

AN EXPERIMENTAL STUDY ON THE LIFE-HISTORY OF HYPOTRICHOUS INFUSORIA.¹

BY

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WITH 3 PLATES AND 12 FIGURES IN THE TEXT.

I. Introduction.....	585
II. General Methods and Technique.	587
III. Description of the Cultures.	590
1. <i>Oxytricha fallax</i> , Culture A.	590
2. <i>Oxytricha fallax</i> , Culture B.	594
3. <i>Pleurotricha lanceolata</i> , Culture A.	594
4. <i>Pleurotricha lanceolata</i> , Culture B.	596
5. <i>Gastrostyla steinii</i> , Culture A.	596
IV. Discussion of the Data of the Cultures.	601
1. Rhythmical and Cyclical Variation in the Rate of Division.	601
2. Artificial Rejuvenescence.	605
3. Conjugation.	606
V. Physiological and Morphological Variation during the Life-cycle.	607
1. Physiological Variation.	607
2. Morphological Variation.	608
VI. Effect of Initial and Daily Stimulation with Salts on the Rate of Division.	614
1. Potassium Phosphate (Monobasic and Dibasic).	615
2. Potassium Chlorid and Sodium Chlorid.	620
3. Potassium Sulphate and Magnesium Sulphate.	621
4. Potassium Bromid.	622
5. Comparison of Results.....	622
VII. Effect of Light on the Rate of Division.	625
VIII. Summary.	626

I. INTRODUCTION.

The first suggestion of the cyclical character of the life-history of Infusoria was advanced by Dujardin as an argument against Ehrenberg's theory that the Protozoa, because of their simple organization and method of reproduction, are not subject to natural death. The observations of Bütschli ('76) and Engel-

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mann ('76), that in infusorian cultures after a number of generations the organisms are reduced in size and show other signs of degeneration, were evidence in favor of Dujardin's theory. As is well known, however, Weismann ('84) greatly elaborated the theory of the potential immortality of unicellular organisms, maintaining, on *a priori* grounds, that the Protozoa, like the germ-cells of higher forms, are not subject to natural death. Maupas ('88; '89) in his classic researches on the life-history of Infusoria brought forward data which weighed heavily against Weismann's hypothesis. In his long-continued cultures he found marked evidence of "senile degeneration" and he confirmed the general conclusion of earlier workers as to the cyclical character of the life-history of certain species. More recently still Joukowsky ('98) and Simpson ('01) have investigated the life-histories of various forms, and Calkins ('02, 1, 2, 3; '04, 1) in a series of papers on *Paramœcium* has submitted strong evidence that this species passes through more or less regular periods of vigor and weakness, the periods of weakness invariably ending in the death of the culture unless the organisms are "stimulated" by conjugation or by changed environment. This work, besides throwing light on the rôle of conjugation in the life-cycle, gave the first experimental proof that various stimuli will "rejuvenate" the lagging functions of exhausted protoplasm and incite the *Paramœcia* to further periods of reproductive activity.

In the light of the previous investigations on the physiology of Infusoria, the following questions seem to be of sufficient importance to warrant still more extensive experimental work on different forms, in order to place the problems involved on a broader foundation:

1. Does the life-history of Infusoria, in general, run in cycles?
2. If so, will changes in the environment bring about renewed activity during depression-periods?
3. Will conjugation effect "rejuvenation"?
4. What are the physiological and morphological changes, if any, characteristic of declining vitality?
5. What effect has initial and daily application of various stimuli on the division-rate, *i. e.*, on the metabolic activity of protoplasm?
6. Is the division-rate affected by light?

The present investigation is an attempt to answer these ques-

tions as far as possible for hypotrichous Infusoria. With this in mind, experiments on five cultures of hypotrichous Ciliata, including *Oxytricha fallax*, *Pleurotricha lanceolata*, and *Gastrostyla steinii*, have been carried on during the last three years. Anticipating the conclusions, it may be stated briefly that the experiments offer affirmative evidence upon the first two points and negative evidence upon the last, while owing to failure of the infusorians to conjugate there is no evidence upon the third point. Regarding the fourth and fifth points, it may be said that morphological changes, particularly such as concern the cytoplasmic and macronuclear structures, are characteristic of declining vitality, and that initial and daily stimuli have a marked effect upon the metabolic activities of the forms studied.

I take pleasure in acknowledging my great indebtedness to Professor Gary N. Calkins, at whose suggestion this investigation was undertaken, for his advice and criticism throughout its prosecution. I also wish to express my thanks to Professor Edmund B. Wilson for many helpful suggestions.

II. GENERAL METHODS AND TECHNIQUE.

In the experiments on Protozoa here described, which have been followed continuously for the past three years, I have employed, with but slight change, the method used by Calkins ('02, 1) which is itself an improvement on the method of Maupas. As this method is described in detail by Calkins, a brief outline of it with my own modifications will suffice.

The organisms were cultivated on slides having a central circular concavity with a capacity of about five drops of water. Cover-glasses, used by Maupas and Calkins, were not employed, as it seemed to me that a more natural condition was obtained without them, and as I found that unless great care was exercised in cleaning the slips they afforded a possible source of contamination. The slides were kept in moist chambers to prevent evaporation of the preparations. These were ordinary stender dishes about ten inches in diameter and three inches deep. In the bottom of the dish was placed about two inches of wet sand. Over the sand was placed a glass plate on which rested four parallel strips of glass and on these the depression slides with the Protozoa were arranged. The whole was covered with a ground-glass top.

The Infusoria were handled with a pipet drawn out to a fine point. Each pipet was used for one purpose and only one. All of the Infusoria employed are of sufficient size to be seen readily with a lens having a magnification of about ten diameters, and as it is far more easy to operate with this than with a compound microscope, it was used almost entirely in transferring specimens with the pipet from one slide to another.

At first hay-infusion was employed as a culture-medium, but later it was found that an infusion of fresh grass gave equally good results and had the advantage that one kind of grass could be selected and used to the exclusion of all others, thus securing a more uniform culture-medium. The infusions were prepared as follows: About three grams of grass or hay was washed in tap-water and then placed in a beaker containing about 200 cc. of tap-water; this was boiled for one minute. In most cases this infusion was used shortly after it had cooled but occasionally it was allowed to stand for twenty-four hours. Except at certain periods of physiological depression and during certain experiments, to be described, this type of culture-medium was used throughout the work.

As pointed out by Bütschli and Calkins, Maupas's method was inaccurate in that he assumed the rate of division of all individuals of a culture to be the same and allowed a large number of specimens to accumulate before computing the number of bipartitions. Protozoa, like all other animals, have their individual physiological peculiarities, as is shown by my own and similar experiments. In order to obviate this source of error as far as possible and to exclude the possibility of endogamous conjugation occurring in the direct line of the culture, one individual from each line of the culture was isolated almost every day. In the great majority of cases not more than four individuals, representing two generations, were present at the time of transference. At each isolation the single infusorian was put in fresh culture medium, the remainder being kept as a reserve, or "stock," in case, through accident or otherwise, the individual isolated did not live.

Following the earlier workers, the maximum and minimum temperature of the laboratory in the vicinity of the cultures, as recorded by a registering thermometer, was noted daily. This is, of course, but a rough method as the temperature within the moist chambers is more constant than in the room itself; still, it

gives the greatest variation which could possibly occur, and by averaging the maximum and minimum points of each day for ten-day periods the result is quite satisfactory for comparative work.

For the purpose of following as closely as possible the changes in cell structure during the life of the cultures, permanent preparations of individuals from different lines were made from time to time. Here again I employed with little change the method used by Calkins, which is briefly as follows: The specimen to be preserved is isolated by means of a fine-pointed pipet on a clean depression slide (which is kept just for this purpose) with as little of the culture-medium as possible. To this is added three or four drops of bichlorid of mercury in saturated solution with 5 per cent of glacial acetic acid. After about five minutes the specimen is transferred to another slide and a few drops of 75 per cent alcohol is added. A slide is now smeared with a trace of egg-albumin and the specimen is taken from the 75 per cent alcohol and gently spurted onto the albumin. After a short time, when the alcohol has coagulated the albumin, the slide with the specimen adhering to it is transferred to a jar of 75 per cent alcohol and is thereafter treated by the ordinary slide method.

For staining, Ranvier's picrocarmin was used, although Delafield's hematoxylin gives quite satisfactory preparations. Clearing was done with xylol, and damar was used in mounting.

For convenience in description the main cultures are designated by letters, and the individual lines (four in number) which make up each of these cultures are designated by figures. Thus, the two cultures of *Oxytricha fallax* are designated respectively A and B, and the lines under them as A-1, A-2, A-3, A-4, and B-1, B-2, B-3, B-4. In each case the culture was started by isolating one wild individual and when this had divided twice, giving four individuals, these were isolated to start the four lines. These four lines thereafter were kept distinct except in cases where one died out through accident or through the isolation of a weak individual, in which case its place was supplied by a specimen from one of the three closely related lines. Of course, the more lines of a culture that are carried on, the closer their average rate of division will approach the true one for the culture. I have found that four lines is all that can be reasonably carried without undue labor and the average here is probably near enough to give

the general result. Throughout this work, as in that of my predecessors, the rate of cell-division is taken as the indication of the physiological status of the cultures; it being generally accepted that this is a just criterion of metabolic activity.

The experiments were started in the Zoölogical Laboratory of Columbia University, New York City, and carried on there continuously (except for a short period during the summer months) during the first two years of the work. The last year of the work was done at the Thompson Biological Laboratory of Williams College, Williamstown, Massachusetts.

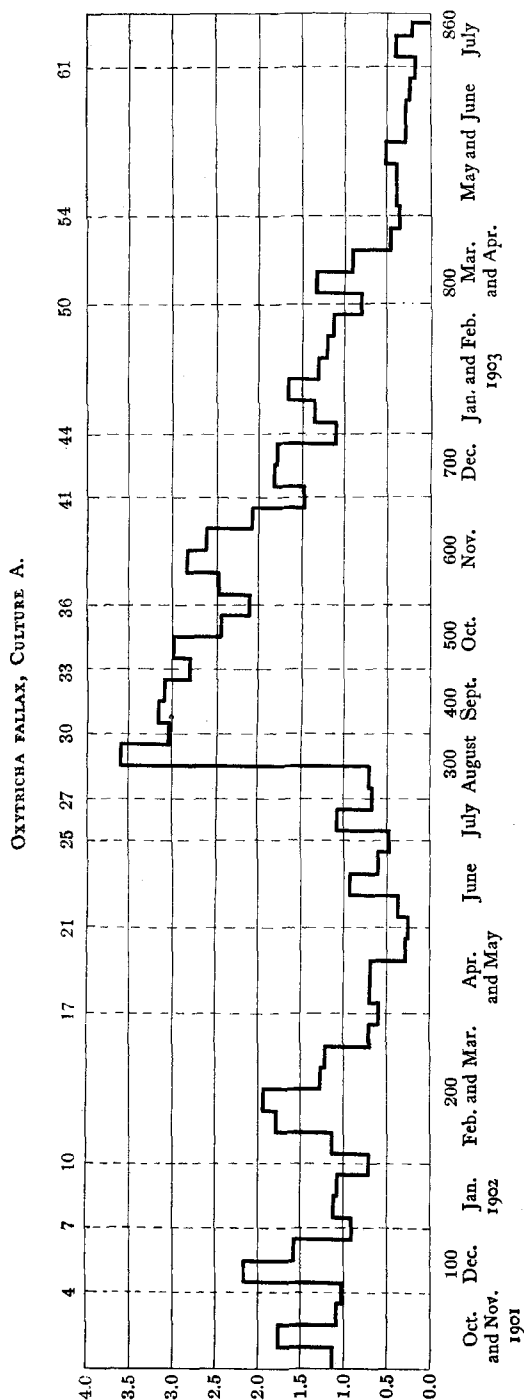
III. DESCRIPTION OF THE CULTURES.

I. *Oxytricha fallax*, Culture A.

On October 26, 1901, a specimen of *Oxytricha fallax* was found in an aquarium, in the Columbia laboratory, containing water and superficial slime taken a few weeks before from a stagnant pond at Van Cortlandt Park, New York City. The individual was isolated on a depression slide in a few drops of hay-infusion as previously described. Two days later the infusorian having divided twice, each of the four individuals was transferred to a separate slide, thus starting the four lines of Culture A which are designated respectively A-1, A-2, A-3, and A-4. The accompanying diagram shows the daily record of divisions of all four lines averaged together and this again averaged for each ten-day period of the life of the culture.

As is indicated in the diagram, the culture started with an average rate of a little over one division per day for the first ten-day period. This was increased to one and three-quarters divisions for the second ten-day period, after which there were two periods in which the rate fell each time below that of the first period, *i. e.*, in period four to exactly one division per day.¹

¹From November 20 to 24, in the third period, A-1 and A-2 were changed from the hay-infusion to a medium of flour and water, prepared by boiling a pinch of flour in about 25 cc. of tap-water for fifteen minutes. This was used about an hour after cooling. This change of medium was made because I became alarmed at the rapid fall in the division-rate—not having become acquainted, as yet, with the general life-cycle of hypotrichous forms. That the use of the flour had no apparent effect was shown by a comparison with the division-rate of A-3 and A-4 which were continued on the hay-infusion diet.



Complete history of *Oxytricha fallax*, Culture A, from start (October 26, 1901) to extinction (July 14, 1903) in the 860th generation. Rate of division averaged for ten-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. The broken lines designate the limits of the various rhythms. Above, the numbers of the ten-day periods which limit the rhythms are indicated; below, the months in which the rhythms chiefly fell. The figures, 100, 200, etc., represent generations and are placed in the periods in which they were attained.

During period five there was a marked rise for no apparent cause to over two and one-eighth divisions, and then a fall to about seven-eighths of a division per day in the seventh period, which was below the lowest rate so far attained by the culture. The next fall, however, was still lower, when seven-tenths of a division per day was recorded. After this there was another rising period extending over about a month and attaining a maximum rate of nearly two divisions. From here there was a gradual decline for four periods when a minimum of six-tenths of a bipartition per day was averaged—the lowest point so far attained. Again a slight rise for twenty days, and then a fall at the twenty-first period (210 days since the culture was started) to one-quarter of a division per day, the lowest point reached in the division-rate, which had been gradually diminishing since the beginning.

It was apparent that unless something was done to stay this decline or “rejuvenate” the culture at this point that it would soon die out. Calkins had succeeded in reviving *Paramœcium* cultures with an extract of beef, and acting on this clue all four lines were transferred to weak beef-extract¹ for five days and then changed back to the regular hay-infusion diet. As the beef-extract showed no immediate results, flour and water was tried again, apparently to no purpose. I now returned to beef-extract, this time making it stronger and varying the strength from day to day, and this treatment was continued up to June 1. This time a very slight rise in the division-rate for the period occurred (*cf.* Diagram I, period 22) and during the following ten days it increased to almost one division per day. Then it fell again for two periods, but the slowest rate here attained was considerably faster than the previous low mark of period 21. In period 26 the diagram shows a considerable rise which was brought about by a sudden springing into activity of one line (A-1) of the culture. Instead of dividing at the rate of about once in two days, it started off on July 7 at the rate of three times per day. When I first noted this sudden change I thought that possibly in some way, in spite of all precautions, an adventitious specimen, perhaps as a cyst, had

¹The beef-extract was made by boiling for a few minutes a piece of lean beef about the size of a silver half-dollar in 200 cc. of tap-water. This was allowed to settle for a number of hours and then the clear extract was used.

vitiating the culture, and I immediately examined the stock of the line—some of which had not been touched for a number of days. I found that this also had started dividing at the same rapid rate, and as there was apparently no way in which all the preparations could have become contaminated simultaneously, I was convinced that the increase in rate was due to some change in the culture itself—a conviction which was substantiated by a study of the cytological changes in the permanent preparations; but to leave no chance for error I removed the rapid line (A-1) to another moist-chamber and thus isolated it from all the rest. This condition of affairs—A-1 dividing about three times each day and the other lines once in two days—continued for just a month when the three other lines sprang into activity. This at once, of course, brought up the average of the four lines as is seen in the twenty-ninth period (Diagram I) when the average rate of multiplication reached over three and one-half divisions per day. This twenty-ninth period was the high record for the division-rate of this culture. During the next ten days the rate fell to three divisions per day; then occurred a slight rise above this for twenty days, and then another drop to about two and three-quarters divisions per day for the thirty-third period. This rising and falling of the division-rate continued to the very end of the life of the culture, or from August, 1902, to July, 1903, nearly a year.

At the fifty-third period it was clear that the culture was again approaching extinction and, accordingly, two lines, A-1 and A-2, were transferred for a day to beef-extract, leaving A-3 and A-4 in the normal hay-infusion. This had no visible effect on the lines treated and both died out at different times, and their places were supplied by individuals from the other two lines.

During period 54 the culture medium for all lines was changed from hay-infusion to an infusion made with fresh grass in order to see what effect a change in medium would have on the behavior of the culture. The very slight rise in the division-rate which followed for the next three periods may be due to this change, but I think it is more probable that it is due to a decided rise in temperature which took place at this time (*cf.* Diagram VII). During the next two periods no attempt was made to revive the culture, and as the fission-rate remained quite uniform I postponed all experiments in order to see what would take place if the culture was

allowed to run its natural course. A very slight falling of the division-rate occurred in the next twenty days; but in the ten-day period after that there was a more decided rise than had taken place for a long while. This proved to be only temporary for the Infusoria suddenly began to die off and within four days only six specimens were left. Efforts to stimulate by artificial means (K_2HPO_4 , return to the usual hay-infusion, etc.) were unavailing, and the last individual died on July 14, in the 860th generation—626 days after the first isolation.

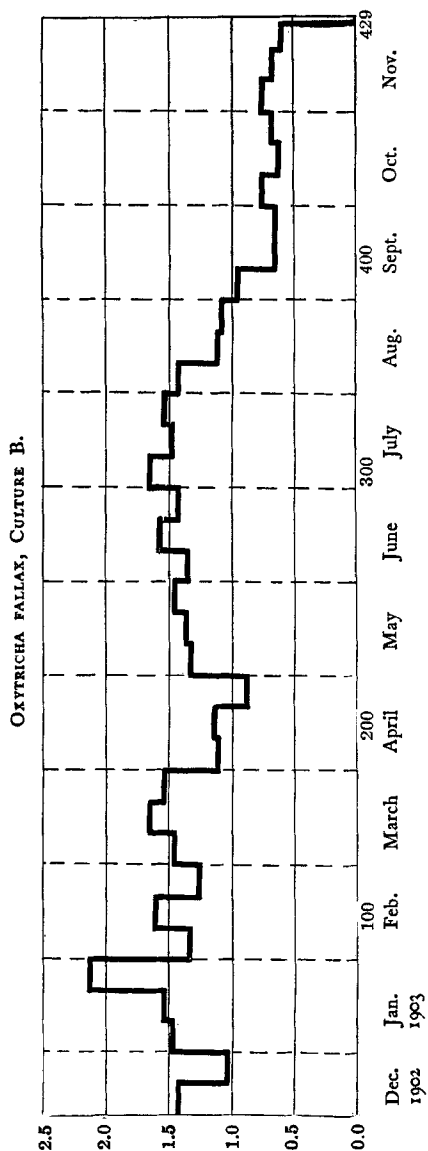
2. *Oxytricha fallax*, Culture B.

A second culture of *Oxytricha fallax* was started on December 10, 1902, with an individual found in a hay-infusion, made with boiled water, in the Columbia laboratory. The method of procedure was the same as that already described for the A-culture. The accompanying diagram shows the history of the fission-rate of all four lines averaged together and this again averaged for each ten-day period of the life of the culture.

Culture B was continued for a period of 348 days during which time it attained 429 generations. Its loss was due entirely to an accident resulting in the drying up of the preparations. The general rate of division for the first twenty-three periods averages about one and one-half divisions per day, and compared with the curve of Culture A, the curve of B is considerably more uniform. From period 24 on (August), however, the rate shows a considerable falling off and it was averaging about one division in two days—the lowest rate in its history—at the time that the culture was lost.

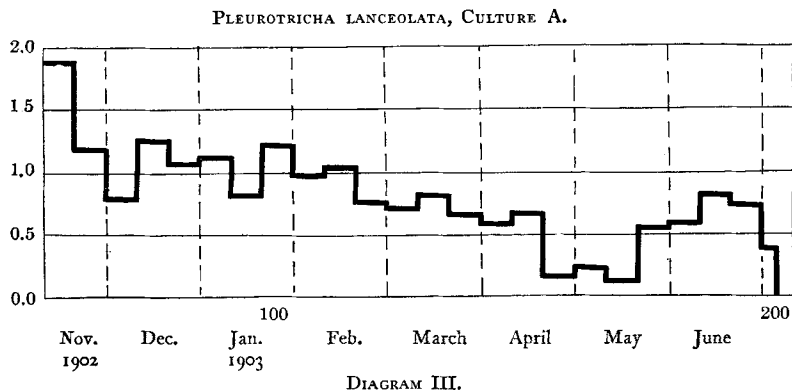
3. *Pleurotricha lanceolata*, Culture A.

A culture of *Pleurotricha lanceolata* was started November 10, 1902, with an individual from an aquarium in the laboratory of Columbia University which contained material collected during the previous month at Fort Lee, New Jersey. The treatment of the culture was the same as that already described for the *Oxytricha* cultures. The general trend of the division-rate, as shown by Diagram III, was steadily downward from the beginning to the nineteenth period (May), when the low rate of one division in eight days was reached. During the next three periods a marked



Complete history of *Oxytricha fallax*, Culture B, from start (December 10, 1902) to finish (November 22, 1903). The broken lines indicate the limits of the various *months* over which the culture extended. The other details are the same as for Diagram I.

rise took place for which there is no apparent cause, but this recovery was not lasting and the rate fell somewhat during the next period, while in the period following this all the infusorians encysted, thus bringing the culture to an end at the two-hundredth generation, and after being under observation for two hundred and thirty-five days.



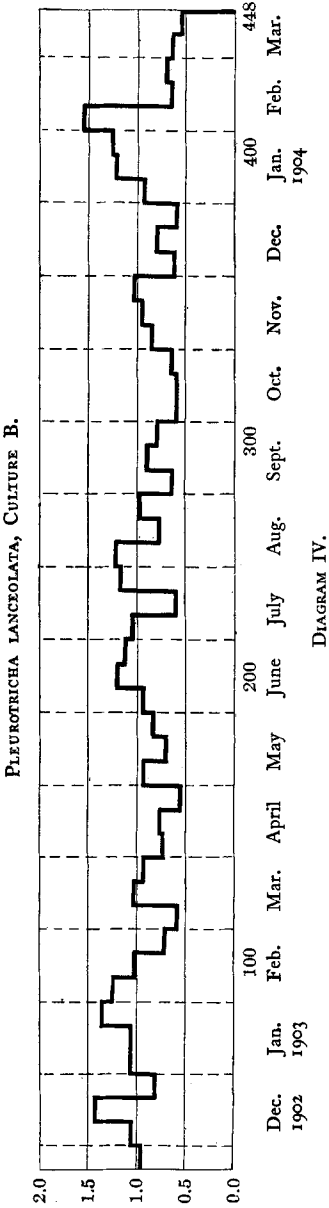
Complete history of *Pleurotricha lanceolata*, Culture A, from start (November 10, 1902) to finish (July 3, 1903). For method of plotting, see Diagram II.

4. *Pleurotricha lanceolata*, Culture B.

A second culture of *Pleurotricha lanceolata* was begun November 25, 1902, with an individual found in some material in the Columbia laboratory which had been recently collected at Van Cortlandt Park, New York City. This culture was carried on by the method used in all previous cultures for 480 days, and reached during this time the 448th generation, when it was lost by an accident similar to that which terminated the *Oxytricha A*-culture. The culture-curve plotted in Diagram IV shows that throughout the life of the culture a general average rate of nearly one division per day was maintained.

5. *Gastrostyla steinii*, Culture A.

A culture of *Gastrostyla steinii* was started on May 28, 1904, with a specimen which was captured in a hay-infusion in the Williams College laboratory. For convenience I have desig-



Complete history of *Pleurotricha lanceolata*, Culture B, from start (November 25, 1902) to finish (March 13, 1904). Method of plotting is the same as for Diagram II.

nated this Culture A, although but one culture of this species has been studied. The culture was put at once on a grass-infusion diet and continued on the same during its life.

From Diagram V it will be seen that the rate of division for the first three periods was very close to one division per day. On June 25, which fell just at the end of the third period, I moved the culture from Williamstown, Massachusetts, to New York City. The greatly increased division-rate, which appeared in the following period and was augmented in the period succeeding that to almost two and one-half divisions per day, is difficult to account for with any certainty. The jolting which the animals received

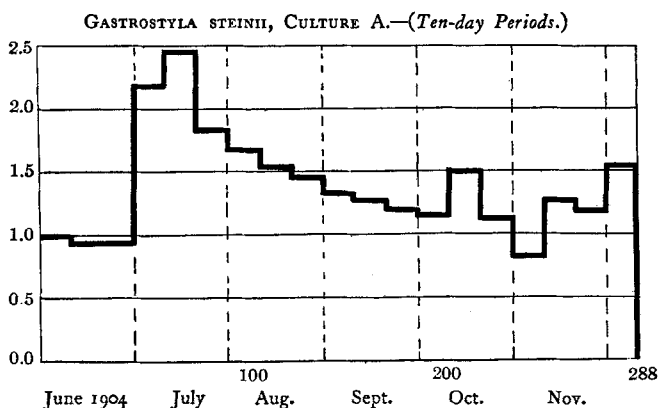


DIAGRAM V.

Complete history of *Gastrostyla steinii*, Culture A, from start (May 28, 1904) to its extinction (December 5, 1904) averaged for *ten-day* periods. Method of plotting the same as in previous diagrams.

on the trip to the city, the change to city tap-water, the change of grass with which the infusion was made, and the increased atmospheric pressure are prominent among the factors which may have tended to stimulate the fission-rate. Further, the treatment of the culture was exceedingly uniform beginning with its location in New York as I wished to see if the minor fluctuations in the division-rate, so prominent in the earlier cultures, could be modified or entirely eliminated by still more stable conditions. Again, I employed this culture as a "control" for certain experiments on the effects of salts on the division-rate and

GASTROSTYLA STEINII, CULTURE A.—(Five-day Periods.)

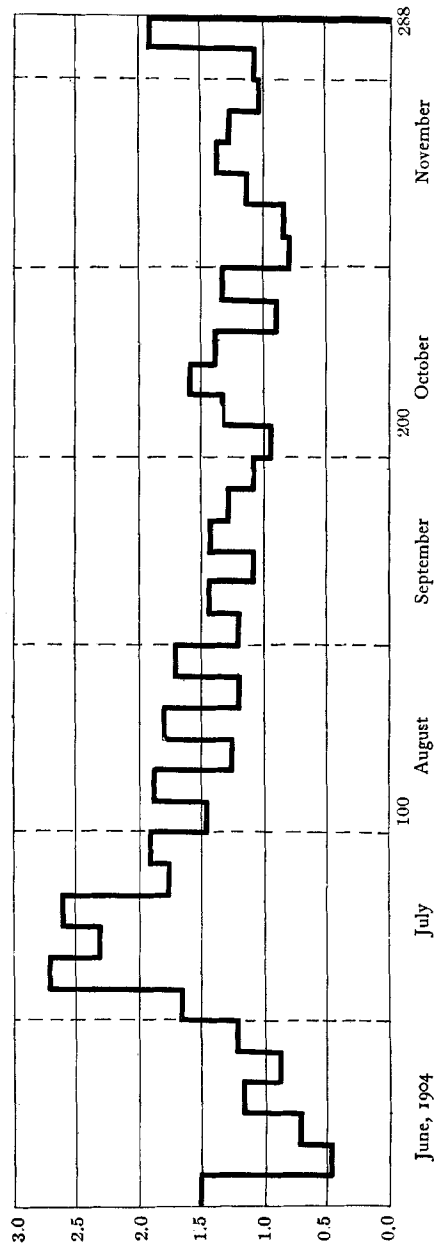


DIAGRAM VI.

Complete history of *Gastrostyla steinii*, Culture A, averaged for five-day periods. Compare with Diagram V.

for this purpose exactness was most essential.¹ Whatever factor or factors caused the high division-rate of the fifth period, the effect was not lasting for in all of the succeeding periods up to, and including, the thirteenth, the rate steadily decreased and at a remarkably uniform rate. During the twelfth period (end of September) I moved the culture back to Williamstown. No effect is to be seen in the succeeding period but the rise in the fourteenth period undoubtedly is due to this change. This time the rise was by no means so marked and it was evident only after a latent period of ten days or more. This possibly can be explained by the fact that the "potential of vitality" of the infusorians was considerably less than when the first removal took place.² Like the acceleration at the first removal, this second one was not lasting as, during the following ten-day period, the fission-rate settled down to where we should expect to find it if the culture had been carried along without any disturbing influence. Beginning with the next period (No. 16) the very exact treatment which I had employed was discontinued and the change of liquid was made only every other day, and then not at exactly forty-eight hour intervals. The effect of this is at once apparent in the considerable fluctuations in the fission-rate shown in the culture-curve during the remaining four periods of the life of the culture. At the beginning of period 20, *i. e.*, at the 191st day of the life of the series, when the animals were dividing on the average three times in two days, the culture suddenly died out, stock and all, at the 288th generation. I noted that the infusorians were exceptionally active on the slides just previous to their extinction. This sudden death of the culture cannot be attributed to any accidental change in the liquid medium as the stock was affected similarly at the same time.³

¹I endeavored to secure this uniformity of treatment and culture medium: (1) By changing the culture medium daily and at the same hour, thus making the daily records of just twenty-four-hour periods. (2) By using the same kind of grass and grass grown in the same place. (3) By washing the grass very thoroughly and boiling it for one minute. This was given as soon as it reached the room temperature.

²Calkins ('02, 1) found, however, that a journey which he made with his *Paramoecium* cultures when they were on a descending cycle accelerated the fission-rate, while a return journey made when the cultures were on the ascending cycle produced a retarding effect.

³It will be recalled that the death of Maupas's culture of *Stylonychia pustulata* was preceded by a period of more rapid division of almost three weeks' duration.

IV. DISCUSSION OF THE DATA OF THE CULTURES.

1. *Rhythmical and Cyclical Variation in the Rate of Division.*

One has but to glance at the plotted curves of the various cultures (Diagrams I to VI) to see that all the species of Infusoria studied pass through periods of greater and less dividing activity when subjected to a stable environment. These periods, upon analysis, are resolved into two kinds: First, the short, more or less rhythmical fluctuations in the fission-rate which I shall refer to as "rhythms"; and second, the long downward trend of the cultures (especially prominent in the *Oxytricha* A-culture) from their beginning to end, or, in the case of *Oxytricha* A, from its start to its recovery by stimulation at about the 250th generation, and again from this point through the second long downward sweep which ended with its extinction. This second type of change of fission-rate I regard as the "cycle." I am satisfied that these two kinds of variation are due to different causes. I believe the rhythms to be somewhat superficial in character and due *in part* to slight variations in the environment, the most important of which is change in temperature. This belief is based on the remarkable agreement which obtains between the rhythms and the fluctuations in temperature. In Diagram VII there is plotted a section of the culture-curves of all the four cultures which were carried on simultaneously, and above them the temperature curve. The agreement is seen to be more marked in the *Oxytricha* cultures; in the *Pleurotricha* series, the similarity, while not as striking, is too exact to be a mere coincidence, and serves to emphasize the fact that while temperature does influence the rate of multiplication, it is not the most important element among the factors which cause fluctuations in the rate. It is only natural that temperature variations should affect the division-rate, if not directly, at least indirectly through the effect on the multiplication of bacteria and therefore upon the food-supply, and this has been shown to be the case by Maupas and Calkins.

I believed there was another and more fundamental factor underlying the rhythms, and with this in mind I took still greater precautions to have the environment as nearly constant as possible in the more recent culture of *Gastrostyla steinii* (see note, p. 600). From the results of this series when plotted in periods of ten days (Diagram V), it would seem that my idea

was wrong and that with a constant medium all rhythms could be removed. To test it still further the same results were plotted for five-day periods. This brought the rhythms to view again (cf. Diagram VI) in such beautiful regularity that it seems to me to show beyond doubt that the rhythmical element of the division-rate cannot be caused entirely by temperature changes or by imperceptible fluctuations in the food supply, but that it is due, in the last analysis, to factors of a more complex character. Variation in the rhythm of division is well known in the development of the metazoön egg, and it has yet to be satisfactorily explained. Towle ('04) in a recent paper on the effects of stimuli on *Paramecium* is led to make this interesting statement: "There may even prove to be rhythmical changes in sensitiveness like those described by Lyon ('02; '04) for cleaving eggs, and Scott ('03) for unfertilized eggs. Something of this nature is indicated by the fact that *Paramœcia* from the same culture vary in sensitiveness from day to day." In my work on the effects of chemicals on Infusoria I have found that individuals react differently at various times to a given stimulus (cf. p. 616 *et seq.*) and I believe we have the clue to these "changes in sensitiveness" manifested in the rhythms of the fission-rate.

A point of some interest in regard to the rhythms in the *Oxytricha* A-culture is the fact that the slowest fission-rate of each rhythm in the descending cycle is less than that of the slowest rate of the preceding rhythm. In the ascending cycle also, the slowest rate in each rhythm is greater than the slowest rate of the preceding rhythm.

So far we have not considered the long trend of the division-rate, which I regard as the cycle as I believe that this is directly comparable with the cycle of Calkins's *Paramecium* cultures. The cycle obviously extends over more generations in *Oxytricha* than in *Paramecium* though in both cases it is a variable number both in the same culture and in different cultures of the same species. Maupas's rather definite limits to the life-cycle are not substantiated by this work as will be readily seen by comparing the various culture-curves. It must be borne in mind, however, that neither Maupas's chief cultures nor my own were started with ex-conjugants, and therefore the number of generations does not afford a just basis of comparison, since they indicate merely the number of bipartitions since the culture began and in no sense

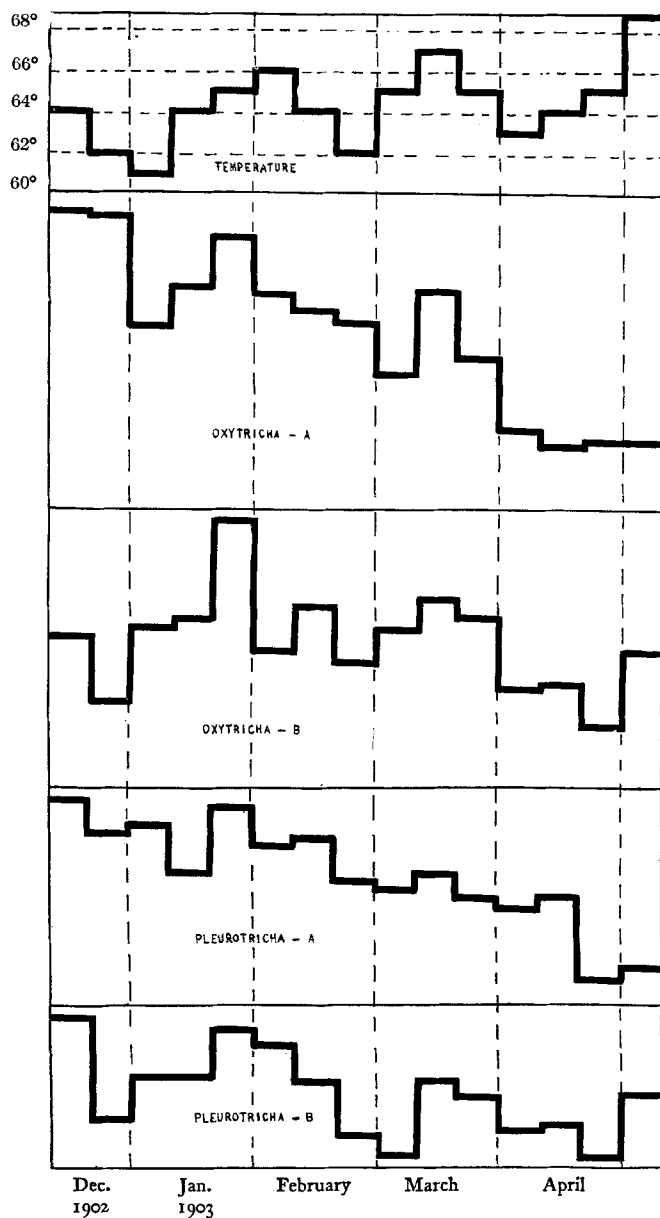


DIAGRAM VII.

Sections of the culture-curves of *Oxytricha* A, and B, and *Pleurotricha* A, and B, together with the temperature curve for the same period (December 10, 1902 to May 9, 1903), showing the correspondence of the "rhythms" of the division-rate with the fluctuations in temperature.

the "age" of the culture with reference to the last conjugation period. The number of generations from the recovery of my *Oxytricha A*-culture to its extinction gives the number of divisions in a cycle of an artificially stimulated line, but it remains to be shown that this is directly comparable to "rejuvenescence" by conjugation.

Joukowsky carried a series of *Pleurotricha lanceolata* through 458 generations and found no signs of degeneration, and he suggested that degeneration depends not on the number of divisions only, but on the rapidity with which they succeed each other. This conclusion, on *a priori* grounds, would seem reasonable, but my cultures give no evidence to substantiate it. Calkins, on the other hand, lays more emphasis on the duration in time of the cycles than on the number of generations passed through, and he showed ('04) that about six months is the period of the cycle in *Paramœcium*, but it would seem that about three months was the result reached by other workers on this species. Calkins ('02, 1, 2, 3) himself, in his earlier studies on *Paramœcium*, believed that the cycle in this species was approximately of three months' duration, as he interpreted the smaller trimonthly fluctuations as the cycles. I am satisfied that these periodic lesser changes in vitality which are so conspicuous in his culture-curves are identical with what I have termed rhythms in my cultures, and in the light of his results with *Paramœcium*, it is probable that the earlier workers on this species, Joukowsky and Simpson, have been dealing with rhythms rather than cycles. I believe that it is essential to recognize a sharp distinction between "rhythms" and "cycles," which may be defined as follows:

A rhythm is a minor periodic rise and fall of the fission-rate, due to some unknown factor in cell-metabolism, from which recovery is autonomous.

A cycle is a periodic rise and fall of the fission-rate, extending over a varying number of rhythms, and ending in the extinction of the race unless it is "rejuvenated" by conjugation or changed environment.

The question of the number of generations, as well as the time duration, of a life-cycle, is very uncertain and extremely difficult to determine as it is probably dependent upon more than one factor. My cultures lead me to believe, with Simpson, that the personal equation, if I may use that term, of the individual selected to start a culture has the most influence in determining the number

of generations attained before "the initial potential of vitality" is exhausted. Calkins's discovery of what he calls "incipient fertilization" in *Paramœcium*—that is, of two ex-conjugants which continue to live "one is invariably far more vigorous than the other"—would seem to bear out this point and to show that the number of generations or the period over which a cycle extends is not a point of great moment.

Taken as a whole my cultures show conclusively that the three species of hypotrichous ciliates studied are subject to periods of greater and less dividing activity, and since the fission-rate is probably a fair criterion of the metabolic activity of the protozoan cell, that ciliates pass through alternating periods of greater and less general vitality. This is also the general conclusion reached by Engelmann, Bütschli, Maupas, Joukowsky, Simpson, and Calkins, and from the range of species investigated it can probably be accepted as of quite general occurrence among the Infusoria.¹

2. *Artificial Rejuvenescence.*

Calkins ('02, 1) showed conclusively that *Paramœcium* cultures when becoming extinct can be revived by the application of various

¹Peters ('04) working on *Stentor*, states that "neither direct observation nor the experiments made, furnish evidence of any *inherent* periodicity of division. The present experiments show that, *except* when some special modification of the medium exists (*e. g.*, presence of potassium chlorid in excess), multiplication runs, in the main, parallel to metabolism." Peters's experiments were not planned directly to investigate this point and I fail to see, from his description of the methods employed, how cyclical variation in the fission-rate, unless very pronounced, would be apparent. That "multiplication runs, in the main, parallel to metabolism" is, I take it, not open to question, and is in no way opposed to periodic fluctuations of the fission-rate. Peters says further, in regard to the culture medium employed in determining periodicity of division, that "such promiscuous mixtures as hay infusion of *unknown composition* will not suffice. Since frequent chemical analyses are impracticable, it will be necessary to construct by trial artificial media of known composition." Undoubtedly hay infusion is not an ideal culture-liquid, but when the hay or grass is carefully selected and thoroughly washed and otherwise treated uniformly, and when this is prepared fresh each day and employed as soon as it has reached the room-temperature, there is little chance for fermentation, and I believe that about as near a perfect medium is obtained as is practicable. Undoubtedly the ideal culture-liquid would be one artificially combined so that its salt content, etc., is accurately known; but as Peters himself says, ". . . a food supply must be added to the salt solution, and this requirement has proved to be a difficulty. For the addition of any food that has been found available utterly changes the salt content both qualitatively and in its proportions." To supply this demand Peters added to the artificial medium which he concocted, "some dry leaves or dead reeds, or both. . . . The final step is to 'seed' this culture with a mixture of all sorts of Infusoria, and other living material from thriving cultures." This done, I do not see how a hay-infusion could be a more promiscuous mixture.

stimuli, and he found that an extract of beef, among others, was most effectual. As previously stated, I employed beef-extract as a stimulant during the first depression period of the *Oxytricha* A-culture which was at its height in May, 1902, and in July, 1902, after a latent period of about six weeks, one series suddenly sprang into new life. It is certain that something "rejuvenated" the culture at this time and I have every reason to believe that it was brought about by the salts of the beef-extract, and that we have here a case of stimulation analogous to "artificial parthenogenesis" as Calkins suggests in his *Paramœcium* work.

3. *Conjugation.*

My endeavor to study the effect of conjugation on the life-cycle of *Oxytricha fallax*, *Pleurotricha lanceolata*, and *Gastrostyla steinii* has been in vain, as at no time during the life of any of the five cultures have I succeeded in getting a single syzygy. Numerous individuals from the A and B cultures were placed together at different times in an endeavor to get exogamous conjugations, but to no purpose. The same is true of endogamous conjugations. With Maupas's conditions of conjugation in mind attention has been paid to the amount of food present but without result.

It seems rather remarkable that *Oxytricha* should pass through 860 generations, *Pleurotricha* through 448 generations, and *Gastrostyla* through 288 generations and at no time show any tendency to conjugate. The significance of this is rather difficult to see. Joukowsky, however, found no conjugations in his long culture of *Pleurotricha*, and Maupas secured none in his cultures of *Stylonychia mytilus* or *Oxytricha* sp. though his other series yielded plenty of syzygies. It is not uncommon to find hypotrichous forms conjugating in wild cultures in the laboratory, so that it is evident that some condition must prevail there which does not obtain in the experiments, and it is just possible that an excess of carbon dioxid and other noxious gases in these wild cultures may be the provoking cause; but it seems more probable, since the physical state of the protoplasm of the infusorian undoubtedly plays an important rôle in the conjugating process, that the required "miscible state" is prevented in artificial cultures through the scarcity of certain salts in the liquid medium used. In the

light of Maupas's results with *Oxytricha* sp. and *Stylonychia mytilis*, and of Joukowsky's with *Pleurotricha lanceolata*, and also my own on two cultures of *Oxytricha fallax*, two of *Pleurotricha lanceolata* and one of *Gastrostyla steinii*, it would seem to be questionable whether conjugation is of so frequent occurrence among the Hypotrichida as in some other groups of Ciliata.

V. PHYSIOLOGICAL AND MORPHOLOGICAL VARIATION DURING THE LIFE-CYCLE.

Maupas emphasized the fact that various changes, cytoplasmic and nuclear, take place in Protozoa as "senile degeneration" advances; and he also found physiological evidence in the form of lessened vitality, increase of endogamous conjugation, and infertile syzygies. Joukowsky found no morphological changes in *Paramœcium* but observed that the rate of division decreased as the cultures advanced and that many of the animals became sluggish. In an eight month culture of *Pleurotricha* he found no signs of degeneration. Simpson made some observations on three to four month cultures of *Stylonychia pustulata*, *Paramœcium caudatum*, and *Paramœcium putrinum*, and while he did not find degeneration in such specific form as nuclear changes or loss of external appendages, still he was "convinced of a gradual ebbing of vital energy as the series proceeds, which expresses itself in slower motion, in a tendency to inactivity and general listlessness, if the word be admissible in this connection, as also in a certain diminution of size that was not remedied by any amount of food." Calkins ('04), however, found marked cytoplasmic and nuclear changes in his long *Paramœcium* cultures, and physiological degeneration was manifested by irregular and abnormal divisions, decreased division-rate, tendency to endogamous conjugations, and above all by the "death of all members of a series fed continually on the same diet of hay-infusion."

1. *Physiological Variation.*

In my cultures, physiological changes have been manifested chiefly in the slowing down of the division-rate after a greater or less number of generations, and coincident with this, in a considerable lessening of the general activity of the infusorians.

The general behavior of individuals on the slide is quite different at various periods in the life-cycle, and by it the condition of the culture can be estimated with some degree of accuracy. Although the activity of the animals is considerably lessened during depression periods, I have not found that their power of taking food is diminished since the oral cilia vibrate normally and keep a continuous stream of food particles passing into the mouth opening, and this results in a black appearance of the infusorians due to accumulated and unassimilated food.

Another indication of physiological disturbances during periods of depression is the greater frequency of pathological divisions at this time, and Calkins found this to be the case in *Paramœcium* cultures. An interesting specimen which occurred in the A-culture of *Oxytricha*, when the vitality was extremely low in June, 1902, is shown in Fig. 21.

2. *Morphological Variation.*

For the purpose of determining the morphological changes which occur during the life-history, permanent preparations were made from time to time during the life of each of the cultures.¹ The series of preparations is particularly complete for the *Oxytricha* A-culture, from the time that series was approaching its first depression period through its recovery by stimulation, and then through the second cycle which resulted in death. On this account, the following description is based on this series of some two hundred slides, while the lesser series of *Oxytricha* B, *Pleurotricha* A, and B, and *Gastrostyla* A are used for confirmation and comparison.

The typical cytoplasmic structure of *Oxytricha fallax*, *Pleurotricha lanceolata*, and *Gastrostyla steinii*, is practically identical and is best described as alveolar throughout. As in all the hypotrichida, no distinction is visible between ectoplasm and endoplasm. The ectoplasmic modifications such as cilia, cirri, and membranelles, of course, vary in a characteristic manner for each species, but it is unnecessary to consider these here. In *Oxytricha* and *Pleurotricha*, as is well known, there are two ellipsoidal macronuclei situated more or less symmetrically in the cell, while

¹For description of technique, see section on General Methods and Technique.

in *Gastrostyla* there are four macronuclei similarly placed. The macronuclei in all three species consist of at least two elements: First, a substance, undoubtedly chromatin, having a strong affinity for nuclear dyes; and second, a clear substance, resisting all stains, which may be termed achromatin. The general appearance of the nucleus is nearly homogenous though this is probably caused by the massing of a granular matrix. A membrane surrounds the nucleus and a very delicate commissure apparently connects the macronuclei though it is very difficult to determine. From time to time a *Kernspalt* is observable. Associated with each macronucleus is a small spherical micronucleus; in *Oxytricha* and *Pleurotricha* there are typically two, and in *Gastrostyla* four, micronuclei. The staining reaction of the resting micronucleus is the same as that of the macronucleus. A typical specimen of *Oxytricha fallax* is illustrated in Fig. 15.

During the earlier part of the *Oxytricha* A-culture no preparations were made, so that during the first period of decline up to the sixteenth ten-day period (Diagram I) I am unable to trace the morphological changes. On April 2, 1902, however, two individuals of the 230th generation were preserved. A glance at the photographs of these specimens (Figs. 1 and 2) shows that marked vacuolization of the cytoplasm has occurred in each case. In Fig. 1 the two macronuclei are considerably displaced in the cell, and each shows a peculiar vacuolized condition of the nuclear material, the chromatin being segregated about what appear like bubbles of the achromatic substance. Each macronucleus is surrounded by a clear area which separates it sharply from the cytoplasm. I believe that this clear area is caused by an accumulation of the achromatic substance against the nuclear membrane, which thus produces the appearance of a halo about the nuclear bodies. In this particular specimen there are two micronuclei present, one being nearly invisible in the photograph as it is somewhat below the plane of focus. Fig. 2 shows the same condition of cytoplasm and nuclear material but the two macronuclei are fused and the whole mass is surrounded by the halo. At least three micronuclei are present in the preparation, two of which are visible in the figure, so that we have a case of micronuclear reduplication similar to that which Maupas described in his culture of *Oxytricha* sp. Specimens from A-2 of the 239th generation (Fig. 3) and A-1 of the 241st generation (Fig. 4) show a

fused condition of the macronuclei similar to that in Fig. 2, though the chromatic material appears somewhat more homogenous. Here again the micronuclei, with two exceptions, are out of focus. Other characteristic specimens of this period of declining vitality are shown in Figures 5, 6, 7 and 8, all representing forms from the 243d to the 247th generation (*cf.* Explanation of Plates).

The specimen illustrated in Fig. 9 is of the 250th generation and is the last of the descending cycle of A-1, since on July 7, 1902, this line sprang into renewed dividing activity (*cf.* p. 592). The marked improvement of the cytoplasmic and nuclear condition of the infusorians is shown in an individual of the 256th generation (Fig. 12). Here the macronuclei, in outline and in general appearance, are again approaching the typical condition, and the position of the micronuclei in relation to the macronuclei is also more typical. Lines A-2, A-3, and A-4, however, which remained dividing at the slow rate, show no improvement in their nuclear condition, as is seen in specimens of the 255th generation (Figs. 10 and 11).

The cytoplasm of the "rejuvenated" individual, from line A-1, represented in Fig. 13 is still somewhat vacuolized, but the macronuclei and micronuclei are nearly typical. The specimen is quite small but this is due to the high rate of division prevailing at this period. This reduction in size is still more apparent in preparations of the 331st generation (Fig. 14), but beginning at about the 409th generation (Fig. 15) the size again increases with the slightly decreased fission-rate. This beautifully diagrammatic condition of the nuclear apparatus is the prevailing state in the large majority of specimens at this period of great reproductive activity. One most interesting exception, however, is that in two lines of the culture, specimens from the 361st to 369th generations lack the posterior micronuclear body. This is but temporary and for almost one-hundred generations after this the normal condition prevails. Preparations of the 458th generation again show evidence of a changed condition of the micronuclei since now they appear pale; the chromatin having but little affinity for the stain. This peculiarity reaches a climax at the 473d generation when the micronuclei appear almost perfectly clear; but from this time on they again resume their normal staining capacity.

Starting at about the 542d generation the cytoplasm shows signs

of vacuolization, and this increases steadily and at approximately the 600th generation the nuclear apparatus begins to differ from the normal. An early stage is shown in Fig. 16, and a later stage exhibiting nuclear fragmentation in Fig. 17. The last stage in this cytoplasmic and nuclear degeneration is shown by specimens of the 853d and 854th generations (Figs. 18, 19 and 20) in which the cytoplasm is greatly vacuolated, the ventral cirri reduced, the macronuclei distorted and fragmented, and the micronuclei increased beyond the typical number; a condition closely similar to that which obtained at the 230th generation (Figs. 1 and 2). The series died out at the 860th generation (*cf.* p. 594).

An interesting feature is the marked variation in size of the infusorians at different periods of the life-cycle. Previous workers have found that a gradual decrease in size occurred as "old age" ensued. This certainly does not hold for the species in question. Fig. 15 shows about the typical relative size of a normal specimen, and a comparison of this with the figures of the succeeding generations and with Figs. 1 through 8 shows that *the size gradually increased as the rate of division decreased*. This, however, is true only up to a certain point for, during the last two days before death, the size decreased quite rapidly, a decrease due to a shrinking of the cytoplasm which produced a more or less abnormal contour of the individuals. This condition is shown somewhat inadequately in the specimen illustrated in Fig. 9, which is the last of the line before the culture was "rejuvenated" in July, 1902. After this recuperation, however, the size of the infusorians decreased remarkably (from the normal) with the high rate of division (*cf.* Figs. 13 and 14).

The B-culture of *Oxytricha* (*cf.* Diagram II), which was lost by accident at the 429th generation, shows far less fluctuation in vitality than does culture A, indicating that the potential of vitality of the B-series was considerably greater. Cytological study of the preparations made from time to time shows that, beginning at about the 140th generation and extending over approximately the ensuing seventy-five generations, the anterior micronucleus was not present. This was the only morphological change apparent during the life of this culture. A typical specimen in the 365th generation is shown in Fig. 22.

In the two series of *Pleurotricha* I have found no nuclear variation at any time. Although neither of these cultures was actually carried to natural death, still from the large number of generations attained it would seem that nuclear changes should have appeared if they occur in this species. Joukowsky's culture of 458 generations of this species, however, gave the same result and that this has been held unjustly as opposed to Maupas's conclusions is evident from my cultures. A slight cytoplasmic vacuolization appeared in both of my cultures as the series advanced. A specimen, in a late division-stage, from the 413th generation of culture B is shown in Fig. 23.

The *Gastrostyla* culture showed morphological changes in the form of vacuolized cytoplasm and distortion of the macronuclei during the later generations; but at the time of the sudden death of this series "degeneration" was by no means so marked as in the *Oxytricha* A-culture long before death ensued.

Briefly reviewing the chief morphological changes apparent during the various cultures, we have: *Oxytricha* A, cytoplasmic vacuolization, disappearance of one of the micronuclei for a period, and later an increase in their number beyond the norm, distortion and fragmentation of the macronuclei, degeneration of part of the ciliary apparatus, and, finally, a gradual increase in the size of the infusorians as degeneration advances; *Oxytricha* B, one of the micronuclei was not present during a number of generations; *Pleurotricha* A and B, slightly vacuolized cytoplasm; and *Gastrostyla* A, cytoplasmic vacuolization and distortion of the macronuclei.

Wallengren ('01) made a careful study of the morphological changes which occur in starved *Paramœcia*, and discovered that in the later stages the endoplasm is distorted by huge vacuoles and finally the macronucleus is deformed and broken. The micronucleus, however, remains unscathed throughout the starvation changes. Calkins confirmed these starvation observations and also found that quite similar morphological changes occur in degenerating *Paramœcia* cultures, and he believes that the similarity of the changes in the two cases indicates that it is the digestive function which becomes impaired in the declining series, since when in this condition the organisms still take food but apparently are unable to utilize it. In my own cultures it has been clear that the power of taking food is not diminished appreciably

during depression periods and the very similar morphological changes which occur in the hypotrichs studied, justifies, I believe, the assumption that the power of assimilation becomes diminished as the culture proceeds and that the effect of the beef-extract is essentially that of concentrated nutrition, resulting in the rapid assimilation of the salts, etc., necessary for the continued life of the animal.

It has been customary to regard the macronucleus as relatively vegetative in function and the micronucleus as reproductive; and this accords well with the results of these experiments, in so far as the morphological variation of the macronucleus may be regarded as an indication of the apparent lack of assimilation of the food taken. Throughout the culture no form-changes were apparent in the micronuclei themselves, but they showed a tendency to numerical reduction when the fission-rate was at the highest, and to reduplication when the lowest rate of multiplication ensued. This may be explained by supposing that the exceedingly rapid rate of assimilation, calling for such frequent bipartitions, results in the exhaustion of the micronuclei during these periods; but when assimilation is at a low ebb, the little demand for the dynamic forces of the cell results in the reduplication of the micronuclei beyond the typical number. Thus Maupas's observations that in certain hypotrichous forms the micronuclei are reduced in number, and later appear again in greater number, is entirely substantiated by these cultures. Variations in the number of micronuclei is not unknown in other forms. Johnson ('93), for instance, working on *Stentor*, found that from one to eight may be associated with each node of the macronucleus. However, the disappearance of all the micronuclei in certain forms, as described by Maupas, has never occurred in my cultures, and the continuance of his series for many generations without this cell-organ I believe is open to question.

Whatever may be the correct interpretation of the nuclear changes taking place in the life-history of the hypotrichida, these cultures strongly suggest that it is customary to regard the structure most frequently observed in "wild" Infusoria as too fixed in character, and to overlook the fact that under varying conditions, modifications may occur which are in no way abnormal. Bütschli ('83) comments on the frequent presence of a coarsely alveolar or vacuolar structure of the protoplasm of certain ciliates

and believes that this should be sharply distinguished from the fine honey-comb structure which obtains in other forms, such as many of the hypotrichida; and he regards the observations of Sterki ('78), that *Stylonychia mytilus* has a markedly vacuolized structure, as an indication of abnormality. Simpson ('01, 2) made sections of what he regards as "absolutely normal" *Stylonychia*, and he states that they "showed the vacuolization fairly well developed. . . ." Again, the question of the fixity of form and position of the macronucleus has been variously discussed since Balbiani more than forty years ago observed a shifting in *Paramœcium*, to its recent consideration by Simpson through observations on various species. My own cultures give conclusive proof that the cytoplasm becomes considerably more vacuolated at certain periods in the life-cycle; but further, daily observation has shown that hardly any two individuals are identical in their cytoplasmic condition, and the same can be said of the position of the macronuclei and the accompanying micronuclei.

The fact that subjection to beef-extract gradually revived the cellular activity and caused the resumption of the normal condition of cytoplasm and nuclei, shows that up to the verge of extinction the cell-life can be revived. I think this indicates that we are hardly justified in assuming that Protozoa, when dividing at a low rate, with nuclei fragmented, etc., are exactly "abnormal." The fact that it is possible to restore such remarkable types as I have figured to the text-book "normal" condition suggests that we are justified in regarding these changes as phases in the life-history of the Infusoria which occur under certain conditions after a considerable period of vegetative reproduction.

VI. EFFECT OF INITIAL AND DAILY STIMULATION WITH SALTS ON THE RATE OF DIVISION.

The first essential for experimental work with salts on the fission-rate of Protozoa is to have a constant subject on which the stimuli are to be applied so that the results obtained shall be directly comparable. This condition is admirably fulfilled by cultures of Infusoria fed daily on the same diet and carried on in this way for many weeks; and such cultures probably afford as near a perfect "control" as it is possible to get for work of this kind.

The results obtained with beef-extract as a stimulant for worn-out Protozoa led me to test the effect of some of the more common salts on the fission-rate, since, as Liebig claimed, the stimulating property of beef-tea is probably due to the extractives and not to the small amount of proteid which it contains. For this work potassium phosphate (monobasic and dibasic), potassium chlorid, potassium bromid, and potassium sulphate; sodium chlorid, and magnesium sulphate were chosen. The series of experiments with these seven salts extended from the early part of July to the middle of September, 1904. The work with each salt extended over twenty days. The salts were made up into equivalent normal solutions¹ and these were then diluted as indicated in the descriptions of the individual experiments. In each case two solutions of different strength were employed, and each of these was applied both as an initial and as a daily stimulus. The culture of *Gastrostyla steinii* was used in this work (*cf.* Diagrams V and VI).

1. Experiments with Potassium Phosphate (Monobasic and Dibasic).

On July 6, 1904, eight cultures (each consisting of four lines) of *Gastrostyla* were started with individuals isolated from my culture A of this species, which had been under observation since May 28. Four of these cultures were used for experiments with the monobasic and four with the dibasic salt. Of these cultures, half were used for initial stimulation and half for daily stimulation; and of each half, one was used for $\frac{n}{100}$ solutions and the other for $\frac{n}{1250}$ solutions of the salt in question.

The method of applying the salt was, briefly, as follows: In the case of initial stimulation, one individual was placed on a slide with as little of the culture-medium as possible. To this was added the solution of the salt to be tested and this was removed again immediately and fresh salt solution put on. Each transference was performed with a pipet used only for this purpose. The length of each initial stimulus was thirty minutes and when this had expired the specimen was transferred back to the grass-infusion. In the case of daily stimulation the method of procedure

¹The solutions were made according to the definition of "normal" solutions as given in Sutton's Volumetric Analysis, Eighth edition, 1900.

was identical except that the duration of the stimulation was ten minutes instead of thirty minutes.

The immediate effect of immersion in the monobasic salt was to cause the infusorian to rotate rapidly on its short axis for a couple of minutes, after which it began to move slowly about the slide, and by the end of the thirty minutes normal locomotion was entirely resumed. Practically the same behavior was caused by the application of the dibasic salt. I found, however, that this typical reaction varied somewhat with different individuals at various times; sometimes, for instance, the duration of the whirling motion was very much shorter and sometimes it was entirely absent. This is true not only for stimulation with potassium phosphates but also for stimulation with the various other salts tried. Slightly different reactions occurred with some of these other salts, but I have noticed the same variability. I am inclined to believe that the explanation of this variability in the reaction to a given stimulus at different times is in some way correlated with the slight changes in vitality which I have described as rhythms. I found also that when the salts were applied daily they soon ceased to cause any abnormal movements, even when their effect on the vitality of the animals, as determined by the division-rate, was detrimental. Here again there were occasional exceptions which point to periodical fluctuations in sensitiveness. This holds true for all the salts employed.

A glance at the diagram shows that the culture stimulated initially with K_2HPO_4 in $\frac{n}{1250}$ solution divided more rapidly than the control during three out of the four five-day periods of the experiment, and produced a greater effect than any of the three other experiments involving initial stimulation. Culture $K_2HPO_4 \frac{n}{100}$, initial stimulation, showed the next greatest effect, but this was manifested in a slowing of the rate in three out of four periods. In initial doses, then, the dibasic salt proved to be more effective—the greater dilution producing an accelerating effect and the lesser dilution producing a retarding effect. An examination of the data of the experiments on daily stimulation shows that $K_2HPO_4 \frac{n}{1250}$ again produced the greatest change in rate, though this time it had a retarding influence.¹ Summarizing the results of the

¹The curve for daily stimulation is not plotted for $KH_2PO_4 \frac{n}{100}$ and $\frac{n}{1250}$, during three periods because the individuals stimulated were lost accidentally at these times.

twenty-day experiments with the two potassium salts, it is apparent that the dibasic salt had in every case a greater "net effect" than the monobasic salt; and that the more dilute solution of the dibasic salt produced a greater acceleration than the less dilute solution when used as an initial stimulus, and also produced a greater depressing effect when given daily. This is a clear-cut example

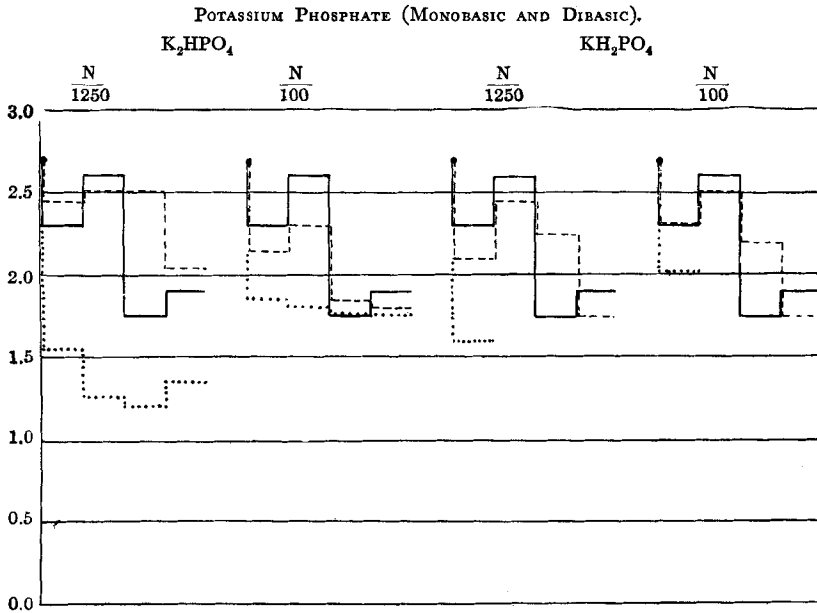


DIAGRAM VIII.

Effect of initial and daily stimulation with K_2HPO_4 ($\frac{n}{100}$ and $\frac{n}{1250}$) and KH_2PO_4 ($\frac{n}{100}$ and $\frac{n}{1250}$) on the division-rate of *Gastrostyla*. Averages are for five-day periods. Control (*Gastrostyla* A, on regular medium) is indicated by a continuous line; initial stimulation, by a broken line; and daily stimulation, by a dotted line. The eight experiments plotted in this diagram were carried on simultaneously from July 6 to July 26, 1904. (Cf. text.)

of a chemical being beneficial in a single small dose but detrimental when used frequently.

In view of the interesting results with the dibasic salt the K_2HPO_4 $\frac{n}{1250}$ initial-stimulus culture was continued for some two months after the twenty-day experiment was over. The result of this work is plotted in Diagram IX. From the curve it will be seen that in the sixth period of the experiment the rate fell

below that of the control, and at this point the infusorians were again stimulated, as previously, for thirty minutes. This apparently accelerated the rate (as compared with the control), but only temporarily as in the eighth period it was again below the control. Another stimulation at this time again raised the rate, but as in the previous case the effect was not lasting. After

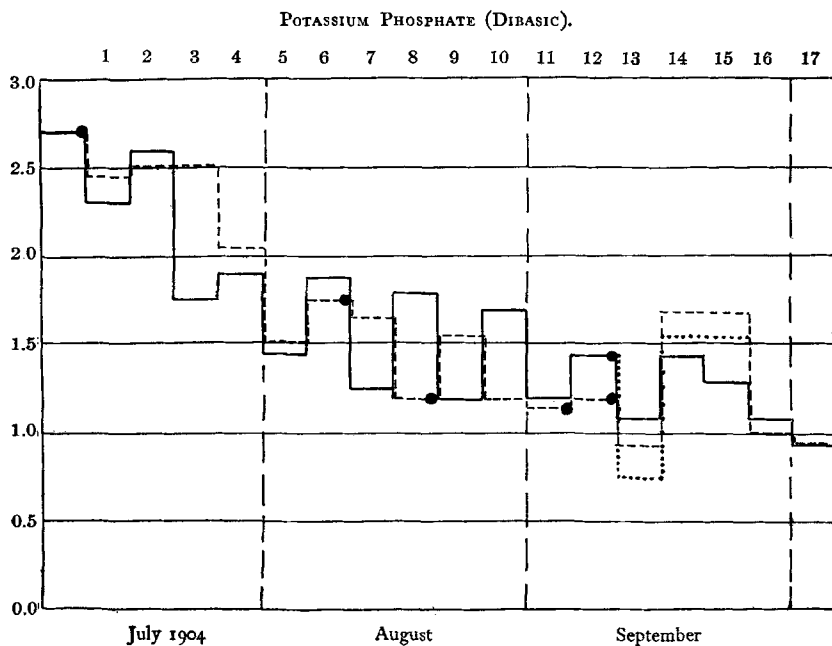


DIAGRAM IX.

This diagram shows the continuation of the experiment with K_2HPO_4 ⁿ/₁₂₅₀ (cf. Diagram VIII) illustrating the effect of stimulation with this salt at different periods in the life-cycle. Method of plotting, as in Diagram VIII. Control = continuous line; regular K_2HPO_4 series = broken line; new series stimulated = dotted line; time of stimulation = •. The figures above the diagram indicate the five-day periods. (See text.)

two more periods had passed, in each of which the stimulated line was dividing at a rate below the control, they were treated still another time with the salt-solution; but this time no acceleration was produced, but instead the rate, as compared with the control (cf. Diagram IX), fell still lower. The behavior of the culture here suggested the possibility that the lack of effect of the salt

was due to the series becoming accustomed to it, and accordingly I started a new series from the control and stimulated both this and the old series at the same time. From the results (see Diagram IX) it was evident that this hypothesis was not substantiated, for the new series showed even a greater drop in the fission-rate than did the old. Instead, it was apparent that the difference in effect of the salt at these later periods must be sought in the change in the general vitality of the culture itself. When the salt was first used the vitality of the series was considerably

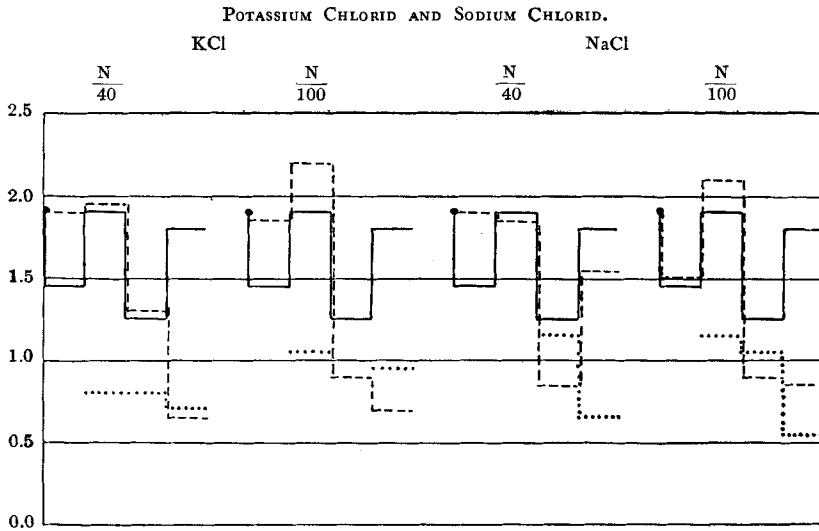


DIAGRAM X.

Effect of initial and daily stimulation with KCl, $\frac{n}{40}$ and $\frac{n}{100}$, and NaCl, $\frac{n}{40}$ and $\frac{n}{100}$, on the division-rate of *Gastrostyla*. Averages are for five-day periods. The eight experiments plotted in this diagram were carried on simultaneously from July 26, to August 15, 1904. Method of plotting is the same as in Diagram VIII.

greater than toward the end of the experiment, as is indicated by the comparative fission-rates of the two times; and the conclusion seems to be justified that a given stimulus produces different effects at different periods in the life-cycle. This result shows how complicated is the whole problem of the effect of stimuli on protoplasm, and the great amount of work that will have to be done before it will be possible to attain any satisfactory knowledge of the part played by a particular salt in the economy of the protozoön.

2. Experiments with Potassium Chlorid and Sodium Chlorid.

The experiments with KCl and NaCl were conducted precisely the same as those with the phosphates of potassium, except that an $\frac{n}{40}$ solution was used in place of the $\frac{n}{1250}$ of the phosphates. The accompanying curve (Diagram X) gives the results of the experiments.

The striking point about the effect of initial stimulation with KCl and NaCl in each dilution used is that they all accelerated the fission-rate during the first part of the experiment, and had a still greater opposite effect during the latter part, so that the "net

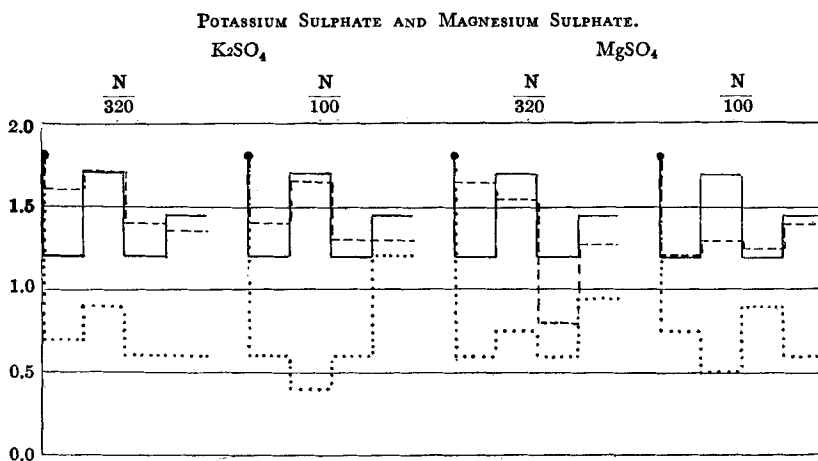


DIAGRAM XI.

Effect of initial and daily stimulation with K_2SO_4 , $\frac{n}{100}$ and $\frac{n}{320}$, and $MgSO_4$, $\frac{n}{100}$ and $\frac{n}{320}$, on the division-rate of *Gastrostyla*. Averages are for five-day periods. The eight experiments plotted in this diagram were carried on simultaneously from August 15, to September 4, 1904. Method of plotting is the same as in Diagram VIII.

effect" for the twenty-day experiment was a marked falling off in the number of divisions. A closer analysis of the results shows that KCl produced a greater variation from the control than did NaCl both when used as an initial and as a daily stimulus. In every case also the greater dilution produced the greater variation. Daily subjection to each of the salts proved to be uniformly

detrimental,¹ as was seen to be the case in the work with the two potassium phosphates. As far as these experiments go they would seem to indicate that potassium has more effect than sodium on the metabolic activity of *Gastrostyla*.

3. *Experiments with Potassium Sulphate and Magnesium Sulphate.*

The third series of experiments was with $\frac{N}{100}$ and $\frac{N}{820}$ solutions of K_2SO_4 and $MgSO_4$. The results of the eight cultures together

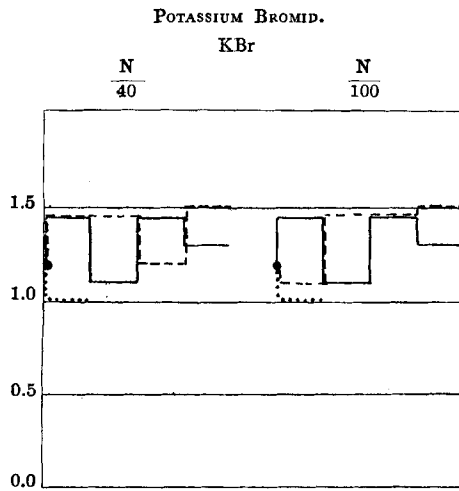


DIAGRAM XII.

Effect of initial and daily stimulation with KBr, $\frac{N}{40}$ and $\frac{N}{100}$, on the division-rate of *Gastrostyla*. Averages are for five-day periods. The four experiments plotted in this diagram were carried on simultaneously from August 30, to September 19, 1904. Method of plotting is the same as in Diagram VIII.

with the control are shown in Diagram XI, and they indicate that an initial application of K_2SO_4 in both dilutions used produced a slight acceleration in the division-rate, whereas $MgSO_4$ under the same conditions produced a retardation. It is evident here again that the daily use of the salts was invariably detrimental; and also

¹The omission of the curve at certain points is due to accidental loss of the specimens under experimentation.

that the greater dilution produced the largest variation in the fission-rate, except in the MgSO_4 daily stimulus experiment.

4. *Experiments with Potassium Bromid.*

The results obtained with potassium bromid in $\frac{n}{40}$ and $\frac{n}{100}$ solutions, are plotted in Diagram XII. This shows that KBr in both dilutions had on the whole a very slight accelerating effect on the division-rate, and also that the greatest variation from the control was caused by the $\frac{n}{100}$ solution. The chief effect of KBr, however, seems to have been to change the rhythm of division as shown when plotted in periods of five days. The daily application of this salt also was deleterious, and I had especial difficulty in maintaining the culture for more than two days when subjected to daily stimulations, which accounts for the omission of the daily curve in three out of the four periods of the experiment.

5. *Comparison of Results.*

Comparing the results of all the experiments with the seven salts when used as initial stimuli, it is clear that K_2HPO_4 $\frac{n}{1250}$ caused the greatest acceleration of the division-rate, while NaCl $\frac{n}{100}$ produced the greatest slowing of the rate. The largest variation from the control, when plotted in five-day periods, was shown by KCl $\frac{n}{100}$. All the salts tested agreed in having a marked deleterious effect when employed daily: K_2HPO_4 $\frac{n}{1250}$ being slightly the most active in this regard. The table on the opposite page gives the actual status of each experiment in relation to the control for each five-day period of the work.

Calkins tried stimulating his *Paramœcium* cultures with various salts, among them the dibasic potassium phosphate and found that it not only produced an acceleration of the division-rate, but also that there were less fluctuations in the rate. His results show far more uniformity with this salt than do my own. Greeley ('04) investigated the effects of a number of salts on the physical structure of protoplasm and incidentally on the division-rate of *Paramœcium*, and he arrived at the general conclusion that "with *Paramœcia* from alkaline cultures, anions or liquefying agents stimulate cell-division, cations or coagulating agents inhibit it. Thus I have frequently observed in my experiments that when the liquefying solution is too weak seriously to modify the structure

TABULATED RESULTS OF SALT EXPERIMENTS.

SALT USED.	SOLUTION.	FIVE-DAY PERIODS.				TOTAL VARIATION IN FOUR LINES.	NET EFFECT IN FOUR LINES.	AVERAGE VARIATION.	AVERAGE NET EFFECT.
		1st	2d	3d	4th				
KH ₂ PO ₄	$\frac{N}{100}$	0	-2	+9	-3	14	+4	3½	+1
	$\frac{N}{1250}$	-4	-3	+10	-3	20	0	5	0
K ₂ HPO ₄	$\frac{N}{100}$	-3	-6	+2	-2	13	-9	3½	-2½
	$\frac{N}{1250}$	+3	-2	+15	+3	23	+19	5½	+4½
KCl	$\frac{N}{100}$	+8	+6	-7	-22	43	-15	10½	-3½
	$\frac{N}{40}$	+9	+1	+1	-23	34	-12	8½	-3
NaCl	$\frac{N}{100}$	+1	+4	-7	-19	31	-21	7½	-5½
	$\frac{N}{40}$	+9	-1	-8	-5	23	-5	5½	-1½
K ₂ SO ₄	$\frac{N}{100}$	+4	-1	+2	-3	10	+2	2½	+½
	$\frac{N}{320}$	+8	0	+4	-2	14	+10	3½	+2½
MgSO ₄	$\frac{N}{100}$	0	-8	+1	-1	10	-8	2½	-2
	$\frac{N}{320}$	+9	-3	-8	-6	26	-8	6½	-2
KBr	$\frac{N}{100}$	-7	+7	0	+4	18	+4	4½	+1
	$\frac{N}{40}$	0	+7	-5	+4	16	+6	4	+1½

Record of the variation in the number of divisions of each initial stimulus experiment from the control, during each five-day period; and also the net effect for the whole twenty days of the experiment. For example: the KH₂PO₄ $\frac{N}{100}$ culture, during the first five-day period, divided exactly the same number of times as the control; during the second period, two times less; during the third, nine times more; and during the fourth, three times less than the control. For the four periods of the experiment, then, there was a total variation of fourteen divisions, or a "net effect" of four more divisions than the control.

of the protoplasm it will however, greatly increase the motility of the protoplasm and the rate of cell-division." Among the electrolytes employed by Greeley are three of the salts which I have used: KCl and MgSO_4 with predominant cations and NaCl with the anion predominant. He found that $\text{KCl } \frac{n}{40}$ and $\text{MgSO}_4 \frac{n}{320}$ each exerted an inhibiting influence on the fission-rate, through a coagulating of the protoplasm. Referring to these salts he remarks that "the less active solutions, such as KCl and MgSO_4 do not produce quite so dense a coagulum as the others, and the reaction is considerably slower." As already stated, my work with an initial stimulation of thirty minutes with $\text{KCl } \frac{n}{40}$ and $\text{MgSO}_4 \frac{n}{320}$ produced a quickening of the rate of fission for the first five days or more; the total result, however, for the twenty days of the experiment showed an inhibiting influence. With $\text{NaCl } \frac{n}{40}$ Greeley found an increase in the rate and this agrees with the first period of my NaCl experiment, but here again I found a slowing of the rate for the total twenty days. It is impossible, though, to make a direct comparison of Greeley's results with my own, both on account of the great difference in the methods employed and because he gives no details of the individual experiments. Peters ('04) describes some experiments with KCl on *Stentor* in which he found that initial stimulation for ten minutes produced an increased division-rate for the three days over which the longer experiments extended. This accords with my results for the early periods of stimulation with the $\frac{n}{40}$ and with the $\frac{n}{100}$ solutions of this salt.

To draw any general conclusions from my experiments with salts on the division-rate of *Gastrostyla*, I think, would be hazardous. Before this can be safely done it will be necessary to perform many experiments on different forms. Work on this subject up to the present time, while affording a nucleus of data as a basis for future investigation, is too meagre and the methods employed by different workers too varied to make the results at all comparable. As Towle ('04) aptly remarks: "the first step toward a clearing of the haze that envelops the subject will be found, I believe, when an effort is made to unify the conditions under which different investigators are working." From this work on the Protozoa, I am persuaded that the most adequate method of attacking the problem is by breeding long cultures of Infusoria on a fixed diet. While this is a tedious process, it is the only way

in which it is possible to know with any degree of certainty exactly what the pedigree of the subjects of the experimentation is, and unless one has the daily record of the ancestry of each protozoön and knows its status in the life-cycle, any results obtained lose a large part of their value. Nothing emphasizes this point more forcibly than the record of my experiments with the dibasic potassium phosphate.

VII. EFFECT OF LIGHT ON THE RATE OF DIVISION.

Maupas ('88) made some interesting experiments on the effect of light on the division-rate of various Infusoria, by keeping cultures for one month in the light and then for one month in the dark and then comparing the rate of division during the two periods. But it would seem that his method is open to criticism for it is clearly impossible to keep the conditions absolutely constant during the two months of the experiments, not to mention the fact that, according to Maupas himself, "senescence" is increasing. Consequently it is impossible to say that the difference, or absence of difference, in the rate during two consecutive months shows the effect, or non-effect, of light on bipartition. I would call attention to the fact that he found less difference in light and darkness than my records show for any two consecutive months of any of the cultures when light and all other factors have been apparently constant.

With this in mind I made an experiment on the effect of light on the division-rate of *Oxytricha fallax*, and endeavored to eliminate the factors which seem to vitiate Maupas's experiments. This was accomplished by isolating an individual from each line of *Oxytricha* A-culture, and starting with them a second culture (designated A¹) in absolute darkness.¹ By this method the light and dark series were carried on simultaneously and this ruled out the question of relative "senescence"; and at the same time variation in the food was reduced to a minimum, since the same infusion was supplied to both cultures simultaneously. Temperature differences were avoided also. It would seem, therefore, that light was the only factor removed in the case of culture A¹, and

¹The culture was necessarily, of course, subjected to light for two or three minutes each day when the record of divisions was being taken.

that this had a very insignificant influence on the fission-rate is shown by the accompanying table. The experiment certainly substantiates Maupas's result, however obtained, that light is of little or no direct importance in the economy of the ciliate.

Oxytricha A (light)	{	A -1	23 divisions.
		A -2	28 "
		A -3	17 "
		A -4	20 "
		—	
		Total, 88 divisions.	
Oxytricha A ¹ (darkness)	{	A ¹ -1	22 divisions.
		A ¹ -2	26 "
		A ¹ -3	16 "
		A ¹ -4	18 "
		—	
		Total, 82 divisions.	
		—	
		Excess in light, 6 divisions.	

VIII. SUMMARY.

1. The chief object of the work was to ascertain if the life-history of hypotrichous Infusoria is characterized by "cycles," and if so, the cytological changes which occur and the effect produced on the cycles by changes in environment.

2. Two cultures of *Oxytricha fallax*, two of *Pleurotricha lanceolata*, and one of *Gastrostyla steinii* have been carried on. *Oxytricha* culture A extended from October 26, 1901, to July 14, 1903, during which time 860 generations were attained. Culture B was started December 10, 1902, and died out through an accident November 22, 1903. *Pleurotricha* culture A was isolated November 10, 1902, and became extinct July 3, 1903. Culture B was carried continuously from November 25, 1902, to March 13, 1904, when it was lost by an accident. The culture of *Gastrostyla* was started May 28, 1904, and died out December 5, 1904. The life-history of each culture is represented graphically by a curve which is plotted by averaging the number of divisions per day of the four lines constituting each culture, and then averaging this for five- or ten-day periods.

3. All the cultures give incontestable proof that the species studied pass through periods of greater and less general vitality

as measured by the rate of division. This cyclical change is most prominent in the *Oxytricha* A-culture. The periods of depression lead to death if the culture is subjected continuously to the same environment.

4. Minor fluctuations occur in the division-rate which I have termed "rhythms" and which are to be clearly distinguished from cycles. The rhythms are probably indicative of a rhythmical change in the metabolism of the organism, though they are influenced somewhat by almost imperceptible changes in the environment.

5. The results of the experiments seem to indicate that "rhythms" and "cycles" should be defined as follows:

A rhythm is a minor periodic rise and fall of the fission-rate, due to some unknown factor in cell-metabolism, from which recovery is autonomous.

A cycle is a periodic rise and fall of the fission-rate, extending over a varying number of rhythms, and ending in the extinction of the race unless it is "rejuvenated" by conjugation or by changed environment.

6. Changes in the environment will revive the lagging functions during the descending cycle, as is shown conclusively by the sudden recuperation of *Oxytricha* A during July, 1902. There is every reason to believe that this "rejuvenescence" was produced by treatment with extract of beef.

7. Seasonal and temperature changes have no apparent influence on the cyclical fluctuations of vitality. Variation in temperature, however, undoubtedly affects somewhat the daily rate of division, if not directly, at least through the food supply.

8. The number of generations which constitute a cycle is not at all constant; and there is no evidence to show that duration in time is of any significance in the forms studied.

9. Periods of extreme depression of vitality are manifested on the physiological side chiefly by a greatly decreased division-rate, and by the comparative frequency of pathological divisions. Morphological changes are apparent chiefly in (1) an increased vacuolization of the cytoplasm; (2) distortion and fragmentation of the macronuclei; (3) numerical increase of the micronuclei; and finally (4) in a reduction of the ciliary apparatus.

10. Variation in the size of the infusorians during the life-cycle is marked; the organisms being very small during periods

of high reproductive activity and progressively increasing in size as "degeneration" advances. In the last couple of generations before death ensues the size is secondarily reduced by a shrinking of the cytoplasm.

11. A disappearance of one of the micronuclei occurred at certain periods of high reproductive activity.

12. These cultures strongly suggest that it is customary to regard the structure most frequently observed in "wild" Infusoria as too constant in character, and to overlook the fact that, under varying conditions, modifications may occur which are in no way abnormal.

13. Throughout the entire period of the cultures no tendency to conjugate was shown in any of the series, and experiments for endogamous and exogamous syzygies failed to produce a single case.

14. Experiments with KH_2PO_4 , K_2HPO_4 , KCl , KBr , K_2SO_4 , MgSO_4 , and NaCl gave evidence of the extreme sensitiveness of Protozoa to solutions of electrolytes. Initial stimulation with KH_2PO_4 , K_2SO_4 , and KBr in $\frac{n}{100}$ solutions caused in each case a slight acceleration of the division-rate; while initial stimulation with $\frac{n}{100}$ K_2HPO_4 , KCl , NaCl , and MgSO_4 caused a slowing of the rate. Daily stimulation with the same solutions of each of these salts invariably caused a marked inhibition of the fission-rate. Initial stimulation with KH_2PO_4 $\frac{n}{1250}$ showed no change in the rate while K_2HPO_4 $\frac{n}{1250}$ produced a marked increase. K_2SO_4 $\frac{n}{320}$ accelerated division; and KCl and NaCl each in $\frac{n}{40}$ solutions, retarded it; while KBr $\frac{n}{40}$ accelerated the fission-rate. Comparison of the effects of the two solutions of each salt shows that, almost without exception, the more dilute solution produced the greater variation in the rate from the control.

15. Stimulation with K_2HPO_4 $\frac{n}{1250}$ gave different results at various periods of the life-cycle, which indicates that the state of the general vitality of the culture, and also the rhythms, are factors which must be taken into account in experimental work of this nature.

16. Light has little or no direct effect on the division-rate of *Oxytricha fallax*.

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EXPLANATION OF PLATES.

The photographs were taken by Dr. Edward Leaming, of Columbia University, from permanent preparations stained with picrocarmin. The magnification is the same in every case and the relative sizes, therefore, represent absolute differences. The figures, unless otherwise specified, are of *Oxytricha fallax*, Culture A.

PLATE I.

Figs. 1 and 2. Two individuals in the 230th generation, period 16, April 2, 1902. (Cf. Diagram I.) The cytoplasm is vacuolated and the macronuclei are vacuolated and displaced in the cell. A characteristic "halo" is visible about the macronuclei. The individual shown in Fig. 2 has three micronuclei.

Figs. 3 and 4. Individuals in the 239th and 241st generation respectively. Period 24, June 1902. The two macronuclei in each are fused and their structure appears somewhat more homogenous than is the case in those illustrated in Figs. 1 and 2.

Fig. 5. Specimen in the 243d generation, period 25, June 24, 1902, showing an extreme case of cytoplasmic vacuolization. The nuclei are exceptionally normal for this period of the cycle.

Fig. 6. Specimen in the 246th generation, period 25, July 1, 1902.

Fig. 7. Individual in the 246th generation (A-2), period 25, July 2, 1902. The macronuclei are surrounded by a "halo" (cf. Fig. 1).

Fig. 8. Individual in the 247th generation (A-1), period 25, July 2, 1902. Note the condition of the cytoplasm.

PLATE II.

Fig. 9. Specimen in the 250th generation (A-1), period 26, July 6, 1902. The cell is shrunken and the cytoplasm considerably vacuolated. Note the somewhat reduced size and irregular contour of the cell. This is the last of the line A-1 before it was "rejuvenated."

Figs. 10 and 11. Specimens in the 255th generation, period 27, July 21, 1902. These individuals are from line A-2 which remained dividing, at this time, at the slow rate. The specimen photographed in Fig. 10 has ingested a *Trachelomonas volvocina*.

Fig. 12. Specimen in the 256th generation (A-1), period 26, July 8, 1902. This line had divided six times within the past forty-eight hours. Note the normal condition of cytoplasm and nuclei as compared with the preceding specimens.

Fig. 13. Specimen in the 287th generation (A-1), period 27, July 20, 1902. Size is reduced. Compare with Fig. 12.

Fig. 14. Individual in the 331st generation (A-1), period 29, August 7, 1902. Size is reduced. Nuclei are proportionately large.

Fig. 15. Specimen in the 409th generation, period 32, September 1, 1902. Apparently a "normal" individual in every respect.

Fig. 16. Specimen in the 542d generation, period 36, October 17, 1902. Cytoplasmic vacuolization begins to appear.

Fig. 17. Individual in the 829th generation, period 56, April 29, 1903. Nuclear fragmentation has begun.

Fig. 18. Specimen in the 853d generation, period 62, July 2, 1903. Nuclear and cytoplasmic degeneration is far advanced. The size of the cell is greatly increased.

PLATE III.

- Fig. 19. Same as Fig. 18, Plate II.
- Fig. 20. Specimen in the 854th generation, period 62, July 4, 1903.
- Fig. 21. A double monster from A-1, 238th generation, June 16, 1902.
- Fig. 22. *Oxytricha fallax*, B-culture. Specimen in the 365th generation, August 25, 1903. Condition is normal.
- Fig. 23. *Pleurotricha lanceolata*, B-culture. Individual in the 413th generation, January 29, 1904. Late division-stage.

