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The Protoplasmic Connection in *Volvox*

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SYNOPSIS. Electron-microscopic observations were performed on 2 species of *Volvox*, one similar to *V. globator*, the other to *V. aureus*. The former has distinct protoplasmic connections in the adult coenobium and specific structures, named "medial bodies," in the connections just at the intersection with the middle lamella. The medial body is disk shaped, about 800 mμ in diameter, and is composed of 3 parts, 2 dense outer layers and an intermediate less dense zone. In the latter species, the connection and medial body were not seen. On the other hand, it was commonly seen in both of them that in younger, dividing gonidia neighboring protoplasts were connected with each other by protoplasmic bridges. The bridges are undoubtedly formed due to incomplete cell separation in the division of a gonidium. The structural difference in the adult coenobium between the 2 species emerges just after inversion of the coenobium. In the *globator* type the medial body appears just after inversion, and the connection remains unruptured all thru life. In the *aureus* type, it seems that the connections are withdrawn or degenerate immediately after inversion. It is discussed whether protoplasmic continuity is really maintained by the connection or not in the free-swimming coenobium of *Volvox*.

IN light-microscopic observations of various species of *Euvolvox* and *Janetsphaera*, it has been found that the constituent zooids are connected with each other by protoplasmic strands. The protoplasts of 2 neighboring cells seem to be directly connected by the strands. However, it has also been pointed out that the structure of the strands is not always similar in all of the species(4,9). The strand has a homogeneous structure in *Volvox aureus*, while in *V. globator* it does not, but one or a few granules are always found just in its middle(9).

On the other hand, it has been found that none of the species of the *Merrillosphaera* section has protoplasmic connections in mature coenobia. This is one of the most marked structural differences between *Euvolvox* and *Merrillosphaera* (6). However, notwithstanding such a difference, no marked difference in the swimming pattern of the 2 coenobia has ever been seen. This suggests some questions on the protoplasmic connection in *Volvox*. First, are the connections really functional in transmitting impulses from cell to cell to coordinate flagellar action? Second, are the connections completely deficient in the species of *Merrillosphaera?* The possibility that neighboring protoplasts are connected by very fine strands should not be denied, even tho they cannot be recognized by observations on the light-microscopic level.

In order to investigate the functions of the protoplasmic connection, it is necessary to clarify its fine structures. The present report is concerned mainly with electron-microscopic observations of the origin and structure of the connection.

MATERIALS AND METHODS

Two different species of *Volvox* can be obtained in ponds near our school. The coenobium of one consists of about 10,000 cells. The protoplast is stellate, with several coarse processes traversing the cell wall. When the coenobium is viewed from the surface, the middle lamellae form a hexagonal pattern. Judging from these structural characteristics, this is undoubtedly a species of *Euvolvox* and resembles *V. globator*. In the other one, the coenobium consists of about 1,000 cells. The protoplast is ovoid and occupies the periphery of a broad mucilaginous layer in which the boundary between neighboring cells cannot be seen. These features seem to indicate that this is *V. aureus*. However, the coenobium does not have the marked protoplasmic connections which are characteristic of *V. aureus*. In order to identify the species of *Volvox*, structural features of the sexual phase of the life history must be seen in addition to those in the asexual phase. However, under the cultural conditions in our laboratory, the protozoa could not survive long enough to reach their sexual phase. Consequently, it was impossible to determine the specific names of the present forms. Because of this, they are named provisionally *"globator* type" and *"aureus* type."

Specimens for electron microscopy were fixed for 30 minutes in ice-cold 1% OsO_4 in phosphate buffer at pH 7.0 or 8.0. The latter fixation gave better results. Permanganate or glutaraldehyde fixation

was also used in a few cases. After fixation the specimens were dehydrated rapidly in a graded series of ethanol and embedded in methacrylate or Epon.

Thin sections were cut with the LKB Ultrotome. In some cases, they were stained in uranyl acetate or lead citrate(11,13). Observations were carried out with a Hitachi H-7 microscope, and micrographs were taken at original magnifications of 2,000-8,000.

OBSERVATIONS

1) Structures of protoplasmic connections in the *globator* type.

In this type the stellate protoplasts have 4-6 coarse protoplasmic processes. It seems likely that each process merges with the corresponding one of the adjacent cell just at the middle lamella which is the boundary between the 2 cells. In the present electron-microscopic study, it was found that a particular structure was always in the connection just at the intersection with the middle lamella (Figs. 1, 2). This structure is undoubtedly the same as the granule (Körnchen) that Meyer(9) previously pointed out. We are calling it the "medial body." The medial body is presumably disk-shaped, about 0.8 μ in diameter and about 0.2 μ thick. It is composed of 3 parts: 2 dense outer layers, each 40 mμ thick, and an intermediate less dense zone about 120 mμ thick. At higher magnification (Figs. 3, 4), holes and hollows about 40 mμ in diameter are seen in the dense layers. Canaliculi of similar diameter occur in the intermediate zone. From these figures, it seems probable that many canaliculi pass thru the medial body.

In young, free-swimming coenobia, the protoplasmic process is tapered and narrowest at the medial body. As the coenobia get older, the distance between the 2 adjacent protoplasts extends farther and farther, and the processes simultaneously become more and more delicate. In old coenobia a slender process always becomes suddenly broad just at the point where it traverses the middle lamella, namely at the site of the medial body (Fig. 5a). Peculiar configurations of the connection are often seen even in light microscopy in

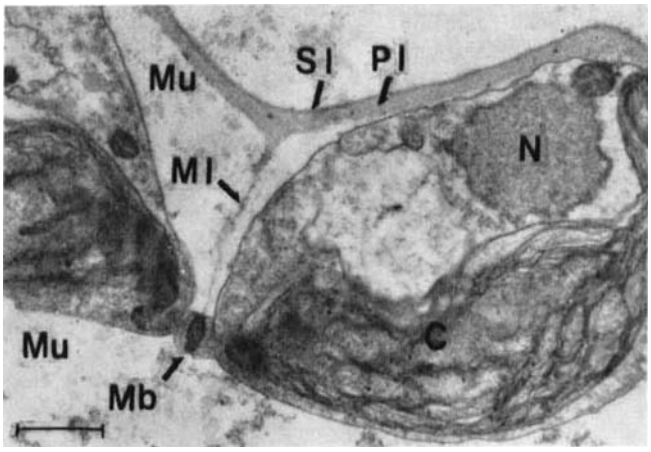

Fig. 1. Electron micrograph of a section thru the peripheral part of a free-swimming coenobium of the *globator* type of *Volvox*. The coenobium is surrounded by a continuous superficial layer (Sl) and a peripheral lamella (Pl). The protoplast is enveloped by mucilage (Mu) and is connected with the adjacent one by a protoplasmic connection. A medial body (Mb) is shown in the connection just at the intersection with a middle lamella (Ml). N—nucleus, C—chloroplast. × 11,000. The scale indicates one micron.

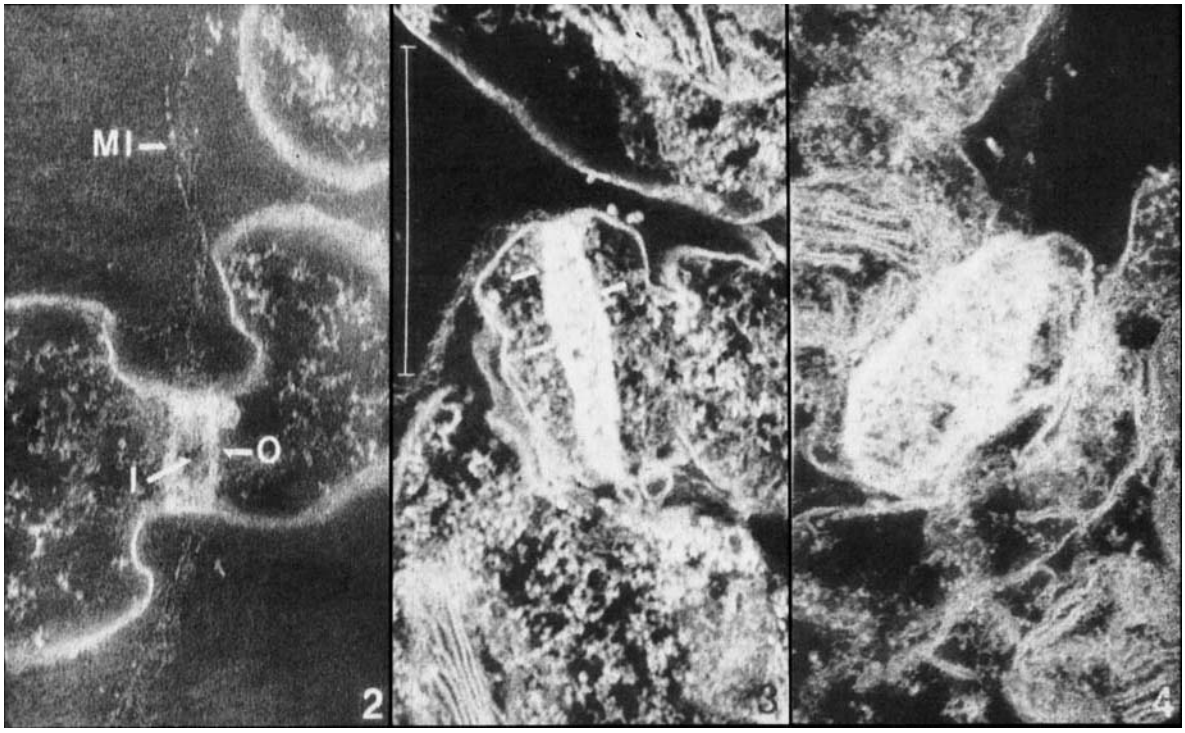

Fig. 2. Electron micrograph of a section thru a medial body showing 2 dense, outer layers (O) and an intermediate less dense zone (I). Ml—middle lamella. × 48,000.

Fig. 3. Electron micrograph of a section thru a medial body showing holes and hollows (indicated by arrows) thru the dense outer layer. × 43,000. The scale indicates one micron.

Fig. 4. Electron micrograph of an oblique section thru a medial body showing canaliculate structure in the intermediate zone. × 35,000.

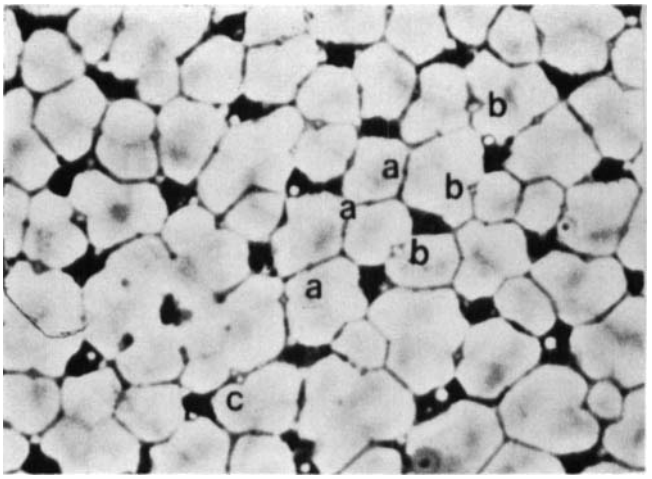

Fig. 5. Phase-contrast microscopic surface view of a free-swimming coenobium of the *globator* type of *Volvox* showing the branching of the connection. a—connection of unbranching process, b—connection of bifurcated tip of a process, c—connection of trident tips of a process. × 420.

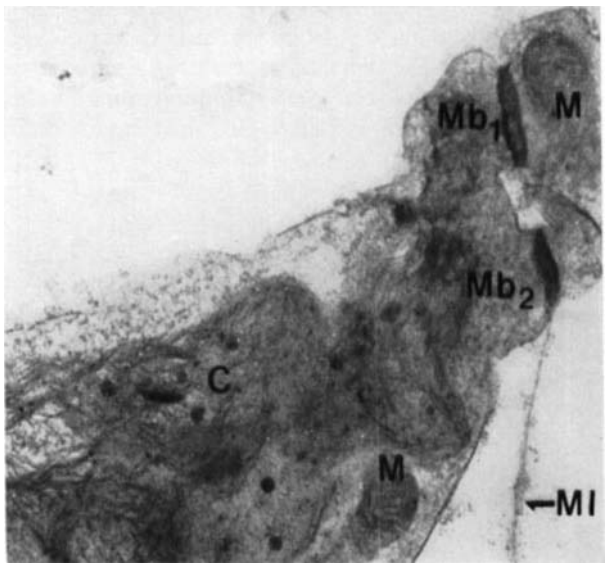

Fig. 6. Electron micrograph of a section thru the bifurcated connection in a free-swimming coenobium of the *globator* type, showing 2 independent medial bodies (Mb_1 and Mb_2) each in a connection. C—chloroplast, M—mitochondrion, MI—middle lamella. × 16,000.

such a coenobium, *i.e.*, the tip of the process becomes broad near the middle lamella, then divides into 2 or 3 branches (Fig. 5b, c). Since the branching occurs similarly in the opposed process, a configuration results in which 2 or 3 protoplasmic strands enclose one or 2 empty areas along the middle lamella. The medial body is always present in every one of the branched connections (Fig. 6).

2) Origin of the protoplasmic connections and formation of the medial body in the *globator* type.

In asexual reproduction of the *globator* type, a gonidium begins to divide soon after the birth of a coenobium. After more than 10 successive cell divisions, it stops division and begins inversion. During the period of cell division, the protoplasts are naked and are arranged in close contact with each other. The mucilaginous and lamellar portions of the cells are produced over the spherically arranged protoplasts, as soon as inversion is completed.

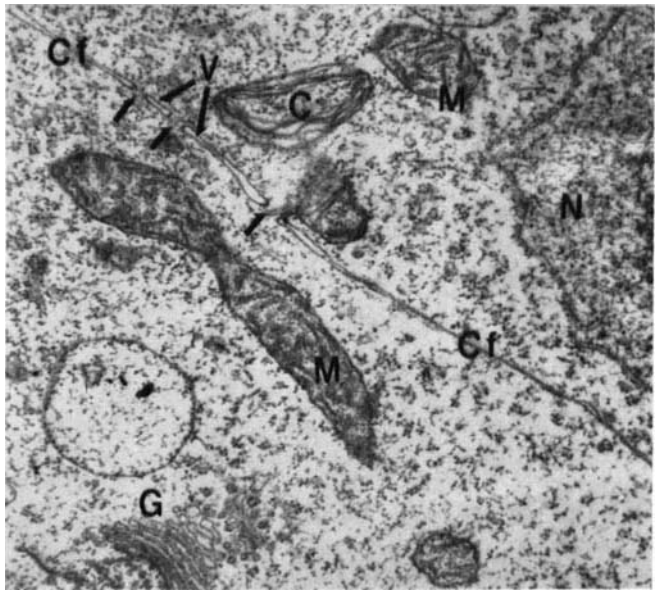

Fig. 7. Electron micrograph of a section thru the boundary area between 2 neighboring cells in a cleaving gonidium of the *globator* type of *Volvox*. Between the tips of the cleavage furrow (Cf), 2 slender vesicles (V) occur in a row, and 3 protoplasmic bridges (indicated by arrows) between the furrow and the vesicles directly connect the 2 neighboring protoplasts. C—chloroplast, G—Golgi complex, M—mitochondria, N—nucleus. × 16,000.

Fig. 7 shows the boundary area between 2 neighboring cells. This is part of a very early gonidium in cleavage. The cleavage furrow has constricted the cell, and membrane-bounded, slender vesicles appear in a row between the tips of the furrows. In this figure, it may be seen that protoplasmic continuity between the 2 daughter cells is maintained thru the gaps between furrows and vesicles. In the present study these protoplasmic bridges were invariably seen between protoplasts in every thin section of the coenobium before inversion. From these facts, it seems reasonable to consider that the bridges are the origin of the protoplasmic connections in the adult coenobium, namely, that the connections result from incomplete separation of daughter cells at the end of cytokinesis during cleavage of a gonidium.

However, we should point out other observations which are not necessarily consistent with the above consideration. In the cleaving gonidium before inversion, the gaps between furrows and vesicles are about 100 mμ wide at the maximum, whereas the protoplasmic connection in the adult coenobia is generally 800 mμ wide at the site of the medial body. Such a difference in width makes it difficult to anticipate that a connection in the adult corresponds directly to a single protoplasmic bridge between cells in a dividing gonidium. For this discrepancy the micrograph shown in Fig. 8 may offer a possible explanation. It shows the protoplasmic connection in a young coenobium just before inversion. The connection is composed of 4 bridges and contains 3 empty vesicles. Each of the bridges is about 100 mμ

wide. This coincides exactly to the width of the bridge in the younger, cleaving gonidium. The empty vesicles may presumably correspond to the ones which appear in a row between the tips of the cleavage furrow shown in Fig. 7. Accordingly, it seems probable that the connection in the adult does not correspond to the single bridge but to the area encircled by the margin of the cleavage furrow between 2 daughter cells in the dividing gonidium. In other

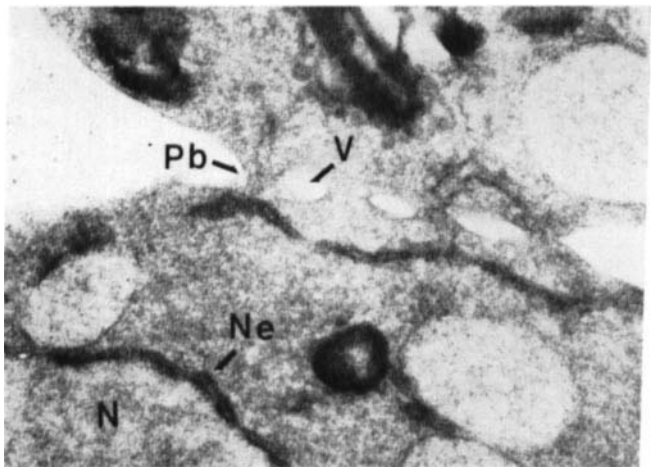

Fig. 8. Electron micrograph of a section thru a protoplasmic connection in a coenobium just before inversion of the *globator* type of *Volvox*. The connection is composed of 4 protoplasmic bridges (Pb) and 3 empty vesicles (V). The medial body is not yet formed in the bridges. N—nucleus, Ne—nuclear membrane. × 42,000.

words, the connection in adult coenobia may correspond in the dividing gonidium to the one which is composed of a few protoplasmic bridges and vesicles. This assumption may be supported by the fact that the width between the tips of the cleavage furrow shown in Fig. 7 (1 μ) is similar to the diameter of the medial body (0.8 μ). However, in this line of consideration, it should be questioned how the bridges and vesicles transform into the medial body. Insofar as the present observations are concerned, it is evident that the medial body is never found in the protoplasmic bridge of the dividing gonidium. It is found exclusively in the adult coenobium after inversion in which the lamellar and mucilaginous portions of cells are already formed. In addition to this fact, it must be mentioned that the medial body always appears in the connection just at the intersection with the middle lamella. These facts seem to indicate that the formation of the medial body begins immediately after inversion and proceeds in close correlation with that of the lamellar and mucilaginous portions of cells especially of the middle lamella. On the formation of the medial body, the present results cannot offer any further detailed information.

3) Protoplasmic connection in the *aureus* type.

In light-microscopic observations of the free-swimming coenobium, no protoplasmic connections are found in this type of *Volvox*. In electron-microscopic observations, it is also true that the connections are not recognized. In some cases, protoplasmic processes are seen projecting from the surface of the protoplast. The projection has a cord-like configuration about 0.2 μ in diameter. This configuration is quite different from the tapered one of the processes in the *globator* type. Moreover, even in these cases, it is impossible to recognize a distinct connection between 2 neighboring cells thru the projections. In this *aureus* type, the cell wall is also quite different in its structure from that of the *globator* type. The middle lamella which forms the boundary between cells in the latter is not found at all in the former. The spherically arranged protoplasts are surrounded by a mucilaginous, continuous layer.

On the other hand, protoplasmic bridges are clearly visible between cells in the young coenobium before inversion. This is shown in Fig. 9. Before inversion it is true in this type too that the lamellar and the mucilaginous portions of cells are not yet formed and the naked protoplasts stand side by side in close contact with each other. Two or 3 protoplas-

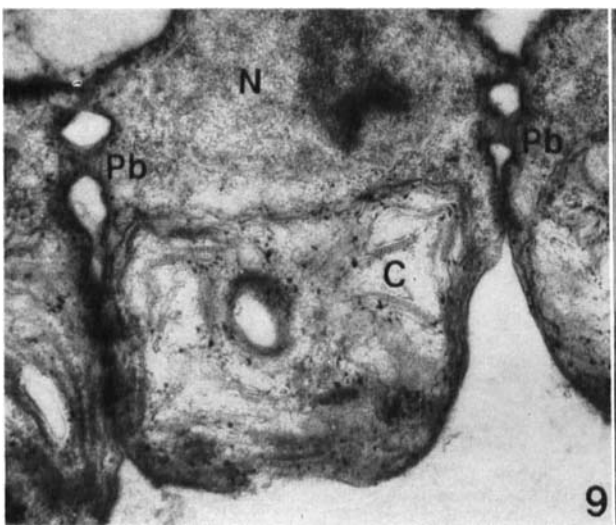

Fig. 9. Electron micrograph of a section thru a coenobium of the *aureus* type of *Volvox* just before inversion, showing protoplasmic connections. The neighboring protoplasts are connected with each other by 2 protoplasmic bridges (Pb). N—nucleus, C—chloroplast. × 18,000.

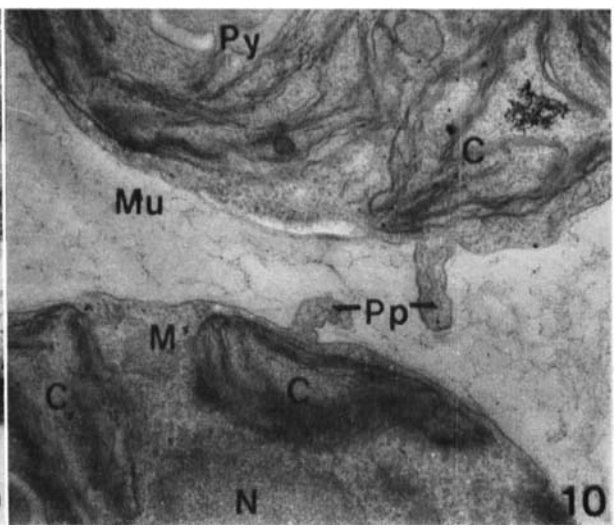

Fig. 10. Electron micrograph of a section thru 2 neighboring protoplasts in a free-swimming coenobium of the *aureus* type, showing protoplasmic projections (Pp). C—chloroplast, M—mitochondrion, Mu—mucilaginous portion, N—nucleus, Py—pyrenoid. × 16,000.

mic bridges can be seen between 2 neighboring protoplasts. These bridges undoubtedly occur in consequence of incomplete separation of 2 daughter cells at the end of cytokinesis.

In looking thru these results, we may state that in this type of *Volvox* the protoplasts in the dividing gonidium are connected with each other by protoplasmic bridges, but after inversion the bridges are drawn out and then torn off as the coenobium gets older.

The medial body which appears in the *globator* type is not found in this type.

DISCUSSION

In the cleaving gonidium of *Volvox*, neighboring protoplasts are connected with each other by intercellular, protoplasmic bridges. This agrees quite well with the light-microscopic observations of Janet(7). Such intercellular protoplasmic bridges have been seen not only in *Volvox*, but also in other Protozoa and in Metazoa too. The constituent cells of a colony of *Dangeardinella*(10) and of young coenobia of colonial Chlamydomonadaceae such as *Gonium*(1,5), *Pandorina*(1) and *Eudorina*(2) are connected with each other by protoplasmic strands. In the cnidoblast of *Hydra*, intercellular bridges are found connecting cells which arise from a single undifferentiated interstitial cell and occur in groups of 8-16 cells(12). In mammalian tissues, similar protoplasmic bridges occur especially in spermatogenesis, where 8 spermatids are connected by bridges and form a syncytial cluster(3). All thru these cases, it is commonly stated that at the end of cytokinesis the cleavage furrow does not completely separate 2 daughter cells, so that protoplasmic connections remain unruptured between the 2. In the present cases too, it seems undoubted that the protoplasmic connection occurs as a result of incomplete cell separation.

However, this does not mean that the connection invariably persists all thru the life of the coenobium. In the *aureus* type, the connection is never seen in the free-swimming coenobium. Similar results have previously been reported on various colonial Chlamydomonadaceae. In light-microscopic observations on *Gonium*(1) and *Pandorina*(1), it was found that the connections which are distinct in young coenobia become obscure as the coenobia mature and approach the phase of reproduction. In the present case, too, it seems probable after inversion the connection is stretched, torn off and then withdrawn or degenerates as the coenobium gets older.

On the other hand, the connections in the *globator* type are maintained all thru the life of a coenobium. But a marked change occurs with the formation of lamellar and mucilaginous portions of cells after inversion. This is the formation of the medial body in the protoplasmic connection. The appearance of the medial body poses a new question as to whether the protoplasts of neighboring cells are really continuous thru the body or not. In appearance, the dense layers of the body seem to be such complete partitions in the bridge that they interrupt protoplasmic continuity between the 2 protoplasts. At the same time, it should be noted that pores and canaliculate structures are found in the medial body. The possibility that protoplasmic continuity remains thru these structures cannot be denied. On this problem, the present structural observations cannot give a clear solution. Further investigations are necessary.

The medial body has some structural resemblance to the desmosome of metazoan cells. But marked differences can also be pointed out between the 2. According to Kelly(8), a desmosome is always formed in an area of approximation of plasma membranes of adjacent cells, and this nearly circular region of approximation is underlain on each side by a dense intracellular plaque toward which numerous tonofilaments converge. In the present electron micrographs, it is impossible to recognize a plasma membrane between the 2 dense layers of the body and tonofilaments. This dissimilarity in organization seems to indicate that the medial body is not homologous to the desmosome. Presumably, the medial body is a characteristic structure of species of *Euvolvox* which have broad protoplasmic connections and lamellar boundaries between neighboring cells.

The present forms of *Volvox*, the *globator* and the *aureus* type, both swim about in water always rotating around a body axis and simultaneously moving forward in the direction of the axis. This swimming behavior seems to be explained only by supposing that the flagellar action of all the zooids are coordinated. If the coordination of flagellar action is really established in the coenobium, it would be natural to consider that the protoplasmic connections do the coordinating by transmitting impulses from cell to cell. The present results indicate that the connections disappear in the free-swimming coenobium of the *aureus* type. From these facts, it should be questioned again whether the protoplasmic connection is an indispensable structure for coordination of flagellar action. Further investigation from different points of view is necessary on the function of the connection and on the mechanism of coordination of flagellar action.

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