A prominent feature of mass conjugation is its simultaneity, comparable in this respect with the simultaneous cleavage of embryonic cells. At the annual changes from winter to spring and from summer to autumn, the Paramecium population responds en masse to the exigencies of the times. The normal summer fission rate is likewise fairly uniform in its incidence, so that the multiplication of an actively feeding community is essentially a synchronous reaction of large numbers of individuals to alterations in the internal medium or endoplasm, while the so-called epidemics of conjugation are mass demonstrations following upon alterations in the external medium. Conjugation, which can be induced experimentally in a mixed culture without the addition of salts, thus appears to result from the interaction or concurrence of two sets of factors, external and internal, not one to the exclusion of the other; and under natural conditions, the seasonal changes are enough to provoke similar responses.

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# IS THERE A NEUROMOTOR APPARATUS IN DIPLODINIUM ECAUDATUM?

DURING the course of recent investigations on a hitherto undescribed ciliate occurring in the cecum of cattle, the writer made comparisons with Balantidium coli from the pig. The centralized neuromotor apparatus described and figured by McDonald<sup>1</sup> in the latter protozoan could not be differentiated. In order to check the staining reactions Diplodinium ecaudatum was studied, because Sharp's<sup>2</sup> excellent description and figures give the impression that the neuromotor apparatus of the latter ciliate is easily demonstrated. According to this author a mass of material which occurs just above the base of the left skeletal area and between the left extremities of the dorsal and the adoral membranelle zones has the staining reactions of amphibian nervous tissue. Fibers run out from this body to all the motor membranelles, to the membranelles for food taking and also to the esophagus, forming a ring around the latter from which branches extend longitudinally. The reasons which are stated for assigning a neuromotor function to the above structure are: (1) Its general similarity to primitive metazoan nervous systems; (2) its position within the body, not being connected to adjacent structures; (3) its freedom from attachments and other characteristics that are required for an organelle of contraction or support, and (4) its connection by nerve-like strands to all mobile territory. The writer was greatly surprised to find the relationships of this area not as Sharp described them and therefore questions his interpretation.

#### METHODS OF INVESTIGATION

A method was devised of orienting Protozoa in the paraffin so that complete serial sections could be made either transversely or longitudinally through isolated individuals. This method is described in another publication (1930). One especially good series contains fifty-four cross-sections cut at three microns and another series contains forty-two longitudinal sections of the same thickness, though usually there were about twenty-five to thirty longitudinal sections. It is much easier to trace tissues and layers in series thus prepared than in sections through masses of Protozoa which have been imbedded in gelatin capsules, because the observer knows definitely the orientation of a particular organism and is not confused by adjacent sections of other individuals. The technique described by Sharp of differentiating with Mallory's triple connective tissue stain and with iron hematoxylin was followed.

# POINTS OF DIFFERENCE FROM SHARP'S INTERPRETATION

On the whole, the correctness of the accurate, painstaking description by Sharp of the arrangement and anatomy of the various organelles and tissues was fully confirmed. However, there occurred in my material organisms that are very closely allied to Diplodinium which were not mentioned by Sharp and were described and figured, though very inaccurately, by S. Awerinzew and Mutafowa.<sup>3</sup> To include these forms the above authors founded the genus Metadinium. Other very closely allied ciliates which Crawley<sup>4</sup> included in the genus Epidium were not noted either by Sharp or by myself. In my studies, the layer of ectoplasm lying directly under the pellicle was not observed to be alveolar. It stained very deeply and persistently with iron hematoxylin and appeared to be of very compact material. Directly under this layer was another

<sup>&</sup>lt;sup>1</sup>James Daley McDonald, "On Balantidium coli (Malmsten) and Balantidium suis (sp. nov.), with an Account of their Neuromotor Apparatus," Univ. Calif. Publications in Zool., Berkeley, v. 20 (10), May 8, pp. 243– 300, figs. A-O, pls. 27–28, figs. 1–14. 1922. <sup>2</sup> Robert G. Sharp, "Diplodinium ecaudatum, with an

<sup>&</sup>lt;sup>2</sup> Robert G. Sharp, "*Diplodinium ecaudatum*, with an Account of its Neuromotor Apparatus," Univ. Calif. Publications in Zool., Berkeley, v. 13 (4), May 4, pp. 43-122, figs. A-D, pls. 3-7, figs. 1-33. 1914.

<sup>&</sup>lt;sup>8</sup>S. Awerinzew and Mutafowa, "Material zur Kenntnis der Infusorien aus dem Magen der Wiederkäuer," Arch. f. Protist., 33: 109, 1914.

<sup>&</sup>lt;sup>4</sup> H. Crawley, 'Evolution in the Ciliate Family Ophryoscolecidae,'' *Proc.* Acad. Nat. Sci. Phila., 75: 393, 1924.

layer which is described by Sharp as alveolar, but which in my material exhibited the same staining reactions as the inner boundary layer of the ectoplasm. This layer could be followed into the folds that comprise the various outer and inner lips and furrows at the anterior end of the body. It is also thicker here than in the posterior end of the body. In a number of places it is reflected back upon itself for considerable distances especially in the area where Sharp describes the motorium. In fact, in the thin and well-destained sections a fold of this layer resembled in size, shape and position the motorium figured by Sharp, and no other tissue could be differentiated which could be made to answer for this body. Sharp's drawings are all semidiagrammatic, made by superimposing camera lucida sketches of several sections. It is, therefore, difficult to compare them with sections on slides. From my point of view his microphotographs of hematoxylin preparations indicate that his slides were not sufficiently destained. For example, his Fig. 15, plate 6, shows in addition to the motorium another mass equally dense on the ventral side of the cytostome and still another in the dorsal membranelle zone. This author states that the inner boundary layer of the ectoplasm stains very deeply. The writer has noted repeatedly that in sections of three microns the hematoxylin stain disappears from this layer after treatment for from three to five minutes with 2 per cent. iron alum, whereas the nuclei, the pellicle with its underlying ectoplasmic layer, the myonemes and the basal granules of the cilia remain black or bluish black for nearly an hour. The mass which appears to correspond to the motorium is completely destained in all my hematoxylin preparations, though, as Sharp reported, it usually retains the acid fuchsin of Mallory's stain as does also the inner boundary layer of the ectoplasm and the micronucleus. On the basis of my interpretation the motorium is a fold of an ectoplasmic layer which forms a cylinder surrounding the esophagus and also underlies the ciliary rootlets of the membranelles because it extends into all the lips and furrows at the anterior end of the body. It is obvious that sections through this layer would appear as strands. The conditions set forth by Sharp for a coordinating nervous mechanism do not, therefore, appear to be fulfilled in the structure that the writer finds in this ciliate.

## OBSERVATIONS CONCERNING THE DIET OF Diplodinium ecaudatum

According to Sharp this ciliate lives solely upon bacteria, but my observations indicate that it ingests much larger food particles. Individuals taken from a steer that had been feeding on green grass appeared to have eaten some of this material. One case of cannibalism was noted. The smaller ingested ciliate was not crushed by passing through the esophagus of its captor; digestive processes had not at the time of fixation progressed sufficiently to interfere with the staining reactions. The wall of the esophagus of *Diplodinium* appears to be folded into longitudinally extending accordion-like pleats which permit marked distention. Sharp's figures indicate this folding but he makes no comment. It is difficult to conceive how the passage of the ingested ciliate through the esophagus of its captor could have occurred without breaking the esophageal ring as Sharp figures this structure.

### COMMENT AND CONCLUSIONS

My interest was directed to the observations herein reported by the fact that disruptions of certain intestinal ciliates in unfavorable environments occurred by an outflow of the endoplasm through the cytostome. The digestive systems of the Protozoa, unlike those of the Metazoa, are characterized by a break of the layers. The pellicle and ectoplasm which line the mouth and esophagus are broken through at the distal end of the latter organelle. What prevents under normal conditions the outflow of the fluid endoplasm? This question led to the development of the abovementioned method of sectioning individual Protozoa in the same way that embryos are sectioned. It is obvious also that the presence or absence of nervelike fibers will have a bearing on the answer to the question. The writer realizes fully the responsibility for the stand that is here maintained because Sharp's researches led the way to a number of successful investigations of neuromotor systems of various ciliates from widely separated groups.

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