

# THE EFFECT OF EXCRETION PRODUCTS OF PARAMAECIUM ON ITS RATE OF REPRODUCTION

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ELEVEN FIGURES

Evidence from many sources points to the fact that a considerable amount of the products of metabolism of a cell is frequently injurious to the cell. As is well known, alcohol in excess of a certain amount inhibits the reproduction of yeast, while the same amount forms a favorable medium for the organism which produces vinegar. Again, vinegar in excess of a certain quantity is toxic to the cells which are responsible for its formation, but serves in turn as a favorable environment for organisms which give rise to another type of fermentation.

De Candolle<sup>1</sup> nearly eighty years ago apparently suggested that low crop production, following continued growth of one crop in the same soil, is due to the accumulation in the nutrient medium of deleterious organic substances originating in the growing plants themselves. Liebig<sup>2</sup> also held this view for a time but later abandoned it in favor of the idea that the beneficial effect of crop rotation is due to the several crops requiring different substances or varying proportions of the same substances, and that the disturbance of the balance in the soil produced by one crop is not unfavorable to the growth of some other crop. The trend of much recent research is distinctly in support of the view that substances are produced by the higher plants which are speci-

<sup>1</sup> DeCandolle, *Physiologie végétale*, Paris, 1832.

<sup>2</sup> Liebig, *Complete works on chemistry, Familiar letters on chemistry*, letter 12, p. 35, 1852. Cf. Gilbert, *Bull. 22, Office Exp. Sta., U. S. Dept. Agr.*, 1895.

fically toxic to themselves and which form an unfavorable medium for the continued growth of a succession of the same species.<sup>3</sup> Recent work on various species of bacteria and molds points in the same direction. Eijkman,<sup>4</sup> for example, observed that these forms growing on artificial nutrient media form waste products which inhibit growth and also that these products of a given species are, as a rule, more toxic to that and closely related species than to those more distantly related. Further, Kuester<sup>5</sup> noted that molds grown in a solution produce substances which inhibit the growth of further inoculations.

Considerable experimental work on various vertebrates has demonstrated that fatigue is due to the accumulation of metabolic products. Ranke, and later Mosso<sup>6</sup> observed that the blood of fatigued animals when injected into the circulation of fresh ones brought about all the symptoms characteristic of fatigue, and Weichart<sup>7</sup> was able to isolate a toxin from fatigued muscle which, when injected into animals, gave rise to similar conditions.

Semper<sup>9</sup> in 1874 made a series of experiments with snails, in which he found, for example, that snails bred singly in two liters of water attained to more than three times the size of those grown in groups of twenty in the same amount of water. In all the experiments the amount of available food was maintained at the optimum. In other experiments the number of snails was constant and the volumes of water unequal, and the same result was obtained. Semper concluded that the results were in some

<sup>3</sup> Cf. Schreiner and Sullivan, *Journ. Biol. Chem.*, vol. 6, pp. 39-50, 1906, and later papers; also Cameron, *Journ. Phys. Chem.*, vol. 14, p. 425, 1910, and *U. S. Farmers' Bull.*, 257, 1906.

<sup>4</sup> Eijkman, *Centralbl. f. Bakt.* 1, 37, p. 436, 1904. Cf. also Rahn, *Centralbl. f. Bakt.* 2, 16, p. 417, 1906.

<sup>5</sup> Kuester, *Ber. d. d. Bot. Gesell.*, Bd. 26a, p. 246, 1908.

<sup>6</sup> Ranke, *Tetanus: Eine physiologische Studie.* Leipzig, 1865. Mosso, *Arch. f. Anat. u. Physiol., Physiol. Abth.*, p. 89, 1890.

<sup>7</sup> Weichart, *Münch. Med. Wochenschr.*, Bd. f 2, p. 2121, 1904.

<sup>8</sup> Cf. Lee, *Journ. Amer. Med. Assn.*, vol. 46, 1906, and Slade, *Journ. Physiol.*, 35, 1907.

<sup>9</sup> Semper, *Arb. a.d. Zool-Zoot. Inst.*, Würzburg, Bd., 1, 1874. *Animal life*, pp. 159-167, 1879.

way due to the volume of water available for each snail, but he was unable to determine how the volume produced the effects noted. The same question was considered in 1894 by De Varigny<sup>10</sup> with considerably elaborated methods, and he found that the size of the snails was affected by the number of individuals in a given volume of water, but he did not observe that they were so markedly sensitive to differences in volume of the water. He concluded that the area of the surface exposed to the atmosphere had more influence than the volume. Snails were bred by Whitfield<sup>11</sup> in a small aquarium for four generations and he found that the size of the shell decreased during each succeeding generation and that various other morphological changes occurred.

Yung<sup>12</sup> in 1885 made experiments with tadpoles and found that, within limits, the larger the area of the exposed surface of the water in which they were bred the more rapid their growth took place. He concluded that the result was due to the water absorbing a larger proportion of oxygen from the air.

An extensive series of experiments was made by Vernon,<sup>13</sup> in 1895, in which, for example, he allowed eggs of several species of sea-urchins to develop in water which had previously been the environment for a considerable time of another batch of eggs, and he found that in every case the larvae of the second batch were diminished in size as compared with the control. He concluded that products of metabolism excreted by the first batch retarded the growth of the second. Other experiments apparently showed that the growth of larvae was decreased by their own metabolic products, and that the products of excretion of adult echinoids acted more adversely both on the life and on the growth of embryos if these belonged to the same species than if they belonged to other species—in fact he actually found that the

<sup>10</sup> DeVarigny, *Journ. de l'Anat. et de la Physiol.*, p. 147, 1894. *Experimental evolution*, pp. 79–88, 1892.

<sup>11</sup> Whitfield, *Bull. Amer. Museum Nat. Hist.*, vol. 1, p. 21. *Amer. Naturalist* vol. 14, p. 51.

<sup>12</sup> Yung, *Arch. des Sci. Phys. et Nat.* T. 14, p. 502, 1885.

<sup>13</sup> Vernon, *Mittheilungen a.d. Zool. Sta. z. Neaple*, 13, p. 389 et seq. *Proc. Royal Soc. London*, vol. 186, B. p. 603, 1895. *Variation in animals and plants*, 1903.

excretion products of two less closely related species were favorable to growth.

More recently, Warren,<sup>14</sup> working with *Daphnia magna*, noted that, by continued breeding in small aquaria in which the water remained unchanged, the rate of reproduction and the number of young in a brood was markedly decreased, and also that the length of the spine of the carapace was considerably shortened. This result he attributed to the excretion products of the daphnids and, since ostracods and copepods flourished when the daphnids were waning, he concluded that the products of metabolism of *Daphnia* are specifically injurious to *Daphnia*.

Little work has been done to determine the effects of limited volumes of culture medium on the reproduction of Infusoria. Balbiani<sup>15</sup> in 1860 briefly reported a single experiment on *Paramecium* from the results of which he concluded that the organism must be in not less than two or three cubic centimeters of infusion for the greatest reproductivity to be realized. Kulagin,<sup>16</sup> studying the generations of certain infusoria with reference to 'senile degeneration,' suggested that this was due largely to the fact that when the organisms have lived for a number of generations in the same water, they have contaminated the water by the excretion of substances analogous to toxins, and these gradually accumulate until the nuclei are affected.

The chief products of the destructive metabolism of the infusorian are in all probability water, urea, carbon dioxide and various salts, and these are eliminated chiefly by means of the contractile vacuole. A considerable amount of experimental evidence as well as analogy with higher forms points to the conclusion that  $\text{CO}_2$  is voided in appreciable quantities by the contractile vacuoles of the Infusoria. For example, Jennings<sup>17</sup> determined that an acid is present in the vicinity of active paramecia which is not sufficiently strong to attack  $\text{CaCO}_3$ , but which will

<sup>14</sup> Warren, Quart. Journ. Mic. Sci., vol. 43, p. 212, 1900.

<sup>15</sup> Balbiani, Comptes Rendus de l'Academie des Sci., Paris, T. 50, pp. 1191-1195, 1860.

<sup>16</sup> Kulagin, Le Physiologiste russe, T. 1, pp. 269-279, 1899.

<sup>17</sup> Jennings, Journ. Physiol., vol. 21, pp. 258-321, 1897.

decolorize rosol, and he therefore concluded that carbon dioxide is excreted by the organisms in quantities sufficient to be detected with proper reagents. The work of Barratt<sup>18</sup> may also be mentioned in which he found that the daily carbon dioxide production of *Paramecium* varies between 1.3 and 5.3 per cent of the weight of the protoplasm, the variations in amount being largely due to temperature and the food supply of the animals.

Beyond the fact that CO<sub>2</sub> is eliminated by the infusorian cell and chiefly by the contractile vacuole, there are comparatively few data as to the form in which other products of katabolism leave the animal. The so-called excretory granules found in practically all groups of Protozoa have been quite carefully studied in *Paramecium*. They were early supposed by Entz and others, by analogy with higher forms, to represent concretions similar to uric acid crystals. Schewiakoff<sup>19</sup> claimed, as the result of a series of careful tests, that they consist of calcium orthophosphate, and, as calcium is one of the most abundant metallic elements in cells, some probability is attached to his conclusion, especially since Schaudinn<sup>20</sup> determined the presence of calcium and phosphoric acid in the excretory granules of *Trichosphaerium*. Rhumbler,<sup>21</sup> however, considering the excretory granules of various Infusoria concluded that they consist of uric acid, and Griffiths<sup>22</sup> believed that he had demonstrated by microchemical tests the presence of uric acid in the contractile vacuoles of several types of protozoa, including *Paramecium*.

Rosbach<sup>23</sup> in 1872 observed that temperature affected the frequency of the contractions of the contractile vacuole and this was confirmed by Maupas<sup>24</sup> in 1883. The French author also computed the relative volume of the contractile vacuole and cell of several Infusoria and determined, for example, that at a temperature of 27° C. *Paramecium aurelia* evacuates a quantity of

<sup>18</sup> Barratt, Zeit. f. Allgen. Physiol., Bd. 5, pp. 166-172, 1905.

<sup>19</sup> Schewiakoff, Zeitschr. f.-wiss. Zool. Phys., Bd., 17, 1893.

<sup>20</sup> Schaudinn, Abhandl. d. k. Akad. d. Wiss., 1899.

<sup>21</sup> Rhumbler, Zeitschr. f. wiss. Zoolog., 1899.

<sup>22</sup> Griffiths, Proc. Royal Soc. Edinburgh, vol. 16, 1889.

<sup>23</sup> Rosbach, Arb. a.d. Zool.-zoot. Inst. Würzburg, pp. 9-72, 1872.

<sup>24</sup> Maupas, Archiv. de zool. exp. et gen. (2), 1 p. 648, 1883.

water equal to that of the entire organism in forty-six minutes, and similarly *Cryptochilum nigricans* does the same in two minutes. In 1907 Kanitz<sup>25</sup> found that, within certain limits, a rise of 10° of temperature caused a doubling of the rate of contraction, while the results of Khainsky,<sup>26</sup> 1910, showed that in *Paramecium caudatum* the vacuole contracted 2.86 times per minute at 16° C., 6 times per minute at 23° to 25° C., and 10 times per minute at 33° to 34° C., under the conditions of the experiments.

It is clear then that excretion products in the case of many organisms have a profound effect on cell division and growth, and it is also clear that under favorable conditions of food and temperature the Infusoria excrete considerable amounts of carbon dioxide, together with various other end-products of metabolism, which may reasonably be expected to be evident through biological as well as chemical tests.

The ordinary 'hay infusion' teeming with animal and plant life is a microcosm in which every organism may and probably does in some degree affect the well-being of every other organism present. Besides the obvious influence exerted by animals in feeding on other forms and by green plants through photosynthetic processes, one would expect the effects of organisms on their environment by the elimination of products of their metabolism, or excretion products, to be one of the most important. The interdependence of the organisms of a hay infusion is so complex that, taken as a whole, it is almost beyond the possibility of analysis, and accordingly the logical method of approach to the subject is to study the interaction of isolated organisms and small groups of organisms on themselves and on each other. The present paper presents the results which have been obtained from the study of the effects on the rate of reproduction of *Paramecium* of:

1. Different volumes of culture medium;
2. Changing the culture medium at twenty-four hour and at forty-eight hour intervals;
3. Culture medium in which rich growths of paramaecia have occurred.

<sup>25</sup> Kanitz, Biol. Zentralblatt, Bd. 27, 1907.

<sup>26</sup> Khainsky, Archiv. f. Protistenkunde, Bd. 22, I, p. 1, 1910.

## EXPERIMENTS

The organisms used in this work were from my pedigree cultures of *Paramecium aurelia* and *Paramecium caudatum*. The experiments were begun on July 24, 1910, when the *P. aurelia* culture was at the 1903d generation and the *P. caudatum* was at the 113th generation, and were concluded on October 4, 1910, when the *P. aurelia* culture was at the 2070th generation and the *P. caudatum* culture was at the 288th generation. Emphasis is placed on the fact that the animals which formed the subjects for the experiments had been under daily observation for over forty months in the case of *aurelia* (which was the main culture employed), and over three months in the case of *caudatum* (which was used in certain experiments for comparison). Consequently their rate of reproduction, and the exact conditions to which they had been subjected for nearly three and a half years, were known in the case of the main culture. Further, since the pedigree cultures were each originally started with a single individual, all the *P. aurelia* used in this work were 'sister cells,' and all the *P. caudatum* used were 'sister cells.' Therefore all the experiments were performed on the 'same protoplasm' of the respective species. Fig. 1 shows graphically the average daily rate of division of the four lines of the *P. aurelia* culture again averaged for each month of its existence to the time it was employed for this work.<sup>27</sup>

1. *The effect of different volumes of culture medium on the rate of reproduction of Paramecium*

A series of four experiments, two of sixteen days duration and two of twenty days duration were made with *P. aurelia*. In all the work it was found that sixteen to twenty days was the most suitable length of time for the experiments, because those of less than sixteen days appeared too short to give conclusive results, and in those which were extended beyond twenty days, the ani-

<sup>27</sup> For details of these cultures see Woodruff, Biol. Bull., 16, 4, 1909; Archiv f. Protistenkunde, Bd. 21, 3, 1911; and Jour. Morph., vol. 22, 2, 1911.

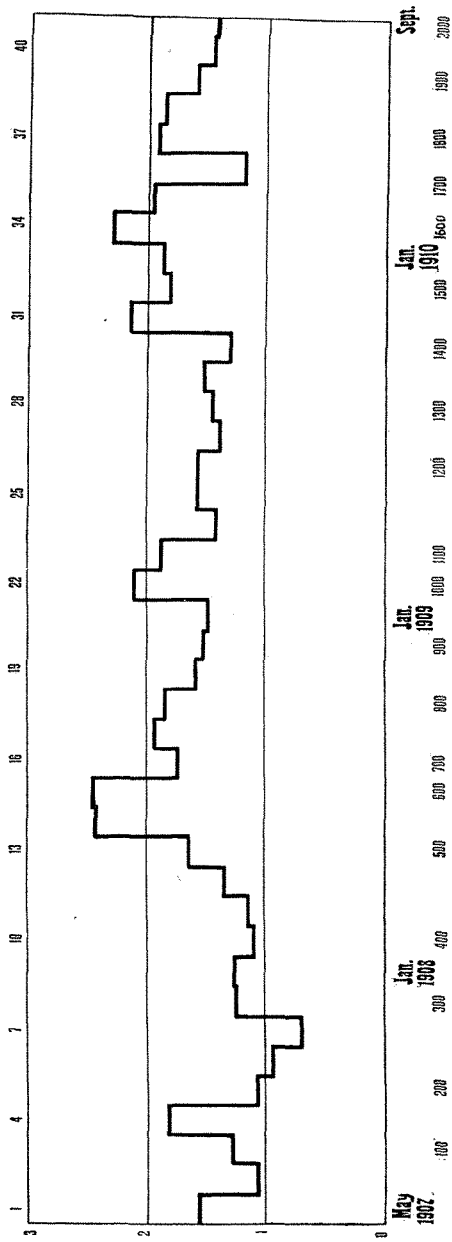


Fig. 1 Complete history of *Paramaecium aurelia*. Culture 1, from start on May 1, 1907, to September 21, 1910, at the 2000th generation. The ordinates represent the average daily rate of division of the four lines of the culture, again averaged for each month of the life of the culture to date.



mals on the various amounts of medium were about thirty generations apart, so that it might be suggested that this offered an objection.

The volumes of medium selected were two, five, twenty, and forty drops. The same pipet was used in measuring the liquid in all the work, and so practical uniformity was attained. Infusions of hay were used as a culture medium and the organisms were isolated on slides of different capacities, depending on the amount of liquid employed. The slides were kept in moist chambers to prevent evaporation.

The daily rate of division of the organisms in two, five, twenty, and forty drops of culture medium changed every *twenty-four hours* showed that, for example, those in five drops divided 2.4 per cent more rapidly than those in two drops, those in twenty drops divided 6.4 per cent more rapidly than those in two drops, and those in forty drops divided 7.4 per cent more rapidly than those in two drops (fig. 4, part A). The details of each of the four experiments (A, B, C, D) are evident in fig. 2 which shows the rate of division of each of the lines on the different amounts of medium averaged for each four days of the experiment. Experiments B and C are the most important ones because these comprised cultures on all the volumes of medium. A was carried to test the general method to be used, and D was carried to check up certain data.

It is believed that the experiments are sufficiently comprehensive clearly to establish the fact that *the rate of reproduction of specimens from pure lines of paramaecia, when bred under identical conditions of temperature and culture medium, is influenced by the volume of the culture medium (within the limits tested in the experiments), and that the greater the volume the more rapid is the rate of division.*

It being clear that in an increased volume of culture medium there is an increased division rate, the next point of importance is to determine to what factor or factors this is due. It is evident that it may be brought about by variations in 1, temperature; 2, pressure; 3, surface of medium exposed to atmosphere; 4, food supply; 5, excretion products of bacteria; or 6, excretion products of paramaecia.

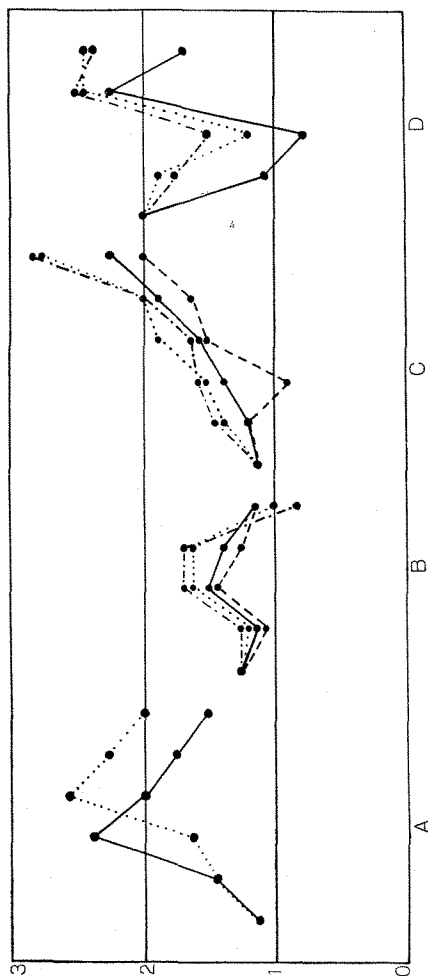


Fig. 2 Record of the rate of division of *Paramecium aurelia* in the series of four experiments (A, B, C, D) to determine the effect of different volumes of culture medium, changed every *twenty-four* hours, on their rate of reproduction. The ordinates represent the average daily rate of division of the four lines of organisms in the respective volumes of medium, again averaged for four day periods. Rate of division in two drops = ----, five drops = —, twenty drops = ...., forty drops = .-.-.-.

The experiments were conducted simultaneously and in the same place for all the volumes of medium, and therefore the temperature was the same.

It is believed that the exceedingly slight pressure variations in the different volumes of water was without appreciable effect. There is certainly no evidence extant that free-living protozoa are sensitive to such exceedingly small changes, and therefore this factor will be dismissed as not entering into the effects noted.

The question of increased surface exposure to the atmosphere in the larger volumes of media employed facilitating the exchange of gases, as oxygen and carbon dioxide, is possibly one of more importance, and accordingly care was taken to use receptacles of different capacities and shapes for the different volumes in an attempt to equalize the proportion of surface to volume of the different amounts of medium. Obviously it is practically impossible to make them actually equal, but certainly the precautions taken were sufficient to render this factor negligible.

The food supply is obviously a factor of great importance. The culture medium consisted of an infusion of hay which was raised to the boiling point to eliminate the possibility of contaminating the culture with 'wild' paramaecia, and was used after it had cooled. No precaution was taken to make the infusions exactly the same each time, but the same infusion was used for all cultures at the same time. This was made up fresh every forty-eight hours, beginning with the first day of each experiment, thus, in those experiments in which the medium was changed every twenty-four hours, the organisms were transferred to some of the medium still remaining in the stoppered flask from the day before. This flask was shaken before it was used, and there is every reason to believe that the organisms in each amount of medium received exactly the same food. Slight variations in the bacterial flora were avoided, it is believed, by the fact that at the beginning of each experiment all the paramaecia came from the same environment, and consequently the bacteria transferred with them when they were isolated would presumably be the same in each case, and if they were not the same, or if the new infections from the air, which must have occurred in the course of

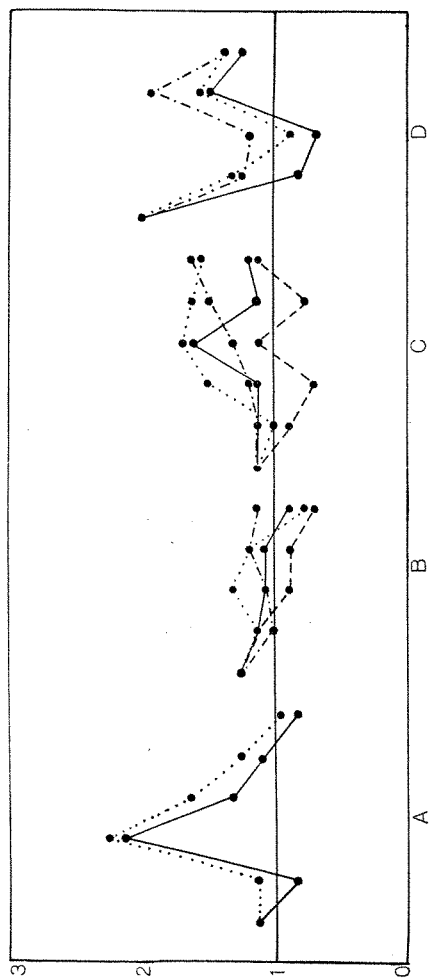


Fig. 3 Record of the rate of division of *Paramaecium aurelia* in the series of four experiments (A, B, C, B,) to determine the effect of different volumes of culture medium, changed every *forty-eight* hours, on their rate of reproduction. The ordinates represent the average daily rate of division of the four lines of organisms in the respective volumes of medium, again averaged for four day periods. Rate of division in two drops = ----, five drops = - . - . , twenty drops = . . . . , forty drops = . . . . .

the experiment, tended to render the media of the lines different, this variation was eliminated by cross infections of all the lines of all the cultures daily at the time of isolation. It is believed then, that every practicable precaution was taken to ensure as near absolutely the same food conditions as it is feasible to obtain, and consequently that the results have not been influenced by variations in nutrition.

It is not apparent that the consistent variations in the division rate in the different volumes of medium can be the result of the products of metabolism of bacteria, in view of the great care taken to maintain identical flora in all the preparations. It is possible, however, that slight irregularities in the curve may be explained on this basis and this point will be mentioned later.

The remaining factor to be considered is the possible effect of the excretion products of the paramaecia themselves. That the infusoria in their ceaseless activity are continually voiding appreciable amounts of carbon dioxide and other products of metabolism is evident from the work of previous investigators. The question then is—is the amount excreted sufficient to affect the division rate appreciably under the conditions of the experiments? Taking the average rate of division during the experiments as one and one-half divisions per day, the average number of organisms in a preparation during the first twenty-four hours would be one and one-third—one during the first sixteen hours, and two during the following eight hours. However improbable it may seem that this number of individuals can excrete sufficient toxic substances to have an appreciable effect on the division rate when diluted, for example, by twenty drops or by forty drops,<sup>28</sup> I believe the experiments outlined make this highly probable, and point to the conclusion that *the variations in the daily division rate in the different volumes of water is due to the excretion products of the paramaecia themselves.*

<sup>28</sup> With the division rate increasingly more rapid with increase of volume, there would actually be more excretion products in the larger volumes than in the smaller. This however can be disregarded.

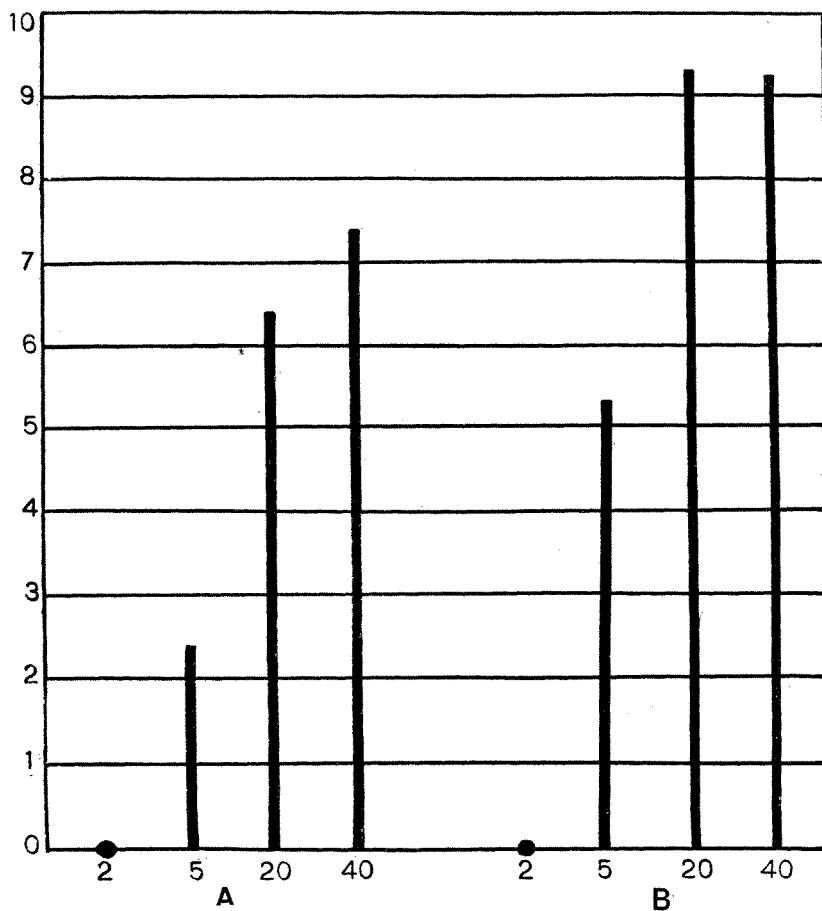


Fig. 4 Summary of the results of the experiments plotted in figs. 2 and 3. *A* shows the per cent gained in division rate by *Paramaecium aurelia* in five, twenty, and forty drops of medium changed at *twenty-four-hour* intervals, over the division rate of those in two drops changed also daily. *B* shows the per cent gained in the division rate by *P. aurelia* in five, twenty, and forty drops of medium changed at *forty-eight-hour* intervals over the division rate of those in two drops changed also on alternate days.

2. *Effect of changing the culture medium at twenty-four and forty-eight hour intervals, in each of the several volumes, on the rate of reproduction of Paramaecium*

If the conclusion reached from the previously outlined experiments is true, the effects of the excretion products should manifest themselves more clearly in cultures in which the organism remained in the medium a longer period, than in those in which the organism remained in the medium for a shorter period of time. To test this a second series of experiments was carried out *simultaneously* with those already described, and in this second series the animals remained in the same medium for forty-eight hours instead of twenty-four hours. There were, then, the following series of cultures involved in this experiment:

- Ad 2 = *P. aurelia* in 2 drops of medium changed daily.
- Ad 5 = *P. aurelia* in 5 drops of medium changed daily.
- Ad20 = *P. aurelia* in 20 drops of medium changed daily.
- Ad40 = *P. aurelia* in 40 drops of medium changed daily.
- Cd 5 = *P. caudatum* in 5 drops of medium changed daily.
- Aa 2 = *P. aurelia* in 2 drops of medium changed on alternate days.
- Aa 5 = *P. aurelia* in 5 drops of medium changed on alternate days.
- Aa20 = *P. aurelia* in 20 drops of medium changed on alternate days.
- Aa 40 = *P. aurelia* in 40 drops of medium changed on alternate days.
- Ca 5 = *P. caudatum* in 5 drops of medium changed on alternate days.

Each of these cultures comprised four separate lines, *e.g.*, *Ad2-1*, *Ad2-2*, *Ad2-3*, and *Ad2-4*, and the number of divisions of each of these four lines was recorded daily, at which time a single organism was isolated in fresh culture medium. Consequently the rate of division of *Ad2* is the average rate of division of the four lines comprising it.

The culture in which the medium was changed at forty-eight-hour intervals showed that the organisms in a volume of five drops divided 5.3 per cent more rapidly than those in two drops; those in twenty drops divided 9.3 per cent more rapidly than those in two drops, and those in forty drops divided 9.25 per cent more rapidly than those in two drops (fig. 4, B). The details of each of the four experiments are graphically shown in fig. 3,

which gives the rate of division of each of the lines in the different volumes of medium averaged for each four days of the experiment.

This series of experiments covered seventy-two days and was run simultaneously and coextensively with those already outlined with which it is compared, and all the conditions to which each was subjected were identical, the only factor requiring special mention being the food content of the culture medium. It is stated in the description of the cultures of the *Ad* series of experiments that the culture medium was made up on alternate days—so that the organisms in this series were continued for two days on infusion that was made up at the same time, but which was supplied fresh (from the stock flask) at the end of the first twenty-four hours. Thus the only difference in the treatment of series *Aa* and *Ad* was that the change to a second supply of the culture medium was not made at the end of twenty-four hours. Therefore the only difference in the environment of series *Aa* and *Ad*, at the beginning of the second twenty-four hours, was that *Ad* was put in a fresh portion of the medium in which it had been for the past day, and consequently a medium not contaminated by its own excretion products, whereas *Aa* was continued for the next twenty-four hours in the same portion of medium in which it had been living for the previous day. It is believed that cross infection rendered the bacterial flora of the infusion in the supply flask and that on the *Aa* culture slides essentially the same and observation also showed that the supply of bacteria on the culture slides was ample.

*The results, then, of this series of cultures in which the organisms were isolated every forty-eight hours, confirms the general result derived from the series isolated every twenty-four hours, i.e., that an increased volume of medium is conducive to more rapid multiplication,<sup>29</sup> and further it clearly shows that the gain in rate of division*

<sup>29</sup> Attention is called to the fact that *Aa-20* gained 0.05 per cent more over *Aa-2* than did *Aa-40* and therefore is an exception. Cf. fig. 4, B. It is possible that another factor enters, or at least becomes perceptible after twenty-four hours in a volume as large as forty drops. The bacteria in this quantity of culture fluid may



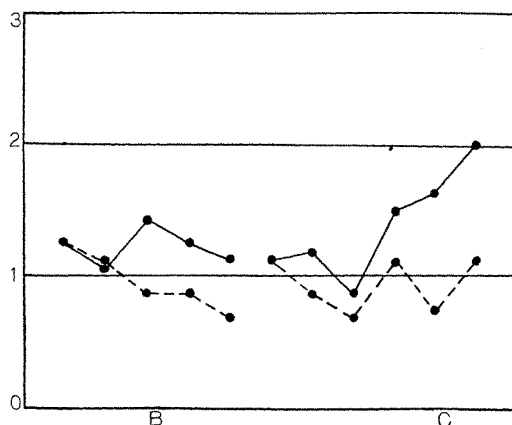


Fig. 5 Record of the experiments on the effects of changing the culture medium at twenty-four-hour intervals and at forty-eight-hour intervals, when *Paramaecium aurelia* is bred in *two* drops of hay infusion. The ordinates represent the average daily rate of division of the four lines of organisms, again averaged for four day periods. Medium changed at twenty-four-hour intervals = —; changed at forty-eight-hour intervals = ----.

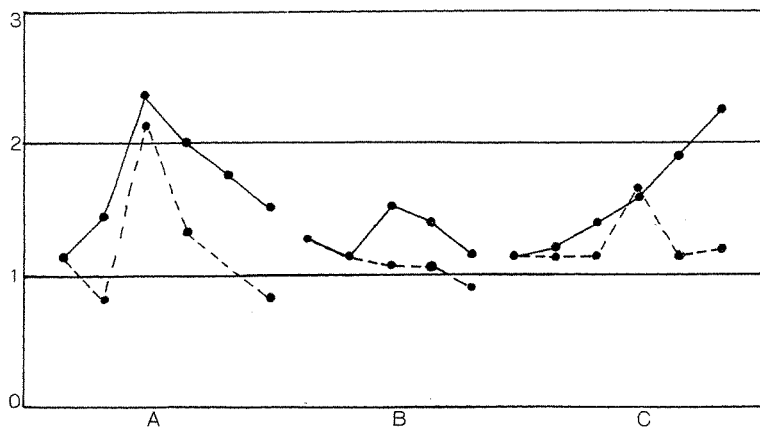


Fig. 6 Record of experiments on the effect of changing the culture medium at twenty-four-hour intervals and at forty-eight-hour intervals, when *Paramaecium aurelia* is bred in *five* drops of hay infusion. The ordinates represent the average daily rate of division of the four lines of organisms, again averaged for four day periods. Medium changed at twenty-four-hour intervals = —; changed at forty-eight-hour intervals = ----.

of *Aa-5*, *Aa-20* and *Aa-40* over *Aa-2* is in every case greater than the gain of *Ad-5*, *Ad-20* and *Ad-40* over *Ad-2*, (cf. fig. 4). Again, from a consideration of the data of comparable cultures changed daily and that of cultures of equal volumes of media changed on alternate days, it is found that the gain of the series changed daily over those changed at forty-eight hour intervals is over eight per cent in the case of two drops and slightly over six per cent in the case of five, twenty, and forty drops. Consequently, as one would expect, changing the medium on alternate days has most influence in the smallest volume of medium (for details cf. figs. 5, 6, 7 and 8).

The results of some of the experiments performed, for control and comparison, on the *P. caudatum* culture are plotted in fig 9, and a glance at this will show that they are perfectly consistent with those derived from the work on *P. aurelia*. A further check on the work is also brought out in the first series of experiments (A) plotted in fig. 9. At the end of the fifth period of this experiment, another culture was isolated, line by line, from *Ca* which was designated *Cad*, and thereafter in this the medium was changed daily. The result, as shown in the diagram, was that the organisms within the following eight days attained the same division rate as that of the culture *Cd*, thus clearly indicating that the variation in the division rate between *Cd* and *Ca* was effected by the duration in time to which they were subjected to the culture medium.

Further, as a control to test the accuracy of the general method employed in all the work, a duplicate control culture, designated *Ad-5dup*, was carried and the number of divisions in this culture at the conclusion of the work was exactly the same as *Ad-5*. Of course it was an 'accident' that there should have been no variation during the long series of experiments, but this indicates that such error as exists in the method used is very slight, and

develop so fast that they exhaust their own food and produce excretion products in sufficient amount to be detrimental to the paramaecia, whereas in the smaller volumes of medium the animals keep the bacteria reduced so that they do not exhaust their food and so continue to multiply, providing food for the paramaecia, but not sufficient excretion products to have a perceptible effect.

Fig. 7 Record of experiments on the effect of changing the culture medium at twenty-four-hour intervals and at forty-eight-hour intervals, when *Paramecium aurelia* is bred in *twenty* drops of hay infusion. The ordinates represent the average daily rate of division of the four lines of organisms, again averaged for four day periods. Medium changed at twenty-four-hour intervals = —; changed at forty-eight-hour intervals = ----.

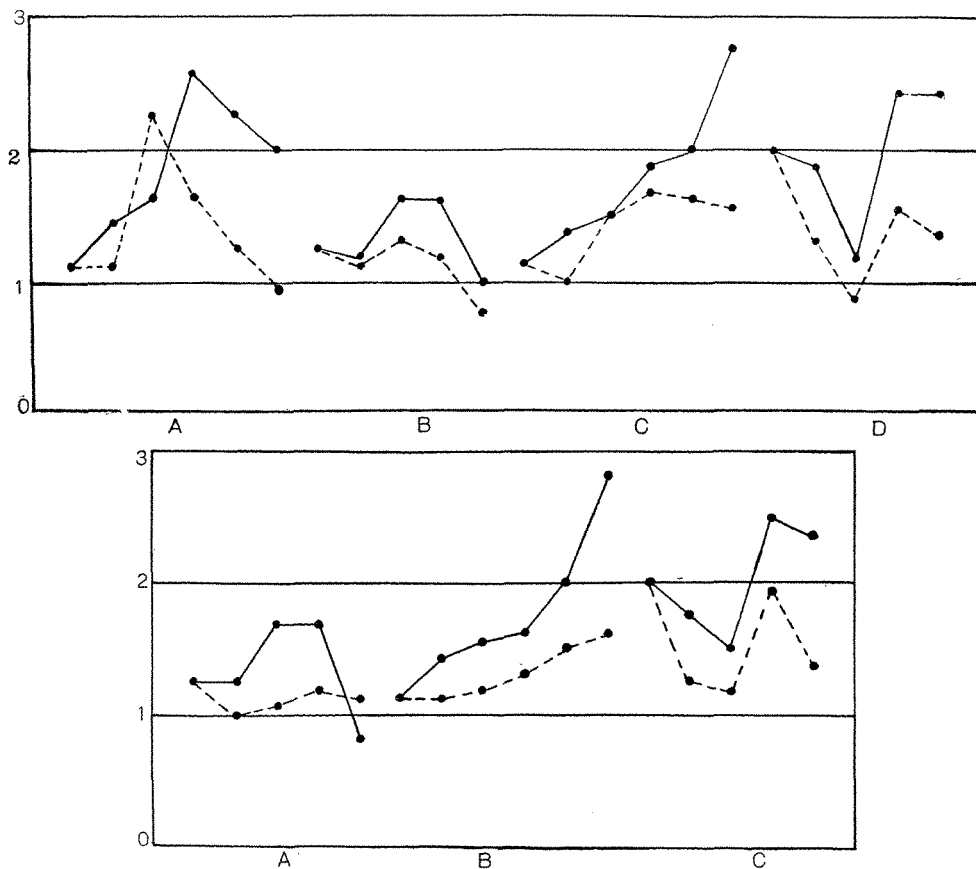


Fig. 8 Record of experiments on the effects of changing the culture medium at twenty-four-hour intervals and at forty-eight-hour intervals, when *Paramecium aurelia* is bred in *forty* drops of hay infusion. The ordinates represent the average daily rate of division of the four lines of organisms, again averaged for four day periods. Medium changed at twenty-four-hour intervals; = —; changed at forty-eight-hour intervals = ----.

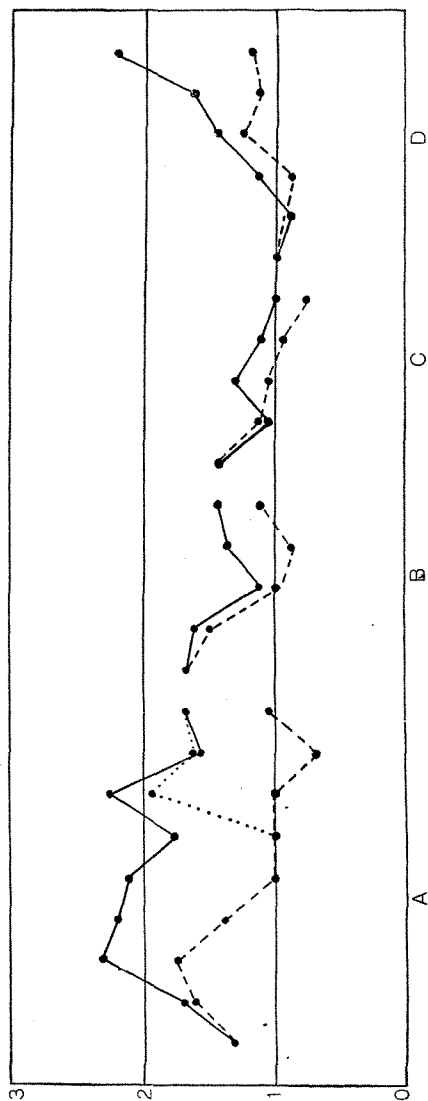


Fig. 9 Record of experiments on the effect of changing the culture medium at twenty-four-hour intervals and at forty-eight-hour intervals, when *Paramaecium caudatum* is bred in *five* drops of hay infusion. The ordinates represent the averaged daily rate division of the four lines of organisms, again averaged for four day periods. Medium changed at twenty-four-hour intervals = — and .....; changed at forty-eight-hour intervals = - - - -.

that the differences in the division rate in the experiments are far beyond the limits of error.

It is clear then that all the data derived from the experiments outlined point to the conclusion that *paramaecia excrete substances which are toxic to themselves and that these substances are more effective, as one would expect, when the organisms are confined in limited volumes of culture medium*. The logical method of procedure is to determine the influence of media known to be contaminated with the excretion products of large numbers of *paramaecia*.

3. *The effect of media in which rich growths of paramaecia have occurred on the rate of reproduction of Paramecium*

As a culture medium for this experiment an infusion of chopped hay was made and boiled. After a thorough stirring, equal volumes were poured into sterile flasks of a capacity of 250 cc. One of these flasks was then seeded with five cc. of infusion containing twenty-five *P. aurelia* from the 'stock' left over from the pedigree culture, and the other flask was seeded with five cc. of the same infusion, from which the *paramaecia* had been removed. The flasks were kept plugged with cotton. There were then two flasks containing precisely the same culture material and bacterial flora, and the one differed from the other only in the presence of *paramaecia*. After these infusions had stood for ten days there was a heavy growth of *paramaecia* in one and none in the other, and the media were ready for the experiments. From the four lines of the pedigree culture of *P. aurelia* two separate cultures were isolated (designated  $A+P$  and  $A-P$  respectively), one of which was bred on hay infusion from the *paramaecia*-free flask ( $F-P$ ), and the other on material from the flask inoculated with *paramaecia* ( $F+P$ ). It was necessary, of course, to remove the *paramaecia* from the culture medium before it was used in the experiments, and this was done daily by filtering it through filter paper before it was used, and then examining it carefully under the microscope, and picking out with a pipet the few animals which had not been retained by the filter paper. As a precau-

tion, the infusion from the flask minus the paramaecia was similarly filtered so that there could be no possibility of error through the filtration process affecting the bacterial content or contaminating the infusion. Further, since in the culture medium  $F+P$  the paramaecia had undoubtedly decreased the number of bacteria present through feeding on them, the fluid, after the animals were strained out, was inoculated with bacteria from the medium  $F-P$ , and as an added precaution  $F-P$  was inoculated with bacteria from  $F+P$ . Thus, while the bacteria were reduced in  $F+P$  through being eaten by the animals, this medium undoubtedly contained more food for bacteria, and therefore, when inoculated, should develop bacteria more rapidly than the  $F-P$  culture medium, and therefore there would be at least as much food for the organisms on which the experiments were made in  $F+P$  as in  $F-P$ .

It is believed then that, when the media were employed in the experiments, they were practically identical except that one contained the products of metabolism of a heavy growth of paramaecia while the other was absolutely free from such contamination.

The results of this experiment are shown graphically in fig. 10. *A* shows the results of a preliminary observation which includes the daily isolations for four days. *B* illustrates the results from another and longer experiment made by the reisolation of both series from the main pedigree cultures *A*. *C* gives clearly the same general results which were obtained when the whole experiment was repeated, this time with new flasks of culture medium, etc.

As a further check on the results, fig. 10, *B* shows that, when after eight days subjection to the  $F+P$  medium, another culture was isolated line by line from it and put on the  $F-P$  medium, the division rate approached the rate of that continuously on the  $F-P$  medium. And again when still another culture was isolated from the culture recently transferred from the  $F-P$  medium, and put back on the  $F+P$  medium, the rate of division of this new culture approached that of the series which had been continually on the  $F+P$  medium.

A short series of experiments for comparison was made with

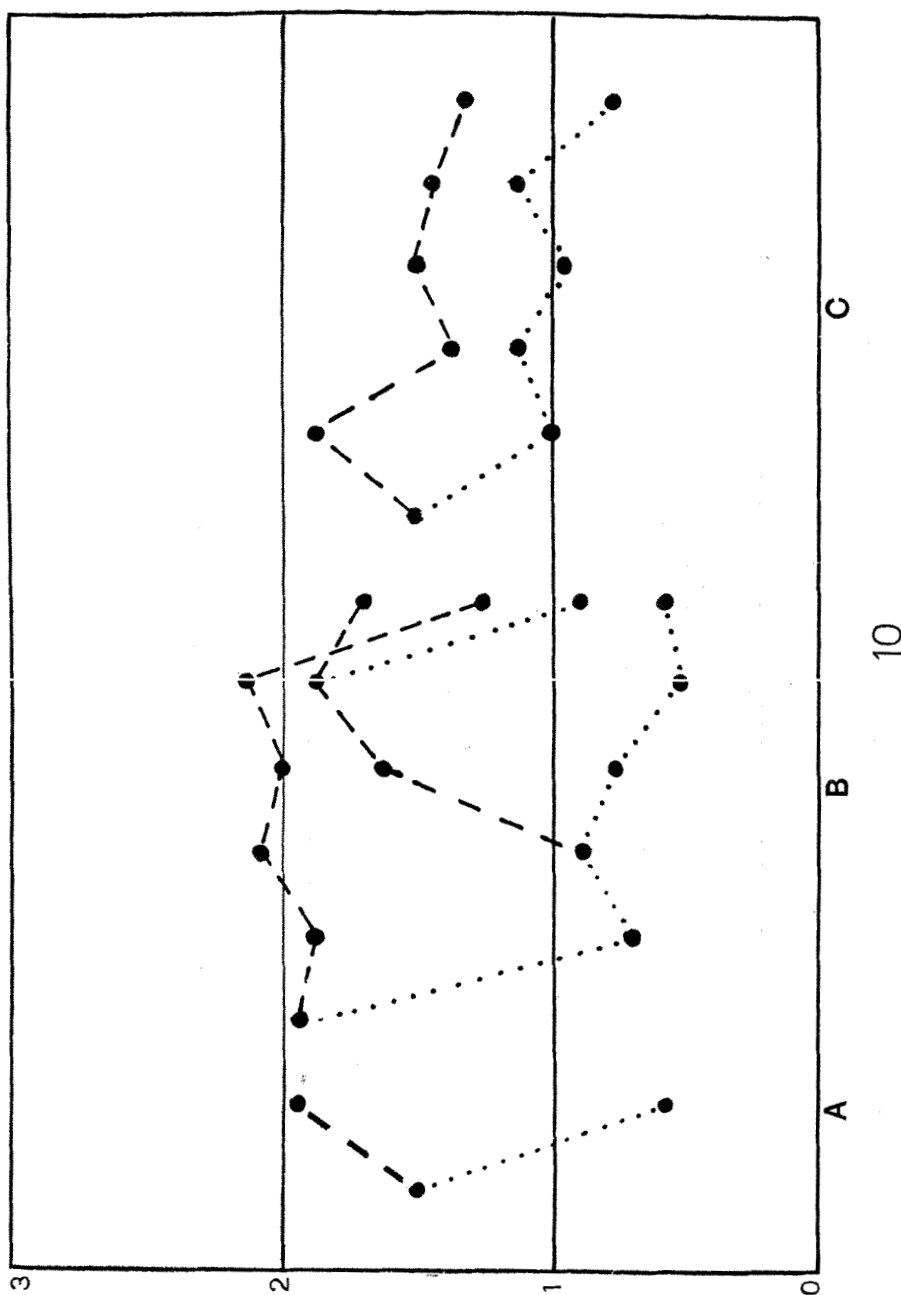


Fig. 10 Record of the experiments to determine the effect of a medium, which has supported a rich growth of *Paramecium aurelia*, on the rate of reproduction of *P. aurelia*. The ordinates represent the average daily rate of division of the four lines of organisms, again averaged for 10 day periods. Rate of division of organisms in culture medium free from the excretion products of *Paramecium* (—P) = — — — —, culture medium with excretion products (+P) = .....

the same two culture media on the pedigree culture of *P. caudatum*, and this gave the same general result. In this experiment the animals on the *F+P* medium died out (fig. 11).

It is obvious then from these data that *culture media in which*

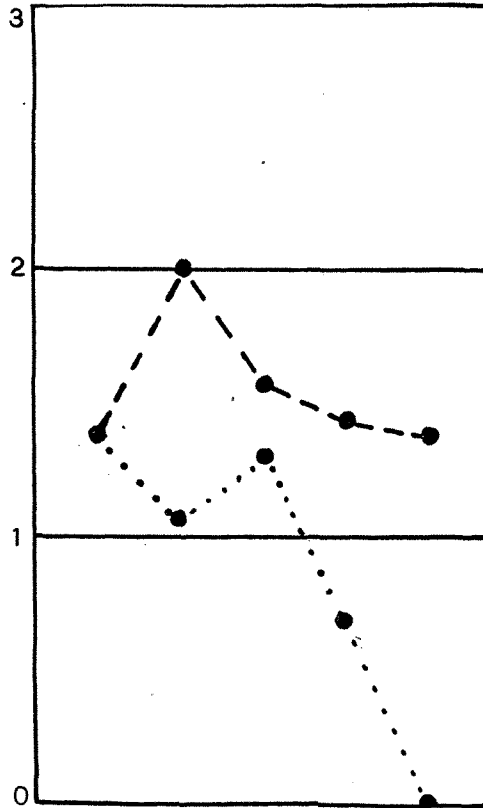


Fig. 11. Record of an experiment to determine the effect of a medium, which has supported a rich growth of *Paramaecium aurelia*, on the rate of reproduction of *P. caudatum*. The ordinates represent the average daily rate of division of the four lines of organisms, again averaged for four day periods. Rate of division of organisms in culture medium free from the excretion products of *P. aurelia* ( $-P$ ) = ---, culture medium with excretion products ( $+P$ ) = ....

*paramaecia have been living has a decidedly depressing effect on the rate of reproduction of paramaecia of the same pure pedigree stock as the contaminating animals, as well as on the rate of reproduction of another species, P. caudatum.*



## CONCLUSIONS

In this paper are presented the initial experiments of a series which is planned to elucidate, if possible, some of the complex factors at work in a 'hay infusion,' for example, such as those which determine the interdependence of the organisms, their sequence, time of appearance and disappearance, etc. The data outlined were derived from the study of the following points:

(1) The effect of different volumes of culture medium on the rate of reproduction of *Paramecium*; (2), The effect of changing the culture medium daily and on alternate days on the rate of reproduction of *Paramecium*; and (3), The effect of culture medium, in which many *paramecia* have been living, on the rate of reproduction of *Paramecium*. It is believed that the results obtained justify the following conclusions:

1. The rate of reproduction of *Paramecium aurelia* and *Paramecium caudatum* is influenced by the volume of the culture medium, within the limits tested, and the greater the volume the more rapid is the rate of division.

2. *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.

3. The excretion products of *paramecia* play an appreciable part in determining the period of maximum numbers, rate of decline, etc., of this animal in 'hay infusions.'