Kleinere Mitteilungen.

(Miller School of Biology, University of Virginia.)

An Investigation of the Question of Cytoplasmic Fusion in *Amoeba proteus.*

By

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Contents.

Introduction.

The reappropriation of cytoplasmic fragments in certain of the Sarcodina has been reported by a number of investigators. The phenomenon was first noted in the Foraminifera. VERWORN, in 1892, observed that in *Orbitolites* enucleated fragments may adhere to the ends of pseudopods of the parent organism, and in 1896, JENSEN reported occasional fusion of severed fragments with the parent body in *Orbitolites* and *Amphistegina.*

In the Testacea, reappropriation of fragments by the parent organism was first observed by PENARD, 1899, who worked with *Difflugia lebes* and *D. pyriformis*. KEPNER and REYNOLDS, 1923, studied the process in *Difflugia acuminata*, and *D. vulgaris*; they found that reappropriation is the usual reaction under favorable conditions in these species, but that cross-fusion of cytoplasm will not take place beetween different species. These results were confirmed by OKADA, 1930. REYNOLDS later observed fusion between cell bodies and severed fragments in ten species of the genus *Difflugia*, in *Arcella polypora*, *Centropyxis aculeata*, and other forms, and concluded that the phenomenon is fairly common among the Testacea. He demonstrated (1924) that, in *Arcella polypora*, fusion will take place between one individual and a protoplasmic fragment of a closely related specimen, and that, when two lines of a clone are kept under similar environmental conditions, their ability to cross-fuse may continue indefinitely. When, however, the environments of the two lines are dissimilar, cross fusion no longer takes place, and this fact was taken as an indication that physiological changes, probably due to environmental influences, occur among the descendants of a single *Arcella* reproducing vegetatively. BURCH, 1930, used cytoplasmic reappropriation in *Arcella vulgaris* and *A. rotundata* in an attempt to vary division rate of the organisms by varying the karyoplasmic ratio. E. DE WITT MILLER, 1930, whose observations are as yet unpublished, found in an investigation of the physical nature of the phenomenon of reappropriation, that *Arcella discoides*, when cultured in media of varying hydrogen ion concentration or when subjected to an electric current, showed no tendency toward acceleration of contact and fusion rates, and concluded that cytoplasmic fusion is very possibly wholly beyond the control of hydrogen ion concentration or the electric current.

Among the Amoebida, OKADA, 1930, observed cytoplasmic fusion in *Pelomyxa*. He found that fusion took place between two individuals or between two fragments from the same individual. In *Entamoeba testudinis*, an isolated case of possible cytoplasmic reappropriation has been observed.

Among the Actinopoda, cytoplasmic fusion has been observed in *Actinophrys* and *Actinosphaerium*. LOOPER, 1928, investigated the formation of temporary colonies by fusion, in *Actinophrys sol*, and was able to induce fusion of enucleated fragments with nucleated individuals or with other enucleated fragments. He used this reaction to demonstrate that division rate increases with increase in cytoplasmic bulk and correspondingly decreases with decrease in

cytoplasm. HOWLAND, 1928, observed that *Actinosphaerium eichhornii* reappropriates fragments from its own body or from that of another organism of the same species.

In general, the Sarcodina in which cytoplasmic reappropriation occurs are forms in which the cytoplasm shows considerable viscosity. It is possible that viscosity is a property upon which cytoplasmic fusion is based. *Amoeba proteus* is a form of less viscous cytoplasm than those already referred to. Cytoplasmic fusion has not been reported in *Amoeba proteus* and conclusive evidence that it does not take place should be a significant point in the consideration of a possible relation of cytoplasmic viscosity to cytoplasmic fusion.

I wish to extend my thanks to Prof. B. D. REYNOLDS for suggesting this problem and for his helpful interest and advice during its progress.

The organism.

These experiments have been made on specimens of *Amoeba proteus* varying in size from 160 microns to 350 microns (greatest diameter). A collection was made, in the autumn of 1930, from a freshwater pool near the campus of the University of Virginia, and the amoebae obtained in this collection were kept in mass culture in a wheat medium. In June, 1931, the culture was taken to the Mountain Lake Biological Station of the University of Virginia, where the following experiments were carried on.

A wheat medium was used in the culture of the amoebae. In preparing the medium, one liter of spring water, containing 25 grains of wheat, was brought to a boil and allowed to cool. About two and one half in ches of theliquid and two of the wheat grains were placed in a glass butter dish, and a quantity of green algae was introduced. This preparation was allowed to stand at least 24 hours before inoculation with the amoebae. Various ciliates were soon found in the culture and served as additional food for the Amoebae. The cultures were kept in a shaded room at normal room temperature (about 20° C).

Apparatus and technique.

The organisms were located under a binocular microscope. Observations and experiments were made under a compound microscope with a 16 mm. objective and a # 4 ocular. The microscope was calibrated and equipped with an ocular micrometer. Camera lucida drawings were made throughout the experiments.

The amoebae were removed from the culture dishes by means of a capillary pipette, and placed in a drop of the culture medium on a clean glass slide. Fragments were cut with a hard glass needle.

Observations.

I. Reaction of the organism to a cytoplasmic fragment of its own cell body.

The following experiments were carried out in an attempt to determine whether, in *Amoeba proteus*, the organism will fuse with cytoplasmic fragments from its cell body. In each case, the organism was placed in a drop of culture medium on a glass slide and the experiments performed under the compound microscope. When severed from the parent body, the enucleated fragments showed almost immediate cessation of cyclosis. In fifteen minutes or more, the fragments rounded up and assumed the form of a wrinkled sphere. To avoid using fragments in which normal cytoplasmic reactions were no longer taking place, observations were made immediately after cutting.

In seventy-five experiments, a cytoplasmic fragment was cut from the organism and allowed to remain at a distance of from 1 micron to 20 micra. Under such circumstances, in the Testacea, the parent cell will soon proceed toward the fragment and fuse with it. In *Amoeba proteus*, the organism is apparently not attracted by the fragment. The parent cell, moving normally on the slide, made temporary contact with the fragment in 43 % of the cases. The number of contacts to be expected if the amoebae moved entirely by chance, the fragments exerting neither an attracting nor a repelling stimulus, was estimated for this group of experiments by the formula $\frac{S}{C} = \%$ of contacts. (S = diameter of fragment; C = circumference of a circle having for its center the point on the parent cell nearest the fragment, and for its radius the distance from this point to the fragment.) The number of contacts to be expected was 38 %. Contact was actually made in 43 % of the experiments. This result indicates that the parent cell is neither attracted nor repelled by the fragment, and encounters it entirely by chance.

The behavior of the cell body toward the fragment was in every case that which would be expected of an organism encountering a particle of inorganic material, which served as an obstruction. The reaction of an amoeba to the presence of food material, such as algae, is definitely positive, and this type of reaction was never observed in these experiments.

In one hundred and ten experiments, the organism was placed in contact with the cytoplasmic fragment. Fusion was never observed to take place. In every case, the parent cell moved away from the

fragment. The following table is an estimate of the speed of this reaction:

Table 1.

Organism and cytoplasmic fragment placed in contact.

Number of organisms	Time required to separate 100 micra	Number of organisms	Time required to separate 100 micra
1	½ min.	1	5 min.
27	1 „	3	6 „
44	2 „	1	7 „
23	3 „	0	8 „
14	4 „	1	9 „

In two cases, the cell body remained for several hours at a distance of about 30 micra from the fragment, and subsequently moved away.

These observations indicate that the fragment has no attraction for the parent cell, and serves as a mechanical obstruction to its locomotion. This conclusion is in agreement with that drawn from the first group of experiments.

Throughout this series of experiments, the size of the cytoplasmic fragment was varied, from about one and one third the area of the nucleated portion to about one fiftieth of its area. There was no apparent difference in the results. In several cases, the fragment used was the second or third which had been cut from the same organism; no difference in the reaction was observed. It was observed by KEPNER and REYNOLDS, 1923, that cytoplasmic fusion in *Difflugia* occured at the midregion of the pseudopods of the parent organism. In the above experiments, the fragment was placed in contact with the base, tip and midregion of the pseudopod, and with the central mass of protoplasm, with no variation in the results.

In three cases, the organism was fragmented by shaking. The reaction was the same as in the experiments where cutting was performed. The nucleated portion moved away from the cytoplasmic fragments as described above.

In an attempt to determine whether cytoplasmic fusion was delayed in *Amoeba proteus*, rather than absent, fifty experiments were carried out, in which the parent cell and enucleated fragment were placed together in the culture medium on a depression slide immediately after cutting. Observations were made at intervals until the fragment degenerated. Cytoplasmic fusion did not take place in any case, nor was the fragment used for food. The parent cell appeared healthy in each experiment, and divided about once in two days. The fragments required from one to five days to degenerate.

II. Reaction of the organism to a cytoplasmic fragment of its own cell body compared with its reaction to a foreign fragment.

In twenty experiments, an attempt was made to determine whether a cytoplasmic fragment from the body of the organism exerts a stimulus on the parent cell different in any way from that exerted by a foreign fragment. The organism in ten cases was placed in contact with its own fragment and its reactions observed; it was then placed in contact with a fragment from another amoeba, and observed. In ten cases, the order of observations was reversed. The reaction of the organism to the foreign fragment was found to be the same as its reaction to its own fragment. In each case, the organism moved away at about the same rate of speed from the two fragments. The comparative rates are shown in the following table:

Table II.

Number of organisms	Time required to separate 100 micra from foreign fragment, compared with rate from own fragment
11	Same
1	½ min. longer
2	1 „ longer
3	½ „ less
3	1 „ less

There is therefore no apparent difference in the reaction of an organism to its own fragment from its reaction to a foreign fragment.

III. Reaction of an uncut organism to a cytoplasmic fragment.

It is known that in *Actinophrys* and the Testacea, a nucleated individual will appropriate a cytoplasmic fragment from another organism of the same species. It seemed possible that cutting might so upset the physiological balance of an organism that fusion, if it took place in *Amoeba proteus*, might be prevented, while the phenomenon might occur in uncut individuals.

Fifty organisms were tested with cytoplasmic fragments from other individuals. In each case, the organism and the fragment were placed in contact, and in each case the organism moved away. The reaction was the same as that observed in cut individuals. The speed of reaction is recorded in the following table p. 309:

Fifty organisms were placed in depression slides with foreign cytoplasmic fragments, as described above, and observations were made until the fragments degenerated. Fusion did not take place in any case.

Table III.

Reaction of uncut organism to foreign fragment.

Number of organisms	Time required to separate 100 micra	Number of organisms	Time required to separate 100 micra
8	1 min.	0	4 min.
12	2 „	1	5 „
4	3 „		

It is evident from this series of experiments that fusion does not take place in *Amoeba proteus* between uncut individuals and cytoplasmic fragments.

IV. Reaction between cytoplasmic fragments.

In *Actinophrys*, Looper, 1928, noted fusion of cytoplasmic fragments with each other. Kepner and Reynolds, 1923, observed that this phenomenon does not take place in *Difflugia*. It has possibly been observed in *Arcella* in one or two cases.

To determine whether cytoplasmic fragments will fuse with each other in *Amoeba proteus*, twenty experiments were carried out in which a pair of fragments from the same individual were placed at a short distance from each other, and observed. In no case did the pair react to each other. In cases in which cyclosis had stopped, no movement was made. In cases in which cyclosis had not stopped, the fragments moved about but did not come in contact with each other. In twenty experiments, the fragments were placed in contact. Where cyclosis had stopped, the fragments drew apart by rounding up; where cyclosis continued, the fragments moved apart.

There was no evidence in these experiments that enucleated fragments in *Amoeba proteus* undergo cytoplasmic fusion.

V. Reaction of the organism to a fragment partially cut off.

It has been stated by Dr. F. O. Holmes, in an unpublished observation on *Entamoeba testudinis*, that cytoplasmic fusion was seen to take place in one case. In this case, a large granule was found in the cytoplasm, which appeared difficult to egest; the amoeba eventually constricted off the portion of cytoplasm containing the granule. The fragment subsequently egested the granule and was itself reappropriated by the parent cell. It seems probable that a thin strand of ectoplasm was present, connecting the fragment with the parent cell, and escaping the notice of the observer. In a effort to demonstrate this possibility, a piece of cytoplasm was cut from an amoeba, leaving a thin strand of ectoplasm connecting the cell

and the fragment. The thread of ectoplasm was invisible under the binoculars, and was seen with difficulty under low power of the higher compound microscope. Almost immediately, cytoplasm from the cell body surged into the connection, and the fragment became indistinguishable from the rest of the cell. This experiment was carried out twice, with the same results. It seems probable that such an ectoplasmic connection is the explanation of the supposed cytoplasmic fusion seen by Holmes.

Summary.

1. In *Amoeba proteus*, cytoplasmic fusion does not take place between an organism and its own fragments, between an organism and a fragment from another individual, or between two cytoplasmic fragments. The fragment does not seem to attract or repel the organism.

2. The reaction of an organism to its own fragment does not differ from its reaction to a foreign fragment.

Bibliography.

Becker, Ellery R. (1926): The rôle of the nucleus in the cell functions of Amoebae. Biol. Bull. Vol. 50.

Burch, Paul R. (1930): The effect on the division rates of Arcella vulgaris and A. rotundata. Arch. f. Protistenk. Bd. 71.

Howland, Ruth (1928): Grafting and reincorporations in Actinosphaerium eichhornii. Biol. Bull. Vol. 54.

Jensen, Paul (1898): Über individuelle physiologische Unterschiede zwischen Zellen der gleichen Art. Pflüger's Arch. Bd. 62.

Kepner, Wm. A. and Reynolds, Bruce D. (1923): Reactions of cell bodies and pseudopodial fragments of Difflugia. Biol. Bull. Vol. 44.

Looper, James B. (1928): Cytoplasmic fusion in Actinophrys sol, with special reference to the karyoplasmic ratio. Journ. Exp. Zool. Vol. 50.

Okada, Yo K. (1930): Transplantationsversuche an Protozoen. Arch. f. Protistenk. Bd. 69.

Parsons, C. W. (1926): Some observations on behavior of Amoeba proteus. Quart. Journ. Mic. Sci. Vol. 70.

Penard, E. (1902): Faune Rhizopodique du Bassin du Leman. H. Kundig, Libraire de l'Institute. Geneve.

Reynolds, Bruce D. (1924): Interaction of protoplasmic masses in relation to the study of heredity and environment in Arcella polypora. Biol. Bull. Vol. 46.

Verworn, Max (1892): Die physiologische Bedeutung des Zellkerns. Pflüger's Arch. Bd. 51.
