The Effect of Fission on the Wavy Path of the Ameba Mayorella bigemma

by

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Introduction

It is the purpose of this paper to analyze the factors involved in the wavy path of the ameba, Mayorella bigemma in so far as the division process is concerned. In order to do this it is necessary to consider many related factors which bear directly or indirectly on the formation of the waves. Among the factors considered are light, temperature size, speed and Y and V forms of the ameba. Although a large mass of data has been collected the limitations on this paper require the use of a relatively small number of amebas actually studied; i. e. those which chanced to divide during the five or six hours they were observed. Because of this fact, much of the following discussion concerns itself with individual casesrecords which appear to be signiffcant but must be considered as suggestive of future lines of study rather than definite conclusions.

However in many cases where a high degree of correlation among all the individuals was noticed the conclusions may be considered as fairly well established.

Historical Review

Rosel V. Rosennof, (1755) who wrote the first description of amebas, was also the first to notice it's peculiar method of movement. He recognized that the form changes were accompanied by the streaming of endoplasm. This was the first and most important observation on ameboid movement.

Corti, (1774) discovered a similar streaming in chara and other plants but this streaming was not accompanied by locomotion. Wallich (1863) discovered that a new pseudopod was formed by a break in the ectoplasm through which the endoplasm flowed, and that some of the endoplasm was changed to ectoplasm.aButschli (1880) showed that endoplasm was changed to ectoplasm at the anterior end of each pseudopod and that endoplasm was formed from ectoplasm at the posterior end of the ameba. Blochman (1894) demonstrated the presence of a third layer. This layer however seems to have no particular significance in locomotion.

Many attempts have been made to explain the cause of ameboid movement but no theory as yet suggested can satisfy all the conditions. Differences in surface tension, between ectoplasm and endoplasm or between the ameba and the surrounding medium have been chiefly relied upon. Contractility, imbibition and change of colloidal state have been commonly suggested. All of

these theories have been based on the assumption "that if one could explain movement at any particular cross section of time one understood the whole process." Schaeffer '20 discovered that "the path of an ameba as it moves on a flat surface free from particles consists of a succession of gentle right and left hand curves alternating with each other". The general appearance of the path is that of a flattened spiral. Having observed a part of an amebas path, therefore one can predict with considerable accuracy in what direction the ameba will continue to move.

Although a new observation in so far as amebas are concerned, there exist many earlier records of spiral movement in both plants and animals. The earliest record is that of Nageli (1860) who observed the spiral paths of flagellates and swarm spores. Kent (1882) records a similar condition in ciliates but seemed to regard it as of no special importance. Jennings (1901) who was first to study into the cause of spiral swimming, regarded it as an acquired habit to keep the animals from swimming in circles due to their asymmetrical form.

Schaeffer (1920) found that a tendency to move in spirals was present in a large number of forms, both plant and animal, (including male protozoa sperm cells a nd larvae of various animals and man) and suggested that "all moving organisms are subject to a tendency to move in a spiral path". He concluded that all animals without

orienting senses or equiliberating organs, moved in orderly paths due to the presence of some automatic directive mechanism.

Bullington (1925) who studied movement in one hundred sixty species of ciliates, found the kind of spiral was characteristic for each species, the direction of turning was constant for each species, (i. e. always either to the left or to the right) and that left spiraling is more characteristic and therefore more perfect expression of the spiralizing mechanism.

This tendency to move in spirals manifests itself in three ways; it may be a helical spiral, as in free swimming protozoa, it may be a true spiral on a flat surface as in man, or it may be a projection of helical spiral on a flat surface as in the ameba.

Schaeffer (abs. 1926) placed amebas inside of and on glass tubes in order to determine whether or not the sinusoidal path of an ameba on a plane surface represents a tendency to move in a helical spiral. He found a tendency to move around either the inside or the outside surface at an angle of about thirty degrees. These results not only furnish evidence that an ameba would move in helical spiral if it were not limited to movement on the surface but offers a possible explaination for the ameba in contrast to the clock spring spiral demonstrated in man. The ameba moves either to the right or to the left in direct contrast to the single direction found by

Materials

The ameba used in these experiments is Mayorella bigemma Schaeffer, a fresh water ameba, found commonly in quiet water where large masses of vegetation are undergoing decay. The cultures used in these experiments were started from cultures growing in the laboratory. The Mayorella bigemma found in this locality are smaller than the description would lead one to expect. The size varies considerably but is usually between 60 and 100 microns long.

The shape of this ameba during locomotion is triangular with the broad end foreward and flattened. The pseudopods are determinate (i. e. limitted in size and do not give direction to locomotion) and the anterior end of the ameba seems to advance at about the same rate as the pseudopods, presenting a web-like appearance. The ameba advances by a series of waves, the ectoplasm pushing forward and connecting several pseudopods then pausing momentarily while the pseudopods advance or new ones are formed.

The flowing of the endoplasm is jerky and irregular, indicating the presence of numerous obstructions to the steady flow. This is more readily understood when one notices that the upper surface is extremely irregular being a disorderly mass of high ridges and deep depress-

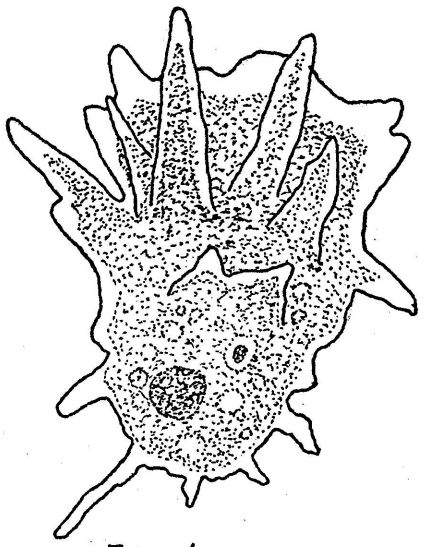


Fig 1.
Mayorella bigemma
(after Schaeffer)

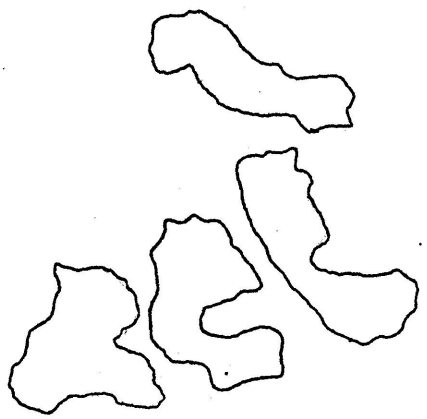


Fig. 2.

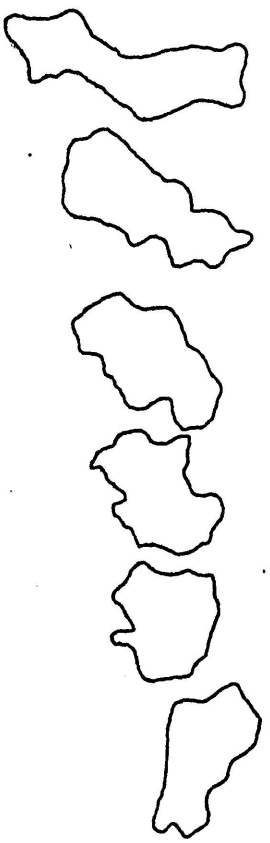


Fig 3.

ions. More or less permanent pillars of stiffened ectoplasm connect these depressions with the lower surface of the ameba. When these pillars give way the bottom of the depressions is suddenly pushed upward indicating that the endoplasm is under considerable internal pressure.

Occasionally during locomotion it assumes a Y or V shape and as the posterior end advances the ameba becomes stretched to several times its normal length. After a time one end breaks loose from the substratum and the ameba continues in a new direction usually at right angles to the former path. This seems to come about in two ways. In the first instance a split is noticed in the web and both ends continue to flow forward and outward until only a slender strand connects the two halves. (Fig. 3) But one half always gives way and the whole ameba starts off in the opposite direction. Another less common condition is brought about in this way. A part of the posterior end becomes securely fastened to the substratum and some of the endoplasm starts to flow in that direction until the connecting strand is so small that it looks as though it would split apart at any moment. But always as in the preceeding case one end gives way and the flowing of the endoplasm becomes coordinated.

It would seem that under these conditions a well ordered path would be out of the question but this is not the case. As will be shown later this ameba presents throughout practically its whole path a succession of

curves, alternating to the right and left.

Methods

Methods of Collecting Data:

Two microscopes were used in these experiments: a Spencer binocular and a Bauch and Lomb compound. A camera lucida was attached to the eyepiece and outlin drawings were made at regular intervals. (the frequency was varied according to the speed of the ameba but was usually one drawing per minute.)

All drawings were made by beginning on the side of the ameba and going around the posterior end first. About six seconds were consumed in any one drawing. In the first few ixperiments the ameba was placed in a large drop of water on a two by three inch slide. This necessitated replacing water as it evaporated so was later discontinued and a glass box was made from a two by three inch slide with sides cemented on with Dekhotinsky Cement. In the course of a single experiment it was necessary to move the slide many times in order to keep the ameba in the field. Squares were marked on the stage of the microscope which were used as guide lines when it was necessary to move the slide. This made it possible to keep the slide paralell to the edge of the stage. The paper was kept parallel to the edge of the table and the camera lucida adjusted to the same angle.

This made it possible to fasten together several segments of the path of a single ameba keeping the direction uniform.

Unless otherwise stated the ameba was placed in dialized culture medium from the culture in which the ameba grew. To dialize a fluid a celloidin tube filled with distilled water is placed in the culture medium for several hours. This allows for the equalization of dissolved salts but no solid particles can pass through.

As "pedigreed" amebas are grown normally in a few drops of fluid it is necessary to use dialized media from similar culture when these are used in experiments. This was done in order to furnish a medium as nearly as possible like the normal fluid but omitting food debris and all solid particles in order to prevent interference with the normal movement by formation of food cups, ingestion or other contacts.

Culture Methods:

The first attempts toward making pedigreed cultures of Mayorella bigemma were made by following the method of Miss Botsford '22 using standard beef extract (see Woodruff and Baitsell 1911) as a culture medium, but although although several attempts were made no satisfactory pedigrees were obtained. It is probable that no satisfactory bacterial flora was developed in these cultures and the ameba could not secure sufficient food.

Chilomonas cultures were tried with varying degrees of success. Chilomonas growing in the laboratory in

hay infusion cultures were fairly successful but in any given culture they would die down in a few days and it was necessary to replace these with new cultures constantly. Certain differences existed in these cultures causing a constant necessity to readjustment on the part of the ameba and they were early discontinued. The same conditions prevailed in cultures of Chilomonas which developed after a few days in water brought in from outside ditches, etc. However when Chilomonas were raised in a solution, made by boiling six grains of wheat in a test tube of water with a few cracked grains left in, they grew well. The bacteria that grew in this fluid had a tendency to gum on the bottom of the dish and it was hard to tear the ameba loose and clogged the pipette when trying to pick them up. As a simpler satisfactory method was found later these tests were discontinued.

Yeast furnished the most satisfactory medium. About two cubic millimeters of yeast dissolved in 50c.c. of distilled water furnished a very satisfactory food for the ameba, with the added convenience of easy handling. In these cultures a small naked foraminiferan often appeared. These were the cultures in which the ameba grew best. A small chilodon was also seen commonly. The source of these forms was never quite clear as they were not common elsewhere in the laboratory.

The general cultures were grown in watch glasses or deep petri dishes and records made regarding the

size of the amebas and the condition of the culture over a period of about two months while the above tests were carried on.

An attempt to raise pedigree stock by the isolation method met with varying success, according to the food culture, etc. One line was pedigreed for nearly two months and barring accidental contamination almost all the amebas used were descended from this line. For isolation pedigrees a few drops of culture media were placed on a concave slide and the slides were dept in a moist chamber to prevent evaporation. Every time an ameba divided the daughters were transferred to another slid and a record kept of each division.

Measuring Data:

All measurements were made with a standard metric rule and were correct to the nearest half-millimeter. A scale was made for each lens combination used in order. To reduce this apparent distance as represented in the drawings and graphs to actual distance in microns. A slide was placed on the stage of the microscope at the same elevation as the ameba had been and a standard metric ruler on the paper in order to determine the magnification of the apparatus. As several lens combinations were used all figures in Table I were reduced to microns in order to get them on a comparative basis. Graphs and drawings, however, show apparent figures only.

In this paper the length of the ameba while in locomotion was considered a criterion of its size.

In order to determine as nearly as possible the size of the ameba a measurement was made of every fifth drawing of any one series and the average was calculated. In very short tracks more frequent intervals were used. An attempt was made to avoid crescent or elongated shapes in measuring for size.

In arriving at the actual size of the ameba certain difficulties were incountered. In the first place, the ameba was always moving while the drawing was being made. This, in itself, made a slight error unavoidable. However, all drawings were made by drawing around the posterior end first making the error always in the direction of increased size. In a fairly rapidly moving ameba drawn with the low power of the compound microscope the ameba moves about one-fifth its length in the period required to draw it. This indicates that each ameba is measured about one-tenth larger than its actual length.

A second difficulty is apparent when one notices the unusual V and Y forms of this ameba but these shapes were ordinarily avoided in determining the size of any ameba.

The uneveness of the upper surface was a third factor for which no allowance could be made in the calculations but which must be kept in mind when attempting to determine the ameba's actual size.

Incidental Observations

Fission Process:

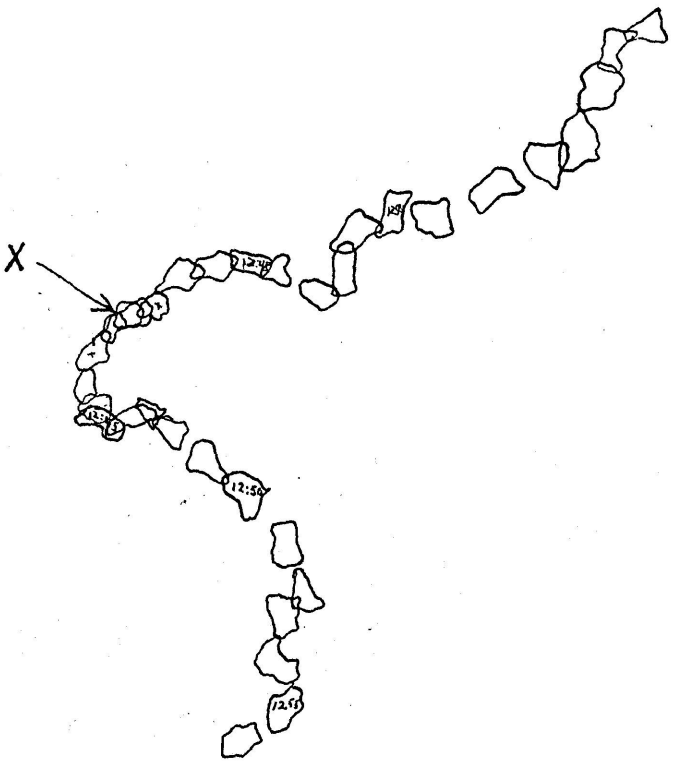
Miss Botsford ('22) who kept isolation pedigree cultures of Mayorella bigemma through one hundred generations found;

1. That vegetative division was never interrupted by spor or gamete formation.
2. That the rate of division fluctuates considerably (it usually required from one to three days for a division as often as three times in a day or as seldom as once in twelve days.)
3. That by dividing its entire pedigree into five day periods a rhythmical character in fission rate could be observed. The low periods in fission came about every twentieth day.

In the course of these experiments the actual process of division was observed many times. The first observable characteristic of the onset of the division crisis is a gradual decrease in the speed of movement of the ameba. The ameba soon ceases to move altogether and forms into a spherical mass with many small projections extending in all directions. After a few minutes a light line appears across the middle or equatorially to the mass indicating the beginning of cytoplasmic division. A short time later the two daughter amebas move apart and division is complete. The actual time that the ameba remains in this rounded state is usually between ten and fifteen minutes.

The two daughter amebas are practically equal in size and seem to be considerably more than half the size of the original ameba in so far as surface appearance is concerned. In order to determine the expected relationship in size or the parent to the daughter amebas a simple formula was employed. $\sqrt[3]{\frac{\text{Length of ameba}^3}{2}}$ equal size of daughter amebas if they divided equally and are measured while in a similar shape geometrically.
As mentioned earlier the "actual length" of any ameba was determined from averages of many measurements and in this manner the chances for error are reduced. By comparing the actual length of each ameba, as measured, with the expected length determined by calculation a remarkable correspondence in size may be noted. (Table I items 8, 5A and 5B.)

In occasional instances a rapid increase in size after division as represented in the drawings is noticable. As the ameba was in a medium free from food particles this could not be an ordinary growth process. Fig. 4 shows rapid increase in size of ameba four during first few minutes after division. Two explainations are suggested. This increase in size may be <u>actual</u> due to the imbibition of water or it may be only <u>apparent</u> due to the flattening of the ameba against the slide a few minutes after division. It is quite possible that both factors influence this increase during the first hour. As an illustration it is interesting to note that in ameba 4B (which was drawn for only sixteen minutes


X
12:30
12:50
12:55

after division and the average length determined from these drawings) the actual length is exactly equal to the expected length and that in ameba 4A. (40 min long) the actual size is about eight minutes longer than the expected and longer than the sister ameba.

Light Reactions:

Numerous investigators have shown that light affects the direction and rate of movement in amebas. Verworn ('89) who was the first to test the effect of light on amebas however working with different intensities and different colors of light, found no effect upon direction or rate when the beams of light were perpindicular to the slide on which the ameba moved. But Davenport ('97) who used horizontal beams of very intense light found that the ameba moved away from the source of light. Mast ('10) likewise found Amoeba proteus (Chaos deffluens?) negative to strong light. Schaeffer ('17) found Chaos deffluens sometimes positive and sometimes negative depending upon intensity and area of the beam and other comditions, to small horizontal beams of light. Schaeffer ('20) studied the influence of light on the spiral path of Metachaos discoides and Chaos deffluens. By alternating dark and light periods (turning on the light about once in five minutes only long enough to make a camera lucida drawing) he determined that when no light was present these amebas followed a definitely wavy path.

Early in the course of these experiments it was

observed that Mayorella bigemma moves directly away from the light. The work was done in front of a large window with northwest light and never with the direct rays of the sun.

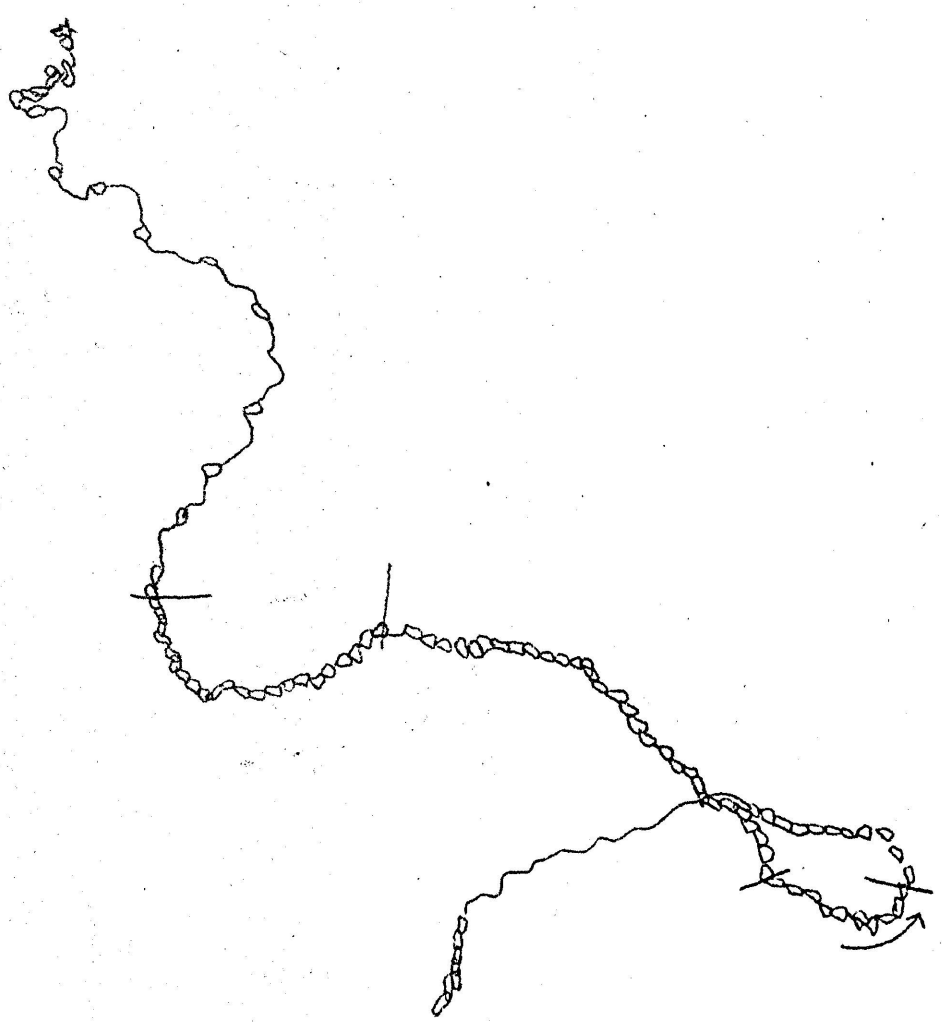
In order to determine the influence of light on the path the following tests were made. After the path of the ameba was established in the direction away from the light, a small book was placed on the stage and retained in this position until the direction of movement was changed from the original. The book was then removed and the ameba again orinted itself in the former direction. This was repeated several times in succession with the same results. Fig. 5 illustrates the change of direction of one individual under these conditions.

In order to make certain that there were no localized differences in the fluid on the slide or other external reasons for continuing in one direction, the box was turned half around so that the ameba was turned in the direction toward the light. But it soon reversed its direction so that it was again negative to ordinary diffuse light.

To avoid this directing influence of horizontal beams of light a dark box was constructed of heavy cardboard to cover the slide on the stage. A hole was made on the upper surface for the insertion of the objective of the microscope. The box was painted

Black inside and edged with felt to insure its fitting tightly against the stage of the microscope. A covering was also made for the objective which fitted smoothly against the top of the box. Thus the only light which could enter were the perpindicular rays reflected from the mirror of the microscope. When using this apparatus the direction of movement varied, that is no ameba continued in the same general direction for the whole of its path. However, no difference could be detected on the shape and size of the waves between those made while influenced by horizontal light and those made while illuminated only by the mirror. So that although horizontal light influenced the general direction of the path as a whole it did not influence the character of the rays in any way. As the use of the dark box caused considerable trouble when it was necessary to move the slide and did not influence the accuracy of the observation it was discarded.

There was on the other hand, one additional advantage in continuing the work in ordinary light. When an ameba divides, the two daughter amebas ordinarily move in opposite directions making it necessary to discontinue following one in a few minutes after division, because of its getting out of the field, but when light is a factor both daughter amebas soon become oriented and travel in the same direction away from the light. This makes it possible to trace both



amebas for as long as two hours without either getting out of the microscopic field.

Temperature:

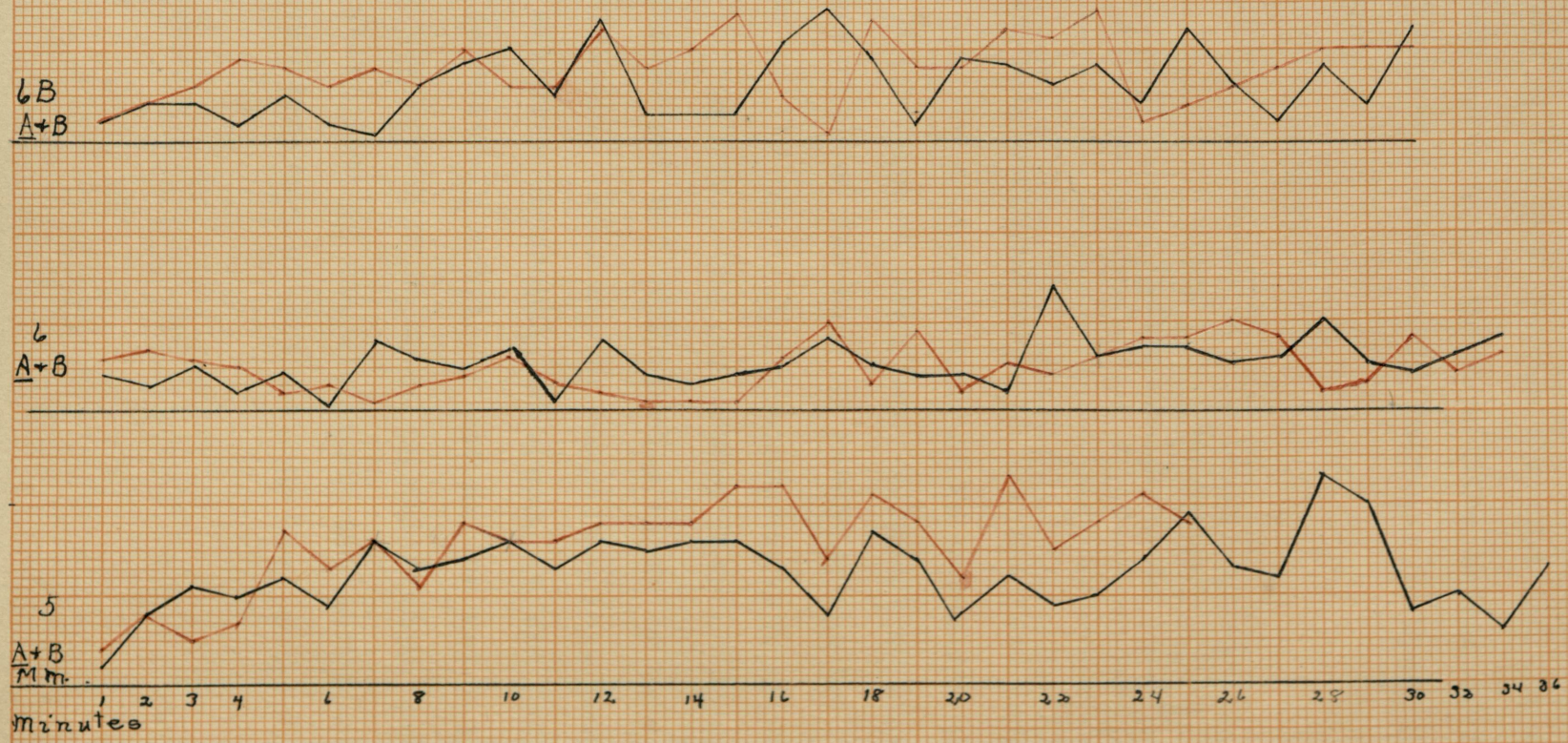
Pantin ('24) has shown that the velocity of an ameba varies with the temperature in a similar manner to many other biological processes. The velocity is greatest at an optimum temperature above which some part of the mechanism is possibly of the nature of an enzyme though the optimum is low. He found the optimum temperature for two species of limas ameba to be 22 C. for one and 20 C. for the other species. Schaeffer ('24) found that the optimum for a species of Cochliopoduim was near 37 C. and Allen ('27) found the optimum for Mayorella Conipes about 39 C. Allen also noticed a much greater variation of speed from minute to minute near the optimum than at lower temperatures and in connection with this greater variation an increased tendency to form the "crescent" shapes so characteristic of the Genus Mayorella.

The frequency of these shapes in certain paths of Mayorella bigemma suggested the idea that room temperature was probably near the optimum for this ameba. A preliminary test on one ameba (only two generations removed from one (6B) which showed an exceptional number of crescents) showed that the optimum for Mayorella bigemma is close to 27 C.

TABLE I

Ameba No.	1	2	3	4	5	6	6B	7
1. % shortening of curves in 2 hrs. ⟶ X	110%							
2. Asymmetry in length of curves.	+2	+ 2		87				
	120min.	8"		83			50min.	
3. Rate in μ per min. for period. ⟶ X	46	87		87			66	
4. Rate in μ per min. for 15 min. ⟶ X	44	70		83			51	
5. Actual length of ameba.	63μ	93μ		83μ			119μ	
6. Temperature.		27°-31°C						
7. Division time.	18min	8min.		9½min.			8 min.	
8. Expected length of daughter amebas	50	74		65			94	
2A. Asymmetry in length of curves. ⟵ X	—	+	+1					
	69min.	20 min.	120 min.	40min.	70min.	84 min.		
3A. Rate in μ per min. for 15 min. ⟵ X	43	76	87	81	97	63	50	51
4A. Rate in μ per min. for period ⟵ X	27	80	63	66	92	44	52	52
5A. Actual length of ameba.	52	61	59	73	100	107	109	
2B. Asymmetry in length of curves. ⟵ X	+1	+3	+1					
	152 min.	43min.	120min.	46min.	28min.	84min.		
3B. Rate in μ per min for period. ⟵ X	39	93	95	73	93	63	59	54
4B. Rate in μ per min for 15 min. ⟵ X	38	59	98	72	84	31	60	47
5B. Actual length of ameba.	49	73	54	65	99	110	101	

Graph I


6B
A+B
6
A+B
5
A+B
Mm.
1 2 3 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Minutes

Experimental Results

Rate of Movement before division:

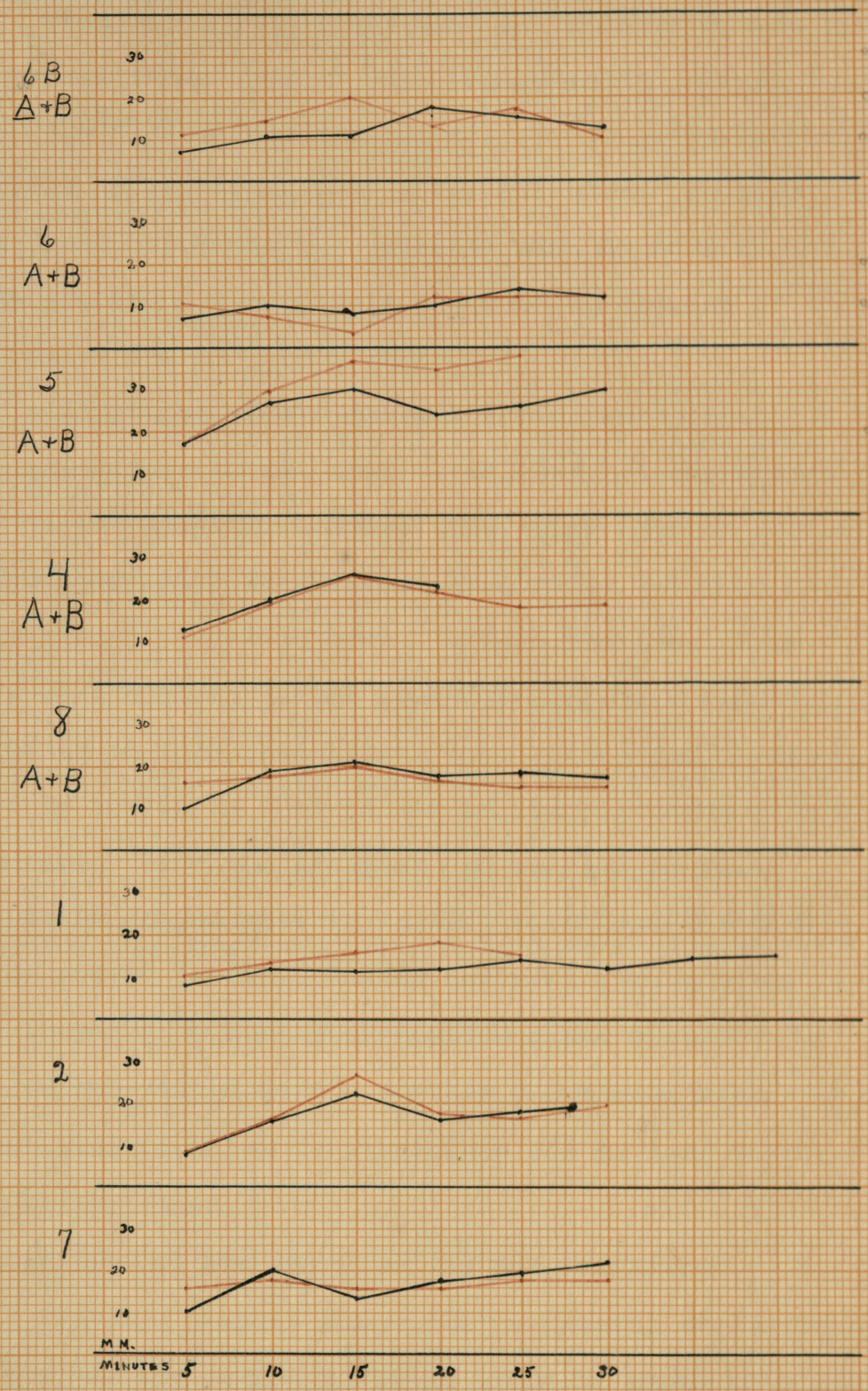
The rate of movement per minute was calculated for the whole period of the path before division and compared with the average speed for fifteen minutes before division. In each case there was a noticable decrease in speed just before fission.

Table II

Individual	Length	Total av. speed	15min.av. speed ~~decrease~~	% decrease
1	63 μ	46 μ	44 μ	4%
2	93 μ	87 μ	70 μ	20%
3	83 μ	87 μ	83 μ	5%
4	119 μ	66 μ	51 μ	23%

Table two shows this comparative rate for the four individuals in which all the nexessary details of movement were watched up to the minute of division. Although no significant average rate of decrease of movement can be arrived at from so few cases, the decreasing rate nevertheless holds throughout. The percentage rate of decrease is however variable and not sufficiently close to consider it at all significant. This may be corrected for, however, when the data are more thoroughly analyzed, as the fifteen minute period is entirely arbitrary and may be too long to account for the decrease in speed in all amebas, owing to individual variations as compared with the entire rate where these variations cancel out more or less completely.

Graph II


6 B
A+B
30
20
10
6
A+B
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5
A+B
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8
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10
1
30
20
10
2
30
20
10
7
30
20
10
M.M.
MINUTES 5
10
15
20
25
30

Rate of movement after fission:

When the speed of movement is measured minute by minute one finds more or less irregularity. Graph I shows the rate of movement in sixteen individuals for a period of thirty minutes after division. In each case the two individuals plotted side by side on the graph are the two daughters produced by fission of one parent ameba.

All graphs show a general variation in speed from one minute to the next which does not correspond for the two daughter amebas. They also show an initial slow period which is followed by a rapid increase in speed over a period of about fifteen minutes. This shows more clearly if one compares graph one with graph two which is identical except that each point is determined by averaging five points on the preceeding ~~xxxx~~graph. With the exception of one single ameba (6B) these graphs show an initial rise from the first to the second period. During the next five minutes this increase is sometimes continued and occasionally lowered but within twenty minutes a definite rate is established which is maintained for as long as the path is continued.

A comparison of the speed of the two daughters.

Although Graph I shows only a general correspondence between the two daughter amebas Graph II shows an almost perfect correlation in speed of movement of the two

amebas. Both daughters increase in speed until they are moving at about the same rate and then move at practically equal speed for the remainder of the path. Ameba five is an exception in so far as actual speed per minute is concerned. That is ameba 5B establishes its rate about 25 a minute faster than 5A. Nevertheless the general shape of the curves hold. A tentative explaination will be considered later. Ameba 6B shows an initial decrease in speed but an examination of the actual path shows a piling up of Y and V forms which always tend to make movement slower. The presence and influence of these forms has been discussed earlier.

Table III presents an interesting study of inheritance of the speed of movement. Ameba one which is 63 long moves at the rate of 46 per minute, which is 73% of its body length. The daughters 1A and 1B are 52 and 49 long and move at the rate of 43 39 respectively or 83 and 78 of body length. Ameba four however was moving at an average rate equalling 105% of its body length and its daughters 4A and 4B both moved 111% of body length. Thus the two individuals have not only identical hereditary characters, but are under identical conditions of temperature, light culturemedium etc. and moving on the same surface.

An effort was made to make all drawings with the amebas in the same type of medium. Therefore it is assumed

that the close correspondence of speed between individuals in each line (parent and two daughters) together with the variation in speed between one line and another is a definite proof of the necessity of isolation pedigrees in physiological experiments of this nature. To insure correct conclusions one must know the genetical history of the individual as well as the conditions under which it exists.

Wavy path:

It is well to bear in mind that the path of an ameba on a flat surface is not a perfect expression of the spiral mechanism and probably for this reason, it is difficult to find any high degree of correlation in the length of waves in the path of a single ameba.

In the case of almost every ameba used a definite series of waves could be detected throughout practically its whole length. (although a great many more were drawn this paper is based on the actual measurements of about twenty-five individuals) However, these waves were not a regular series but were often skewed or varied in length and depth.

To illustrate this statement several sections from the paths of various amebas are inserted. Fig. 6 illustrates the effect of numerous V and Y forms on the spiral path. An occasional form of this nature does not seem to influence the path to any great extent, but in some cases these forms pile up, cause the ameba to move very slowly for a time and act "undecided" as to the direction it wishes to take. These fast and slow periods alternate at about fifteen minute intervals for the entire length of the path. Split forms are also connected with the irregularities in the waves illustrated in Fig. 7.

When an ameba for any reason makes an unusually long wave this wave is almost invariably followed by a correspondingly short one. (waves 2 and 4) When this happens the curve opposite is naturally skewed (3).

Graph III

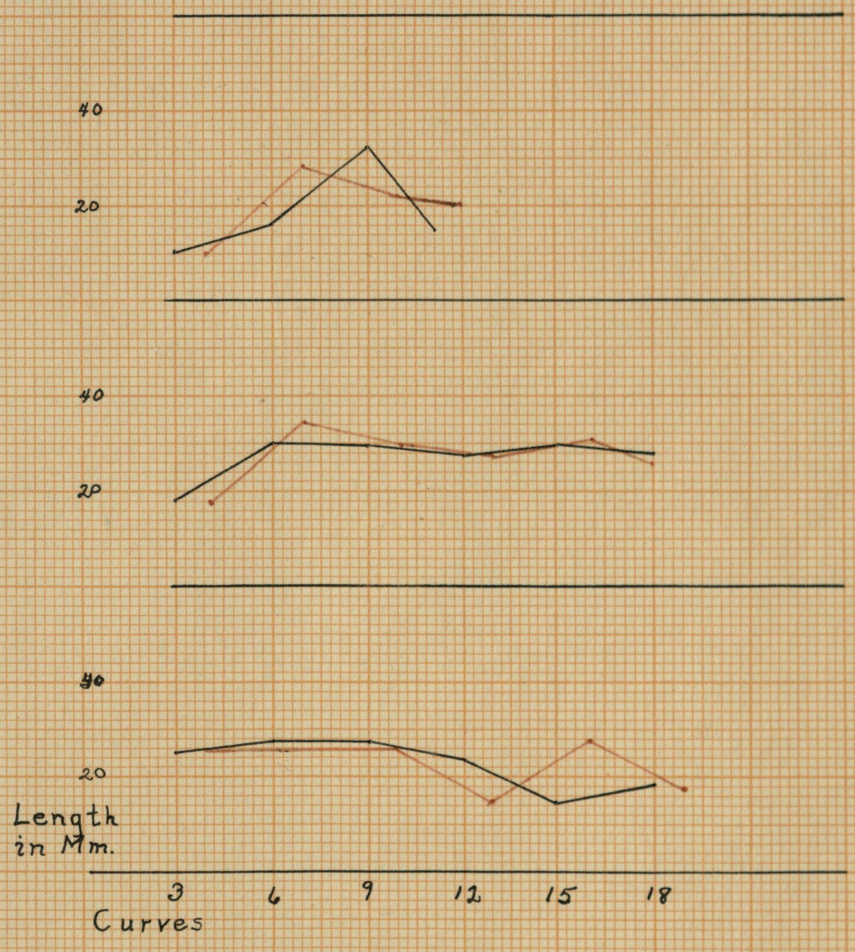


Fig. 6.

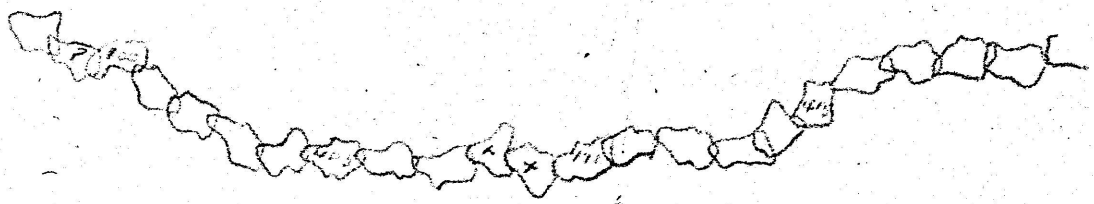


Fig. 7

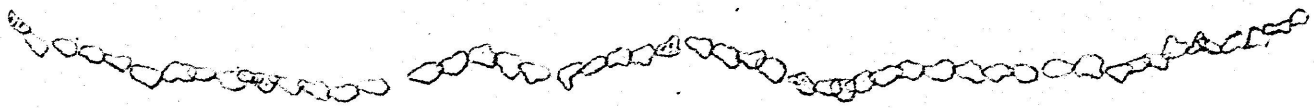
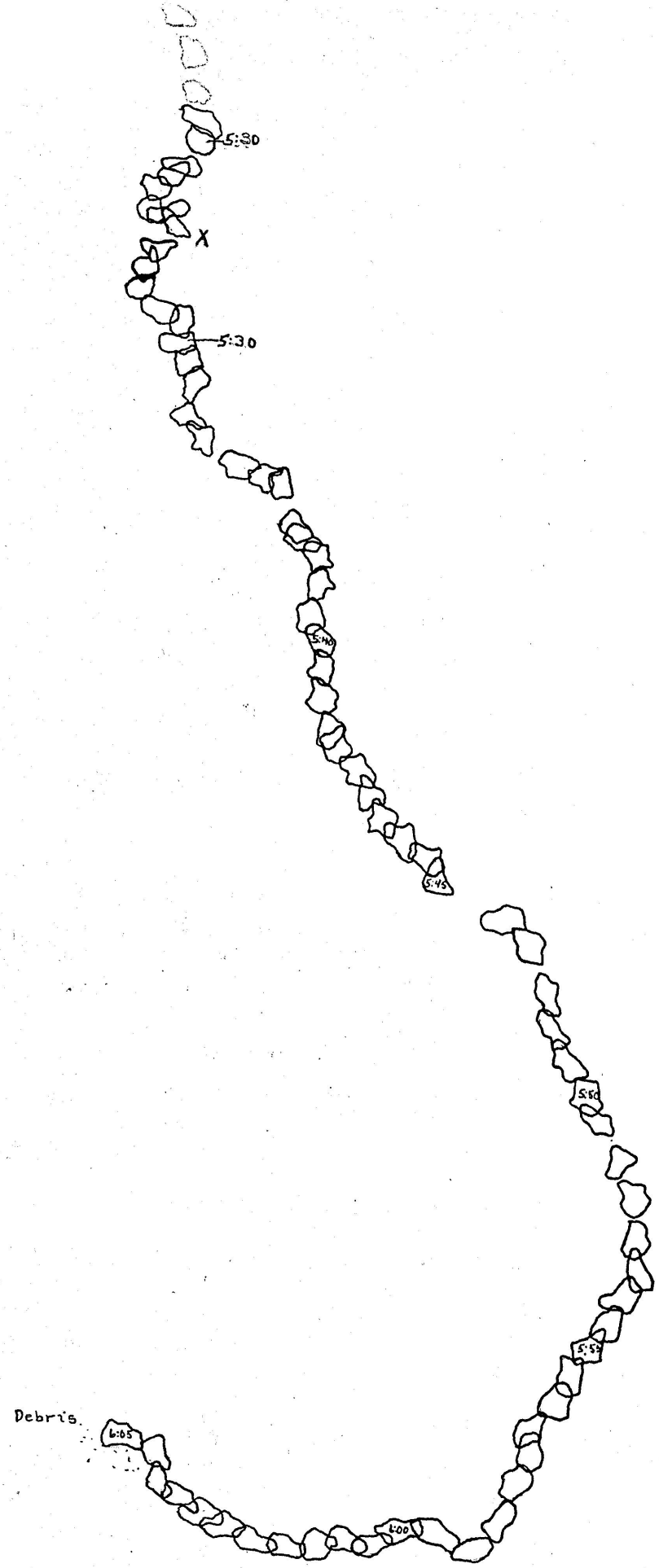


Fig. 8.


5:30
X
5:30
5:40
5:45
5:50
5:55
Debris
6:05
6:00

Another interesting condition is the flatness of the waves on the outside of the circle when the ameba tries to change its direction (wave 1 and 2) If the change of direction is more rapid a double curve is formed on the inside (see Schaeffer '20, Fig.33)

As explained in the discussion of temperature many crescent forms are present near the optimum. As these forms interfere with the working of the wave mechaniam one would expect that the wavy path was more irregular than where these did not appear. A rapidly moving ameba in which these forms do not appear shows the most regular curves.

The influence of division on the size of the waves is well shown in fig. 10 which shows one individual from the time of division till the path was ended.

As shown in fig. 7 the ends of the waves were connected by straight lines and the distance the ameba moved between these points was measured. These were plotted successively on a graph, one line being used for the odd numbered waves and another for the even numbered waves (in other words the waves on one side of the ameba were plotted in a straight line and alternated with those on the other side). Graph 3 shows a reduction of the original graph of one individual before and after division. This is typical of all individuals.

It shows a sudden reduction in the length of curves just before fission and an increase in length of curves of two daughter amebas after fission. As in speed this lasts just about 15 minutes or through the first two or three waves of the daughter amebas.

Conclusions

1. The wavy path is the normal method of locomotion in the ameba. This tendency to wave formation continues until the time of division and begins in the daughter amebas as soon as they separate.

2. The character of the waves is only slightly changed by the approach of fission, the only difference being a marked shortening of the waves for about fifteen minutes before.

3. After fission the opposite statement holds; the first 5 or 6 waves are short the others longer.

4. A rapidly moving ameba usually forms longer and more regular waves, except when a large numberof V. and Y. forms are noticed. The speed of movement is slower just before and after division. This is associated with small waves.

5. The tendency to V and Y forms usually maintains a balance with the tendency to form waves.

6. No difference was observed between the paths of amebas grown in chilomonas, wheat and those in yeast but in water the path showed a marked straightening.

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