. In *Noctiluca* this region is very short, indicating that there is little, if any, of the displacement which normally forms the intercingular region. (3) The remainder of the sulcus between the base of this flagellum and the tentacle is the homologue of the antapical or posteingular section of the sulcus.

The girdle is best seen in the ventral view (pl. 18, figs. 3, 5, *g.*). It is a short trough, making less than 0.25 of a turn around the body in the smaller individuals and often obliterated in the larger ones. It originates very near, or slightly anterior to, the base of the longitudinal flagellum (see Robin, 1878, pl. 37, fig. 6; pl. 41, fig. 24; reproduced in our pl. 18, fig. 5). It is narrower than the sulcus and leaves it in an arching curve, convex anteriorly, which narrows and fades out distally before reaching the left side of the body, that is, in about 0.08 to 0.20 turn in Robin's (1878, pl. 36, fig. 4; pl. 37, fig. 6; pl. 41, fig. 24) smaller individuals, 400–425 microns in diameter.

This structure has precisely the morphological relations of the proximal end of the girdle and its characteristic form, but has never before been noted or interpreted, though sketched without comment by two investigators. It was clearly figured by Robin (1878) but he calls no attention to it and does not designate it. A bit of its proximal end appears in one only of Webb's (1855, pl. 6, fig. 6) figures but without designation or comment. There appears to be no other suggestion of this structure in the literature of the subject.

The two flagella typical of the dinoflagellates are both represented in *Noctiluca*. The longitudinal flagellum is the short, threadlike, vibratile structure in the sulcus trailing posteriorly from its origin in the depths of the sulcal recess a short distance posterior to the junction of the proximal end of the girdle with the sulcus. It is relatively small with respect to the size of the body, and appears to have little if any appreciable effect on the movements of the body.

The degeneration of all the girdle except its proximal end apparently accompanies the complete disappearance of the transverse ribbon-like flagellum in the zoöspore stage in which the longitudinal flagellum is present and functional. There appears, however, to be an organ in the adult which, from its position, variability, and structure, may represent the degenerated transverse flagellum. This organ is the so-called "tooth" of Huxley (1855), or "prehensile organ" of Brightwell (1857). It is a projection of the surface, and in the proximal end of the girdle on the left side of the sulcus. In life it forms an overarching projection anterior to the base of the longitudinal flagellum. It varies greatly in outline, having sometimes a row (pl. 18, fig. 3) of three or more equidistant equal prominences significantly suggestive of undulations of the transverse flagellum.

In other cases there may be a single prominent projection and other minor ones passing distally into the girdle. In a few instances observed by me and rarely shown in published figures, this structure appears to stand out as a long projection whose length is from several to as many as ten times its width. This appears in two of Huxley's (1855, pl. 5, figs. 2, 5) figures. It may be the structure which Allman (1872, pl. 18, fig. 2, reproduced in modified form in our pl. 18, fig. 2) figures as a tube, its vibratile (?) action suggesting to him his interpretation. Traces of it appear in Brightwell's (1857, pl. 12, figs. 9, 13) figures and in those of Cienkowsky (1873, pl. 3, fig. 1, 6).

The interpretation of this toothlike organ as the transverse flagellum is significantly confirmed in Ishikawa's (1899) account of mitosis. This investigator shows that this structure persists as a prominent projection during mitosis and, in common with the longitudinal flagellum, arises immediately adjacent to the large centrosphere of "archoplasm" (see his pl. 19, figs. 1, 4), exactly where a flagellum would be expected to have its origin. The morphological evidence is plainly conclusive that the tooth is the degenerate transverse flagellum.

The evidence from the functional standpoint is, perhaps naturally, less convincing. Movements in this organ, as I have thus far observed them, are slow and spasmodic. No rapid undulations have been seen by me but only very slow almost imperceptible retractions of the projections, and more rapid, spasmodic, somewhat rhythmical, downward beats into the sulcus on the part of this overarching projection or flagellum. The rhythmical feature is suggestive of flagellar activity.

The morphological evidence is thus conclusive that *Noctiluca* is a dinoflagellate highly modified through distention by hydrostatic

vacuoles. It retains the sulcus, modified anteriorly into the apical trough, but only the proximal part of the girdle persists. With the degeneration of the girdle the transverse flagellum is reduced to the prehensile tooth. The posterior tentacle is the homologue of the tentacle of *Pavillardia* and *Erythroopsis* and is not a modified flagellum.

RELATIONSHIPS OF *Noctiluca* AND STATUS OF THE CYSTOFLAGELLATA

The order Cystoflagellata was established by Haeckel (1873) to receive *Noctiluca* whose aberrant structure seemed to detach it from other flagellates. Later the genera *Leptodiscus* Hertwig (1878), *Craspedotella* Kofoid (1905) and *Radiozoum* Mingazzini (1904) were added. The last named is undoubtedly a skeletonless radiolarian allied to *Globicella* Borgert (1907). *Leptodiscus* and *Craspedotella* are so little known that the decision as to their final affinities may well await further investigation. They may equally well be left, pending new data on their relationships, as an undistributed residue in the thus emended "Cystoflagellata," whose nomenclatural status is imperilled by the removal therefrom of its type genus *Noctiluca*.

The evidence adduced in this paper establishes the affinities of *Noctiluca* to the Dinoflagellata and to the tribe Gymnodinioidae, since *Noctiluca* has in common with this tribe the girdle and sulcus and the two flagella and lacks a theca or cuirass.

In view of the high specialization induced by hydrostatic inflation and of the presence of the tentacle it seems justifiable to utilize in the Gymnodinioidae the family Noctilucaeidae Saville-Kent for the genus *Noctiluca* and the other tentaculate non-ocellate genus *Pavillardia* Kofoid and Swezy MSS.

There appears thus to be no adequate morphological justification for the separate order of Protozoa for the reception of *Noctiluca*, such as Haeckel (1873) created in his Cystoflagellata. This order can only be maintained in emended form to exclude *Noctiluca* and retain *Leptodiscus* and *Craspedotella* tentatively.

SUMMARY

1. *Noctiluca* is a tentacle-bearing dinoflagellate with a sulcus, girdle, and longitudinal and transverse flagella.
2. The sulcus is longitudinal and midventral. It includes the apical trough and the recessed oral pouch and cytostome.
3. The tentacle arises from its posterior end.

4. The girdle has hitherto been overlooked. It is a shallow trough at the left of the sulcus and at right angles to it. It is seen best in small individuals.

5. The longitudinal flagellum is reduced and lies within the oral pouch. The transverse flagellum is represented by the prehensile tooth at the proximal end of the girdle at the left of the base of the longitudinal flagellum. This organ exhibits structural undulations, and spasmodic or rhythmical contractions.

6. Distention by hydrostatic vacuoles, with flotation replacing active locomotion, has led to degeneration of the flagella and their reduction in size, and to the almost complete disappearance of the girdle.

7. *Noctiluca* belongs in the Noctilucaeidae, a family of the tribe Gymnodinioidae, with *Pavillardia*, another tentaculate, naked, non-ocellate dinoflagellate.

8. There is no morphological justification of a separate order of flagellates to hold *Noctiluca*, such as the Cystoflagellata Haeckel.

9. The Cystoflagellata may be retained as thus emended to receive *Leptodiscus* and *Craspedotella* pending discovery of their affinities.

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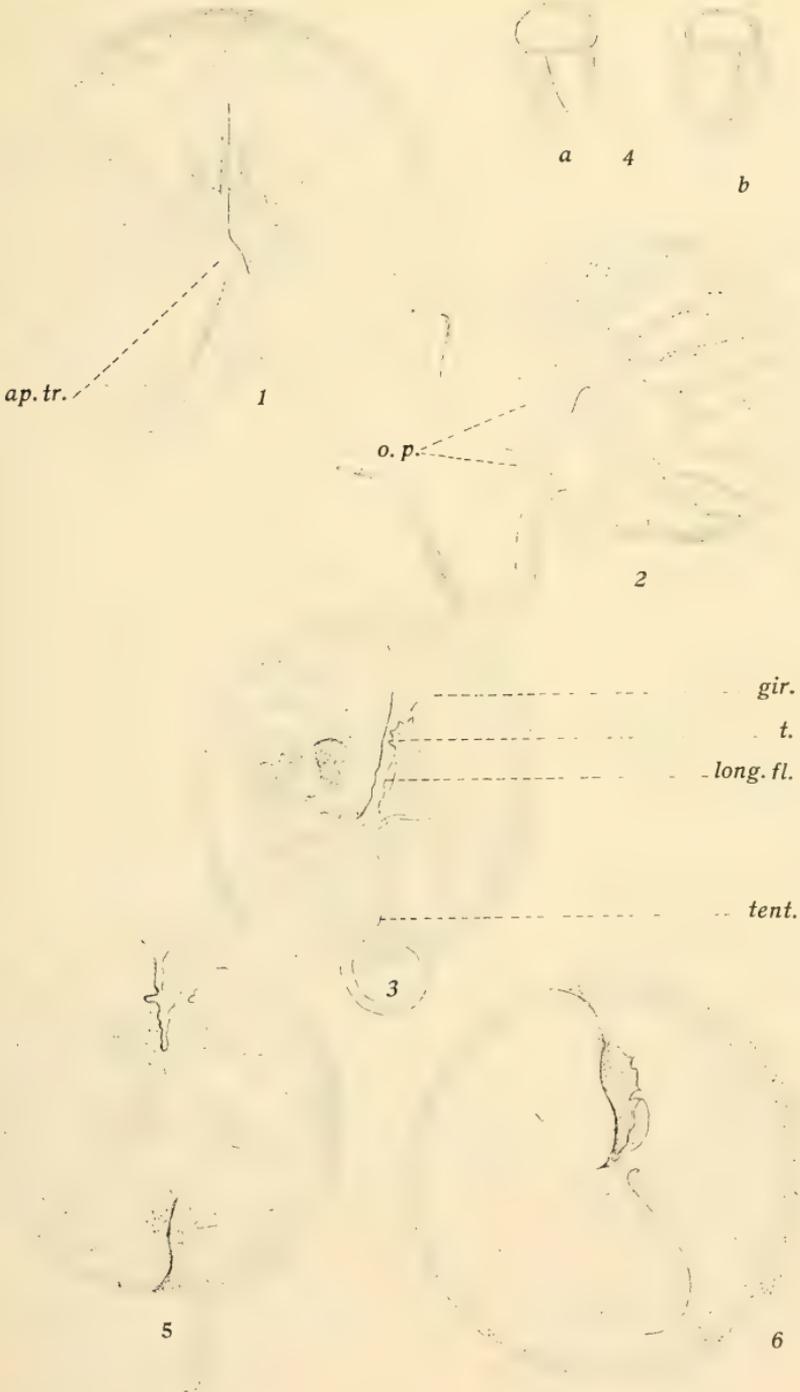
EXPLANATION OF PLATE 18

Noctiluca scintillans (Macartney)

1. Dorsal view showing apical trough. After Allman (1872, pl. 18, fig. 1).
× 125.
2. Lateral view from the left side showing the deep oral pouch. Modified
after Allman (1872, pl. 18, fig. 2). × 125.
3. Posteroventral view showing sulcus, girdle, undulating membrane or tooth,
flagellum and tentacle. The anterior lip is at or near the upper margin of the
figure. Modified slightly after Robin (1878, pl. 36, fig. 4). × 100.
- 4a, 4b. Zoöspores. After Cienkowsky (1873, pl. 6, figs. 38, 42). × 500.
5. *Noctiluca* in chain at mitosis showing girdle in the anterior schizont. After
Robin (1878, pl. 41, fig. 24). × 125.
6. Midventral view showing sulcus, rudimentary girdle, transverse flagellum
or tooth, longitudinal flagellum and tentacle. Modified after Webb (1855, pl. 6,
fig. 7). Magnification not given.

ABBREVIATIONS

- ant. l.*, anterior lip.
ap. tr., apical trough.
gir., girdle.
long. fl., longitudinal flagellum.
o. p., oral pouch.
t., tooth or transverse flagellum.
tent., tentacle.



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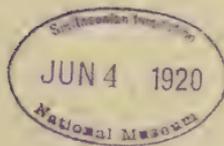
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THE LIFE CYCLE OF ECHINOSTOMA
REVOLUTUM (FROELICH)

BY
JOHN C. JOHNSON



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INTRODUCTION

Although many different species of adult echinostomes have been recorded, nowhere in the literature do we find the description of all the stages of their life cycle. In 1909, Lühe (1909, p. 65) clearly stated that up to that time the life cycle was insufficiently known and that no cercaria had been identified as belonging to a definite adult echinostome. The nearest approach to finding all the stages of the life cycle was made by Nicoll (1906, 1906*a*) and Lebour (1908) in their work on *Echinostomum secundum* Nicoll. However, these workers definitely state that no stages before the redia with cercariae inside were seen. Since that time neither in Europe nor America have all

the missing stages been traced. This lack of information is due principally to the complex life cycle. The chief difficulties involved are those of connecting exactly, by experimental feeding, the encysted agamodistome with the adult echinostome and of finding the exact species or genus of snail into which the miracidium will enter.

Knowledge concerning the entire excretory system of any stage of the life cycle of an echinostome is even less complete than that of the life history. This lack of knowledge is due largely to wrong methods of study which formerly consisted of using only preserved specimens.

At the suggestion of Professor W. W. Cort, the writer undertook in August, 1917, to work out the excretory system of an echinostome cercaria which later proved to belong to *Echinostoma revolutum* (Froelich). After tracing the ramifications of the tubules of the excretory system of the cercaria, work on this system in the redia was taken up. This led to the search for other stages suspected to exist in the life cycle of this species. The species chosen proved to be favorable for both types of work.

METHODS OF STUDY

The solution of the life cycle was undertaken in four ways. The first method was that of examining carefully a large number of snails, *Physa occidentalis* Tryon, from Stow Lake, Golden Gate Park, San Francisco, in which the cercariae were present to see if there might not be found other stages if studied during the entire year. Careful examination of four hundred of these snails showed that 86.75 per cent were infected with either the encysted agamodistomes or rediae or both. In addition, one other stage, the daughter redia, previously never reported for echinostomes, was found.

The second method was experimental. It consisted of feeding large numbers of encysted agamodistomes to white mice, New Zealand rabbits, white leghorn chicks, and mongrel ducklings. They were all protected in every way from other trematode infections, the last two being hatched in incubators and raised in brooders. The ducklings and chicks were never allowed near water or soil, there being always a board floor in the brooder. Their food was also carefully selected. Negative results were obtained from the experiments with the mice, rabbits and chicks, but the ducklings gave positive results.

The third method was also experimental. It consisted in hatching the echinostome eggs in syracuse dishes in which most of the water

was changed daily. Shortly before the miracidia were due to emerge, young snails were placed in the syracuse dishes with the eggs. These young snails were raised from eggs in aquaria which in no way could have become infected. They were about six months old at this time.

The fourth method was that of examining the various suspected water birds from the lake from which all the snails were taken, and particularly those birds which stay on the lake nearly or all the year round. The following water birds were examined: one specimen of *Olor columbianus* (Ord.); one of *Anseranus semipalmata* (Lath.); three mud hens, *Fulica americana* (Gmel.); one shoveller duck, *Spatula clypeata* (Linn.); one mallard, *Anas platyrhynchos* Linn.; two ruddy ducks, *Erismatura jamaicensis* (Gmel.) and two bluebills or more accurately American scaup, *Marila marila* (Linn.). All but the American scaup gave negative results for echinostomes. From one of these two ducks, adult echinostomes were obtained, which proved to be the same species as those obtained from the experimentally fed ducklings.

The tracing of the excretory system of the cercaria was accomplished by using the method described by Cort (1918). This method of using living specimens for study by placing them under a No. 1 cover slip, although on the whole advantageous, has a decided disadvantage, since most of the water must be withdrawn to retard the movement of the animal. When a slight amount more of water is withdrawn, due to evaporation, the body wall quickly breaks in many places. For this reason great numbers of cercariae had to be studied before the system was completely worked out.

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I wish here to express my thanks to Mr. Bryant Walker of Detroit, Michigan, and Mrs. Oldroyd of Stanford University for identification of snail hosts; to Mr. John Maclaren, Superintendent of Golden Gate Park, for permission to obtain water-fowl in said park; to Mr. Charcutte of Golden Gate Park and those under his charge, and to Mr. C. G. Budd, special game warden and policeman of San Francisco, for collecting and shipping the swans, mud hens and ducks; to Mr. F. H. Ballou, of the Department of Zoology, for collecting snails and other materials used in this study; to Dr. Joseph Grinnell and Mr. Tracy I. Storer, of the Museum of Vertebrate Zoology, for identification of vertebrate hosts; and to Mrs. Johnson for directing the experimental feeding of chicks and ducklings and for help in various ways.

To Professor W. W. Cort, under whose direction this study has been made, I wish here to express my appreciation for his continued interest and many helpful suggestions.

EGGS AND DEVELOPMENT OF MIRACIDIA

From adult specimens of *Echinostoma revolutum* found in the intestine of the duck, *Marila marila* (Linn.), about forty-five mature eggs were obtained, nearly all of which produced miracidia. From adults of the same species raised experimentally in ducklings, several hundred eggs were also obtained, some of which did not mature since they were forced out by pressure from the uterus. The eggs from both sources were alike in color, shape, and size variations, as would be expected since the adults belonged to the same species. They measured in length from 0.094 to 0.119 mm., the majority being about 0.108 mm. These figures fall within the range of variation given by Looss (1899, p. 679-684). His measurements show larger and smaller eggs than mine, which is to be expected since he measured a larger number of eggs from more adults. The color of the egg is usually yellow or yellowish brown.

In order to watch the eggs from day to day, they were placed in syracuse dishes containing tap water. To keep the eggs in a favorable medium part of the water was changed daily. About fifteen eggs were placed in each dish in a room where the temperature varied little from 70° F.

At one end of the egg is usually to be found a slight thickening of the shell which appears to be of a darker color. At the other end is the operculum or cap. When the eggs emerge from this echinostome they are in the one cell stage (fig. 1). This condition can also be readily determined by studying the eggs while still in the uterus. Although little work has been done on echinostome eggs, it seems probable that the one cell stage of development at the time of emerging is a family characteristic. Looss' drawing (1896, fig. 92) clearly shows eggs of *Echinostomum liliputanum* in the one cell stage near the genital pore. Usually the two cell (fig. 2) and the four cell (fig. 3) stages are formed within the first twenty-four hours. During this time the yolk mass appears somewhat cellular. It is made up of two materials, one of opaque granules, the other of an oily and semi-transparent fluid. The granules are prominent and at this stage are numerous and small. These two materials seem to be mixed with each

other, making a medium the nature of which is difficult to show in a drawing. As the number of cells of the miracidium increases the granules of the yolk material collect into larger units, but again decrease in prominence until by the twelfth day they have almost disappeared. By the seventh day the oily material has formed many well defined globules (fig. 7). On the second or third day the eight, ten, and twelve cell stages are reached (fig. 4). On the fourth day about twenty cells are represented (fig. 5). On the sixth day the number of cells have increased to about thirty (fig. 6), each cell being somewhat smaller than in the stages where there are fewer cells. During this time the embryo remains quite distinct from the yolk mass, although the exact boundary line is sometimes difficult to determine. On the seventh day (fig. 7) angular epithelial cells of the miracidium are clearly distinguishable. On the eighth day (fig. 8), the condition differs from the preceding day principally in the great reduction of the granules of the yolk material. Also on this day the epithelial cells are less distinct. On the tenth day (fig. 9), the embryo has attained a shape which is somewhat like that of a fully developed miracidium, the nuclei of the epithelial cells are less distinct and the oily globules are fewer but larger in size. On the eleventh day (fig. 10), the embryo is still longer and on the posterior end a tail-like structure appears which possibly represents the region of the primitive germ balls. This projection disappears in a few days. The first movement of the embryo is also to be seen on this day, which consists of contraction and expansion at the anterior end. On the twelfth day (fig. 11), the movement has increased, while the muscular nature of the body wall has become evident. The oily globules are still fewer in number yet larger. At the end of two weeks (fig. 12), germ balls can be seen at the posterior end of the embryo miracidium, while the activity has still further increased. On the sixteenth day (fig. 13), the embryo is nearly as long as the egg, the eye spot has appeared and more germ balls are visible. For the first time flame cells are to be seen. They are two in number, one being anterior to the other. Also the primitive digestive tract is visible. On the seventeenth day (fig. 14), the germ balls have increased to about twelve or fifteen in number, the primitive digestive tract is more clearly outlined, the epithelial cells are becoming quite opaque and thus the flame cells can hardly be seen. The activity of the miracidium has also greatly increased. On the twentieth day (fig. 15), the embryo has greatly increased in length, being now about 0.120 mm., but this is accompanied by a decrease in width.

Because of this increase in length, the posterior end is often doubled back in the egg. The primitive digestive tract is larger and the germ balls are more conspicuous. For the first time the cilia on the surface of the body are clearly distinguished. The activity now is often violent, the miracidium lunging back and forth against the operculum. The egg is now ready to hatch. Figure 16 shows another mature miracidium also ready to hatch, in which the central nervous system is apparent.

I am inclined to believe that studies on the development of other echinostomes will show the above description of development to be characteristic of the family. Because of the opaque condition I was unable to determine whether the two flame cells are joined by short, nearly straight capillaries or if the capillaries are long and much coiled. The excretory pores are located on each side near the posterior end. The excretory system of most miracidia seems to consist of but two flame cells and two capillaries. Looss (1896, pls. XI and XII) finds the excretory system of the miracidium of *Gastrodiscus aegyptiacus* (Cobbold), *Gastrothylax gregarius* Looss, and "*Distomum hepaticum* variet. *aegyptica*," following this plan. Looss (1892, pl. XLX) again shows that in "*Amphistomum subclavatum*," the excretory system consists of but two flame cells and two capillaries.

A few minutes after figure 15 had been drawn, I was fortunate enough to observe the miracidium escaping from the egg. After many and vigorous lunges the operculum opened as if on a hinge (fig. 17). Within three seconds the whole miracidium was out and swimming vigorously about in search of its proper host. The operculum measures 0.023 mm. in diameter. Inside the newly hatched egg (fig. 17) is to be seen a considerable amount of waste and oily material. This material as well as the operculum is lost, however, inside of a few hours (figs. 18 and 18a).

Although the echinostome eggs are much alike in shape they differ considerably in size. Even in the same species there is considerable variation. Perhaps the largest echinostome egg of unquestionable record is that of *Pegosomum saginatum* (Ratz), the length of which is 0.130 mm. and the width 0.089 mm., but even here there is great variation in size. One of the smallest echinostome eggs is produced by *Monilifer spinulosus* (Rud.), measuring but 0.069 to 0.072 mm. in length by 0.048 to 0.050 mm. in width. Although variation in size of eggs is common to nearly all digenetic trematodes, yet no species seems to have greater variation than the records given for *Echinostoma*

revolutum. The greatest length yet recorded for this species is by Looss (1899, p. 682), who has obtained the extreme measurement of 0.134 mm. Throughout the whole genus *Echinostoma*, however, the mean is not far from 0.110 mm. The great variation in size of eggs in *Echinostoma revolutum* suggests that Looss (1899, pp. 679-684) and others, have placed several species under this one name. This will be more fully discussed under the heading of the adult echinostome.

INFECTION OF SNAILS AND DEVELOPMENT OF MOTHER-REDIAE

Young snails of *Physa occidentalis* were placed in syracuse dishes with the eggs of *Echinostoma revolutum* in which the miracidia were almost fully developed. These young snails were known to be free from any trematode infection since they were raised in small aquaria from eggs produced during the months of August and September, 1918. To make doubly sure, many of the young snails were killed and carefully examined but all these examinations gave negative results.

At the time of the first experiments the snails were about six months old. Usually eight snails were placed in each syracuse dish with about fifteen eggs. During the first day almost all the eggs were eaten by the snails, but they passed through the digestive tract of the snails unharmed. They remained in the feces until they hatched, which occurred at the end of twenty-one days.

In one syracuse dish in which no young snails were placed, the movement of the miracidia outside the egg was studied. Their activity is much like that of other miracidia for about twenty-four hours. At the end of that time the vigorous activity quite rapidly subsides, followed in a short time by a breaking of the body wall and the extrusion of some of the contents.

In the other syracuse dishes the miracidia must have penetrated the young snails almost immediately since they were never to be found free in the water except for a few minutes after hatching. The penetration of the young snail by the miracidium I was never fortunate enough to observe. Because of its large anterior papilla, which is capable of being greatly retracted and then forcibly extruded, I judge that it would encounter little difficulty in piercing the thin body wall of the viscera or even the more rigid foot of the snail.

From time to time, these young snails were carefully examined to see what stage followed. All the evidence points to the fact that the

miracidia metamorphose directly into rediae, which in this paper I shall call mother-rediae. On several occasions very small mother-rediae were obtained, measuring in length from 0.148 to 0.302 mm. After thirty-five days in the tissues of the young snails some mother-rediae had only increased 0.28 mm. beyond the length of the miracidium, thus being in all only 0.148 mm. long in their most contracted condition. The smallest contracted free daughter-redia ever seen measured 0.180 mm. in length, or 0.032 mm. longer than mother-rediae when five weeks old. If these young mother-rediae had been daughter-rediae instead then there would also have been present large rediae, which never were found even after the most careful examination. Also if I had missed a generation, and these mother-rediae were really daughter-rediae, then large numbers of daughter-rediae should have been found. But never were more than three found in a snail and that only once, the rest contained two or one mother-rediae or none. Also the shape at this time is somewhat different from that of the daughter-rediae. These four facts taken together make me feel certain that there is no intervening stage between the miracidia and the rediae found in the experimental snails.

The variation in the size of these small mother-rediae is due partially to the degree of contraction. They are more sensitive than when mature since they contract and expand if slightly interfered with on a slide. Figure 19 shows a mother-redia in four different shapes due to the degree of contraction. Figure 19*a* shows the mother-redia to be almost sac-shaped with no trace of the posterior protrusions. Figures 19*b*, *c*, and *d* show the posterior protrusions becoming more prominent as the length increases. The blind intestine also shortens and lengthens with the body.

Because of the opaqueness of the body of the young mother-redia, the germ balls could not be made out. How long it takes the mother-redia to develop to maturity and to produce another parthenogenetic generation is as yet undetermined, but probably it is accomplished in about three months during the warmer weather.

REDIAE CONTAINING DAUGHTER-REDIAE

In thirteen snails of *Physa occidentalis*, taken from Stow Lake, San Francisco, rediae with daughter-rediae inside, were found. Although I have never obtained very small rediae containing daughter-

rediae in these snails, and although I have not traced the cycle of the daughter-rediae, yet I feel quite certain that there is only one such generation. Because of the fact that I believe only one generation of daughter-rediae to exist, I shall call the rediae enclosing daughter-rediae the mother-rediae. These mother-rediae, I feel quite certain, have been derived by metamorphosis from miracidia, as were the mother-rediae found in the young experimental snails.

This viewpoint is supported by the fact that of the thirteen snails found to harbor the mother-rediae, eleven contained only a few rediae, and of these there were never more than four or five mother-rediae, usually less. In the two cases where mother-rediae were found in snails having many rediae producing cercariae, these mother-rediae were limited to three or four in number. Had there been more than one generation of daughter-rediae, then numerous rediae with daughter-rediae inside would have been found since the daughter-rediae and germ balls inside of one redia often exceed seventy.

Further only one generation of daughter-rediae is necessary to produce the average number of rediae found in a single snail. The cases of two or three hundred rediae being found in one snail could be explained by two, three, or four miracidia finding their way into a single snail, which undoubtedly happens many times. Figure 26 shows a mother-redia containing ten daughter-rediae in which the pharynx and digestive sac is clearly distinguishable besides thirty-one good sized germ balls and many smaller ones. In another mother-redia were found seventy-one daughter-rediae and good sized germ balls as well as many smaller ones.

When the daughter-rediae emerge they measure from 0.180 to 0.250 mm. in length, depending on the degree of contraction, and contain small germ balls (fig. 29). At the time of emerging the activity is usually quite pronounced. The shape is nearly like that of a fully developed redia with quite prominent posterior projections, but the "collar" and birth pore are very small or entirely absent.

The daughter-redia stage was not found before December, 1918, but this is readily explained by the fact that only those snails having a heavy infection were examined carefully, since the material was so abundant. It is also likely that the daughter-redia stage is somewhat seasonal and thus the chance of finding it is greatly reduced.

The daughter-rediae find their way out through the birth pore one at a time, thus allowing room for other germ balls to increase in size.

REDIAE CONTAINING CERCARIAE

The rediae that produce the cercariae of *Echinostoma revolutum* are, I believe, those that have emerged directly from the mother-rediae, which were probably derived by metamorphosis from miracidia. In all structural characters the rediae producing daughter-rediae and the rediae producing cercariae, seem to agree at maturity.

The rediae are quite characteristic even though they vary greatly in size, shape, color, activity and number of cercariae and germ balls enclosed.

Rediae containing active or mature cercariae vary from 0.31 to 2.80 mm. in length, the average being about 1.60 mm. The smaller rediae contain few, usually only one, active cercariae and a small number of germ balls. The larger ones may contain as many as eleven active cercariae with perhaps seventy good sized germ balls, besides many other smaller ones. Active cercariae are nearly mature and always contain prominent concretions. The count of the active cercariae was often made upon this characteristic, which was found to be wholly reliable. Occasionally a large redia may contain only one or two active cercariae and a few germ balls, and on a very few occasions only two or three germ balls and no active cercariae. These I judge to be old rediae producing their last cercariae before final deterioration, as several entirely empty and apparently lifeless rediae were seen. From fifty rediae taken at random the average number of active cercariae proved to be five with also at least fifty fair sized germ balls.

This great variation in size of rediae is accounted for in two ways, first, that there is no very definite adult size, and second, that mature cercariae are produced long before the rediae are full grown. They possibly continue to grow and to produce cercariae until the last of the germ balls are matured. I am inclined to think that the first explanation accounts for most of the variation.

The great variation in number of germ balls contradicts Ssinitzin's theory (1911) that the numbers of cercariae and germ balls of a given species fall within a certain definite yet quite a large range. To a certain extent the number of germ balls is determined by the size of the redia, but there are plenty of exceptions.

The shape of the redia is usually that of a stocking when viewed from the side (figs. 26 and 27) and apparently straight when viewed

dorsally or ventrally. Many other shapes may be found, however, such as nearly spherical, very long and slender, or even constricted in one or more places. The characteristic echinostome marks are present in all, however, if viewed from the proper angle. The paired posterior protrusions are usually located near the middle of the body. They extend ventrally, slightly laterally, and protrude about one-half the width of the redia. They are rounded and non-muscular, apparently never moving in a forward direction. Upon these protrusions the redia usually rests when placed in water, the anterior end often swaying from side to side. At the anterior end a circular projection or "collar" is found. This "collar" is somewhat rigid and into it the neck of the redia may be partially withdrawn. Figures 24 and 25 show the elongated and contracted anterior end of the redia. Just behind the "collar," to the left, to the right or dorsally the birth pore is located. It is usually quite prominent. The mouth is located at the anterior end, immediately followed by a pharynx varying greatly in diameter as a result principally of the sizes of the rediae. However, the average diameter is about 0.08 mm. The blind intestine is club-shaped and about twice the length of the diameter of the pharynx.

The color of the redia varies from almost a transparent condition to a heavy dark brown. The former condition is only to be seen in young or active rediae, the latter usually in inactive and apparently very old rediae. The light orange or light brown color, which is the more common, is to be found in both the large and small, the young and old, the active and inactive rediae.

The activity, as stated, varies much, the greatest being in the younger rediae. In one case a newly freed daughter-redia measured only 0.180 mm. in its most contracted form (fig. 22), while extended it had a length of 0.405 mm. (fig. 23). There is, however, as stated no locomotion, rather only a swaying from side to side, using the posterior protrusions as a pivot.

Because the variation of these rediae is so great, it might be asked if all belonged to one species. The answer is that without a question they all are the rediae of *Echinostoma revolutum*. The two main proofs are that from every type of rediae the same cercaria emerged having the definite shape, size, number of spines, and arrangement of excretory system characteristic of *E. revolutum*, and that in a single snail nearly as great variations in each of the above discussed characters were found as in the sum total larval stages from all the snails examined of this species.

The excretory system of the redia (fig. 31) is difficult to see and I found it impossible to analyse it completely. The most difficult part to make out proved to be the capillaries and smaller tubules at either end of the collecting tubules. The excretory pore is located on the side and slightly in front of the posterior projection of the body. Immediately underneath is a small bladder, which gives off two branches, the collecting tubes, one extending posteriorly and the other anteriorly. Close around the posterior protrusion eighteen flame cells were seen. No plan of branching or attachment of the capillaries could be made out, due to the extreme coiling. At the anterior end, a short distance behind the intestinal sac seventeen flame cells were seen. These capillaries and accessory collecting tubes were also much coiled and so the pattern could not be determined here either. Possibly other flame cells are to be found at either end. It is quite probable that this condition of the excretory system prevails on both sides although in a given specimen it was never so seen. Other rediae, whose excretory system has been worked out, show it to be bilateral and the pattern to be quite definite. This bilaterality is described by Looss (1896, p. 199) in the redia of *Cercaria distomatosa* Sons. This redia also has its capillaries and flame cells arranged in groups of threes. He also shows the young redia of "*Amphistomum conicum*" Rud. (fig. 130) arranged in a definite plan. Again Looss (1892, pl. XX, fig. 9) shows the redia of "*Amphistomum subclavatum*" to be bilateral, having two flame cells on a side. Cort (1918c, pl. VII, fig. 2) also finds *Cercariaeum mutabile* to be bilateral and as far as he could make out, with definite capillary arrangement. From this evidence it seems safe to say that probably the excretory systems in all rediae are bilaterally symmetrical and may have a definite grouping of the capillaries and flame cells.

CERCARIAE

The fully developed cercariae of *Echinostoma revolutum* has a total length of from 0.82 to 1.25 mm., according to the degree of contraction, averaging about 1 mm. when moderately extended and from 0.19 to 0.30 mm. in width, averaging about 0.25 mm. at its widest part. The body alone varies in length from 0.39 to 0.63 mm., depending on the amount of contraction; the tail from 0.43 to 0.62 mm., depending on the same condition. Occasionally in extreme contraction or expansion of the body and tail there is an even greater range of variation.

Generally the length of the body and tail are about equal when moderately extended, each being about 0.50 mm. The width of the tail under average extension is about 0.05 mm. at the widest point. Actual variation in the size of cercariae is not observable, the difference being due entirely to the degree of contraction. The ventral sucker is somewhat larger than the oral sucker; the former measuring on the average 0.094 mm. and the latter 0.072 mm. When seen from the side the ventral sucker is often to be found greatly extruded (fig. 44). It is located just back of the middle of the body.

The number of collar spines is probably always forty-three. These spines have been counted on a large number of cercariae and that number seems to be specific. Although the possibility of missing count by one is easily understood by those who have attempted such counts, I feel quite safe in saying that the collar spines are forty-three in number. The ventral and lateral spines of the collar are from 0.016 to 0.018 mm. in length, being a little larger than the dorsal spines which are 0.014 mm. long. The spines are arranged in two alternate rows, although the ventral ones show more of a clumping than of an alternate arrangement. These collar spines do not appear until perhaps the very last period in the development of the cercaria. Often apparently mature cercariae were found within the redia with these collar spines either absent or faintly developed. This probably explains why cases of echinostome cercariae have been recorded lacking the collar spines.

The surface of the body is partially covered with spines which extend dorsally just a short distance back of the oral sucker. Ventrally they reach well past the ventral sucker where they gradually lose their definite size, shape, and arrangement. Figure 45 shows these spines as arranged on the side of the cercaria between the oral and ventral suckers. They measure in this region 0.006 mm. in length.

The digestive system (fig. 41) is quite easily seen and of considerable size, the intestinal caeca extending nearly to the posterior end. The mouth is slightly ventrad, the typical echinostome position. The pharynx is 0.035 mm. in length by 0.025 mm. in width, and is preceded by a prepharynx about 0.028 mm. in length. The oesophagus under average extension is about 0.14 mm. long, while the bifurcations of the intestine are about 0.23 mm. in length.

The movement of the cercaria is accomplished in two ways, one of which is much slower than the other. The slower movement is leech-like and is used only on a surface. This is the only movement possible

under the cover glass. When free in the water, however, the movement is vigorous. The body, which is concave ventrally between the suckers, is doubled in upon the ventral sucker. The posterior end also is doubled in so that the body is almost in a ball. The tail then lashes vigorously back and forth, usually in one plane, driving the cercaria at a high rate of speed but in no definite direction. The duration of this vigorous movement is usually less than twenty-four hours although longer periods of activity have occasionally been seen. This movement is almost identical with that described by Cort for *Cercaria trivolvis* Cort (1915, p. 37).

EXCRETORY SYSTEM OF CERCARIA

The excretory system of the cercaria of *Echinostoma revolutum* (fig. 47) proved to be extremely difficult to work out. The difficulties were due principally to two things. First to the fact that the entire body from the region of the pharynx to the posterior end is filled with cystogenous glands which are somewhat opaque. The longer the cercariae are free in the water the more opaque these glands become. The second difficulty was due to the complexity and progressive modification of the accessory collecting tubules from the posterior end towards the oral region. Also the exact place of attachment of the capillaries was hard to determine. The fact that only a few flame cells are likely to be active at a given time, some only during or after the bursting of the body wall, and the fact that a cercaria lasted only a few minutes, also added to the complexity of the situation. However, the abundance of material greatly offset these difficulties. In order to work out this system in this cercaria, one hundred and twenty snails were examined, of which eighty-six contained mature active cercariae. From these eighty-six snails probably over two thousand cercariae were studied before the complete ramifications of the tubules and the pattern could be made out. This one system was studied for a period of five consecutive weeks before this pattern was determined.

Since the limits of the bladder are poorly defined, I shall adopt the following nomenclature. The tubes in the tail I shall call the caudal division of the bladder (*1a*); the part commonly called the bladder I shall call the muscular sac of the bladder (*1b*), which may or may not be divided into two parts as drawn in figure 47; the two large winding tubes joining the anterior end of the muscular sac of the bladder I shall designate as the muscular descending tubes of the bladder (*1c*);

the divisions of the bladder containing the concretions I will term the concretionary descending tubes of the bladder (*1d*); the parts of the bladder attached to the concretionary descending tubes (*1d*) and extending backward to the posterior end of the body where each is joined by three collecting tubules, will be called the ascending tubes of the bladder (*1e*); the collecting tubules are numbered *2a*, *2b*, *2c*, and *2d*, respectively, starting from the most anterior one; the accessory collecting tubules are designated as *3a*, *3b*, *3c*, *3d*, *3e*, *3f*, *3g*, and *3h* respectively, also starting from the anterior end; the flame cells are numbered individually from one to twenty-four, while their capillaries correspond, only with the "x" attached. The numbers of the flame cells appear on the left-hand side, while the other designations are on the right side. However, since the two sides are alike it will be easy to transpose to the opposite side when necessary.

The muscular sac of the bladder (*1b*) is located at the extreme posterior end of the body. It may contract quite frequently to force out waste. It is usually of medium size and from its posterior end is given off a branch (*1a*), running into the tail which quickly subdivides into two parts. The subdivisions run nearly at right angles to the main branch, opening to the outside. Through the excretory pores all the body waste seems to pass. As stated, the muscular sac of the bladder usually appears in two parts connected by a narrow neck, but this is far from being constant, so these parts will be spoken of as forming a single part of the bladder (*1b*).

The muscular descending tubes of the bladder (*1c*) join the muscular sac of this organ (*1b*). These tubes of the bladder are also muscular. I have seen them contract and expand to about twice their average size nearly up to the ventral sucker. In the region of the acetabulum the coiling of the muscular tubes of the bladder (*1c*) nearly ceases, and from there to the region of the pharynx the tubular descending division of the bladder widens considerably. Inside these tubes are to be found many prominent opaque concretions. This part of the bladder was not seen to contract and is thus quite distinct from the muscular tubes of the bladder (*1c*) and so is designated "*1d*." The concretions are small at the anterior end, becoming larger and larger until the region of the forking of the intestine is reached, when they gradually become smaller again. These concretions, which I believe to be gradually and continuously formed from the waste liquid, enlarge as they are pushed farther down the concretionary tubes of the bladder (*1d*). Why they again decrease in size, I cannot determine,

but that they do so is certain and that wastes pass out only in liquid condition is almost equally certain. Evidently the concretions are produced about as fast as dissolved and eliminated, since I have never seen an active cercaria without these concretions filling this entire part of the bladder. In the region of the anterior end of the pharynx these tubular divisions of the bladder turn completely round, forming a very characteristic area (fig. 47, *t.a.*).

Here the ascending tubes of the bladder (*1e*) join the concretionary tubes of the bladder (*1d*). The difference between these two divisions of the bladder is principally physiological, i.e., in terms of the presence or absence of concretions. These latter divisions, as stated, are the ascending tubes of the bladder (*1e*). These divisions of the bladder pass ventral to the concretionary tubes of the body (*1d*) at the anterior end, but later come to lie on the outside of and parallel to it. In the region of the posterior end of the ventral sucker, a collecting tubule (*2a*), joins the ascending tubes of the bladder (*1e*). This collecting tubule is joined shortly by a bulblike accessory collecting tubule (*3c*), into which three capillaries enter that drain the wastes by means of three flame cells from the region of the acetabulum. A short distance anteriorly three other capillaries join this collecting tube (*2a*). These capillaries unite singly some distance apart with the collecting tubule. However, we might consider that the part of the tubule between capillaries *4x* and *6x* represents the accessory collecting tubule (*3b*). This seems probable because the accessory collecting tubule (*3c*) is reduced to a mere bulb and because even here the capillaries do not join exactly at one place. At the anterior end of the collecting tubule (*2a*), three other capillaries are received which drain the wastes from the oral region. Here again the accessory collecting tubule (*3a*) has either been eliminated or is represented by the tubule between capillaries *2x* and *3x*. In this latter group the capillaries are even farther apart than the ones forming the group just preceding. This loss or modification of the accessory collecting tubules *3a* and *3b* is shown graphically by comparing text figure No. 1 with figure 47. Thus to collecting tubule (*2a*) three groups of three flame cells each are joined, directly or indirectly, by accessory collecting tubules.

At the posterior end of the body, the ascending tube of the bladder (*1e*) receives three collecting tubules (*2b*, *2c*, and *2d*). One of these collecting tubes (*2b*) is considerably longer than the others. It receives two sets of three capillaries and flame cells, numbers 10, 11, 12, and 13, 14, 15. The most anterior group of capillaries arises nearly

from the same point and drain the region at the posterior end of the acetabulum, while the other set is just a short distance behind, each capillary being attached singly. If the accessory collecting tubule for the first group exists it may be said to be between the capillaries *11x* and *12x*, while the latter group might be said to have for its accessory collecting tubule the part between *13x* and *15x*. Another collecting tubule (*2c*), if such can be said to exist, receives a very short accessory collecting tubule (*3f*), which in turn receives three capillaries almost uniting at the same point. The flame cells in this group are numbers 16, 17, and 18 and are located just behind the group previously mentioned, being about half-way between the acetabulum and the posterior end of the body. The other collecting tubule (*2d*) is very short. Into it flow the wastes of the accessory collecting tubules (*3g* and *3h*). To the former of these (*3g*) join capillaries of flame cells 19, 20, and 21; to the latter (*3h*) capillaries of flame cells 22, 23, and 24.

Thus the ascending tubes of the bladder (*1e*) are joined on each side by four collecting tubules. One receives three sets of three flame cells each; two receive two sets each, and one only one set. On each side of the body are twenty-four flame cells. The formula for this system is then " $2 \times 8 \times 3$," totalling forty-eight flame cells, according to the plan used by Looss (1894, p. 68) and lately adopted by Cort (1919, p. 2). There are no flame cells found in the tail. The anterior half of the body contains only six flame cells on each side, while the posterior half has eighteen. In terms of the cercaria this arrangement is hard to explain, but in the light of the adult echinostome the number at either end is quite readily understood. In the adult (fig. 49) the region of the body back of the ventral sucker is several times longer than that anterior to it. Since I believe that the number of flame cells of the cercaria is the same as that of the adult, it is plain that a stretching of his system to meet the adult's growth at the posterior end would result in a uniform distribution of the flame cells throughout the entire body when the worm is full grown.

As stated previously, a significant progressive modification of the attachment of the capillaries is noticeable throughout the cercaria. At the posterior end of the body the groups of three capillaries join at a common point on the accessory collecting tubule and form very definite capillary groups. The more anteriorly the capillaries join the more widely separated are the groups on the collecting tubule or its accessory. At the anterior end, unless the plan of arrangement of the capillaries into threes were known to exist throughout the rest of the

body one would certainly say that each joined the collecting tubule or its accessory singly instead of in threes. However, knowing of this grouping into threes at the posterior end, it is not difficult to make the proper association here. This widening of the distance between the attachment of the capillaries in a group is accounted for by the gradual loss of the accessory collecting tubules or by the merging into one of this and the main collecting tubule.

These groups of three flame cells are arranged dorsally and ventrally in a definite way. The most anterior group is essentially a dorsal group appearing quite close to the upper surface. The next group of threes is a ventral one, the next one is dorsal, the next ventral, and so on to the posterior end. I cannot be absolutely sure of the dorsal-ventral arrangement of the last two groups, however, because the groups are much coiled, yet I am reasonably certain that they are so arranged. This arrangement is probable if the entire body is to be drained equally. Again within the group of threes there is a definite arrangement. Starting at the anterior end the first flame cell is dorsal, the next ventral, the next dorsal; then in the next group of threes the first is ventral, the next dorsal, the next ventral, and so on. This again is a proper arrangement if dorsally and ventrally the body is to be equally well drained of wastes. These two plans, or some modification of them, I should expect to find in all echinostome cercariae and adults.

From a study of the literature and the drawings of the excretory systems of cercariae that have been worked out, and from my own work, it seems probable that the flame cells and their capillaries are arranged in definite groups connecting with the accessory collecting tubules, and that each group though modified through increase, reduction, or loss of parts has come from the mitotic division of a single flame cell. In some cercariae, such as those of *Schistosoma japonicum* or *Cercaria douthitti*, the capillary groups have not yet arisen, since the capillaries are arranged singly, but I should expect to find in the adult worm each flame cell in the cercaria represented by a group of flame cells. In *Echinostoma revolutum* the groups are already present, hence in the adult I should expect to find the same number of flame cells. The excretory system of the cercaria of *Echinostoma revolutum* has been modified somewhat and is hard to interpret as a definite unified arrangement throughout. This theory of the formation of capillary groups was first given by Looss (1894) and later accepted by Cort (1918a). Even in 1881 (pl. 1, fig. 4) Fraipont shows the formation of two flame cells by mitotic division.

In the cercaria of *Echinostoma revolutum* sometimes the collecting tubules and again the accessory collecting tubules are much reduced or have entirely disappeared, as shown in figure 47, 3c. This reduction or loss is probably due to several causes, which might all be summed up by calling it adaptation to the position they occupy in the cercaria's body or to the position they will eventually occupy in the body of the adult echinostome.

1a, caudal division of bladder;
 1b, muscular sac of bladder;
 1c, muscular descending tube of bladder;
 1d, concretionary descending tube of bladder;
 1e, ascending tube of bladder;
 2a, 2b, 2c, 2d, collecting tubules;
 3a, 3b, 3c, 3d, 3e, 3f, 3g, 3h, accessory collecting tubules;
 1-24, flame cells.

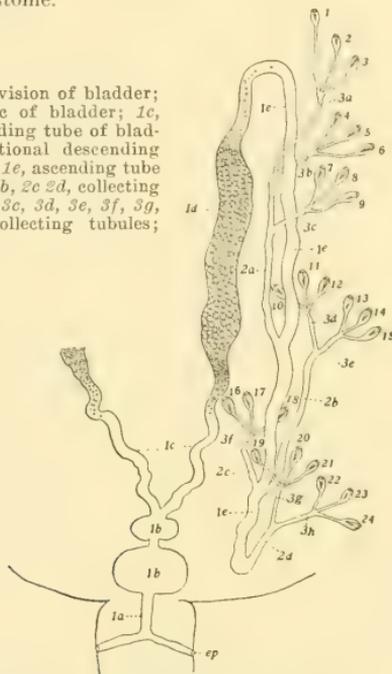


Fig. 1. A diagrammatic drawing showing the probable arrangement of the excretory system of the primitive echinostome cercaria.

The above figure represents graphically what I consider to be the generalized system of an echinostome cercaria from which the present system probably arose. The comparison of this diagram with figure 47 illustrates a method by which the capillary grouping of a system could be progressively modified until the exact pattern is somewhat obscured.

The drawing (text fig. No. 1) shows particularly what I think has happened to the accessory collecting tubule (3c), to which are attached the capillaries draining flame cells 7, 8, and 9. Compare with figure 47

which represents the actual condition. As shown graphically this accessory collecting tubule is quite long, whereas in the actual specimen it is reduced to a mere bulb, to which the capillaries join. In figure 47 we find that the accessory collecting tubule (3b), to which are attached capillaries 4x, 5x, and 6x, has entirely disappeared or perhaps better, is stretched out between these capillaries. The same condition exists in the case of the accessory collecting tubule of capillaries 1x, 2x, and 3x. Near the posterior end of the ascending tubular division of the bladder (1e), two collecting tubules (2b and 2c) are attached, one on the left and the other on the right-hand side. The one on the right side (2b) receives, as shown graphically, two accessory collecting tubules (3d, 3e), which in figure 47 are entirely absent or are stretched out between 10x, 11x, 12x, and 13x, 14x, and 15x respectively. The collecting tubule on the left (2c) merges directly into accessory collecting tubule 3f as shown graphically; in figure 47, 3f has disappeared unless it is to be found between the capillaries (17x and 18x). The last collecting tubule (2d), in the graph and in the living specimen is short, and is joined by accessory collecting tubules (3g and 3h). Thus it seems that what I have suggested as the primitive pattern has been considerably modified, possibly to meet the shape and needs of the cercaria or of the adult echinostome.

The study of the development of the bladder in the cercaria of *Echinostoma revolutum* is of peculiar interest and yielded some definite results. The first appearance of the bladder or any part of the excretory system was seen in a germ ball 0.134 mm. long and 0.104 mm. wide (fig. 37), which had just commenced to change from the typical round germ ball. At this time two widely separated tubes extending nearly the entire length of the body were present. At the anterior end the tubes appeared to end in flame cells which could not be clearly distinguished, due to the lack of movement of their cilia. Each tube clearly must function independently of the other since the two excretory pores are now 0.045 mm. apart. When the embryo reaches a length of 0.280 mm. the suckers are distinct; the digestive system is fairly well developed; the tail has commenced to differentiate from the body, and the excretory pores are closer together, being only 0.018 mm. apart (fig. 38). When the embryo is 0.360 mm. long the digestive system is complete. At this time the inner surface of the walls of the excretory tubes, a short distance above the excretory pores, nearly touch each other (fig. 39). The tail is now quite decidedly differentiated from the body. At about one-fourth of the distance from

the base of the body to the end of the tail the excretory tubules bend almost at right angles and empty laterally. Figure 42 shows the right half of this primitive muscular bladder in various shapes, drawn within a few seconds of each other. These swellings or bulbs give evidence of a process similar to peristalsis in the elimination of excretory waste and show that in this early stage the bladder is quite muscular. When the embryo is 0.710 mm. long the final shape of the cercaria is nearly attained. At this stage the union of the two tubules of the primitive muscular bladder is partially accomplished (fig. 40) just at the juncture of the body and tail. In cercariae 0.765 mm. in length (fig. 41) the process of union is nearly completed, extending both into the body and tail. Thus the tubule found in the tail of mature cercariae is really a part of the bladder. Principally upon similar data in regard to the development of the bladder tubules in the tail, Ssinitzin (1911) based his theory that the body proper of many cercariae really extends to the region of the excretory pores in the tail and not to where the slender part ordinarily called the tail joins the body. He then holds that the true tail is an outgrowth from tissue found just posterior to and between the excretory pores. There is considerable evidence, aside from the work of Ssinitzin, to uphold this theory. Looss (1896, figs. 147, 155, 172, 173, 174) shows that in both monostomes and distomes such a formation of the bladder takes place. Also Looss (1892, pl. XX) shows several stages in the development of an amphistome cercaria portraying the same development of the bladder. From this evidence it seems to me quite logical to say that the body proper of such cercariae extends to the excretory openings on the side of the tail and that the true tail extends only from this point on.

Since the bladder sac is derived from the union of two tubes in the embryo, then certainly, if necessary, they could unite for a longer distance and form a much larger bladder. Also since the muscular descending tubes (*1c*) have the power of contraction and expansion it is obvious that to limit the bladder to the muscular sac is not correct. Also since the concretionary tubes of the bladder (*1d*) are continuations of the muscular tubes (*1c*), and since the ascending tubes (*1e*) are directly continuous with the concretionary tubes (*1d*), then it would appear to follow that all these parts form the complete bladder.

No record can be found of the complete analysis of the excretory system at any stage of an echinostome. The nearest approach is the work of Looss (1894, fig. 191), in which he describes this system of

the cercaria, which he believes to belong to *Echinostoma revolutum* (= *Distomum echinatum*). This cercaria, however, is not the cercaria of *Echinostoma revolutum*, as will be proved under the discussion of the adult. The smaller tubules and capillaries as well as the large tubules, divisions of the bladder and excretory pores, correspond with the cercaria of *Echinostoma revolutum*. The posterior part of the excretory system he fails to show in detail. This failure can now readily be understood because of the cramped condition of the tubules and the density of the cystogenous glands. The anterior arrangement of the tubes of the bladder is nearly the same as in the cercaria of *Echinostoma revolutum*. The only difference is that at the anterior end the triangular arrangement of the tubes of the bladder as they bend to proceed posteriorly is not present in Looss' form. Lebour (1912, pl. XXVIII) shows three cercariae in which the several divisions of the bladder, as far as worked out, resemble the bladder of the cercaria of *Echinostoma revolutum*.

Faust (1917 and 1918) describes four new species of echinostome cercariae, *Cercariae trisolenata*, *Cercaria biflexa*, *Cercaria chisolenata*, and *Cercaria acanthostoma*. He figures parts of the excretory system of each of these species but in no one does he find a definite pattern for the collecting tubules, accessory collecting tubules, capillaries, and flame cells. Even the part which I have called the complete bladder he has described very differently in his forms.

The excretory system of the digenetic trematode is quite conservative. Wherever the excretory system for different members of the same family has been worked out a marked uniformity is found. Looss (1894, pl. VIII, figs. 157 nad 163) found two distomes, *Opisthoglyphe ranae* (Froelich) and *Haplometra cylindriacca* (Zeder), which belong to the same family, built on the "2 x 6 x 3" plan. Cort (1917) found four fork-tailed cercariae, *Cercaria douthitti*, *Cercaria emarginatae*, *Cercaria douglasi*, and *Schistosoma japonicum*, with the flame cells arising singly. Since the echinostome family is so uniform in regard to other characters, it seems very improbable that such a conservative system as the excretory should vary greatly. The echinostome excretory systems described by Faust are almost entirely different from those described by Looss (1894), Lebour (1912), and Cort (1915), and from that of *Echinostoma revolutum*. Faust makes the following statement concerning the excretory system of echinostomes: "This family of distomes is characterized by simplicity of detail in the excretory system except at the head of the main lateral vessel." From

my study of the cercaria of *Echinostoma revolutum* I am inclined to think that just the opposite is true. Certainly the excretory system is very complex in this one species and since it is such a conservative system I can hardly believe the species of the Echinostomidae differ much on fundamental points. I even venture to suggest that when this system is studied in detail in more echinostomes the detailed arrangement of parts will be found to be quite similar in pattern.

The parts of the excretory system found in the tail of *Echinostoma revolutum*, although very simple in arrangement, were found hard to work out on account of the deceptive appearance of the muscles in this region. This part of the excretory system of *Echinostoma revolutum* corresponds with the description given by Looss (1894, fig. 191), by Lebour (1912, pl. XXVIII, figs. 9, 13, 17) and by Cort (1915, figs. 39 and 43). This makes a total of at least seven echinostomes showing the same arrangement of the bladder tubules in the tail of the cercariae. The four echinostome cercariae described by Faust differ decidedly on this characteristic from any of the above. They also differ much from each other. He shows none of them with excretory pores in the tail, and the excretory system in the tail of *Cercaria trisolenata* is shown as a single tube. Faust makes the following statement in regard to *Cercaria biflexa* (1917, p. 79): "The excretory tube in the tail is a single median tube for about two-fifths of the way distad, at which point it forks and continues double the remainder of the way distad, with numerous cross anastomoses. It does not open to the outside either on the sides or end." The excretory system in the tail of *Cercaria acanthostoma* Faust finds to be still different, as is shown by the following quotation: "The excretory system in the tail is confined to a long sac-like reservoir, extending the entire length of the organ without any definite wall or lining. Near the proximal end it frequently bulges out on each side to form a lateral reservoir." He shows this sac-like reservoir in the tail to be fully four times the size of the muscular sac of the bladder. *Cercaria chisolenata* he finds to be somewhat like *Cercaria trisolenata*, although the ending of the excretory tube is not quite so definite. I feel quite certain that he confused the tail excretory parts with the tail muscles. This statement is borne out by the fact that in *Cercaria chisolenata* (1918, fig. 9) he shows the tube in the tail and the muscles of the tail with the same type of line and then loses the excretory tube in the muscles. This apparent extension of the excretory tube in the tail as shown in *Cercaria chisolenata* and *Cercaria biflexa*, I have observed in the

cercaria of *Echinostoma revolutum* many times, but upon a more careful study the two excretory tubes and pores could also be seen. This proved to be one of the most difficult points to determine because of the deceptive appearance of the museles. That the wide variety of excretory parts in the tail of echinostomes exists as described by Faust, seems very improbable in such a well defined family and especially so since Looss, Lebour, Cort, and myself have found a uniform type with two excretory pores in the tail.

Nowhere in the middle or posterior parts of three of his cercariae does Faust find flame cells. That they exist there I feel quite positive. The failure to see flame cells and tubules in these regions is probably due to the heavy cystogenous glands in these three species. Only at the anterior end where there are no cystogenous glands does he find the flame cells. In *Cercaria acanthostoma*, however, he shows flame cells directly connected singly with tubes which are equivalent in the other species to the anterior tubes of the bladder. That these flame cells join directly to the tubular division of the bladder is improbable, since in no cercaria where the excretory system has been carefully and completely worked out does this occur. Rather do the flame cells, by means of their capillaries, unite with accessory collecting tubules.

At the anterior end in each of these four new echinostome cercariae described by Faust, he shows three flame cells on a side. These three flame cells are probably present in all echinostome cercariae. Their arrangement and attachment is probably a family characteristic. I feel quite sure, however, that these three flame cells do not unite with the concretionary tubes of the bladder as shown in *Cercaria trisolenata* and *Cercaria chisolenata*, or even as in *Cercaria acanthostoma*. As stated above, capillaries are not known to unite with parts of the bladder. In the arrangement and attachment of these three anterior flame cells, *Cercaria biflexa* (Faust, 1917, fig. 138) is much like the cercariae of *Echinostomum revolutum* and "*Distomum echinatum*" as described by Looss. But even in *Cercaria biflexa* I am inclined to believe that the tubule to which the three anterior flame cells are attached does not bend forward and join the tube of the bladder but proceeds much farther posteriorly, joining the tube of the bladder in the region of the acetabulum (fig. 47. 2a). The arrangement shown in *Cercaria biflexa* (Faust, 1917, fig. 135) by which the fine capillaries join the tubes of the bladder is also an improbable arrangement. These capillaries are probably equivalent to the branching of the tubes of the bladder as shown by Lebour (1912, pl. XXVIII, figs. 9, 13, 17).

Concerning any of the criticisms of Faust's work, I will say in fairness to him, that since none of his species has been studied, I may have carried my criticisms based on comparison too far. It must be said, however, that his arrangement of the excretory parts violates some of the most fundamental homologies of excretory systems. Also since details are so lacking in Faust's work the conclusion is reached that he really did not completely solve the pattern of the excretory system of any one of the four echinostome cercariae.

Because of the numerous flame cells,* the complexity of the arrangement of the collecting tubules and accessory collecting tubules and the definite arrangement into capillary groups throughout the body, it seems quite probable that the excretory system of the echinostome cercariae represents much more closely the adult condition than the condition found in the schistosomes or fork-tailed cercariae, in which the flame cells arise singly and are few in number, and in which the pattern is quite simple.

The general knowledge of the excretory system of trematodes is comparatively meager. In nearly every cercaria described, the bladder and larger tubules are shown, whereas the collecting tubules, accessory collecting tubules, capillaries and flame cells are missing or nearly so. This is, as stated before, due to working on preserved material.

* Shortly after this manuscript was completed, Faust published in the Biological Bulletin for May, 1919, an article which included data on the excretory system of echinostome cercariae. In the summary of this article, "The Excretory System in Digenea, II," he makes the following statements:

1. "The number and distribution of groups of flame cells is the fundamental basis of structure of the excretory system of the distomes." This agrees with my findings in all cercariae that I have studied.

2. "The group of flame cells is typical of all members of a family or at least of a sub-family." My work also suggests the fundamental significance of the capillary group.

3. "Seven new species of distome cercariae are described, which are of special importance in affording evidence of the orderliness of the excretory system of the Digenea." This statement also is substantiated not only by my study of *E. revolutum* but also by unpublished work on fork-tailed, stylet, and monostome cercariae. If the above statement concerning the orderliness of the excretory system is universally true, then much of the previous work on this system in Digenea indicates incompleteness.

The descriptions of the excretory systems of echinostome cercariae in this paper agree more closely with my findings in *E. revolutum* than did Faust's earlier work. *Cercaria complexa*, which he figures, shows the tail arrangement of the excretory system to be identical with that of *E. revolutum*. The flame cells of *C. complexa* likewise are arranged much like those of *E. revolutum*. Probably a larger number of flame cells are present in *C. complexa* than are shown and would have been seen except for the numerous heavy cystogenous glands. The arrangement of the flame cells at the anterior end of *C. complexa* is particularly like that of *E. revolutum*. I should expect, upon thorough study of other echinostome cercariae to find that the anterior grouping of flame cells is very similar in them to that in *C. complexa* and *E. revolutum*.

It is interesting to note that as more thorough studies of the excretory system in Digenea are made, more uniform results are obtained.

But even though the larger parts of the system have been long known, yet for a considerable time they were confused with parts of the digestive tract, the oviduct and other parts of the genital system (Fraipont, 1880, pp. 415-420). The flame cell itself was perhaps first seen by Bütschli in 1879, so it is improbable that earlier workers thought of a unified system. Even after the flame cells and capillaries had been found, they were considered for a time as distinct from the larger tubules and bladder. That this confusion could readily exist can now be well understood, because of the minuteness and complexity of the parts of this system.

In reviewing the literature on the excretory systems of cercariae that have been completely worked out, the conclusion, I believe, can be safely drawn that there is a definite plan of arrangement of the collecting tubules and the accessory collecting tubules, when developed, and that the capillaries and flame cells are found in groups, nearly always of an equal number throughout the body. This grouping of the flame cells, naturally can occur in cercariae only where the excretory system in the cercaria approximates to the same condition as that found in the adult.

Looss, in 1892, thirteen years after the discovery of the first flame cell in a digenetic trematode, worked out quite completely the excretory system of "*Amphistomum subclavatum*." In the cercaria he found the excretory system, as well as other parts, to be bilaterally symmetrical and the capillaries and flame cells to have a definite pattern. Looss (1894) also found that the excretory system of the adult worm, *Allocreadium isoporum* (Looss) was on a definite " $2 \times 6 \times 4$ " plan; that *Opisthoglyphe ranae* (Fröl.) was built on the " $2 \times 6 \times 3$ " plan; that *Haplometra cylindracea* (Zeder) was also built on the " $2 \times 6 \times 3$ " plan. Several other species included in Looss' paper showed a definite grouping of flame cells on each side as far as he could trace them. Sometimes the anterior end and again the posterior end would be too opaque to trace the fine capillaries. Again he found (Looss, 1896) in *Anchitrema sanguineum* (Sons.) a definite arrangement, although some of the capillary groups are in twos while others are in threes. However, in each case both sides of the body are the same. Cort (1918a, fig. 2) shows *Agamodistomum marcianae* (La Rue) to be built on the " $2 \times 10 \times 6$ " plan. Cort (1918c, pl. VII) shows *Cercariaeum mutabile* to have a definite " $2 \times 8 \times 4$ " plan. He (1919a) also shows *Cercaria polyadena* to have a definite formula, namely " $2 \times 6 \times 3$." Dollfus (1911, figs. 1 and 2) shows the excretory system of *Gymnophallus*

somateriae Levinsen to be built on the "2×4×2" plan, and that of *Cercaria pectinata* Huet on the "2×7×2" plan. The cercaria of *Echinostoma revolutum* is built on the "2×8×3" plan. Two stylet cercariae excretory systems (unpublished) that I have worked out also show a definite arrangement of the tubules and grouping of the capillaries.

When many more excretory systems have been worked out I believe that a sound classification of the families, genera, and species can be formulated. However, I am not suggesting that a classification should be built upon this system only.

ENCYSTED AGAMODISTOMES OR CYSTS

The cercariae of *Echinostoma revolutum* form encysted agamodistomes or cysts which are most commonly found in *Physa occidentalis*. They vary in diameter from 0.147 to 0.181 mm., but the great majority are about 0.165 mm. Except for the numerous opaque concretions in the tubular divisions of the bladder, the agamodistomes as well as the cyst wall are nearly colorless. These agamodistomes may be found encysted in any part of the body of the snail, from the foot to the extreme end of the viscera, with apparently no preference as to their location. Two hundred and eighty-two snails out of the four hundred examined, or 70.5 per cent, were infected with this stage. Out of the two hundred and eighty-two so infected, ninety-five, or nearly 34 per cent, harbored only the cyst. In this respect this species is different from the majority of digenetic trematodes in that the secondary intermediate host is the same as the intermediate host. However, Lebour (1912, p. 442) states that, "in exceptional cases the echinostome cercariae may encyst in its first host." Cort (1910, p. 37) finds both the cercariae and cyst of *Cercaria trivolvis* in the tissues of *Planorbis trivolvis*. He also found that *Cercaria reflexa* encysted in the same snail, *Lymnaea reflexa* (p. 42). Faust (1917) found *Cercaria trisolenata* and its cyst in the same host, occurring both in *Physa gyrina* and *Planorbis trivolvis*. He also found the cyst of *Cercaria biflexa* in the tissues of its host beside the cercariae. From the above and other similar evidence, I believe that as more echinostome life cycles are partially or completely known that encystment in the same host with the cercariae will be found to be a common family characteristic.

It often happens that the cercariae escape from the snail only to reënter the same specimen to form the cyst. However, since 34 per cent of the snails containing cysts had only this stage, it is evident

that the cercariae need not reënter the same snail from which they came. This leaving and reëntering seems to be an unnecessary condition, equivalent to going up hill to go down again. It is even worse than that, since there is a decided waste of cercariae, and thus the possibility of the continuation of the life cycle is lessened. It is certainly safe to say that many more cercariae fail to find their way to another suitable host or back to the same snail than those that succeed. Such a waste of cercariae would be entirely avoided if the cercariae encysted in the same snail. Faust (1917, p. 80) found that *Cercaria biflexa* encysted within the host immediately upon breaking through the birth pore of the redia. Such a step seems an obvious and probable shortening of the process. I believe that it does take place quite often with the cercaria of *Echinostome revolutum*. The evidence is from two sources: first, from the fact that in some cases the number of cysts exceed four hundred, while three hundred and two hundred cysts in the same snail is not uncommon. That four hundred or three hundred cercariae could find their way into a single snail from the open water seems impossible. Even for two hundred cercariae so to enter seems extremely improbable. I should say that fifty cercariae so finding entrance to one snail would be unlikely and in fact in the majority of cases less than fifty cysts are present. The second evidence is more direct, since a still further shortening of the process of encystment has been seen many times. When this shortening occurs the cercariae encyst within the rediae without ever emerging through the birth pore. Almost by accident fifty-eight rediae were found with cysts inside, since in the great majority of snails examined no special attempt was made to see how many could be so obtained. Of these fifty-eight cysts inside rediae, twenty-six occurred singly (fig. 32), six rediae had two cysts each (fig. 33), four contained three cysts each (fig. 34), and two had four cysts each inside (fig. 35). That cercariae coming from the open water could penetrate the rediae and there encyst, two, three or four in number is entirely impossible. In fact, that one cercaria should penetrate a redia and encyst is quite improbable. These rediae containing cysts were found in thirteen different snails. The rediae containing these cysts were of different shapes and sizes, being small, large, of normal shape or constricted at one or more places. They also differed much in color and activity. The majority of these rediae, however, were almost lifeless and of a dark brown color. In the rediae with the cysts were sometimes found active cercariae nearly ready to escape and germ balls of different sizes.

This encysted agamodistome is not specific to *Physa occidentalis* since once I found it in *Lymnaea traski*. Also out of twenty planariae taken from the same rocks as the snails, six contained cysts, one having seventeen embedded in its muscular tissue and another ten. The other four, however, had only one, two, or three cysts. Leeches taken from the same rocks gave negative results, as did the tadpoles of *Hyla regilla* Baird and Girard, and *Notophthalmus torosus* (Rathke), into whose aquaria active cercariae were placed. I predict that a thorough canvass of Stow Lake or other places where this cercaria is found would reveal other hosts for this stage. This lack of specificity of the encysted agamodistome is quite common in digenetic trematodes.

There are at least three ways in which the cyst may be taken into the primary host. The first and perhaps the usual way is when infected snails are eaten by ducks, geese, etc. McAtee (1918) reports, after a careful study of the food habits of seventeen hundred and twenty-five mallard ducks of the United States, that 9.47 per cent of the food consists of animal matter. He says, "Mollusks, the most important element of animal food of the mallard, comprises three-fifths of this and 5.73 per cent of the total. Fresh water snails are represented most numerous, no fewer than fifty sometimes being taken at a single meal." That the American scaup duck, *Marila marila* (Linn.) eats snails is certain, but the exact amount has, so far as I know, never been tabulated. The mallard ducks eat over 90 per cent vegetable food in the wild, but it is likely that a still larger percentage is of vegetable matter in parks where they are fed daily. For this reason I should expect to find ducks living on ponds where additional food is not supplied and where the echinostome infection of snails is equally heavy, to be more parasitized with the echinostome adults than those fed in a park.

Dead snails also are probably eaten by ducks, geese, etc. Hundreds of specimens of *Physa occidentalis* were seen dead on the surface of Stow Lake. Since this water is not used for drinking purposes, and therefore no chemicals are used, and because there were also many healthy active snails, I feel sure that their death was caused largely by the echinostome parasites. The dead snails were found upon examination to be heavily parasitized in nearly every case. Sometimes the infection was so heavy that in quantity it was about one-third the size of the viscera of the snail. If only cysts were present, little harm would probably result, but with an abundance of rediae absorbing food and giving off wastes into the body, considerable injury is bound

to be done, and in extreme cases I believe death results. Lebour (1912, p. 423) makes this statement concerning the effect of the parasite on the host: "The presence of sporocysts and rediae certainly injures the molluscan host. The outer appearance of the digestive gland is usually enough to show if it is inhabited by these parasites. It looks unhealthy and is a grey, sickly yellow or a peculiar unnatural orange. It is generally completely riddled with the sporocysts or rediae which feed upon its substance. If the gonad is infected it is quite destroyed. On the other hand encysted cercariae seem to do little or no harm to their hosts, even though present in large numbers." From my own observations, and from statements by other workers, I am inclined to believe Lebour is right. During the fall of the year dead snails are most abundant. This fact is significant in light of the fact that the rediae and cercariae are also most abundant at this time. The snails detach themselves at death from the rocks and float to the surface, having nearly always a large part of their body extruded from the shell and thus they are very accessible as food to the water birds. Probably in this way many birds are infected.

Another method of infection is that cysts are scooped up from the bottom of the pond by ducks, geese, etc., in their search for food in shallow lakes. Rediae and cercariae seldom live more than twenty-four hours in a dead snail, but the encysted agamodistomes are known to live through the decaying of the snail apparently unharmed by the putrefying process. These cysts then sink to the bottom of the pond, where they remain alive for a considerable time. A somewhat similar condition is illustrated by the behavior of the cysts of *Paragonimus ringeri* which break loose from the gills of the fresh water crabs and crayfish and sink to the bottom of the pond or stream.

Still another method is that of ducks, geese, etc., eating planaria and possibly other secondary intermediate hosts. However, because of the prevalent infection of this snail with the cysts (70.5 per cent) I feel that the planaria and other forms of animal life play a minor rôle in the infection of the host of the adult echinostome.

The cyst wall is not hard or impervious to water, as was proved by accident when 5.8 per cent salt solution was used instead of normal. In each case within three minutes every cyst, and of course every redia and cercaria, was dead. This probably means that the cyst is absorbing moisture all the time and possibly a small amount of food. The cyst wall is usually round, although it is capable of being changed in shape slightly by the movement of the enclosed agamodistome.

In order to discover how long cysts could live outside the body of snails or in the tissues of dead snails, many infected specimens were picked up at death, placed in small glass containers and examined from time to time. In the handling of the dead snails, the body wall was often ruptured and in this manner freed cysts were obtained. The water in these small glass containers was not changed but more water was added as evaporation took place. If the water had been changed occasionally, I feel sure that the cysts would have lived longer than they did because the bacteria and protozoa infection would not have been so heavy. At the end of ten days every cyst appeared to be in a healthy condition. The containers by this time had a very offensive odor. At the end of thirty-six days about 75 per cent of the cysts were alive, although in some cases part of these showed signs of deterioration. At the end of forty-two days only 11 per cent of the cysts were alive. Two days later only 5 per cent were alive. Thus cysts in this unnatural condition sometimes live longer than six weeks in dead snails or in the water. However, for the majority their life was about five weeks. That this would be twice as long in the open pond or stream, I do not doubt in the least. These results substantiate the previous belief that cysts are quite hardy and long lived. Since this echinostome cannot encyst in water, it means that any cysts that reach the bottom of the pond must survive the decay of the snail.

ADULTS

Since the adult, *Echinostoma revolutum*, has been well described by Looss (1899), Lühe (1909), Dietz (1910), and others, very little description of this stage will be given.

The adults of *Echinostoma revolutum*, as previously stated, were obtained in two ways, first, by examining different species of water birds commonly found on the lake from which the snail, *Physa occidentalis*, was taken. One species of duck, *Marila marila*, was found to contain three adult echinostomes in its intestines. These adults survived about fifty-five hours in the intestine of the duck, and twenty-four hours in normal salt solution. During the last twenty-four hours about forty-five eggs were produced, which were incubated and traced to the full-grown miracidia stage. The second method of obtaining adults was by feeding the encysted agamodistomes (cysts) found in *Physa occidentalis* to mongrel ducklings which were carefully protected from other trematode infections. In this way, eight full grown

worms and sixty-five partly grown worms of *Echinostoma revolutum* were found in the intestine. Upon careful study the adults from both sources were found to be of the same species.

The half grown adults 3.80 mm. long were found to have a few eggs in their uteri.

Perhaps the most significant thing about the adult *Echinostoma revolutum* as compared with its cercaria, is that the number of collar spines, forty-three, is not carried over in total number to the adult. The adults have only thirty-six or thirty-seven collar spines. In those with thirty-seven collar spines the last one on one of the sides is only about one-third the size of the others. In those with thirty-six spines this small spine is lacking. This reduction in number of spines from the cercaria to the adult, I believe, can be readily explained by the ease with which any of the spines can be lost unless the worms are handled very carefully, and the fact that there is not enough room on the collar for more spines. The spines in the adult are arranged in alternate rows as in the cercaria and also have the same clumping arrangement on the ventral side, but the number in each clump in the adult is reduced about three on a side. The size of the innermost spine, the very small one above mentioned excluded, is usually about two-thirds the length of the larger ones, which are 0.099 mm. in length. Since the largest spines on the collar of the cercaria are only 0.018 mm. long this means that the majority of the collar spines have grown about five and a half times. This explains the fact above stated that there is not enough room for the total number of spines on the adult collar when compared with the increase in size of the collar of the adult over the cercaria. The collar of the cercaria measures about 0.125 mm. in diameter while the collar of the adult only measures 0.495 mm.

The body spines of the adult are much like those of the cercaria (fig. 45) but have increased about four times in size, those on the side between the oral and ventral suckers measuring 0.025 mm. in length. The arrangement of the body spines is also the same. Ventrally, between the oral and ventral suckers, the spines almost completely cover the body. On the sides of the ventral sucker, blunt irregularly arranged spines were also found.

The great increase in the size of the ventral sucker is an outstanding fact. In the cercaria it is 0.094 mm. in diameter, whereas in the adults 8 to 10 mm. long it averages about 1.075 mm. in diameter. The size of the oral sucker, however, has increased only from 0.072 to

.270 mm. This points to the fact that the ventral sucker in the adult plays a very important function in attachment.

The distance between the oral and ventral suckers in the adult is about 1.05 mm., whereas in the cercaria it is about 0.023 mm. This is a gain of less than four and a half times. Since the body of the cercaria is about 0.5 mm. in length, and the body of the adult is about 9 mm. or eighteen times larger, it is evident that the greatest region of growth of the adult body is posterior to the ventral sucker.

The bladder of the excretory system of the adult is interesting in that the muscular sac (*1b* in the cercaria) measures more than one-fourth of the total length of the worm, whereas in the cercaria it is less than one-tenth of the length of the body. The muscular tubes (*1c* in the cercaria) are also much elongated, measuring nearly three-fifths of the total length of the body of the adult, whereas in the cercaria they measure but one-third of the body length. The concretions in the tubes of the cercaria's bladder (*1d* in the cercaria) are not to be found in the adult, although the tubes are about as great in diameter as in the cercaria. The ascending tubes of the bladder (*1e* in the cercaria) are long in the adult as in the cercaria, extending nearly the whole length of the body. Several other collecting tubules and accessory collecting tubules were also seen (fig. 49). The muscular sac division of the bladder was found to branch to either side a number of times, these branches in turn breaking up into smaller and smaller tubules that extend almost all over the body. Flame cells were never seen but the cilia in the main collecting tubule were very evident, being arranged in bunches and thus appearing much like large flame cells.

So far as the excretory system in the adult can be traced it is much like that of the cercaria. However, each part has been modified in length and size to meet its place in the adult; and the bladder is very much branched. If the flame cells, capillaries, accessory collecting tubules and collecting tubules could be accurately traced in the adult I should judge that they would be the same in number and similar in pattern. The great increase in the size of the adult over the cercaria would have to be met, so far as draining the wastes from the body is concerned, by the fine tubules of the branches of the muscular sac of the bladder.

As stated previously, Looss (1899), Lühe (1909), and others have undoubtedly placed two species, perhaps more, under the name *Echinostoma revolutum* (= *Distomum echinatum* Zeder).

Looss (1899, p. 684) sums up the discussion concerning the knowledge of this species and, in my opinion, places at least two different species together. In the first place the hosts in which he finds the two types are quite different. One type he finds in a shore bird of the Limicolidae, *Machetes pugnax*; the other he finds in the domestic goose. If this were the only difference it would hardly be enough to make two distinct species, since specificity of hosts is probably not as great as is usually believed. But the number of collar spines is found to be entirely different also. The echinostome found in the goose has thirty-seven spines, while the one from the shore bird has but thirty-four spines. Furthermore, the length, the shape, and the arrangement of the spines are entirely different. In one the smaller inner ventral spines are not present. The clumping or crowding of the ventral spines on each side is to be found in one and not the other. The range of variation in egg size is also different. One range of variation is from 0.092 to 0.134 mm., the other from 0.101 to 0.111 mm.

Since *Echinostoma revolutum* was first found by Froelich in the goose, and later by Zeder, Looss and others, including myself, in the goose and duck, the type with the thirty-seven spines, I think, should be called by the above name and a new name chosen for the one with the thirty-four spines found in the shore bird, *Machetes pugnax*. I suggest the name *Echinostoma limicoli*. The large number of primary hosts assigned to this species by different workers I feel quite sure is due to placing two or more species under the one name.

THEORETICAL DISCUSSION OF THE LIFE CYCLE

Briefly stated the stages of the life cycle of *Echinostoma revolutum* are these: egg, miracidium, mother-redia, daughter-redia, cercaria, encysted agamodistome (cyst) and adult. To accomplish this life cycle only two hosts are necessary, the snail *Physa occidentalis* and a duck or goose. Apparently any duck and perhaps any goose forms a suitable host, since the adult echinostomes were raised experimentally in mongrel ducklings, were found in the American scaup duck, *Marila marila*, on Stow Lake, and have been reported from many other ducks and geese in many places in the northern hemisphere. As stated, *Physa occidentalis* usually serves as both the intermediate and secondary intermediate host. This stage, however, is not specific, since the encysted agamodistome stage has also been found in another species of snail and in *Planaria*.

Although all the stages in the life cycle have been found and the necessary hosts are known, yet the time of the appearance of each stage and its duration are not known.

In regions where there is a definite winter or freezing period, I believe, each stage will be found during a certain season of the year. In the San Francisco Bay region of California, where light frosts may sometimes occur, the seasonal distribution of each stage is hard to solve. For this reason, California is not a good place to work on this particular part of the problem of the life cycle, although very favorable in every other respect. I should expect to find, and do find here considerable overlapping of the stages taking place.

During the migration of water birds in the spring and fall of the year, the great majority of the eggs are doubtless dropped in California ponds and streams. Although a few ducks and geese nest in the bay region of California, and thus some eggs are probably dropped in the summer time also, the great majority of the ducks and geese raise their young farther north. If the eggs are dropped into the ponds and streams during the fall migration, they doubtless remain practically dormant during the winter season. This conclusion is reached because the temperature of the water, although seldom at the freezing point, is sufficiently low to keep the germ balls in the redia from developing. This is borne out by the work of Nakagawa (1917) in connection with the development of the eggs of *Paragonimus westermani* Kerbert. He makes this statement concerning the incubation period of these eggs: "The rate of development of the miracidia varies with the temperature and is retarded by cool weather. During the summer in Shinchiku (Formosa), i.e., from May to October, the melon seed-like miracidia develop in 14 to 15 days, begin to move in 19 to 22 days, and hatch in 23 to 28 days. In March and April they take some weeks to hatch, and the miracidia remain for a long time within the egg even though they are as lively as in the warm season. From November to February or March no development was noted, though the eggs were watched constantly. According to my observations, the temperature for hatching is 25-31° C., and embryonic development ceases below 25° C. Manson gives 26-34° C., Nakahama 30° C., and Garrison and Leynes 25-34° C. At 37° C. the eggs seem to disintegrate." If the eggs are vented during the spring migration, doubtless they begin development immediately. Thus the eggs produced during the fall and spring seasons of the year would develop into miracidia at about the same time. These miracidia probably appear about the first to the

middle of May. By the process of metamorphosis and growth the mother-rediae should have daughter-rediae inside ready to escape through the birth pore by the middle of July. These daughter-rediae in turn, I should expect to have grown to maturity and to have cercariae escaping through the birth pore by the middle of August or the first of September, a rapid development due to the warmer weather. From the middle of August until November the cercariae are known to develop rapidly and to be continually escaping from the daughter-rediae to form cysts in other snails or planaria or to return to the same snail. Since many cercariae do not leave the snail but encyst just after emerging from the birth pore of the redia, some encysted agamodistomes are certain to be formed. Also since from September to November is the fall migration season, it is clear that the cysts are formed just in time to be eaten with the snails by migrating ducks and geese.

As shown by experimental feeding of encysted agamodistomes to young ducklings, the mature adult echinostomes appear in four weeks, having at this time about five hundred eggs in the uterus. In the winter home of the ducks and geese, numerous eggs undoubtedly are being given off. This would make it possible for *Echinostoma revolutum* to develop far south.

The adult worms probably live for several years in the intestine of the ducks and geese, producing eggs all the while. The length of life of this adult echinostome could possibly be determined by feeding the cysts to young ducklings and examining the feces from month to month.

Although the mother-rediae stage doubtless can be found during any season of the year in California, the winter season is probably its dormant period, as will be proved in case of the daughter-rediae. Though probably found during any season of the year in California, because some ducks and geese stay here all the year round, yet, as before stated, I should expect to find this stage most abundant during May, June and July.

The daughter-redia stage, i.e., the rediae that produce cercariae, is known to exist at all seasons of the year in California, yet it is found most abundantly in the fall. That the germ balls grow little or not at all during the months of December, January, February, and March is evidenced by the fact that during the sixty-three days from December 6th to February 7th not a single cercariae was to be found, and from February 7th to the first of April they were very rarely seen,

although the rediae were abundant. The temperature of the water is probably the controlling factor. The temperature of water of three lakes about the size of Stow Lake has been kept daily for several years. These three lakes are close to Stow Lake and are about the same size, so that their temperature probably differs but little. During the three winter months the average temperature of the water is 52° F, occasionally dropping as low as 48°. Beginning the first of March there is a quite rapid and steady rise in the temperature, which is accompanied by a corresponding increase in the number of active cercariae. During the three warmest months, July, August, and September, the temperature averages 67° F, occasionally reaching 70°. During these three months and the month following the active cercariae continually increase in number. It seems obvious that the degree of temperature controls the development of cercariae, the mother rediae and the enclosed daughter-rediae.

There are four ways by which stages other than the adult of *Echinostoma revolutum* can pass through the so-called winter season in the bay region of California. The first way is by means of the egg. The second way is by means of the mother-redia. This could only result from eggs being produced during the late summer or early fall. During the process of metamorphosis from the miracidium into the mother-redia, or after the enclosed daughter-redia had grown nearly to full size, the coming of winter would force the mother-redia to remain dormant until spring. The third way is by means of the daughter-rediae, i. e., rediae containing cercariae. This could result only from eggs produced during mid-summer. There would be sufficient time for the mother-redia stage and nearly enough time for the daughter-rediae to produce active cercariae. The lack of time to mature the cercariae would force the daughter-rediae to remain dormant until spring. The fourth way is by means of the encysted agamodistomes. In a climate where there is a definite winter season, I doubt if the second and third ways of surviving the winter exist.

The length of life of echinostome rediae has never been determined, but doubtless they exist for a year or more. The reason for this conclusion is, that since the average number of active cercariae in the rediae is five, and the total number of germ balls of all sizes is over a hundred, probably closer to two hundred, it seems impossible that all the germ balls could grow to maturity in less than a year's time. That they exist in a dormant or semi-dormant condition for the four months of the winter season is known.

How long an encysted agamodistome may live in a living snail has also never been determined. Since cercariae are not produced during the winter months, and since cysts are found throughout the year, it is obvious that they live for at least four months. Doubtless they exist for a year and perhaps several years in the tissues of the snail before deterioration and death. By raising eggs of *Physa occidentalis* in aquaria and by placing with these young snails the cercariae of *Echinostoma revolutum*, the duration of the cyst could be determined by examination of the snails from month to month.

The following table gives an analysis of the various stages of *Echinostoma revolutum* found in the four hundred snails of various sizes of *Physa occidentalis* taken from Stow Lake, Golden Gate Park, San Francisco:

Mother-rediae	13
Daughter-rediae and cercariae	234
Encysted agamodistomes or cysts	282
Cysts only	95
Cysts within rediae	13
Uninfected snails	53

These snails were examined during every month of the year except July. The cyst and daughter-redia stages were found during every month, but the mother-redia stage was found only in the months of December, January, and February. Since the mother-redia stage was not known or even suspected until December, 1918, it is obvious that more than thirteen snails were so infected. Also, since as stated, no effort was made to see if all the snails had some rediae that contained cysts, the number so parasitized was also probably much greater than recorded. The outstanding feature of the above table is that such a large percentage of *Physa occidentalis* contained some stage of the parasite. Perhaps the next most important feature is that more snails were found to be infected with cysts than with rediae. This, as previously stated, is due to this snail being both the intermediate and secondary intermediate host.

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EXPLANATION OF PLATES

PLATE 19

Figs. 1-9. Stages of development of miracidia. × 34.

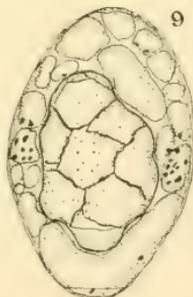
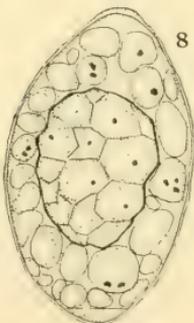
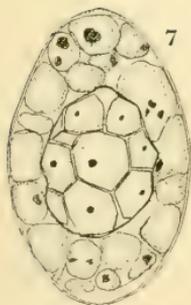
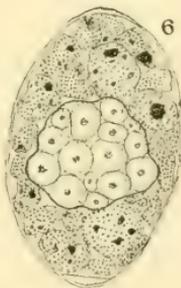
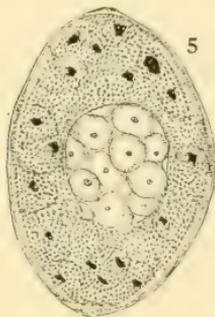
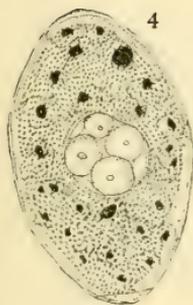
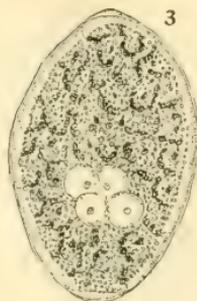
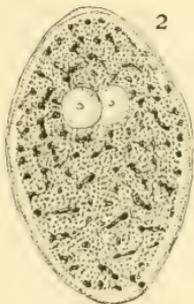
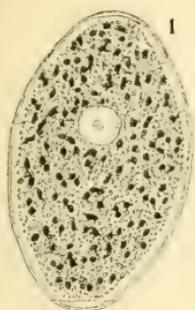


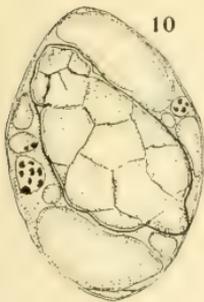
PLATE 20

Figs. 10-16. Stages of development of miracidia. $\times 34$. 14. *pdt.*, primitive digestive tract; 15. *ap.*, anterior papilla; 16. *ens.*, central nervous system.

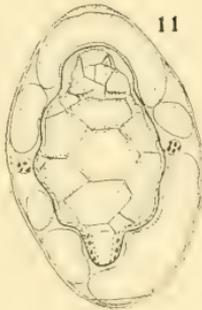
Fig. 17. Egg just after escape of miracidium, showing operculum attached and waste material inside. $\times 34$.

Fig. 18. Eggshell with operculum detached. $\times 34$.

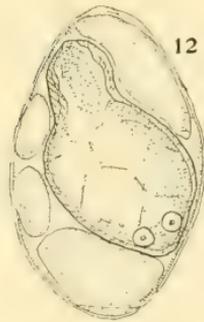
Fig. 18a. Operculum of egg. $\times 34$.



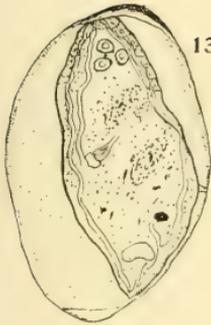
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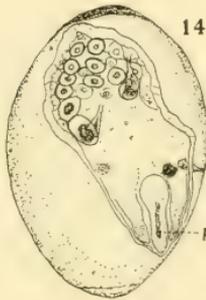
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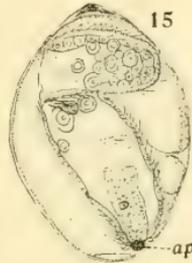


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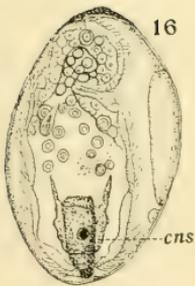
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pdt



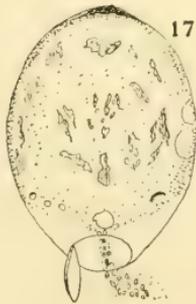
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ap



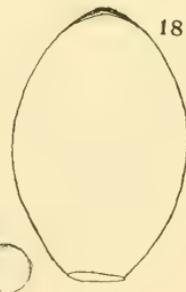
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PLATE 21

- Fig. 19, *a, b, c, d*. Mother-redia in various stages of extension. $\times 429$.
ir., intestine of redia.
- Fig. 20. Immature mother-redia. $\times 429$. *ph.*, pharynx.
- Fig. 21. Immature mother-redia. $\times 333$. *ir.*, intestine of redia.
- Fig. 22. Immature daughter-redia contracted. $\times 372$.
- Fig. 23. Immature daughter-redia extended. $\times 372$.
- Fig. 24. Anterior end of mature daughter-redia extended. $\times 140$.
- Fig. 25. Anterior end of mature daughter-redia contracted. $\times 140$.
- Fig. 26. Mature mother-redia containing daughter-rediae and germ balls.
 $\times 112$. *dr.*, daughter-redia; *drgb.*, daughter-redia germ ball; *ph.*, pharynx.
- Fig. 27. Mature daughter-redia containing cercariae and germ balls. $\times 66$.
bp., birth pore; *c.*, cercaria; *cgb.*, cercaria germ ball; *ir.*, intestine of redia.

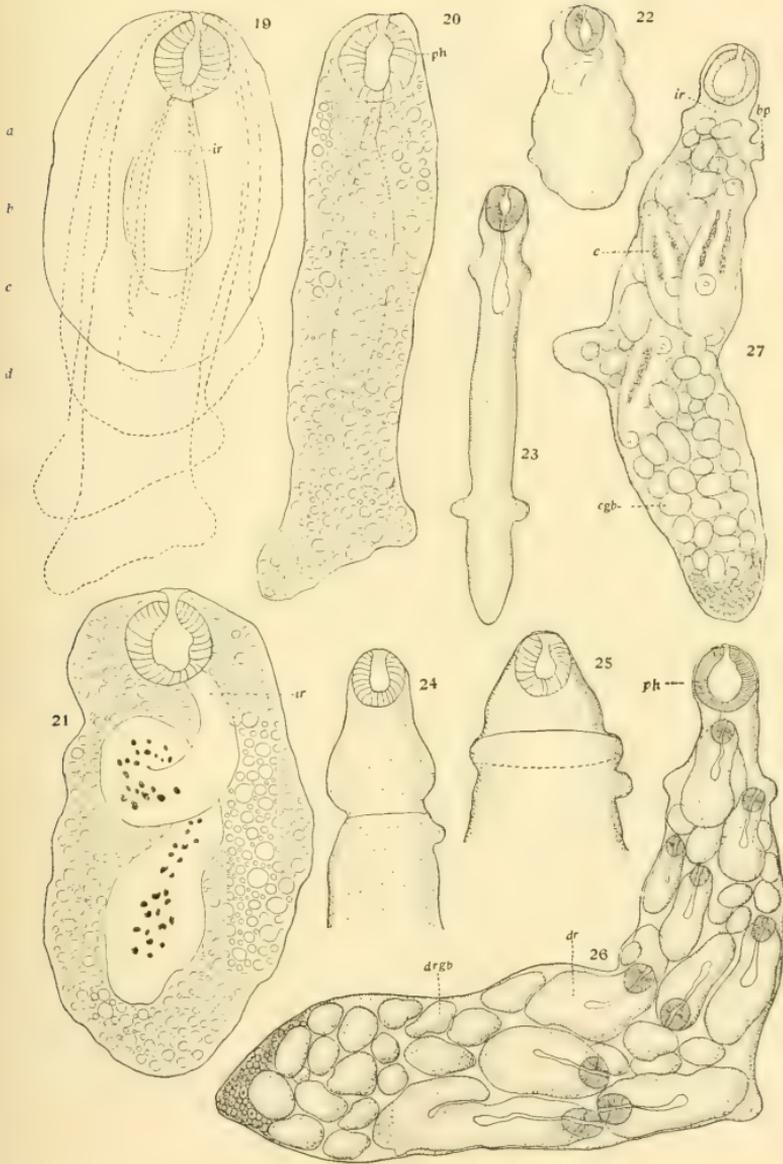


PLATE 22

Fig. 28. Lateral view of daughter-redia just after escaping from mother-redia. $\times 446$.

Fig. 29. Immature daughter-redia with germ balls inside. $\times 181$.

Fig. 30. Flame cell of mature daughter-redia. $\times 313$.

Fig. 31. Mature daughter-redia, showing excretory system. $\times 66$.

Fig. 32. Mature daughter-redia containing one encysted agamodistome (cyst) and germ balls. $\times 49$.

Fig. 33. Mature daughter-redia containing two encysted agamodistomes. $\times 44$.

Fig. 34. Mature daughter-redia containing three encysted agamodistomes and germ balls. $\times 76$.

Fig. 35. Mature daughter-redia containing four encysted agamodistomes, two mature cercariae, and a germ ball. $\times 36$.

Fig. 36. Mature daughter-redia parasitized by one mature tetracotyle, several immature tetracotyle, three mature cercariae, and several germ balls. $\times 60$.

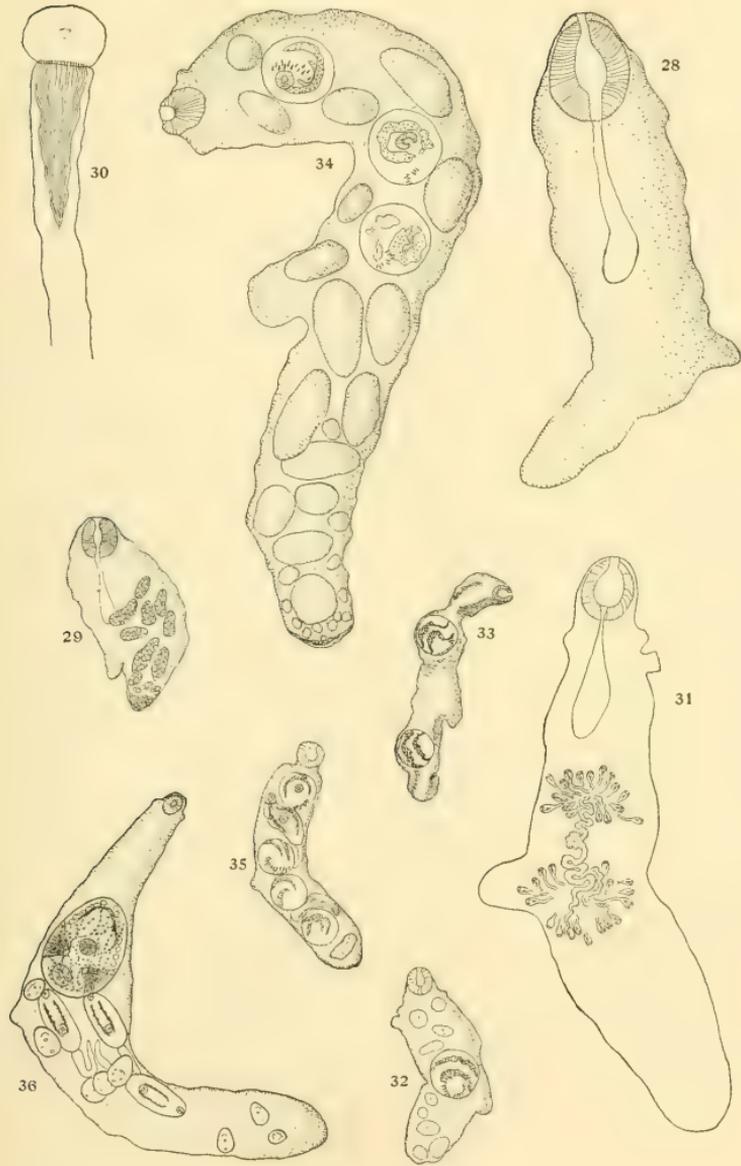


PLATE 23

- Fig. 37. Cercaria germ ball, showing beginning of excretory system. $\times 190$.
- Fig. 38. Cercaria germ ball, showing formation of tail and further development of excretory system. $\times 190$.
- Fig. 39. Further development of cercaria. Note bulblike enlargements in region of bladder. $\times 190$.
- Fig. 40. Immature cercaria, showing union of bladder tubes. $\times 190$.
- Fig. 41. Immature cercaria, showing further union of bladder tubes. $\times 190$.
- Fig. 42. Various stages of movement of tube bulbs of bladder as shown in figure 39. $\times 350$.
- Fig. 43. Lateral view of cercaria, showing formation of oral and ventral suckers. $\times 178$. *os.*, oral sucker; *vs.*, ventral sucker.
- Fig. 44. Lateral view of mature cercaria. $\times 127$. *os.*, oral sucker; *vs.*, ventral sucker.
- Fig. 45. *a.*, body spines of cercaria on side between oral and ventral suckers. $\times 529$; *b.*, dorsal view of one spine. $\times 2117$; *c.*, lateral view of one spine. $\times 2117$.
- Fig. 46. Eneysted agamodistome (cyst). $\times 194$.

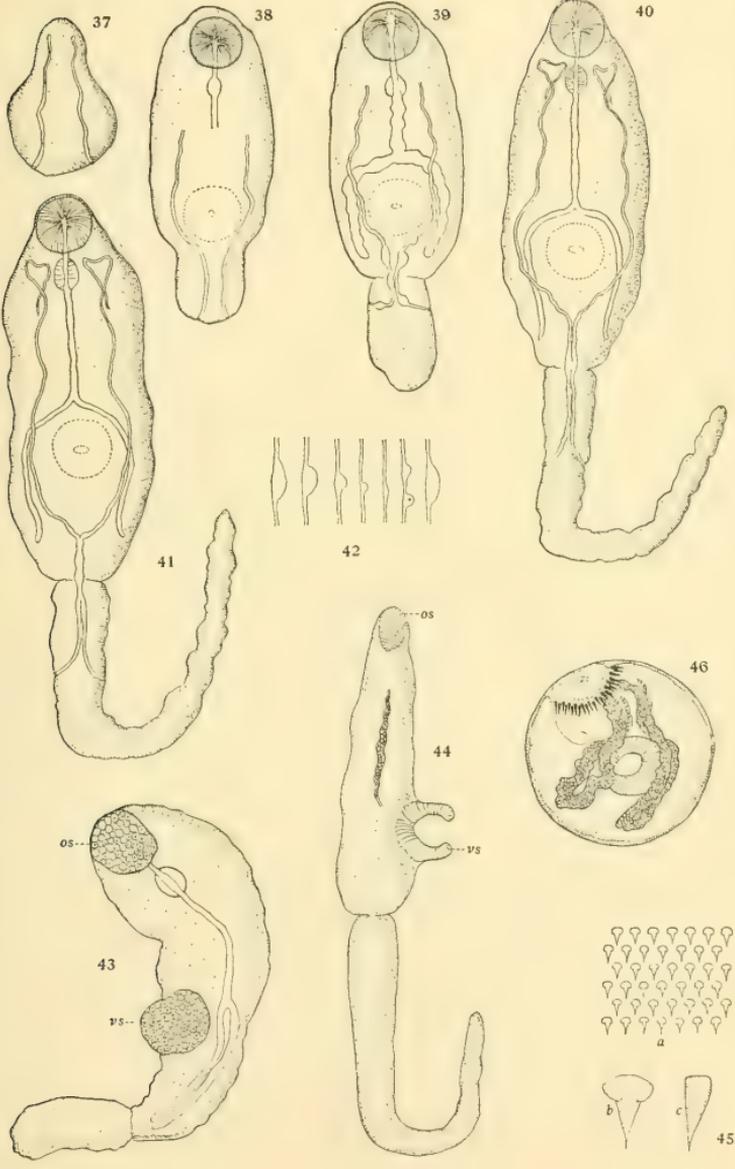


PLATE 24

Fig. 47. Mature cercaria showing detailed arrangement and pattern of excretory system. $\times 335$. *1a*, caudal division of bladder; *1b*, muscular sac of bladder; *1c*, muscular descending tube of bladder; *1d*, concretionary descending tube of bladder; *1e*, ascending tube of bladder; *2a*, *2b*, *2c*, *2d*, collecting tubules; *3a*, *3b*, *3c*, *3d*, *3e*, *3f*, *3g*, *3h*, accessory collecting tubules; *1-24*, flame cells; *cs*, collar spines; *ta*, triangular area.

Erratum. On the lower right-hand side of figure 47, the figure "3" should be inserted before the letter "f."

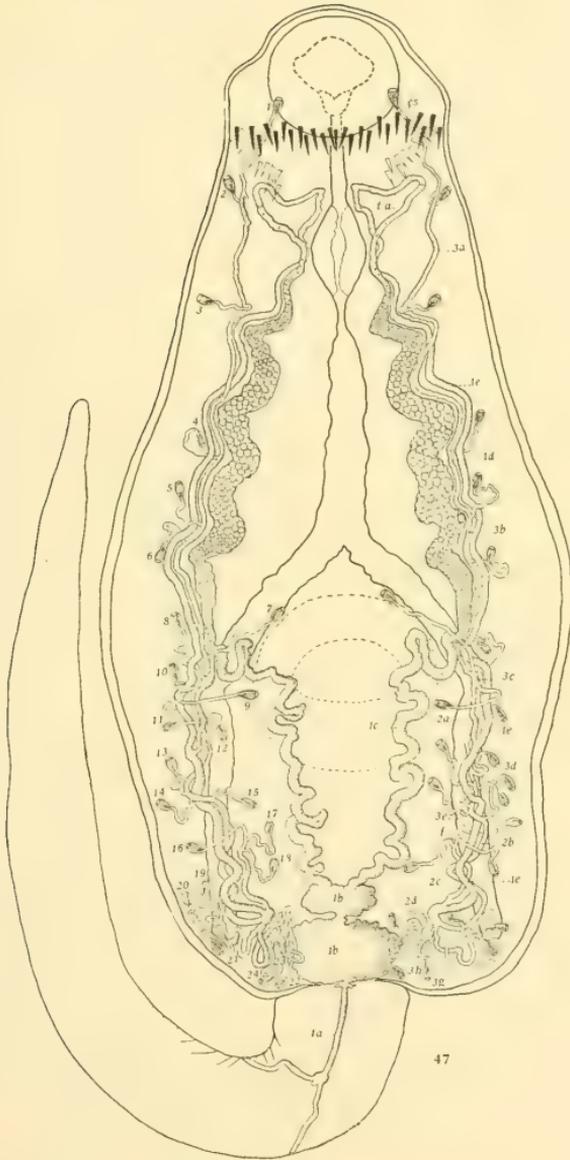


PLATE 25

Fig. 48. Adult, showing collar spines, suckers, digestive and genital parts. $\times 22$. *ac.*, acetabulum; *es.*, esophagus; *gp.*, genital pore; *i.*, intestine; *o.*, ovary; *os.*, oral sucker; *ov.*, oviduct; *ph.*, pharynx; *t₁*, *t₂*, testes; *u.*, uterus; *vd.*, vitelline duct; *vr.*, vitelline reservoir.

Fig. 49. Adult, showing parts of excretory system. $\times 22$. *ac.*, acetabulum; *1b.*, muscular sac of bladder; *1c.*, muscular descending tube of bladder; *1d.*, concretionary descending tube of bladder; *1e.*, ascending tube of bladder; *ep.*, excretory pore; *o.*, ovary; *os.*, oral sucker; *t₁*, *t₂*, testes.



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IN

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Vol. 19, No. 12, pp. 389-402, plates 26-28 August 21, 1920

ON SOME NEW MYRIOPODS COLLECTED IN
INDIA IN 1916 BY C. A. KOFOID

BY

RALPH V. CHAMBERLIN

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August 21, 1920

SEP 1920

ON SOME NEW MYRIOPODS COLLECTED IN
INDIA IN 1916 BY C. A. KOFOID

BY

RALPH V. CHAMBERLIN

The chilopods and diplopods noted in this paper comprise a small collection sent to me for identification by Professor C. A. Kofoid. All but two of the species represented were collected in 1916 at Coonoor, which is in the Nilgiri Hills district of southern India, presidency of Madras. One of the remaining forms, the spirostreptid, was taken at Nellakota, in the Wynaad, near Ootacomund, presidency of Madras, and one at the Marine Biological Station, Misaki, Japan. Six of the species are here described as new, one of these being made the type of a new genus, *Titsonobolus*, related to the East Indian *Trachelomegalus*.

These collections were made possible by a grant from Mr. W. H. Crocker, regent of the University of California, for parasitological research in the Orient by Professor C. A. Kofoid.

CHILOPODA

MECISTOCEPHALIDAE

1. *Mecistocephalus nilgirinus*, sp. nov.

Description.—Olive brown, the body under the lens seen to be densely mottled or marbled with black, spots of black occurring also on the anal legs. Prehensorial segment and head chestnut, the latter darker laterally. Antennae chestnut. Legs fulvous of slight chestnut tinge.

Head nearly 1.66 times longer than wide, being thus relatively broader than in several allied species. Widest at level of submarginal teeth or spurs, narrowing caudad, more abruptly so toward posterior end, as usual, the posterior corners rounded. Moderately and sub-uniformly punctate with coarse and more numerous finer punctae (cf. pl. 26, fig. 1).

Antennae rather long, gradually attenuated. Ultimate article twice as long as thick, a little longer than the penult.

Labral margins wholly without cilia. Lateral pieces considerably overlapping the median, their free margins crenulate toward mesal ends as shown in plate 26, figure 3. At anterior end of median areolated stripe separating the non-areolated clypeal areas is a small chitinous area and on each side of this and farther forward are two larger non-areolated areas. The small median area bears two short seta, and each of the posterior lateral areas mentioned bears two, each anterior bearing one (pl. 26, fig. 2).

Mandibles each bearing nine lamellae, of which the ultimate is modified, not being truly pectinate but bearing several fingers or filaments at the distal end (pl. 26, fig. 6). The first lamella bears seven stout teeth which are uniform or nearly so, and are stouter than the teeth of the other lamellae (cf. pl. 26, fig. 5). The ordinary lamellae present numerous long teeth which are of nearly uniform length throughout, much as in *insularis*. A median lamella bears twenty-three teeth, or nearly that number.

Coxosternum of second pair of maxillae characterized by the truncate posterior corners; a median non-areolated region or band present, but more or less broken by areolated cross-bands. Ectal angles of coxae of first maxillae elevated or produced forward, acute (cf. pl. 26, fig. 4).

Free portion of prosternum seven-eighths as long as wide. Anterior margin armed with two distinct teeth, obtusely rounded. Length of mesal side of exposed part of femuroid of prehensors is to outer height as six to one. Femuroid armed with two teeth which are about equal in height but the distal one is a little stouter and more blunt. Succeeding two articles each with a small, nodule-like tooth. Claw armed at base with a broad, low eminence (cf. pl. 26, fig. 7).

First legs reduced and slender, about three-fifths as long as the second legs.

Impressions of anterior sternites furcate, the furcation subrectangular or but slightly obtuse. In going caudad the branches decrease

in length and the angle typically becomes somewhat more acute (cf. pl. 26, figs. 8 and 9).

Sternite of pregenital segment trapezoidal, typically somewhat abruptly narrowed a little in front of caudal end, the posterior portions densely clothed with fine and very short hairs. Coxopleurae densely porose, the pores small and very small, the latter the more numerous. Anal pores distinct. Anal legs slightly attenuated, their length, exclusive of coxopleurae, typically near 1.78 times longer than the penult legs.

Number of segments: forty-nine.

Measurements.—Length of type, 86 mm.; width of first segment (tergite), 4 mm.

Locality.—India: Coonoor. Three specimens collected April 10, 1916.

Comparison.—This form was at first supposed to be *M. insularis* (Lucas), a species which, in general features, it much resembles. It may, however, be readily distinguished from that species by the crenulation of the mesal ends of the free margins of the lateral pieces of the labrum, by the five minor non-areolated areas of the clypeal region, and by the setae of this part, by the characters of the maxillae, and by various other details. In the character of the labral margins this species suggests the Australian *M. kurandanus* Chamberlin (cf. pl. 27, fig. 12).

SCOLOPENDRIDAE

2. *Scolopendra subspinipes* Leach

Trans. Linn. Soc. London, 1814–15, 11, 383.

One specimen taken at Misaki, Japan, July 21, 1916, in the spinning of its legs agrees with the forma *typica* rather than with the forma *japonica*.

OTOSTIGMIDAE

3. *Rhysida longipes* (Newport)

Branchiostoma longipes Newport, Trans. Linn. Soc. London, 1845, 19, 411.

Rhysida longipes Pocock, Biol. Centr. Amer. Chilop., 1896, p. 27.

One specimen of this tropicopolitan species was secured at Coonoor.

4. *Rhysida simplicior*, sp. nov.

Description.—General color olive brown; a narrow stripe across posterior border of each tergite typically paler; pleural region and legs and antennae paler, sometimes in part with a tinge of blue.

Head wider than long; appearing smooth and shining, but under the lens showing some obscure shallow punctae. Antennae short, reaching only to the third segment; composed of but seventeen articles. Prosternum armed on each side with four teeth, of which the two most mesal are closer together, less deeply and widely separated than the other two; the second from ectal end largest (cf. pl. 27, fig. 13).

Cephalic plate wider than long; appearing smooth and shining, but under the lens showing some obscure shallow punctae. Antennae short, reaching only to third segment; composed of only seventeen articles.

Dorsal plates bisulcate from the seventh caudad. Only the twenty-first is strongly margined, but the five preceding ones are more weakly, sometimes vaguely and incompletely margined. Last tergite angularly produced caudad (cf. pl. 27, fig. 15).

Ventral plates without any complete sulci, at most with short anterior traces. Last plate strongly narrowed caudad, the caudal margin excavated (pl. 27, fig. 14).

Coxopleural processes of but moderate length; bidentate at apex, a single marginal spine at base (pl. 27, fig. 16).

Only the first five pairs of legs with two tarsal spines, the others to the twentieth inclusive with tarsal spine single.

Femur of anal legs armed on ventral surface ectally with three spines, mesally with two; on mesal surface a single spine; no distal spine or process (pl. 28, fig. 21).

Measurements.—Length, 44 mm.

Locality.—India: Coonoor. Five specimens collected April 3, 1916. All but two of these have lost the anal legs.

Comparisons.—Resembles *R. longipes* (Newport), but readily distinguished by various details. Antennae shorter and composed of but seventeen articles. Prosternal teeth and femuroidal process obviously different in form and the limiting furrow of prosternal dental plates more obtuse. Differing in anal legs in having no spine at distal corner, etc.

5. *Ethmostigmus coonooranus*, sp. nov.

Description.—Color olive brown, the head darkest. Last dorsal plate and all legs chestnut, or the proximal joints sometimes tending toward olive brown.

Antennae composed of twenty joints, the joints longer than wide, the first three glabrous, and the others shortly pilose, as usual.

Prosternal plate with 3+3 teeth, these much as in *platycephalus* (pl. 27, fig. 17).

Dorsal plates furrowed from the third caudad; margined from the seventh caudad; the margining sulcus of the seventh short. All plates smooth. Last dorsal plate with caudal margin strongly convex as shown in plate 27, figure 19.

Ventral plates with paired furrows from the sixth or seventh caudad, these in general not extending caudad of the middle of the plate, more distinct in posterior region. Last ventral plate with a single distinct, median longitudinal sulcus; posterior margin conspicuously and somewhat angularly excavated (pl. 27, fig. 18).

Coxopleural processes long, exceeding the last ventral plate by more than its length, subparallel or bending but little toward each other; each with but a single spine distally, a little proximad of which on dorsal side is another large spine with a second much smaller dorsal spine farther proximad; laterally a single spine; dorsal line convex, much as in *rubripes* (pl. 27, fig. 20).

First two pairs of legs with two tarsal spines each, the others to and including the twentieth with tarsal spine single.

Distal process of femur of anal legs of but moderate size. Femur with two spines on ventral surface ectally and one, mesally; two spines on mesal surface and two along mesodorsal line.

Measurements.—Length, 57 mm.

Locality.—India: Coonoor. Two specimens.

DIPLOPODA

STRONGYLOSOMIDAE

6. *Prionopeltis indus*, sp. nov.

Description.—Body deep chocolate to black, the keels yellow excepting anteriorly. Legs light brown, with a fulvous background often showing. Antennae dark chocolate or black.

Head with vertigial sulcus very deep down to level of antennal sockets. Densely shortly pilose over lower and lateral regions, more coarsely so above. Antennae long and slender.

Metazonites densely granular both dorsally and below the keels. A distinct transverse sulcus on metazonites from the third caudad, also an obscurely indicated one on the second. A series of short setae along

caudal border of each metazonite and a series across anterior border, there being in addition on the collum and on the several immediately following plates and on the eighteenth and nineteenth plates a third, submedian, and more or less irregular series. Keels elevated or curved up to or a little above level of middorsal region. Margins of keels smooth, or with two slight indentations at most.

Form of collum and arrangement of its setae shown in plate 28, figure 23.

A weak longitudinal or somewhat oblique pleural keel evident on fourth and fifth segments. The usual processes at base of legs.

Cauda of anal segment much exceeding the valves, flattened, distally truncate, slightly convex above; bearing on caudal end four long, double or geminate setae. Dorsal surface of anal scutum not finely granular like the other tergites, though roughened. Plate bearing four long setae along each margin, and two pairs above, one pair being near middle of the plate; the other farther caudad (cf. pl. 28, fig. 24). Anal valves strongly margined mesally; smooth; two setae in usual position, each borne on tubercle. Anal scale with two long setae (cf. pl. 28, fig. 25).

Gonopods of the male of the same general type as those of *kelaarti* Humbert, but differing in details. The end branch of the terminal division longer and more slender, sigmoidally curved, distally bending first mesad and then abruptly caudad instead of simply mesad as in *kelaarti*. Style more slender, with the process across which it lies longer. In ectal view the mesal hook is seen to curve moderately distad instead of bending strongly proximad as it does in *kelaarti* (seq. fig. of Attems, cf. pl. 28, fig. 22).

In the male the fifth sternite between anterior pair of legs bears the usual broad process which is moderately rounded and mesally furrowed. Third joint of legs of fifth and sixth segment with a low angular process proximad of middle on ventral surface.

Measurements.—Length, to about 35 mm.; width, 4.7 mm.

Locality.—India: Coonoor. Two males and three females.

Comparisons.—Obviously close to *kelaarti* Humbert. I have seen no specimens of the latter species; but Attems' redescription and drawings of the type seem to indicate a species different from the one here described. The gonopods differ in a degree equivalent to that between *indus* and the following species, *atopus*, sp. nov. *Kelaarti* is a lighter, more reddish form with yellowish brown legs and antennae.

7. *Prionopeltis atopus*, sp. nov.

Description.—A lighter, more brownish species than the preceding one, with keels entirely fulvous, the light color extending along both anterior and caudal borders instead of being confined to the caudolateral region of keel.

The sculpturing of dorsum the same in general excepting at caudal end. Here, in the type, the nineteenth and anal segments are partially retracted within the eighteenth; but as only one specimen has been observed, this feature may be an artifact or an abnormality. The anal scutum is more distinctly granular. Cauda more rounded at caudal end, not gently decurved as in *kelaarti* but straight or rather slightly upraised at end. Anal valves granular, and each of its setigerous tubercles bearing three setae instead of two. Keels elevated as in *indus*.

Fifth sternite in male with the usual process; its anterior tubercles more prominent. Third joints of legs of fifth and sixth segments with rounded proximal tubercles or processes.

Differing in having no pleural keels on fourth and fifth segments. The usual pegs at bases of legs.

The mesal hook of male gonopods broader, distally more clavate, with its distocaudal angle finely acutely produced, the end in outline from ventral view not unlike a bird's head. Style crossing spinous process, not running nearly parallel with it. Gonopods obviously larger than in *indus* (pl. 28, fig. 27). A more robust species than *indus*.

Measurements.—Length, 53 mm.; width, 5 mm.

Locality.—India: Coonoor. One male.

SPIROBOLOIDEA

Titsonobolus, gen. nov.

Description.—Resembles *Trachelomegalus* in the large size of the collum which similarly extends forward over the base of the head, reaching or a little overlapping the posterior borders of eyes. Collum laterally narrowed, extending low down free from the head and over the antennae. Unlike *Trachelomegalus*, having the second tergite also produced ventrad, in the genotype below level of the collum and below level of sternum. Eyes widely separated, twice their longer diameter or more apart. Cauda much exceeding the anal valves, strongly curved downward. Spiracles in front of sutures.

Characters of male not known.

Genotype.—*T. uncopygus*, sp. nov.

8. *Titsonobolus uncopygus*, sp. nov.

Description.—In general the prozonites fulvous, this color encroaching upon the metazonites below, and the metazonites black, with this color encroaching upon the prozonites at and above the pores. Collum dark, blackish or dusky chestnut, the head and anal segment similar. Legs light chestnut.

Head with vertex roughened by numerous shallow depressions which are densely arranged. Vertigial sulcus distinct to or below level of middle of eyes. Antennae short, lying in a deep excavation below eyes and completely concealed, or nearly so, by the collum (cf. pl. 28, fig. 29).

Collum narrowed down each side, rounded below; not at all margined or striate; finely punctate. Second tergite extending a little below ends of collum and forward beneath latter to its anterior edge, the lower edge nearly straight, longitudinal depressed anteriorly and above lower border for reception of contiguous part of collum; punctate, not at all truly striate (cf. pl. 28, fig. 29). Third tergite also produced a little ventrad, with ventral region of segment also concave. The fourth and fifth segments intermediate between this and the normal form. Each prozonite of the typical segments marked below and on the sides with numerous striae curving forward and then dorsad, and branching, and more or less anastomosing; the striae in the dorsal region all transverse and more lightly impressed, branching and anastomosing; just in front of suture or furrow above are numerous strong punctae and impressed semicircles and crescents. Metazonite crossed by longitudinal striae below, the series not attaining the middle of side, the surface elsewhere smooth. The segmental suture represented by an impression or furrow rather than a sharp line. Pore separated from suture or furrow by from half to its entire diameter.

Cauda cylindrical, acutely pointed, strongly curved downward, its tip at about middle height of valves. Anal valves with mesal borders broadly and strongly elevated, in lateral view subangular at caudoventral corner. Anal scale with caudal edge long and nearly straight (cf. pl. 28, fig. 30).

Number of segments: forty-six.

Measurements.—Length, about 72 mm.; width, 8.2 mm.

Locality.—India: Coonoor, April 16, 1916. Three females.

SPIROSTREPTOIDEA

9. *Spirostreptidarum*, gen. et sp.?

A single female belonging to the Spirostreptidae which cannot at present be more closely placed. The caudal end of the specimen is missing.

Types of all new species described in this paper are in the collection of the Museum of Comparative Zoology, Cambridge, Massachusetts, and paratypes of all except *Prionopeltis atopus* are in the collection of the Department of Zoology, University of California, Berkeley, California.

Transmitted February 16, 1920.

MUSEUM OF COMPARATIVE ZOOLOGY, HARVARD COLLEGE,
CAMBRIDGE, MASSACHUSETTS.

EXPLANATION OF PLATES

PLATE 26

Mecistocephalus nilgirinus, sp. nov.

- Fig. 1. Head in outline, dorsal view. $\times 14$.
- Fig. 2. Anterior region of head, ventral view, with mandibles and maxillae removed. $\times 30$.
- Fig. 3. Median region of labrum. $\times 195$.
- Fig. 4. Maxillae, ventral view. $\times 38$.
- Fig. 5. First lamella of mandible. $\times 315$.
- Fig. 6. Last lamella of mandible. $\times 315$.
- Fig. 7. Left prehensor and part of prosternum. $\times 14$.
- Fig. 8. Sternal impression, tenth segment. $\times 18$.
- Fig. 9. Sternal impression, twentieth segment. $\times 18$.
- Fig. 10. Caudal region of body, ventral view. $\times 18$.
- Fig. 11. Caudal region of body, dorsal view. $\times 18$.

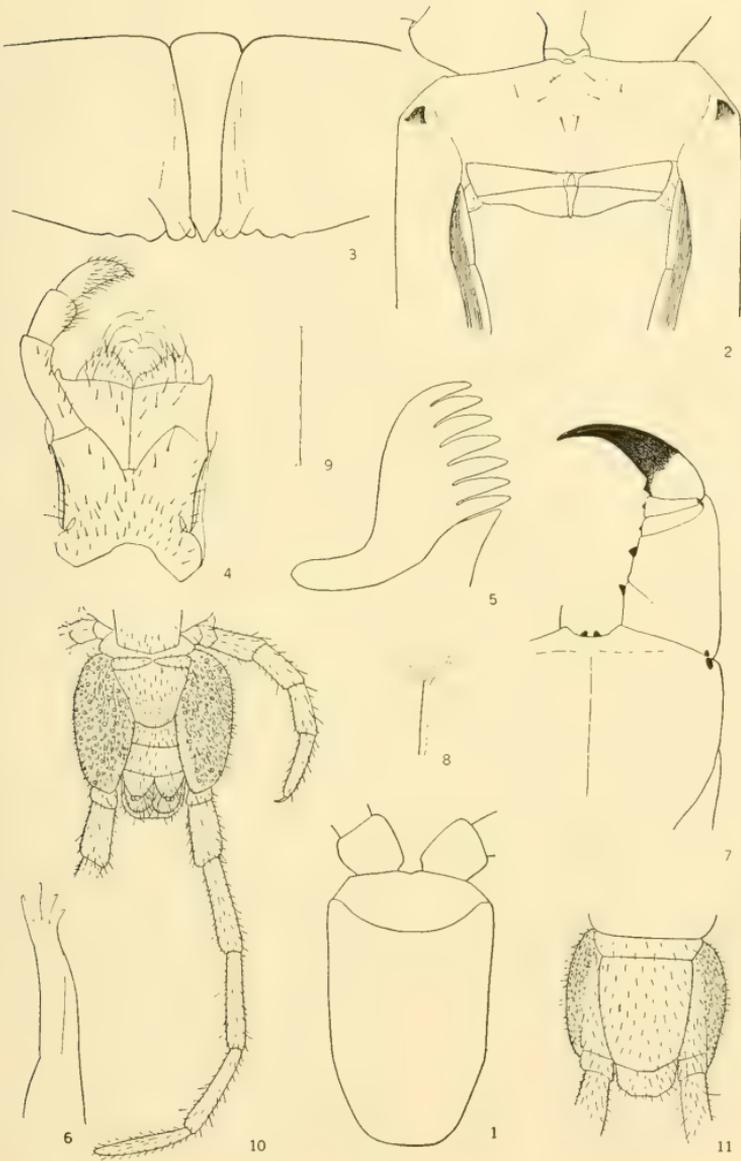


PLATE 27

Mecistocephalus kurandanus Chamberlin

Fig. 12. Median region of labrum. $\times 195$.

Rhysida simplicior, sp. nov.

Fig. 13. Right prehensor and part of prosternum, ventral view. $\times 18$.

Fig. 14. Ventral plate of pregenital segment. $\times 18$.

Fig. 15. Tergite of pregenital segment. $\times 18$.

Fig. 16. Right coxopleura of pregenital segment. $\times 18$.

Ethmostigmus coonooranus, sp. nov.

Fig. 17. Right prehensor and part of prosternum. $\times 15$.

Fig. 18. Ventral plate of pregenital segment. $\times 15$.

Fig. 19. Tergite of pregenital segment. $\times 15$.

Fig. 20. Lateral view of pregenital segment. $\times 15$.

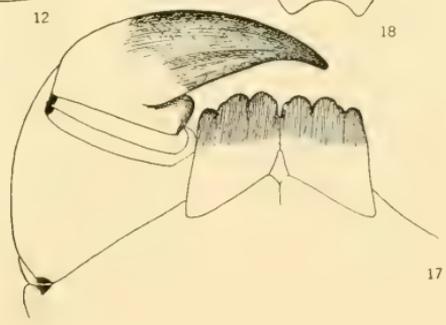
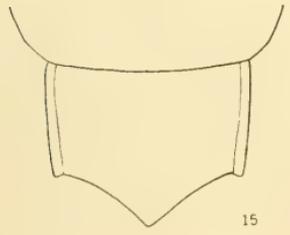
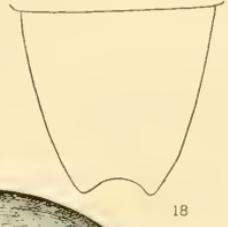
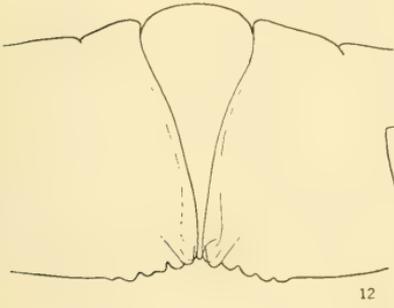
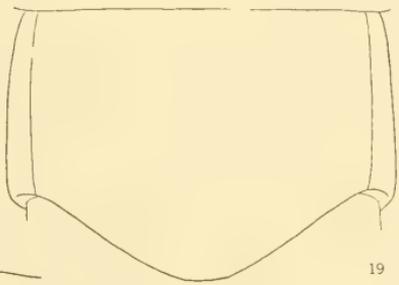
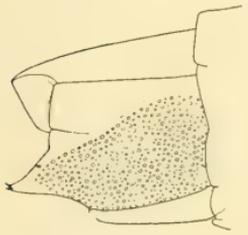
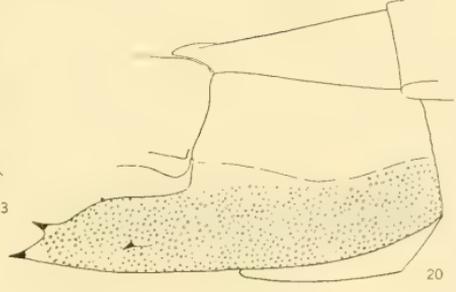
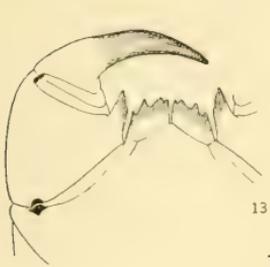


PLATE 28

Rhysida simplicior, sp. nov.

Fig. 21. Femur of anal leg, ventral view. $\times 18$.

Prionopeltis indus, sp. nov.

Fig. 22. Right gonopod of male, ventral view. $\times 30$.

Fig. 23. Collum in outline, showing also the setae, dorsal view. $\times 18$.

Fig. 24. Last two tergites, dorsal view, granulation not represented. $\times 18$.

Fig. 25. Anal scale. $\times 18$.

Fig. 26. Third article of fifth leg of male, caudal view. $\times 30$.

Prionopeltis atopus, sp. nov.

Fig. 27. Right gonopod of male, ventral view. $\times 25$.

Fig. 28. Collum in outline, dorsal view. $\times 13$.

Titsonobolus uncopygus, sp. nov.

Fig. 29. Head and first two tergites, lateral view. $\times 6$.

Fig. 30. Caudal end of body, lateral view. $\times 6$.



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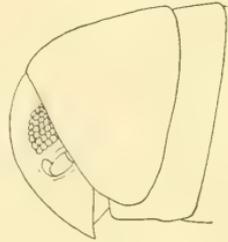
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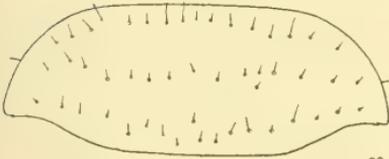
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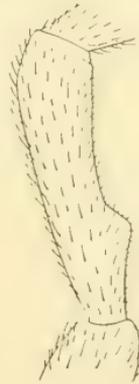
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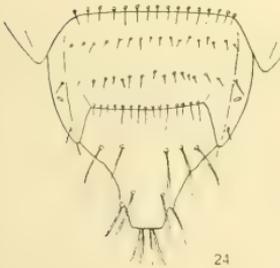
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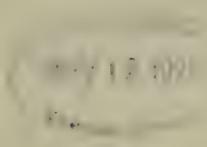
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DEMONSTRATION OF THE FUNCTION OF
THE NEUROMOTOR APPARATUS IN
EUPLOTES BY THE METHOD
OF MICRODISSECTION

BY

CHARLES V. TAYLOR



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INTRODUCTION

Protozoa are commonly regarded as representatives of the most primitive and simplest forms of life. The most salient feature of the phylum is conceded to be their unicellularity, each individual being the morphological equivalent of a single cell. That these characteristics indiscriminately applied to this very large and diversified group of organisms are not only inadequate but somewhat misleading is particularly evident from several recent investigations on various flagellates and ciliates. The results of these researches point toward complexity rather than simplicity and stimulate inquiry into the nature and function of certain intracytoplasmic structures that these animals possess, which may indicate an organization more highly evolved than is usually assumed.

These structures in both flagellates and ciliates are intimately associated with ectoplasmic organelles (flagella, cilia, cirri, etc.), a fact which strongly suggests that they share some rôle in the animal's motor mechanism. Accordingly, investigators are generally agreed in designating the structures with their attached organelles "the motor apparatus."

Of the organisms possessing such a motor apparatus a larger number of flagellates than of ciliates has been studied and comparatively described. In the former class a series has been worked out that indicates a progressive evolution of this mechanism. A simple type of motor apparatus appears in the biflagellate stage of the soil amoeba, *Naegleria gruberi* (Schardinger). It consists of two flagella attached to a basal corpuscle, the blepharoplast, which in turn is connected by a fine fibril to the nucleus. This organism spends most of its excysted life as an amoeboid trophozoite, but it may become transformed for a brief period of twenty-four hours or less into a very active flagellate. This interesting change has been described by Whitmore (1911), Alexieff (1912), and more completely by Wilson (1916), who has shown that variations in temperature, media, and other factors may induce the change. The actual transformation may be followed in living forms and its stages analyzed in fixed material.

It was thus observed that the motor apparatus arises by an outgrowth from the karyosome, "presumably from the centriole," Wilson

states, "which crosses the clear nuclear zone, emerges through the nuclear membrane" whence arises a plastic thread, the rhizoplast, that ends near the periphery in the blepharoplast. The two flagella grow out from this blepharoplast.

The origin of the apparatus from the centriole is not clearly established. This centriole can be seen within the karyosome during the entire development of the flagella, although its division may give rise to these structures. Dr. Swezy (1916) offers a critical discussion of this point.

A less primitive motor apparatus is met with in *Prowazekia lacertae* (Grassi), a parasitic flagellate found within the intestine of amphibians. The form is described by Alexeieff (1912), and Janicki (1915), and its motor apparatus critically compared with that of other flagellates by Swezy (1916). One stage in the life-cycle shows a motor mechanism quite similar to that of the flagellated soil amoeba, including two flagella attached to the blepharoplast which is connected by the rhizoplast to the nucleus. This condition becomes modified by an enlarged blepharoplast that elongates and buds off its larger portion to form the parabasal body. The latter structure remains attached to the blepharoplast by a rhizoplast and so shares a part in an integrated motor apparatus that is typical for several other forms of the series. The parabasal body is described in some protozoological literature under the name "kinetonucleus." Protozoologists using this nomenclature designate the nucleus "trophonucleus." The former is held to be a product of the latter, is composed of nuclear chromatin, and divides mitotically. But more recent researches do not substantiate these claims (Doflein, 1911, Kofoid, 1915, Swezy, 1916). The origin of the body, as in *Prowazekia lacertae*, from the blepharoplast to which it remains attached, is a fact which in itself establishes the structure as a component of the motor mechanism. In this relation it has been regarded as an accessory kinetic reservoir which supplies oxidizable material to the locomotor organelles (Kofoid and Swezy, 1915).

In *Trypanoplasma congri* (Martin, 1913), occurs a further complexity in the motor apparatus with the attachment of a trailing flagellum to the body by a fairly well developed membrane. It is also significant that the parabasal body is here considerably elongated, extending from near the blepharoplast lateral to and beyond the nucleus. These variations are regarded (Swezy, 1916) as representing a step in the evolutionary series toward the conditions that obtain

in certain trichomonads. Of this genus, *Trichomonas augusta* (Kofoid and Swezy, 1915) possesses a motor mechanism to whose blepharoplast are attached: (1) three equal anterior flagella, (2) one intracytoplasmic flagellum, the axostyle, (3) a trailing flagellum attached laterally along the margin of the undulating membrane, and (4) an elongated, chromatoidal rod, the parabasal body, which lies along the proximal edge of the undulating membrane. Recalling the occurrence of a trailing flagellum and the elongated parabasal body in *Trypanoplasma congri*, an homology between these and structures (3) and (4) in *Trichomonas augusta* appears obvious.

But the complexity of the motor apparatus does not end with the trichomonads. In the other genus of the Polymastigina, the Octomitidae, appear further advances in the series. An amphibian parasite, *Octomitus dujardini* (Dobell, 1909) claims for its motor mechanism a pair each of blepharoplasts, parabasal bodies and axostyles, and three anterior flagella attached to each blepharoplast. Omitting the undulating membranes, *Octomitus* is really the equivalent of two trichomonads. This duplex condition does, in fact, become complete in *Giardia* (Kofoid and Christiansen, 1915*b*, Boeck, 1917), the remarkable motor mechanism of which rivals in complexity that about to be described for certain ciliates. There is a duplication here of each structure found in the apparatus of *Trichomonas*. But with the connection of the two blepharoplasts by a commissure and with a chiasmal crossing of the anterior lateral flagella, two organisms become integrated into one individual (Kofoid and Christiansen, 1915*b*).

It was suggested by Professor Kofoid (Kofoid and Christiansen, 1915*b*) that this integrating fibrillar complex in *Giardia*, associated with the blepharoplasts, parabasal bodies and the very active organelles of locomotion, was neuromotor in function. To the system he assigned the name "neuromotor apparatus" which has since been applied to homologous fibrillar systems in other flagellates of the above series.

The series indicated in the foregoing examples has been considerably amplified by a number of other flagellates (Swezy, 1916) which show greater or less complexity in their motor apparatus. For the ciliates, however, no such assemblage has yet been made, although this large field would seem only to await further investigation. Numerous forms of this class have long been known to possess intracytoplasmic structures associated with their motor organelles, but the morphological relationship of these structures has been completely worked out in only two organisms.

Sharp (1914) was the first to succeed in this endeavor. Working upon a parasitic ciliate, *Diplodinium caudatum*, common in the stomach of the ox, this investigator discovered a system of fibrils connecting all the motor organelles of the oral region. Owing to the shape, position, relations, and staining properties of this system, Dr. Sharp regarded it as having an unusual significance.

The organism in several respects is one of the most complex among all known Protozoa. The body, which resembles "a short, plump banana," bears all the organs of locomotion and food-taking at the anterior end. This region is more or less flexible and decidedly contractile, while the remaining portion of the body is rigid, free from appendages and, for the most part, firmly supported by an exoskeleton. At the anterior extremity, toward the ventral side of the body, is located the cytostome. This is an elliptical aperture surrounded by an oval disk that bears on its inner border a circle of oral cilia. The cytostome opens directly into the oesophagus, a short tube which ends blindly beside the anterior end of the macronucleus. Around the outer border of the oral disk appears a row of heavy adoral membranelles that function chiefly in locomotion. Encircling these membranelles are an inner and an outer adoral lip dorsal to which lies a prominent operculum. The latter structure is continued dorsally into the dorsal disk which is surrounded by the dorsal membranelles. These, like the adoral membranelles, are locomotor organelles.

The relation of the above structures has been very briefly stated only to facilitate a review of the excellent description Dr. Sharp has given of the complex motor apparatus found in this ciliate. The constituent parts of this mechanism embrace (1) a motorium lying deep in the ectoplasm beneath the operculum, (2) a dorsal motor strand, (3) a ventral motor strand, (4) a dorsal lip strand, (5) opercular fibers, (6) oesophageal fibers, and (7) a circumoesophageal ring. The relation of these parts to the organelles with which they are associated is best described in Dr. Sharp's own words. In a specimen stained with his modification of Mallory's connective tissue stain, the so-called motorium was first observed as a mass "which had stained rather intensely and showed by transmitted light the same bright red color which was noted in the case of the micronucleus. Further investigation along this line revealed the fact that not only was this mass constant but (1) that it was connected dorsally, by means of a delicate strand, i.e., dorsal motor strand, with the bases of the dorsal membranelles, also a branch strand ran along the base of the inner dorsal

lip, i.e., the dorsal lip strand; (2) that a fine strand, the ventral motor strand, ran from it to the bases of the adoral membranelles, also that a branch strand left this ventral motor strand and passed along the base of the inner adoral lip, the adoral lip strand, and that many well-defined fibers passed from it, following the contour of the operculum toward the right to become lost in the immediate vicinity of the base of the right skeletal structure. These are the opercular fibers. Most interesting of all, however, was the apparently perfectly definite connection with a ring of the substance surrounding the oesophagus at just about the level of the outer adoral furrow. This ring, which is designated as the circumoesophageal, as well as all of the fibers described as leaving the motorium, showed in all regions the same bright red color. Other fibers also staining bright red are found in the oesophageal walls. These are found in the oesophageal walls. These are called the oesophageal fibers, but thus far it has not been definitely decided whether they take their origin from the motorium or directly from the circumoesophageal ring, probably the latter, however" (Sharp, 1914, p. 83).

Inasmuch as this complex system of motor mass and strands is intimately associated with the motor organelles, one is justified here, as in the case of the flagellates, in regarding these structures as a part of the animal's motor mechanism, whatever their specific rôle may be. But just what is their specific function? Three possibilities were obvious: (1) this intracytoplasmic system may be skeletal, for support; (2) it may be muscular, the strands representing primitive contractile fibrils; or (3) these strands may have conductive properties with the motorium functioning as a coördinating center for impulses passing over the primitive neural fibrils. After weighing the evidence which his investigations had disclosed, Sharp concluded that the last hypothesis was in nearest agreement with the facts.

The skeletal hypothesis, adopted by Braune (1913) for a similar system found in *Ophryoscolex purkynjei* of the same family as *Diplodinium ecaudatum*, was believed by Sharp to be insufficient for his species. The diminutive size of the "motor mass," its nonconformity in shape to the particular region of its location, and the want of attachment of the several strands to any fixed structures were conditions unfavorable to such an interpretation.

Nor did it seem probable that the mechanism is contractile in function. If it were so, it should appear attached to fixed structures, on the one hand, in order to affect movable structures, on the other,

which is not the case. Furthermore, the organelles with which the strands are associated are never translated in "the direction of the strands leaving the motorium, but rather in a direction at right angles to the course of the fibers, thus militating against a contractile function for the fibers" (Sharp, 1914, p. 86).

The perfect coördination in the activity of mobile parts, all of which are supplied by strands from the centrally placed motorium, and the advantageous location of the system to function "as a center of motor coördination in an animal which is exceedingly active, exceedingly responsive to external stimuli and one, moreover, which exhibits a high degree of selective feeding," are phenomena which could be most satisfactorily explained on the hypothesis that this apparatus functions as a primitive type of nervous system whose coördination is effected through the central motor mass, the motorium. Accordingly, Sharp gave to this system the name "neuromotor apparatus."

In a fresh-water ciliate, *Euplotes patella*, a fibrillar system comparable with that of *Diplodinium ccaudatum* has recently been worked out and described by Yocom (1918). It is noteworthy that these two forms are of different orders and habitats as well, the latter an Oligotrichan parasite common in the stomach of ruminants, while the former is free-living and a member of the order Hypotricha. The presence of these homologous systems in ciliates so varied in mode of life and kinship indicates the possible widespread occurrence of comparable systems in numerous other forms of this exceedingly interesting and important group of protozoans.

Let us now consider the nature of this "neuromotor apparatus" in *Euplotes patella*, as found and described by Dr. Yocom. Prefacing this consideration, it will be convenient to offer a very brief account of the external form of the animal and the relative positions of its ectoplasmic organelles. The body in general contour roughly resembles the bowl of a tablespoon, the convex surface of which represents the dorsal side of the organism, and the concave surface its ventral side. For the anterior end, to complete the figure, one should picture a mere stub of a very broad handle still attached and well rounded to suit the contour of the bowl. The stub would then represent the oral lip of the animal. This lip forms an anterior projection of the dorsal side over a wide triangular cytostome at whose posterior apex is the pharynx situated on the left about halfway down the body. A series of membranelles borders the dorso-posterior margin of the oral lip and on the left turns ventrad to continue along the left side of the cytostome into

the pharynx. The remaining external organelles embrace eighteen styliiform cirri. Of these, four are caudal and fourteen ventral in position. The right anterior ventral surface bears nine cirri, of which six are termed frontal and three ventral cirri. The remaining five of those ventral in position, known as anal cirri, are the largest and longest and are the most important. These have their origin at the ends of the five ventral grooves about twenty-five microns from the posterior end, and extend backward beyond the caudal margin of the body.

All the cirri were observed by Yocom, in agreement with Maupas (1883) and Griffin (1910), to be composed of cilia with distinct basal granules. The component cilia are imbedded in a dense plate of ectoplasm just beneath the pellicle, the plate serving as a firm support for the cirrus. Now from the basal plate of each anal cirrus there extends a fiber toward the anterior end. These fibers were first seen and figured by Maupas (1883) who briefly described them as joining the five anal cirri and extending forward to converge and unite into a single thread which disappeared near the anterior end of the animal. In 1903 Prowazek found similar fibers in *Euplotes harpa* and Griffin (1910) described such fibers for *E. worcesteri*. Yocom, however, was able to trace the fibers in *E. patella* farther forward to where they join one end of a very small bilobed body, "the motorium." "It was first seen as a dark body in animals stained with iron-alum haematin, lying close to the right anterior corner of the triangular cytostome. In specimens which are well destained this body is seen to be composed of very fine granules closely grouped together, but if too dark it has the appearance of an almost homogeneous body. When stained with Mallory's stain the motorium becomes bright red from the acid fuchsin and lacks the granular appearance characteristic of specimens colored with haematin. Plate 14, figure 5 (*mot.*) shows that this motor mass does not have a smooth contour, but rather that it has ragged edges with processes extending out into the surrounding ectoplasm" (Yocom, 1918, p. 355). The motorium is about eight microns long and, as figured, about one-fourth as wide as it is long. Joining its left end are the five long fibers from the anal cirri. These fibers converge and appear to unite with the motorium as a single strand.

From the right end of the motorium another fiber, the anterior cytostomal fiber, was found to pass anteriorly and to the left along the proximal border of the oral lip and the bases of the membranelles throughout the entire series. Within the oral lip was observed a

conspicuous "lattice-work structure" whose bases, like those of the membranelles, very closely approximate the cytostomal fiber. Thus is formed (Yocom, 1918) "an unbroken fibrillar complex between the heavy anal cirri which are used chiefly in locomotion and the membranelles of the adoral zone which function as organs of food getting, organs of locomotion, and as tactile structures." Several finer and shorter fibers pass out from the base of each of the other thirteen cirri but Yocom found no indication that these fibers connect with any part of the complex uniting the membranelles, the lattice-work structure of the oral lip, and the anal cirri.

The anatomical continuity of this fibrillar system, its selective staining properties, the anterior, free position of the motorium and the intimacy of its several branches with the large, vigorous anal cirri, with the peculiar diffused lattice-work of the oral lip and with the ever active membranelles, these were significant features which strongly suggested that the whole, unique arrangement must have a function more highly specialized than merely that of support or even one of contractility. Rather, the system here, as the one in *Diplodinium ecaudatum*, should be regarded as possessing properties of conductivity functioning to coordinate the movements of the organs with which it is associated. It, accordingly, was also designated "neuromotor apparatus."

The morphological evidences which Yocom's researches have yielded lend strong support to this "neuromotor" hypothesis. Yet, however significant may be the foregoing evidences favoring the function of conductivity for this novel apparatus in *Euplotes*, to establish this or any interpretation of organic function, methods beyond the bounds of morphological inquiry must be introduced. In this endeavor, the investigator enters another field of labor, viz., that of experimental biology, the need and importance of which has, in comparatively recent years, become more fully recognized among biologists. Phenomena studied and described by the morphologists are of primary importance. A comprehensive knowledge of a structure and its relations is prerequisite to an understanding of its function. But functions can not be ascertained by exploring and mapping parts. Experimental means must also be provided, otherwise further progress is impeded and may even be rendered impossible.

In view of this and because of the important significance that attends the theory of the presence in certain Protozoa of structures which are neural in function, it was thought advisable to undertake the task of which this paper is an account.

During the winter of 1916-17 when Dr. Yocom had found and was studying the fibrillar system in *Euplotes patella*, it seemed to me that the experimental method of microdissection might be successfully employed to aid in determining the actual function of this system and that Yocom's excellent morphological studies might be supplemented by experimental evidence.

The value and necessity of experimentation was duly recognized by Dr. Yocom, who has already added several experiments of another sort to this essential phase of the problem. "In studying *Euplotes patella*" (Yocom, 1918, p. 363) "that have been treated with very weak solutions of certain chemicals, such as neutral red, methylene blue and especially nicotine, it has been noticed that the anal cirri and cytostomal membranelles are the last to cease moving. The other cirri become quiet but the membranelles and anal cirri have been seen to move even after the cytoplasm has begun to break up. Such phenomena favor very strongly the idea that the motorium serves as a coordinating center between the anal cirri and the cytostomal membranelles. However, other observations on living animals give even stronger evidence in favor of the neural function. It has also been noted in specimens subjected to a very weak solution of nicotine that the frontal, ventral and marginal cirri continue moving even after the animal has ceased to swim about. The membranelles also move but more slowly than in normal animals. Occasionally one or more of the anal cirri may be seen to make a feeble movement not sufficiently strong to cause the animal to move. However, as the animal revives from the effects of the narcotic and begins to swim about by vigorous kicks of the anal cirri, a decided increase in the rate of movement of the membranelles may be noticed."

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This experimental investigation has been made under the very helpful direction of Professor Charles A. Kofoid, whose kindly and stimulating criticisms have contributed much to any merits the results may possess.

My thanks are also due to Professor S. S. Maxwell for several valuable suggestions on methods and useful literature.

METHOD AND MATERIAL

The method of microdissection has been greatly improved with the use of glass needles manipulated in a three-movement holder introduced several years ago by Dr. M. A. Barber and later extensively employed by Kite and Chambers (1912), Kite (1913*a* and *b*), Chambers (1914, 1915, 1917*a*, *b*, and 1918) and Seifriz (1918). The technique used by these investigators makes possible the dissection and observation of ova, spermatozoa, fresh tissues and Protozoa under the highest magnification of the microscope. A detailed description of the method is given by Barber (1914) which has been elaborated by Chambers (1915, 1918). I have made use of the principal features of this method in these studies on *Euplotes patella*.

The efficiency of the Barber instrument is indeed remarkable. Considerable experience was found necessary for drawing the finer and most serviceable needles, but their manipulation in the three-movement holder is a comparatively simple matter. One learns the adjustment of the screws controlling the needle almost as readily as the operation of a mechanical stage. After some practice the facility with which the apparatus may be manipulated and the feats thus made possible with a glass needle are rather surprising.

Moist chambers.—Two forms of moist chambers have been successfully employed. A Bausch and Lomb monocular microscope having a rotary stage was first used. For this stage a convenient round moist chamber was devised as follows: The base of a heavy, extremely shallow petri dish, in diameter slightly less than that of the rotary stage, was fastened upon the latter by means of two brass posts 20 mm. long screwed into the clip holes of the stage. The upper ends of these posts firmly supported the top of a large stender dish, this top or roof having a diameter equal to that of the bottom or floor. It will be observed that both roof and floor were thus securely fastened to the rotary stage. On the other hand, the wall of the moist chamber, made from the upper portion of the stender dish mentioned above, was solidly attached by two brass arms to the shank of the microscope and was of such height as to permit free movement of the roof and floor. A hole through the wall on the right allows the insertion of the needle into the moist chamber. Also, a circular hole 20 mm. in diameter

appears in the center of both the roof and the floor. Inside the chamber around the lower hole was sealed a glass ring which completed a shallow enclosure for water or moist cotton. A heavy steel shank supported the Barber holder. The shank was clamped to a metal base upon which the microscope also was fastened. This sort of moist chamber on a rotary stage has one advantage of much importance for the microdissection of Protozoa: while the animal is being held by water-glass surface tension it may be rotated and so cut through any part at any desired angle.

The other moist chamber, constructed on a plan very similar to those described by Barber (1914) and Chambers (1915, 1918), was used with a mechanical stage on a Bausch and Lomb binocular microscope. The mechanical stage was reversed and fitted onto the left side of the microscope stage, the Barber instrument being attached to the right side. The combined use of the mechanical stage and the Barber holder is often advantageous and sometimes necessary. This arrangement just stated, which allows the free use of both hands, has been found very convenient. For a detailed description of the rectangular type of moist chamber, the articles of Barber (1914) and Chambers (1915, 1918) may be consulted, and further account of it here is unnecessary.

Binocular microscope.—Most of these experiments have been performed with the aid of a binocular (Bausch and Lomb) microscope. Especially for microdissection purposes, this instrument is much superior to the monocular microscope. To observe clearly the position and adjustments of the needle in the vertical dimension was found to be very essential in several experiments. As will be described later, all the anal cirri were successfully removed from a few animals without any apparent injury to the body. This, it seems, would have been impossible with only monocular vision. Furthermore, the general contour of the organism, the relative positions and movements of its organelles, the cyclosis of the granules of the endoplasm, the contractions of its vacuole and its forms of behavior in creeping and swimming are much more satisfactorily studied under the binocular microscope. The use of both eyes soon becomes fully as desirable in microscopical as it is in unaided vision.

Glass needles.—Much of one's success or failure in microdissection can be attributed to the quality, shape and size of the needles employed. Soft glass needles are of little value in making incisions, but because of their flexibility they may be used to hold the protozoan without

undue pressure and without injury in order to study the movements of organelles and cyclosis phenomena. Needles made of Jena glass were found to be very suitable for general purposes and especially useful in performing transections and the excision of parts. But even more serviceable were the needles drawn from a hard quality of Pyrex and glass tubing. A special mixture of this glass may be obtained from the Corning Glass Co., Corning, N. Y. This is less flexible than the Jena glass and apparently not as fragile. For making narrow incisions and the excision of organelles, quartz needles are quite

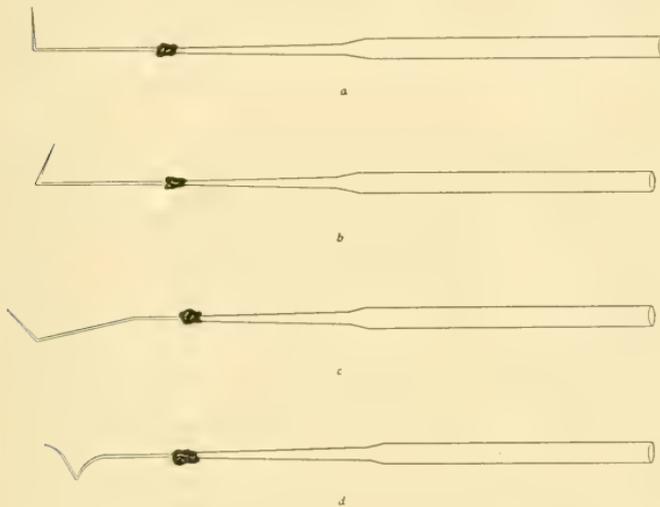


Fig. A. Types of glass needles used in microdissection.

superior in every respect. The costliness of this material becomes negligible if the needles are drawn from tubing that is about one half the diameter of a pin, then sealed with "orange stick" in a handle made of ordinary glass tubing. In fact, I have come to use this method also in making needles of Jena and Pyrex glass. These were drawn in a very small alcohol flame, but an oxygen-gas or oxy-acetylene flame is necessary for making quartz needles. The finest points were obtained by completing the needle not in but quite near the flame. Muscular sense is more dependable than eyesight for drawing very fine points, some of which, especially those of quartz, are less than a micron in diameter. The shape of what may be called the shank of the needle was found to be of considerable importance. The figures

above (p. 415) illustrate four forms of shanks which were found most serviceable. For convenience I have named these: *a*, right angled; *b*, acute angled; *c*, obtuse angled, and *d*, V-shaped shanks. Needles *a* and *b* have been used for probing or tearing regions or dissecting off parts; needles *c* and *d* for making incisions and, particularly *d*, for bisecting the organism, making wide incisions or snipping off organelles. The V-shaped shank affords more flexibility, which may be increased by lengthening the V.

Control.—To provide for the control of the organism during operation, several methods were tried. Fine fibers of silk and of cotton, also very finely ground particles of glass were sealed with agar to the surface of the cover-slip. These afford helpful means of holding the animal in place for the beginner until he has learned the rather difficult but by far the most satisfactory method of control, namely, water-glass surface tension, suggested by Kite (1913, p. 146). I have used a very small pipette with a rubber tube attached for the mouth as a means of transferring the animals and reducing the volume of the hanging drop to afford just the necessary amount of surface tension. This amount one learns only after considerable practice. Allowing slight degrees of evaporation also facilitated this proper adjustment. The animal must be held in place but a further increase in surface tension may cause it to disintegrate, often with explosive violence. A perfectly clean surface of the cover-slip and a wide hanging drop, say 10 mm. in diameter, aid greatly in obtaining proper surface tension. Another fairly satisfactory and more simple method of control is to confine the protozoan within a very small hanging drop, the surface tension of which with the glass is greatly reduced by applying a mere trace of paraffin or some other harmless oil.

In making an incision, the needle was applied suddenly and rather firmly by means of the up-and-down movement screw. After an interval of a few seconds, this screw was slowly turned back and forth, which caused a seesaw movement of the needle-point. With proper care and if the needle be not too flexible, a surprisingly clean cut may thus be made without any loss of endoplasm. Chambers (1917*a*) has very helpfully suggested the use of a needle not exceedingly fine and the importance of slow movement and sufficient time in making an incision. Otherwise a loss of endoplasm usually results and this may be followed by rapid and complete disintegration. This outflow of endoplasm may, however, be regulated to advantage by applying a V-shaped needle near an animal in which a careful incision has been

made and which is held near the edge of a wide but very shallow hanging drop. By slowly turning the screw for the up-and-down movement, delicate changes in the degree of stress of the surface film are thus effected, an outflow of endoplasm may be induced and its rate of discharge varied more or less at will. As will later be described, this affords a study of several interesting features including the nature and extent of the ectoplasm and of the pellicle.

Staining.—Several vital stains have been employed with varying success. For the study of the external organelles, a .0001 per cent solution of haematoxylin gave the most satisfying results. This was also useful in staining the fibrillar system; certain new features of this system, in fact, were first seen after the animals had been subjected for about eighteen hours to this stain. It was incidentally discovered that a very weak solution (.001–.0001 per cent) of tannic acid, after eight to ten hours, distinctly sharpens the outline of the fibrillar apparatus. This is apparently due rather to its effect upon the cytoplasm, affording a contrast which discloses more clearly the apparatus. Neutral red (Grübler), new methylene blue R (C. C. Co.), toluidin blue (Grübler) are among other vital dyes which enhanced the view of the system of fibers. Usually for dissecting, however, the anal cirri fibers and not infrequently the motorium with its attached fibers, may be seen clearly enough under oil immersion (2 mm. Zeiss apochromat), without the aid of intra vitum dyes.

For studying specimens fixed and stained before or after dissection, the several fixatives and stains employed by Yocom (1918, p. 342) were used with good results. The method of pieromercuric fixation followed with Mallory's stain or with iron-haematoxylin was especially valuable for the study of the fibers before and after they were cut. Delafield's haematoxylin stains the fibrillar apparatus even more distinctly. There was some evidence that the cut fibers do not stain so deeply with the iron-haematoxylin, but this has not, as yet, been definitely ascertained. Much care is necessary in staining single specimens. Fixatives were applied, usually hot, by means of the pipette (above referred to) under the low power binocular. The specimen was then transferred to a cover-slip or slide which had been treated with Meyers albumen fixative. After a distinct film had formed, the slide was passed through the alcohols and stains, usually without detachment and loss of the specimen.

Material.—The fresh water ciliate, *Euplotes patella*, possesses certain morphological features that make it an unusually choice subject

for microdissection studies. Plate 29, fig. 1, illustrates several structures which are very favorable for operative work on the neuromotor apparatus. The large C-shaped nucleus permits the cutting of the anal cirri at several points with no injury to the nucleus. Also, the cyto-stomal fiber may be cut at various angles and the motorium destroyed likewise without injuring the nucleus. The stiff, fairly tough pellicle which envelops the body ably maintains the normal form after an incision, often very deep, has been made. The remarkable firmness of this structure makes possible the removal of cirri with no apparent injury to the body. The projection of the oral lip and of several cirri affords successful excision of these parts, and the definite grouping of the frontal, ventral, anal, and marginal cirri permits various transections and combinations of transections and excisions that have proven to be exceedingly useful in studying the functions of these groups of organelles in creeping and swimming movements. The location of the single micronucleus at the anterior end of the body is especially favorable for ascertaining more accurately the specific rôle of this interesting and important organ.

THE LIVING ORGANISM

Owing to the invaluable aid of water-glass surface tension for the control of Protozoa in a hanging drop, it is now possible to study active, living organisms in minute detail under the highest magnification. With a properly constructed moist chamber, the time limit for this study depends rather upon the endurance of the observer. A living *Euplotes* was held continuously within the field of a 2 mm. Zeiss apochromat lens for more than two hours, at the end of which time, when a drop of water was added, the animal swam slowly about; within half an hour its movements were apparently normal. This allotment of time is ample for a complete, detailed review of all the structures and movements of the organism that may appear within the range of microscopical vision. By properly adjusted, transmitted light, the binocular microscope with an apochromat lens affords here the view of a living, active form that rivals any of nature's finest displays. The study of living organisms always lends increased interest and adds the essential complement to our knowledge of the structures and relations disclosed in fixed material.

ENDOPLASM

In his microdissection studies on living ova of certain marine invertebrates, Chambers (1917a) finds their cytoplasm to consist of "a hyaline fluid matrix in which are imbedded granules of various sizes." The granules, classified into microsomes and macrosomes, differ considerably not only in size but also in number, shape, solubility, refractive indices and in chemical reactions. Rapid tearing of the internal cytoplasm with the needle induced in that region the dissolution of the macrosomes and liquefaction of the cytoplasm in which the microsomes exhibited distinct Brownian movements. Such injuries sometimes spread throughout the entire cell. Also, a rapid dissolution of the macrosomes occurred with the outflow of the cytoplasm into the sea-water "if no protective membrane intervened." The microsomes were much more resistant and displayed the dancing Brownian movement for a considerable time after the complete disappearance of the liquefied cytoplasm. A protective membrane frequently formed around a mechanically injured, disorganized area within the cell or on the surface of endoplasm exuding through a rupture of the surface-film or ectoplasm. This membrane is directly comparable with the ectoplasm. Both represent a colloidal gel enclosing the endoplasm which usually exists in the sol state but may come to form temporary organs such as the cell asters (Chambers, 1917b) by a reversal of the sol to the gel state.

A similar consistency of cytoplasm can be identified in *Euplotes patella*. Here, however, the general appearance of the endoplasm is considerably modified by food vacuoles which are of various sizes and sometimes numerous. But with high magnification and well-regulated light, hosts of small granules, comparable with Chamber's microsomes, appear throughout the entire body. Larger granules or macrosomes are less conspicuous and have been observed only in the endoplasm. Both the large and small granules are larger than the macrosomes and microsomes described by Chambers. The small granules are fairly constant in size, with a diameter of about one micron or more. Here and there within the endoplasm they exhibit Brownian movement. They appear round and are highly refractive. The large granules vary from three to five microns in diameter, are usually opaque and often irregular in shape. They have not been identified in the ectoplasm and never appear within the cyclosis currents of the endoplasm. Mechanical

injury by rapid movements of the needle-point causes their disappearance in that region of the body. They may be observed for a time within small globules of the endoplasm which have flowed out along the sides of the needle and become enveloped with a "protective membrane." But if the outflow of the endoplasm is sufficiently rapid and of such quantity as to prevent the formation of a membrane, these large granules quickly swell and burst or otherwise disappear, and the hyaline, liquefied endoplasm disappears leaving only the small granules, which may remain for hours constantly in Brownian movement. In one instance this dancing movement of the granules continued throughout part of an afternoon and evening, a period of about six hours.

ECTOPLASM

The outflow of endoplasm and disintegration of the organism from incisions made abruptly or from other causes is generally rapid and sometimes explosive. To prevent this sudden disruption and regulate the rate of outflow of endoplasm, a method which has been previously described (p. 416) was used. This method permits a careful study of the ectoplasm and pellicle. The ectoplasm consists of a comparatively thin, gel matrix with densely packed small granules of a dimension similar to that of the smaller granules of the endoplasm. These ectoplasmic granules appear equally numerous throughout, closely approximating the pellicle on one side and the endoplasm on the other. Griffin (1910a) describes similar granules in the ectoplasm of *E. worcesteri* which, however, vary in size and appearance more than do these granules.

Frequently, there is evident a fairly definite boundary between the ecto- and endoplasm but this condition apparently varies. If its outflow be not too rapid, the endoplasm separates from the ectoplasm and pellicle, sometimes leaving large areas that may remain intact for several seconds. With further disintegration of such areas, the pellicle and matrix of the ectoplasm quickly disappear, but the granules here, like the "microsomes" of the endoplasm, may persist for several hours in continuous Brownian movement. Plate 33, fig. 23, shows a portion of ectoplasm with a frontal cirrus attached. The position of granules on one side illustrates the manner of disintegration.

A further discussion of the pellicle appears under the caption "Experimental."

MACRONUCLEUS

The outline and structure of the large C-shaped macronucleus appears in the living, unstained organism very much as in the fixed material figured and described by Yocom (1918). The "contraction phase" of this organ with its reconstruction bands may be clearly observed in animals free from too many food vacuoles. But these features and particularly the granular, mesh-work consistency of the macronucleus can be much more satisfactorily studied after the latter has been dissected out with the needle. It is then found to be a highly

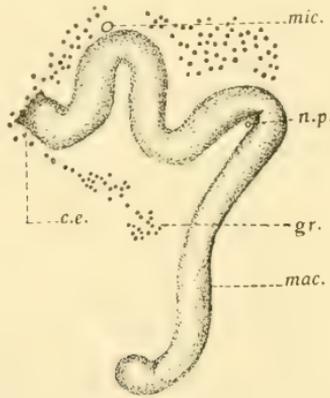


Fig. B. The nuclei of *Euplotes*. *c.e.*, cut end; *gr.*, ectoplasmic granules; *mac.*, macronucleus; *mic.*, micronucleus; *n.p.*, needle point.

gelatinous, rather rigid structure composed of small granules imbedded in a viscous, hyaline matrix (text fig. B). The organ is enveloped by a very thin, structureless membrane. Upon exposure to the water, the macronucleus increases slowly in size; within half an hour or so small blisters of the membrane slowly appear over the surface; the rate and extent of swelling increases and, upon rupture of the membrane in one or several places, there follows a rapid dissolution of all except the small granules, which for several hours exhibit a dancing Brownian movement. These granules vary somewhat in size, with an average diameter about one fourth that of the microsomes found in the endoplasm.

MICRONUCLEUS

This organelle is much less conspicuous than other organs in living, unstained animals, but when once clearly identified it may always be readily located with suitable magnification and properly regulated light. The variations in position and size depending upon its several phases (Yocom, 1918) have been definitely verified even without the aid of vital dyes. With a .0001 per cent aqueous solution of neutral red, haematoxylin, methylene blue or Bismarck brown, both micro- and macronucleus become sharply outlined and the visibility of their structure is considerably enhanced. Several times the macronucleus has been dissected out with the micronucleus attached and lying in a very shallow pocket (Griffin, 1910; Yocom, 1918). In two cases, the micronucleus was brushed with the needle-point out of the depression in which it was feebly held evidently by a viscous, ductile substance that stretched only for a few microns in fine, retractile threads. These properties of this substance indicate that it may be comparable with the hyaline, gelatinous matrix of the macronucleus. Other interesting features of the micro- and macronucleus will be treated in a later paper.

CONTRACTILE VACUOLE

My observations on the size and position of this organelle are in general agreement with Yocom's (1918), although I have observed an abnormal increase in its size after certain vital stains such as neutral red, Bismarck brown, Congo red, gentian violet, fuchsin S, etc., have been added to the water. Moreover, any distinct mechanical disturbance, e.g., rapid movement of the needle to and fro through the hanging drop, continually jarring or shaking the moist chamber, etc., effects very noticeable changes in the size and period of pulsation of the vacuole. One should then expect, as is the case, that incisions, transections, or excisions would similarly affect this organelle. It would appear, indeed, that the contractile vacuole in *Euplotes patella* is exceedingly sensitive to various stimuli. The average size of this vacuole in ten carefully handled animals was 29 microns at maximum diastole. Its diameter upon disturbance or after incisions may become 45 or even 50 microns, and its period of pulsation, which is normally about forty seconds between systoles, may thereupon vary from three to fifteen minutes.

The discharge of the vacuole is clearly on the ventral side within three or four microns of the right margin of the pellicle. This may be observed with careful focusing when small food vacuoles are lying just posterior to the point of discharge. The relative position of the last trace of a systole, as compared with that of the food particles and ventral pellicle, appears distinctly ventral. Also, this position may be verified by applying the needle-point very lightly against the ventral surface near the point of discharge, whereupon the position of the discharge is ventral.

ANAL APERTURE

This has been located in *E. patella* on the ventral side slightly posterior to the discharge pore of the contractile vacuole and within five microns of the margin of the pellicle. It was first observed when two frustules of *Navicula* were seen to pass successively from an animal held in a shallow hanging drop. The emission of various other fecal particles, mostly very small, has since been noted in several individuals. Voiding apparently seldom occurs when the stress of surface tension or the pressure of an applied needle is increased. In only three cases has the emission of particles thus been observed. However, pressure from the needle or from surface tension may sometimes cause the protrusion of a small area including the anal pore. Griffin (1910a) describes the location of an anal opening in *E. worcesteri* immediately to the right of the outermost anal cirrus. This closely approximates the position of the pore in *E. patella*. But the pore in *E. worcesteri* is anterior to the contractile vacuole, whereas in *E. patella* it is posterior. It should be said, however, that the position of the contractile vacuole varies considerably in different *E. patella* which, of course, somewhat alters the relations above referred to.

CIRRI

The eighteen styliform cirri of *Euplotes patella* appear on the ventral side in four fairly well defined groups. Yocom (1918) classifies these into six frontal cirri, three ventral cirri, five anal and four marginal cirri, which is in agreement with Stein (1859). While the groups of frontal and ventral cirri are less clearly defined than are the others, it is at least more convenient and, I believe, more accurate to regard the frontal group as composed of seven, and the ventral group of two cirri. Also, for further convenience in describing the

microdissection experiments, I should subdivide the seven frontal cirri into an anterior group of three and a more posterior group of four. Accordingly, these will hereinafter be referred to as the "group of three" and the "group of four" frontal cirri.

The ciliary composition of the cirri of various *Euplotes* is a well established fact. The component cilia with their basal granules have been described for the cirri of *E. vannus* by Minkiewicz (1901), of *E. harpa* by Prowazek (1902), of *E. worcesteri* by Griffin (1910), and of *E. patella* by Yocom (1918). This feature of a cirrus may be readily demonstrated in a shallow hanging drop by means of a V-shaped dissection needle. Here a detached cirrus may be pushed to the edge of the hanging drop for greater surface tension and gently rolled to and fro between the needle and cover-slip. Soon the cirrus splits into loose bundles of its numerous cilia. But this method reveals other features: the cilia are embedded in a gelatinous matrix that is highly viscous, as may be seen by pushing the bundles about with the needle. These remain attached at one or several places even after rather rough handling. They frequently adhere to the needle and so may be pulled a considerable distance through the water. Upon exposure to the water for a few minutes, the cilia of the bundles further separate and show adhering to their sides minute globules of the coagulated matrix. The question here arises whether this coagulation of the viscous, hyaline matrix may not account for the extreme rigidity that overtakes the cirrus soon after its detachment, when it may be pushed about and even beyond the margin of the shallow hanging drop without any apparent bending. Furthermore, after examining numbers of these cirri by the above method, one becomes rather convinced that the matrix-cilia complex is invested with an extremely thin, structureless membrane that is fairly tough but very flexible. I have not been fully satisfied about this structure since I have not clearly seen it apart from its enclosure. This final evidence may later appear. However, if present, the membrane rapidly dissolves from a recently detached cirrus, which then splits into its component cilia.

Except the anal cirri, all are round at their base and gently taper to a rather sharp point. The two right marginal cirri are fimbriated (Yocom, 1918). Not infrequently the second and third (numbering from left to right) anal cirri are also fimbriated. The shape of the base of the anal cirri differs considerably from the others. Figures 19 and 18 show the comparative dorso-ventral width and lateral thickness of an anal cirrus base, the former being six to eight microns and the latter about three microns.

The attachment of the cirri will be discussed in connection with a description of the neuromotor apparatus. It remains here to describe briefly the several movements that are common to the different groups of cirri. Pütter (1903) discusses these general types of ciliary movements among Protozoa: (1) the "hook-like" type, found in cilia or flagella used for food-taking; (2) the "whip-like" type, exemplified by the flagellum of *Euglena*, and (3) the "infundibular or funnel-like" type, very common among most flagellates and ciliates. The anal cirri of *E. patella* frequently exemplify types both (1) and (2), while types (2) and (3) are common for their frontal, ventral, and marginal cirri. Yocom's observation (1918, p. 363), that the anal cirri "move in only one plane, that parallel to the median plane of the body," is hardly adequate. As will be described presently, these cirri are very frequently used in guiding the animal to the right or left, and are especially active as the chief means for making sharp turns to the right, which is not an uncommon reaction during swimming. In the latter instance, particularly cirri 3, 4, and 5 (numbering from left to right) are flexed rather abruptly near their base and lash close along the ventral surface of the body. Griffin (1910, p. 301) regarded the anal cirri of *E. worcesteri* to have "only a single, strong motion: a vigorous kick directed backwards." In *E. patella*, however, this backward stroke is by no means the only effective movement, nor even the most important. The "avoiding reaction" of this species, which will be described further on, is effected chiefly by means of the anal cirri. Furthermore, these cirri, together with the frontal and ventral groups, are the animal's "feet" for creeping and, as we shall see later, upon removing the anal cirri, creeping becomes impossible.

Another common use of these anal cirri may be observed in their attachment to suspended débris in the water and swimming about with it sometimes for several minutes; or, less frequently, in holding on to floating débris or even to the dissecting needle and suspending the body dorsal side down, occasionally at an angle of fifteen degrees or more. The attachment, to the needle at least, is usually with two or three and often four anal cirri. In such cases I have observed clearly a slight flexure of the tip of one or more cirri about the needle and had concluded this to be the means of supporting the body; but later, an attachment by only one cirrus was seen with the tip several microns in length lying along the under side of the needle. This latter observation has since been made a number of times and in two instances I was able to move the needle slowly back and forth without disturbing

the animal, when the support was sufficient to carry the body along with the needle. As to how this curious feat may be accomplished I can only conjecture the possibility of a secretion present on the cirrus.

MEMBRANELLES

Projecting anteriorly from along the dorsal base of the oral lip, the series of membranelles turn ventrad on the left in a gracefully twisting curve and continue along the left side of the cytostome and pharynx to end in a hooklike turn at the apex of the pharynx. Yocom (1918, p. 4) has aptly likened the twisting and general shape of this continuous series to the collar and lapel of a coat. His splendid detailed description may be referred to for the more minute structure of these organelles. A further description concerning only their attachment and their relation to the neuromotor apparatus will be given later under the heading "Experimental." However, the considerable discussion on the actual relations of the cilia which compose the membranelles described for various *Euplotes* is here worthy of note. Obviously, these relations condition the shape of the membranelles. For *E. harpa*, Wallengren (1901) describes and figures the membranelles as triangular in shape. Minkiewicz (1901) found those of *E. vannus* to be of a similar shape. Yocom's discussion of this point would seem to favor the view of the above authors, although he does not refer to the particular shape of a membranelle. Griffin (1910a), on the other hand, states that after repeated examination of these structures in *E. worcesteri*, he is inclined to believe that the membranelles which are nearly rectangular in shape are composed of distinct cilia "with movements so perfectly coördinated that they act and ordinarily appear as a single and delicate band" (p. 299). Möbius (1887) had come to the same conclusions regarding both the shape and structure of the membranelles of *E. harpa*.

From the present studies on *E. patella*, I am convinced that the cilia composing a membranelle in this species are definitely fused and that they are so arranged as to give each membranelle the shape of an elongated triangle. Indeed, those extending over the oral lip (fig. 16) approximate the form of a short cirrus with a very wide base. By means of a dissecting needle, several of those dorsal to the oral lip may be excised, together with a portion of that organ from which they readily separate, and thus the features mentioned above may be exhibited. They may very soon split into bundles of component cilia

that show basal granules distinctly, while later there appear along the cilia minute, coagulated globules comparable with those described for the cirri. Also, excellent views of the shape and arrangement of the entire series of membranelles may be had upon transferring an organism to a hanging drop of 0.1 per cent solution of tannic acid. The animal usually dies within a few minutes but in the meantime the membranelles become stained and their movements are slowed so as to afford a splendid study of each membranelle of the entire series.

The primary function of the membranelles of the cytostomal and pharyngeal region is food-taking. Yocom (1918) has discussed the manner of the intake of food, but he does not refer to the expulsion of particles from the pharynx after they have been "sampled" and refused. This ejection may be sometimes rather violent and is effected by a reversal of the membranelles which may involve only those of the pharynx, or also the cytostomal membranelles, or occasionally even the entire series. The chief function of the adoral membranelles is their indispensable service in swimming. An account of this important feature is given in later paragraphs.

NEUROMOTOR APPARATUS

The system of fibers connecting the series of membranelles, the lattice-work structure of the oral lip and the five anal cirri to a small bilobed body lying in the extreme anterior right of the animal, together with other fibers radiating from the base of the remaining thirteen cirri were found and described by Yocom (1918) as the neuromotor apparatus of *Euplotes patella*. In preceding paragraphs I have given a brief but fairly complete review of Dr. Yocom's account of this apparatus. It is my purpose here to reconsider certain parts of his account and in following paragraphs (see "Experimental") offer a few minor modifications and additions (fig. 13).

Following Yocom's figures and descriptions, I have been able to identify in the living organism all the structures of this interesting and complex mechanism. The anal cirri fibers are usually distinctly visible throughout most of their length. The presence of food vacuoles dorsal to the frontal cirri frequently interferes with the tracing of these fibers to their junction with the motorium, but this interference may be obviated by keeping the animals in well-filtered water for several hours, at the end of which time most of the food vacuoles will have disappeared. It is then possible to observe not only all five fibers

throughout their extent but also the motorium and from its outer end the membranelle fiber passing to the oral lip and membranelles. After they are once clearly identified with the aid of vital dyes, the motorium and its connecting fibers may be recognized usually with little difficulty in unstained animals.

The several fibers associated with the base of the frontal, ventral, and marginal cirri are much less distinctly visible. Very careful focussing and regulation of light are necessary, and even then it is usually impossible to make sure of these fibers without the aid of vital dyes. This may be said also of the membranelle fiber along the base of the membranelles. Here the presence of the basal corpuscles of the cilia composing the membranelles and of a compact row of large ectoplasmic granules (fig. 17) renders this fiber so obscure that a distinct and satisfactory view of it may be had only after dissecting off the membranelles and oral lip and allowing the ectoplasm to disintegrate. Most of the lattice-work complex within the oral lip may be distinctly seen in ventral view. The basal attachments of this to the membranelle fiber are indistinct if at all visible, due to the basal corpuscles and large granules of the ectoplasm.

MOVEMENTS

So far as I have been able to ascertain, the creeping and swimming movements of the genus *Euplotes* have not been described. In this species, *Euplotes patella*, there are evident three specific creeping and six swimming movements. Of the latter, two are much less common than are the other four.

Being typically of creeping habit, this animal is usually found moving about on the bottom of an aquarium or over various débris and vegetation or on the under-surface of scum or of the surface film of the water. Its creeping movements, therefore, are readily observable. This method of locomotion is effected by means of all the cirri on the ventral surface, aided more or less by the ever active membranelles. The three kinds of creeping movements are: (1) locomotion straight ahead or slightly to the left (orally), (2) a quick, backward movement, usually for a distance about equivalent to the length of the body, or less, and (3) a turn to the right (aborally) through an angle of thirty to sixty degrees. Movements 2 and 3 are comparable with Jennings' "avoid-reaction." The accomplishment of movement 2 probably involves

all the cirri to some extent but chiefly the anal cirri and adoral membranelles, as experiments later will show. Movement 3 is affected also with the aid of all cirri and aboral membranelles, but chiefly by means of the frontal cirri, except when the turn is very rapid and through a large angle, say 90 degrees; then all the cirri, but principally the frontal and anal cirri and the adoral membranelles, are brought into play. Creeping is rendered impossible upon excising the anal or the frontal cirri. This feature will be described later under the head "Experimental."

Euplotes patella's swimming habits are less common than are its creeping movements but the animal utilizes this special advantage by no means infrequently. Also, the variety of its swimming movements indicates considerable proficiency in this valuable mode of locomotion. The six movements in swimming, above referred to, are as follows: (1) straight ahead without rotation, (2) straight ahead in spiral rotation, (3) circus movement to the right, without rotation, (4) circus movement to the left, without rotation, (5) a sharp turn to the right, similar to creeping movement (3), and (6) movement directly backwards, comparable with creeping movement (2).

It will be convenient here to recall the special, effective strokes of the membranelles and of the various groups of cirri: (a) The effective strokes of the membranelles may pull the animal forward or by reversal drive it backward. Without the interplay of cirri, the tendency of direction by means of the membranelles is neither straight ahead nor straight backwards but in a circuit, as will be shown later. (b) The caudal cirri may function as rudders or as propellers. The two on the right usually function as propellers; the two on the left, as rudders. Griffin (1910), for the caudal cirri in *E. worcesteri*, finds the tendencies of their movement to be just the reverse of those which I have ascribed to corresponding cirri in *E. patella*. (c) The anal cirri may lash directly backward, individually or simultaneously, and so drive the animal forwards; or they may lash directly forward, not always but often synchronously, driving the animal backwards; or they may lash to the right side with the effective stroke backwards or forwards, thus aiding to turn the animal to the left or right respectively. (d) The frontal and ventral cirri commonly show infundibular movement, with the effective stroke directed variously; only occasionally has the lashing, whiplike movement been observed in these cirri.

(1) The "straight ahead" swimming movement is of short duration, but occurs rather frequently, particularly after the animal has been

strongly stimulated mechanically, e.g., by stirring the water violently. This movement is often observed upon transferring an animal to the hanging drop by means of a capillary pipette. The anal and marginal cirri may aid in this movement but they are not essential. (Experimental evidence will be given for this and following positive statements.)

(2) Spiral movement is the one most frequently observed. For this movement, the marginal cirri are not essential, the anal cirri are useful and the frontal cirri very valuable. Without the adoral membranelles the movement normally is quite impossible.

(3) Circus movement to the right is frequently seen after the animal has been confined in a narrow hanging drop which may be either comparatively deep or shallow. The anal and marginal cirri may aid in this movement but they are quite unessential. The frontal cirri, particularly the "group of three" are useful here but the movement is performed chiefly by means of the adoral membranelles.

(4) Circus movement to the left is so infrequent that the means for its accomplishment have not been studied. It has been observed only when the animal was confined in a hanging drop.

(5) The sharp turn to the right is performed chiefly by means of the adoral membranelles and anal cirri. The marginal cirri are here useful but not essential. The movement is very common. Usually the spiral movement does not proceed far without this sharp turn intervening to divert the animal's course.

(6) The backward movement is effected chiefly also by the anal cirri and *always* concomitantly with the reversal of the membranelles. It is wholly an avoiding reaction and is distinctly comparable with the creeping movement 2. Indeed, it may be regarded as merely the augmentation of that movement 2, as shown by disturbing the creeping animal sufficiently with the needle-point or by applying some chemical, such as methylene blue. The animal may thereupon dash backwards a distance several times its length, even repeating the movement again and again. In this respect *E. patella* strongly reminds one of its relative, *Uronychia*, whose avoiding reaction brings into play the large posterior cirri which are seldom if ever otherwise used (Calkins, 1911, p. 98).

EXPERIMENTAL

The following results are from experiments made on several hundred *Euplotes patella*. Of these experiments, 315 were recorded with fairly extensive notes on the exact location and nature of the cut and on the animal's reactions before, during, and after the operation, allowing several minutes for its recovery from the shock effects. The various cuts include: (1) transections (dividing the animal in any plane at right angles to its long axis), (2) excisions of external organelles with or without a portion of the body, and (3) incisions in the body or oral lip in any plane. Efforts were made also to ascertain some of the physical properties of the pellicle and of the fibrillar system.

PELLICLE

This membrane which completely envelops the body and oral lip of *Euplotes patella* is firm, fairly tough, and sufficiently rigid to maintain constantly the normal form of the body and lip, even when the animal is subjected to a considerable stress from changes in water-glass surface tension or to the applied pressure of a flexible needle. Figure 1 shows the extent of an incision fully two-thirds the width of the body, yet this animal continually kept its normal shape during a half-hour of devious movements through the water. In making dissections the toughness of the pellicle requires the use of needles with rather stiff, short points. Long-pointed, very flexible needles are ineffective.

The extensile property of the pellicle is quite obvious in an animal which has gorged itself with food until the body is conspicuously bulged. If such an animal be subjected to a gradually increasing pressure by the surface tension method previously described, just sufficient to cause the egestion of a few food particles through the pharynx, then as the needle is slowly removed the pellicle may here and there become wavy or wrinkled. Within a few minutes the wrinkles usually entirely disappear. The elasticity of the membrane may be readily demonstrated by applying a fairly flexible needle the full width of the body when, with due pressure there occurs a conspicuous bending of the body over the needle. Upon releasing the pressure the body at once resumes its normal shape. This may be repeated successively many times. If, however, the animal has been

well flattened out by surface tension for about an hour, the flatness persists for a time after a drop of water has been added, but gradually the body recovers its normal form, usually within half an hour or less. During an incision, short furrows frequently appear on either side of the needle (fig.1). These may remain for some time but eventually disappear.

Any apparent modification in the shape of *Euplotes patella* occurs only from extraneous pressure. That the animal of itself is unable to vary its shape may be observed when it is hemmed in by cotton or silk fibers partially sealed to the cover-slip. Paramecia in the same hanging drop force their way among the fibers through narrow passes with constrictions of the body, a feat quite impossible to *E. patella*. Further contrast in the pellicles of these two forms is seen upon adding a weak solution (.1 per cent) of tannic or acetic acid. "Blisters" quickly appear on *Paramecium* but not on *E. patella*, although both may die in the solution within a few minutes.

FIBRILLAR SYSTEM

Studies of the fibers and their relations were made by means of various dissections but the most satisfactory observations were had when a slow disintegration of the body was brought about by inducing delicate changes of surface tension with a V-shaped needle. Thereupon the fibrillar system and its attached organelles would often remain intact and were always the last part of the body to undergo disintegration.

The anal cirri fibers normally lie upon the inner surface of the ectoplasm just above ventral grooves which are formed by clearly defined ridges. Each ridge is chiefly composed of a single row of very large ectoplasmic granules (fig. 20) that at times present internally a finely granular appearance and often persist several minutes after the body has entirely disintegrated. Sometimes they have been seen to swell and burst explosively, disappearing entirely from view. These and surrounding smaller ectoplasmic granules lie embedded in a hyaline, gel matrix which apparently is continuous with the basal plates of the anal cirri. This region of ectoplasm resists disruption longer than the adjacent portions and so it frequently happens that the anal cirri fibers, which lie upon the inner surface of the ectoplasm, all remain intact after the complete disintegration of the body. This condition, however, does not long prevail. Soon the ectoplasm here

shows signs of dissolution by a gradual dispersion of its granules and the anal cirri fibers, with or without their cirri attached, are at length set free, their spatial relations occasionally remaining unchanged. Careful observations during this tardy disintegration of ectoplasm, along with the explorations by means of the needle, make it fairly certain that the anal cirri fibers do not lie within the ectoplasm but upon its inner surface, being supported there by a very thin, hyaloplasmic sheath which may be a continuation of or comparable with the hyaloplasmic matrix in which are embedded the granules of the ectoplasm. The critical focus for a fiber does not appear to be identical with that for the ectoplasmic granules along (below) the fiber. Furthermore, the fibers are more or less readily displaced by means of the needle, although when undisturbed they remain adherent to the ectoplasm.

When set free from all attachments, the anal cirri fibers may be bent variously with the needle (fig. 15). They are then found to be fairly flexible, in no wise brittle and almost wholly irresilient. However, before the ectoplasm has completely dissolved, the fibers are much less flexible and generally recover after being bent. Figures 14 and 15 illustrate several permanent shapes into which the fibers were bent by means of the needle. They may adhere to the needle and so be pulled about through the water. They do not long resist dissolution and so disappear usually within fifteen minutes or less time after their exposure to the water.

Apposed dorsally to the basal plate of each anal cirrus, the corresponding fiber is modified into a "fan shaped structure" (Yocom, 1918) which I shall here designate the "anal fiber plate." This small plate is distinctly rectangular (fig. 14), and not oval as figured by Yocom. Its attachment to the fiber proper is secure, as may be readily ascertained by pulling or pushing the fiber about through the water with the needle-point. An interesting and significant feature is its intimate association with the basal plate of the anal cirrus. Figure 14 is a camera drawing of a cirrus in the process of detachment from the "anal fiber plate." It will be observed that the cirrus has rotated 90 degrees on its long axis and that the gelatinous extensile basal plate, which is a highly viscous gel, remains attached to the anal fiber plate. This attached condition is rarely found, owing to the readiness with which the basal plate detaches from the anal fiber plate. While attempting to make this drawing with the parts *in situ*, the separation ensued so readily that I succeeded in outlining only the partial detachment as shown in the figure.

Just as the anal cirri with their attached fibers frequently persist intact after the remaining cytoplasm has dissolved, so also do the membranelles with the membranelle fiber resist immediate disintegration. Furthermore, in seven recorded instances I have observed the anal cirri, their fibers, the motorium, the membranelle fiber, and the membranelles, all remain united for several seconds to about three minutes after the disruption of the body. In three of these cases, the anal cirri and membranelles continued lashing, but feebly and for a few seconds only.

The motorium with its attached membranelle and anal cirri fibers has been distinctly identified after more or less complete disintegration of the body. Much more frequently, however, only the fibers are evident. It would appear, therefore, that the motorium readily detaches itself from its connected fibers or otherwise vanishes, perhaps by rapid dissolution. In its normal position the motorium may be readily displaced with the needle-point. However, it resumes its usual position upon the removal of the needle. But if it be pushed too far, say ten microns, out of place it may become detached from its fibers, or apparently injured to such an extent that it dissolves or otherwise disappears.

In unstained animals, as stated previously, the membranelle fiber may be distinctly seen only a short way from its attachment to the motorium. Thereafter it becomes concealed among the ectoplasmic granules along the basal plates of the membranelles (fig. 17). It may be observed only after these granules have dispersed with the dissolution of the ectoplasm. Its general physical properties are apparently the same as those above stated for the anal cirri fibers. That description may suffice for this fiber also.

However, associated with the membranelle fiber and membranelles, certain plates have been found which I shall here call the "membranelle fiber plates" (fig. 13). These were first clearly observed upon partial disintegration of the series of membranelles which had been dissected from an animal vitally stained for about eighteen hours in a .0001 per cent aqueous solution of haemotoxylin. The membranelles proper had been set free, thus exposing these plates, one for each double row of membranelles. Figure 17 is a camera drawing of the plates and the membranelle fiber. The spokelike formation shown in the figure is usually assumed by the series of plates upon detachment of the membranelles and disintegration of the ectoplasm. This arrangement is clearly occasioned by their individual attachment at

only one end to the membranelle fiber. Explorations with the needle show this connection to be fairly secure. Also, the relation of each membranelle to its corresponding membranelle fiber plate has been found to be the same as the relation of the anal cirrus to its anal fiber plate. Of this one may be fully convinced upon observing the membranelle peel from its plate, a process which occurs not infrequently about one minute after the disruption of the ectoplasm. Thereupon, the basal plate of the membranelle, in which the basal corpuscles of the component cilia and the ciliary rootlets are imbedded, completely separates from the membranelle fiber plate which, like the anal cirrus plate, shows a smooth, clean surface, with no evidence of any ciliary rootlets having been attached.

The "dissociated fibers" described by Yocom (1918) as radiating at the base of each of the thirteen cirri (i.e., excluding the anal cirri), have been found to be definitely connected with a plate somewhat similar to the anal cirri plate, although of a shape (fig. 21) corresponding to that of the base of the cirrus. These were first observed upon the disintegration of an animal likewise stained with a .0001 per cent aqueous solution of haematoxylin. Several radiating fibers were distinctly seen to be united to each plate. As yet, I have not definitely observed the separation of one of these plates from its cirrus. Indications in two cases where the separation was almost complete point toward a relation between cirrus and plate here that is similar to the relation of an anal fiber plate to its corresponding cirrus. I shall designate these plates the "dissociated fibers plates." Figures 21 and 21a show several such plates from the same organism which vary slightly in size and shape. These variations are apparently common.

TRANSECTIONS

Between the "group of three" and "group of four" frontal cirri (fig. 2).—The anterior part of the animal swims rapidly (cf. swimming movement 3, p. 429), the inner side, that with the three frontal cirri, performing a small circle and the opposite side a correspondingly larger one. This performance continues the same after more water is added to the hanging drop. The part infrequently revolves, as on the long axis of the normal animal, and it occasionally reverses the effective stroke of the membranelles to drive itself a short distance backwards (cf. swimming movement 6, p. 429). In either case the circus movement to the right is soon resumed and continues with few such interruptions until the part apparently becomes fatigued and dies. Death

generally results within an hour after the transection, but in three cases this ceaseless activity continued for more than four hours. Any indications of regeneration have not been observed.

The posterior piece is much less active. It usually swims straight ahead and occasionally in circuits to the right (cf. swimming movements 1 and 3, p. 428) and very frequently revolves, then with obvious difficulty, on the long axis. It remains most of the time inactive, seldom creeps and always very slowly and feebly, and responds more or less readily to jarring or to stimuli effected by the needle-point. In the latter case its anterior end is very little if any more sensitive than other parts of the body. A distinct avoiding reaction (cf. swimming movement 6, and creeping movement 2, p. 428) has at no time been observed. Use of the frontal and marginal cirri is conspicuously more normal than is its use of the anal cirri, especially when attempting to creep, as on the under surface of the inverted cover-slip.

Experiment 106 (fig. 2).—Anterior piece swims very rapidly in right circus movement with the three frontal cirri as a "moving center." Occasionally it whirls and tumbles deviously about; it may then swim straight ahead revolving two or three turns on its long axis, but very soon returns to circus movements. It very seldom reverses the effective stroke of membranelles, even when needle is thrust in its way. Frontal cirri beat continually as also do the membranelles.

Posterior part mostly at rest with occasional movements of frontal and marginal cirri less often of the anal cirri, except when another *E. patella* runs into it; then it dashes for a short distance usually straight ahead. It then bumps into piece of debris which it pushes straight ahead but does not go far. Jarring starts its movements, which soon cease. Anal cirri are commonly moved upon any lashing of the other cirri which is sufficient to move the body. It would appear that they initiate such movement, which is then taken up by the anal cirri. When stimulated with a needle point, its anterior end is little if any more sensitive than are the sides or posterior end.

Transection just posterior to the "group of four" frontal cirri (fig. 3).—Anterior part swims in circus movements, although somewhat less completely than does the anterior piece above described. It resorts more frequently to rotation on the long axis and to the reversal of the organelles to drive the part backwards, although here, too, the "rotation" movement is more common than the reversal reaction. The part never creeps but swims rapidly and continuously for hours after the operation. It does not turn sharply to the right (cf. swimming movement 5, p. 429) and very seldom shows the avoiding reaction upon being stimulated by the needle. After several hours the part may come to rest on debris, on the surface film of the water or on the cover-glass. It is then generally very sensitive to slight jarring or other mechanical stimuli, whereupon its rapid swimming may be resumed

for long periods. Its oral lip is more sensitive to a stimulus by the needle-point than are the membranelles over the oral lip, or its posterior, cut surface, or the frontal cirri or any other part.

The posterior part is generally very much less active. However, in ten recorded instances this piece revolved on its cut surface as an axis exceedingly rapidly (about two revolutions per second) for a half minute or less just after the transection was completed. This revolving performance, generally at a much slower rate, is a common reaction of the posterior piece following this operation. The direction of these revolutions is clockwise when viewed from the left side. For some time after the cut is made the anal cirri are quite active with their effective stroke in such a way as chiefly to induce the revolutions. This part swims in circus movements to the right infrequently and very seldom rotates on the long axis; when it does so, it moves quite clumsily and imperfectly. Within about an hour movement ceases. It is then much less responsive to mechanical stimuli than is the anterior part, after coming to rest. When thus stimulated its movements, which are for the most part revolutions, are effected chiefly by means of the two ventral and the two right, fimbriated marginal cirri, the anal cirri remaining more or less passive.

Experiment 209 (fig. 3).—Anterior part swims violently in various devious movements, sometimes rotating on the long axis or reversing to swim a short distance backwards, but most of time it moves in right circus movements. This ceaseless swimming continued from 11:50 A.M. until about 5 P.M., when its movements were considerably slower, and at 5:30 P.M. the part was resting on débris. Readily responded to touch of the needle point against the oral lip, but less so when the adoral membranelles, frontal cirri or posterior cut surface was similarly stimulated. Slight jarring induced violent swimming, which lasted about thirty seconds, after which it again became quiet. At this time rotation on the long axis was more common than previously. Following morning, this part had died.

Posterior part not very active from the first. Anal cirri beat slowly, irregularly and with little effectiveness. Occasionally swam in circus movement to the right and sometimes showed imperfect rotations on the long axis, but more often its movements were revolutions about the cut surface as an axis. Within forty minutes, it had become passive, the two right marginal cirri infrequently showing movements which were always infundibular but without effect. Only slightly responsive to jarring, then generally revolved as before but very few times, after which it again became quite inactive. These revolutions were effected mainly by the two right, fimbriated marginal cirri with infundibular movement; the two on the left lashed with the effective stroke upward, thus inducing the revolutions. The two ventral cirri were active most of the time, also showing conspicuously the infundibular type of movement. The anal cirri, on the other hand, were mostly inactive; irregularly one or two might lash feebly, but never more than one at a time. Their effective stroke was always backward, therefore tending to aid the part in its rotations clockwise as viewed from the left. This part also died within about thirty-six hours.

Between the two ventral and five anal cirri (fig. 4).—Here, the movements of the anterior piece are quite similar to those of the same part described just previously. There is, however, less tendency to swim in circuits to the right and spiral swimming movements are considerably more frequent. Indeed, in the two respects just mentioned, this part closely approximated the corresponding swimming movements of the normal animal. There are, on the other hand, some notable differences: (1) Avoiding reactions (swimming movement 6, p. 429) are seldom observed even when the oral lip region is strongly stimulated by means of the needle, or when some disagreeable solution, such as methylene blue, is introduced. (2) Creeping is very infrequently attempted and is conspicuously more or less impossible. In a few cases, the piece has been observed to crawl slowly and awkwardly for a short distance over débris or along the needle, but this ability is distinctly impaired. (3) Sharp turns to the right have not been seen. Tortuous, random movements that involve turning both to the right and to the left are sometimes resorted to but these are readily distinguishable from the short, sharp turns to the right which are common for the normal animal (swimming movement 5, p. 429). (4) The creeping "avoiding reaction" (number 2, p. 429) has at no time been observed even when mechanical stimuli are applied.

The principal characteristic movement of this posterior piece is its rotating on the cut surface as an axis, a rotation which is not uncommonly very rapid (two or three times per second) following the completion of a transection. This performance is of brief duration and the piece comes to rest on the surface film of the hanging drop or upon débris. Thereafter, it is usually very irresponsive to mechanical or chemical stimuli. If aroused, its revolving movements are performed chiefly by means of the marginal cirri, the anal cirri functioning individually and spasmodically, or not at all. But previously, just after the operation, the anal cirri were very active and mostly responsible for the rapid revolutions. Imperfect circus movements to the right and aboral spiral movements are quite uncommon for this part. These have never been observed after the marginal cirri were excised, as a later experiment will show.

Experiment 160 (fig. 4).—Anterior piece immediately swims in circus movements to right, but as often or more often moves in aboral spiral straight ahead. Sometimes performs winding, devious movements but returns to spiral swimming or to circus movements. During ten minutes it reversed three times to swim a short distance backwards; this reaction fairly normal. Two hours later, resting on débris. Very responsive to jar, also to needle. Oral lip much more sensitive than membranelles, cirri, or any part of the body. Slowly and awkwardly

crawls a short distance over débris. Does not show creeping "avoiding reaction" when stimulated with needle-point or by means of methylene blue. Also, applied weak acetic acid, when part swam straight forward, then reversed the effective stroke of organelles, swimming backwards a short distance, but again swam in circuit or in spiral apparently beyond the influence of the acid solution.

Posterior part turned over and over very rapidly, two times per second, with cut surface as axis. This continued about twelve minutes. Slowed and came to rest on surface film of hanging drop. Half hour later, very irresponsive to jarring or needle-point. Aroused and for few seconds revolved as before, but very slowly and chiefly by means of the marginal cirri. Anal cirri mostly passive or individually and irregularly active. Again came to rest. From jarring, slowly moved in circuit, very awkwardly, by irregular pushing of one or two anal cirri; other anal cirri were passive.

EXCISIONS

Removal of oral lip with adoral membranelles.—Anterior piece always shows one and only one reaction, viz., circus movement to the right. This movement is very rapid and continues until the part becomes fatigued and dies, which generally occurs within fifteen minutes or less after the excision. The effective stroke of the membranelles is the same here as when the normal animal swims in a circuit or spirally straight ahead. In many specimens observed, there was at no time any indication of the reversal of membranelles, and the path of the circuit was continuously about the same. This part did not possess the motorium.

The reactions of the posterior part are mostly very similar to those described for the same part when a transection was made between the "group of three" and "group of four" frontal cirri. In the former, however there are present the "group of three" frontal cirri and the motorium. This piece is able to creep fairly normally and in a few instances gave the creeping "avoiding reaction," although the anterior end here was much less sensitive than the oral lip of a normal form. Swimming is uncommon and abnormal. Spiral rotations on the long axis are infrequent and quite imperfect. The part more frequently revolves with the cut surface as an axis, but this movement is generally combined with a sort of spiral movement on the long axis. Of five such posterior parts carefully observed, one regenerated an oral lip after budding off about twenty microns of its anterior end; the bud, containing three frontal cirri, was more or less spherical, quite active for about an hour, but died some time during the night. The other four of these five regenerated normally but only after eighteen to thirty hours.

Removal of marginal cirri.—These, due to their exposed position, were readily snipped off with the needle. The excised parts thereafter were never observed to beat. But when one, two, three or all were removed with some of the body plasm the cirri continued lashing very rapidly and driving the piece deviously through the water until death, which followed a few minutes later. When an excision (properly, a transection) of the caudal end was made to include all four marginal cirri, the piece revolved very rapidly with the cut surface as an axis but in such manner as to move speedily through the water with the left side foremost. Such removal or the snipping off of some or all of these cirri did not apparently modify the several swimming or creeping movements of the normal animal. If, however, these cirri had been removed from an animal which was then transected just anterior to the anal cirri, the posterior part usually rotated rapidly, the cut surface as its axis, for several minutes. Circus or spiral movements were at no time observed. But within an hour after becoming quiet it was irresponsive to mechanical stimuli and did not resort to rotating movements again. The anal cirri lashed feebly, very irregularly and ineffectively.

Excision of frontal cirri.—Owing to the length of these cirri and the extension of four of them beyond the right lateral margin of the body the four may be snipped off with a V-shaped needle. Thereafter, the animal very seldom attempts creeping and its spiral movements are abnormal. In the latter instance, its anterior end rotates in a larger spiral than that of the posterior end. In two cases all but one frontal cirrus were either snipped off or removed by inserting the needle-point at the base of each cirrus, thus to gouge them loose. These cirri came off rather readily. Three of them beat several times following their detachment. In each case the animal could not creep, but frequently swam slowly in circuits, or in spiral movements with the anterior end describing spirals which were about twice the diameter of those of the posterior end. No discernible injury resulted from gouging out these cirri; the movements of both the membranelles and anal cirri were apparently normal. In a few instances, the animal gave the avoiding reaction upon stimulation of the oral lip with the needle-point, although these were feeble and abnormal. However, both animals died within forty-eight hours without regenerating new frontal cirri. Their death may have been due directly to injuries from the excisions or indirectly to infections or other causes.

Excision of anal cirri.—Infrequently, *E. patella* were found with fully half of the anal cirri extending beyond the caudal margin of the

body. In several cases the anal cirri, to about two-thirds of their length, the marginal cirri and a small piece of the caudal end of the body were excised. Creeping was thus made practically impossible, the animals resorted more frequently to circus movements to the right, and sharp turns to the right were not evident. Spiral revolution on the long axis was apparently normal. Four such animals regenerated the excised parts, including the anal cirri.

Several attempts were made to gouge off the anal cirri just as the frontal cirri had been removed. In two experiments (nos. 215 and 216) all the anal cirri were successfully removed, with little or no injury to the body. In each case, there followed several significant results: (1) the animal was unable to creep, (2) it did not turn sharply to the right, (3) the avoiding reaction was never observed, and (4) circus movements to the right were performed more frequently.

Experiment 216.—Removed anal cirri 4 and 5 (see p. 424). Released the animal by adding water to the hanging drop. It then performed all the major swimming and creeping movements, including the avoiding reaction.

Again drew off the water and removed the remaining anal cirri. Upon adding more water the animal was observed to revolve in spirals on the long axis and to swim in circuits to the right, but at no time was it seen either to creep, to turn sharply to the right, or to give the avoiding reaction. Its efforts to creep on the under side of the cover-glass were unsuccessful, the posterior end being suspended so as to incline the body at an angle of about 30 degrees with the cover-slip.

INCISIONS

Through the oral lip without cutting the cytostomal fiber.—There was no apparent decrease in the sensitivity of any part of the oral lip, and no change in the normal movements and functioning of the adoral membranelles. The results were wholly negative. The cut usually healed completely within an hour.

Through the oral lip, severing the cytostomal fiber (fig. 5).—In seventeen cases there resulted abnormal swimming movements and distinct changes in the movements of the membranelles on either side of the incision: (1) the progress of the animal forward was impeded, (2) in its spiral revolutions, commonly the anterior end described a wide spiral, (3) circus movements to the right were markedly less common as was also (4) the occurrence of the avoiding reaction, and (5) periods of quiet were more frequent and of much longer duration. Upon examining with high power the movements of the membranelles, a difference in rhythm was frequently conspicuous between the series on the left side of the cut and those on the right side. The former

were always active with that effective stroke which normally tends to drive the animal forward. The membranelles on the right side of the cut occasionally moved in coördination with the former or sometimes did not move in the least but projected straight out from their bases. Not infrequently, they were distinctly seen to beat with the effective stroke in the opposite direction to that of the series on the left side of the cut. Carmine granules or india ink which had been introduced into the water clearly indicated these three changes in the behavior of the adoral membranelles on the right side of the cut. It will be noted that the membranelle fiber at the base of these membranelles on the right side of the incision was continuous and in connection with the motorium. These results of such experiments were very obvious and remarkably uniform.

Incision through the membranelle fiber at any point posterior to the oral lip (fig. 6).—Differences between the rhythm and direction of the effective stroke of the membranelles anterior to the incision and of those posterior were apparently identical with those just described above. However, the swimming movements following such incisions were practically normal. Some animals (three especially were noted) were less active after the incision and showed more tendency toward cirrus movements; otherwise, their swimming and creeping reactions were comparatively normal.

Incisions on the right or left side or at the posterior end did not sever the cytotomal fiber or any of the anal cirri fibers.—Following such incisions made in many animals at various angles through the macronucleus or not (figs. 10–12) I have never as yet observed any noteworthy change in their normal swimming or creeping reactions or in the perfect coördination between the series of membranelles and the anal cirri.

Incisions severing all the anal cirri fibers.—Incisions were made (1) on the right side between the group of three and the group of four frontal cirri (fig. 7); (2) on the right side between the group of four frontal cirri and the two ventral cirri (fig. 8); and (3) on the right side between the two ventral cirri and the five anal cirri (fig. 9).

After severing the anal cirri fibers at any one of these three regions, two significant changes were evident in creeping and three in swimming movements: (1) there was distinctly less tendency to creep; the animal when not swimming was more frequently found quiet on the surface of the cover-slip, on the surface film of the hanging drop or upon débris. But when creeping, the anal cirri were used with less sureness and facility than normally. That much was commonly evident

upon careful observation. Nevertheless, the lack of coördinated movement between the frontal and ventral and the anal cirri (these being the animal's creeping feet), was not at all times conspicuous. In some cases (four recorded instances), this coördination was apparently about normal. Even here, however, it was obvious that the frontal cirri sometimes initiated the movement which was then taken up by the anal cirri, but this succession was not always evident; (2) the avoiding reaction was very seldom observed. If the oral lip were touched by the needle-point (a stimulus which normally induces the avoiding reaction) the incised animal would infrequently give this reaction, but more often would turn to the right anteriorly (cf. third creeping movement, p. 428), thus avoiding the stimulus without performing the preliminary backward movement (cf. second creeping movement, p. 428). The three notable changes in swimming movements after the incision were: (1) A tendency to swim in circuits to the right. This reaction was particularly noticeable just after the incision was completed, when it became for a time the only swimming movement. The tendency, nevertheless, persisted even until the wound was more or less fully healed. (2) Sharp turns to the right were quite infrequent and in a few cases were at no time observed (three of these were recorded). This movement is effected chiefly by a strong, quick lash of the outermost three or all five anal cirri. The performance of this stroke is possible for these anal cirri after the fibers are cut, as will later be shown, but apparently such strokes are not readily or simultaneously linked up with corresponding beats of the membranelles; (3) The backward swimming movement (number 6, p. 429) has not been definitely observed, as yet, in any of these incised animals, even when stimulated mechanically by means of the needle or chemically with such reagents as methylene blue or acid solutions. In one instance, this or a similar reaction was apparent, but of this I could not make certain. It occurred upon adding a solution of methylene blue with a needle pipette. The movement backward was only a short distance, two or three times the animal's length; this was followed by rapid circus movements to the right and was not repeated, as is usually the case with a normal *E. patella*.

Cutting the anal cirri fibers or the membranelle fiber or both near the motorium, or destroying the motorium.—The general effects upon swimming or creeping movements were definite, fairly constant, and much the same after performing any of these incisions. These movements have, in fact, already been described in the foregoing paragraph. It is important, therefore, to note that the destruction of the motorium

by means of the needle-point produces modifications in the animal's several movements which, so far as I have yet been able to ascertain, do not differ markedly from the effects that follow severing the membranelle fiber or the anal cirri fibers or both near the motorium, or the anal cirri fibers at any point. There is, then, no certain evidence from these experiments that the function of the motorium is more specific than that of its attached fibers. These *negative* results may be attributable, however, to faulty or insufficient technique. On the other hand, the differences in the behavior of the membranelles on the left and right sides of the incision severing the membranelle fiber, which were previously described, might indicate there some rôle peculiar to the motorium.

Perhaps the clearest evidence for the want of coördination and of concomitancy of movements between the membranelles and anal cirri appeared in these incised animals upon supporting one of them against the under surface of the cover-glass with a very flexible needle. To the hanging drop had been added a trace of india ink or a carmine solution; thereupon, any changes in the direction of the effective stroke either of the anal cirri or of the membranelles were quite conspicuous in the corresponding movements of the particles of india ink or of carmine. Infrequently the carmine granules were driven in the same direction by the membranelles and by the anal cirri, and the effective stroke of these organelles varied synchronously. This concomitancy however, did not long continue. Their phases of rhythm, it would seem, changed so that now while *the membranelles* were driving some particles anteriorly, other particles were being driven posteriorly by the *anal cirri*, or *vice versa*. These changes were conspicuous and frequent.

DISCUSSION

These experimental studies have yielded some evidences on the nature of organelle movement in *Euplotes patella* which are here worthy of consideration. The significance of the general problem of ciliary structure and movement, probably due to the prevalence of cilia in both protistan and metazoan organisms, was early recognized (Stuart, 1867) and has occasioned the writing of a large literature, most of which has been reviewed by Pütter (1903), Prenant (1914), and Saguehi (1917). Aside from minor modifications, the structure of cilia, wherever found, appears much the same. A cilium is composed

of two different parts (Maier, 1903), an elastic axial filament covered by a sheath which, according to Khainsky (1910), is continuous with the pellicle. Each cilium arises from a basal granule situated in the ectoplasm beneath the pellicle (Pütter, 1904). The theory of Henne-guy and Lenhossek, that this granule in metazoan cells is a derivative of the centriole, has recently been opposed by Saguchi (1917), who regards the granules as having their origin in mitochondria. Continued from each basal granule into the cell-plasm is a fibril, the ciliary rootlet, which in certain ciliates has been found to unite with other such rootlets by means of a basal fibril running parallel to the periphery beneath each row of cilia (Maier, 1903). In flagellates, the basal granule or blepharoplast may show two such rootlets, one uniting the blepharoplast to the nucleus and the other connecting the blepharoplast and parabasal body (Swezy, 1916).

The component cilia of the cirri and membranelles in *Euplotes patella* clearly possess each a basal granule and ciliary rootlet (fig. 17). As previously stated, the granules and rootlets lie within the basal plate of each cirrus and membranelle, which in turn is united to the corresponding fiber plate. There is, as yet, no evidence that the ciliary rootlets are united to the fiber plate and they are here regarded only as contiguous with that plate. The ease with which the basal plate detaches from the fiber plate and the want of indications on its surface that there were ciliary attachments favor this interpretation.

As regard the movement of cilia, there appears in the literature a considerable difference of opinion as to how this movement is produced. Certain investigators regard the cilium as wholly passive, its movement being effected either by way of the basal granule (Henne-guy, 1898; Lenhossek, 1898; Peter, 1899; Joseph, 1903; Saguchi, 1917), or by the contractility of the ciliary rootlets (Simroth, 1876; Benda, 1899). There are others who believe the cilium itself to be active (Engelmann, 1879; Klebs, 1881; Bütschli, 1885; Schilling, 1891; Fischer, 1894; Kölsch, 1902; Prowazek, 1903; Pütter, 1903; Gurwitz, 1904; Erhard, 1910; Kolačev, 1910). Its power of contractility lies either in the axial filament or in the protoplasmic sheath surrounding the filament.

Favoring the latter view are the observations of several authors who have noted that cilia may continue to contract after they have become detached. Klebs (1881) saw in the long flagella of *Trachelomonas* that contractions and extensions continued after the flagella were detached from the body. Bütschli (1885) describes movements

of a detached flagellum of *Glenodinium cinctum*, which rolled up in corkscrew fashion, remained quiet for a moment, then straightened out and soon turned over in an up-and-down movement. These movements lasted for only a minute or less, after which the detached flagellum came to rest and did not move again. Schilling (1891) observed similar reactions in detached flagella of *Peridinium* and Fischer (1894) saw that the detached flagellum of *Polytoma* continued its movements for some time after it had separated from the body. In an isolated cilium of Phycomyceten zoospores, Rothert (1894) clearly observed several movements. Kölsch (1902) saw cilia on a blister of paramecium that continued to beat rapidly. He thought that to these cilia the basal corpuscles remained attached. In detached cirri of *Euplotes harpa*, Prowazek (1900) observed repeated movements.

It is not uncommon, during the disintegration of the body of *E. patella*, to see frontal or marginal cirri continue several contractions upon being set free. Occasionally, but less frequently, I have distinctly observed detached anal cirri to show similar movements. Sometimes the movements of detached frontal cirri, even after being gouged out by the needle, were quite vigorous, and continued so for several seconds. As formerly stated, frontal and marginal cirri have been snipped off with a V-shaped needle. In very few cases was it possible to cut these cirri off and carefully observe any reactions of the excised parts. However, such parts were never seen to contract. Their failure to show any movement may have been due to injury which resulted in rapid death. Anyhow, from the various ways in which the cirri of this animal are used, not only in creeping and swimming but also in attachment to objects, which in several instances were observed to involve distinct flexures (as over the needle or about pieces of débris) it would seem that contractility inheres throughout the cirrus.

Furthermore, rather more frequently, the movements of the membranelles may be distinctly seen to continue after separation from a disintegrating body, even for longer periods than those of detached cirri. As few as four membranelles have been cut off which afterwards showed several fairly normal movements. Attempts to excise a single membranelle and observe any contractions were unsuccessful, but the failure would appear to be due rather to inferior technique.

It is obvious that the contractions of anal, frontal or marginal cirri or of membranelles of *E. patella* are not conditional upon attachment to the body and, therefore, not upon any mechanism within the body.

Another matter of considerable importance here concerns any specific function which a group of organelles in *E. patella* may perform. Are there indications of a division of labor among the several groups of cirri and membranelles? Since in many ciliates the body is definitely differentiated and frequently bears several sorts of organelles, such as cilia, cirri, membranelles, etc., some authors have regarded this differentiation in the form and position of organelles as representing a division of labor among the several groups. Pearl (1900) concluded from observations on *Colpidium* that the effective stroke of a group of anterior cilia, which is always toward the oral side when the animal is stimulated by the electric current, caused the body to turn toward the aboral side. Similarly, Pütter (1900) observed that the peristomial cilia in *Stylonychia*, with their effective stroke toward the oral side, produced the swerving of the body toward the aboral side.

If these usual movements are effected wholly by a special group of organelles, then the movements should disappear upon the removal of those structures. Accordingly, Jennings and Jamieson (1902) undertook to ascertain the effect of the removal of one or more groups of organelles in *Stylonychia*, *Stentor*, *Spirostomum*, and *Paramoecium*. These investigators found that when any of these ciliates were cut into pieces, "if they are not too minute or too irregular in form, the pieces swim in a spiral, swerving continually toward a certain side, just as do the entire organisms" (p. 232). It became evident, therefore, that the usual reactions of these animals could not be attributed to any particular set of structures, but that all the organelles have a share in the production of these characteristic movements.

The several transections made on *E. patella* indicated a similar tendency in the movements of each of the two pieces. Here, however, the reactions were not so definite or so invariable as were those for ciliates described by Jennings and Jamieson (1902). It will be recalled that the swimming movements of *E. patella* are more varied than are those described for the above animals. In addition to the spiral swimming movement which is, indeed, very common in this ciliate, at least five other characteristic swimming movements have been identified, three of which—the circus movement to the right, a sharp turn to the right, and the backward, avoiding reaction—are by no means uncommon. Furthermore, the transections have shown that the anterior piece possessing only the group of three frontal cirri and adoral membranelles swam almost constantly in circuits to the right,

although the piece reverted occasionally to the spiral movement and to the backward, avoiding reaction. Now, since the excised oral lip reacts only in right circus movements, it would appear evident that these organelles are chiefly responsible for the same movements when only the three frontal cirri are added. And one may enquire whether the circus movements to the right by the normal animal may not be effected mainly by these adoral membranelles. The fact that when such movements are performed the anal and marginal cirri not infrequently remain wholly passive, and that these movements are more common after the anal and marginal cirri have been removed, would lend support to such a conclusion.

It was also observed that a sharp turn to the right was accompanied, if not mainly produced, by the quick lateral flexure of the anal cirri, and that when these cirri were removed, this reaction was never distinctly observed.

Again, the usual reaction of the posterior part resulting from a transection just anterior to the anal cirri, was a rotation with the cut surface as an axis. Circus movements to the right were infrequent and still less frequent were the spiral, revolving movements on the long axis. In fact, neither of these two movements was seen if the marginal cirri had been snipped off previous to the transections.

From these observations, therefore, it appears that in *E. patella* one of the several swimming movements prevails in a piece formed by a transection, or that one of these movements becomes less frequent and may not appear at all upon the removal of a group of organelles, such as the anal cirri.

These facts, nevertheless, are not contradictory to the more general truth, viz., that all the locomotor organelles cooperate in the performance of any characteristic movement. The very significance of organization precludes any other interpretation. But are we to regard each group of organelles *equally* effective in producing any one of these movements? If so, then the removal of the marginal cirri should impair a given movement in the same manner and to the same extent as excision of the adoral membranelles impairs that movement. But it can be said with certainty that the same results in each case do not follow. Were we to assume that all the locomotor organelles of *E. patella* function to the same end with equal effectiveness, it would be necessary to regard both the adoral membranelles and the marginal cirri as distinctly creeping organs, which they are not. In this respect, therefore, we may speak of a division of labor among the locomotor organs of *E. patella*.

None would question the evidence for a division of labor among the intracytoplasmic organelles in this ciliate, and the several experiments previously described would indicate that the extracytoplasmic organelles, also, may share a degree of specific, but none the less coördinated, functions in the animal's normal behavior. Accordingly, in accomplishing such swimming movements as the sharp turn to the right or the quick backward, avoiding reaction, we may regard the anal cirri as especially effective if not normally indispensable, much as the large caudal cirri in *Uronychia* are largely responsible for that animal's very rapid, backward movements (Calkins, 1911, p. 98).

In this consideration, it is important to note that the feature of coördinated activity is in all respects evident in the normal *E. patella*. The claim here made is that the perfection of both creeping and swimming movements is dependent upon the coöperation particularly of those organelles (e.g., the frontal and anal cirri in creeping) which contribute most effectively to the performance of any usual movement. Therefore, the elimination of any important group of organelles, or the interference with any mechanism by which they operate or coöperate with another similarly important group, should result in perceptible changes in swimming or in creeping movements.

We may now enquire: Does the fibrillar system in *Euplotes patella* represent a mechanism that affects the external organelles individually? Or does this complex, unified apparatus function in the coördination of all the several groups of organelles with which it is intimately associated? An affirmative reply to the first question would assign either a supporting or a contractile function to this system, and to affirm the second question is to attribute to the system the function of conductivity.

The experimental evidences set forth in previous paragraphs support an affirmative answer to the second question, viz., that this fibrillar apparatus exhibits features of conductivity functioning to coördinate the groups of external organelles with which its unified and dissociated parts are directly or indirectly intimately associated. These evidences, furthermore, do not support the assumption that the system is either contractile or supporting in function.

The facts which concern these three propositions may be stated as follows:

The fibrillar system in E. patella is not skeletal or supporting in function.—The rigid, fairly tough pellicle is amply sufficient to maintain the normal shape of the body under considerable stress. It was

shown that the pressure of a very flexible needle when applied to the full width of the body did not alter the normal shape of the animal. Also, when the body was flattened for a few minutes by applying a stiffer needle, or by surface tension, upon releasing the stress the body at once recovered. It was also stated that the pellicle was sufficiently tough to require in dissections the use of needles with fairly stiff, sharp points. Other needles were ineffective. Furthermore, the firmness of the pellicle is sufficient to preserve the normal shape of the body after an incision fully two-thirds its width had been made. The friction of water, induced by the animal's continuous and devious swimming movements, effected no visible change in its shape. Any momentary modification in the shape of *E. patella* can result only from extraneous pressure. Unlike *Paramoecium*, which readily forces its way through narrow meshes of silk fibers with distinct constrictions of the body, this animal, owing to the consistency of its pellicle, is of itself unable to alter its form.

The basal plate and not the fiber plate is the means of secure attachment and support for both the cirri and the membranelles. The rootlets of the component cilia of both membranelles and cirri are imbedded in the gelatinous ectoplasmic basal plate and are only contiguous with, but not attached to, the fiber plate. The readiness with which the basal plate becomes detached from the fiber plate and the want of any indications that the ciliary rootlets had been attached to the smooth, clean fiber plate, was previously described.

The consistency, solubility, size, and shape of the fibers are incompatible with efficient structures for support. Particularly are the anal cirri fibers frail, readily flexible, and irresilient. They may be pulled in two or bent variously with the needle-point. When entirely free from the ectoplasm they are not resilient and, by means of the needle, they may be readily distorted. They may adhere to the needle and thus be pulled about through the water. Their dissolution is sometimes rapid and usually occurs within fifteen minutes or less after being exposed to the water. It is probable that they are not imbedded in the ectoplasm but lie upon its inner surface, being supported there by a thin, hyaloplasmic sheath. This loose attachment, together with the extensive length and the minuteness of these fibers, indicate that they do not function as supporting structures either for the pellicle, which is of itself distinctly firm and resistant, or for the cirri, whose component cilia are not attached to, but only contiguous with, the basal plate.

This fibrillar system is not contractile in function.—The contractility either of cirri or of membranelles is not conditioned upon their attachment to the body and consequently not upon any mechanism within the body. All the frontal, ventral marginal and anal cirri and membranelles have distinctly been observed to continue contractions for a considerable period after their detachment from the body. These reactions have already been discussed somewhat at length, and need not be further elucidated here. It is now only worth while to emphasize that their capacity of contraction inheres within these external organelles themselves. Whether this contractility is effected by the basal corpuscles, the axial filament of the component cilia, or the plasmic sheath enclosing the filaments, is not for our consideration.

The loose attachment of the basal plate to the fiber plate indicates that the fibrillar system differs both in structure and in function from the contractile, external organelles. The ease and completeness with which the basal plates become detached from the fiber plates and the want of evidence that the ciliary rootlets and fiber plate are more than merely contiguous structures are significant features supporting this conclusion.

The consistency of the anal cirri fibers and their feeble attachment to the ectoplasm and to the easily displaced motorium would suggest their meager effectiveness in functioning as contractile structures. The fibers tend to remain straight when undisturbed. They do not become kinked or curled upon the disintegration of the ectoplasm. It is only by means of the needle or some other external agency that they may readily become distorted. They may be pulled in two with the needle-point but at no time have they shown any indications of stretching.

The reversibly effective strokes of the anal cirri preclude the possibility that the anal cirri fibers are contractile in function. The four effective strokes of these cirri have been described in foregoing paragraphs. These are: (1) directly backward strokes parallel to the sagittal plane, (2) directly forward strokes parallel to that plane, (3) laterally backward strokes hardly parallel to the frontal plane, and (4) similar lateral strokes directed forward. All these strokes have been seen many times in the anal cirri of a transected posterior piece as well as after an incision which had clearly severed the anal cirri fibers. Since contractile fibers can operate effectively only in one direction, it is inconceivable that an anal cirri fiber can function as a contractile organelle.

The fibrillar system in Euplotes patella does possess properties of conductivity functioning to coördinate the movements of the external organelles with which it is associated.—Normal, coördinated activity of the series of membranelles is effected through the motorium, the membranelle fiber and its attached membranelle plates. An incision at any point through the oral lip, which did not sever the membranelle fiber, gave negative results. But when the membranelle fiber was severed, there were conspicuous changes in rhythmic movements of the membranelles on either side of the incision and distinct modifications in the animal's swimming movements. It was stated that the membranelles on the right side, whose fiber remained connected with the motorium, at times became inactive and projected straight out from their base; only occasionally were they seen to move in apparent coördination with those on the left side of the incision. The latter, the fiber of which had lost its connection with the motorium, showed continuous movements with their effective stroke mostly such as normally tends to drive the animal forward. This tendency in the rate of movement and in the direction of the effective stroke is comparable with the unchanging, ceaseless movements of the adoral membranelles of the excised oral lip which continually moved in circuits to the right and was never observed to reverse the effective stroke of the adoral membranelles. This constancy in the behavior of membranelles whose fibrillar connection with the motorium is severed might suggest that their usual modifications in direction of stroke and rate of movement may in some way be effected through the motorium. It is furthermore evident that the unusual swimming movements which followed such incisions resulted from the severing of the membranelle fiber.

Efficient, coördinated behavior of the five anal cirri is effected through the normal functioning of the five anal cirri fibers with their attached fiber plates. The effects of severing these fibers at any one of several regions (see Incisions, page 441, above) were distinct and more or less constant. The infrequency and lack of facility in creeping which was, at times, obviously initiated by the frontal cirri, and the rare occurrence of the avoiding reaction were noteworthy changes in the animal's creeping movements. But more evident were its modifications in swimming. There was a marked tendency toward performing circus movements to the right. Sharp turns to the right were infrequent and in three cases at no time observed. The rapid, backward, avoiding reaction has never been clearly identified after

the anal cirri fibers had been severed. It is important and significant in this connection to recall that the severing of the anal cirri fibers did not incapacitate any of the four movements of the anal cirri. Each of these movements has been clearly observed in the anal cirri upon supporting such an incised animal, ventral side down, by means of a very flexible needle against the under surface of the cover-glass. Occasional, usual creeping or swimming movements by the incised animal might, therefore, be expected as occurring incidentally. Accordingly, it is the infrequency of these occurrences and not their absence that suggests the want of coördination and warrants the conclusion that the fibers are conductive in function.

Perfect and efficient coördination between the series of membranelles and the five anal cirri is accomplished through the normal functioning of the motorium and its attached fibers. Whether the fibers were cut on both sides of the motorium or the motorium destroyed by means of the needle-point, the effects were very much the same. The usual swimming movements were more distinctly altered than were the creeping movements. Changes from normal conditions were the rarity of creeping movements, their slow rate, very infrequent avoiding reactions and the tendency of the animal when not swimming to remain quite passive on débris and unusually irresponsive to mechanical stimuli. The most common reactions in swimming were the right circus movements, which, here, were more often combined with abnormal, spiral revolutions in which the anterior described much wider spirals than did the posterior end. In no case was the backward swimming reaction observed, although, as described above, the reversal of both the adoral membranelles and the anal cirri was clearly seen. It will be recalled that when the anal cirri fibers were cut very conspicuous effects were seen in the want of concomitancy and coördination between the movements of the membranelles and of the anal cirri. This, perhaps, showed more clearly than any other experimental evidences that this fibrillar complex is coördinative in function.

Perfect and efficient coördination between the series of membranelles and anal cirri is contingent essentially and only upon the motorium with its attached fibrillar complex. Any incisions through any region of the body, which did not sever or injure this fibrillar apparatus neither impaired the perfect coördination of the membranelles and anal cirri, nor modified the animal's normal creeping and swimming movements. Whether these incisions did or did not pass through the macronucleus, the results were always without

noteworthy consequences. It is apparent, then, that the destruction of the motorium or the severing of some or all of its attached fibers is alone accountable for modifications in the perfect and efficient coördination between the series of membranelles and the anal cirri. We may, therefore, regard these normal, morphological relationships as conditioning the animal's usual behavior both in creeping and in swimming.

Previous to the researches of Sharp (1914) and Yocom (1918), several other investigators had found fibers in certain ciliates, which they believed to represent nervous elements. Engelmann (1880) described distinct fibers associated with the peripheral and anal cirri of *Stylonychia* and concluded that they were nervous in function. Neresheimer (1903) found two separate fibrillar systems in *Stentor coeruleus*, one of which possessed muscular and the other nervous properties. Their shape, size, selective staining, and relative positions suggested these distinct functions. Moreover, this author found experimental evidence supporting his interpretations. Lebedew (1908) describes two systems of fibrils in *Trachelocerca phoenicopterus*. On one side and running parallel to each row of basal corpuscles appeared a smooth, structureless fiber staining light, while another larger, less even and densely staining fiber also ran parallel to the row of corpuscles but on the opposite side. The latter was believed to be a myoneme and the former was perhaps of nervous function.

Other authors (Bütschli, 1889; Schuberg, 1891; Schröder, 1906; Maier, 1903; Prowazek, 1903; Griffin, 1910) have discredited the "nerve hypothesis" for protozoans and have attributed to such systems of fibers either the function of support or of contractility.

It would seem that these discordant interpretations may owe their origin largely to differences in the more general conception of the nature of organization and degree of specialization among the Protozoa. And it is in the forming of this general conception that the qualifying attributes—unicellular, primitive, and simple—assert themselves. In the light of the complex, embryogenic processes that give rise to skeletal, muscular and neural tissues in the many-celled animals, it is not easily conceivable how a single, undivided, simple and primitive "cell"—the protozoan—could evolve organs performing these specialized functions. Furthermore, it is evident that many protozoans are similar in general appearance and in method of division to a single metazoan cell; both are defined as "a mass of protoplasm containing nuclear substance (chromatin) concentrated into one or more

nuclei" (Minchin, 1912, p. 1). Metazoan organs are composed of many cells which have become modified and often highly specialized to form tissues, of which several kinds may appear in the same organ. On the other hand, organs of the Protozoa are not composed of cells but are modifications of a single cell. We might, therefore, regard the protoplasm within the organs of the protozoan as having the same general physiological properties as the protoplasm throughout the protozoan body and these general properties should be possessed in common with those of any protoplasm wherever found.

Now one general property of all protoplasm is the propagation throughout all its substance of an excitation effected by a stimulus. The morphological continuity of this substance into all the parts or organs of the protozoan body would appear to be the only essential condition for the conduction of an excitation, wherever initiated, to any such part or organ. If this condition is evident in all protozoans, it would seem that specialized, conductive structures for the transmission of excitations were unessential and useless. Accordingly, caution in ascribing a nervous function to a structure or a system of structures in a protozoan body is justifiable.

However, may not as much be said for other general properties of protoplasm? Chambers (1917*a*) has shown that the surface layer of marine eggs may be pulled out into long strands "without otherwise disturbing the contour of the cell. On being released the strands tend to curl and retract slowly until they disappear" (p. 6). Similar phenomena may be readily demonstrated in the endoplasmic globules of *E. patella* that frequently form with the escape of the endoplasm into the water. Also, the proverbial amoeba and many of its relatives display the phenomenon of contractility in normal behavior, as do also all amoeboid cells of the Metazoa. And the cytoplasm of amoebae possesses no fibrils or other specialized structures, so far as is known, by which it effects contraction. Nevertheless, this general property of the cytoplasm is not functioned by such simple and primitive means in many protozoans. It is a well-established fact that in the so-called higher forms contractility is effected mainly, though perhaps not exclusively, by specialized structures, the myonemes.

If, therefore, in the "unicellular" protozoan the general property of contractility has become more or less localized in special organelles, what should restrain conductive protoplasm from the specialization of structures to facilitate conductivity? The extreme rapidity with which many protozoans react to stimuli suggests the presence of

specialized, conducting elements in their protoplasm. That such elements in the ciliate, *Euplotes patella*, have become unified into an efficient, integrated system for the coördination of its associated organelles, is supported, it is believed, by experimental evidences set forth in foregoing paragraphs.

Should further experimentation substantiate these results, then their significance is clear. The most salient feature of structures and functions in the Protozoa as in the Metazoa is not cellularity but organization. The external organelles of a protozoan body are not mere continuations of the protoplasm as the fingers are a part of the glove. They are rather modifications which are sometimes distinctly specialized, as the cirri and membranelles of *E. patella* clearly indicate. Moreover, the complex, integrated fibrillar apparatus of this organism signifies higher specialization in its intracytoplasmic structures. From these considerations it would follow that any general conception of the Protozoa which assumes that any and all of this extensive and diversified group of organisms are so simple and primitive as to lack specific organization—the specialization of intra- and extracytoplasmic organelles—is inadequate and will assuredly be abandoned.

SUMMARY

The fibrillar system in *Euplotes patella*, found and described by Yocom (1918) as a "neuromotor apparatus," has been identified in the living organism both with and without the aid of vital dyes.

Other structures of this system not previously described are: (a) membranelle fiber plates, each of which is contiguous with a membranelle basal plate and is attached at one end to the membranelle fiber; (b) dissociated fiber plates contiguous with the basal plates of the frontal, ventral and marginal cirri, to each of which are attached the "dissociated fibers."

The rectangular anal fiber plates, a modification of the posterior ends of the anal fibers, directly approximate the basal plates of the anal cirri.

The fairly rigid pellicle is amply sufficient to maintain the normal shape of *Euplotes* under considerable stress and after an incision fully two-thirds the width of the body has been made.

The contractility of cirri or of membranelles is not contingent upon their attachment to the body and consequently not upon any mechanism within the body.

The normal locomotion of *Euplotes patella* includes three creeping movements: (1) straight ahead, (2) a quick backward movement, (3) a turn to the right (aborally); and six swimming movements: (1) forward without spiral revolutions, (2) forward in spiral revolutions, (3) circus movement to the right, (4) circus movement to the left (orally), (5) a sharp turn to the right, (6) rapidly backwards without revolutions.

It was evident from transections of the body and excision of parts that the frontal cirri or anal cirri are indispensable to normal creeping movements, that the adoral membranelles are largely responsible for swimming movement 3, that the anal cirri function chiefly in performing creeping movement 2 and swimming movement 5, and that the adoral membranelles and anal cirri cooperate to effect swimming movement 6.

Cutting the membranelle fiber results in conspicuous differences in the behavior of the adoral membranelles on either side of the incision and in abnormal spiral revolutions in swimming.

Severing the anal cirri fibers affects both creeping and swimming. Creeping movement 2 is infrequent. Swimming movement 5 was seldom observed and 6 was never seen after the fibers had been severed.

Destroying the motorium or cutting its attached fibers interrupts coördination in the movements of the adoral membranelles and anal cirri.

Any incision not severing either the membranelle fiber or the anal cirri fibers does not impair normal creeping or swimming movements.

These experimental evidences do not support the assumption that the fibrillar system in *Euplotes patella* is either contractile or supporting in function, but they indicate that this complex system of fibers does possess conductive properties functioning in the coördination of the movements of the locomotor organelles with which it is intimately associated.

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EXPLANATION OF PLATES

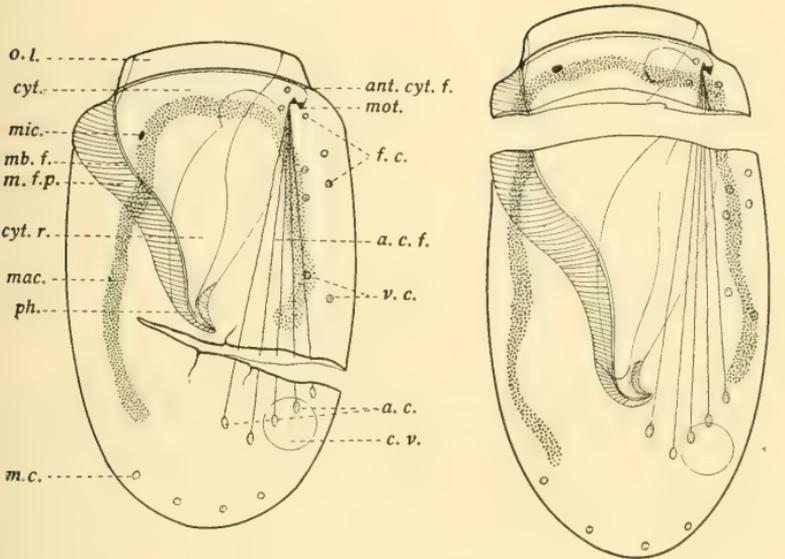
PLATE 29

Fig. 1. Transverse incision through three-fourths of the body, after which the animal maintained normal form. $\times 800$. *a.c.*, anal cirrus; *a.c.f.*, anal cirri fiber; *ant. cyt. f.*, and *mb. f.*, membranelle fiber; *c.v.*, contractile vacuole; *cyt.*, cytostome; *f.c.*, frontal cirri; *m.c.*, marginal cirri; *mac.*, macronucleus; *m.f.p.*, membranelle fiber plate; *mic.*, micronucleus; *mot.*, motorium; *o.l.*, oral lip; *ph.*, pharynx.

Fig. 2. Transection between "group of three" and "group of four" frontal cirri. Dorsal view. $\times 800$.

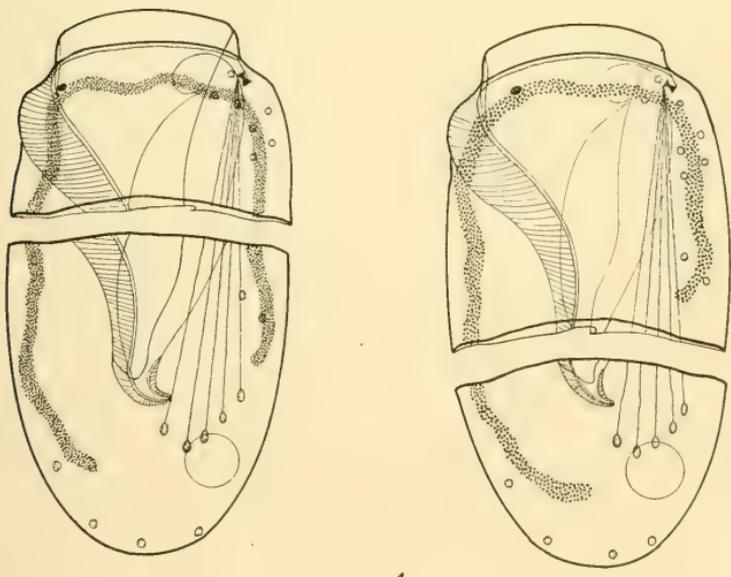
Fig. 3. Transection between "group of four" frontal cirri and the two ventral cirri. $\times 800$.

Fig. 4. Transection between the two ventral cirri and five anal cirri.



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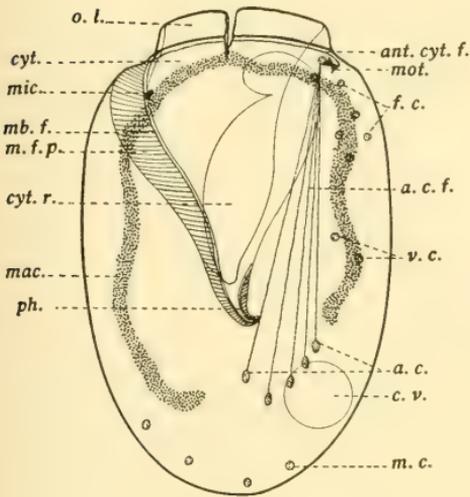
PLATE 30

Fig. 5. Incision through the oral lip severing the membranelle fiber. $\times 800$. *ac.*, anal cirrus; *a.c.f.*, anal cirri fiber; *ant. cyt. f.* and *mb. f.*, membranelle fiber; *c.v.*, contractile vacuole; *f.c.*, frontal cirri; *m.c.*, marginal cirri; *mac.*, macronucleus; *m.f.p.*, membranelle fiber plate; *mic.*, micronucleus; *mot.*, motorium; *o.l.*, oral lip; *ph.*, pharynx.

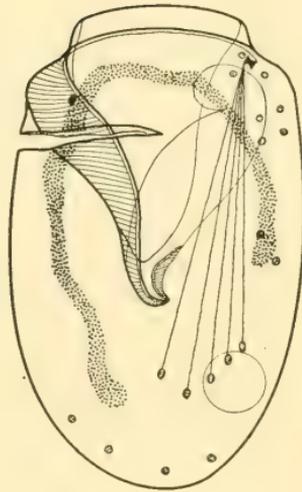
Fig. 6. Incision through the cytostomal membraneles, cutting the membranelle fiber. $\times 800$.

Fig. 7. Incision cutting the anal cirri fibers between the "group of three" and "group of four" frontal cirri. $\times 800$.

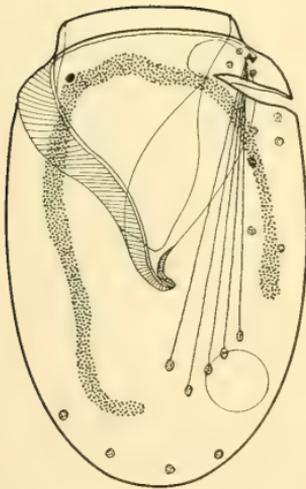
Fig. 8. Incision between the "group of four" frontal cirri and the two ventral cirri, severing the anal cirri fibers. $\times 800$.



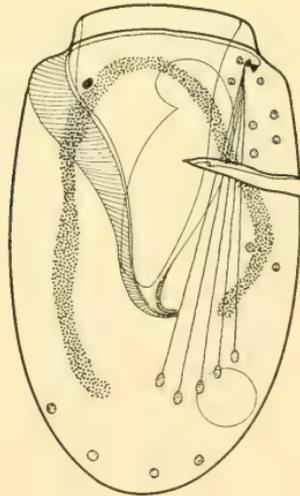
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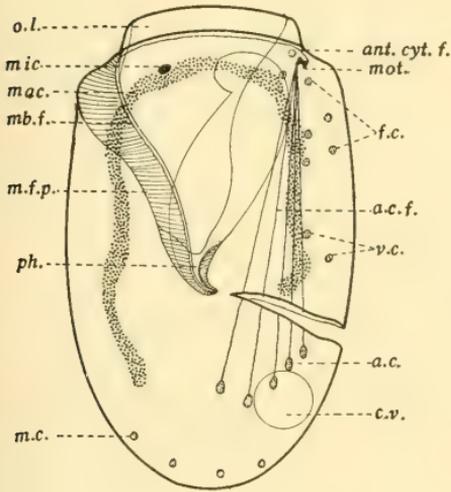


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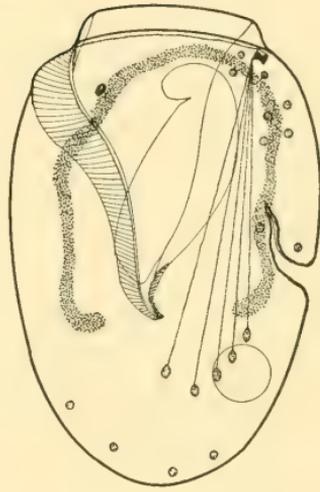
PLATE 31

Fig. 9. Incision anterior of the anal cirri, cutting the anal cirri fibers. $\times 800$. *a.c.*, anal cirrus; *a.c.f.*, anal cirri fiber; *ant. cyt. f.* and *mb.f.*, membranelle fiber; *c.v.*, contractile vacuole; *f.c.*, frontal cirri; *m.c.*, marginal cirri; *mac.*, macronucleus; *m.f.p.*, membranelle fiber plate; *mic.*, micronucleus; *mot.*, motorium; *o.l.*, oral lip; *ph.*, pharynx.

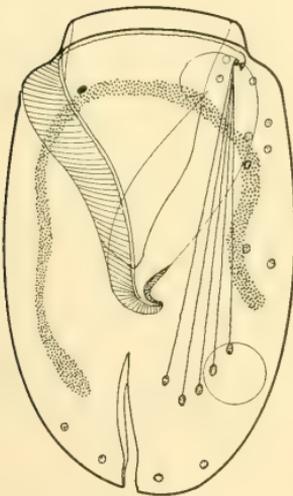
Figs. 10, 11, and 12. Incisions not severing the anal cirri fibers or the membranelle fiber.



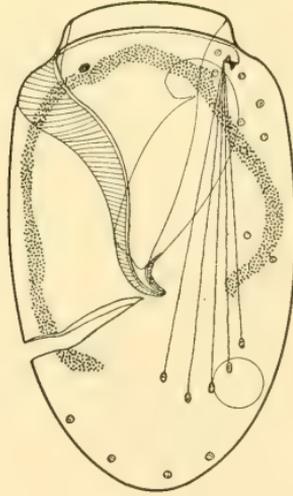
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PLATE 32

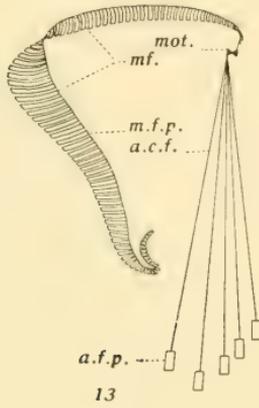
Fig. 13. Diagram of the neuromotor apparatus. $\times 1600$. *a.c.f.*, anal cirri fiber; *a.f.p.*, anal fiber plate; *m.f.*, membranelle fiber; *m.f.p.*, membranelle fiber plate; *mot.*, motorium.

Fig. 14. Anal cirrus detaching from its fiber plate. The cirrus has rotated 90 degrees on its long axis. $\times 1450$. *a.c.f.*, anal cirri fiber; *a.f.p.*, anal fiber plate; *b.p.*, protoplasmic basal plate; *e.g.*₂, large ectoplasmic granules; *n.p.*, needle point.

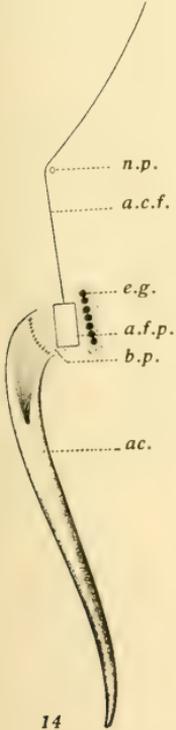
Fig. 15. Anal fiber plate with a portion of its attached fiber distorted by the needle point. $\times 1450$.

Fig. 16. Diagram of a membranelle showing its relation to the membranelle plate. $\times 1450$. *b.g.*, basal granule; *c.r.*, ciliary rootlet; *f.p.*, fiber plate.

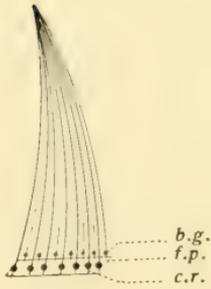
Fig. 17. Dissected portion of disintegrating membranelle fiber plates attached to the membranelle fiber. $\times 1450$. *m.f.p.*, membranelle fiber plate; *m.f.*, membranelle fiber; *e.g.*₂, large ectoplasmic granule; *e.g.*₃, small ectoplasmic granule.



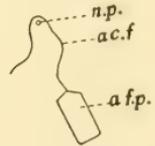
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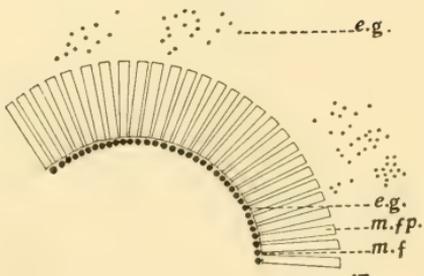
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PLATE 33

Fig. 18. Dorsal view of anal cirrus. $\times 1450$.

Fig. 19. Left lateral view of anal cirrus. $\times 1450$. *a.c.*, anal cirrus; *b.g.*, basal granule; *c.r.*, ciliary rootlet; *p.gl.*, coagulated protoplasmic globule.

Fig. 20. Ventral view of anal cirri, fibers and plates lying among the disintegrating ectoplasm. Anal cirri have turned 90 degrees on their long axis. $\times 1450$. *a.c.*, anal cirrus; *a.c.f.*, anal cirri fiber showing portion of its plate dorsal to the cirrus; *e.g.*₁ and *e.g.*₂, small and large ectoplasmic granules.

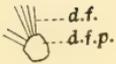
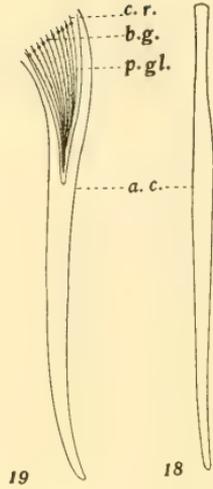
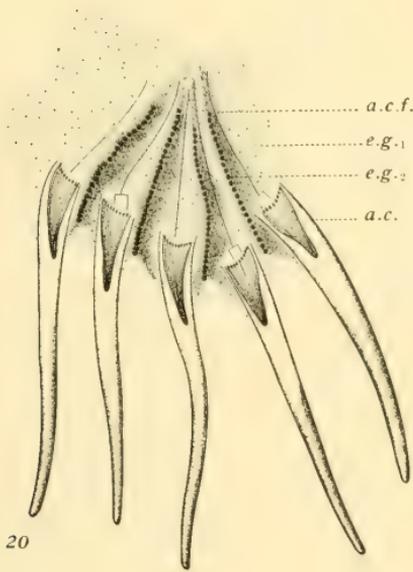
Fig. 21. Plates and fibers of five frontal cirri. $\times 1450$.

Fig. 21a. Dissociated fiber plates of the ventral cirri with their attached fibers. $\times 1450$.

Fig. 22. Lateral view of a detached membranelle previous to disintegration. $\times 625$.

Fig. 22a. Disintegrating membranelle showing the component cilia each with its basal granule and ciliary rootlet. $\times 625$. *b.g.*, basal granule; *c.m.*, membranelle cilium; *c.r.*, ciliary rootlet; *p.gl.*, protoplasmic globule.

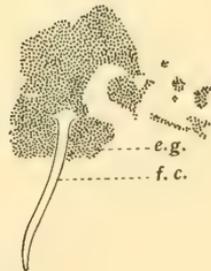
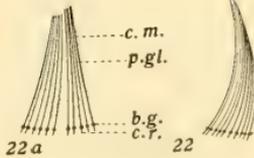
Fig. 23. A frontal cirrus attached to the disintegrating ectoplasm. $\times 625$. *e.g.*, ectoplasmic granule; *f.c.*, frontal cirrus.



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