STUDIES ON REPRODUCTION IN *AMOEBA PROTEUS*¹

JOSEPH LEVY

Medical School, The Johns Hopkins University, Baltimore, Maryland

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INTRODUCTION

Fundamental significance in so many different directions is commonly attributed to Amoeba that detailed knowledge of its genetic phenomena is desirable. The following paper, dealing with Amoebae of the "*proteus*" types, contributes observations on the constancy of characteristics in diverse types when cultivated under similar conditions; on the rate and rhythmic character of vegetative reproduction in different strains; on the effects of diverse cultural conditions upon reproduction; and on the origin and methods of reproduction of polynucleate forms.

MATERIAL AND METHODS OF CULTURE

The Amoebae employed were partly obtained from the laboratory cultures of Dr. J. G. EDWARDS; partly from a pond in the Botanical Garden of the JOHNS HOPKINS UNIVERSITY. Pedigree cultures were followed

¹From the Zoölogical Laboratory of THE JOHNS HOPKINS UNIVERSITY.

by isolating single individuals in the concavities of hollow-ground slides, each concavity containing 0.2 cc of culture fluid. The slides were kept in moist chambers and examined daily as to fission. Fresh culture fluid was supplied at different intervals in different experiments, as will be set forth. Pedigree records were kept by giving a designation to each individual, on the following system: The parent individual is designated N. 1. (N being the number of the line). The products of the first fission are designated N.2 and N.3. The offspring of N.2 are N.4 and N.5; those of N.3 are N.6 and N.7. This process is continued for ten generations, the individuals of which have a range of numbers from N.1024 to N.2047. The designation of the individuals which are to be continued in the pedigree is then reduced to N. Ia1, N. Ib1, etc. The formula used above is employed again and continued for another ten generations. The designation of the individuals of the twentieth generation is reduced to N. IIa1, N. IIb1, etc. The pedigree can be continued indefinitely, each member of the pedigree having as its identification mark the number of the family, the number of the generation reached, and the line of descent. All pipettes, slides, bottles and dishes were washed in boiling water after having been used once, to eliminate any possibilities of contamination from unknown sources.

A hay-infusion infusoria culture medium was employed, as by STOLC (1905) and SCHAEFFER (1917). It is prepared in the following manner: Stalks of dry timothy hay are cut into half-inch strips and autoclaved. Two hundred cubic centimeters of distilled water are placed in a finger bowl (diameter 4 inches) and on the surface of the water is spread one gram of the sterilized hay. This is left exposed to the air. In a few days the hay begins to decay, the surface of the liquid serving as a nutrient medium for a variety of bacteria, yeasts and moulds. At ten-day intervals, the evaporated liquid is replaced by equal amounts of distilled water (50-75 cc). Fifteen days after the preparation of the culture, it is inoculated with 50 cc of a dilute hay-infusion fluid which contains numerous *Chilomonas* and smaller infusoria, but no rotifers. The infusoria feed on the micro-organisms, and multiply rapidly. Fifteen days after inoculation, the medium is filtered through a coarse filter paper into one-half pint milk bottles. It is now ready to serve as a culture fluid for *Amoebae*. These cultures were renewed at ten-day intervals.

These studies were undertaken at the suggestion of Professor H. S. JENNINGS in November 1921, and were attacked by the method of isolation pedigree cultures used by the investigators on inheritance in unicellular organisms at THE JOHNS HOPKINS Zoölogical Laboratory. The writer is indebted to Professor H. S. JENNINGS for his valuable counsel and aid.

CONSTANCY OF THE CHARACTERISTICS OF DIVERSE TYPES

The Amoeba figured in text-books of elementary biology was discovered by RÖSEL in 1755. He named the specimen that he had found "der kleine Proteus." PALLAS in 1766 redescribed and renamed this organism, "*Volvox proteus*." EHRENBERG found the same form in 1830, but described it under a new name, "*Amoeba diffluens*." One year later he described a large Amoeba of the same type, and differentiated it from his first find by giving it a new specific name, "*Amoeba princeps*." EHRENBERG's Amoebae became confused by later investigators. A number of observers described the same organism, or slight variations of it, under new specific names, e.g., *Amoeba ramosa* Fromentel; *Amoeba communis* Duncan, and *Amoeba villosa* Wallich. Thinking that the environment had a great deal to do with the size and the general appearance of the Amoebae, also seeking to eliminate this confusion of description and nomenclature, LEIDY first grouped all these forms under the name *Amoeba chaos*, but finally, in his survey of 1879, decided on the name *Amoeba proteus*. This simplified matters a great deal, but several students of Amoebae protested at this wholesale grouping of all the variations under one name.

With each protest came new specific names. PENARD (1902) divided the group into three species, viz., *Amoeba proteus*, *Amoeba nobilis* and *Amoeba nitida*, using nuclear differences as the basis of his classification. CASH and HOPKINSON (1905) found that twenty-three names had been applied to the *Amoeba proteus* described in LEIDY's work.

SCHAEFFER (1916) found that the morphological differences in various forms of *Amoeba proteus* were accompanied by corresponding differences in behavior to many food substances. He consequently broke the group up into (a) the granular type and (b) the raptorial type, and divided *Amoeba proteus* (Leidy) into three species, as follows:

(a) Granular type.

1. *Amoeba proteus* Pallas (Leidy). About 600 microns; longitudinal ridges on pseudopods; discoidal nucleus which may be folded in old individuals.

2. *Amoeba discoides* sp. nov. About 400 microns; no ridges; discoidal nucleus; numerous, small crystals.

(b) Raptorial type.

3. *Amoeba dubia*, sp. nov. About 400 microns; no ridges; few, large crystals; ovoidal nucleus.

CARTER (1919) accepted SCHAEFFER's separation of *Amoeba proteus* into the granular and raptorial types, but protested against the addition

of new specific names to the already overburdened nomenclature list of the organism. She put forward the hypothesis that all these Amoebae belong to a single species and submitted the following classification for use until the discovery of the complete life-cycle of *Amoeba proteus*.

(a) Granular Amoebae.

1. *Amoeba proteus* Pallas, var. *granulosa*.

Amoeba princeps Ehrenberg

2. *Amoeba nobilis* Penard, multinucleate

} *Amoeba proteus* Y.

Amoeba proteus Z.

(b) Raptorial Amoebae.

3. *Amoeba proteus* Pallas.

Amoeba proteus X.

The opinion has at times been expressed that the many diverse types of *Amoeba* are constitutionally the same, the diversities being merely the result of diverse environment. Others hold that the diverse forms are permanently differentiated, so that they may properly be characterized as different species.

SCHAEFFER (1917) pedigreed each of his three types under controlled conditions and found that the morphological as well as the physiological characteristics of each type remained constant for 15 generations. Previously, STOLC (1906), in a pedigree study of a single line of a granular *Amoeba*, found that the characteristics of the organism did not change during 292 generations, more than 1 year and 8 months, in isolation culture. CARTER (1919) found a constancy of characteristics existing in a raptorial *Amoeba*, multiplying by fission for more than 6 months.

To further test the constancy of the differences existing between these two types, seven granular *Amoebae* (each with longitudinal ridges on the pseudopods), and seven raptorial *Amoebae*, were isolated by the writer for pedigree study. All the *Amoebae* were transferred daily to clean slides containing fresh culture fluid. One line of each type was pedigreed for 40 generations, the other lines were studied for shorter periods.

It was found that the marked differences that existed between the two types remained constant, as SCHAEFFER had previously demonstrated. Slight variations in the cytoplasmic and nuclear characteristics in the granular *Amoebae* arose during the course of the pedigree. The individuals that did not divide for long periods became more densely granular and much larger than the parent form, while those that divided rapidly were, as a rule, less granular and smaller than the parent. The larger individuals were occasionally binucleate and even polynucleate. The nuclei of others of the larger *Amoebae* were oftentimes bent or wrinkled. But all the modifications were, without exception, of a temporary nature. The

different forms were interchangeable; one could pass into the other, depending upon the condition of the organism and the nature of the culture medium. The raptorial Amoebae showed no apparent signs of divergence from the parent form, throughout the pedigree.

A consideration of table 1 shows that the total number of recorded divisions in all the pedigree lines of the raptorial Amoebae was 626 and the average interval between two successive divisions was 1.6 days. The total number of recorded divisions of the granular Amoebae was 846 and the average interval between successive divisions was 2.5 days. In addition to this difference in the division rate, there was a significant differ-

TABLE 1

Data on the division rate of all the individuals in 7 lines of granular Amoebae and 7 lines of raptorial Amoebae. The different lines were cultivated simultaneously from Feb. 13, 1922 to June 14, 1922. Each individual was transferred daily to fresh culture fluid.

NUMBER OF DAYS BETWEEN DIVISIONS	THE TOTAL NUMBER OF INDIVIDUALS THAT DIVIDED, IN ALL LINES OF	
	Granular Amoebae	Raptorial Amoebae
1	343	377
2	243	187
3	111	37
4	47	6
5	31	5
6	19	4
7	6	4
8	13	5
9	5	...
10	7	1
11		...
12	6	...
13	1	...
14	1	...
16	2	...
17	1	...
18	1	...
19	1	...
Average interval between divisions in days	2.5	1.6

ence between the two types in the length of the period of inactivity (interval during which there is no division). The maximum period of inactivity of the raptorial Amoebae was 10 days, while the granular Amoebae went without dividing for periods of 18 and 19 days (table 1). The raptorial Amoebae differed in still another respect from the granular Amoebae. While a long period of inactivity proved fatal to the former, it had no such

effect on the latter. The net result was a higher death rate among the raptorial forms.

Another difference between the two types was observed when the intervals between successive divisions were averaged for five-day periods (figure 1). This method of presenting results was used by WOODRUFF and BAITSELL (1911) in their studies on the division rates in *Paramecium*. The graph shows that during the first half of the pedigree the granular

FIGURE 1.—Graphs for the division rates of the individuals in a line of granular *Amoebae* (a), and a line of raptorial *Amoebae* (b). The vertical scale shows the average number of days between the divisions of the individuals that divided within each 5-day period; the horizontal scale shows the number of successive 5-day periods. The dotted lines represent periods during which no division occurred. a, graph for the granular *Amoebae*; b, graph for the raptorial *Amoebae*.

Amoebae divided very slowly, while the raptorial *Amoebae* divided daily. The latter divided about four times as rapidly as the granular type. In the last half of the pedigree, the granular *Amoebae* showed a decided increase in the division rate, while the raptorial *Amoebae* showed a slight

decrease. Thus the division rate of the two types at the end of the pedigree was about the same. The death of all the raptorial Amoebae prevented a further comparison of the two lines. These results are indicative of an increase in the vitality (fission rate) of the granular Amoebae and a decrease in the vitality of the raptorial Amoebae when both are kept under the same culture conditions.

A further examination of figure 1 shows an interesting rhythmic character in the division rate of each type. It shows that there is an alternation of periods of high and low reproductive activity, which appeared to be of the same nature for both types; also that the value of the several maxima and minima varied, and the interval between two successive maximum division rates was about 25 days. BOTSFORD (1922) found similar rhythms in the reproduction of *Amoeba bigemma*. The exact nature and the importance of these rhythms in the life cycle of the Amoebae has not yet been determined. It seems probable that cytological study will show that the rhythms result from processes similar to endomixis in *Paramecium*.

My results from pedigree cultures thus confirm those of SCHAEFFER in showing that the differences between the granular and the raptorial forms are constant for long series of generations under identical conditions.

INFLUENCE OF DIVERSITIES IN ENVIRONMENTAL CONDITIONS ON THE
RATE OF REPRODUCTION AND THE RHYTHMIC CHARACTER OF
THE DIVISIONS IN *Amoeba proteus*

Several investigators have presented evidence that the excretion products of an organism have a profound effect on its growth. WOODRUFF (1911, 1913) shows that the excretion products of *Paramecia* apparently lower the division rate of this protozoan. ROBERTSON (1921, 1922), on the other hand, maintains that the excretion products of the infusoria have no effect on their division rate. It appears of interest to investigate, not only the effect of excretion products and other changes in the culture medium on the division rate in *Amoeba proteus*, but also the influence of such changes on the rhythmic character of the divisions. WOODRUFF (1917) found that normal changes in the environment of *Paramecia* have no effect on the length or the character of the rhythm in the divisions of these organisms.

The organisms used in the present investigation were isolated from cultures of Amoebae which had been grown under pure-line conditions for 9 months. The Amoebae were placed in concavities of hollow-ground slides, each concavity containing 0.3 cc of a hay-infusion infusorian culture prepared as described above. Diversities in the environmental conditions

were produced by keeping the Amoebae isolated in the same culture fluid for varying lengths of time. The series used were as follows:

A. Culture medium changed daily. The Amoebae were transferred every 24 hours to clean slides containing fresh culture fluid. (The fresh culture fluid was obtained from a stock medium which was prepared anew every 10 days).

B. Culture medium changed every 2 days.

C. Culture medium changed every 3 days.

D. Culture medium changed every 5 days.

E. Culture medium changed every 10 days. In this group, water which evaporated was replaced at the fifth day by the addition of distilled water.

An examination of the mortality rates (number disintegrated divided by total number isolated) in all the lines of each series showed that certain changes had occurred in the various culture media. In general, the data indicate that within the limits tested (1 to 10 days) the longer the period that an Amoeba is kept in the same culture fluid, the more detrimental the effect on the vitality of the isolated organism (see table 2).

TABLE 2

The relation between the length of time that an Amoeba is kept in the same culture fluid and the mortality rate. The data include individuals of all lines during the entire period of the experiment.

INTERVALS IN DAYS BETWEEN TRANSFERS	TOTAL NUMBER OF INDIVIDUALS ISOLATED	NUMBER OF INDIVIDUALS THAT DISINTEGRATED	MORTALITY RATE IN PERCENT
1	353	21	5.95
2	752	38	5.05
3	591	62	10.51
5	449	55	12.25
10	409	87	21.27

With relation to the rate of multiplication, the five sets of experiments above described were conducted simultaneously for five months, from October 6, 1922, to March 6, 1923. These allowed comparisons to be made of the effects of the same and of different cultural conditions, on different lines, some belonging to the same races, some to diverse races. The results of the various comparisons are as follows:

(1) Effects of diverse cultural conditions on lines belonging to the same race are shown in table 3, in which are summarized the results of keeping under different cultural conditions a set of lines, all isolated from the fifth generation of a single line in series B. The table is to be read as

follows: In the lines that were changed every day, 43 divisions occurred at one-day intervals, 56 at 2-day intervals, and so on; in those changed at 2-day intervals, 58 divided at 1-day intervals, etc., etc. The data given in table 3 as to the intervals between the divisions,—particularly the averages—indicate that there is a progressive decrease in vitality as

TABLE 3

The effect of different environmental conditions on the division rates of individuals of various lines of the same race of Amoeba proteus. Each line is designated by a capital letter that represents the environmental condition to which it is being subjected, and a subscript which indicates the source from which the parent form was isolated.

INTERVAL IN DAYS BETWEEN SUCCESSIVE DIVISIONS	NUMBER OF AMOEBAE THAT DIVIDED, WHEN THE CULTURE MEDIUM WAS CHANGED EVERY			
	Day, in line A ₁₉	2 days, in line B ₁₉	5 days, in line D ₁₉	10 days, in line E ₁₉
1	43	58	26	27
2	56	92	46	28
3	25	32	17	16
4	17	14	8	6
5	6	15	5	5
6	8	9	5	4
7	2	10	2	2
8	2	7	2	3
9	..	6	..	1
10	2	2	2	1
11	4	8	1	..
12	1	4	4	1
13	..	2	1	1
14	5	5	1	..
15	3	..	..	1
16	..	2	..	1
17	1	..	..	..
18	..	2	..	..
19	..	..	1	1
20	..	..	..	2
24	..	..	..	1
25	..	..	1	..
30	..	..	1	..
32	..	..	1	..
39	..	..	..	1
Average interval between divisions	3.52	3.90	4.01	4.42

the period between transfers to fresh culture fluid is made longer. The graphs in figure 2 show that in all the lines there is a similarity in the succession of high and low periods of reproductive activity, though there is some irregularity in the lengths of the rhythmic periods.

(2) When for the experiments on the effects of changing the animals at different intervals we select lines derived from different original cultures, (and thus from diverse original progenitors) the results are those shown in table 4. The results are more variable than when lines all derived from

TABLE 4

The effect of different environmental conditions on the division rates of lines of different races of Amoeba proteus. Each line is designated by a letter representing the condition to which it is being subjected, and a subscript which indicates the source from which the parent form was isolated.

INTERVAL IN DAYS BETWEEN SUCCESSIVE DIVISIONS	NUMBER OF INDIVIDUALS THAT DIVIDED, WHEN THE CULTURE MEDIUM WAS CHANGED EVERY			
	1 day, in line A ₁	2 days, in line B ₂	5 days, in line D ₂₅	10 days, in line E ₂
1	52	18	21	6
2	47	23	22	34
3	15	7	14	11
4	7	16	13	9
5	3	2	12	12
6	8	4	10	14
7	4	1	2	5
8	1	1	3	10
9	5	..	5	4
10	4	..	3	2
11	3	..	2	3
12	1	..	3	2
13	3	..	3	5
14	3	..	1	2
15	..	1	1	3
16	..	1	1	1
17	1	..	1	1
18	..	1	2	..
20	..	..	..	1
21	..	..	1	..
26	..	1	..	..
Average interval in days between divisions	3.42	3.92	5.06	5.16

the same original parent are employed, but they show on the whole the same decrease in reproductive vigor as the animals that are kept longer in the same culture fluid. Graphs of the progress of these experiments show the same rhythms of high and low reproductive rates, with the same irregularity in the length of the rhythms, that are exhibited in figure 2.

(3) Difference in division rate in the progeny of two sister cells. In a number of cases two sister Amoebae were cultivated separately, but

under identical conditions. Some of these twin cultures were changed every 2 days, others every 3, 5 or 10 days. In the B series of experiments (changed every 2 days), two sister cells were used that were derived from a parent *Amoeba* having a bent nucleus. At its division one of the two

FIGURE 2.—Graphs for the divisions of 4 lines of *Amoebae* belonging to the same race, but kept under different environmental conditions. The vertical scale shows the average number of days between the divisions of the individuals that divided within each 5-day period; the horizontal scale shows the number of 5-day periods. A, culture medium changed daily; B, medium changed every 2 days; D, medium changed every 5 days; E, medium changed every 10 days.

Amoebae produced had a smooth nucleus, the other a bent nucleus. The former gave rise to the line B2 the latter to the line B2b; the two were cultured under the same conditions. The division rate of the two lines thus derived differed greatly. The line B2b derived from the parent with

bent nuclei multiplies much more slowly (table 5). In line B2 the individuals were larger and had smooth nuclei; in line B2b most of the individuals had bent nuclei. Graphs of the reproduction in the two lines show the usual rhythms.

FIGURE 2, C and D. Legend on preceding page.

Three other cases in which sister lines were kept under identical conditions are shown in table 6. The average rate of fission differed somewhat

in the case of the sister lines, C and D, but were identical in the two lines of E. In all these cases the reproduction showed rhythmic alternations of high and low reproductive rates, as seen in the graphs of figure 2.

TABLE 5

The variation in the division rates of the individuals in two lines of the same race of Amoeba proteus when kept under the same environmental conditions. The lines differed in the size and shape of the nucleus. The Amoebae of line B2b invariably had bent nuclei, while those of line B2 usually had smooth nuclei; both were derived from a parent form that had contained a bent nucleus.

NUMBER OF DAYS BETWEEN SUCCESSIVE DIVISIONS	NUMBER OF INDIVIDUALS THAT DIVIDED, WHEN THE CULTURE MEDIUM WAS CHANGED EVERY TWO DAYS	
	In line B2	In line B2b
1	23	4
2	46	7
3	18	8
4	15	5
5	21	3
6	7	7
7	7	3
8	3	1
9	2	1
10	1	2
11	4	..
12	2	..
13	2	1
14	3	1
15	3	..
16	..	1
17	..	1
21	..	1
22	2	..
23	1	..
30	..	1
Average number of days between divisions	4.34	6.12

It is thus evident that a change in environmental conditions alters the division rate in *Amoeba*, but does not alter the rhythmic periodicity of high and low rates. This agrees with what WOODRUFF (1917) found as to the effects of environmental changes on the periodicity of endomixis in *Paramecium*. Whether some process similar to endomixis occurs periodically in *Amoeba proteus* remains to be determined; further study is needed both of the rhythms themselves, and of possible accompanying cytological changes.

THE ORIGIN AND THE METHODS OF REPRODUCTION OF POLYNUCLEATE
FORMS OF *Amoeba proteus*

It has generally been accepted that the polynucleate condition of an amoeboid cell is produced by the fusion of two or more mononuclear cells. STOLC (1906) observed the fusion of two sister mononucleate individuals

TABLE 6

Variation in the division rates between the individuals in two lines of the same race of Amoeba proteus when kept under the same environmental conditions. Two sister lines were kept under three different sets of culture conditions.

NUMBER OF DAYS BETWEEN DIVISIONS	NUMBER OF INDIVIDUALS THAT DIVIDED, WHEN THE CULTURE FLUID WAS RENEWED EVERY					
	Three days, in lines		Five days, in lines		Ten days, in lines	
	C ₁	C _{2a}	D _{1b}	D _{1a}	E _{1c}	E _{1a}
1	61	66	26	27	27	9
2	58	67	46	39	28	27
3	26	48	17	27	16	11
4	19	19	8	18	6	13
5	7	27	5	18	5	9
6	12	21	5	10	4	7
7	11	11	2	6	2	4
8	5	13	2	8	3	4
9	1	7	..	1	1	5
10	7	3	2	2	1	1
11	5	6	1	3	..	..
12	1	2	4	3	1	1
13	3	2	1	1	1	2
14	1	2	1	2	..	..
15	3	4	..	1	1	1
16	2	..	..	1	1	..
17	3	..	..	..	1	1
18	2	1	..	..	..	..
19	..	2	1	1	1	..
20	..	..	..	1	2	..
22	..	..	..	1	..	..
24	..	..	..	..	1	..
25	..	..	1	..	..	..
29	1	..	..	..	..	..
30	..	..	1	..	..	..
32	..	..	1	..	..	..
39	..	..	..	..	1	..
Average in- terval between divisions	4.42	4.12	4.01	4.40	4.42	4.42

of *Amoeba proteus*. LAMBERT (1912) has shown that giant polynucleate cells *in vitro* are produced by the fusion of several wandering cells. Re-

cently, however, LEWIS and WEBSTER (1921), working with tissue cultures, while uncertain as to how the polynucleate cells arise, found evidence for the amitotic division of the nucleus without a division of the cytoplasm, but could find no evidence for a fusion of cells. HEGNER (1920) explains nuclear doubling in *Arcella dentata* by a failure of one nucleus and half of the cytoplasm to separate into the new shell which is formed when the parent nucleus divides. STOLC (1906) had previously observed that by an alteration in the culture medium, the binucleate form of *Amoeba proteus* could develop directly from the mononucleate condition. This occurred when the mononucleate Amoeba was placed in a culture fluid containing very little food, or an over-abundance of food; when the mononucleate Amoeba was kept for a long period in the same culture fluid; and twice in a line that was pedigreed for more than 20 months. From these observations, he concluded that the binucleate form arises from the mononucleate, by a division of the nucleus without a simultaneous division of the cytoplasm; but he did not determine how this took place.

Occurrence of polynucleate Amoebae

During the past year, forms with two, three and four nuclei have appeared in each of 18 isolation pedigree lines of *Amoeba proteus*. The frequency of their appearance varies considerably. It does not depend upon the quantity of available food material, for they may arise in cultures that have an abundance or a lack of food. The length of time that the mononucleate Amoeba is kept in a given quantity of culture fluid seems to affect the number of polynucleate forms that may appear. They rarely appear when the mononucleate organism is transferred from day to day into a fresh culture medium. However, if the transfers are made at intervals of two, three, five or ten days, the tendency for the Amoebae to become polynucleate is very strong. It was found that a constant supply of these large Amoebae could be obtained by the following procedure. An Amoeba is placed in a cavity of a hollow-ground slide which contains 0.3 cc of a dilute hay-infusion Chilomonas culture. The slide is placed in a moisture chamber. The small amount of liquid that evaporates is replaced periodically by the addition of fresh culture fluid. Under such conditions the Amoeba divides at irregular intervals, producing in a short time a large population. About thirty days after the start of the experiment, polynucleate Amoebae begin to appear. Careful examinations of the culture failed to reveal any signs of fusion of one individual with another. At about the period of the appearance of the polynucleate

forms, the total number of Amoebae in the cavity remains constant for a while, and then begins to decrease. The conditions existing in the cavity are therefore unfavorable for the multiplication of the organisms. Such unfavorable conditions may be brought about by the accumulation of the excretion products of the Amoebae and the other organisms that have populated the culture, by a concentration of the organic substances which are left behind when the slight but continuous evaporation of water occurs, or, by a combination of these changes.

Origin of the polynucleate Amoebae

Since the polynucleate Amoebae did not arise in these cultures by a fusion of cells, their origin was probably due to an irregularity in the division process of the mononucleate forms. SCHULZE (1875) has shown that an Amoeba reproduces by a simple division, the two portions moving apart until the very thin strand of protoplasm, which bridges the gap between them, finally breaks. The mononucleate forms of *Amoeba proteus* divide in the same manner, but the division of the nucleus, so clearly seen in SCHULZE'S *Amoeba polypodia*, could not be discerned in *Amoeba proteus*. In order to make intelligible the experiments later described, an account is given of the external features of fission.

The process of division may be considered as taking place in two stages:

1. *Resting stage.* The Amoeba is a spherical mass of protoplasm with short blunt pseudopods projecting from its surface in all directions (figure 3, a to c). There is a very slow movement of the protoplasm, but the Amoeba does not change its shape or position. The nucleus can not be seen in the living specimen. Whole mounts of the Amoebae in this resting stage, prepared by fixation in Yocum's fluid and stained with iron haematoxylin indicate that the nucleus has become completely disorganized. Spherules of chromatin material are scattered throughout the cytoplasm. I have not determined whether the absence of the nuclear membrane is due to the destructive action of the fixing agent, or whether it is characteristic of the method of nuclear division.

2. *Cytoplasmic division.* The Amoeba begins to elongate by a slow streaming of the protoplasm, which flows first in one direction, then in the opposite direction. As the Amoeba elongates, the central portion becomes narrower than the parts on either side of it (figure 3, d to h). Pseudopods radiate from each of the dividing parts. They are projected for a short distance only. Some of these are withdrawn and larger ones move out. This process of pushing out a pseudopod and then withdrawing it, continues throughout the division of the cytoplasm. The movement is not

limited to the pseudopods. It is seen in the constricted central portion which becomes a narrow strand of protoplasm. For several minutes there is an interchange of protoplasmic materials between the two dividing parts through this connecting strand. At first there is a slow movement of granules from A to B, (figure 3, d to h), which stops, and then the streaming proceeds from B to A. This alternating movement continues as

FIGURE 3.—Camera lucida sketches of successive stages in the division of a mononucleate individual of *Amoeba proteus*. a, b, c, stages in the quiescent period, during which nuclear division occurs; a, at 10:30 a.m.; b, 10:35 a.m.; c, 10:45 a.m.; d to i, stages in the division of the cytoplasm, with the separating parts marked A and B; n, nucleus; v, vacuole; d, at 10:47 a.m.; e, 10:50 a.m.; f, 10:53 a.m.; g, 10:56 a.m.; h, 11:00 a.m.; i, 11:15 a. m.

the parts move away from each other, and ceases when the strand between them comes to be too narrow to permit the passage of even the smallest granules. The connection, now consisting almost entirely of ectoplasm, breaks, and two mononucleate individuals are the result (figure 3, i).

The process of cytoplasmic division is of long enough duration (15 to 30 minutes) to permit of experimental study. Can the process in any way be

altered so as to prevent the cytoplasm from dividing? It was found that the alternating streaming of the protoplasm, which is so characteristic of the division process, could be modified by local application of certain chemicals and even by a mechanical stimulus applied to several of the short pseudopods. The pseudopods invariably moved away from the stimulus, and by the continued application of it, the movement of the protoplasm was made to proceed more in one direction (away from the source of stimulation) than in the other. It was possible to stop the alternation in the streaming of the protoplasm during the cytoplasmic division and to force the streaming to proceed in one direction only by the following method: A small drop (diameter 1 mm) of culture fluid is placed on a glass slide; a dividing *Amoeba* is transferred with a fine capillary pipette to the outer edge of this drop so that only a portion of the added fluid merges with the drop. The organism is so oriented that one of the dividing parts, B (figure 3, d), fits snugly in the space enclosed by the minute quantity of liquid that was transferred with the *Amoeba*. The other portion, A, projects into the larger drop. The movement of the protoplasm alternates through the connecting strand for a few minutes. When a pseudopod of the part B reaches the edge of the drop, it is quickly withdrawn. As the drop evaporates, its edge approaches B, and prevents it from projecting any more pseudopods. The only part of B that is not affected by the evaporating drop is the portion attached to the connecting strand. Further evaporation of the drop that surrounds B forces its protoplasm to flow with increased rapidity into A. This streaming of one part into the other continues as long as the movement of B in any other direction is prevented. Eventually all of the part B flows into A, and the resultant *Amoeba* does not attempt to divide again. An organism is produced which is identical in both morphological and physiological characteristics with the binucleate *Amoebae* found in the isolation cultures, and in the material collected from ponds. The trinucleate and the tetranucleate *Amoebae* can be produced by the same method.

Since the nucleus divides while the *Amoeba* is at rest, (figure 3, a to c) the polynucleate form is produced by a failure of the cytoplasm to divide with it. The process of cytoplasmic division begins, but is prevented from going to completion by a mechanical or a chemical condition existing in the culture medium. Such conditions are more frequent in old concentrated cultures than in freshly prepared, dilute ones.

Methods of reproduction of polynucleate Amoebae

The question as to the permanence of these nuclear modifications leads to a study of the methods of reproduction of the polynucleate forms.

STOLC (1905) made an extensive investigation of this problem. In an experimental study on 62 binucleate Amoebae, 5 trinucleate Amoebae, and 1 tetranucleate Amoeba, he found that the methods of division of protoplasm in the polynucleate condition could be classified as follows:

1. Protoplasmic division without a simultaneous division of the nuclear material.
2. Protoplasmic division with a simultaneous division of all the nuclei.
3. Protoplasmic division with a simultaneous division of only part of the total nuclei.

Furthermore, he described, but did not classify, a case in which a binucleate Amoeba became tetranucleate, apparently by a division of the nuclei without a division of the cytoplasm.

Division of binucleate Amoebae

Of the 248 binucleate Amoebae isolated for the present investigation, 11.3 percent, after living in the culture medium for an average period of 10 days, died. This mortality among the binucleate forms is significant when compared with that of only 5 percent among the several hundred mononucleate Amoebae that were isolated. The method of division of the nuclei and of the cytoplasm, and the distribution of the nuclei to the cytoplasm occur in a number of different ways:

1. *Division of the cytoplasm without a division of the nuclei.* In 13 cases out of the total 248, or in 5.2 percent, the Amoeba existed in the binucleate condition for an average period of 8.2 days, and then divided into two large Amoebae, each of which was mononucleate. These continued to reproduce by an ordinary division into two parts, the products being always mononucleate.

2. *Division of the cytoplasm with an incomplete amitotic division of the nucleus.* In 3.2 percent of the cases, the Amoeba, after existing in the binucleate condition for an average period of 5.5 days, divided into two parts, each of the products being mononucleate. These nuclei, however, instead of being smooth discoids, as were those of the parent, were bent or folded. The amount of the abnormality in the shape of the nucleus varied from a small protuberance at one end to a complete folding of the nucleus on itself. In the latter condition, two nuclei seemed to be joined together, or conjugating. But that this is not the case is shown at the next division, the products of which continued to be two Amoebae, each with a bent nucleus. One line was pedigreed for 35 generations, and a race of Amoebae with a tendency to possess bent nuclei was produced. Some of the members of this line had normal nuclei, but the majority possessed bent nuclei.

These Amoebae are large,—about 1.5 times the size of an Amoeba with a smooth nucleus. They are less viable and have a slower rate of division than the forms with normal nuclei. It is assumed that this condition is brought about by an incomplete amitotic division of the nucleus. In a recent publication, TAYLOR (1923) finds cytological evidence which indicates that an incomplete amitotic division of the nucleus occurs in *Amoeba proteus*.

3. *Division of both nuclei without a division of the cytoplasm.* In 4 cases, or 1.71 percent of the total number of binucleate Amoebae that were isolated, examination after about five days showed that the organism contained four small nuclei instead of the two large ones that were present upon the previous examination. There had evidently taken place in the interim, a division of the nuclei without a division of the cytoplasm.

FIGURE 4.—Camera lucida sketches of the division of a binucleate Amoeba into a larger and smaller portion, the former with 3 nuclei (n), the latter with but 1. a, parent, about an hour before division; b, progeny, about an hour after division.

4. *Division of the cytoplasm with a division of both nuclei.* (a) In 5.7 percent of the cases, the Amoeba, after existing in the binucleate condition for an average period of 8.4 days, divided into 2 binucleate Amoebae.

(b) In 4.5 percent of the cases, the Amoeba, after existing in the binucleate condition for an average period of 8.7 days, divided into two unequal parts; the smaller one being mononucleate, the larger portion trinucleate (figure 4).

(c) In 138 cases, or 55.6 percent the Amoeba, after existing in the binucleate condition for an average period of 6.3 days, divided into three Amoebae, the largest one being binucleate, the two smaller ones mononucleate (figure 5). This, as the figure shows, occurred by a direct division

FIGURE 5.—Camera lucida sketches of the division of a binucleate Amoeba into three,—two each with one nucleus, the third with two nuclei. a, one day before division. b, during division, with separating parts marked A, B, C; c, progeny, 25 minutes after division.

into three parts, confirming the earlier observations of STOLC (1905). One of the mononucleate parts was the first to break away from the connecting strand; the remaining two parts separated within the next minute.

(d) In 32 cases, or 12.9 percent of the total 248, the Amoeba after existing in the binucleate condition for an average period of 7.3 days,

divided into four mononucleate Amoebae. Although a simultaneous division into four parts was not observed, it is probable that this is what actually occurred.

Resumé

The most common method of reproduction of the binucleate Amoebae is that in which a division of the cytoplasm and both of the nuclei occurs. 78.6 percent of the 248 binucleate forms divided in this manner. Two general classes of divisions can be distinguished in this group, depending upon the rates of division of the two nuclei. The nuclei may divide simultaneously, or the division of one may lag behind the division of the other. The production of four mononucleate Amoebae, by a direct division, which occurred in but 16 percent of the 195 cases in this category, is probably the result of a simultaneous division of the nuclei. More than 70 percent of the remainder produced, by a direct division into three parts, one binucleate and two mononucleate Amoebae. This method of division is probably brought about by a slight difference in the rate of division of the two nuclei. One of the nuclei divides, producing a trinucleate Amoeba, which immediately begins to separate into three unequal mononuclear parts. During the triple division of the cytoplasm, the other nucleus divides, but a further division of the cytoplasm is prevented by the one already in progress. The products of such a division, if permitted to go to completion, are one large binucleate form, and two smaller mononucleate forms.

Experimental modification

This process, however, can be modified by the use of the evaporating-drop method (page 141). While the triple cytoplasmic division is in progress (figure 5, b) the parts which will eventually form the binucleate portion, A, and the mononucleate portions, B and C, can be identified by their differences in size. If the dividing Amoeba is so oriented in a minute drop that the mononucleate part, B, can not send out its pseudopods, the streaming of its protoplasm can proceed in only one direction, through the connecting strand into either A or C. If it flows into the mononucleate part, C, division into two binucleate Amoebae results. If, on the other hand, the protoplasm of B flows into the binucleate part, A, again a division into two Amoebae occurs, one of which is mononucleate, the other trinucleate (figure 4). The frequency with which these two methods of division occur under natural conditions is approximately the same. Finally, the protoplasm of both the mononucleate portions, B and C, can be made to flow into the binucleate part, A, causing the production

of a tetranucleate Amoeba. This occurs in very few cases. Although these experiments were modified in various ways, no other methods of division than those already described could be obtained. It seems, therefore, that the trinucleate and the tetranucleate Amoebae originate, like the binucleate, by conditions that prevent the process of division of the cytoplasm from going to completion.

Division of the trinucleate Amoebae

Only 22 trinucleate Amoebae were available for the study of the methods of reproduction of these forms. Five of these Amoebae did not divide, but disintegrated, having lived in the culture medium for an average period of 29.4 days. The length of life (from the date of origin to disintegration) of a trinucleate Amoeba is therefore about three times that of a binucleate form, and nearly six times that of a mononucleate Amoeba. The mortality is also greater than that of the binucleate and mononucleate forms. The methods of division of the trinucleate Amoebae are even more variable than are those of the binucleate Amoebae:

1. *Division of the cytoplasm with the division of only one of the nuclei.* In one case, the Amoeba existed in the trinucleate condition for 8 days. It then divided into two binucleate Amoebae. Each of the parts contained one large undivided nucleus and one of the products of the divided nucleus.

2. *Division of the cytoplasm with the partial division of only one of the nuclei.* In another case, the Amoeba existed in the trinucleate condition for 8 days. It then divided into two Amoebae. One of the parts was mononuclear, the other contained one smooth and one bent or folded nucleus.

3. *Division of the cytoplasm with a division of all the nuclei.* (a) In one case, the Amoeba existed in the trinucleate condition for 4 days. It then divided into three unequal binucleate Amoebae.

(b) In three cases, the Amoeba existed in the trinucleate condition for an average period of 7 days; it then divided into three Amoebae, one trinucleate, one binucleate and one mononucleate (figure 6).

(c) In two cases, the Amoeba existed in the trinucleate condition for an average period of 15 days; it then divided into three parts, two of which were mononucleate, the third was a large tetranucleate Amoeba.

(d) In six cases, the Amoeba existed in the trinucleate condition for an average period of 7.3 days; it then divided into four parts. The largest portion was trinucleate, the three smaller Amoebae were mononucleate.

(e) In another case the Amoeba existed in the trinucleate condition for 5 days; it then divided into four parts. The larger two were binucleate, the other two were each mononucleate.

(f) In two cases the Amoeba existed in the trinucleate condition for an average period of 7 days; it then divided into five Amoebae. Four of these were mononucleate; the other was binucleate.

FIGURE 6.—Camera lucida sketches of the division of a trinucleate Amoeba into three progeny, containing respectively 1, 2 and 3 nuclei. a, parent 3 days before division; b, progeny about an hour after division.

Resumé

Thus the most common method of reproduction in the trinucleate Amoebae is again a division of the cytoplasm accompanied by a division of all the nuclei. 68.2 percent of the 22 trinucleate Amoebae that were isolated divided in this manner. The great variation in the number of cytoplasmic products, and the distribution of the daughter nuclei to them is probably determined by the differences in the rates of division of the three parent nuclei. The three nuclei may divide simultaneously; two of them may divide together; or, all three may divide at different times. The tendency seems to be for two of the nuclei to divide simultaneously. A pentanucleate Amoeba would thus be produced and it will immediately begin to divide into five parts. While the cytoplasmic division is in progress, the third of the parent nuclei divides, but a further division of the cytoplasm does not occur. The products of such a division would be four mononucleate and one binucleate Amoeba. This has actually occurred in only two of the cases. In the majority of the cases (80 percent of the 15 divisions described in group 3) the cytoplasm divides into only three or four parts, and generally at least one of these portions is either trinucleate or tetranucleate. There are, therefore, indications that processes similar to those going on during an evaporating-drop experiment, modify the

method of division of the trinucleate Amoebae. The observations, which were limited to the divisions of but 17 organisms, represent but a small proportion of the total possible methods of reproduction of the trinucleate Amoebae. There are many more, depending upon the nature of the nuclear division, the number of nuclei that divide, and their relative rates of division, the number of cytoplasmic products, and the distribution of the nuclei to these parts.

Division of the tetranucleate Amoebae

Five out of the eight tetranucleate Amoebae that were isolated died, having lived in the culture medium for an average period of 19.8 days. The remainder divided in an irregular manner:

(1) *Division of the cytoplasm without a division of any of the nuclei.* In one case, the Amoeba existed in the tetranucleate condition for 1 day. It then divided into one large mononucleate Amoeba and a still larger trinucleate form.

(2) *Division of the cytoplasm with a division of only one of the nuclei.* In another case, the Amoeba existed in the tetranucleate condition for 14 days. It then divided into one trinucleate and two mononucleate Amoebae.

(3) *Division of part of the nuclei without a division of the cytoplasm.* Lastly, an Amoeba existed in the tetranucleate condition for 8 days. It was now found to contain 6 nuclei. Two of the four nuclei of the parent had divided but the cytoplasmic division had been prevented, resulting in the production of a hexanucleate Amoeba. The latter organism disintegrated after a length of life of 22 days.

The rarity of appearance of the tetranucleate Amoeba, and the high mortality (62.5 percent amongst those isolated), make an adequate study of the method of reproduction in these forms extremely difficult.

SUMMARY

1. The granular Amoebae (*Amoeba proteus* according to SCHAEFFER) and the raptorial Amoebae (*A. dubia* SCHAEFFER), pedigreed for 40 generations, were found to retain, throughout, their differential characteristics (confirmation of SCHAEFFER and others).

2. In the raptorial Amoebae (626 cases) the average period between fissions was 1.6 days. In the granular Amoebae (846 cases) it was 2.5 days.

3. In all the lines studied, the reproduction was rhythmic, periods of high reproductive rate alternating with periods of low reproductive rate,

as in infusoria. This seems to indicate that some process akin to endomixis occurs periodically in *Amoeba*.

4. The rates of division of sister lines of the same race of *Amoeba proteus* when kept under the same environmental conditions vary; but these variations are less than those between different races kept under the same conditions, and less than those between lines of the same race kept under different conditions.

5. An abnormal (bent) condition of the nucleus has a considerable lowering effect on the division rate, which persists even after the normal condition of the nucleus has returned.

6. The division rate and the vitality of the organism are lowered, when the *Amoebae* are kept for long periods in the same culture fluid. The optimum conditions for reproductive activity were obtained by daily transfers.

7. Four sets of long-continued experiments show that the rhythmic character of the divisions in *Amoeba proteus* is similar for all the races that were studied, both under the same and under different environmental conditions.

8. A change in the environmental condition, therefore, affects the division rate, but not the periods of high and low reproductive activity. This is similar to the condition found in infusoria.

9. Polynucleate *Amoebae* appeared in isolation pedigree cultures of *Amoeba proteus*. They also appeared in mass cultures upon certain modifications of the culture medium.

10. There is no evidence to show that the polynucleate forms arise by a fusion of mononucleate cells. The evidence indicates that there has been a division of the nucleus without a division of the cytoplasm.

11. An experimental method is presented by which it is possible to prevent or modify the division of the cytoplasm, and thus to cause the production of polynucleate *Amoebae*. It is suggested that something similar to this is the method of origin of these forms in nature and in laboratory cultures.

12. An extensive study was made of the methods of division in polynucleate *Amoebae*, based upon observation of 248 binucleate, 22 trinucleate and 8 tetranucleate individuals. In general this confirmed the earlier observations of STOLC (1905), but yielded certain methods not observed by STOLC. These very numerous ways of dividing are all modifications of the following:

- (a) Division of the cytoplasm with incomplete division of all the nuclei.

- (b) Division of the cytoplasm with incomplete division of only part of the nuclei.
 - (c) Division of all the nuclei without a division of the cytoplasm.
 - (d) Division of part of the nuclei without a division of the cytoplasm.
13. An increase in the number of nuclei is accompanied by a decrease in the division rate. An increase in the number of nuclei is accompanied also by an increase in the mortality rate.

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