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Reappropriation of cytoplasmic fragments.

1. In different concentrations of hydrogen-ions and 2. Under the influence of the electric current.

By

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Introduction.

The phenomenon of cytoplasmic fusion, as it exists among certain genera of the RHIZOPODA and ACTINOPODA, has attracted the attention of a number of investigators during the past few years. This is especially true of the thecamoebae. The fact that an organism will reappropriate a lost part of its own, or that of a closely related individual of the same species, should be of interest. Experiments have been carried out with the hope of ascertaining some of the underlying factors involved in the action, as well as some of the changes taking place within the organism.

PENARD (1899) first observed cytoplasmic fusion with the parent body in *Difflugia lebes* and *D. pyriformis*. KEPNER and REYNOLDS (1923) showed that among *Difflugia* this performance occured quite frequently. REYNOLDS (1924) experimented in a similar manner with ten species of *Difflugia*, *Arcella polypora*, *Centropyxis aculeata* and other protozoa. Among the *Arcella* he was able to demonstrate that fusion will take place between an individual and a fragment of a closely related specimen and that this tendency to fuse may be prolonged indefinitely by keeping two lines of a clone under identical environmental conditions. LOOPER (1928) showed that in *Actinophyrs sol* temporary colonies were often formed by fusion of individuals. According to observations made by HOWLAND (1928), *Actinosphaerium eichornii* will reincorporate severed axopodia of its own body or that of a different

individual. BURCH (1930) in dealing with *Arcella vulgaris* and *A. rotundata* took advantage of this phenomenon of fusion of severed fragments of protoplasm in an effort to determine its effect upon the division rate. OKADA (1930) in his work with *Pelomyxa*, *Difflugia* and *Actinosphaerium* demonstrated by a process of staining that when a fragment of protoplasm was appropriated by fusion, undifferentiated plasma resulted. Two actinosphaeria could easily be induced to fuse while separate fragments would not fuse. Two pelomyxae, by means of special technique, as well as two fragments from an individual would fuse and the protoplasm mix, while in the case of two individuals of different types there was fusion but no mixing of protoplasm. Among *Difflugia* fusion was observed where the fragment was from its own cell body but if from another individual the result was negative.

It was by way of some of the aforementioned experiments, especially those dealing with *Arcella* and *Difflugia*, that the present problem suggested itself. REYNOLDS (1924) page 136, infers that the phenomenon of cytoplasmic fusion probably involves the molecular structure or ionization of participating masses of protoplasm, for he states: "It seems most reasonable to think of it (the failure to fuse under certain conditions) as being due to a difference in molecular structure, or ionization; at least the sudden violent shattering reaction is suggestive of an electrical phenomenon." In order to test this hypothesis experiments were devised in which the fusion phenomenon could be studied under the influence of an electric current as well as under the influence of different hydrogen-ion concentrations. If for instance, it could be shown that through the agency of the electric current, or the hydrogen-ion, control to any degree could be made manifest, then a better understanding of the principles involved would have been brought about.

I wish here to thank Prof. B. D. REYNOLDS, who proposed this problem, for aid and many valuable suggestions and for the kindly interest he has shown in my work.

The organism.

The following observations have been made on Arcella, a freshwater Rhizopod. According to the description give by LEIDY (1879), PENARD (1902) and DE FLANDRE (1928), it belongs to the species *discoides* EHRENBERG. In this species the shell is nearly circular, having a diameter of 70 to 210 micra (the average for those used in present experiments being about 80 micra); the dome is low;

the color is pale, being almost transparent in a young individual. The contractile vesicles may number as many as twelve. Ordinarily there are two nuclei present. The mouth opening is large in comparison to that of some of the other species; which, in part perhaps, accounts for the fact that pseudopods are liberally extended. These features, especially flatness, transparency of shell, large size and lengthy pseudodods make this species favorable for this type of experimentation. The original animals used in these experiments were collected from a pond of fresh, running water near the University of Virginia. A jar of the water containing some leaves and pond ooze was allowed to stand and settle for a day or so, after which time the material was examined and the animals isolated and placed in Petri dishes containing a wheat culture medium.

Methods.

A wheat medium was found to be very well adapted for culturing the arcellae. This was prepared by bringing 300 c. c. of distilled water to a boil and introducing fifteen selected grains of wheat, after which, the material was set aside to cool. Twenty-four hours were allowed to elapse and all except five grains were removed. Twenty-four more hours were allowed before using for culturing purposes. During the 48 hours sufficient bacteria had accumulated to serve as food material. Fresh material was prepared frequently, since there was a tendency for an over-accumulation of bacteria, a fact which made the material undesirable to work with.

A mass culture from the original stock was kept on hand at all times, and all cultures were protected from direct sunlight and maintained at a temperature around 20 degres C.

In order to obtain a supply of suitable material for these observations, media of the desired p_H were prepared by the addition of dilute tartaric acid for the acid cultures and of dilute sodium hydroxide for the alkaline cultures. The colorimetric method was used in making the determinations, making it possible to record variations as large as 0.2 p_H with accuracy. These prepared culture media were kept in small 40 c. c. glass containers, each supplied with a hard rubber stopper and a small pipette. Several drops of medium of the desired p_H was placed in each concavity of a slide, which had previously been sterilized, and a single animal introduced. These were covered and placed in shallow moist-chambers to prevent undue evaporation. Time was then allowed for several generations (never over 20) to develop before using them.

The difficulty arising at this point, with reference to the culture media centers around the maintenance in constancy of the p_H of these solutions, since no buffers were employed. Under normal laboratory conditions the p_H of the media remained at approximately 6.5. If, by the addition of an acid or an alkali, the p_H of the solution was increased or decreased, there was a subsequent shift, with time, in the hydrogen-ion concentration of the medium, tending to restore the p_H to 6.5. This shift took place more rapidily in solutions which had been made more strongly acid or alkaline than it did in solutions adjusted within a narrower range of hydrogen-ion concentration. This condition was overcome in so far as it was reasonably possible by determining the proper concentrations each time before changing to fresh culture media. This change was made daily and with as much regularity as to time of day as was possible. When removing the medium from a concavity advantage was taken of the fact that arcellae attach themselves rather firmly to the slide and by inverting the slide rapidily with a sudden jerk removal was accomplished without loss of organisms.

In Part II of this experiment all the organisms used were cultured in wheat medium, no account being taken of the p_H. In all other respects identical technique was used, except that fresh culture medium was usually added to the cultures, instead of being changed.

Observations were made on specimens under normal conditions, as controls, before subjecting other individuals from the same culture to the influence of the electric current.

Apparatus and technique.

A compound microscope equipped with a 16 m. m. objective and a 15 eyepiece was used in making all operations and observations. With the aid of a camera lucida, sketches, distances, and sizes pertaining to the organism and fragment were obtained. By means of glass needles pseudopods were severed and the organisms placed in position. Ordinary dry cell batteries connected in series supplied the current. The voltage was regulated by means of a sliding contact tube rheostat having an approximate resistance of 920 ohms and a capacity of 0.6 megohms; (a swing of one division of the scale being equal to about .3 microampere). The current readings are given in terms of microamperes. Electrodes of platinum were employed in these experiments by fastening them to the ends of the insulated copper lead wires, the latter being firmly clamped to the microscope stage. The mode of construction allowed easy adjustment of the

electrodes in reference to the position of the organism. In order that potential differences of the electrodes might be eliminated as far as possible, compensating currents were run through them at regular intervals.

The organisms to be used were transferred from the desired culture by means of a small capillary pipette to a clean slide containing several drops of a like medium. Usually three individuals were placed in the same concavity. One was selected from which pseudopods were severed, then removed from the field. A second one was selected and placed near the severed fragment, the distance ranging between 10 and 150 micra, any remaining organisms having been previously removed. Observations of various facts were then made and tabulated.

Part I.

Reappropriation of cytoplasmic fragments: in different concentrations of hydrogen-ions.

In all, over 1000 observations have been considered in obtaining the results shown in Tables I and II. Table I indicates the average reactions of ARCELLA with respect to reappropriation and fusion when cultured in media of varying H-ion concentration. The facts to be observed are; (1) the dimensions of the fragment; (2) time required for organism to make contact with fragment; (3) the number of failures to make contact; (4) time required for organism to accomplish complete fusion after making contact; (5) the number of failures to fuse, which includes the number of failures to make contact; (6) the distance separating organism and fragment; and (7) the number of observations made. Tabulations are stated in terms of minutes and micra.

It is quite necessary when considering these results that account be taken of the facts that uniformity in the dimensions of the fragment and the distance separating fragment and organism were impossible, which largely accounts for the failure in every instance to show a gradual incline or decline in the rate, as the case may be.

Optimum conditions for cytoplasmic fusion appear to exist among those organisms cultured at H-ion concentrations between 6.2 and 7.6. Within this range the rate of growth and development were most rapid and very little difference, if any, was noticed. However, slight difficulties were encountered in cultures of p_H 5.0 and p_H 8.0 and more time had to be allowed for the organisms to multiply to sufficient numbers to constitute a desirable culture.

Table 1.

pH of media	Average dimensions of fragments in micra	Average number of minutes required to make contact	Average percentage of failures to make contact	Average number of minutes required to complete fusion after contact	Average percentage of failures to fuse	Average distance separating organism and fragment in micra	Number of observations
4.6	27.0 × 18.0	2.8	.41	1.2	.49	54.0	38
5.0	29.3 × 16.6	2.12	.11	1.2	.30	64.6	60
5.6	28.4 × 14.3	1.8	.15	.97	.28	57.2	70
6.2	17.5 × 10.4	1.5	.00	.43	.08	43.0	30
6.6	24.5 × 12.7	1.8	.02	.72	.10	46.4	50
7.0	27.0 × 14.0	1.9	.04	.80	.09	48.5	26
7.4	36.0 × 17.0	1.42	.00	1.1	.00	34.4	40
7.6	24.0 × 13.0	1.03	.00	1.25	.00	26.4	25
8.0	26.0 × 13.0	2.1	.11	.93	.16	42.4	45
8.4	24.0 × 13.0	2.65	.06	.91	.10	48.4	35

Table 2.

Amount of current in microamperes	Average dimensions of fragments in micra	Average number of minutes required to make contact	Average percentage of failures to make contact	Average number of minutes required to complete fusion after contact	Average percentage of failures to fuse	Average distance separating organism and fragment in micra	Number of observations
.3	28.0 × 13.0	1.7	.23	.56	.35	58.8	25
.6	29.0 × 14.0	2.0	.36	.80	.51	59.6	45
1.2	28.0 × 16.0	2.2	.16	.62	.16	69.0	25
1.5	31.0 × 15.0	2.2	.30	.81	.47	54.6	50
2.1	25.0 × 16.0	2.2	.26	1.00	.40	56.0	25
Controls:	27.0 × 14.7	1.5	.06	.47	.18	52.00	50

Likewise, the contact rate appears to have been somewhat retarded; the number of failures to make contact also seemed to have increased and the same may be said of the number failing to fuse. This same general tendency appeared to prevail at higher and lower concentrations, i. e. at p_H 4.6 and p_H 8.4. The difficulties encountered in culturing at these concentrations were very pronounced. All attempts to culture ARCELLA at concentrations of p_H 4.4 and p_H 8.6 were unsuccessful.

Discussion of Part I.

One will note that organisms cultured in media of increasing acid and alkali concentrations tend to show an increasing dilatory response, signified by the time required to make contact and the number of failures to do so.

An explanation, at least in part, of what occurs here seems to lie in the change produced in protoplasmic viscosity brought on by the application of acids and bases. There are diverse opinions as to whether an acid causes an increase or a decrease in viscosity, and same may be said of an alkali; but, according to HEILBRUNN (1928), there is general agreement that acids at certain concentrations do produce coagulation. The same authority states that the liquifying action of acids, when it does occur, is perhaps due to the fat solvent action of carbonic acid, or that it may possibly be due to a direct effect of the H-ions in increasing the electric charge at the surface of the protoplasmic granule. JACOBS (1920) in his treatment of various protozoa with carbonic acid found a decreased viscosity in the protoplasm. The observations of DARWIN, C. (1875) and BOKORNY (1888) indicate coagulation of protoplasm when exposed to dilute solutions of alkalies. GREELEY (1904), however, noted that the protoplasm of PARAMECIUM was liquified by anions, or negatively charged ions and coagulated by cations, or positively charged ions.

Whatever the effects of acids and bases upon the viscosity of the protoplasm in ARCELLA, whether a decrease or an increase, the effects upon the rate of contact and fusion appear to be very similar, that of retardation. In no case can it be said that accleration of the rate has been produced by increasing or decreasing the H-ion concentrations.

Part II.

Reappropriation of cytoplasmic fragments: under the influence of the electric current.

As previously stated, organisms for the following experiments were closely related and cultured in ordinary wheat medium. The technique, in general, was indentical with that used in Part I, except that after pseudopods had been severed and the organism placed in position the desired amount of current was gently applied and the observations recorded in Table II were made.

Table II shows the average results of the same observational facts considered in Table I. In so far as rates are concerned very similar responses occured under the influence of current as resulted from the effects of the H-ion; i. e. a general tendency to decrease as the amount of current increased.

If the averages for the controls are considered, and these represent the rates occuring under normal conditions, it becomes quite evident that a checking factor soon present itself upon the appli-

cation of the electric current. The significant point here, perhaps, is seen in the distribution of fairly uniform effects. Here, as in the case of the H-ion concentration, there is no evidence suggesting acceleration of the rates but only a retarding effect.

Discussion of Part II.

Such a resemblance in results as is shown in Tables I and II at once suggests a possibility of similar effects caused by the H-ion and the electric current upon the organism and fragment. This has been found true. Many experiments have been made in the past to determine the direct effect of the electric current upon various protozoa. Among those who have worked in the field are GREELEY (1904), KÜHNE and VERWORN (1889), PEARL (1900) and others. GREELEY, in his work on *Paramecium*, found that when this animal was subjected to a weak, constant current either a contraction of the whole cell into a dense, opaque mass of protoplasm occured, or else, the cell contents were liquified which ultimately caused the cell to burst, depending upon whether the cell was in the region of the anode or the cathode. Also, that a microscopical examination revealed the structural changes produced to be identical with those produced by electrolytes. In other words, he states, that the coagulating reaction of protoplasm occuring about the anode and on the anodal side of the cell is equivalent to that brought about by the cations in weak solutions; while the liquifying reaction taking place near the cathode corresponds to the effect of anions in weak solutions. BAYLISS (1920) observed that a weak and proper amount of electric current caused Brownian movement in the protoplasm of *Amoeba* to cease almost instantly as if it had been frozen. Movement was resumed, however, when the current was stopped. HEILBRUNN (1928) thinks that from our knowledge of the effects on Brownian movement it becomes apparent that the electric current can produce a reversible gelation or coagulation of the protoplasm. It will be recalled that a like condition evidently took place under the effects of the H-ion.

General discussion.

Previous mention has been made of the fact that reappropriation by fusion of severed fragments is the usual course among *Arcella*. Furthermore, an organism will fuse with fragments severed from closely related individuals. REYNOLDS (1924) observed in *A. polypora* a shattering of the severed fragment upon contact with the cell

body in cases where the individual organisms concerned were too distantly related and not cultured in the same receptacle. This shattering has been observed on only three different occasions during the course of these present experiments, due, perhaps to the fact that only closely related individuals have been used.

Briefly, it may be stated that workers in this field have shown that *Arcella* will do one of four things with respect to a fragment from another individual of the same species; (1) straightway make contact and fuse, (2) produce a shattering of the involved protoplasm upon making contact, (3) make contact without fusing, (4) treat it as mechanical obstruction

Under the effects of the electric current there are still other types of responses to be added; (I) the organism will approach and make contact without fusing, but instead of directing itself away, as normally happens, it remains within close proximity to the fragment, either apparently motionless or sauntering, until after the current has been removed. Normal movement may be resumed within 2 to 30 minutes, depending upon the strength and duration of the current. A second, third, or even a fourth contact may be made and fusion accomplished. (2) Initial movement of the organism relative to the fragment exhibits no visible evidence of attraction. This movement may be in an opposite direction or it may be within close proximity to the fragment until a change or a sudden reversal of direction takes place, and then the organism will straigthway make contact and fuse. When a fragment is near the center of the field of force the reaction between the cell-body and it is not influenced by the position of the former; i. e. it makes no difference whether the cell-body is between the fragment and one of the poles, or to one side in any intermediate position.

Arcella shows a tendency to move in the direction of the anode when subjected to the electric current. This is not always the case, however, as one out of every five or six moves toward the cathode. When the current is first applied definite pseudopodial formation is in the direction of the cathode, but after 30 seconds to a minute has elapsed there is a more uniform distribution of pseudopods. Greeley (1904) states, "It is interesting to note also that in the protozoa a movement of the protoplasmic particles within the cell toward the anode has been observed."

From the results shown in these experiments one is inclined to favor the idea that cytoplasmic reappropriation is wholly beyond the control of H-ion concentration or the electric current. However,

there yet remains a vague possibility that with a reduced amount of current (less than .3 microamperes) evidence of some electrical control might be encountered. There is likewise a possibility that any satisfactory explanation of this phenomenon would involve more than a single motivating factor.

Summary and conclusions.

1. When cultured in media of varying H-ion concentrations *Arcella discoides* shows no tendency toward acceleration of contact and fusion rates.

2. A tendency toward retardation in contact and fusion rates is produced when cultured in media having a p_H greater than 5.0 or less than 7.6.

3. The rate of multiplication is retarded in cultures having a p_H greater than 5.0 or less than 7.6.

4. Culture media having a p_H of 4.4 or 8.6 are not suitable for culturing *A. dicoides*.

5. Effects produced by the electric current upon the contact and fusion rates are very similar to those produced by the H-ion concentration and suggest a related protoplasmic reaction.

6. Cytoplasmic reappropriation is not hastened by the electric current.

7. When subjected to the electric current *A. discoides* usually moves in the direction of the anode.

8. Arcellae may become motionless under the effects of the electric current, but usually recover within 2 to 30 minutes, depending upon the strength and duration of the current.

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