AN EXPERIMENTAL ANALYSIS OF THE FACTORS
CONCERNED IN DIVISION IN AMEBA

by

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^{now}
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AN EXPERIMENTAL ANALYSIS OF THE FACTORS
CONCERNED IN DIVISION IN AMEBA.

The main question of this research was; What are the factors which lead to division in ameba ? Paramecia and Stylonychia divide at fairly regular intervals. That is a paramecium will divide to form two daughter cells and these two paramecia then divide into two each at the same time. The resulting four paramecia then divide again all at about the same time (fig.1).

On the other hand the individuals of collateral lines from the same original ameba are known to divide very irregularly. Thus for example, the original ameba may divide within thirty-six hours after isolation or it may go as long as ten days without division. Moreover when it does divide into two, one of these amebas is very likely to divide the next day and the other may perhaps go for three days before it undergoes division. Thus we have "fast" lines and "slow" lines of amebas originating from the same individuals. The decided difference between amebas and ciliates in regularity of fission rate in collateral lines originating from the same individual, suggests that an experimental analysis of the factors leading to division (fig.2) should be possible. One of the first things to do in order to shed light upon the matter, was to study normal division in ameba. After this was done, the following questions were considered; Is it increase in size due to growth that causes an ameba to divide ? If so, can division be avoided if increase in size be prevented by

starvation ? Can division be prevented by cutting off enough cytoplasm at intervals to compensate for growth ? What are the effects if any, on such an ameba, in general appearance, number and size of crystals, size and appearance of nucleus, etc. ? Does the ameba grow a larger amount of protoplasm when dividing normally than when a certain amount is cut off regularly ? What effect is produced by cutting off two-thirds or so of the protoplasm of a large ameba; does the nucleus become smaller in size as a result of such cutting ? Would such reduction in size result in change of shape of the nucleus ? These questions and many others suggested themselves in working out this problem and for some of them at least, a fairly definite answer can now be given as a result of experiments directed on this general question.

HISTORICAL REVIEW.

In order to understand the setting which these experiments have in connection with other observations and experiments which have been made on closely related problems in amebas and other Protozoa, a short historical account may not be out of order here. Such an account will also permit a more connected exposition of the experiments and observations given below, in that extended discussion of related work done by other investigators will not then be necessary. The following list of authors includes most of the workers whose work bears more or less directly on the subject of the experiments in this thesis;

LIST OF AUTHORS CITED IN HISTORICAL REVIEW.

Roesel-----	1758.
Carter-----	1863.
Wallich-----	1863.
Hussbaum-----	1885.
Gruber-----	1885.
Verworn-----	1889.
Hofer-----	1890.
Balbiani-----	1891.
Lillie-----	1897.
Penard-----	1899.
Morgan-----	1901.
Stole-----	1909.
Schaeffer-----	1916.
Willis-----	1916.
Carter, L.-----	1919.
Taylor-----	1919.
Botsford-----	1922.
Reynolds-----	1923.
Taylor-----	1923.
Howland-----	1924.
Hartmann-----	1924.
Sokoloff-----	1924.

In passing, it is probably of interest to note the definite waves of interest which this subject has excited in the minds of biologists. Thus between the earliest work of Roesel, and that of Carter and Wallich, a lapse of one hundred and three years appears, in which almost nothing new was discovered about amebas. Between the time of Carter and Wallich, and that of Nussbaum and the others of that period, there is a lapse of twenty-two years in which investigation in this field slows down. Similar lack of interest was shown in the decade from 1901 to 1910. From 1916 onward investigations have been underway continuously.

All historical accounts of ameba must begin with Roesel, who discovered the first ameba. He was also the first to experiment upon the ameba. For example, he was the first to employ any kind of an instrument to further his knowledge of structure and internal mechanisms among protozoans. He used a finely pointed feather to draw the ameba upon the edge of the dish and hold it "fast" and also to cut its ectoplasm. He discovered the more conspicuous properties of the ectoplasm and the endoplasm.

In 1863 Carter studied the ameba and described the parts of the ameba in detail. The outer covering, he calls "pellicula" (ectosarc or diaphne); the inner substance, he calls endosarc or sarcode (following Dujardin, 1841). Then, with much precision he describes granules of various sorts. First he speaks of granules which are rounded and dark in outline, being probably of organic nature. Besides the rounded ones he finds

ellipsoids, angular crystalloids, and others which are octahedral in form. The latter are of a pinkish color and because of their dissolving in nitric acid Carter suggests they may be a form of oxalate of lime. The presence of these granules was noticed in old as well as young animals and the size and number is not in any way related to the age and size of the ameba. (Observations and experiments recorded in this paper do not confirm the latter statements) (~~see page~~) Also, Carter says, that as the animal increases in size and advances in age the crystals change from a rounded to a crystalloid and angular form, the largest (octahedral in shape) measured twelve microns. These crystals are found not only in amebas, but occur in rhizopods generally. Those found in Myxagastres are small, round, colorless, compound, crystalloid masses. Their ready effervescence with nitric acid, Carter thinks, indicates that they are carbonate of lime. Carter also describes another sort of granule designating it as a fat globule which, he says, occurs most frequently in "robust" amebas.

G.C. Wallich in 1863 devoted his time to studying the nucleus and the crystals. He was the first to discover that the shape of the nucleus in Ameba proteus was discoidal. He described it as of a yellow color and located on one side of a transparent capsule, which forms a pellucid halo around the nucleus. Regarding the crystals, he noted that they were of rhombohedral shape and found in specimens in full vigor of growth. The length varies from thirteen microns to five and five-tenths microns. The breadth from one and two tenths

microns to five microns. The most obtuse angle measured one hundred and forty degrees. All traces of the crystals are lost when HCl is added. This reaction together with the rhombohedral form leads Wallich to conclude that the crystals are carbonate of lime. In addition to these crystalloids Wallich finds two kinds of spheres, the first of which he describes as not discoidal and varying in diameter from seven microns to fifteen microns. In these spheres no cell wall was evident and the presence of a nucleus doubted. The second kind of a crystal had no nucleus and was devoid of a cell wall. The spheres measured $\frac{1}{2000}$ inches ~~microns~~ to $\frac{1}{16500}$ inches ~~microns~~ in diameter. Wallich thinks these spheres exercised the function of ovules in some rhizopods. Both sorts of spheres are distributed equacly in endosarc and share equally in endocyclosis.

A. Gruber's work in 1885 on the cutting and ensuing regeneration in Stentor marks the beginning of modern experimental work involving the cutting of living protozoa. In his work he cut stentors into two pieces and concluded that the anterior piece regenerates as rapidly as the posterior, and that any piece is capable of regeneration as long as it has a nucleus. Likewise the part of the animal containing the largest pieces of the nucleus regenerate most rapidly and pieces completely devoid of nuclear material fail to regenerate. Gruber also noted that pieces of stentor might be grafted together if the operation was begun immediately after separation of the pieces. Somewhat similar observations were

made by Penard (1899) who worked on Diffugia and Reynolds (1923) who worked on the same animal. In regard to the nucleus Gruber believed it to be the "species maintaining character" of the cell, and of the highest significance in heredity and fertilization. The presence or absence of food in "operated" organisms is without influence on division. Probably the seat of diffuse nervous potentialities is chiefly in the cortex of stentor.

Nussbaum worked at the same time that Gruber did and upon the same general subject, but he used Oxytricha for his experiments. He found that it made no difference whether the oxytrichas were cut in two longitudinally or transversely: the parts regenerated completely and produced two new individuals. Irregularly shaped pieces also regenerated into whole animals, but the nucleus seems to be necessary. Regenerated oxytricha multiply normally by division and the offspring appear normal in every respect. Because of experimental division, no influence on the "modus" of subsequent reproduction processes could be demonstrated.

Verworn in 1889 confirmed (using rhizopods) the results of Nussbaum and Gruber. Verworn concluded that enucleated pieces of rhizopods are more sluggish and die after a time while nucleated pieces remain alive; and that the nucleus is in no way the psychic center of the cell, but that each piece of protoplasm has its own "Psyche".

Hofer in 1890, quotes Gruber as saying that enucleated pieces have the same power to move as nucleated pieces (or

normal individuals) and quotes Balbiani as saying that the movement of cilia persists after cutting, but gradually becomes feebler until the death of the piece. Hofer himself experimented with nucleated and enucleated parts of ameba in an attempt to analyze the effect of the presence and the absence of the nucleus on digestion of food, water diffusion processes, and the formation of a contractile vacuole. In generalizing his results one may say that he found the presence of a nucleus necessary to carry on the fundamental body processes. In detail his results show; (1) that the nucleus makes the cut piece more resistant to water diffusion processes, in that a new ectoplasm can be more easily built; (2) when ~~only~~ a little protoplasm was cut off with the nucleus the piece died within one and one half hours*;

*This result will be discussed further on page 33 ff.

ameba in the process of engulfing a food organism was cut, the nucleated piece succeeded in killing the organism, but the enucleated piece allowed the captured organism to go free; (4) the nucleus influences the digestive power of protoplasm in so far as it is able to produce digestive secretions by cooperating with the protoplasm; (5) a new contractile vacuole arises in the enucleated part, but the ~~rythm~~ slows down; (6) it is quite impossible to keep an ameba in a medium lacking oxygen over a few hours; (7) an enucleated piece may live as long as fourteen days. Balbiani worked with Frontonia (Erhenberg) (Cyrtostomum Stein). He found that pieces containing a nucleus regenerate in from twenty-four to

to forty-eight hours, and that the regenerated individuals are just like the originals excepting perhaps a little smaller. However, this did not hold true for enucleated pieces. He also concluded that the nucleus secretes the cuticular membrane and that the nucleus is the "trophic" center of the cell. Although the nucleus is a most necessary component of the cell, the following observations were made on enucleated pieces which show that certain functions may proceed with the nucleus absent: (1) movement of cilia; (2) pulsation of contractile vacuole; (3) prehension and ingestion of food elements; (4) and Balbiani states with some doubt that the processes of assimilation and digestion are interfered with by the absence of the nucleus. Concerning the length of nucleated and enucleated fragments, Balbiani found the former to live almost a month (which amount of time they consumed in regeneration) and the latter lived from two to eight days. In some subsequent work on Prorodon, Balbiani was able to verify the results he obtained from his work on Frontonia namely: (1) that a nucleus or a part of a nucleus is necessary for regeneration of separate parts of the body; (2) the absence of a nucleus affects neither ciliary movements nor pulsations of the contractile vacuole, only slowing them down until death; (3) the defecation and probably the absorption of alimentary contents continues in spite of the absence of the nucleus; (4) death is always a result of the absence of this cellular element; (5) in three or four hours the nucleated fragments become complete individuals and a new contractile

is formed.

Frank R. Lillie in 1896 offered an explanation as to why very small parts of animals failed to develop. In the first place, perhaps the organization is deficient; that is the whole organization of the species cannot be included in so small a space. Secondly he suggests that perhaps so small a volume of matter cannot fulfill the mechanical conditions consequent on cell division, formation of a segmentation cavity ~~in~~ (in metazoans), invagination, etc., owing perhaps to the increased surface tension, not to mention other conceivable alterations ~~on the~~ ~~extrinsic~~ factors of development. Thirdly, Lillie suggests that maybe such a small part is not able to set free a sufficient amount of energy which would be required for its transformation into a gastrula or a planula. However, Lillie himself immediately ^{discards} ~~discredits~~ the third alternative by saying that "masses may continue to live for a considerable period of time and display an amount of energy in atypical form changes and rapidly movement, which would suffice to produce the phenomena of normal development, did not other factors included in the first or second of the above alternative prevent". Moreover, it is well known that ^{an} exceedingly minute protoplasmic body very much smaller than one-eighth the echinoderm ovum may produce a relatively enormous amount of energy, e.g., bacteria and spermatozoa. Finally, we do not know how much of developmental energy is of intrinsic and how much of extrinsic origin. Surface tension and other extrinsic factors of development of metazoa have

not been shown to exercise a controlling influence in the regeneration of such an organism. Ciliate infusoria offer conditions for decision between these two alternatives. If it should be shown that a nucleated portion below a certain minimal size is incapable of regeneration, the first alternative would receive support. If on the other hand the smallest nucleated fragments of the body are capable of regeneration with restoration of the normal form, the first hypothesis would fall and the second tend to be established. In his attempt to establish one of these alternatives experimentally, Lillie took a number of Stentor polymorphus and Stentor coeruleus, put them in a bottle and shook them well thus getting many pieces of varying shapes and sizes, some with nuclei and some without. And from numerous experiments involving many hundreds of S. polymorphus it was found that the smallest parts capable of regeneration possessed the volume of a sphere of about eighty microns in diameter. A smaller number of experiments on S. coeruleus yielded results almost identical with these. By far the greater number of nucleated parts which possessed a spherical diameter of less than one hundred microns did not regenerate.

In conclusion, Lillie summarizes the results of all work on the merotomy of protozoa up to the time of the publication of his paper, as follows: (1) cytoplasm without the nucleus is incapable of regeneration (Nussbaum, Gruber, Verworn, Balbiani, and others) (Gruber has shown that if a stentor in the process of fission be transversally divided so that the poster-

ior part receives no nucleus, this part is nevertheless able to regenerate); (2) the nucleus without cytoplasm is incapable of regeneration (Verworn and Balbiani); (3) portions of the body consisting of a nucleus and cytoplasm are capable of regeneration. To this I (Lillie) must add: provided that the amount of cytoplasm exceeds a certain minimal volume. (Which in the case of Stentor at any rate, is quite considerable.)

This amounts to a demonstration of Verworn's view that regeneration in Protozoa is due to the "reciprocal interaction of nucleus and cytoplasm". Organization resides in the cytoplasm as well as in the nucleus. How, otherwise, are we to explain the fact that a difference in volume between two spheres, one of eighty microns, the other of seventy microns respectively determines the occurrence of regeneration? There is probably for each species of animal a minimal mass of definite size consisting of nucleus and cytoplasm within which the organization of the species can just find its latent expression. This is the minimal organization mass.

In Protozoa this is the smallest part capable of complete regeneration. Q

In 1901, Morgan performed his experiment on Stentor. He did not deal particularly with nucleated and enucleated parts, but worked with regenerations of the organs in general.

Stolk came out in 1909 with the statement that enucleated pieces can live thirty days and that enucleated plasma can pick up food and eat it. He also believed that enucleated pieces can digest food, ^{and} but not assimilate it.

Penard (1899) made some very interesting observations on Diffflugia pyriformis and Diffflugia lobes (Penard). His experiments consisted in cutting pseudopods from a diffflugia and noting the reaction of the mother pseudopod. He found that these fragmentary pseudopods are rejoined to the mother, it making no difference where the pieces were placed, so long as they were within a distance equalling one or two times the diameter of the mother diffflugia. The mother changes the viscosity of the surface contiguous to the piece cut off so as to enable the piece to flow back readily into the mother diffflugia. Tests were also made to see how the diffflugia would react to inorganic substances such as stone, a piece of diffflugia shell apparently of the same species, and also to crustacean eggs, but on no occasion were these substances rejoined to the animal. In one of his experiments, Penard secured some unique results. He, one day found a Diffflugia lobes in the process of division and when the operation was completed, he separated the individuals and immediately cut a pseudopod from one (a), and placed it near the other (b) and in a short time it was rejoined to the other ameba (a). This was repeated several times the next day for verification. The same result could not be brought about by feeding pieces of Diffflugia pyriformis to Diffflugia lobes.

In 1916 Willis published a paper on the "Influence of the nucleus on the behavior of ameba." He found that those pieces having a nucleus behaved like normal individuals. Those without behaved differently. In spite of this he held the nucleus had no direct influence on protoplasmic movement. Hofer's results were similar to those of Gruber in that he found that movements in parts with nuclei are much like those in normal individuals. For a period of from fifteen to thirty minutes after experimental division parts without a nucleus behave like normal specimens, but later these differ in the rate of locomotion, in regularity of movement, and in number and length of pseudopods. Willis discusses Hofer's statement that these differences might be due to injury received in operation, but agreed with Hofer that this is possibly untrue because both pieces have received the same treatment. Willis therefore believes that the cause must be found in the nucleus. The influence of the nucleus may be direct or indirect, that is that behavior may be due to an impairing of the ^{fundamental} elementary functions, (digestion, respiration, and excretion) controlled by the nucleus, but Hofer found the process of digestion in the absence of the nucleus continues for several days after division; respiration takes place in the absence of the nucleus; and that the excretory function in enucleated continues until death. He therefore comes to the conclusion that the nucleus secretes a chemical substance and that behavior is controlled through this. He thinks a certain amount of this substance is stored up in

the different parts of the protoplasm and that the normal movement of the enucleated segments for fifteen to thirty minutes after operation is due to the influence of the substance thus stored. Hofer further concludes since movement occurs in parts without a nucleus, the cytoplasm has the power of movement. But since the movement in these parts is more irregular and haphazard than it is in nucleated parts, he holds that the nucleus must have a regulatory function. In other words he thinks the nucleus serves as a regulatory centrum for behavior. Verworn agrees with Hofer in holding that there is a difference in the behavior of the parts of ameba with a nucleus and parts without a nucleus, but does not agree with him in the conclusion that the nucleus exerts a direct influence on movement; that is he does not think that the nucleus is a regularity centrum for movement. In discussing these conclusions of Hofer and Verworn, Willis says, that judging from the results of his own experiments it is clear that there are distinct differences in the behavior of parts of ameba which contain a nucleus and parts which do not contain a nucleus. Difference in such parts have been observed in the character of movement, in the accuracy of orientation in light, and in the rate of locomotion. It is clear also that these differences are traceable to the nucleus. In summarizing, Willis says that⁽¹⁾ locomotion in segments of ameba with a nucleus is of the same general character as movement in normal amebas.⁽²⁾ In a horizontal beam of light, normal specimens direct their locomotion from the source of stimulation,

i.e. they orient and are negative. Parts containing a nucleus respond in the same manner; those without a nucleus however do not orient. (3) The rate of locomotion varies greatly in different individuals, large specimens move more rapidly than small specimens, the rate of locomotion bearing a definite ratio to the size of the specimen. The rate of locomotion in nucleated specimens bears essentially the same ratio to their size as it does in normal individuals. Segments without a nucleus show very little locomotion and this is always very slow and irregular. (4) The size of the parts, ~~the contractile~~, and the position which the parts occupied in the intact specimens before division seemed to be in no way responsible for differences in the behavior of nucleated and enucleated parts of ameba. (5) The only other known respect—aside from those mentioned in which the two parts differ, concerns the nucleus. Consequently the differences in the behavior of these parts are in all probability in some way related to the nucleus. (6) The regulatory influence on behavior in ameba seems to be brought about by some sort of influence upon the attachment of the organism to the substratum.

Schaeffer published a paper in 1916 in which he describes A. proteus Pallas (Leidy) and divides it into three distinct species. I shall deal with this paper only incidentally at this point. Schaeffer found while carrying on some feeding experiments with common fresh water amebas that the individuals differed in the nature of the ectoplasm and the endoplasm and in their reaction to food substances. In order to better

enable him to study these differences he pedigreed these amebas and as a result he found that A. proteus Pallas (Leidy) consisted in reality of two species with definite characters which breed true during vegetative reproduction. The species proteus correctly described is not autonomic, as the work on breeding them has shown. There are two species that have discoid nuclei, one of which, the larger, possesses longitudinal folds or ridges on the pseudopods while the smaller does not. The best test of a species is in breeding it--each of the species described in this paper were pedigreed fifteen to twenty linear generations together with many collateral lines. The shape and size of the nucleus, crystals, excretion spheres, the color of the protoplasm, and the character of the streaming of the endoplasm remained uniform through the pedigree.

The remainder of the paper which deals with diagnosis of the above mentioned species will be discussed later.

Miss Lucy Carter (Sister Bernardine) made some observations in 1920 upon division in ameba and described her findings as follows. She actually observed the division of an ameba into two multinucleate amebas. In these multinucleate forms she was able to count as many as seventy nuclei and of necessity in individuals containing so many nuclei they must be very small. ~~In 1919 Sister~~

In 1919 Sister Monica Taylor published a paper in which she analyzed and described the processes which take place when a culture of amebas begins to recuperate after dying down in the spring. It is well known that cultures die down

occasionally and that soon after very many small amebas are found in the culture. Nuclear division is of two sorts resulting in binary fission and in sporulation. Arranged around the periphery of the nucleus is the chromatin, then in the center of the nucleus is the karyosome containing a ground substance and chromatin blocks. In the turning over of the nucleus which occurs in the streaming of the protoplasm, the chromatin, karyosome, and chromatic network become folded. A fold appears at the proximal and distal ends to make it dumbbell shaped. It is a common experience among cytologists that whenever an organism is developing rapidly, mitotic figures are sure to be forthcoming. Hence, it would seem that published figures of mitosis in A. proteus belong to the sporulation cycle of the life history. The following is Miss Carter's description of halving of the nucleus into two hemispheres. A patch of chromatin blocks divides each into two, the process gradually extends to the whole chromatin mass. Simultaneously the karyosome divides into two, each daughter chromosome assigning itself to one daughter set of chromatin blocks. The outer set of daughter products sloughs off gradually from the inner by increase of nuclear sap and by the formation of necessary additional areas of nuclear membrane and two daughter nuclei thus result. The lobed or folded nuclei are the result of interference in separating daughter nuclei.

In 1922, Miss Botsford published some interesting results regarding rate of division in her pedigreed cultures of

Ameba bigemma. She records; (1) daily divisions, (2) two divisions in one day, (3) three divisions in twenty-four hours, (4) no fission in from one to twelve days. "An average of the daily divisions in eight lines for a period of ninety days showed the rate to be slightly above one division in two days ... By averaging the number of divisions in a single line for 5 five day periods. A rhythmic character of the fission rate was apparent in the form of alternating periods of high and low productive activity; one low point to the next comprising about twenty days."

Reynolds in 1923 carried on a series of experiments with Diffugia which extended and confirmed those of Penard- and are identical with Penard's work in most respects.

Ho land, 1924, by means of microdissection shows that the ectoplasm of Ameba verrucosa is much firmer and more persistent than that of Ameba proteus or the other closely related forms.

In December, 1924, after I had begun the present series of experiments, M. Hartman published some interesting results which probably bear as directly on the problem at hand as any of the above mentioned papers. He kept stentors without dividing for a number of generations by successive excisions. He wished to answer the question whether the rejuvenating effect of division can be brought about in some other way than by division (regeneration). He worked on two species of ameba, Ameba proteus and Ameba polypodia. Of the two species A. polypodia proved to be much more suitable in that it was

much more resistant and its division rate was much more uniform. Hartman says that since he did not succeed in securing over eight regenerations (in fifteen days) in the first few months of experimenting (January to April 1923) with Ameba proteus, after numerous trials he carried on the experiments thereafter with A. polypteria, with which his results deal exclusively. Controls were carried along and the temperature was kept at 21 degrees C. As a rule division occurred every two days, sometimes one in three or more days or one every day. Irregularities which occurred were due in part to variations in food and differences in the dishes. The results from the amebas at hand contrast with the earlier results from Stentor in that the amebas are more difficult to work with, for one has to reckon with many more deaths in connections with the amputations. The operations were performed in such a way that only a small piece of indefinite size - one fourth or one third - was cut from the ameba before it reached normal division size. "Both pieces were retained in order to determine which contained the nucleus. A large number of accidents occurred and a great many excised amebas died. It often happened that the piece was hurt too much or was too small to regenerate, in spite of possessing a nucleus, so it is necessary for one to run large series of parallel experiments. At first most of them died and later many died. Hartman in two experiments only carried two amebas for a long periods. (1) April 23 to May 18, and another one, (2) May 9

to June 6, the first one being operated on twenty-one times, the other thirty-two times. During this time the controls divided respectively eleven and thirteen times. Operations were made, for the most part, daily, but sometimes intervals of from two to four days occurred. Hartman also "shows that nucleated amebas may be kept alive for thirty-two regenerations during forty-two days, while controls divided fifteen times". "One may assume also that they might have lived a longer time, indeed potentially ad infinitum, without the necessity of division".

Sokoloff published a large paper on regeneration in Protozoa in 1924. His main results are as follows; (1) the minimal organization mass is between $1/53$ and $1/69$ in Spirostomum ambiguum. In Dileptus, it is $1/70$ -- $1/75$, (2) the larger the piece the more rapid the process of regeneration, (3) pieces in which the nucleus is excessive in size, proportionately regenerate with more difficulty than those in which the nucleus and cytoplasm retain the normal proportions. Regeneration is delayed until excessive nuclear matter is resorbed. (4) The proper (normal) relation between ectoplasm and endoplasm must also be established before regeneration can proceed. (5) The nucleus is not necessary to rebuild the form of the body; the ectoplasm does that. The nucleus is necessary for life processes. (6) Hunger (2 or 3 days) first affects the structure of the nucleus (Bursaria). It does not markedly affect form regeneration. If hunger leads to deformation of body, no regeneration occurs. Calcium ions possess

a specific influence on regeneration of form. (8) Hydrogen ion concentration pH 8 is of no importance for Dileptus, but very deleterious for Bursaria

Sokoloff worked on Opalina, Dileptus, Spirostomum, Frontonia, Bursaria, and Gregarina. He gives an extensive historical account of regeneration experiments in Protozoa.

These are the main papers dealing with merotomy in Protozoa which have special bearing on the main question about which this research is concerned.

REPRODUCTION IN AMEBA PROTUS AND RELATED FORMS.

It is necessary to bear in mind the general reproductive processes in rhizopods(fresh water) in order to correctly interpret results obtained in experiments in regeneration in these forms. For that reason a short account of the main points in the reproduction of Ameba and closely related rhizopods follows.

Reproduction is of two kinds, sexual, and asexual. Among freshwater rhizopods however, sexual reproduction has not definitely been observed and described, although there is reason to believe that it may exist. Ordinary fission is the means of reproduction found in Ameba, Arcella, and Diffugia, representative members of this class of Protozoa. Just as an Ameba prepares for division, it forms itself into a hemisphere with very minute pseudopods all over the free surface. In a few moments, a thin clear line appears along the diameter of the ameba and in the course of the ensuing moments the line becomes clearer and clearer until the division is complete, and the two daughter amebas send out pseudopods preparatory to moving away. Previous to the appearance of any clear line the nucleus has divided into two parts by mitotic division. The process of binary fission in Arcella and Diffugia is very similar to this excepting in these two genera the animal is enclosed in a shell and it is interesting to note how the shell is divided. Arcella is an interesting kind of animal possessing a shell frequently furnished with spines. In some experiments Hegner (1919) performed he found the

minimum number of spines to be seven and maximum seventeen, while the mean was eleven. The animal crawls along the dish with the shell uppermost which makes it very hard to observe division or for that matter any processes which go on in the animal. When division is about to take place, the parent Arcella sends out enough cytoplasm from the mouth of the shell to secrete a new shell. After the new shell is completed, enough more cytoplasm flows into the new shell to make the division half and half and gradually the bridge of cytoplasm between the two individuals gets thinner and thinner until it breaks into two. The spines are probably produced by pseudopods laying down a shell around themselves. In Diffugia, we observe a process of shell formation which is similar and which was described by Jennings in 1920. "The shell consists of sand grains, embedded in a hard chitinous substance. As the parent creeps about in its daily life, it takes up sand grains and stores them in the interior part of its body. At reproduction the parent swells and projects from the mouth of its shell. This projecting mass takes a form similar to that of the parent. The sand grains within the parent body pass out into the projecting mass, come to its surface, and spread over it. They are embedded in a fluid secretion which now turns hard, so that they form a shell like that of the parent. The two shells are in contact at their mouths. Now the common mass of protoplasm divides into two, and the two individuals separate—one retaining the old shell, the

the other with the new one

It will then be seen that the main features in reproduction in Littlugia and Arcella are brought about by the same process of streaming of protoplasm and that regeneration phenomena in all three would necessarily be very similar.

DESCRIPTION OF AMOEBA PROTEUS (LEIDY).

Amoeba proteus consists of three "plasm's. One, a very thin outer covering--noticeable only by the fact that small granules found in the cultures are carried along by some sort of a membrane, possibly the plasma membrane. The middle layer is the ectoplasm, a viscous substance which lies underneath the outer thin layer. The word "ectoplasm" is used here to designate the more viscous layer of protoplasm, not merely clear protoplasm. Inside of the ectoplasm, occupying the whole of the inside of the amoeba is the endoplasm, which carries the cellular contents--nucleus, contractile vacuole, food vacuole, crystals, and spheres.

It is probably of interest here to tell something of the streaming of protoplasm, especially as regards the manufacture of new ectoplasm. "The most distinctive morphological characteristic of this species of amoeba is the possession of longitudinal grooves on the surface of the pseudopods when in locomotion." (These ridges were described by Leidy, 1879 and Penard 1902, as well as by Schaeffer 1916). Ordinarily when describing the formation of pseudopods, writers say that that it is an outpushing of "ectoplasm" (i.e. clear protoplasm) followed by a continual stream of endoplasm. The enlargement of pseudopod in length and diameter is gauged by the amount of endoplasm which flows into it. As thus stated, this process is comparatively simple and holds for the majority of amebas, but is somewhat modified in the case of A. proteus. As Schaeffer has observed; "There is not only one current in the

peritoplasm, flowing along the long axis of the pseudopods, but numerous small ecto- and endoplasmic currents flow at acute or right angles or parallel to the surface of the pseudopods. These side currents frequently flow out as a thin ridge along the side or top of a pseudopod of more or less even width..... A wave or ridge, once formed, usually does not disappear except at the posterior end where the ectoplasm is changed into endoplasm". In this ameba longitudinal depressions of ectoplasm occur at the base of a lateral wave, because the ectoplasm and adjacent endoplasm of the pseudopod or older wave along which a new ridge flows, do not melt away rapidly. "The endoplasm of a pseudopod may be conceived of as a single mass or stream flowing around and about numerous ridges^{of} ectoplasmic (plus endoplasmic) obstructions of greater or less extent".

The ectoplasm forms the outer layer and envelopes the endoplasm in the form of a cylinder, the posterior end being covered with ectoplasm and the anterior not. As the ectoplasm flows out at the anterior end of a pseudopod, it flows around the end of a pseudopod and continues its course posteriorly for a short distance. New ectoplasm is continually being manufactured at that point on the ameba where the straight sides of the cylinder of ectoplasm ^{fuse with} ~~the~~ the new ^{which} pseudopod is pushing out. Now, if we know the rate of locomotion of an ameba, its length and diameter, we can calculate how rapidly the ectoplasm is formed. At the cut surface, which is of considerable interest in connection with the

question of wound healing after an ameba has a piece cut from it. Let us assume that our ameba is of average size 600 microns long and 100 microns in diameter and that it moves at the rate of 600 microns per minute. The area of the cut surface would be 7,864 microns and the area of the cylinder disregarding the ends would be 188,496 square microns (or area formed per minute in locomotion). Thus by dividing 7,864 by 188,496 we find that it takes the ameba .041 minutes or 2.460 seconds to renew the ectoplasm on the cut surface if it formed at the same rate as in ordinary locomotion. There is no reason to suppose that in an emergency of this kind, the ameba might ^{not} be able to manufacture its ectoplasm much faster. This short time in which to repair the cut surface may explain the inactivity of cut pieces for a fraction of a minute after excision. These results show that the shock of excision with consequent healing of the wound could not possibly affect the reaction of the cut pieces for but a fraction of a minute.

The nucleus together with the cytoplasm is the life of the cell and as we have seen in the above historical account and as we shall see in the results I have obtained in my experiments, none of the fundamental reactions except locomotion are observed in enucleated amebas. The nucleus contains the chromatin which is the basis of heredity and the nucleus is that part of the cell that initiates cell division.

The contractile vacuole is a clear spherical body found usually in the posterior part of the animal. It contains a

clear liquid and when it reaches about the size of the nucleus, it bursts, the liquid escaping to the exterior through a small pore. As soon as this contractile vacuole has burst, another is seen to arise as a small collection of droplets growing gradually in size. Soon, these merge into one which grows until it approximates the size of its predecessor, when it bursts in similar fashion. It is thought that probably this vacuole serves as some sort of excretory organ for the ameba.

Food vacuoles are simple small "pockets" in the ameba which contain a recently captured food organism and a little water.

Concerning the crystals found in the endoplasm of A. proteus, not a great deal is known. They originate and increase in size in the endoplasm, never being dissolved or excreted so far as is known, though they are probably a waste product. They are found in all amebas, but differ in shape in different species and increase in number with the age of the ameba. In this species (A. proteus) the largest ones are between four and five microns long. Their shape is like that of two frustums of square pyramids placed base to base. Regarding the chemical composition of the crystals, only speculations can be made, the probability is that they consist of lime in combination with phosphorus.

Aside from the above described crystals found in A. proteus numerous spheres are found. They are several times the size of the largest crystals, but nothing is definitely known of their ^{origin} composition or fate.

MATERIALS AND METHODS.

In starting these experiments I isolated four individuals of Ameba proteus and kept daily records of their condition. In a few days two of these had divided several times and from these several were selected to be the parents of the pedigree-lines. The stock amebas were taken from cultures of ~~cultures of~~ amebas which had been kept in the laboratory continually by such culturing for nine years. The food animals that I used were Uronema marina (Dujardin) and Chilomonas paramecium (Ehrenberg). Quite frequently Paramecium caudatum was in the culture, but how much the amebas fed on it was not determined. The food organisms were raised in culture dishes on hay infusion.

The method of pedigreeing the amebas was as follows: Each individual ameba was kept in a small German watch glass about thirty millimeters in diameter which was placed on a small black solid watch glass with a square base. In order that the individual amebas could be designated one from the other, a piece of paper with its number written on ~~was placed~~ on was placed in the concavity of the black dish and could of course be seen readily through the German watch glass which contained the ameba. A very simple system of numbering was used. The parent ameba for instance was known as one--1 then the products of division were numbered 11 and 12 and the third generation 111 and 112 in the 11 line and 121 and 122 in the 12 line (See chart). These small black dishes, used as bases for the watch glasses were kept in a medium sized

culture dish with a little water over the bottom of the dish to prevent rapid evaporation. The dish was kept covered with plate glass and was kept out of direct sunlight. The day's operations began by washing the reserve watch glasses thoroughly in distilled water and turning them upside down on a piece of filter paper. It may not be out of place to state here that ^{Valuable} (sad and expensive) experience was gained in not having the dishes and instruments chemically clean. While the dishes were drying, the culture fluid was filtered through coarse filter paper that allows Chilomonas and Uronema to go through. The culture fluid was collected in a Syracuse watch glass. Various sorts of materials were used for filtering the culture with varying degrees of success. Some of these were coarse filter paper one thickness, filter paper two thicknesses; bolting cloth of the finest weave; fine long-cloth, and worn damask linen. When pure culture fluid free from Protozoa was used for starving experiments--absorbent cotton was saturated with distilled water and choked into the neck of the funnel. This prevented anything ^{solid} (visible in a compound microscope) from going through. For cursory examination of the contents of each watch glass, the Greenough-type binocular was used and the watch glass was placed upon the (mechanical) stage (the watch glass was rested on a slide), thus facilitating the moving of the watch glass without any danger of upsetting and spilling the contents. The contents of each little watch were examined daily. When the ameba was located in the watch glass, its condition was noted and recorded in a notebook kept for that specific purpose. This being done,

a new dish was prepared with food organisms and culture fluid, and the ameba removed from the old dish with a very fine pipet and placed in the new one. This dish was then placed on the binocular stage for examination, to make sure that the ameba had actually been placed in the dish and not lodged in a pipet. This process was repeated for every ameba and when excisions were to be made the high power of the binocular was used. For excision, instruments consisting of very fine glass needles were prepared by drawing glass rods out to hair-like rods over a gas flame. In order to obtain the finest sort of a flame a very small needle from an injecting apparatus was fitted into the end of a piece of rubber tubing. The cutting is done by a sawing movement on that piece of the ameba which is to be cut off.

In those experiments where the crystals were to be counted, a compound microscope with a number ^{6 C} ~~six~~ compensating micrometer eyepiece and an oil immersion lens was used. After an excision of a quarter or a half of an ameba was made, the piece was placed on a special slide made for the purpose as follows. An ordinary slide was used as a foundation. Then, three rectangular pieces of coverslip glass were fastened on the slide with balsam, separated from each other by a distance of about four and one half millimeters. The piece of coverslip in the center was thirty microns thinner than the other two. The fragment of the ameba was placed on the center piece of coverslip and another coverslip placed over all three pieces of coverslip. The observations were made with an oil immersion.

lens. To aid in the actual counting of crystals a "crystal-ometer" was made to place in the eyepiece just above the eyepiece. The crystalometer was made by cementing a piece of a slide on the ^{stage} edge of a binocular and scratching minute lines on its surface by means of a carborundum crystal cemented to a brass arm. The piece of brass was placed on a mechanical stage and by turning the screws first in a horizontal then in a longitudinal direction, and pressing the crystal against the slide a checkered design was scratched, the lines ^{being} one millimeter apart. The instrument is in some respects similar to a haemocytometer. During the winter months (January and February), I kept the ameba cultures in a constant temperature oven which was constructed in the laboratory. The oven was made by placing a cracker box in a wooden case about two or three inches larger than the tin cracker box, the intervening space being filled with hard, packed sawdust. The lid was hinged on and closely padded to aid in maintaining a constant temperature. In the top of the crackerbox a "home made" thermostat of zinc and copper strips was fastened to regulate the temperature. The range of temperature in the oven was from nineteen to twenty-two degrees and averaged ^{slightly less than} twenty-two degrees. During March, April, and May when the cultures were not kept in the oven the room temperature varied from fifteen to twenty-nine degrees.

EXPERIMENTAL RESULTS.

Various methods were employed in the process of analyzing the factors concerned in division in ameba. First, a half was cut from one of the vigorous amebas, one piece with the nucleus and the other without. Both pieces were kept to note changes that occurred. From the results thus obtained, it was decided that one half was too large a piece of an ameba to excise at one time. So, beginning with a new specimen one fourth was cut off daily or just often enough to prevent division. These amebas seemed to stand the operation without any noticeable effects and it was decided that one quarter was probably not the most that could be cut at one time, consequently the remainder of the experiments were performed in an effort to see how large a piece could be excised and still have the ameba completely regenerate.

In continuing I may say that many observations in my experiments confirmed in general those results obtained by authors mentioned in the "historical review". In my study of the crystals, I found Carter's and Wallich's description to coincide with my observations as regard crystal shape. I can confirm Wallich's original observation also, that the nucleus is discoidal in shape. Gruber found in his work that any piece of ^{1/4} one-celled animal possessing the nucleus, or a piece of the nucleus, was capable of regeneration. This was exactly as I found it. Nussbaum also states that the nucleus is necessary to regeneration, likewise that excisions do not affect the subsequent normal division. Several

examples of this observation appear in my chart. Verworn finds also that enucleated pieces of rhizopods are more sluggish and die after a time while nucleated animals continue to live. I also observed as did Hofer that when an ameba in the process of engulfing a food organism is excised, the excised ameba will be able to hold on to the food organism provided the piece of the ameba possesses a nucleus. In the absence of the nucleus, the food organism is able to make its escape from the ameba. Willis is very general in his statements, saying that the nucleated amebas behave like normal animals and that there is a distinct difference between parts which contain and parts which do not contain a nucleus. As mentioned above, these observations confirm my results. I am able also to agree with Botsford in regard to periodicity in division in ameba--though she worked with Ameba bigemma and I with A. proteus--a striking similarity exists. Hartman's experiment on keeping Stentor and Ameba polypodia alive by excision taking the place of normal division is quite like mine on A. proteus.

My results show quite conclusively that after ^{suc} excessive excisions covering a period of thirty-two days, amebas are able to regenerate perfectly. ~~/In no way does the excision affect~~
That is, if no further excisions are made, their ability to recuperate from the shock of excision in no way affects regeneration, and subsequent reproduction (see chart).

From the results obtained in starvation* experiments

*Actual starvation ^{conditions} ^{possible} was not accomplished on account of the
Perfect ^{ere}

presence in the culture fluid of bacteria and ultra-microscopic organisms.

(excessive accumulation of crystals evidently--because of lack of opportunity to divide which in turn is due to lack of food) and those obtained in excision experiments, the conclusion may be reached that excision (artificial division) to a certain extent takes the place of normal division. I observed that when amebas were starved, they did not divide, and at the same time the crystals increased in numbers to such an extent that the animal was practically opaque at the end of the starvation period. This would lead one to conclude that excessive production of crystals under normal conditions influences division, that is, negatively. And knowing that amebas under normal conditions, which are not able to divide, soon die; and that by successive excisions of cytoplasm from an ameba we can keep it alive (for long periods of time, see chart) we may say that artificial division in some manner supplants natural division.

Having discussed experimental cutting in a general way, we shall now deal with specific experiments performed. The first experiment to be performed was the cutting of an ameba into halves, leaving the nucleus intact in the one half. Both pieces were kept until the presence of a nucleus in one could be positively established. This cutting was done regularly every day except in cases where it looked as though the ameba might divide before the next day. As a rule, the nucleated halves live from nine to twenty-five days, but all of them finally died. After an excision was made, both pieces of the animal remained inactive for a fraction of a minute, and as we

have seen above, the pieces probably consumed this time in healing the wound made by excision. After such a period had elapsed, the nucleated piece always became attached to the bottom of the dish and proceeded to throw out pseudopods, much as a normal amoeba does. On the other hand, the enucleated piece may have become attached to the dish in a few seconds, or more frequently this act was delayed for several minutes. Even after the attachment was made, the slightest jarring of the dish dislodged the enucleated fragment. Having been able to fasten itself to the bottom of the dish, the piece was very slow to throw out pseudopods and move about. In the course of ten or eleven days (in the case of the longer lived pieces) the amoeba succeeded in partially regenerating the excised protoplasm during this time, maintaining the appearance and carrying on the habits of a normal amoeba. But after this period, ten or eleven days, degeneration set in. The piece was often found unattached to the dish, a phenomenon indicative of retrogression. It threw out few if any pseudopods, ate practically nothing, and finally assumed the shape of a sphere and rolled about in the dish for four or five days. When the dish was examined on the fifth or sixth day, all traces of the piece were gone. The enucleated fragments behaved very much like the nucleated ones, after retrogression had set in, that is, on the day after excision the enucleated pieces appear to move only sluggishly, sending out very many pseudopods. Captured food organisms were not seen often in the cytoplasm of the enucleated piece; the inference being

that food organism are not often captured. These observations cause me to take issue with Stolk--referring to his statement that enucleated pieces can live thirty days. The longest possible time that I was able to keep an enucleated ameba alive was ten days. Also I doubt very much the ability of enucleated fragments to assimilate food. If this could be true, why should they not live as long as nucleated pieces? Stolk may have had a multinucleate ameba or a fragment of nuclear material such as chromidial bodies may have been present in the "enucleated" piece to give it the extraordinarily long life of thirty days and to enable it to digest food and assimilate it. On the other hand my results disagreed with those of Balbiani, that enucleated fragments die within one and one half hours. The pieces I excised always lived over two days after excision. Hofer says that enucleated pieces have the same power of locomotion that nucleated amebas have. This is not at all in accordance with the results of my experiments as explained above.

Judging from the results obtained by excising one half the cytoplasm of an ameba every other day, this amount must be too large an amputation for the ameba to recover completely. Accordingly, excisions of one quarter were made daily, but this proved to be too small an amount, because one of these fragments grew rapidly and divided before I expected it to. This ameba, I had kept for thirty-two days excising one quarter of its cytoplasm ^{almost} daily thereby prohibiting normal division. On the thirty-third day, when I examined the

dish, I discovered that the ameba had divided. This experiment shows conclusively that successive excisions of one quarter of an ameba's cytoplasm (for a period of thirty-two days at least) has no effect on subsequent division--for from the thirty-third day on, this ameba continued to live and divide just as any normal ameba.

Having discovered that one half an ameba's cytoplasm is too much to excise at any one time, and that one quarter was not enough, I set about to find out whether there is a definite amount of cytoplasm which could be cut daily from an ameba to keep it from dividing and which would still permit it to carry on normal division when excisions were ceased. Owing to the pressure of other work I have not yet been able to secure sufficient evidence on this point to speak definitely, but I have some very interesting data which will probably help to arrive at some conclusion. (Work is now in progress on this point)

The next thing I tried was to cut just as much of the protoplasm from an ameba as was possible with my glass needle. The first two amebas experimented upon in this way were lost in transferring from the dish in which they were cut to the culture dish. In one case I estimated that the ratio between the nucleus and cytoplasm was as twenty is to one. After more efficient ^{technic} had been developed, I succeeded in keeping the amebas under inspection and was able to cut a little more cytoplasm each day. Before any excisions were made in this

experiment on the ameba, the nucleus measured forty microns in the longest diameter. Three days later, when it was measured, after two more excisions were made, the nucleus was thirty-six microns in long diameter and two days later, it measured twenty-eight microns. The nucleus took on more of an ovoidal shape as it decreased in size. At the beginning of the experiment, it was of a typical discoidal or biscuit shape. These results lead me to conclude that, with the aid of a microdissector, progressive excisions could be made until the fragmentary ameba would be of infinitesimal size--because this allows the nucleus to reduce in size to accommodate itself to the reduced amount of cytoplasm.

Records were kept of the number of times excisions were made and the amount excised each time in the case of the ameba that was carried thirty-two days (without normal division occurring). These excisions show that the total amount of cytoplasm excised equalled that of two and four tenths amebas while that laid down by the controls at the same time was twelve times as great as one ameba. In other words, the controls produced five times as much cytoplasm as the excised ameba. This leads us to assume that division is the direct result of increase in size and at the same time the rate of growth is definitely retarded, ^{in some manner} by the act of excision. Furthermore, I have also observed that a change in amount and kind of organisms used as food, for the amebas, sometimes speeds up division for a day or two. Thus, we see that division is not dependent on one factor, but a combination of three

(at least) (1) increase in size, (2) increase in crystal number, (3) and amount and kind of food organism used.

In the case of one of these last mentioned excised amebas, careful observations were made regarding the quantitative relation of the cytoplasm to the nucleus. The ratio of nucleus to cytoplasm in a normal ameba is about one to two hundred and seventy. In this excised ameba, after several excisions of considerable amounts of cytoplasm were made, the ratio ^{of} was found to be nucleus to cytoplasm as 1 is to $2 \frac{3}{8}$. In other words, this ameba is $1/80$ as large as a normal ameba, but there is no reason to suppose that this is the smallest ^a part of an ameba that can regenerate into a whole ameba. But even as it is, this is quite a contrast to Lillie's minimal reorganization mass as he found in the Stentor, which was one hundred microns (Stentor coeruleus) or eighty microns in Stentor polymorphous. It is also smaller than Sokoloff's minimal reorganization mass of $1/53$ to $1/59$ in Spirostomum ambiguum or $1/70$ to $1/75$ in Dileptus. This ($1/80$) is in fact the smallest proportional part of a protozoan that has been found capable of regeneration

Regarding Hartman's casualties and inability to keep Ameba proteus alive, it seems to me that his technique must have been somewhat deficient. Owing to the fact that both A. polypodia and A. proteus are inadequately described^{by Hartman}, it is ^{somewhat} doubtful, judging by the results obtained, that Hartman actually worked on A. polypodia and A. proteus. It is known that A. polypodia is probably less resistant than A. proteus and from the ease with which I was able to keep pedigree lines of Ameba proteus going, it seems that Hartman should have had better success with his pedigree lines. In every case where death occurred I was able to assign a cause. On the other hand, Hartman says he was able to secure only eight regenerations in a period of a month and that casualties were very frequent. He states that in his work on A. polypodia, he was able to secure thirty-two regenerations covering a period of forty-two days, substituting excision for normal division. He further adds that these regenerations might be carried on ad infinitum. Is this not quite a broad statement to make when the life history of A. polypodia has not been completely worked out up to this time? It is very probable that a process may exist in the life history of A. polypodia, such as endomixis which occurs in paramecia. Undoubtedly, such a method of rejuvenation^{might} would completely upset the ~~continued existence~~^{regeneration} of such an excised ameba.

Note.

Spirostomum ambiguum was used at first in this experiment to study the experimental factors concerned in division, but owing to slow rate of division and difficulties in raising the animals isolated, Ameba proteus was substituted. The results obtained agree with those obtained by others who worked on the same species and performed similar experiments.

When amebas were not allowed to divide, as when starved, a marked increase (50%) in crystals found in the protoplasm, occurs. When excisions of protoplasm of sufficiently large size are made, for thirty-two days, to prevent division, there is only a slight or no increase in crystal numbers. The numbers of crystals seems to be a good indication of metabolic changes occurring in an ameba and perhaps cells in general. The final^{but} results of this experiment have not yet been obtained, ^{but} further observation will be made as soon as possible.

CONCLUSION.

Although Spirostomum ambiguum was first used in this experiment, it proved to be unsuitable because of its slow rate of reproduction and the difficulty with which it is raised in isolation. Having experienced these difficulties with Spirostomum ambiguum, I isolated several individuals of Amoeba proteus^{and} found them very suitable for experimental purposes. They are relatively large and of convenient size. Culturing in pedigree lines was accomplished with comparative ease after suitable technique had been developed (which consumed only a short time). A. proteus is also very convenient for experimental purposes because of its rapid rate of division (This rate of division varying with the temperature). Neither is it difficult to find suitable food for proteus, it being able to thrive equally well on any one of several small ciliates.

1. The act of excision only temporarily (2.46 seconds) incapacitates fragmentary amoebas.

(a). This explains the inactivity of cut pieces immediately after excision.

(b). The shock of excision could not possibly affect the recuperation of cut pieces for more than a fraction of a minute.

(c). Enucleated amoebas are not able to carry on any fundamental body processes, excepting locomotion.

(d). The presence of the nucleus is necessary for a fragmentary amoeba to recuperate from excision.

2. One half of the cytoplasm of an amoeba is too large an amputation to be made at one time.

(a). Nucleated pieces of amoebas, having one half the cytoplasm excised, live from nine to twenty-five days.

3. One quarter of an amoeba is too small an amount to be excised at one time.

(a). Regeneration is so rapid that complete recuperation takes place within twenty-four hours and fission occurs.

4. Successive excisions of one quarter of an amoeba's cytoplasm (for a period of thirty-two days at least) has no effect on subsequent division.

(a). From the thirty-third day on, that is after excision had ceased, and regeneration was complete, the amoeba continued to live and divide just as any normal amoeba.

5. After successive excisions of the largest amounts possible of an amoeba's cytoplasm, the nucleus is shown to reduce in size to accommodate itself to the decreased amount of cytoplasm.

(a). The nucleus changes from a typical discoidal or biscuit shape to an ovoidal shape as it decreases in size.

6. Division in proteus is a direct result of increase in size and at the same time, the rate of growth is definitely retarded by the act of excision.

(a). A change in amount and kind of organisms used as food speeds up division for a day or two.

7. The minimal reorganization obtained thus far in the experiment is one eightieth as large as the normal amoeba.

Heretofore the smallest minimal reorganization mass recorded was one-seventy-fifth of the original protozoan.

There is no reason to suppose that this is the smallest part capable of regeneration.

8. Artificial division (excision), in some manner, supplants natural division.

(a). In amebas in which crystals are found in excessive amounts producing opacity, division does not occur and death soon results.

(b). Starved amebas show excessive accumulation of crystals, a phenomenon accompanied by absence of division.

(c). Examination of excised portions of the protoplasm of an ameba show only a slight or no increase in crystal numbers.

(d). Numbers of crystals appear to be a good indication of metabolic changes occurring in an ameba and perhaps in cells in general.

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Key

- ⊕ = Dead
- = Cut
- = Divided
- ⊗ = Nucleated
- ⊙ = Starved
- ⊖ = Enucleated
- ② = 1/2 cut off