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# The relation of density of population to rate of reproduction in the ciliates *Didinium nasutum* and *Stylonychia pustulata* [1].

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## Contents.



## Introduction.

In 1921 ROBERTSON reported the occurrence in the ciliate *Enchelys farcimen* of a phenomenon which has since aroused great interest among protozoologists, namely, that of the allelocatalytic effect. The nature of this effect may be quite satisfactorily eluci-

[1]) I am indebted to Prof. MAX HARTMANN for valuable suggestions in connection with the methods of culture employed and for his kindness in placing the facilities of his Abteilung at my disposal.

dated by certain excerpts from ROBERTSON's own papers. "When two infusoria (*Enchelys farcimen* EHRBG.) are isolated into the same drop of culture medium, the rate of production of new cells is much more rapid than it is when a single individual is isolated into a drop of the same size. It may be 16 times as great. This has been designated 'allelocatalytic effect'" (1922, p. 411). "Evidently some substance issues from the cells which has the effect of accelerating multiplication. When but one individual is initially present the concentration of this substance is small and the rate of reproduction low. When two individuals are initially present the concentration of this substance is higher and the rate of multiplication is, in consequence, enhanced. The process of multiplication is, for this reason, autocatalyzed" (1924 a, p. 108).

ROBERTSON (1923, p. 95) considered the nucleus to be the source of the allelocatalyst and maintained that the catalyst is freed into the fluid medium at the time of cell division. He based this conclusion on evidence of the following type: When one specimen of *Enchelys* was introduced into a drop of hay infusion, only two individuals were produced in the first 24 hours, though 14 were produced in the second 24 hours. When two individuals were isolated into a drop of equal size, three were produced in the first 24 hours, and 117 in the second 24 hours. The assumption is that once a cell division has occurred, the catalyst is free in much greater quantities in the fluid medium where it may act on all the cells of the community, thus accelerating appreciably the rate of reproduction.

An allelocatalytic effect was likewise observed by ROBERTSON (1924) in cultures of the ciliate *Colpidium* sp., some of which were made up with hay infusion and others with synthetic media.

This work was much at variance with earlier work on factors affecting reproduction in infusoria. WOODRUFF (1911) in studies on *Paramecium caudatum* and *P. aurelia* reported that as the density of population increases, the fission rate decreases. Speaking of the relation of the reproductive rate to the volume of culture fluid, he states (p. 581) that "the greater the volume the more rapid is the rate of division". This fact he accounts for as follows: "Paramecia excrete substances which are toxic to themselves when present in their environment, and these substances are more effective when the organisms are confined in limited volumes of culture fluid." Similar results were obtained by WOODRUFF (1913) when his investigations were extended to the hypotrichous forms *Stylonychia*

*pustulata* and *Pleurotricha lanceolata*. Hence, the general impression up to the appearance of Robertson's results was to the effect that the vitality of infusoria is appreciably diminished by their own excretion products when they are confined to small environments.

It is therefore not surprising that Robertson's work occasioned a somewhat extensive inquiry into the relation of density of population to rate of reproduction in infusoria. Up to the present the results of this inquiry have shown the allelocatalytic effect to be a somewhat elusive phenomenon. Cutler and Crump (1923 a), upon testing for allelocatalysis in *Colpidium colpoda* by isolating from one to four individuals into small drops of a synthetic medium which they had previously devised (1923), failed to obtain positive evidence of the phenomenon. Within the limits of their experiments, they found that neither the initial number of animals nor the size of the drops employed (0.5 cmm. to 8.5 cmm.) influenced the reproductive rate appreciably. Robertson (1924 b) criticised these results on the grounds that the authors did not wash their animals before establishing the test cultures. Whereupon, Cutler and Crump (1925) repeated their experiments using washed animals, but with results not unlike their first ones. Greenleaf (1926) in a series of experiments on *Paramecium caudatum*, *P. aurelia*, *Pleurotricha lanceolata*, and *Stylonychia pustulata* in which he used volumes of hay infusion varying in size from 0.05 cc. to 1 cc. not only obtained negative results as regards allelocatalysis but found that the smaller volumes of fluid actually retarded the fission rate. Peskett (1924, 1925) failed to obtain evidence of allelocatalysis in his studies on sterile cultures of yeast, whether the cells were unwashed or washed. Calkins (1926, p. 205) also obtained negative results when different numbers (one to four) of the hypotrichous ciliate *Uroleptus mobilis* were isolated daily over a period of 60 days into drops of standard size. He obtained the highest division rate in the drops that contained initially only one individual. Myers (1927) in an extensive study of certain factors which affect the reproductive rate in *Paramecium caudatum* found that "increasing the number of individuals present in a given volume does not increase the rate of reproduction either from the beginning of the cultures or after the first fission. On the contrary, it decreases it markedly". Jahn (1929) successfully circumvented the disadvantages of small drops of culture medium by adopting in tests for allelocatalysis in *Euglena* a mass culture method which embraced the employment of relatively large volumes of an autotrophic medium and of counts made from samples of the

medium. His results failed to demonstrate the existence of the allelocatalytic effect. PETERSEN (1929) also investigated the question of allelocatalysis in *Paramecium caudatum* and obtained in most cases negative results, though there were certain exceptions. YOCOM (1928), however, in contrast to the results just cited obtained quite definite evidence of the effect in *Oxytricha* sp. The division rate in different experiments was from 12 to 25 per cent. higher in four drops of 0.05 per cent. beef extract solution than in ten drops, though the difference was by no means so marked as that recorded by ROBERTSON. (Reference may be made to LUDWIG, 1928, for a review of much of this work from the standpoint of biometrics.)

The studies of DARBY (1930) on *Paramecium caudatum*, *P. aurelia*, and *Stylonychia pustulata* deserve special comment. DARBY not only obtained no evidence of allelocatalysis in these forms, but also offers a likely explanation of the results obtained by ROBERTSON. Upon examining critically ROBERTSON's method of preparing culture fluid, he concludes that ROBERTSON's medium had an unfavorable $p_H$ and was weakly buffered. The infusoria which ROBERTSON introduced into this medium tended to change the $p_H$ in the direction of the optimum, according to DARBY, and the greater the number of animals which ROBERTSON introduced at one time, the more rapid the change in $p_H$ was effected. The buffering power of a few infusoria in a large amount of medium would be inadequate, DARBY states, to alter the $p_H$ appreciably, whereas that of a large number of infusoria in a small amount of medium would be sufficiently pronounced to alter the $p_H$ rapidly toward the optimum, thus accelerating the reproductive rate.

From the foregoing it is evident that most of the studies on allelocatalysis in infusoria have dealt with forms which feed upon bacteria. It is entirely possible, to be sure, that in such cases the catalyst may originate in the bacteria. Indeed, ROBERTSON in his earlier work (1921) maintained that the allelocatalyst of cellular multiplication, while originating p e r s e in the infusoria, is produced only in the presence of a so-called "x-substance" previously formed by the bacteria. Later he places less emphasis on the "x-substance", probably because of the fact that a bacterised hay infusion is already of sufficient chemical and physical complexity without the postulation of a specific "x-substance" therein. In general, ROBERTSON (1927) discountenances the view that the allelocatalyst is formed by the bacteria, as does YOCOM.

Nevertheless, it cannot be denied that the bacteria, while an indispensable component of the culture medium in many cases, constitute an ever-present potential source of error. Not only may the numbers of bacteria available as food fluctuate, but the kinds of bacteria present may vary, and either of these variables may influence the reproductive rate of the infusoria. The fact that most of the work on allelocatalysis has been done on forms that feed on bacteria and the patent desirability of more information on the phenomenon have prompted the present study in which a definite attempt is made to relegate the bacteria to a position of comparative insignificance. This has been accomplished in two ways: (1) By testing for allelocatalysis in *Didinium nasutum*, which can be cultivated in an inorganic medium with *Paramecium* as the associated food organism, and (2) by testing for it in *Stylonychia pustulata*, employing the phytomonad *Chlamydomonas* as food and sterile Knop solution as the fluid medium.

## *Didinium nasutum.*

### A. Methods.

All tests for allelocatalysis were carried out in depression slides. A measured amount of fluid containing *Paramecium caudatum* was placed into each depression, one or more didinia were added, and records were made of their reproductive rates. The smallest amount of fluid used was 0.02 cc. To test the effect of increased volume of fluid, several times this amount was added to each depression or "test culture". The slides were kept in moist chambers having tightly fitting ground-glass tops, so that evaporation was to all practical purposes prevented. Once the didinia were introduced into the fluid, the latter was in no case renewed, nor was any additional fluid added to the original amount.

Records were made at 12-hour intervals of the number of progeny and general condition of the test culture. To prevent evaporation, it is needless to say that the recording of such data was effected with as much dispatch as possible. The culture fluid, 0.01 per cent. modified Knop solution of $p_H$ 6.8, was in no sense a nutrient medium, but rather a natatorial medium having a tonicity and reaction suitable for *Didinium* and *Paramecium*. Furthermore, it was a medium poorly suited to the growth of bacteria. I have described elsewhere (1932) the details of its preparation.

In the preparation of the food, well-fed paramecia (all of a single clone) were taken from flourishing hay-infusion cultures and were concentrated centrifugally into a small volume of infusion. They were then transferred to a paper filter, in which they were washed repeatedly by passing KNOP solution through the filter. By this method they were got into fresh KNOP solution which was uncontaminated by hay infusion and comparatively free of bacteria. Upon being pipetted out of the filter, they were ready to be used in the tests for allelocatalysis. (Special care was taken in removing the paramecia from the original infusions to avoid all visible masses of bacterial zoogloea. I have elsewhere, 1928, directed attention to the importance of culturing didinia on well-fed paramecia.)

In all tests a definite attempt was made to keep the ratio of the paramecia to the volume of fluid contained in a depression constant. By this procedure any substances which the paramecia might excrete and which might influence the fission rate of the didinia were present in like quantity per unit of volume in all cultures. To do this with absolute accuracy by counting the paramecia would obviously be a task of overwhelming magnitude. In practice the procedure was to add to each depression one drop of KNOP solution containing paramecia to each drop of pure KNOP solution. For example, in the simplest experiments (one *Didinium* to two drops of fluid) one drop of pure KNOP solution was put into a depression and one drop of KNOP solution containing paramecia was added. Then a *Didinium* was introduced. In 8-drop tests, 4 drops of pure KNOP solution and 4 drops of the food mixture were used. It is true that the number of paramecia per unit volume of solution varied somewhat each time a fresh mixture of food was prepared. The final results indicate, however, that this variation was of no significance. In general, each drop of the food mixture contained about 500 paramecia. In all cases the term "drop" signifies a volume of 0.01 cc. Sufficient food was added at the time the test cultures were established to maintain the didinia for the duration of the experiment. Test cultures in which the food supply became exhausted before the formal termination of the experiment were rejected.

All didinia were thoroughly washed in two or more changes of KNOP solution before they were transferred to the depression slides.

All cultures were kept in a constant temperature chamber maintained at 19° C.

## B. Effect of isolating different numbers of didinia into identical volumes of fluid.

### Series I. Clone A.

In this series of experiments from one to four didinia were isolated into two drops (0.02 cc.) of fluid. Each "experiment" of the series embraces the results obtained with four test cultures.

The results of this series of four experiments are presented in Table 1. In order to clarify the method of presenting the data, which is constant throughout the entire paper, we may examine in detail the data of Experiment 1. The numerical designation of the experiment is given in Column I. The fact that each of the 4 didinia was isolated into 2 drops of fluid is shown in Col. II. Records were made at 12-hour intervals (Col. III), as has been said. The number of animals present in each of the 4 cultures at successive 12-hour intervals is given in Col. IV. Upon following through the history of the first culture, we find that 4 animals were present after 12 hours, 8 after 24 hours, 10 after 36 hours, and 25 after 48 hours. At the end of 60 hours no count was made, for some of the animals had encysted, as is shown by "E", and some were conjugating, as is shown by "C". When either encystment or conjugation occurred in one or more test cultures of an experiment, the experiment was considered to be at an end, for either of these processes marks a cessation of reproduction. Hence, Exp. 1 came to a close at the end of 60 hours. The total number of animals present in all 4 cultures at the end of successive 12-hour periods is shown in Col. V, the mean total per culture in Col. VI, and the mean total derived from each initial animal in Col. VII. In this experiment the number of test cultures was the same as the initial number of animals, and therefore the totals in Cols. VI and VII are the same. Finally, the mean number of divisions per initial animal for each 12-hour period is given in Col. VIII. The mean number of divisions for any 12-hour period was reached by converting the total number of animals indicated in Col. VII into the corresponding number of divisions, and by subtracting from this figure the number of divisions that occurred in the preceding 12-hour periods.

With these details of method in mind, we may proceed to an examination of the results given in Table 1. Col. VIII shows that 1.87 divisions occurred in the first 12-hour period in Exp. 1, 1.15 in the second 12-hour period, 0.71 in the third, and 0.98 in the fourth. Then, when nearly five generations had been attained

Table 1.

*Didinium nasutum*. Series I. Clone A. Effect of isolating different numbers of animals (1 to 4) into equal volumes of fluid (2 drops or 0.02 cc. KNOP solution containing paramecia). The totals in Columns V, VI and VII indicate numbers of animals and not numbers of divisions. An experiment was discontinued whenever encystment (E) or conjugation (C) occurred in one or more of the test cultures.

| I | II | III | IV | | | | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of experiment | Ratio of animals to drops | Time interval in hours | Number of animals present in each test culture | | | | Total for four cultures | Mean total per culture | Mean total per initial animal | Mean number of divisions per initial animal |
| 1 | 1:2 | 12 | 4 | 4 | 3 | 4 | 15 | 3.7 | 3.7 | 1.87 |
| | | 24 | 8 | 8 | 8 | 9 | 33 | 8.2 | 8.2 | 1.15 |
| | | 36 | 10 | 16 | 14 | 16 | 56 | 14.0 | 14.0 | 0.71 |
| | | 48 | 25 | 26 | 27 | 33 | 111 | 27.7 | 27.7 | 0.98 |
| | | 60 | E, C | E, C | E, C | 47 | E, C | | | |
| 2 | 2:2 | 12 | 5 | 8 | 8 | 7 | 28 | 7.0 | 3.5 | 1.75 |
| | | 24 | 16 | 12 | 17 | 15 | 60 | 15.0 | 7.5 | 1.12 |
| | | 36 | 28 | 32 | 34 | 30 | 124 | 31.0 | 15.5 | 1.06 |
| | | 48 | E, C | E, C | 44 | C | E, C | | | |
| 3 | 3:2 | 12 | 11 | 12 | 9 | 12 | 44 | 11.0 | 3.6 | 1.83 |
| | | 24 | 26 | 30 | 23 | 22 | 101 | 25.2 | 8.4 | 1.22 |
| | | 36 | 43 | E | C | E, C | E, C | | | |
| 4 | 4:2 | 12 | 16 | 15 | 12 | 16 | 59 | 14.7 | 3.7 | 1.85 |
| | | 24 | 34 | 33 | 27 | 32 | 126 | 31.5 | 7.9 | 1.12 |
| | | 36 | C | C | E, C | C | E, C | | | |

(27.7 animals per culture — Col. VI), conjugation and encystment set in and ended the observations (Col. V). In Exp. 2 it is seen from Col. VIII that 1.75 divisions occurred in the first 12-hour period, 1.12 in the second, and 1.06 in the third. Cysts and conjugants appeared in this case after only 36 hours (Col. V), when the total number of animals in each culture was 31 (Col. VI). The figures given in Col. VIII for Exps. 3 and 4 do not differ significantly from the corresponding figures given for Exps. 1 and 2, though in Exps. 3 and 4 cysts and conjugants appeared after only 24 hours (Col. V) — as formerly, when the total number of didinia in each culture was approximately 30 (Col. VI). Col. VII emphasizes the fact that the division rate was practically the same in all experiments; it shows that at the end of any 12-hour period the total number of progeny derived from each initial animal was practically the same in each experiment, insofar as the contemporaneous life of the cultures of different experiments permits comparisons.

Thus it is seen that no matter whether 1, 2, 3, or 4 didinia were introduced originally into the drop the division rates were essentially the same in each experiment. Increasing the initial number of animals did not accelerate the reproductive rate. When the initial number was doubled, the fission rate remained the same, and twice as many animals resulted in a given length of time — a total of 56 animals in Exp. 1 after 36 hours and 124 in Exp. 2 (Col. V); similarly, 60 animals in Exp. 2 after 24 hours and 126 in Exp. 4. If Exp. 1 is compared with Exp. 3, a similar relation obtains in that three times as many didina were produced in any given length of time — 33 in Exp. 1 after 24 hours and 101 in Exp. 3.

Furthermore, if the results of a particular experiment (Exp. 1 or 2, for example) are considered alone, we find no acceleration of the division rate as the number of animals in the test cultures increased. If an allelocatalyst is actually liberated into the fluid at the time of cell division, as ROBERTSON maintains, we should expect to find a marked acceleration of the reproductive rate immediately after the first division. But we find no such acceleration, either between the first and second periods or between the second and third. We find only a slight deceleration in the later periods, attributable in all probability to an accumulation of metabolic waste in the small test cultures. In conclusion, we find no evidence of the allelocatalytic effect in Series I.

Certain other points are of special interest. Why was the division rate higher in each experiment in the first 12-hour period than in the second? This circumstance is explained by the fact that the initial animals were not products of an immediate fission. They were in all cases animals of uniform size which had fed and grown since the last fission. Such individuals were selected rather than small ones for the reason that small didinia found in stock cultures are often individuals which, instead of feeding and growing, divide after some hours without feeding and produce two conjugants. As has been stated, the production of conjugants in a test for allelocatalysis negatives the experiment. Furthermore, small didinia found in stock cultures may be individuals of low vitality, though they may appear to be structurally normal. In order to demonstrate that the foregoing explanation of the higher division rate in the first 12-hour period is actually the correct one, a similar series of experiments was later carried out with Clone A using small animals

derived from a recent fission. These results will follow under the heading "Series III".

A second point of special interest relates to the onset of conjugation and encystment in the cultures. MAST (1917) and MAST and IBARA (1923) have pointed out that didinia often encyst even though food is present, a fact which I have also observed many times. They attribute the encystment to the accumulation of waste products in the environment. Evidently an excessive accumulation of metabolic waste accounts for the production of cysts in the cultures of the present study. Not only does encystment occur in densely populated cultures of *Didinium*, but conjugation occurs as well, and I have on many occasions obtained conjugants by allowing cultures to become exceedingly thickly populated. It appears, therefore, that conjugation, like encystment, may be induced in *Didinium* by the accumulation of waste in the fluid, and this circumstance accounts for the onset of conjugation in the test cultures. The relatively small number of bacteria present in the KNOP solution of the test cultures, the rapid increase in the number of didinia, and the rapid correlative decrease in the number of paramecia indicate that the waste is derived from the didinia.

It is evident, therefore, that there is a definite limit to the number of didinia which a given volume of fluid can support in a condition of active vegetative multiplication. In Series I it appears that a volume of 0.02 cc. can support a maximum population of about 30 animals. With the attainment of this maximum, vegetative reproduction ceases and conjugation or encystment sets in. An analogous condition is reported by MYERS (1927, p. 39) in *Paramecium caudatum*, as follows: "After introduction of a certain number of parent individuals into a given volume of culture fluid, the population increases to a maximum, then declines, till the animals become extinct."

## Series II. Clone A.

To avoid the criticism that allelocatalysis may have been adversely affected in Series I by culture in volumes of such small size, the experiments were repeated, using volumes four times as large. These experiments constitute Series II, in each culture of which 8 drops (0.08 cc.) of fluid were used instead of only two.

The results are presented in Table 2, which in plan is entirely like Table 1, though in order to eliminate excessive detail Col. IV is omitted. The numerical designation of the remaining columns is

unchanged. It is to be borne in mind that in Series II and in all succeeding experiments with *Didinium* the cultures were discontinued upon the appearance of cysts or conjugants therein, although this fact is not indicated in any of the tables that follow.

Table 2.

*Didinium nasutum.* Series II. Clone A. Effect of isolating different numbers of animals (1 to 4) into equal volumes (8 drops or 0.08 cc.) of KNOP solution containing paramecia. The columns are numbered as in Table 1, though Col. IV is omitted. An experiment was discontinued upon the occurrence of encystment or conjugation in one or more of the test cultures.

| I | II | III | V | VI | VII | VIII |
|---|---|---|---|---|---|---|
| Number of experiment | Ratio of animals to drops | Time interval in hours | Total for 4 cultures | Mean total per culture | Mean total per initial animal | Mean number of divisions per initial animal |
| 5 | 1 : 8 | 12 | 15 | 3.7 | 3.7 | 1.87 |
| | | 24 | 29 | 7.2 | 7.2 | 1.06 |
| | | 36 | 57 | 14.2 | 14.2 | 0.97 |
| | | 48 | 142 | 35.5 | 35.5 | 1.32 |
| | | 60 | 242 | 60.5 | 60.5 | 0.78 |
| | | 72 | 458 | 114.5 | 114.5 | 0.9 |
| 6 | 2 : 8 | 12 | 30 | 7.5 | 3.7 | 1.87 |
| | | 24 | 62 | 15.5 | 7.7 | 1.06 |
| | | 36 | 123 | 30.7 | 15.3 | 0.98 |
| | | 48 | 269 | 67.2 | 33.6 | 1.13 |
| | | 60 | 484 | 121.0 | 60.5 | 0.84 |
| 7 | 3 : 8 | 12 | 46 | 11.5 | 3.8 | 1.91 |
| | | 24 | 97 | 24.2 | 8.1 | 1.09 |
| | | 36 | 186 | 46.5 | 15.5 | 0.93 |
| | | 48 | 379 | 94.7 | 31.6 | 1.04 |
| 8 | 4 : 8 | 12 | 63 | 15.7 | 3.9 | 1.97 |
| | | 24 | 128 | 32.0 | 8.0 | 1.03 |
| | | 36 | 248 | 62.0 | 15.5 | 0.94 |
| | | 48 | 515 | 128.7 | 32.2 | 1.07 |

Reference to Table 2 shows the following: When any particular 12-hour period in the early part of an experiment is considered, it is seen that the division rate was essentially the same in all four experiments, regardless of the initial number of animals (Col. VIII). It is true that in the first 12-hour period the division rate was slightly higher in Exps. 7 and 8 than in Exps. 5 and 6, but a difference of only 0.1 division is scarcely significant in view of ROBERTSON'S findings. Furthermore, this difference was not of consistent occurrence; it is found to be completely absent when the

second and third periods are examined. Upon considering the total number of progeny produced in each experiment at the end of any 12-hour period, we find (Col. V) that this number was directly proportional to the initial number of didinia. This fact may perhaps be more readily discerned upon examining Col. VI, the totals of which are smaller. For example, at the end of 48 hours a mean total of 35.5 animals was present in each test culture of Exp. 5, 67.2 (approximately twice as many) in Exp. 6, 94.7 (three times as many) in Exp. 7, and 128.7 in Exp. 8. If the mean total per initial animal is considered for any 12-hour period, we have practically the same figures in each experiment (Col. VII).

If any one of the experiments is considered without relation to the others, we are unable to observe any acceleration of the reproductive rate following the first, second, or even the third division. In other words, we find no evidence of the elaboration of an allelocatalyst at the time of cell division.

As the initial number of animals was increased, the length of time before the beginning of encystment and conjugation was decreased. Cysts and conjugants first appeared in Exp. 5 after 72 hours, in Exp. 6 after 60 hours, and in Exps. 7 and 8 after only 48 hours.

Since the volume of fluid used in Series II was four times as great as in Series I, we might expect the maximum population attained per culture to be four times as great in Series II. A comparison of Tables 1 and 2 verifies this expectation. In Series I the total number of didinia per experiment varied from 101 to 126, the average being 115. In Series II the total varied from 379 to 515, the average being 459 or almost exactly four times as great. The relation of the volume of the culture fluid to the maximum population is here so striking as to merit special comment, and indeed it is more striking in this particular case than in others. Probably in but few ciliates would the maximum population conform so faithfully to the volume of culture fluid. It must be remembered, however, that *Didinium*, as regards its highly specialized and easily controlled food habits and its ready cultivability, — once its habits and needs are understood — is an exception among ciliates.

### Series III. Clone A.

This series of experiments was in plan entirely like Series II, with but one exception — the didinia initially isolated were products of a recent fission and were therefore of small size. The series

was designed to ascertain whether the higher division rates observed in the first 12-hour period of Series I and II were actually the result of isolating "full-grown" individuals.

Table 3.

*Didinium nasutum*. Series III. Clone A. Effect of isolating from 1 to 4 animals into equal volumes of Knop solution (8 drops or 0.08 cc.). The initial animals were products of a recent fission, and hence the division rates in the first 12-hour period were lower than in Series I and II.

| I | II | III | V | VI | VII | VIII |
|---|---|---|---|---|---|---|
| Number of experiment | Ratio of animals to drops | Time in hours | Total for 4 cultures | Mean total per culture | Mean total per initial animal | Mean number of divisions per initial animal |
| 9 | 1 : 8 | 12 | 10 | 2.5 | 2.5 | 1.25 |
| | | 24 | 22 | 5.5 | 5.5 | 1.12 |
| | | 36 | 51 | 12.7 | 12.7 | 1.22 |
| | | 48 | 114 | 28.5 | 28.5 | 1.19 |
| | | 60 | 236 | 59.0 | 59.0 | 1.06 |
| | | 72 | 514 | 128.5 | 128.5 | 1.16 |
| 10 | 2 : 8 | 12 | 18 | 4.5 | 2.2 | 1.12 |
| | | 24 | 40 | 10.0 | 5.0 | 1.12 |
| | | 36 | 96 | 24.0 | 12.0 | 1.25 |
| | | 48 | 208 | 52.0 | 26.0 | 1.12 |
| | | 60 | 397 | 99.2 | 49.6 | 0.93 |
| 11 | 3 : 8 | 12 | 25 | 6.2 | 2.1 | 1.04 |
| | | 24 | 61 | 15.2 | 5.1 | 1.23 |
| | | 36 | 171 | 42.7 | 14.2 | 1.51 |
| | | 48 | 371 | 92.7 | 30.9 | 1.15 |
| 12 | 4 : 8 | 12 | 38 | 8.5 | 2.4 | 1.18 |
| | | 24 | 86 | 21.5 | 5.4 | 1.16 |
| | | 36 | 189 | 47.2 | 11.8 | 1.14 |
| | | 48 | 413 | 103.2 | 25.6 | 1.12 |

The results are presented in Table 3, examination of which shows the following: In Exp. 9 the division rate was slightly, though not appreciably, higher in the first 12-hour period than in the remaining periods, but in general the animals divided at the same rate in all periods (Col. VIII). In Exp. 10 the division rate was uniform in the first, second, and fourth periods, with but slight departures in the third and fifth periods. In Exp. 11 the lowest rate was observed in the first period and higher rates in the second and third. If such results were obtained consistently in each experiment, we should have a suggestion of allelocatalysis. Such, however, is not the case, as has been shown and as is brought out

again in Exp. 12, in which the division rate showed practically no variation in successive periods.

Thus we see again from the results of Series III that all test cultures exhibited essentially the same rate of division, regardless of the number of didinia originally isolated, and regardless of the increase in numbers of animals per culture in successive 12-hour periods. Furthermore, the division rate in the first 12-hour period was lower in every experiment of Series III than in any experiment of Series I and II, thus showing that the higher division rate observed previously in the first 12-hour period was actually the result of isolating "full-grown" animals.

To resume our consideration of Table 3, Col. VII shows that at the end of any 12-hour period the total number of progeny derived from each initial animal was practically the same in each experiment. If the total number of animals per culture (Col. VI) or per experiment is considered (Col. V), it is evident that doubling, trebling, or quadrupling the initial number of didinia merely multiplied by 2, 3, or 4 the number of progeny on hand at the end of any 12-hour period. Finally, encystment and conjugation appeared earlier in cultures having the largest initial number of animals, as was the case in Series I and II.

One matter of special interest which may fittingly be taken up at this point concerns the lag period, by which is meant a pronounced lapse of time between the introduction of the cells into the new medium and the first cell division. As Robertson (1923, 1924 a) has pointed out, the lag period is a phenomenon of common occurrence whenever cells, whether bacteria, infusoria, or metazoan cells *in vitro*, are introduced into new media. The existence of a lag period is reported by Rorertson, Myers, Greenleaf, Petersen, and others in studies on allelocatalysis. The lag period in infusoria may be due to various causes, among them: (1) A difference in the chemical composition of the old and new media, necessitating an adjustment to the new medium on the part of the infusoria and entailing as a consequence a delay in fission; (2) insufficient bacterial food in the new medium when the infusoria are introduced; and (3) a depressed condition of the animals when they are transferred, due to an excessive accumulation of metabolic waste in the old medium.

Both Greenleaf and Myers found that the lag period may be entirely eliminated by appropriate experimental procedure. Greenleaf states (p. 166) that a lag period is evident when infusoria are

"transferred from old cultures, in which the amount of the products of metabolism in the culture medium is probably very large, to fresh culture media", but that "there is no apparent lag when animals are changed from young cultures to fresh medium". MYERS found that the age of the hay infusion to which the infusoria are transferred has a marked effect on the lag period. Upon transferring the animals (paramecia) to fresh infusion, he observed regularly a lag period which varied in length from 24 to 36 hours; transfer to one-day-old or two-day-old infusions eliminated the lag period entirely. He concludes (p. 24): "It is only introduction into the freshly prepared fluid containing no bacteria that delays fission."

What is the status of the lag period in the present studies on *Didinium*? Briefly, the results show that a lag period is absent. This fact is brought out most satisfactorily in Table 3. Inspection of the table shows that in no case was the division rate appreciably lower in the first 12-hour period than in the second. The greatest difference appears in Exp. 11, and here the difference is only 0.2 division. If a lag period is present in an infusorian which divides about 1.2 times per 12-hour period, we should expect to find a complete suppression of cell division until 18 or more hours have elapsed after the establishment of the new cultures. In none of the test cultures of Series III was such a delay in evidence. This same conclusion in reference to the absence of a lag period is borne out in Series I and II and in certain series of experiments which are yet to be described. The initial animals being larger in size than in Series III, the division rate was higher in the first 12 hours, and there was no evidence of a lag.

The absence of a lag period in the present experiments is to be accounted for by virtue of the favorable, adequate, and similar conditions which characterized both the stock cultures from which the didinia were transferred and the test cultures into which they were isolated. The stock cultures which supplied the animals for the isolation tests were maintained in deep watch crystals (BOVERI-Schalen) containing KNOP solution and washed paramecia. Subcultures were made every other day. By this method excessive accumulation of waste, exhaustion of the food supply, and excessive bacterial growth were prevented in the stock cultures. The didinia were therefore maintained in the best of condition and the transfer to the test cultures was inconsequential.

C. Effect of isolating different numbers of didinia (1 to 8) into different volumes of fluid (0.04 cc. and 0.16 cc.).

Series IV. Clone B.

The 8 experiments of this series were carried out simultaneously with animals of a second clone. The results are summarized in Table 4. Each experiment consisted as formerly of four test cultures, though in Table 4 the total numbers of animals for each experiment (Col. V of the preceding tables) are omitted.

Table 4.

*Didinium nasutum.* Series IV. Clone B. Effect of isolating different numbers of animals (1 to 8) into different volumes of fluid (4 or 16 drops of Knop solution containing paramecia).

| I | II | III | VI | VII | VIII | I | II | III | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of experiment | Ratio of animals to drops | Time in hours | Mean total per culture | Mean total per initial animal | Mean number of divisions per initial animal | Number of experiment | Animals to drops | Time in hours | Mean total per culture | Mean total per initial animal | Mean number of divisions per initial animal |
| 13 | 1:4 | 12 | 3.7 | 3.7 | 1.87 | 17 | 1:16 | 12 | 3.5 | 3.5 | 1.75 |
| | | 24 | 8.5 | 8.5 | 1.19 | | | 24 | 7.7 | 7.7 | 1.19 |
| | | 36 | 17.2 | 17.2 | 1.02 | | | 36 | 16.4 | 16.4 | 1.08 |
| | | 48 | 34.1 | 34.1 | 0.99 | | | 48 | 35.2 | 35.2 | 1.08 |
| | | 60 | 61.3 | 61.3 | 0.85 | | | 60 | 54.7 | 54.7 | 0.61 |
| | | | | | | | | 72 | 132.0 | 132.0 | 1.32 |
| 14 | 2:4 | 12 | 7.5 | 3.7 | 1.87 | 18 | 2:16 | 12 | 7.0 | 3.5 | 1.75 |
| | | 24 | 16.0 | 8.0 | 1.13 | | | 24 | 15.5 | 7.7 | 1.19 |
| | | 36 | 32.5 | 16.2 | 1.02 | | | 36 | 32.6 | 16.3 | 1.08 |
| | | 48 | 69.3 | 34.6 | 1.06 | | | 48 | 74.2 | 37.1 | 1.14 |
| | | | | | | | | 60 | 137.6 | 68.8 | 0.91 |
| | | | | | | | | 72 | 267.2 | 133.6 | 0.96 |
| 15 | 4:4 | 12 | 14.7 | 3.7 | 1.82 | 19 | 4:16 | 12 | 14.6 | 3.6 | 1.82 |
| | | 24 | 31.0 | 7.7 | 1.12 | | | 24 | 29.8 | 7.4 | 1.04 |
| | | 36 | 71.6 | 17.9 | 1.18 | | | 36 | 66.1 | 16.5 | 1.16 |
| | | | | | | | | 48 | 120.4 | 30.2 | 0.86 |
| | | | | | | | | 60 | 239.7 | 60.0 | 0.99 |
| 16 | 8:4 | 12 | 27.3 | 3.4 | 1.7 | 20 | 8:16 | 12 | 29.1 | 3.6 | 1.82 |
| | | 24 | 61.1 | 7.6 | 1.2 | | | 24 | 62.3 | 7.8 | 1.13 |
| | | | | | | | | 36 | 134.9 | 16.8 | 1.1 |
| | | | | | | | | 48 | 214.8 | 26 8 | 0.62 |

If Exps. 13—16 are considered together, there results a unit which in plan is not unlike Series I, II, or III. Examination of Col. VIII shows clearly that increasing the initial number of animals failed to accelerate the reproductive rate. The rate for any parti-

cular 12-hour period was essentially the same in each experiment, insofar as the size of the population permitted the cultures of different experiments to enjoy a contemporaneous existence. Col. VII emphasizes further the uniformity of the division rate in all experiments. With regard to conjugation and encystment, these processes appeared earlier, as in Clone A, in cultures having the larger initial number of animals. Finally, there is no evidence that a second fission is hastened by the completion of the first, that is, there is no evidence of the production of an allelocatalyst at the time of division.

If Exps. 17—20 are considered together, the comments of the foregoing paragraph are equally appropriate. The volume of fluid being four times as large in these experiments, the maximum population per culture and the length of life of the cultures were greater than in Exps. 13—16.

Finally, if Exp. 13 is compared with its companion, Exp. 17, we find no evidence of a higher division rate in the smaller volume of fluid. The animals in both experiments divided at practically the same rate (Cols. VII and VIII) regardless of the size of the volume of fluid. Similarly, a comparison of Exps. 14 and 18 or 15 and 19 or 16 and 20 fails to disclose any significant differences in the reproductive rate.

In conclusion, these experiments afford no evidence of the existence of an allelocatalyst.

## Series V. Clone C.

This series, like Series IV, consisted of 8 experiments, though didinia of a third clone were used. This clone was characterized by a slower rate of division than either Clone A or B. The results are presented in Table 5. The results in all important features were so like those of Series IV that to include in Table 5 all of the data would be needless repetition. Therefore, only the fission rates per initial animal are given.

When Exps. 21—24 or Exps. 25—28 are considered as a unit, there is no evidence that an increase in the initial number of animals accelerates the division rate. Nor is there any evidence that the occurrence of one fission hastens the completion of a second fission. When Exps. 21—24 are compared with Exps. 25—28, respectively, there is likewise no evidence that culture in a smaller volume of fluid accelerates the division rate. Increasing the volume of fluid

merely increased the maximum population of the cultures and extended the time before conjugation or encystment set in, as in Series IV.

Table 5.

*Didinium nasutum.* Series V. Clone C. Effect of isolating different numbers of animals (1 to 8) into different volumes of fluid (4 and 16 drops).

| I | II | III | VIII | I | II | VIII |
|---|---|---|---|---|---|---|
| Number of experiment | Animals to drops | Time in hours | Mean number of divisions per initial animal | Number of experiment | Animals to drops | Mean number of divisions per initial animal |
| 21 | 1 : 4 | 12 | 1.37 | 25 | 1 : 16 | 1.5 |
| | | 24 | 0.82 | | | 1.05 |
| | | 36 | 1.5 | | | 0.96 |
| | | 48 | 1.1 | | | 1.02 |
| | | 60 | 0.69 | | | 0.85 |
| | | 72 | 0.87 | | | 0.77 |
| | | 84 | | | | 0.62 |
| 22 | 2 : 4 | 12 | 1.25 | 26 | 2 : 16 | 1.22 |
| | | 24 | 1.02 | | | 1.04 |
| | | 36 | 0.94 | | | 0.93 |
| | | 48 | 1.0 | | | 0.95 |
| | | 60 | 0.72 | | | 0.84 |
| | | 72 | | | | 0.95 |
| 23 | 4 : 4 | 12 | 1.28 | 27 | 4 : 16 | 1.16 |
| | | 24 | 0.94 | | | 1.12 |
| | | 36 | 1.05 | | | 0.82 |
| | | 48 | 0.83 | | | 0.65 |
| | | 60 | | | | 0.9 |
| 24 | 8 : 4 | 12 | 1.13 | 28 | 8 : 16 | 1.1 |
| | | 24 | 0.98 | | | 1.06 |
| | | 36 | | | | 0.98 |
| | | 48 | | | | 0.88 |

In conclusion, the results obtained with Clone C are entirely in accord with those obtained with Clones A and B. Under the conditions of the experiments, there is no evidence of the liberation into the pericellular medium of an allelocatalytic substance which accelerates the reproductive rate in cell communities of *Didinium*.

## *Stylonychia pustulata.*

### A. Methods.

As we have seen, in studies on allelocatalysis in which the infusoria are dependent on bacterial food, the bacterial population in itself constitutes a factor which must be reckoned with constantly and a factor the real significance of which is as yet a matter of

conjecture. The desirability of eliminating this source of error in the present studies on *Stylonychia* counseled the use of a non-bacterial food and the employment of a medium in which bacteria do not thrive. WEYER'S (1930) success in culturing *Gastrostyla steinii*, a form closely related to *Stylonychia*, on *Gonium* and *Chlorogonium* in KNOP solution led finally to the selection of an undetermined species of the phytomonad *Chlamydomonas* as the food organism for *Stylonychia* and to the adoption of 0.01 per cent. modified KNOP solution of $p_H$ 6.8 as the fluid medium.

As with *Didinium*, all tests for allelocatalysis were carried out in depression slides. Amounts of KNOP solution containing *Chlamydomonas* which varied in size from 2 drops to 12 drops, one drop being 0.01 cc., were placed into the depressions and one or more specimens of *Stylonychia* were introduced.

The chlamydomonads were cultured on KNOP agar under an artificial source of light. When it was desired to prepare culture fluid for the tests, they were removed from the agar surface by scraping with a cover slip and were transferred to BOVERI dishes containing KNOP solution. The dishes were then placed near a north window, and the flagellates, being positively phototropic, soon collected on the side receiving the more intense illumination. They were then pipetted out and were transferred to a second dish of KNOP solution. Upon aggregating in the second dish, they were transferred to a third, and from this they were transferred after aggregation to 1 or 2 cc. for fresh KNOP solution. This final mixture served as the fluid medium for the test cultures. (Reference may be made to HARTMANN, 1928, and to BĚLAŘ, 1928, for the details of this method of culturing and washing phytomonads.) The adoption of this procedure does not mean that all bacteria were rigidly excluded from the test cultures. It does mean, however, that their numbers were quite unimportant and that *Chlamydomonas* unquestionably constituted the principal article of food of the stylonychiae.

Sufficient food was present when the stylonychiae were added to the test cultures to last throughout the experiment. A few cultures in which the food supply became exhausted before the animals stopped multiplying were discarded. Again, an attempt was made to keep the ratio of the food organism to the volume of fluid constant. This was done by agitating the KNOP solution plus flagellates before the drops were pipetted out onto the depression slides. In each series of experiments all test cultures were established with drops of fluid plus food from the same stock dish.

The temperature in these experiments was not constant, and hence comparisons are not made between series of experiments carried out at different times, though all test cultures of a particular series were established at the same time and were cultured in parallel. The temperature varied from 18° to 20° C.

All test cultures were established with animals of uniform size which were approximately half-grown, i. e., their length was intermediate between that of an individual of maximum size which is on the point of dividing and that of a daughter cell just derived from a fission.

Stock cultures of each of the 3 clones of *Stylonychia* studied were maintained in small Boveri dishes containing Knop solution and chlamydomonads. Therefore, the animals upon being transferred from the stock dishes to the depression slides experienced the minimum of environmental change. The equableness of the change was reflected, as will be seen, in the absence of a lag period in the test cultures. The animals were washed in several changes of Knop solution before being isolated onto the depression slides.

Thus it is apparent that in plan and execution these studies were fundamentally like those already described for *Didinium*, though each *Stylonychia* "experiment" embraced the results obtained with 5 test cultures instead of only 4 and records were made at 24-hour intervals. The method of presenting the data in the following tables is unchanged.

An experiment was discontinued whenever one or more of the following events intervened: (1) When cysts appeared in the cultures, though cysts were never produced in great numbers; (2) when the population of a test culture remained constant, or nearly so, on 2 successive days, a condition which, in view of the absence of dividing individuals, was evidently due to the cessation of reproduction and not to the simultaneous occurrence of deaths and cell-divisions in approximately equal numbers: and (3) when the population showed a decrease, as was commonly the case after the attainment of the maximum. The precise reason for discontinuing an experiment is not indicated in the tables. As a rule, all 3 factors were operative.

### B. Effect of isolating different numbers of animals (1 to 8) into different volumes of fluid (0.02 cc. to 0.12 cc.).

#### Series I. Clone A.

The results of the first series of experiments on *Stylonychia* are presented in Table 6. The 8 experiments of the table fall readily into 2 groups, the division resting on the size of the volume. Exps.

1—4 comprise the first group, in which from 1 to 4 animals were isolated into 2 drops. Exps. 5—8 constitute the second group, in which like numbers of animals were isolated into 6 drops.

Table 6.

*Stylonychia pustulata.* Series I. Clone A. Effect of isolating different numbers of animals (1 to 4) into different volumes of fluid (0.02 cc. and 0.06 cc. of Knop solution containing *Chlamydomonas*). Each experiment consisted of 5 test cultures. Columns VI and VII indicate actual numbers of animals and not numbers of divisions.

| I | II | III | VI | VII | VIII | I | II | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of experiment | Ratio of animals to drops | Days of culture | Mean total per culture | Mean total per initial animal | Mean number of divisions per initial animal | Number of experiment | Ratio of animals to drops | Mean total per culture | Mean total per initial animal | Mean number of divisions per initial animal |
| 1 | 1:2 | 1 | 2.8 | 2.8 | 1.4 | 5 | 1:6 | 3.0 | 3.0 | 1.5 |
| | | 2 | 7.4 | 7.4 | 1.45 | | | 7.8 | 7.8 | 1.45 |
| | | 3 | 17.2 | 17.2 | 1.22 | | | 23.5 | 23.5 | 1.52 |
| | | 4 | 18.2 | 18.2 | 0.07 | | | 54.4 | 54.4 | 1.23 |
| | | 5 | | | | | | 53.2 | 53.2 | 0.0 |
| 2 | 2:2 | 1 | 6.0 | 3.0 | 1.5 | 6 | 2:6 | 6.2 | 3.1 | 1.55 |
| | | 2 | 16.0 | 8.0 | 1.5 | | | 15.6 | 7.8 | 1.4 |
| | | 3 | 17.6 | 8.8 | 0.1 | | | 46.6 | 23.3 | 1.51 |
| | | 4 | | | | | | 48.0 | 24.0 | 0.04 |
| 3 | 3:2 | 1 | 8.8 | 2.9 | 1.46 | 7 | 3:6 | 8.8 | 2.9 | 1.46 |
| | | 2 | 21.2 | 7.1 | 1.31 | | | 22.8 | 7.6 | 1.44 |
| | | 3 | 22.8 | 7.6 | 0.13 | | | 66.6 | 22.2 | 1.5 |
| | | 4 | | | | | | 65.4 | 21.8 | 0.0 |
| 4 | 4:2 | 1 | 12.4 | 3.1 | 1.55 | 8 | 4:6 | 11.6 | 2.9 | 1.45 |
| | | 2 | 26.8 | 6.7 | 1.12 | | | 30.8 | 7.7 | 1.47 |
| | | 3 | 26.4 | 6.6 | 0.0 | | | 58.2 | 14.5 | 0.9 |

If Exps. 1—4 are considered as a unit, we are unable to discern sufficient variation in the division rate in different experiments to justify construing the results as indicative of allelocatalysis (Col. VIII). It is true that on the first day the division rate was lowest in Exp. 1 and highest in Exp. 4, but a difference of 0.15 division is scarcely significant in view of the results obtained in other experiments (Exps. 5 and 8, for example). On the second day when the catalyzer, according to Robertson's view, would have been liberated into the fluid medium owing to the completion of a cell division, the fission rate was nearly the same in Exps. 1 and 2, though the initial number of animals was twice as great in Exp. 2. On the second day the rate decreased in Exps. 3 and 4, due evidently to

the crowding of the cultures. On the third day all cultures showed an appreciable decrease, this decrease being least in Exp. 1 and greatest in Exp. 4. (The environments were so small that Exps. 3 and 4 yielded somewhat inconclusive results as regards allelocatalysis.) As the initial number of animals was increased, the length of life of the cultures was decreased. The maximum population which 0.02 cc. of fluid could support varied from 18 to 26 animals (Col. VI). With the attainment of this maximum, reproduction ceased, some animals encysted and some died. These events were in all likelihood due to an accumulation of metabolic waste in the cultures. Certainly they were not due to lack of food, for active chlamydomonads were present in all test cultures at the time.

The results obtained in Exps. 5—8 were more satisfactory, in that all cultures maintained their maximum reproductive rate for 2 days or longer, thereby permitting more extensive comparisons. On the first day the division rate was nearly the same in all experiments, the variation being only 0.1 division (Col. VIII). On the second day it likewise showed but little variation in the different experiments, and, furthermore, it was practically the same as on the first day. On the third day it maintained its former level in Exps. 5—7, though it suffered a decrease in Exp. 8. Since the division rate was essentially the same in all 4 experiments on any particular day, insofar as the life of the cultures permitted comparisons, it is evident that increasing the initial number of animals merely increased the number of progeny proportionately (Col. VI). If the second day is considered as a means of illustrating this point, we find that an average of 7.8 animals were present in each culture of Exp. 5 on this day, 15.6 in Exp. 6, 22.8 in Exp. 7, and 30.8 in Exp. 8. A corresponding relation obtained on the third day in Exps. 5—7, though in Exp. 8, in which the initial number of animals was largest, the reproductive rate showed a decrease on this day, indicative of the attainment of the maximum population. Col. VII emphasizes the point just made, for from this column it is seen that each initial *Stylonychia* produced approximately the same number of progeny per interval of time with total disregard for the presence of varying numbers of neighboring cells.

If the experiments in which equal numbers of animals were isolated into volumes of different sizes are compared with each other (Exp. 1 with Exp. 5, for example), it is evident that increasing the amount of the fluid from 2 drops to 6 drops merely lengthened the life of the cultures and increased the maximum number of

animals per culture (Col. VI), without affecting the division rate appreciably.

Finally, the results show the total absence of a period of lag following the isolation of the animals. In general, the same number of divisions occurred in the first 24-hour period as in any succeeding period, for the variation in temperature was not great, as has been pointed out. The absence of a lag period is to be accounted for, it may be recalled, by virtue of the absence of any abrupt environmental change at the time the animals were isolated into the test cultures and by the favorable and similar natures of the conditions prevailing in both stock cultures and test cultures.

## Series II. Clone B.

The 8 experiments of Series II were carried out with animals of a second clone of *Stylonychia*, the reproductive rate of which was distinctly higher than that of Clone A. The volumes of fluid were twice as large as those employed in Series I. The results are summarized in Table 7.

### Table 7.

*Stylonychia pustulata.* Series II. Clone B. Effect of isolating different numbers of animals (1 to 4) into different volumes of fluid (0.04 cc. and 0.12 cc. of Knop solution containing *Chlamydomonas*). Each experiment consisted of 5 cultures.

| I | II | III | VI | VII | VIII | I | II | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of experiment | Ratio of animals to drops | Days of culture | Mean total per culture | Mean total per initial animal | Mean number of divisions per initial animal | Number of experiment | Ratio of animals to drops | Mean total per culture | Mean total per initial animal | Mean number of divisions per initial animal |
| 9 | 1 : 4 | 1 | 3.8 | 3.8 | 1.9 | 13 | 1 : 12 | 3.6 | 3.6 | 1.8 |
| | | 2 | 14.8 | 14.8 | 1.95 | | | 13.6 | 13.6 | 1.9 |
| | | 3 | 59.4 | 59.4 | 2.01 | | | 57.2 | 57.2 | 2.09 |
| | | 4 | 62.6 | 62.6 | 0.1 | | | 220.0 | 220.0 | 1.93 |
| 10 | 2 : 4 | 1 | 7.2 | 3.6 | 1.8 | 14 | 2 : 12 | 7.6 | 3.8 | 1.9 |
| | | 2 | 27.8 | 13.9 | 1.94 | | | 27.2 | 13.6 | 1.8 |
| | | 3 | 87.4 | 43.7 | 1.63 | | | 98.6 | 49.3 | 1.84 |
| | | 4 | 86.0 | 43.0 | 0.0 | | | 162.2 | 81.1 | 0.73 |
| 11 | 3 : 4 | 1 | 11.0 | 3.7 | 1.84 | 15 | 3 : 12 | 10.8 | 3.6 | 1.8 |
| | | 2 | 43.8 | 14.6 | 1.99 | | | 39.4 | 13.1 | 1.84 |
| | | 3 | 77.6 | 25.9 | 0.79 | | | 161.4 | 53.8 | 2.04 |
| | | 4 | 72.2 | 24.1 | 0.0 | | | 185.2 | 61.7 | 0.25 |
| 12 | 4 : 4 | 1 | 13.6 | 3.4 | 1.7 | 16 | 4 : 12 | 14.4 | 3.6 | 1.8 |
| | | 2 | 52.8 | 13.2 | 1.95 | | | 54.0 | 13.5 | 1.89 |
| | | 3 | 72.2 | 18.1 | 0.48 | | | 214.8 | 53.7 | 1.99 |
| | | 4 | 70.0 | 17.7 | 0.0 | | | 165.6 | 41.4 | 0.0 |

If the results of the first day of culture are considered, it is seen that the division rate was remarkably uniform in all experiments, though the initial number of animals and the size of the environment varied considerably (Cols. VIII). In 7 of the experiments the division rate varied from 1.8 to 1.9 on the first day. In Exp. 12, in which the greatest number of animals was isolated into the smallest volume of fluid, the division rate was, for unexplained reasons, slightly lower.

On the second day the division rate was likewise strikingly uniform in all of the experiments. It was slightly higher in 7 of the experiments than on the first day, due no doubt to an unobserved temperature variation. The significant fact is, however, that on the second day the rate of reproduction was essentially the same in all experiments, in spite of the great variation in number of animals and volume of fluid. This point may be fittingly illustrated by a comparison of Exps. 9 and 12. In Exp. 9 only 3.8 animals were present in each test culture at the end of the first day (Col. VI), whereas 13.6 (nearly 4 times as many) were present in each culture of Exp. 12. Yet the animals of both experiments underwent exactly the same number of fissions on the second day (Col. VIII). This fact receives further emphasis when similar comparisons are made on the division rates of the second day in other experiments (Exps. 13 and 16, or 12 and 16, or 9 and 13, for example).

On the third day the rate of reproduction showed a decrease in Exps. 10, 11, and 12 — experiments in which the initial number of animals was greatest per unit volume of fluid. In the remaining experiments the rate exhibited no decrease on this day, and furthermore it was quite uniform. On the fourth day the cultures of Exp. 13 alone maintained the former reproductive rate, for in this experiment the initial number of animals was least in proportion to the fluid.

From the foregoing it is evident that the experiments of Series II failed to show even a remote suggestion of the existence of an allelocatalytic effect.

The maximum population attained per culture in Series II was noticeably greater than would be expected in view of the results obtained in Series I. Since the volumes of fluid employed in Series II were twice as large as those of Series I, a maximum population of twice the size might reasonably be expected. But as a matter of fact, the maximum populations attained in Series II were from 3 to 4 times as large as those attained in corresponding

experiments of Series I (Cols. VI of Tables 6 and 7). Whether this difference was due to the quality of the food or to other cultural factors, or to an intrinsic difference in the constitution of the animals is not entirely clear. The evidence indicates, however, that the greater tolerance for accumulated metabolic waste displayed by the animals of Clone B represents an actual biotypic difference, for the cultural conditions were essentially the same in both series.

Finally, brief mention may be made of the fact that a lag period was absent in Series II upon isolation of the animals into the test cultures.

## Series III. Clone C.

This series also consisted of 8 experiments, though a third clone of *Stylonychia* was used and the initial number of animals was increased to 8 in certain experiments. The results are presented in Table 8. The results being entirely in accord with those already described for *Stylonychia*, presentation of the data is restricted in Table 8 to the daily division rates.

Table 8.

*Stylonychia pustulata*. Series III. Clone C. Effect of isolating different numbers of animals (1 to 8) into different volumes of fluid (0.04 cc. and 0.12 cc.). Each experiment consisted of 5 test cultures.

| I | II | III | VIII | I | II | VIII |
|---|---|---|---|---|---|---|
| Number of experiment | Animals to drops | Time in days | Mean number of divisions per initial animal | Number of experiment | Animals to drops | Mean number of divisions per initial animal |
| 17 | 1 : 4 | 1 | 1.35 | 21 | 1 : 12 | 1.45 |
| | | 2 | 1.4 | | | 1.42 |
| | | 3 | 1.36 | | | 1.38 |
| | | 4 | 1.48 | | | 1.5 |
| | | 5 | 0.42 | | | 1.32 |
| 18 | 2 : 4 | 1 | 1.4 | 22 | 2 : 12 | 1.5 |
| | | 2 | 1.48 | | | 1.4 |
| | | 3 | 1.46 | | | 1.46 |
| | | 4 | 0.16 | | | 1.25 |
| | | 5 | 0.0 | | | 0.09 |
| 19 | 4 : 4 | 1 | 1.35 | 23 | 4 : 12 | 1.4 |
| | | 2 | 1.5 | | | 1.33 |
| | | 3 | 0.24 | | | 1.45 |
| | | 4 | 0.0 | | | 0.84 |
| 20 | 8 : 4 | 1 | 1.35 | 24 | 8 : 12 | 1.35 |
| | | 2 | 1.12 | | | 1.42 |
| | | 3 | 0.04 | | | 1.05 |

Upon examining the division rates of the first day, it is seen that the variation in the different experiments was slight indeed, and such variation as occurred can not possibly be interpreted as indicative of allelocatalysis. The division rates of the second day did not differ greatly from those of the first day, except in Exp. 20, which showed on the second day the effects of crowding. (The temperature variation in this series was slight from day to day.) On the third day the cultures of three of the experiments (19, 20, and 24) attained their maximum populations and therefore showed a decrease in the division rate. The division rates of the five remaining experiments showed little variation among themselves, nor did they differ noticeably from the rates of the two preceding days. On the fourth day the cultures of two of the experiments (17 and 21) still maintained their former rate, in spite of the great increase in the number of animals per culture. These cultures were those in which the initial number of animals was smallest.

Thus it is seen that the results obtained with Clone C were entirely in accord with those obtained with Clones A and B. The division rates of different experiments showed remarkable uniformity from day to day, though the initial number of animals and the quantity of fluid varied greatly.

The results of each series of experiments have been treated so fully in the foregoing that further general discussion is deemed unnecessary. All results, whether with *Didinium* or *Stylonychia*, point to the absence of the allelocatalytic effect.

## Summary.

This study was undertaken to ascertain whether an allelocatalytic effect (acceleration of the rate of reproduction induced by the presence of a second individual or additional individuals) characterizes the reproductive activities of *Didinium nasutum* and *Stylonychia pustulata.*

Different numbers (1 to 8) of washed specimens of *Didinium* were isolated into different volumes of fluid (0.02 cc. to 0.16 cc.) and their reproductive rates were recorded at regular intervals. The fluid medium employed was 0.01 per cent. Knop solution which contained washed specimens of *Paramecium caudatum* as food for the didinia.

Similarly, different numbers (1 to 8) of washed specimens of *Stylonychia* were isolated into different volumes of fluid (0.02 cc. to

0.12 cc.). The fluid was Knop solution containing washed specimens of *Chlamydomonas* as food.

The comments which follow immediately are generally applicable to either *Didinium* or *Stylonychia*: The rate of reproduction in different test cultures was the same, whether the cultures were established with one individual or with 2, 3, 4, or 8 individuals. The rate of reproduction was the same in small volumes of fluid as in large volumes. There was no evidence that the completion of one fission hastens the occurrence of a second fission, i. e., there was no evidence of the liberation into the fluid medium at the time of cell division of a substance which accelerates the reproductive rate, as Robertson has maintained. Under the experimental conditions, there was no evidence of an allelocatalytic effect.

There was found to be a definite limit to the number of infusoria which a limited volume of Knop solution could support in a condition of active vegetative multiplication. Upon the attainment in *Didinium* of the maximum number of individuals for a given volume of fluid, reproduction ceased and encystment or conjugation set in, even though paramecia were still present in abundance in the cultures. Upon the attainment of the maximum population in *Stylonychia*, reproduction ceased and most of the infusoria died, though some encysted, even though food was still present. These phenomena were attributed to an accumulation of metabolic waste in the cultures.

A lag period following the isolation of the animals into the test cultures was absent in both ciliates studied. This was attributed to the favorable, adequate, and similar nature of the conditions prevailing in both the stock cultures from which the infusoria were removed and the test cultures into which they were isolated.

Three clones of each genus were employed with concordant results.

## Literature cited.

Beers, C. D. (1928): The relation of dietary insufficiency to vitality in the ciliate Didinium nasutum. Journ. Exp. Zool. Vol. 51 p. 121.

— (1932): Diet in relation to depression and recovery in the ciliate Didinium nasutum Arch. f. Protistenk. In press.

Bělař, K. (1928): Untersuchung der Protozoen. Meth. d. wiss. Biol. Bd. 1 p. 735.

Calkins, G. N. (1926): The biology of the protozoa. Philadelphia.

Cutler, D. W., and Crump, L. M. (1923): The rate of reproduction in artificial culture of Colpidium colpoda. Bioch. Journ. Vol. 17 p. 174.

— (1923 a): The rate of reproduction in artificial culture of Colpidium colpoda. Part II. Ibid. Vol. 17 p. 878.

— (1925): The influence of washing upon the reproductive rate of Colpidium colpoda. Ibid. Vol. 19 p. 450.

Darby, H. H. (1930): Studies on growth acceleration in protozoa and yeast. Journ. Exp. Biol. Vol. 7 p. 308.

Greenleaf, W. E. (1926): The influence of volume of culture medium and cell proximity on the rate of reproduction of infusoria. Journ. Exp. Zool. Vol. 46 p. 143.

Hartmann, M. (1928): Praktikum der Protozoologie. Jena.

Jahn, T. L. (1929): Studies on the physiology of the euglenoid flagellates. I. The relation of the density of population to the growth rate of Euglena. Biol. Bull. Vol. 57 p. 81.

Ludwig, W. (1928): Über den funktionellen Zusammenhang zwischen Populationsdichte, Nahrungsdichte und Teilungsrate bei Protisten und über die Zunahme der Bevölkerungsdichte überhaupt. Biol. Generalis Vol. 4 p. 351.

Mast, S. O. (1917): Conjugation and encystment in Didinium nasutum with especial reference to their significance. Journ. Exp. Zool. Vol. 23 p. 335.

Mast, S. O., and Ibara, Y. (1923): The effect of temperature, food, and the age of the culture on the encystment of Didinium nasutum. Biol. Bull. Vol. 45 p. 105.

Myers, E. C. (1927): Relation of density of population and certain other factors to survival and reproduction in different biotypes of Paramecium caudatum. Journ. Exp. Zool. Vol. 49 p. 1.

Peskett, G. L. (1924): Allelocatalysis and the growth of yeast. Bioch. Journ. Vol. 18 p. 866.

— (1925): Studies on the growth of yeast. I. The influence of volume of culture medium employed. II. A further note on allelocatalysis. Ibid. Vol. 19 p. 466.

Petersen, W. A. (1929): The relation of density of population to rate of reproduction in Paramecium caudatum. Phys. Zool. Vol. 2 p. 221.

Robertson, T. B. (1921): Experimental studies on cellular multiplication. I. The multiplication of isolated infusoria. II. The influence of mutual contiguity upon reproductive rate and the part played therein by the "X-substance" in bacterised infusions which stimulates the multiplication of infusoria. Bioch. Journ. Vol. 15 p. 595.

— (1922): Reproduction in cell-communities. Journ. Phys. Vol. 56 p. 404.

— (1923): The chemical basis of growth and senescence. Philadelphia.

— (1924): Allelocatalytic effect in cultures of Colpidium in hay-infusion and in synthetic media. Bioch. Journ. Vol. 18 p. 1240.

— (1924 a): The nature of the factors which determine the duration of the period of lag in cultures of infusoria. Australian Journ. Exp. Biol. and Med. Sci. Vol. 1 p. 105.

Robertson, T. B. (1924b): The influence of washing upon the multiplication of isolated infusoria and upon allelocatalytic effect in cultures initially containing two infusoria. Ibid. Vol. 1 p. 151.

— (1927): On some conditions affecting the viability of cultures of infusoria and the occurrence of allelocatalysis therein. Ibid. Vol. 4 p. 1.

Weyer, G. (1930): Untersuchungen über die Morphologie und Physiologie des Formwechsels der Gastrostyla steinii Engelmann. Arch. f. Protistenk. Bd. 71 p. 139.

Woodruff, L. L. (1911): The effect of excretion products of Paramecium on its rate of reproduction. Journ. Exp. Zool. Vol. 10 p. 557.

— (1913): The effect of excretion products of infusoria on the same and on different species, with special reference to the protozoan sequence in infusions. Ibid. Vol. 14 p. 575.

Yocom, H. B. (1928): The effect of the quantity of culture medium on the division rate of Oxytricha. Biol. Bull. Vol. 54 p. 410.