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DEMONSTRATION OF THE FUNCTION OF
THE NEUROMOTOR APPARATUS IN
EUPLOTES BY THE METHOD
OF MICRODISSECTION

A THESIS ACCEPTED IN PARTIAL SATISFACTION OF
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BY

CHARLES VINCENT 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, eirri, 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 corpusele, the blepharoplast, which in turn is connected by a fine fibril to the nucleus. This organism spends most of its exysted 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), Alexeieff (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 Janieki (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 "kinetoneucleus." 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 intraeytoplasmic 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 intraeytoplasmic 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 ecaudatum*, 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 opereulum toward the right to become lost in the immediate vicinity of the base of the right skeletal structure. These are the opereular 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 intraeytoplasmic 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 *Diplo-dinium 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 caudatum* 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 styliform 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 coördinate 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. Kofoed, 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 Seifrizz (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 vitam 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 pieromereuric 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 eiliary composition of the cirri of various *Euplores* is a well established fact. The component cilia with their basal granules have been described for the cirri of *E. rannus* 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 eilia 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' "avoiding 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) eirens movement to the right, without rotation, (4) eireus 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 haematoxylin. 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 corpuseles 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 cirrus 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 membranules, 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 debris. 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 cirrus 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) cirrus 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) cirrus 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 cytostomal 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 debris. 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 cirrus 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 Saguchi (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 Henneguy 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 (Henneguy, 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 coöperate 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 intraeytoplasmic organelles in this ciliate, and the several experiments previously described would indicate that the extraeytoplasmic 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 corpuseles, 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 cirrus 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" (Minehin, 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 (1917a) 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 coöperate 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.*, macro-
nucleus; *m.f.p.*, membranelle fiber plate; *mic.*, micronucleus; *mot.*, motorium;
o.l., oral lip; *ph.*, pharynx.

Fig. 6. Incision through the cytostomal membranelles, cutting the mem-
branelle 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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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.*₁, small ectoplasmic granule.

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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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