

## Chapter V

## COMPETITION FOR COMMON FOOD IN PROTOZOA

## I

(1) At the end of the last century Boltzmann, considering the struggle for existence in the biosphere as a whole, remarked that there exists a considerable quantity of essential mineral substances needed by all living beings, but that the resources of available solar energy are comparatively more restricted and they constitute the narrow link representing the principal object of competition. This circumstance has since been pointed out by many biophysicists, and we will quote the words of Boltzmann himself ('05): "The general struggle for existence of all living beings is not the struggle for the fundamental substances, for these fundamental substances indispensable for all living creatures exist abundantly in the air, the water and the soil. This struggle is not a struggle for the energy which in the form of heat, unfortunately not utilizable, is present in a great quantity in every object, but it is a struggle for entropy, which is available when energy passes from the hot sun to the cold earth. In order to utilize in the best manner this passage, the plants spread under the rays of the sun the immense surface of their leaves, and cause the solar energy before reaching the temperature level of the earth to make syntheses of which as yet we have no idea in our laboratories. The products of this chemical kitchen are the object of the struggle in the animal world." This idea of Boltzmann that the available solar energy represents the narrow link for the living matter in the biosphere taken as a whole is in a certain agreement with the data of the modern geochemists. Thus Professor Vernadsky ('26) points out that a part of the solar energy which is capable of producing chemical work on the earth is to the very end utilized in the mechanism of the biosphere. In other words, the transforming surface of the green living matter utilizes entirely the rays of a definite wave-length in the process of photosynthesis.

(2) However that may be, the energetic side of the struggle for existence in the biosphere as a whole has as yet been little studied, and at the present level of our knowledge we will have to undertake a detailed analysis of the most simple cases only. But the words of Boltzmann compel us to turn our particular attention to those narrow links in the conditions of our microcosm which constitute the real objects of the competition. In the foregoing chapter we had to deal with a competition of the yeast cells for the utilization of a certain limited amount of energy in the test tube. The limit for the growth of the biomass in these organisms was connected with the accumulation of the waste product (alcohol), and this factor stopped the growth

before the exhaustion of the energetic resources of the microcosm. As a result the entire process of competition could be expressed in terms of the narrow link—alcohol production. Thus the situation in these experiments was a peculiar one.

In the present chapter we will try to approach the regularities which, as Boltzmann supposed, are characteristic for the biosphere as a whole. We will examine the struggle for existence in carefully controlled populations of Protozoa. Here the growth will be limited by an insufficiency of organic nutritive substances, a factor analogous to an insufficiency of available energy. The second peculiarity is that the energetical resources of the microcosm will be maintained continuously at a certain fixed level in the course of the experiment. This approaches somewhat to what exists in nature, where the level of energy is maintained by the uninterrupted influx of solar energy. As before we will be concerned in these experiments with the problem in what proportion the energy of the microcosm will be distributed between the populations of the two competing species. But besides this first stage we shall be enabled to examine here the following fundamental question: *Will one species drive out the other after all the available energy of the microcosm has been already taken hold of? And if so, will one species in these conditions drive the other one out completely, or will a certain equilibrium become established between them?*

(3) It has been already tried more than once to use Protozoa for the study of the communities of organisms and their succession under laboratory conditions. But as an ecologist has recently remarked, the mere fact of a community set up in a laboratory dish does not mean at all that it is simple. Interesting observations have been made on the succession of communities of Protozoa in a hay infusion by Woodruff ('12) Skadowsky ('15) and more recently by Eddy ('28). However, in experiments of this type there exists a great number of different factors not exactly controlled, and a considerable difficulty for the study of the struggle for existence is presented by the continuous and regular changes in the environment. It is often mentioned that one species usually prepares the way for the coming of another species. Recollecting what we have said in Chapter II it is easy to see that in such a complicated environment it is quite impossible to decide how far the supplanting of one species by another depends on the varying conditions of the microcosm which oppress the first species, and in what degree this is due to direct competition between them. In this connection one of the main problems of our experiments with Protozoa has been to eliminate the complicating influence of numerous secondary factors, and to apply such a technique of cultivation as would enable one to form a perfectly clear idea as to the nature of the factor limiting growth. This could not be done at once and the

technique of our first experiments presented all the usual defects. Only later, taking into account certain suggestions of American authors, we made use of a synthetic medium for cultivating the Protozoa, and the result furnished exceedingly clear data to a detailed description of which we will soon pass.

(4) A new property of the infusorian population distinguishing it from that of yeast cells is that the infusorian population constitutes a secondary population living at the expense of bacteria which it devours. Thus here appears an elementary food chain: bacteria @ infusoria. In our initial experiments the standardization of the conditions of cultivation was only a quite superficial one. Without taking any precautions as to an exact control of the physicochemical properties of the medium and the number of growing bacteria, we prepared the nutritive medium in the following manner: to 100 c.c. of tap water 0.5 gr. of oatmeal was added; the whole was boiled for 10 minutes, left to stand and then the upper liquid was carefully poured off, diluted 1.5 times by water, and sterilized in an autoclave. After this an inoculation of *Bacillus subtilis* was made, and the medium was put into the thermostat at 32° for seven days in order to obtain an abundant growth of bacteria. Before using, the medium was diluted twice by tap-water, and without any further sterilization was put into test tubes. (This was the so-called "oaten medium without sediment." The "oaten medium with sediment" mentioned in Chapter VI differs in its not being diluted by water before using, and a small quantity of sediment originating from the oatmeal was allowed to remain.) The cultivation was made in tubes with a flat bottom (about 1 cm in diameter and 5-6 cm high) of the nutritive solution. The tubes were closed by cotton wool stoppers and kept in a moist thermostat at 26°C. Close paraffinized cork stoppers were not found convenient because if we use them the population begins to die off immediately after cessation of growth, and the curves take the form described by Myers (27). At the same time under optimal conditions after the growth of the population has ceased the level of the population is maintained unchanged for a certain time, and only later Paramecia begin to die off.

In the initial experiments no change of the medium in the course of growth of the population was made, and the increase in the number of individuals was studied according to the average values for the test tubes of a definite age. The contents of the tube was destroyed every time after examination just as in the experiments with yeast. The counting was made under a magnifying glass on a slide plate. Figure 18 represents the growth of the number of individuals in pure lines of *Paramecium caudatum* and *Stylonychia mytilus* cultivated separately and in a mixed population. These data are founded on two experiments which gave similar results. At the beginning of the experiment into each tube were placed five *Paramecium*, or five *Stylonychia*, or five

*Paramecium* plus five *Stylonychia* in the case of a mixed population. *Stylonychia* for inoculation must be taken from young cultures to avoid an inoculation of degenerating individuals.

(5) The growth curves of the number of individuals in Figure 18 are S-shaped and resemble our well known yeast curves. After growth has ceased the level of the saturating population is maintained for a short time, and then begins the dying off of the population which is particularly distinct in *Stylonychia*. It is evident that this dying off is regulated by factors quite different from those which regulate growth, and that a new system of relations comes into play here. Therefore there is no reason to look for rational equations expressing both the growth and dying off of the populations.

Figure 18 shows that *Stylonychia*, and especially *Paramecium*, in a mixed culture attain lower levels than separately. The calculated coefficients of the struggle for existence have the following values: a (influence of *Stylonychia* on *Paramecium*) = 5.5 and b (influence of *Paramecium* on *Stylonychia*) = 0.12. This means that *Stylonychia* influences *Paramecium* very strongly, and that every individual of the former occupies a place available for 5.5 *Paramecia*. With our technique of cultivation it is difficult to decide on what causes this depends. As a supposition only one can point to food consumption.

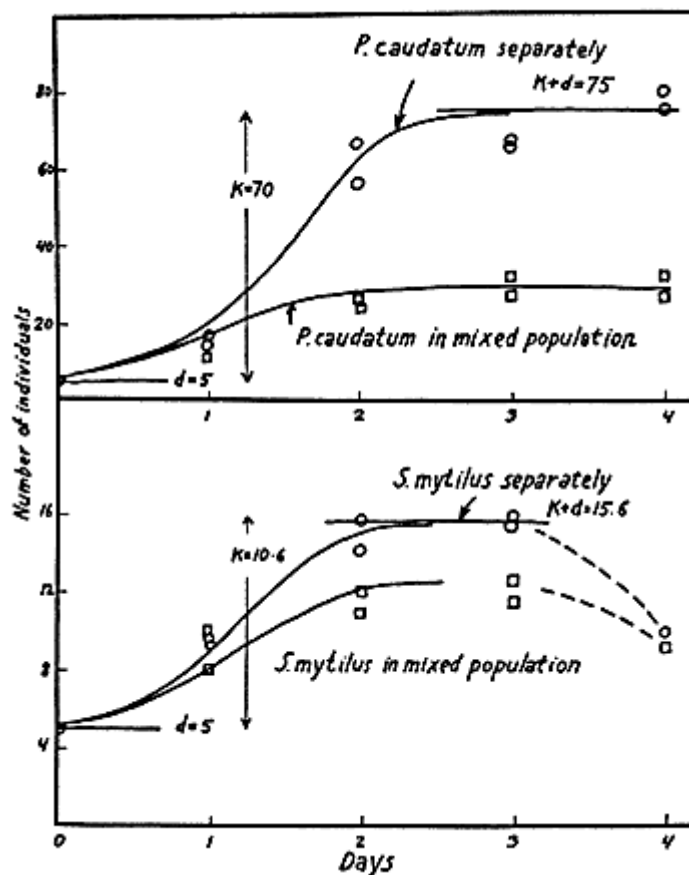


Fig. 18. The growth in number of individuals of *Paramecium caudatum* and *Stylonychia mytilis* cultivated separately and in the mixed population.  $d$  denotes lower asymptote. From Gause ('34b)

(6) We have but to change slightly the conditions of cultivation and we shall obtain entirely different results. Figure 19 represents the growth of populations of the same species on a dense "oaten medium with sediment" sown with various wild bacteria. Here owing to an increase in the density of food the absolute values of the maximal population in both species have considerably increased. The character of growth of the mixed population now essentially differs from the former one: *Paramecium* strongly influences *Stylonychia*, while *Stylonychia* has almost no influence upon *Paramecium*. We simply have here an "alchemical stage" of investigation, and the absence of an exact control of the conditions of the medium creates the impression of a complete arbitrariness of the results of our experiments. Unfortunately many protozoological researches are still in this stage, and the idea is very widespread that "Protozoa are not entirely satisfactory for the study of populations as they require bacteria for food, and it is very difficult to measure accurately and to analyze the relations between protozoan population and the bacterial population."

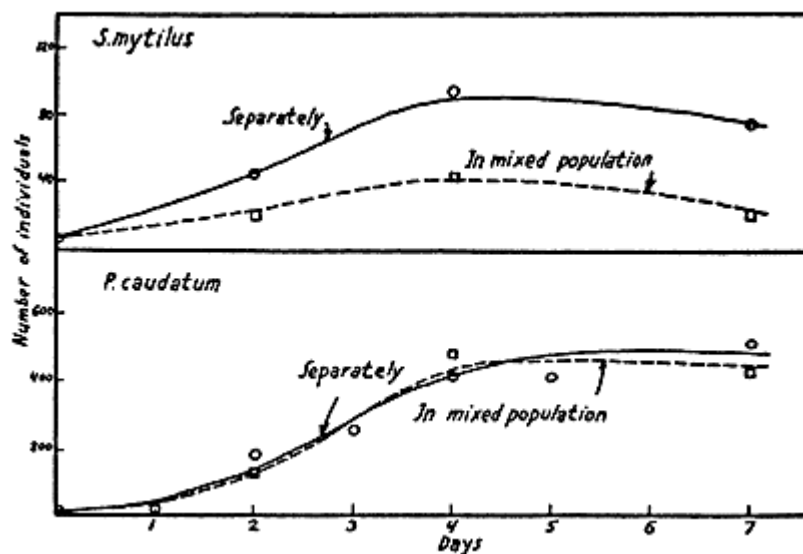


Fig. 19. The growth in number of individuals of *Paramecium caudatum* and *Stylonychia mytilis* cultivated separately and in the mixed population. Medium contains wild bacteria.

In order to draw any reliable conclusions as to the quantitative laws of the struggle for existence in Protozoa we must begin by elaborating the technique of cultivation, keeping in mind the following excellent words of Raymond Pearl: "When the biologist exercises something approaching the same precision and infinitely painstaking care, over all the most trivial details of a biological experiment that the physicist

does over his, the results tend to take on a degree of precision and uniformity not so far short of that usual in the older science, as we are accustomed to expect" ('28, p. 35).

## II

(1) Jennings pointed out the necessity of a careful control of bacteria in the cultures of Protozoa in 1908, and one of the first attempts to grow *Paramecia* in pure cultures of bacteria was made by Hargitt and Fray ('17), Oehler ('20) and Jollos ('21). Since then numerous researches have appeared and a good review of them can be found in the recently published book of Sandon ('32) *The Food of Protozoa* as well as in Hartmann ('27) and Belar ('28). It can be noted in a quite general form that in order to standardize the conditions of cultivation of Protozoa it is necessary: (1) to standardize the quality of food— to cultivate the Protozoa on bacteria of a definite species, (2) to standardize the quantity of food—the number of bacteria per unit of volume must have a fixed value, and (3) to standardize the physicochemical conditions of the medium. These difficult and as one may think hardly realizable problems have been solved very simply for *Oxytrichia* by Johnson ('33), who has been partly preceded by Barker and Taylor ('31). The method is this: a culture of a certain bacterium is made on a solid medium and then a fixed quantity of bacteria is taken off the solid medium and transferred into a balanced physiological salt solution, where these bacteria do not multiply and serve as food for the Protozoa. Like Johnson we used Osterhout's salt solution, the composition of which is given in Table VIII. As will be shown further on, in certain experiments this medium was buffered and kept at a definite hydrogen ion concentration (pH). Special experiments made by Johnson showed that the bacteria do not multiply in this medium, and that their number scarcely changes within 24 hours.

TABLE VIII  
*Balanced physiological salt solution of Osterhout*

NaCl	2.35 g
MgCl <sub>2</sub>	0.184 g
MgSO <sub>4</sub>	0.089 g
KCl	0.050 g
CaCl <sub>2</sub>	0.027 g

Bidistilled water to 100 c.c.	
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This solution is diluted with bidistilled water 225 times.

Beginning our experiments on the growth of pure and mixed populations of *Paramecium caudatum*, *Paramecium aurelia* and *Stylonychia pustulata*, we devoted a certain time to finding a culture of bacteria suitable as food for all three species of Protozoa. Using the data published by Philpot ('28) we chose finally the pathogenic bacterium *Bacillus pyocyaneus*, which was cultivated in Petri dishes at 37°C on a solid medium of the following composition: peptone, 1 g; glucose, 2 g; K<sub>2</sub>HPO<sub>4</sub>, 0.02 g; agar-agar, 2 g, per 100 cm<sup>3</sup> of tap-water. One standardized uniformly filled platinum loop of fresh *Bacillus pyocyaneus* taken off the solid medium was placed in 10 cm<sup>3</sup> of Osterhout's salt solution. This mixture was prepared anew every day, and we will speak of it as the "one-loop" medium.

(2) Such a standard and convenient technique of cultivation enables us to approach the experimental investigation of an important problem: the course of the process of competition for a source of energy kept continually at a certain level. With this object the cultivation was carried on in graduate tubes for centrifugation of 10 c.c. capacity, which were filled with nutritive medium up to 5 c.c. and closed with cotton wool stoppers. Twenty individuals of the corresponding species were placed in every tube, or 20 plus 20 in case of a mixed culture. The medium was changed daily in the following manner. The tube was placed in a centrifuge, and after two minutes of centrifugation with 3500 revolutions per minute the infusoria fell to the bottom, the liquid above was very gently drawn off by means of a pipette with a caoutchouc ball and a freshly made nutritive medium was poured in. Besides this, every other day each culture was washed with the salt solution free of bacteria, in order to prevent the accumulation of waste products in the few drops of liquid remaining at the bottom of the tube with the Paramecia at the moment when the medium was changed. For this purpose after the pouring off of the old medium the tubes were filled with a pure salt solution, centrifuged and the liquid was drawn off a second time. Every day before the medium was changed each culture was carefully stirred up, 0.5 c.c. of the liquid was taken out and the number of infusoria in it counted. After counting the sample was destroyed. All the experiments were made in a moist thermostat at 26°C with pure lines of infusoria.

(3) In an experiment of such a type all the properties of the medium are brought to a certain invariable "standard state" at the end of every 24 hours. Hence, we acquire the possibility of investigating the

following problem: can two species exist together for a long time in such a microcosm, or will one species be displaced by the other entirely? This question has already been investigated theoretically by Haldane ('24), Volterra ('26) and Lotka ('32b). It appears that the properties of the corresponding equation of the struggle for existence are such that if one species has any advantage over the other it will inevitably drive it out completely (Chapter III). It must be noted here that it is very difficult to verify these conclusions under natural conditions. For example, in the case of competition between two species of crayfish (Chapter II) a complete supplanting of one species by another actually takes place. However, there is in nature a great diversity of "niches" with different conditions, and in one niche the first competitor possessing advantages over the second will displace him, but in another niche with different conditions the advantages will belong to the second species which will completely displace the first. Therefore side by side in one community, but occupying somewhat different niches, two or more nearly related species (e.g., the community of terns, Chapter II) will continue to live in a certain state of equilibrium. There being but a single niche in the conditions of the experiment it is very easy to investigate the course of the displacement of one species by another.

TABLE IX  
*Contents of the microcosms in the experiments with Osterhout's medium*

Contents of the microcosm	Number of microcosms
(1) <i>Paramecium caudatum</i> separately.....	4
(2) <i>Stylonychia pustulata</i> separately.....	5
(3) <i>Paramecium aurelia</i> separately.....	3
(4) <i>P. caudatum</i> + <i>P. aurelia</i> .....	3
(5) <i>P. caudatum</i> + <i>S. pustulata</i> .....	3
(6) <i>P. aurelia</i> + <i>S. pustulata</i> .....	3



(4) Two series of experiments were arranged by us in which the process of competition was studied in 21 microcosms for a period of 25 days. Table IX shows the combinations of separate species of Protozoa which were used. Let us first of all analyze the competition between *Paramecium caudatum* and *Paramecium aurelia*. The data on the growth of pure and mixed populations of these species are presented in Table 3 (Appendix) which gives the number of individuals in a sample of  $0.5 \text{ cm}^3$  taken from a culture of  $5 \text{ cm}^3$  in volume. (A separate counting of the number of individuals in every culture was discontinued from the twentieth day, and we began to take average samples from the similar cultures.)

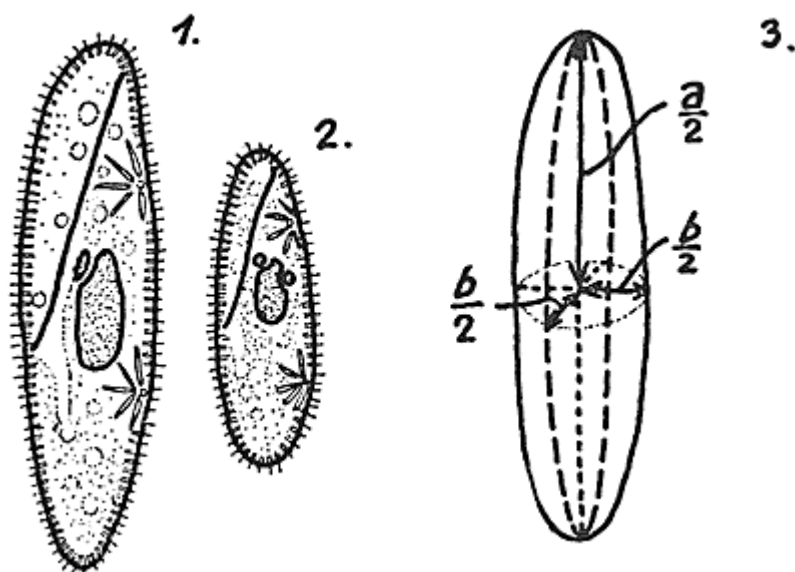


Fig. 20. *Paramecium caudatum* (1) and *Paramecium aurelia* (2) according to Kalmus ('31). (3) Measurements for the calculation of volume of *Paramecium*.

In order to investigate the process of competition, we had to pass from the number of individuals of *P. caudatum* and *P. aurelia* to their biomasses, as these species differ rather strongly in size (see Fig. 20). In order to obtain an idea of the biomass we had recourse to the volumes of these species. *P. caudatum* and *P. aurelia* were measured under the conditions of our experiments (Table X) and on the basis of these measurements the volumes were calculated. As in shape *Paramecium* after fixation approaches somewhat closely to an ellipsoid of rotation with the half-axes:  $\frac{a}{2}, \frac{b}{2}, \frac{b}{2}$  (see Fig. 20), the calculation of the volumes was made according to the formula for this body.

TABLE X

*Measurements of Paramecium caudatum and Paramecium aurelia*  
(after fixation)

Specification of measurements is taken from Figure 20

Origin of Paramecia	Average values for <i>P. caudatum</i> in divisions of ocular micrometer	Average values for <i>P. aurelia</i> in divisions of ocular micrometer	Calculated volume of <i>P. aurelia</i> (volume of <i>P. caudatum</i> = 1)
Growing culture with Osterhout's medium	$\begin{cases} a = 18.3 \\ b = 6.0 \end{cases}$	$\begin{cases} a = 13.8 \\ b = 4.2 \end{cases}$	0.39
Old culture with Osterhout's medium	$\begin{cases} a = 17.1 \\ b = 5.1 \end{cases}$	$\begin{cases} a = 12.6 \\ b = 3.8 \end{cases}$	$\begin{cases} 0.37 \\ 0.41 \end{cases}$
Culture with the buffered medium	$\begin{cases} a = 18.0 \\ b = 6.2 \end{cases}$	$\begin{cases} a = 12.9 \\ b = 4.8 \end{cases}$	0.429

Taking the volume of the larger *P. caudatum* equal to unity, the volume of *P. aurelia* can be easily expressed in a relative form. Under different conditions the relative volume of *P. aurelia* varies somewhat, but for Osterhout's medium it can be taken as equal on an average to 0.39 of the volume of *P. caudatum*. In this way, in order to pass from the growth of the number of individuals of the two species of Paramecia to the growth of their volumes, we can leave without alteration the number of individuals of *P. caudatum*, and only *diminish the number of individuals* of the small *P. aurelia* by multiplying it in every case by 0.39.

(5) Figure 21 represents graphically the growth in the number of individuals and in the volumes of *P. caudatum* and *P. aurelia* cultivated separately in a medium changed daily for 25 days. The general character of the curves shows that the growth of population under these conditions has an S-shaped form. At a certain moment the possibility of growth in a given microcosm is apparently exhausted, and with a continuously maintained level of nutritive resources a certain equilibrium of population is established. The oscillations of population round this state of equilibrium are not governed by any apparent law, and depend on various accidental causes (variation in temperature of the thermostat, a slight variability in the composition of the synthetic medium, etc.). A comparison of the curves of growth of *P. caudatum* and *P. aurelia* shows that as regards the number of individuals the level of the saturating population of *P. aurelia* is considerably higher than that of *P. caudatum*. Nevertheless, the comparison of the volumes shows something completely different; in

this respect *P. aurelia* only slightly surpasses *P. caudatum*, accumulating at the expense of a certain definite level of food resources a scarcely larger biomass. As will be shown further on, the Osterhout salts medium is not quite favorable in its properties for the Paramecia, and this complicates the question as to the factors limiting growth. On the one hand, the insufficiency of food plays a part here which we can judge of by a direct observation of the cultures: with a population in equilibrium the turbid bacterial medium introduced daily becomes quite transparent after a certain time, as the bacteria are entirely devoured by the Paramecia. However, owing to a comparatively high concentration of bacteria and a somewhat unoptimal reaction of the medium a depressory action of certain other influences plays also a role here.

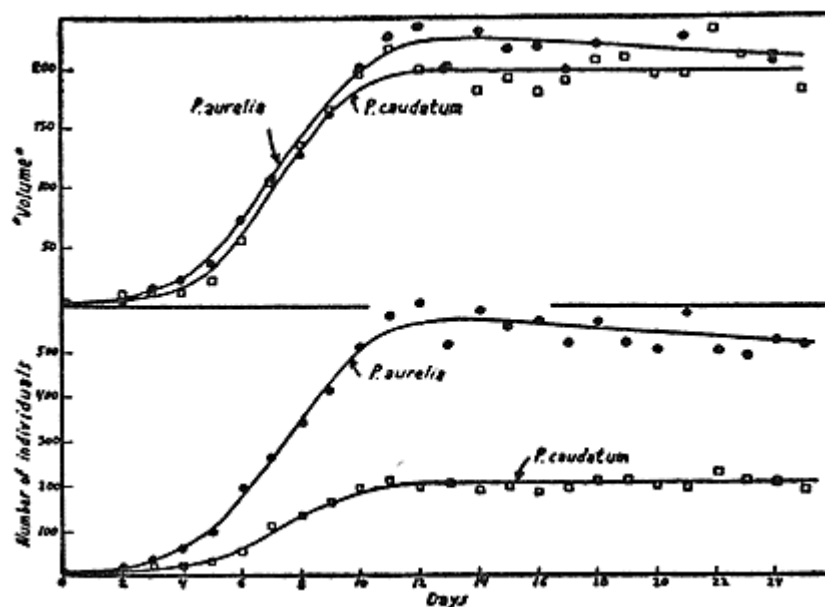


Fig. 21. The growth of the number of individuals and of the "volume" in *Paramecium caudatum* and *Paramecium aurelia* cultivated separately on the medium of Osterhout. From Gause ('34d).

(6) The data on the growth of the volumes of *P. caudatum* and *P. aurelia* in a mixed population are given in Figure 22. The curves of growth of each species in a mixed culture are presented here on the background of control curves corresponding to the free growth of the same species. It is easy to see that the growth of a mixed population consists of two periods: (a) during the first period (till the eighth day), the species grow and compete for the seizing of the still unutilized energy (food resources). But the moment approaches gradually when all the utilizable energy is already taken hold of, and the total of the biomasses of the two species tends to reach the maximal possible biomass under given conditions. (This happens on the eighth day; the total biomass is equal to about 210.) This first period corresponds to

what we have already observed in yeast cells. (b) After this there can only arise *the redistribution of the already seized energy between the two species*, i.e. the displacement of one species by another. Figure 22 shows that such a displacement is actually observed in the experiment: the number of *P. caudatum* gradually diminishes as a result of its being driven out by *P. aurelia*. As several further experiments have shown (see Fig. 24), the process of competition under our conditions has always resulted in one species being entirely displaced by another, in complete agreement with the predictions of the mathematical theory.

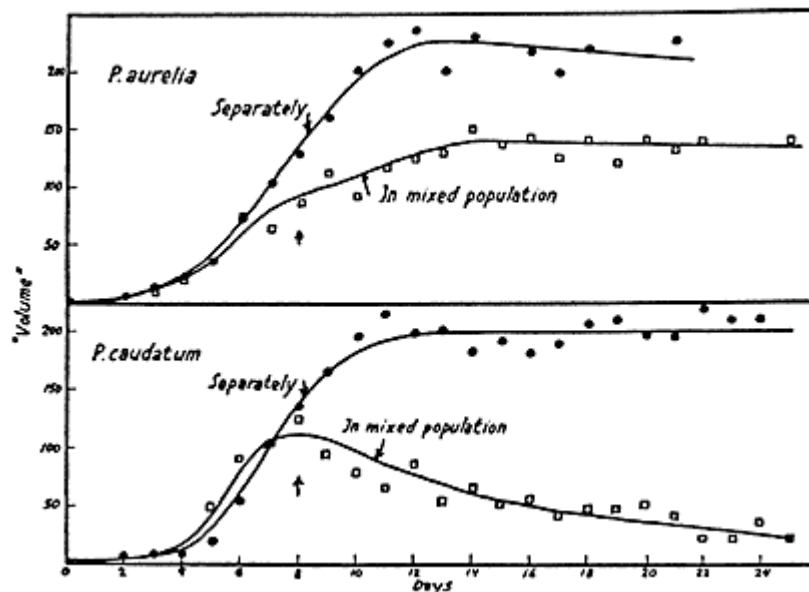


Fig. 22. The growth of the "volume" in *Paramecium caudatum* and *Paramecium aurelia* cultivated separately and in the mixed population on the medium of Osterhout. From Gause ('34d).

If we consider the curves in Figure 22 more in detail, we shall note that they generally are of a rather complicated character. It is interesting to note that *P. caudatum* in a mixed culture at the beginning of the experiment grows even better than separately. This is apparently a consequence of the more nearly optimal relationships between the density of the Paramecia and that of the bacterial food, in accordance with the observations of Johnson ('33).

### III

(1) Although the situation in our experiments with Osterhout's medium has been considerably simpler than in the case of the "oaten medium," it is still too complicated for a clear understanding of the mechanism of competition. In fact, why has one species been victorious over another? In the case of yeast cells we answered that the success of the species during the first stage of competition depends on definite relations between the coefficients of multiplication and the alcohol

production, and that it can be exactly predicted with the aid of an equation of the struggle for existence. What will be our answer for the population of Paramecia?

To investigate this problem we made the conditions of the experimentation the next step in the simplification. We endeavored to make a medium with a very small concentration of nutritive bacteria and optimal in its physicochemical properties for Paramecia. Under such conditions the competition for common food between two species of Protozoa has been reduced to its simplest form.

(2) As Woodruff has shown ('11, '14), the waste products of Paramecia can depress the multiplication and be specific for a given species. In any case we are very far from an exact knowledge of their role and chemical composition. Therefore first of all we must eliminate the complicating influence of these substances. This problem is the reverse of the one we had to do with in the preceding chapter. There in the experiments with yeast we tried to set up conditions under which the food resources of the medium should be very considerable at the time when the concentration of the waste products had already attained a critical value. Now with Paramecia our object is that the concentration of the waste products should still be very far from the critical threshold at the moment when the food is exhausted.

First of all we turned our attention to the hydrogen ion concentration (pH), which in the light of the researches of Darby ('29) can be of great importance for our species. When Paramecia are cultivated in Osterhout's medium, pH is near to 6.8 and unstable, whereas the reaction in our wild cultures is commonly near to 8.0. Therefore we,

like Johnson, buffered Osterhout's medium by adding 1 cm<sup>3</sup> of  $\frac{m}{20}$

KH<sub>2</sub>PO<sub>4</sub> to 30 cm<sup>3</sup> of diluted salt solution, and bringing the reaction

of the medium with the aid of  $\frac{m}{20}$  KOH to pH = 8.0. At the same time

we isolated new pure lines of Paramecia out of our wild culture, as the Paramecia which had been cultivated for a long time on Osterhout's medium could not stand a sudden transfer into a buffered medium.

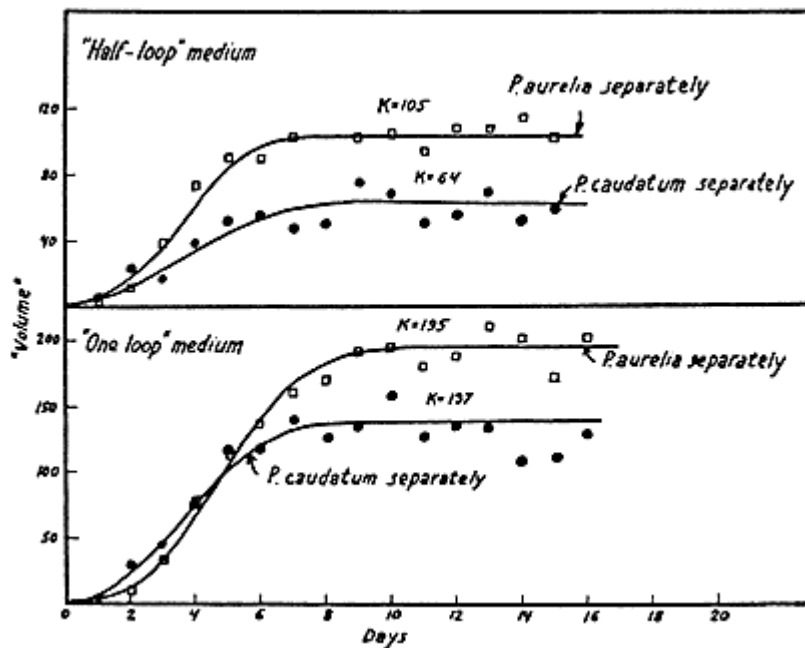


Fig. 23. The growth of the "volume" in *Paramaecium caudatum* and *Paramaecium aurelia* cultivated separately on the buffered medium ("half-loop" and "one-loop" concentrations of bacteria). From Gause ('34d).

In order to diminish the concentration of the bacteria we made a new smaller standard loop for preparing the "one-loop medium," and also arranged experiments in which the one loop medium was diluted twice ("half-loop medium"). The data obtained are given in Table 4 (Appendix) where every figure represents a mean value from the observations of two microcosms. This material is represented graphically in Figures 23, 24 and 25.

Let us examine Figure 23. The curves of growth of pure populations of *P. caudatum* and *P. aurelia* with different concentrations of the bacterial food show that the lack of food is actually a factor limiting growth in these experiments. With the double concentration of food the volumes of the populations of the separately growing species also increase about twice (from 64 up to 137 in *P. caudatum*;  $64 \cdot 2 = 128$ ; from 105 up to 195 in *P. aurelia*;  $105 \cdot 2 = 210$ ). Under these conditions the differences in the growth of populations of *P. aurelia* and *P. caudatum* are quite distinctly pronounced: the growth of the biomass of the former species proceeds with *greater rapidity*, and it accumulates a *greater biomass than P. caudatum at the expense of the same level of food resources*.<sup>1</sup> If we now express the curves of separate growth of both species under a half-loop concentration of bacteria with the aid of logistic equations we shall obtain the data presented in Table XI. This table shows clearly that *P. aurelia* has perfectly definite advantages over *P. caudatum* in respect to the basic characteristics of

growth.

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<sup>1</sup> This is apparently connected with the resistance of *P. aurelia* to the waste products of the pathogenic bacterium, *Bacillus pyocyaneus* (see Gause, Nastukova and Alpatov, '35).

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TABLE XI

*Parameters of the logistic curves for separate growth of Paramecium caudatum and Paramecium aurelia Buffered medium with the "half-loop" concentration of bacteria*

	<i>P. aurelia</i>	<i>P. caudatum</i>
Maximal volume ( <i>K</i> )	$K_1 = 105$	$K_2 = 64$
Coefficient of geometric increase ( <i>b</i> )	$b_1 = 1.1244$	$b_2 = 0.7944$

(3) We will now pass on to the growth of a mixed population of *P. caudatum* and *P. aurelia*. The general character of the curves on Figures 22, 24 and 25 is almost the same, but there are certain differences concerning secondary peculiarities. For a detailed acquaintance with the properties of a mixed population we will consider the growth with a half-loop concentration of bacteria (Fig. 24). First of all we see that as in the case examined before the competition between our species can be divided into two separate stages: up to the fifth day there is a competition between the species for seizing the so far unutilized food energy; then after the fifth day of growth begins the redistribution of the completely seized resources of energy between the two components, which leads to a complete displacement of one of them by another. The following simple calculations can convince one that on the fifth day all the energy is already seized upon. At the expense of a certain level of food resources which is a constant one in all "half-loop" experiments and may be taken as unity, *P. aurelia* growing separately produces a biomass equal to 105 volume units, and *P. caudatum* 64 such units.

Therefore, one unit of volume of *P. caudatum* consumes  $\frac{1}{64} = 0.01562$

of food, and one unit of volume of *P. aurelia*  $\frac{1}{105} = 0.00952$ . In other

words, one unit of volume of *P. caudatum* consumes 1.64 times as

much food as *P. aurelia*, and the food consumption of one unit of volume in the latter species constitutes but 0.61 of that of *P. caudatum*. These coefficients enable us to recalculate the volume of one species into an equivalent in respect to the food consumption volume of another species.

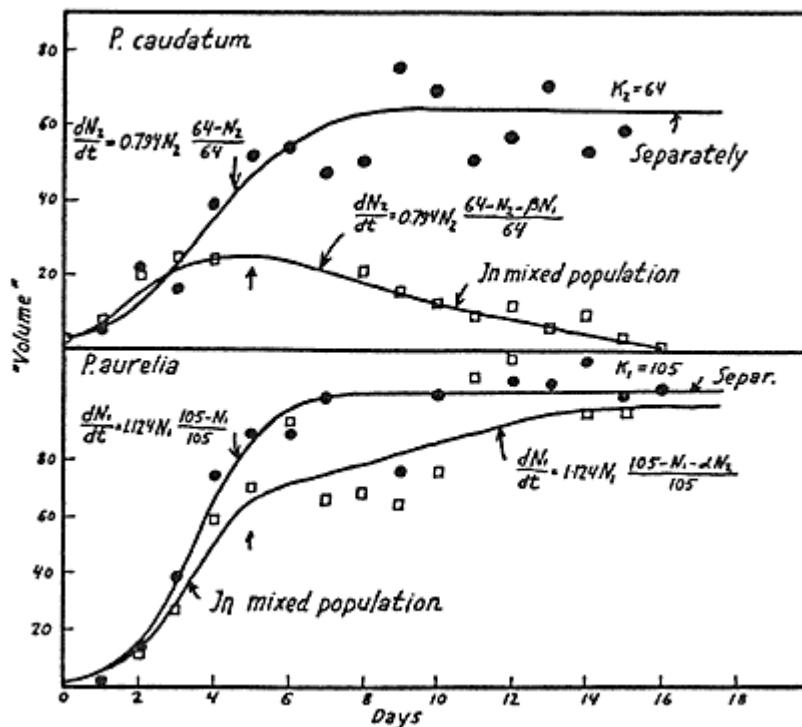


Fig. 24. The growth of the "volume" in *Paramecium caudatum* and *Paramecium aurelia* cultivated separately and in the mixed population on the buffered medium with the "half-loop" concentration of bacteria. From Gause ('34d).



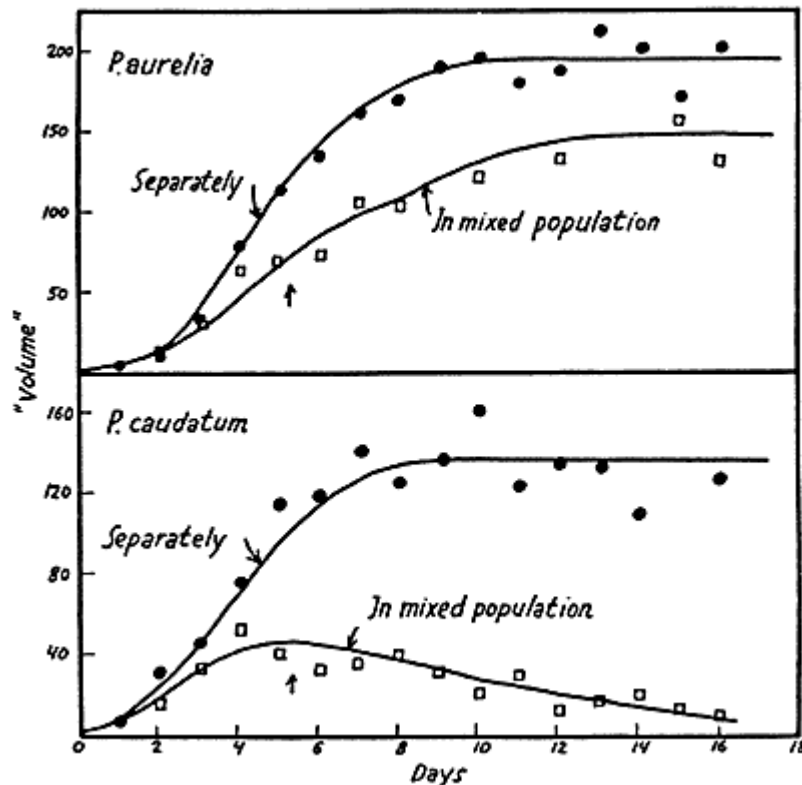


Fig. 25. The growth of the "volume" in *Paramaecium caudatum* and *Paramaecium aurelia* cultivated separately and in the mixed population on the buffered medium with the "one-loop" concentration of bacteria. From Gause ('34d).

On the fifth day of growth of a mixed population the biomass of *P. caudatum* (in volume units) is equal to about 25, and of *P. aurelia* to about 65. If we calculate the total of these biomasses in equivalents of *P. aurelia*, we shall have:  $(25 \cdot 1.64) + 65 = 106$  (maximal free growth of *P. aurelia* is equal to 105). The total of the biomasses expressed in equivalents of *P. caudatum* will be  $(65 \cdot 0.61) + 25 = 65$  (with the free growth 64). This means that on the fifth day of growth of the mixed population the food resources of the microcosm are indeed completely taken hold of.

(4) The first period of competition up to the fifth day is not all so simple as we considered it in the theoretical discussion of the third chapter, or when examining the population of yeast cells. The nature of the influence of one species on the growth of another does not remain invariable in the course of the entire first stage of competition, and in its turn may be divided into two periods. At the very beginning *P. caudatum* grows even somewhat better in a mixed population than separately (analogous to Fig. 22), apparently in connection with more nearly optimal relations between the density of Paramecia and that of the bacteria in accordance with the already mentioned data of Johnson

(33). At the same time *P. aurelia* is but very slightly oppressed by *P. caudatum*. As the food resources are used up, the Johnson effect disappears, and the species begin to depress each other as a result of competition for common food.

It is easy to see that all this does not alter in the least the essence of the mathematical theory of the struggle for existence, but only introduces into it a certain natural complication: the coefficients of the struggle for existence, which characterize the influence of one species on the growth of another, do not remain constant but in their turn undergo regular alterations as the culture grows. The curves of growth of every species in a mixed population in Figure 24 up to the fifth day of growth have been calculated according to the system of differential equations of competition with such varying coefficients. In the first days of growth the coefficient B is negative and near to - 1, i.e., instead of  $-bN_1$  we obtain  $+N_1$ . In other words, the presence of *P. aurelia* does not diminish, but increases the possibility of growth of *P. caudatum*, which proceeds for a certain time with a potential geometrical rate,

outrunning the control culture ( $\frac{64 - N_2 + N_1}{64}$  remains near to unity).

At this time the coefficient a is equal to about +0.5; in other words, *P. aurelia* suffers from a slight depressing influence of *P. caudatum*. Later the inhibitory action of one species upon the growth of another begins to manifest itself more and more in proportion to the quantity of food consumed, because the larger is the part of the food resources already consumed the less is the unutilized opportunity for growth. In our calculations for *P. caudatum* from the second and for *P. aurelia* from the fourth days of growth we have identified the coefficients of competition with the coefficients of the relative food consumption, i.e.,  $a = 1.64$ ,  $b = 0.61$ . It is obvious that this is but a first approximation to the actual state of things where the coefficients gradually pass from one value to another. The entire problem of the changes in the coefficients of the struggle for existence in the course of the growth of a mixed population (which apparently are in a great measure connected with the fact that the Paramecia feed upon living bacteria) needs further detailed investigations on more extensive experimental material than we possess at present.

(5) It remains to examine the second stage of the competition, i.e., the direct displacement of one species by another. An analysis of this phenomenon can no longer be reduced to the examination of the coefficients of multiplication and of the coefficients of the struggle for existence, and we have to do in the process of displacement with a quite new qualitative factor: the rate of the stream which is represented by population having completely seized the food resources. As we have already mentioned in Chapter III, after the cessation of growth a

population does not remain motionless and in every unit of time a definite number of newly formed individuals fills the place of those which have disappeared during the same time. Among different animals this can take place in various ways, and a careful biological analysis of every separate case is here absolutely necessary. In our experiments the principal factor regulating the rapidity of this movement of the population that had ceased growing was the following technical measure: a sample equal to  $\frac{1}{10}$  of the population

was taken every day and then destroyed. In this way a regular decrease in the density of the population was produced and followed by the subsequent growth up to the saturating level to fill in the loss.

During these elementary movements of thinning the population and filling the loss, the displacement of one species by another took place.

The biomass of every species was decreased by  $\frac{1}{10}$  daily. Were the species similar in their properties, each one of them would again increase by  $\frac{1}{10}$ , and there would not be any alteration in the relative

quantities of the two species. However, as one species grows quicker than another, it succeeds not only in regaining what it has lost but also in seizing part of the food resources of the other species. Therefore, every elementary movement of the population leads to a diminution in the biomass of the slowly growing species, and produces its entire disappearance after a certain time.

(6) The recovery of the population loss in every elementary movement is subordinate to a system of the differential equations of competition. In the present stage of our researches we can make use of these equations for only a qualitative analysis of the process of displacement. They will show us exactly what particular species in the population will be displaced. However, the quantitative side of the problem, i.e., the rate of the displacement, still requires further experimental and mathematical researches and we will not consider it at present.

The qualitative analysis consists in the following. Let us assume that the biomass of each component of the saturating population is decreased by  $\frac{1}{10}$ . Then according to the system of differential

equations, inserting the values of the coefficients of multiplication and of the coefficients of food consumption, we shall be able to say how each one of the components can utilize the now created possibility for growth. The result of the calculations shows that *P. aurelia*, primarily

owing to its high coefficient of multiplication, has an advantage and increases every time comparatively more than *P. caudatum*.<sup>2</sup>

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<sup>2</sup> It is obvious that in these calculations it is necessary to introduce varying coefficients of the struggle for existence. At the same time with our technique of cultivation corrections to the "elementary movements" must be also included in an analysis of the first stage of growth of a mixed population (an approximation to the asymptote). But at the present stage of our researches we have neglected them.

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In summing up we can say that in spite of the complexity of the process of competition between two species of infusoria, and as one may think a complete change of conditions in passing from one period of growth to another, a certain law of the struggle for existence which may be expressed by a system of differential equations of competition remains invariable all the time. The law is that the species possess definite potential coefficients of multiplication, which are realized at every moment of time according to the unutilized opportunity for growth. We have only had to change the interpretation of this unutilized opportunity.

(7) It seems reasonable at this point to coordinate our data with the ideas of the modern theory of natural selection. It is recognized that fluctuations in numbers resembling the dilutions we have artificially produced in our microcosms play in general a decisive role in the removal of the less fitted species and mutations (Ford, '30). An interesting mathematical expression of this process proposed by Haldane ('24, '32) can be formulated thus: how does the rate of increase of the favorable type in the population depend on the value of the coefficient of selection  $k$ ? In its turn the coefficient of selection characterizes an elementary displacement in the relation between the two types per unit of time—one generation. Therefore the problem resolves itself into a determination of the functional relationship between the increase of concentration of the favorable type and the elementary displacement in its concentration. A recent theoretical paper by Ludwig ('33) clearly shows how the fluctuation in the population density alters the relation between the two types owing to the fact that one of them has a somewhat higher probability of multiplication than the other. It seems to us that there is a great future for the Volterra method here, because it enables us not to begin the theory by the coefficient of selection but to calculate theoretically the coefficient itself starting from the process of interaction between the two species or mutations.

#### IV

(1) How complicated are processes of competition under the

conditions approaching those of nature can be seen from the experiments made by Gause, Nastukova and Alpatov ('35). They studied the influence of biologically conditioned media on the growth of a mixed population of *Paramecium caudatum* and *P. aurelia*. The analysis of the relative adaptation of the two species at different stages of population growth has shown that *P. caudatum* has an advantage over *P. aurelia* in the coefficient of geometric increase (in the absence of *Bacillus pyocyaneus* in the nutritive medium which in the experiments described above inhibited *P. caudatum* by its waste products) whilst *P. aurelia* surpasses *P. caudatum* in the resistance to waste products. Therefore if the decisive factor of competition is a rapid utilization of the food resources, *P. caudatum* has an advantage over *P. aurelia*; but if the resistance to waste products is the essential point, then *P. aurelia* will take place of *P. caudatum*.

It is interesting to note also that in the complicated situation of these experiments the superiority of one species over another in competition did not simply reflect the properties of these species taken independently, but was often essentially modified by the process of their interaction.

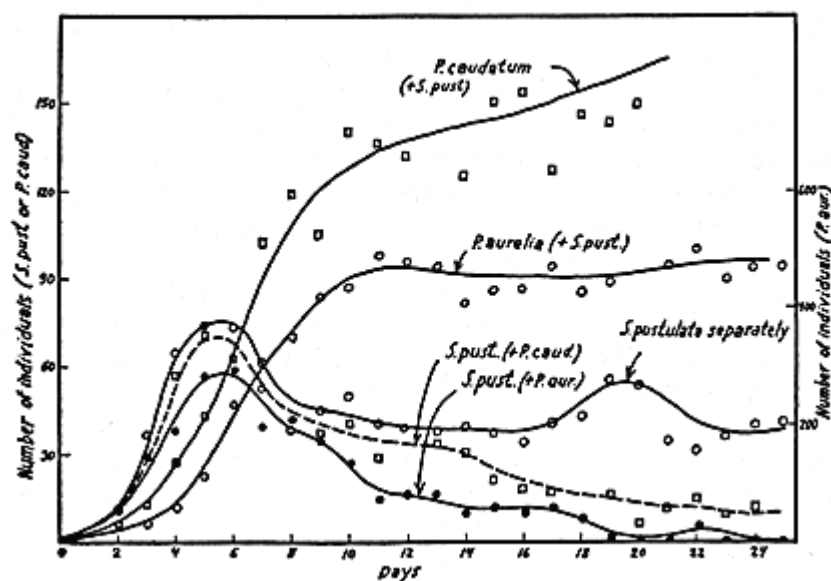


Fig. 26. The growth of the number of individuals of *Stylorzychia pustulata* cultivated separately, and in the mixed populations with *Paramecium caudatum* and *Paramecium aurelia* (on the medium of Osterhout).

(2) If we turn to the population growth of *Stylonychia pustulata* and its competition with two species of *Paramecium*, we shall encounter extremely complicated processes. The corresponding data are given in Table 5 (Appendix) and Figure 26. These experiments were made with Osterhout's medium containing *Bacillus pyocyaneus*, simultaneously

with those mentioned above. Therefore, the data on the separate growth of *P. caudatum* and *P. aurelia* given in Appendix Table 3 serve as a control for these experiments.

First of all, the separate growth of *Stylonychia pustulata* is very peculiar: having attained a certain maximum, the density of population decreases and remains stationary at a lower level. Direct observation shows that the bacteria at the close of the twenty-four hour intervals between the changes of medium remain partly unconsumed, and the limiting factor here is apparently an accumulation of waste products and not an insufficiency of food. The fluctuations in the density of population of the separately growing *S. pustulata* are probably connected with some complex processes of the influence of metabolic products on growth. As to the mixed populations, the same regularity with which we had to deal previously repeats itself here: one species finally completely displaces another, and the species displaced is always *Stylonychia pustulata*.

(3) Let us summarize the data of this chapter. We have studied the competition between two species for a source of energy kept continually at a certain level. This process may be divided into two periods. *In the first period the two species compete for the still unutilized resources of energy.* In what proportion this energy will be distributed between the two species is determined by the system of Vito Volterra's differential equations of competition, but the coefficients of the struggle for existence in these equations change in the course of the growth of the population and are therefore more complicated than in the preceding chapter. *In the second period there is but a redistribution of the completely seized energy between the two species,* which is again controlled by the differential equations of competition. Owing to its advantages, mainly a greater value of the coefficient of multiplication, one of the species in a mixed population drives out the other entirely.

