Kinetics of Growth of the Ciliate *Tetrahymena pyriformis* on *Escherichia coli*

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(Received 23 May 1980)

The growth of the ciliate *Tetrahymena pyriformis* on non-growing *Escherichia coli* has been studied by following the time courses of population densities and protozoan mean cell volume in batch cultures. Viable, non-encysted protozoa always stopped feeding before the bacterial density was reduced to zero and non-feeding ciliates tended to swim faster than feeding ciliates. In addition, the number of bacteria and other particles of bacterial size consumed in the formation of one new ciliate, when averaged over the lag and reproductive phases of a culture, declined toward a limiting value of about 1.6×10^4 particles per ciliate as the initial density of such particles was increased.

INTRODUCTION

The feeding of protozoa on bacteria, unicellular algae and other protozoa constitutes predation on a microscopic scale. Such predation is important in wastewater treatment, since the protozoa collect and concentrate particulate matter in the water and also serve to regulate competition between decomposer populations. It is probably also important to the dynamics of aquatic ecosystems, where the protozoa constitute a link in the food webs. Finally, it is important in that it is an example of a basic natural process that can be isolated and studied under controlled laboratory conditions. A number of such studies have been reported, beginning with the classic work of Gause (1934) – for a review, see Fredrickson (1977) and also Taylor (1978).

Some of the foregoing studies have dealt with the effect of prey density on the feeding rate of the predators and the results have been particularly interesting. Thus, Salt (1967) in his study of predation of the large ciliate *Woodruffia metabolica* on *Paramecium aurelia* found that the predators' feeding exhibited a threshold type of response to prey density. When the prey density was above a threshold value *Woodruffia metabolica* fed at a rate that was independent of prey density but when the prey density fell below the threshold value *Woodruffia metabolica* underwent a morphological change and formed non-motile, non-feeding cysts.

Danso & Alexander (1975) observed similar phenomena when they added various protozoa to cultures of non-growing bacteria. The protozoa (*Tetramitus rostratus*, and strains of *Hartmanella*, *Naegleria* and *Vahlkampfia*) would eat the bacteria (*Rhizobium meliloti*) down to a threshold level but no further. Dive (1975) observed that *Colpidium campylum* would not start eating bacteria unless the bacterial density exceeded a threshold level, and Berk *et al.* (1976) made similar observations with *Uronema nigricans* and

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Potomacus pottsi. Cessation of feeding and failure to start feeding are probably different aspects of the same behavioural pattern.

Bader *et al.* (1976) studied the feeding of the small ciliate *Colpoda steinii* on growing *Anacystis nidulans* (a cyanobacterium) in chemostat culture. *Colpoda steinii* encysted under certain conditions, one of which was reduction of the prey density to a threshold level, and this release of the prey from predation pressure had a strong stabilizing effect on the predator-prey relationship. In particular, Bader *et al.* (1976) never observed sustained oscillations of predator and prey densities in their chemostat cultures, although they did observe damped oscillations of the densities. In contrast, Drake & Tsuchiya (1976) observed what were probably sustained oscillations when they grew *Colpoda steinii* and *Escherichia coli* together in a chemostat under conditions of low energy supply rate and high dilution rate, where *Colpoda steinii* did not encyst.

Jost *et al.* (1973) studied the feeding of the small ciliate *Tetrahymena pyriformis* on *Escherichia coli*, on *Azotobacter vinelandii* and on a mixture of these two bacteria, in chemostat culture. Unlike *Colpoda steinii*, *T. pyriformis* has not been confirmably reported to form cysts (Corliss, 1970). Nevertheless, experimental evidence (Jost *et al.*, 1973) suggested that the feeding behaviour of this ciliate at low prey densities resembles that of encysting protozoa in that a plot of specific feeding rate (number of prey eaten per unit time per predator cell) versus prey density appeared to approach the origin with zero slope rather than with a finite slope. The evidence for this was indirect, but still strong.

Habte & Alexander (1978) provided direct evidence that the feeding rate of *T. pyriformis* falls to zero, or at least to immeasurably low values, at finite values of prey density. Thus, they found that addition of the predator to suspensions of *Klebsiella pneumoniae* in saline solution led to a decrease in the bacterial density, but not to complete consumption of the bacteria. If the initial predator density was very high, feeding of the predators never started. Cessation of feeding, or failure to start feeding, could not be explained by inviability of the protozoa, for Habte & Alexander (1978) showed that viable protozoa, as well as viable bacteria, were present when no feeding was taking place.

Many additional phenomena of interest have been observed in studies of protozoan growth. For example, studies of predation on *Paramecium aurelia* by *Woodruffia metabolica*, *Amoeba proteus* and *Didinium nasutum* showed that the average number of prey consumed by a predator cell during its life cycle declined as predator density increased, even though the prey were replenished periodically and the reproduction rate did not slow down (Salt, 1967, 1968, 1974). An additional study on predation by *Didinium nasutum* (Salt, 1975) revealed that the volume of a typical predator cell, at a given stage of the life cycle, increased during culture, even though the amount of food consumed per predator decreased. Salt (1975) advanced a working hypothesis to explain these seemingly contradictory observations, but full proof of the hypothesis has not yet appeared.

In the present paper we report results of experiments which were designed to provide additional information on the kinetics of growth of protozoa. We chose *Tetrahymena pyriformis* rather than an encysting protozoon as the test organism to avoid the dynamical complications of systems in which encystment occurs.

METHODS

All experiments were started by adding a fixed number of *T. pyriformis* cells to a suspension of *E. coli* cells in a chemically defined medium which contained no carbon/energy source for *E. coli* growth. It is essential in such experiments that the inocula have fixed physiological states, or at least have physiological states that can be controlled. To keep the physiological states of the inocula as constant as possible, bacteria and protozoa were cultivated in chemostats operated under fixed conditions of dilution rate and composition of the feed stream; inocula were taken from these chemostats after they had achieved steady states.

Because one of the initial aims of this research was to study food selectivity by *T. pyriformis*, the chemostat in which it was cultivated was fed with the overflows from two other chemostats, one containing a pure culture of *E.*

coli and the other containing a pure culture of *Azotobacter vinelandii*. The selectivity study was postponed when the phenomena reported below became apparent, but use of two bacterial chemostats to feed the protozoan chemostat was continued. Hence, protozoan inocula taken from the chemostat contained some *A. vinelandii* cells, as well as some *E. coli* cells.

Organisms. The organisms used were *Escherichia coli* B (supplied by Professor J. T. Prince, Department of Microbiology, University of Minnesota), *Azotobacter vinelandii* (supplied by Professor E. Schmidt, Department of Microbiology, University of Minnesota) and *Tetrahymena pyriformis* GL (supplied by Professor A. Hooper, Department of Biological Sciences, University of Minnesota). Stock cultures of *E. coli* B were grown and stored at 9.9 °C on slants containing 5 g tryptone, 1 g glucose, 2.5 g yeast extract and 20 g agar per litre. Stock cultures of *A. vinelandii* were grown at 25 °C in a medium containing 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 5 mg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.1 g CaCl_2 , 1.77 g K_2HPO_4 , 0.335 g KH_2PO_4 and 20 g sucrose per litre. Stock cultures of *T. pyriformis* GL were grown at 25 °C in a broth containing 20 g proteose peptone and 1 g yeast extract per litre.

The chemostats used in growing inocula were patterned after the design of Novick & Szilard (1950) and had capacities of 100 ml. Temperature was maintained at 25 ± 0.2 °C by constant temperature water jackets. The two chemostats for bacterial growth were operated at a dilution rate of 0.02 h^{-1} and were aerated with humidified air at 2.8 l h^{-1} . The chemostat for protozoan growth was fed with the overflows from the other two vessels, and thus operated at a dilution rate of 0.04 h^{-1} . It was aerated with humidified air at 1.4 l h^{-1} . The concentration of glucose in the feeds to the two bacterial chemostats was 1 g l^{-1} . No glucose was detectable by standard analytical techniques in any of the chemostats. When steady states were achieved under the above conditions, the populations obtained were as follows:

E. coli in *E. coli* chemostat: 1.60×10^9 cells ml^{-1} by plate count; $2.2 (\pm 0.2) \times 10^9$ cells ml^{-1} by Coulter count; mean cell volume, $0.27 \mu\text{m}^3$.

A. vinelandii in *A. vinelandii* chemostat: 1.85×10^8 cells ml^{-1} by plate count; $2.6 (\pm 0.2) \times 10^8$ cells ml^{-1} by Coulter count; mean cell volume, $1.4 \mu\text{m}^3$.

E. coli in protozoan chemostat: 1.26×10^7 cells ml^{-1} by plate count; the Coulter count was about 30% larger.

A. vinelandii in protozoan chemostat: 1.93×10^5 cells ml^{-1} by plate count.

T. pyriformis in protozoan chemostat: $6.9 (\pm 0.6) \times 10^4$ cells ml^{-1} by Coulter count; mean cell volume, $2.8 (\pm 0.6) \times 10^3 \mu\text{m}^3$.

Thus, 11 000 viable *E. coli* cells and 1300 viable *A. vinelandii* cells or 15 700 particles of the *E. coli* size range and 1900 particles of the *A. vinelandii* size range were consumed, on average, by the formation of one new protozoan cell.

Medium. To prevent irreversible complexing of the medium constituents, they were autoclaved in four separate portions (all concentrations are final concentrations in the medium): (i) 0.335 g KH_2PO_4 and 1.77 g K_2HPO_4 per litre; (ii) 1.25 g $(\text{NH}_4)_2\text{SO}_4$, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4 mg $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 1 mg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and 1 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per litre; (iii) 0.02 g CaCl_2 and 0.01 g NaCl per litre; (iv) 1 g glucose per litre (not added to the medium for batch culture experiments). Distilled water was distributed among the portions and they were autoclaved separately at 120 °C for 30 to 60 min, except for the glucose solution which was autoclaved for only 15 min. The pH was 7.5 after the cooled solutions were mixed.

Batch culture techniques. Inocula for batch cultures were collected from the *E. coli* and *T. pyriformis* chemostats after they had reached steady states. In some preliminary experiments, addition of *T. pyriformis* to the *E. coli* culture when the bacterial density was large led to death of the protozoa. Qualitative experiments carried out in test tubes confirmed that the *E. coli* broth had a toxic effect on the protozoa. Hence, two series of batch experiments were conducted.

In the first series, measured volumes of cultures from the two chemostats were added to a batch growth vessel and diluted with sterile medium containing no glucose; the volumes of the chemostat cultures used were such as to give the required initial densities in the batch culture.

In the second series, the *E. coli* from the chemostat were washed with 0.85% (w/v) saline solution on a membrane filter (0.45 μm pore size) under sterile conditions. If the bacteria were to be used to begin a batch experiment, they were next resuspended in fresh batch culture medium and counted so that the volume of suspension needed for the inoculation could be calculated. If the bacteria were to be added to the stationary phase of a batch culture, the chemostat organisms were counted, quantitatively delivered to the filter, washed and then resuspended in the stationary phase culture itself. Resuspension was carried out by drawing off a portion of the stationary phase culture medium, adding the filter plus bacteria to the medium, swirling to separate the bacteria from the membrane, and returning all but the membrane to the flask. Thus no dilution of the batch culture occurred when the fresh *E. coli* were introduced.

Since the physiological state of the *T. pyriformis* inoculum was found to have a marked effect on the subsequent behaviour of a batch culture, and since washing of *T. pyriformis* was found to alter this state in an inconsistent way, these organisms were not washed. Because they were not washed, some *E. coli* metabolites from the protozoan chemostat were thereby introduced into the batch cultures. However, by comparing the results of

experiments with washed and unwashed *E. coli*, it was determined that the *E. coli* metabolites added with the *T. pyriformis* inoculum (about 15 ml) had negligible effects on the batch culture.

Batch cultures (265 ml) were propagated in glass vessels maintained at $26 \pm 0.5^\circ\text{C}$ and aerated with humidified air at 111 h^{-1} .

Analytical techniques. To count populations, organisms were suspended in a saline solution (containing 8.5 g NaCl and 0.2 g Na_2EDTA per litre) that had been filtered through a Millipore membrane ($0.22\text{ }\mu\text{m}$ pore size). The *E. coli* densities were evaluated using a model Z_B Coulter counter with a $30\text{ }\mu\text{m}$ aperture. *Tetrahymena pyriformis* was counted and sized on a model B Coulter counter with a $100\text{ }\mu\text{m}$ aperture. Sizing was carried out by varying the lower threshold from 1 to 100, such that an integral curve could be generated. From this curve, a mean cell volume was calculated.

The numbers of viable *E. coli* were determined by plating dilutions on tryptone/glucose/yeast extract agar and incubating at 25°C for about 1 d.

RESULTS

A series of batch culture experiments were performed in which the initial *E. coli* density was varied and the *T. pyriformis* density was fixed. The lowest initial bacterial density, as indicated by Coulter count, was 0.81×10^8 particles ml^{-1} , and the highest density was 5.8×10^8 particles ml^{-1} ; to achieve the latter density required the use of almost all of the cells from the 100 ml *E. coli* chemostat. The initial density of *T. pyriformis* was $4.6 (\pm 0.6) \times 10^3$ cells ml^{-1} in all runs. Since the ratio of *A. vinelandii* to *T. pyriformis* in the protozoan chemostat was 2.8:1, the initial *A. vinelandii* density in the batch runs was 1.3×10^4 cells ml^{-1} . This is only about 0.02% of the lowest initial *E. coli* density used, and so the presence of *A. vinelandii* is assumed to have no effect on the results reported below.

Experiments using washed *E. coli*

Figure 1 shows the time courses of culture variables measured in two runs made at essentially identical initial conditions. Quantities measured include (1) the density of *T. pyriformis* (N_T) by Coulter count, (2) the density of viable *E. coli* (N_E) by plate count, (3) the density of small (of the *E. coli* size range) particles (N_{sp}) by Coulter count and (4) the mean

Fig. 1. Time courses of culture parameters for two replicate experiments (shown by open and closed symbols) using washed *E. coli* at an intermediate initial density. (a) N_T , density of *T. pyriformis* ($\blacksquare$, $\square$); $\bar{v}_T$, mean cell volume of *T. pyriformis* ($\bullet$, $\circ$). (b) N_E , density of viable *E. coli* ($\blacktriangle$, $\triangle$); N_{sp} , density of small particles of *E. coli* size range ($\bullet$, $\circ$); $N_{usp} (= N_{sp} - N_E)$, density of unidentified small particles (---). The smoothed curves were fitted by eye.

cell volume of *T. pyriformis* ($\bar{v}_T$) by Coulter count. The time course of the density of unidentified small particles (N_{usp}) is also shown. The quantity N_{usp} is simply the difference between N_{sp} and N_E and it represents the density of dead *E. coli* and other non-living particles having the same size range as *E. coli*.

The curves of N_T and $\bar{v}_T$ versus time in Fig. 1 show that no divisions of the protozoa occurred during the first 6 h of culture, but that protozoa fed rapidly during this time and increased their mean cell volume by a factor of about 4. Divisions began after about 6 h, and the population doubled in the next 4 h. This was followed by a second doubling which also required about 4 h for completion.

We have drawn the curve of N_T versus time in Fig. 1 to show a reduction in the fission rate between 9 and 11 h. Such a reduction, if it is genuine, would indicate that fissions were partly synchronous and occurred in two episodes which overlapped only partially.

The evidence supporting the conclusion that fissions were partly synchronous in experiments in which *T. pyriformis* fed on washed *E. coli* is not solely the N_T versus time data of Fig. 1. The $\bar{v}_T$ versus time data of Fig. 1 show that there was a reduction in the rate of decrease in mean cell volume between 9 and 11 h in both replicate runs, and there is even some suggestion that mean cell volume increased during this period. This is entirely consistent with the notion that the fission rate was reduced in this period. Figure 2 shows N_T and $\bar{v}_T$ versus time data for a different run in which, again, *T. pyriformis* fed on washed *E. coli*, but samples were taken at about 1 h intervals rather than at 2 to 3 h intervals as in Fig. 1. The N_T versus time data of Fig. 2 show a reduction in the fission rate between 11 and 13 h and this is mirrored by an increase in $\bar{v}_T$ during the same period. Finally, the apparent reductions in the fission rate shown in Figs 1 and 2 occur at times when the population density has doubled and this, again, is consistent with the notion that fission was partially synchronized. It is true that the flattening of the N_T versus time curves during the periods between presumed episodes of fission of *T. pyriformis* is not as pronounced as that which can be obtained by some other techniques of synchronization (see, for example, Zeuthen & Rasmussen, 1972), but we believe that the total evidence available warrants the conclusion that fission was partially synchronous when *T. pyriformis* grew on washed *E. coli*.

Figure 1(b) shows the time courses of the densities of particles which were consumed by the protozoa. Of greatest interest is the levelling-off of all three curves (N_E , N_{sp} and N_{usp} versus time) after about 20 h. This appears to be the phenomenon found by Habte & Alexander (1978), and additional experiments described below show that such was the case.

A second interesting feature of Fig. 1(b) is the consistently more negative slope of the N_E versus time curve compared with that of the N_{usp} versus time curve. This suggests that *T. pyriformis* might have been feeding selectively and taking up living *E. coli* in preference to other particles of the same size range. For, if there were no selectivity, that is, if *T. pyriformis* took up particles at rates that were in the same ratio as their densities in the culture, and if there were no processes that produced unidentified small particles, then the slopes of the N_E versus time and the N_{usp} versus time curves would be the same when plotted on semi-logarithmic paper. In fact, the derivatives $-d(\log N_E)/dt$ and $-d(\log N_{usp})/dt$ are in the ratio 2.3:1 at 10.4 h, when $N_E = N_{usp}$. The data do not prove that feeding was selective, for the same results could have been produced if unidentified small particles were produced during the course of the experiment. We cannot rule out this possibility.

The time courses of N_T , N_{sp} and $\bar{v}_T$ for an experiment using washed *E. coli* and a higher initial value of N_{sp} (5.80×10^8 particles ml^{-1}) are shown in Fig. 2. Time courses of N_E and N_{usp} are not shown because plate counts of *E. coli* were only made at the beginning and end of this experiment. The maximum *T. pyriformis* density achieved corresponded very closely to three doublings but an apparent interruption in the slope of the N_T versus time curve only occurred once, after the population had doubled once. Peaks appeared in the curve of $\bar{v}_T$ versus time at 7 h, when the first episode of division began, and again at about 11.5 h, when the second episode began. No peak was apparent at 14 to 15 h, when the third episode must

Fig. 2. Time courses of N_T ($\square$), N_{sp} ($\circ$) and $\bar{v}_T$ ($\triangle$) (defined in the legend to Fig. 1) in an experiment using washed *E. coli* at high initial density.

have been starting. Possibly, the second and third episodes overlap almost completely, or perhaps the hourly sampling interval was still too coarse. The asymptotic value of N_E , the *E. coli* density in the stationary phase, as determined by plate count, was 2.3×10^5 cells ml^{-1} .

Experiments using unwashed *E. coli*

Figure 3 shows the time courses of N_T , N_{sp} and $\bar{v}_T$ for an experiment in which the initial density of small particles was large (5.4×10^8 particles ml^{-1}). Of greatest interest here is the initial decrease in *T. pyriformis* density. This must represent lysis as well as death of the protozoa, for the density was determined by Coulter count. The data show that about half of the initial population died and lysed. Comparison of Fig. 3 with Fig. 2, where the initial density of small particles was nearly the same, suggests that the decrease in *T. pyriformis* density shown in Fig. 3 was caused by the presence of *E. coli* metabolites in the medium in which *E. coli* were suspended, although the mechanism involved is not clear. The minimum in the curve of $\bar{v}_T$ versus time which occurs at 12 to 14 h in Fig. 3 suggests that a reduction in fission rate occurred shortly after 14 h, but no reduction is apparent in the N_T versus time curve.

Figure 4 shows time courses of N_T , N_{sp} and $\bar{v}_T$ for an experiment in which an intermediate initial *E. coli* density (3.2×10^8 particles ml^{-1}) was used. No initial decrease in *T. pyriformis* density was evident during this run, nor in one using unwashed *E. coli* at an initial density of 4.3×10^8 particles ml^{-1} (data not shown). In the experiment shown in Fig. 4, the *T. pyriformis* density increased by a factor of about 5 between 9 and 16 h, but there is no clear evidence of reductions in the rate of fission and the rate of decrease in mean cell volume during this period.

Factors of increase of *T. pyriformis*

If $N_{T,\max}$ and $N_{T,0}$ are the maximum and initial densities of *T. pyriformis* in a run, then the ratio $N_{T,\max}/N_{T,0}$ is the factor of increase of *T. pyriformis* during the run and

$$G = \log_2(N_{T,\max}/N_{T,0}) \quad (1)$$

is the number of generations which intervene between an average cell of the culture at the end of the reproductive phase and its ancestor cell in the inoculum. The quantity G cannot be so interpreted if some of the cells die during the run so we apply equation (1) only to runs in which there was no initial decrease in *T. pyriformis* density.

Fig. 3

Fig. 4

Fig. 3. Time courses of N_T (■), N_{sp} (●) and $\bar{v}_T$ (▲) (defined in the legend to Fig. 1) in an experiment using unwashed *E. coli* at high initial density.

Fig. 4. Time courses of N_T (■), N_{sp} (●) and $\bar{v}_T$ (▲) (defined in the legend to Fig. 1) in an experiment using unwashed *E. coli* at an intermediate initial density.

Fig. 5

Fig. 6

Fig. 5. Effect of the initial density of small particles ($N_{sp,0}$) on the factor increase of *T. pyriformis* ($N_{T,max}/N_{T,0}$, where $N_{T,max}$ is the maximum density of *T. pyriformis* achieved in an experiment and $N_{T,0}$ is the initial density) in experiments with washed (□) and unwashed (■) *E. coli*.

Fig. 6. Effect of $N_{sp,0}$ on $nN_{T,0}$ (where n is defined by equation 2 and $N_{sp,0}$ and $N_{T,0}$ are defined in the legend to Fig. 5) in experiments with washed (□) and unwashed (■) *E. coli*.

Figure 5 shows the effect of change in initial *E. coli* density on the factors of increase for runs using both washed and unwashed *E. coli*. There is no obvious difference between the results of the two kinds of experiments, and the factor of increase can be taken to be dependent only on the size of the initial food supply.

We have drawn a smooth curve having inflection points at initial small particle densities of about 3×10^8 and 5×10^8 ml⁻¹ through the data points. This suggests that most *T. pyriformis* cells present at the time of their maximum density arose from one division of their ancestor ($G \approx 1$) if the initial density of small particles was between 1×10^8 and 3×10^8 ml⁻¹,

from two such divisions ($G \approx 2$) if the initial density was between 3×10^8 and 5×10^8 ml⁻¹, etc. A curve without inflection points could have been drawn through the data points and no doubt some would argue that that is what should have been done. We have made no detailed statistical comparison of the curve shown with a curve lacking inflection points, but it is qualitatively evident that a curve lacking inflection points will produce markedly greater deviations of the points from the curve near $N_{sp,0} = 3 \times 10^8$ particles ml⁻¹ than at other values of $N_{sp,0}$. This feature is also present in the curve we have drawn, but to a lesser degree. Since there is no *a priori* reason to suppose that the data points are more scattered near some values of $N_{sp,0}$ than near others, and also because we want to emphasize that there might be a most interesting and unusual phenomenon here, we have drawn the curve with inflection points.

The data of Fig. 5 can be replotted to show the effect of the initial density of small particles on the number of such particles consumed in the formation of an average protozoan cell during the lag and reproductive phases of the culture. If this number be denoted by n , then

$$n = \frac{N_{sp,0} - N_{sp,Tm}}{N_{T,max} - N_{T,0}} \simeq \frac{N_{sp,0}}{N_{T,0} [(N_{T,max}/N_{T,0}) - 1]} \quad (2)$$

where $N_{sp,Tm}$, the density of small particles at the time when $N_T = N_{T,max}$, was negligible in comparison with $N_{sp,0}$ in all runs.

Figure 6 is a plot of $nN_{T,0}$ versus $N_{sp,0}$. The ordinate was chosen to be $nN_{T,0}$ rather than n so that the units of ordinate and abscissa would both be (small) particles ml⁻¹. A graph of n versus $N_{sp,0}$ would simply involve a change of the ordinate scale, since $N_{T,0}$ was nearly constant at 4.3×10^3 cells ml⁻¹ in all runs.

The most evident feature of Fig. 6 is the scatter of the data. Some of this scatter is certainly due to errors in the density measurements used to calculate $nN_{T,0}$. An uncertainty analysis making what appear to be liberal estimates of uncertainties ($\pm 10\%$ in $N_{sp,0}$ and $N_{T,0}$; $\pm 25\%$ in $N_{T,max}$) produces estimates of uncertainty in $nN_{T,0}$ which are in rough agreement with the scatter of the data at $N_{sp,0}$ values from 1×10^8 to 2×10^8 particles ml⁻¹ but which are only about one-third as great as the observed scatter at $N_{sp,0}$ values from 2×10^8 to 5×10^8 particles ml⁻¹. Hence, the scatter seems to have some origin in addition to errors in density measurement.

A second feature of Fig. 6 is that there are no values of $nN_{T,0}$ less than about 7×10^7 particles ml⁻¹ so that there are no values of n less than about 1.6×10^4 particles per *T. pyriformis* cell. There is thus a minimum particle requirement for forming a new predator cell and this is about 1.6×10^4 particles with the *E. coli* used.

A third feature of Fig. 6 is that the values of $nN_{T,0}$ tend to converge towards the minimum value of 7×10^7 particles ml⁻¹, so the values of n tend to converge toward the minimum value of 1.6×10^4 particles per *T. pyriformis* cell, as the initial bacterial density is increased. This appears to be the same phenomenon as observed by Salt (1967, 1968, 1974) in his studies of predation by species of *Woodruffia*, *Amoeba* and *Didinium*; namely, that the number of prey consumed per predator per generation decreases as predator density increases.

Cessation of predation

Some experiments were done to test the reality of the phenomenon reported by Habte & Alexander (1978). A number of batch cultures having the same initial densities of *T. pyriformis* and *E. coli* were started, and washed *E. coli* were added to these at different times after a stationary, low bacterial density had been achieved. Densities of *T. pyriformis* and viable *E. coli* were followed throughout the whole course of the batch culture, as before. Addition of *E. coli* to cultures did not dilute them or add any nutrients (see Methods).

Results of these experiments are shown in Fig. 7. The data points for the run on the control batch to which no *E. coli* were added after constant bacterial density had been achieved have

Fig. 7. Effects of addition of washed *E. coli* to declining *T. pyriformis* cultures. The time courses of N_T (---) and N_E (---) after additions of *E. coli* are shown. The data points on time courses for the control culture (—) have been omitted for clarity.

been omitted for clarity (however, the control run was one of those whose results are plotted in Fig. 1, and the data points for the early part of the run are given there). The results show that a substantial fraction of the *T. pyriformis* cells were still viable even after 80 h culture (55 h after cessation of feeding), so that cessation of feeding cannot be explained by loss of protozoan viability. Since no nutrients were introduced with the bacteria, exhaustion of some essential nutrient that the protozoa absorb from the medium cannot explain cessation of feeding. Since no water was added with the bacteria, dilution of some inhibitory substance in the culture did not occur, and the presence of such a substance can also be ruled out as an explanation for cessation of feeding. Hence, these results confirm the validity of the observations made by Habte & Alexander (1978).

Two additional experiments were done in which fresh *T. pyriformis* and *E. coli* from the steady state chemostat were added to batch cultures at 46.5 h. Enough *T. pyriformis* were added to raise their density to $2.3 \times 10^4 \text{ ml}^{-1}$; the *E. coli* added raised their viable count from 1.1×10^5 to $1.8 \times 10^5 \text{ ml}^{-1}$. No reproduction of *T. pyriformis* followed these additions, and feeding was just sufficient to reduce the density of viable *E. coli* to the initial level of $1.1 \times 10^5 \text{ cells ml}^{-1}$ over a period of about 10 h. These results provide additional evidence that cessation of feeding is not due to loss of protozoan viability, and they also show that feeding *T. pyriformis* quickly adapts to low bacterial density and ceases feeding.

Microscopic observations made on the culture showed that *T. pyriformis* cells behave differently when they are feeding from when they are not feeding. During the initial stages of a batch culture, before division has commenced, the cells are large and turn frequently but their motion is such that during a given period they do not move very far from their initial location. However, after feeding has stopped, the cells are small and they move rapidly in straight lines, so that during a given period they move quite far from their initial location. These observations are consistent with recent quantitative observations on swimming rates of *Didinium nasutum* cells made by Salt (1979), in which, under conditions of high *Didinium nasutum* density, the swimming rate of starved cells was significantly higher than that of well fed cells.

Effects of E. coli metabolites on growth of T. pyriformis

Figure 3 shows the initial decrease in *T. pyriformis* density that occurred when the ciliates were introduced into a dense suspension of unwashed *E. coli*. Such decreases occurred in

The tendency of the number of bacteria consumed in the formation of a new ciliate cell to decrease as initial bacterial density is increased appears to be identical with the phenomenon observed by others that the number of prey consumed per predator per generation decreases as the predator density increases. The ecological consequences of this phenomenon (or these phenomena) are not clear.

Our results suggest, although they do not prove beyond reasonable doubt, that reproduction of *T. pyriformis* feeding on washed *E. coli* is partially synchronous, that *T. pyriformis* takes up viable *E. coli* in preference to non-viable *E. coli* and other non-living particles of the *E. coli* size range, and that the curve of the factor of increase of *T. pyriformis* versus initial food availability (Fig. 5) has inflection points. It is well known that fission of *T. pyriformis* can be synchronized (Zeuthen & Rasmussen, 1972), and Dive (1973) has reported that the ciliate *Colpidium campylum* fed selectively on Gram-negative bacteria when presented with a mixture of Gram-negative and Gram-positive bacteria. These observations may render our suggestions that synchrony and food preference were observed in the present experiments somewhat less implausible. However, we know of no other reports of phenomena like that exhibited by the curve in Fig. 5.

This work was supported by the National Science Foundation, grant no. NSF/ENG77-21632.

REFERENCES

- BADER, F. G., TSUCHIYA, H. M. & FREDRICKSON, A. G. (1976). Grazing of ciliates on blue-green algae. Effects of ciliate encystment and related phenomena. *Biotechnology and Bioengineering* **18**, 311–332.
- BERK, S. G., COLWELL, R. R. & SMALL, E. B. (1976). A study of feeding responses to bacterial prey by estuarine ciliates. *Transactions of the American Microscopical Society* **95**, 514–520.
- CORLISS, J. O. (1970). The comparative systematics of species comprising the hymenostome ciliate genus *Tetrahymena*. *Journal of Protozoology* **17**, 198–209.
- CURDS, C. R. & VANDYKE, J. M. (1966). The feeding habits and growth rates of some fresh-water ciliates found in activated-sludge plants. *Journal of Applied Ecology* **3**, 127–137.
- DANSO, S. K. A. & ALEXANDER, M. (1975). Regulation of predation by prey density: the protozoan–*Rhizobium* relationship. *Applied Microbiology* **29**, 515–521.
- DIVE, D. (1973). Nutrition holozoïque de *Colpidium campylum*. Phénomènes de sélection et d'antagonisme avec les bactéries. *Water Research* **7**, 695–706.
- DIVE, D. (1975). Influence de la concentration bactérienne sur la croissance de *Colpidium campylum*. *Journal of Protozoology* **22**, 545–550.
- DRAKE, J. F. & TSUCHIYA, H. M. (1976). Predation on *Escherichia coli* by *Colpoda steinii*. *Applied and Environmental Microbiology* **31**, 870–874.
- FREDRICKSON, A. G. (1977). Behavior of mixed cultures of microorganisms. *Annual Review of Microbiology* **31**, 63–87.
- GAUSE, G. F. (1934). *The Struggle for Existence*, ch. 7. Baltimore: Williams & Wilkins.
- HABTE, M. & ALEXANDER, M. (1978). Protozoan density and the coexistence of protozoan predators and bacterial prey. *Ecology* **59**, 140–146.
- JOST, J. L., DRAKE, J. F., FREDRICKSON, A. G. & TSUCHIYA, H. M. (1973). Interactions of *Tetrahymena pyriformis*, *Escherichia coli*, *Azotobacter vinelandii*, and glucose in a minimal medium. *Journal of Bacteriology* **113**, 834–840.
- NOVICK, A. & SZILARD, L. (1950). Description of the chemostat. *Science* **112**, 715–716.
- SALT, G. W. (1967). Predation in a experimental protozoan population (*Woodruffia-Paramecium*). *Ecological Monographs* **37**, 113–144.
- SALT, G. W. (1968). The feeding of *Amoeba proteus* on *Paramecium aurelia*. *Journal of Protozoology* **15**, 275–280.
- SALT, G. W. (1974). Predator and prey densities as controls of the rate of capture by the predator *Didinium nasutum*. *Ecology* **55**, 434–439.
- SALT, G. W. (1975). Changes in the cell volume of *Didinium nasutum* during population increase. *Journal of Protozoology* **22**, 112–115.
- SALT, G. W. (1979). Density, starvation, and swimming rate in *Didinium* populations. *American Naturalist* **113**, 135–143.
- TAYLOR, W. D. (1978). Growth responses of ciliate protozoa to the abundance of their bacterial prey. *Microbial Ecology* **4**, 207–214.
- ZEUTHEN, E. & RASMUSSEN, L. (1972). Synchronized cell division in protozoa. In *Research in Protozoology*, vol. 4, pp. 9–145. Edited by T. T. Chen. Oxford: Pergamon Press.