Long-term Studies of Ciliate–Bacterial Interactions: Use of a Chemostat Fed with Bacteria Grown in a Separate Chemostat

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Two-stage chemostat cascades have been used to study growth of the ciliate *Tetrahymena pyriformis* on *Escherichia coli* over extended periods of time. Upward drifts (continuous, slow, uni-directional changes) of ciliate number density and downward drifts of ciliate mean cell size and density of residual bacteria occurred for many generations of ciliates before steady states were obtained. Ciliate chemostats were operated at two different dilution rates. Rates of drift were slower, and drifts persisted longer, both in terms of time and in terms of numbers of generations, in the chemostats operated at the higher dilution rate. Some speculations on the origins of the drifts are presented.

INTRODUCTION

One of the potentially useful assemblies for the study of ciliate–bacterial interactions is the two-stage cascade apparatus used in many experiments reported in the literature (e.g. Curds & Cockburn, 1971; Jost *et al.*, 1973; Ashby, 1976; Drake & Tsuchiya, 1977; Swift *et al.*, 1982). The upper chemostat of this assembly contains a pure bacterial culture, and it is operated at a dilution rate that is sufficiently low for the rate-limiting substrate to become essentially completely exhausted. The overflow of the bacterial chemostat feeds the lower chemostat containing the growing ciliates. The original objective in the use of the cascade apparatus was to study the dynamics of ciliate–bacterial interactions under steady-state conditions and without the complicating effects of bacterial growth. However, experimental work by Habte & Alexander (1978) and Sambanis (1985) has shown, the first indirectly and the second directly, that bacterial growth cannot be eliminated. Indeed, in a mixed culture with ciliates, bacteria grow and reproduce on products of lysis and perhaps also metabolism of the ciliates in an interaction termed ‘saprotrophy’ (Sambanis, 1985). In spite of the occurrence of saprotrophy, common sense as well as mathematical models (see, e.g. Sambanis *et al.*, 1987) suggest that the culture in the lower chemostat of a cascade should reach a steady state after some initial transients that last for no more than a week to 10 d. However, experimental results reported in the literature for various pairs of micro-organisms do not agree on whether this actually occurs in practice.

Hamilton & Preslan (1970) grew the ciliate *Uronema* sp. on a supply of non-growing *Serratia marenorubra* at dilution rates (D) ranging from 0.029 to 0.14 h⁻¹. They reported that ‘the ciliates attained a steady state quickly in the chemostat and could, if necessary, be so maintained for weeks.’ Jost *et al.* (1973) studied the interactions of *Tetrahymena pyriformis* and *Azotobacter vinelandii* in the lower chemostats of two cascades operated at $D = 0.04$ h⁻¹ and $D = 0.11$ h⁻¹. At both dilution rates, the population density of the ciliates became constant within

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approximately one week. Swift *et al.* (1982) reported that in the lower chemostats of cascades containing *T. pyriformis* and *Escherichia coli* and operated at dilution rates ranging from 0.02 to 0.20 h⁻¹, the population densities of ciliates and of all particles having roughly the same size as the bacteria did not vary systematically after one week of operation. However, the random changes which occurred were sometimes quite significant. On the other hand, Curds & Cockburn (1971) found that a culture of *T. pyriformis* and *Klebsiella aerogenes* (*K. pneumoniae*) fed with non-growing bacteria and operated at $D = 0.254 \text{ h}^{-1}$ did not reach steady state after 25 d operation. Instead, the various measured quantities exhibited significant unidirectional changes. With the similar system of *T. pyriformis*-*E. coli* interacting in the lower chemostat of a cascade at $D = 0.04 \text{ h}^{-1}$, Glaser (1984) observed slow changes (drifts) of the ciliate population density and mean cell volume and of the density of viable bacteria; the drifts lasted for more than 760 h. During Ashby's (1976) experiment with the system *Uronema marinum*-*Vibrio* sp. at $D = 0.014 \text{ h}^{-1}$, the various measured quantities exhibited cycling variations as well as overall drifts. The culture did not reach steady state even after 100 d operation. Finally, Drake's (1975) data (some of which were also reported in Drake & Tsuchiya, 1977) suggest a correlation which might exist between the occurrence of drifts in the lower chemostat of a cascade and the dilution rate at which that chemostat is operated. Indeed, uni-directional changes seem to have occurred at $D = 0.023 \text{ h}^{-1}$ and $D = 0.060 \text{ h}^{-1}$ but not at $D = 0.125 \text{ h}^{-1}$.

In the above reports of drifts, each of the measured quantities generally changed in the same direction in the various experiments. The ciliate population density increased and the ciliate mean cell volume decreased in all experiments except, perhaps, in the one reported by Ashby (1976). The concentration of ciliate biovolume (that is, the fraction of the culture volume occupied by the ciliate cells) remained constant or nearly constant in all experiments except that reported by Curds & Cockburn (1971), in which the ciliate biovolume increased at an essentially constant rate until the termination of the culture after 25 d operation. The bacterial density in the ciliate chemostats generally decreased with time. Drake's (1975) experiments, however, revealed no unidirectional change of that quantity at any dilution rate.

In some of the experiments reported above, low-amplitude, long-period oscillations might have occurred as well. The overall changes of the measured quantities were, of course, the previously described long-term drifts. Thus, the cycling variations observed by Ashby (1976) could actually represent oscillations of either constant or slowly decreasing amplitude. Similarly, Glaser (1984) reported that the number of ciliate cells, the ciliate biovolume and the yield of ciliate biovolume on bacterial numbers seemed to exhibit damped oscillations with a period of 250–300 h and an amplitude equal to about 35% of the mean.

In experiments with the system *T. pyriformis*-*E. coli*, we too observed long-term drifts of the type described above. Some of these drifts persisted for more than 100 generations of ciliates, and produced a roughly twofold reduction in ciliate mean cell volume. We are not aware that similar phenomena occur with prokaryotic micro-organisms, and therefore they seemed worth investigating in more detail. This paper reports the results of the investigation. Its purposes were to (i) see if the drifts were artifacts produced by secular changes in the inputs to the chemostats or in the physical conditions of the chemostats, (ii) obtain an accurate quantitative description of the drifts, (iii) evaluate the effects of chemostat operating conditions on the rate and duration of the drifts, and (iv) conduct a preliminary study of the causes of the cellular and population changes that occur.

METHODS

Organisms. *Tetrahymena pyriformis* GL was supplied by Professor M. Gorovsky, Department of Biology, University of Rochester, NY, USA. This amiconucleate species should now probably be called simply *T. pyriformis* (Nanney & McCoy, 1976). *Escherichia coli* B was provided by Professor J. T. Prince, Department of Microbiology, University of Minnesota. The organisms were maintained as described by Watson *et al.* (1981) except that the *E. coli* slants were stored at 7 °C instead of 9.9 °C.

Apparatus. All six experiments reported here were done with two-stage cascades of chemostats (cascades 1–6, respectively). The chemostats used were patterned after the design of Novick & Szilard (1950). The cultures in the chemostats were maintained at constant temperature by circulating water from a bath maintained at 25 ± 0.2 °C.

Fig. 1. The tips of the overflow lines of the chemostat vessels: (a) the old tip; (b) the new tip. The inside diameter of the capillary tubes is 2 mm.

The lower chemostats of all six cascades had nominal volumes of 100 ml; this ensured constant surface-to-volume ratio and mixing and aeration patterns in the ciliate chemostats. The upper chemostats of cascades 1–4 had nominal volumes of 100 ml and those of cascades 5 and 6 nominal volumes of 400 ml. The overflow siphon of the bacterial chemostat of cascade 1 had the tip shown in Fig. 1 (a) (old tip), whereas the siphons of the same chemostats of cascades 2–6 had the tip shown in Fig. 1 (b) (new tip). The siphons of the ciliate chemostats had the old tip. Each ciliate chemostat was aerated with 1200 ml (STP) atmospheric air h^{-1} , and each 100 ml bacterial chemostat with 2800 ml (STP) h^{-1} . Each 400 ml bacterial chemostat was aerated with 5400 ml (STP) h^{-1} except during the first 50 h or so when the rate was 2800 ml (STP) h^{-1} . Before entering the cultures, the air was humidified and then sterilized by passage through two sterile tubular filters, the first of which contained glass wool and the second cotton wool.

Medium. The constituents of the medium (BC medium) fed to the upper chemostats of the cascades were autoclaved as four separate solutions to prevent any irreversible complexing. The solutions were mixed after they had cooled to room temperature. The composition of each solution (per litre of distilled water in the final medium) was as follows: (i) 0.335 g KH_2PO_4 and 1.77 g K_2HPO_4 ; (ii) 1.25 g $(\text{NH}_4)_2\text{SO}_4$, 0.10 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1 mg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; (iii) 0.01 g NaCl , 0.02 g CaCl_2 , 1 mg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 2.8 mg $\text{Na}_2(\text{EDTA}) \cdot 2\text{H}_2\text{O}$; and (iv) 0.5 g glucose. Each solution was sterilized by autoclaving at 121 °C for 20–60 min, depending on its volume. Before autoclaving, each solution was filtered individually through a 0.45 μm Millipore filter to remove the particles that increased the background count when electronically counting the bacteria and could also act as nuclei for the crystallization of precipitates in the medium.

Analyses. The population density and size distribution of *T. pyriformis* were obtained with an electronic particle counter and size distribution analyser [model 80 XY Electrozone (Elzone) Celloscope, Particle Data Inc.]. The same instrument was used to measure the density and size distribution of small particles (bacteria + non-living particles of bacterial size). A 95 μm aperture was used for the ciliates and a 30 μm aperture for the small particles. The samples were diluted with a saline solution containing 8.5 g NaCl and 0.2 g $\text{Na}_2(\text{EDTA}) \cdot 2\text{H}_2\text{O}$ per litre of distilled water. To reduce the background count, the diluting solution was filtered continuously through a series of two 0.22 μm Ultipore filters (Pall Trinity Micro Corporation) before use. The dilutions were such that the ciliate count was between 6×10^2 and 5×10^3 with a sampling volume of 501.9 μl and the small particle count between 1.5×10^4 and 4×10^4 with a sampling volume of 100 μl . The density of viable *E. coli* was measured by preparing spread plates on tryptone/glucose/yeast extract agar medium after appropriate dilution of the samples with sterile saline. Samples from pure *E. coli* cultures produced equal (within statistical error) Elzone and viable counts, indicating that essentially all plated *E. coli* cells developed into colonies.

Inoculation procedure. The *E. coli* cells used to inoculate the bacterial chemostats were grown in batch culture in a medium very similar to BC (preculture BC medium). Ciliate inocula were taken from two different sources, either adapted to axenic growth or adapted to monoxenic growth on *E. coli*. Each of the ciliate chemostats of cascades 1 and 2 was inoculated with approximately 50 ml *T. pyriformis* preculture in proteose peptone/yeast extract (PPYE) broth, plus approximately 50 ml preculture BC solution without glucose. This solution reduced the foaming of PPYE and initiated an adaptation of *T. pyriformis* to the medium in which *E. coli* was growing. The ciliate chemostats of cascades 3–6 were all inoculated from the same microbial suspension which was prepared as follows. A two-stage cascade, both chemostats of which had nominal volumes of 100 ml, was started with a *T. pyriformis* preculture in PPYE in the second stage and *E. coli* in the first stage. The chemostats were fed with BC medium and operated at $D = 0.03 \text{ h}^{-1}$ for 10 d; then the culture in the lower stage was harvested and diluted with 500 ml BC medium without glucose. The suspension was homogenized and used to fill the lower chemostats of cascades 3–6.

Sampling procedure. To minimize the disturbance of the cultures caused by the withdrawal of samples (approx. 8 ml), all counts and size distributions were each time taken from a single sample and a period of 2–4 d was allowed to elapse before a chemostat was sampled again. The sampling intervals of cascades 3–6 were 2, 3, or 4 d, and each sampling interval of each of these chemostats was chosen at random, with equal probability being assigned to each

of the three choices. This rule also applied to cascade 2 before the increase in medium flow rate; after that, cascade 2 was sampled every 2 d and at the end every 3 d. The lower chemostat of each cascade was sampled first and the upper second. To prevent the sudden discharge of bacterial culture, the overflow siphons of the upper chemostats of cascades 2–6 were broken just before sampling the lower chemostats; they were restored immediately after sampling. That practice was not followed with cascade 1. After the withdrawal of a sample, the initial dilutions for all counts were completed within 4 min or less, and then the counts and size distributions were taken, one at a time, after completing any necessary additional dilutions. Tests (Sambanis, 1985) showed that the counts and size distributions thus obtained were representative of the cultures at the time of sampling.

Development of the experimental technique. Certain aspects of the technique described above were modifications of the techniques used by Watson *et al.* (1981) and Swift *et al.* (1982). Those modifications were made because of problems caused by the existing techniques during initial cascade experiments: (1) after a few (usually 3–4) days of operation of the cascades, macroscopic biofilms and flocs developed in the bacterial chemostats; (2) the *E. coli* density in the bacterial chemostats exhibited a strong downward drift; (3) an iron precipitate developed on the bottom of the medium jugs, and that precipitate failed to enter the chemostats; (4) there was significant scatter in the data on the density of viable bacteria in the ciliate chemostats.

Problems 1, 2 and 3 were found to be associated with aspects of the medium composition. To alleviate them, the medium of Watson *et al.* (1981) was modified as follows: (i) the concentration of glucose was reduced to 0.5 g l^{-1} ; (ii) all iron was added in the ferric instead of the ferrous form; (iii) the amount of $\text{Na}_2(\text{EDTA}) \cdot 2\text{H}_2\text{O}$ was increased to twice that stoichiometrically needed to chelate all the iron. The medium which resulted from these changes is the BC medium described above. Furthermore, to ensure that the bacterial growth rate was limited by glucose, the bacterial chemostats were always operated at a low dilution rate of approximately 0.03 h^{-1} . [An analysis of the effect of the dilution rate on the identity of the rate-limiting substrate is given by Sambanis (1985) and Baltzis & Fredrickson (1987).] After the above changes were made, no macroscopic biofilms or flocs of biomaterial were apparent in the bacterial chemostats. Furthermore, no precipitate was ever observed on the bottom of the medium jugs, and the downward drift of the bacterial density was drastically reduced. For example, in a bacterial chemostat operated at $D = 0.03 \text{ h}^{-1}$, the density of viable *E. coli* fell to 40% of the maximum value after 10 d operation and to 80% of the maximum value after 33 d operation when BC medium contained $\text{Na}_2(\text{EDTA}) \cdot 2\text{H}_2\text{O}$ at 0.4 mg l^{-1} and 2.8 mg l^{-1} respectively.

Problem 4 was found to be associated with the irregular operation of overflow siphons having the tip design shown in Fig. 1 (a) (Tunkel, 1983). In particular, those siphons would deliver as many as 15 to 20 drops at a time – corresponding to about 1 ml culture – followed by a long period of zero overflow. With the bacterial densities used, it can be calculated that the irregular operation of a siphon would cause the density of viable *E. coli* in the ciliate chemostat to vary by a factor of 10 or more. Thus, if the lower chemostat were sampled at random times relative to the discharge of the overflow, the scatter in the viable counts would be very significant. Such a scatter was reported by Swift *et al.* (1982), and although those authors attributed it to other factors, it seems that in that case also the scatter was caused by the irregular operation of the overflow. The operation of the siphons was improved by adopting the tip design shown in Fig. 1 (b) (suggested by D. P. Lavin – personal communication), which gave a constant rate of discharge. For example, for a 100 ml bacterial chemostat fed with $3.2 \text{ ml BC medium h}^{-1}$, the standard deviation of the intervals between drops was 24% of the mean. The volume of a typical drop remained approximately 0.06 ml, so that the density of viable bacteria in a ciliate chemostat was expected to vary by a factor of 1.6 or less.

RESULTS

Figs 2–4 show the results obtained during experiments with cascades 1–6: the quantities presented are as follows. N_T is the population density of *T. pyriformis* and $\bar{v}_T$ is the mean cell volume of the ciliate cells; $N_T \bar{v}_T$ is thus the concentration of ciliate biovolume. N_{E_2} and N_{sp_2} are the densities of viable *E. coli* and of small particles, respectively, in the ciliate chemostats, and N_{E_1} and N_{sp_1} are the same quantities in the bacterial chemostats. Q is the volumetric flow rate of medium into the cascades, and ΔQ represents a step increase in Q . Table 1 reports the dilution rate at which each chemostat was operated. Arrows f_1 and f_2 in Figs 3 and 4 show the times at which the culture in the lower chemostat of a cascade was harvested and filtered aseptically through a $0.45 \mu\text{m}$ filter. The filters withheld all organisms, and the sterile filtrates thus obtained were inoculated with *E. coli* to measure the amount of bacterial growth supported by each solution.

Fig. 2 shows the results obtained during cascade experiments 1 and 2. To ensure that all data were collected with ciliates well adapted to monoxenic growth, the cascades were operated for at least a week prior to the withdrawal of the first samples. In the bacterial chemostat of cascade 2,

Fig. 2. Results obtained during cascade experiments 1 (filled symbols) and 2 (open symbols).

Table 1. Dilution rates (h^{-1}) at which the upper and lower chemostats of cascades 1–6 were operated

Cascade no.	Upper chemostat	Lower chemostat
1	0.033	0.033
2 before ΔQ	0.034	0.033
2 after ΔQ	0.115	0.109
3	0.036	0.037
4	0.035	0.037
5	0.031	0.116
6	0.032	0.121

N_{E_1} and N_{sp_1} drifted slowly downwards until about 700 h, and then remained constant until the step increase in medium flow rate, ΔQ . After ΔQ , N_{E_1} and N_{sp_1} increased, indicating that maintenance is important to a pure culture of *E. coli* at the low dilution rates used. In the ciliate chemostat of cascade 2 before ΔQ , the measured quantities behaved as follows. Between 170 and 420 h, N_T increased and $\bar{v}_T$ decreased in an essentially linear fashion. After 420 h, both quantities remained nearly constant except for a decrease in N_T around 800 h. $N_T \bar{v}_T$ exhibited a very slow downward drift, apparently as a result of the downward drift of N_{E_1} . N_{E_2} decreased slightly around 350 h, followed by a very slow upward drift. The data on N_{sp_2} revealed no

Fig. 3. Results obtained during cascade experiments 3 (open symbols) and 4 (filled symbols).

unidirectional change; however, they were considerably scattered, possibly because of small-amplitude oscillations having a period of 200–250 h. After ΔQ , N_T decreased and then started to drift upwards whereas $\bar{v}_T$ increased and then started to drift downwards. $N_T \bar{v}_T$ approximately doubled, as did N_{E_2} , but N_{sp_2} was not affected significantly by ΔQ .

The data on N_{E_1} , N_{sp_1} , $N_T \bar{v}_T$ and N_{sp_2} from cascade 1 compared well with those from cascade 2. However, the data on N_T and $\bar{v}_T$ from cascade 1 were shifted to later times by 3 d relative to those from cascade 2. That shift was probably the result of the older (by approximately 3 d) axenic ciliate culture used to inoculate cascade 2 relative to that used to inoculate cascade 1. The values of N_{E_2} measured during cascade experiment 1 were two to three times those measured during cascade experiment 2. That difference was caused by the discharge of bacterial culture which occurred when the ciliate chemostat of cascade 1 was sampled.

During cascade experiment 3 (Fig. 3), N_{E_1} and N_{sp_1} exhibited downward drifts very similar to those seen during cascade experiments 1 and 2. In the ciliate chemostat of cascade 3, the populations underwent initial transients, manifested as initial increases of $\bar{v}_T$, $N_T \bar{v}_T$ and N_{E_2} , that lasted for approximately 80 h. N_T increased until about 400 h and then stabilized at a value smaller than that at the end of the upward drift. $\bar{v}_T$ decreased until 260 h and remained constant thereafter. $N_T \bar{v}_T$ remained essentially constant except for a small decrease around 450 h. N_{E_2} declined until 360 h and remained constant thereafter. The data on N_{sp_2} were significantly scattered, but revealed no unidirectional change. The scatter in the data on N_{sp_2} and $N_T \bar{v}_T$ could have been the result of small-amplitude oscillations.

The data from cascade 4 reproduced well those from cascade 3 during the time that the former cascade was operated. Operation of cascade 4 was terminated at the time indicated by arrow f_1

Table 2. Amounts of *E. coli* growth supported by filtrates F3, F4, F5 and F6

F3 = filtrate from cascade 3, etc. N_{E_i} and N_{E_f} are the population densities of viable bacteria at the beginning and during the stationary phase of each test culture, respectively.

Filtrate	$10^{-2} \times N_{E_i}$ (cells ml ⁻¹)	$10^{-7} \times N_{E_f}$ (cells ml ⁻¹)	Bacterial growth [$10^{-7} \times (N_{E_f} - N_{E_i})$] (cells ml ⁻¹)
F3	6.56	2.82	2.82
F4	7.73	3.49	3.49
F5	7.09	2.85	2.85
F6	8.45	3.40	3.40

Table 3. Mean rates of change (calculated by the least-squares method) of the ciliate population density and mean cell volume during the long-term drifts in three cascade experiments

Cascade expt	Ciliate inoculum adapted or non-adapted to monoxenic growth	Dilution rate of ciliate chemostat (h ⁻¹)	Mean rate of increase of N_T		Mean rate of decrease of $\bar{v}_T$	
			(cells ml ⁻¹ h ⁻¹)	(cells ml ⁻¹ per generation)	(μm^3 h ⁻¹)	(μm^3 per generation)
2	Non-adapted	0.033	170	3570	5.18	109
4	Adapted	0.037	185	3470	4.36	81.7
5	Adapted	0.116	76.7	458	3.59	21.5

in Fig. 3; the culture in the ciliate chemostat was then filtered and the resulting broth (filtrate F4) was tested for its ability to support bacterial growth. The ciliate culture of cascade 3 was filtered at the time indicated by arrow f_2 , yielding filtrate F3. Amounts of bacterial growth supported by the filtrates are reported in Table 2.

Data from cascades 5 and 6 are shown in Fig. 4. In the bacterial chemostat of cascade 5, N_{E_1} and N_{sp_1} exhibited an initial increase but then drifted slowly downwards and later stabilized as in the bacterial chemostats of cascades 1–4. In the ciliate chemostat of cascade 5, $\bar{v}_T$, $N_T\bar{v}_T$, N_{E_2} and N_{sp_2} underwent initial transient increases. The upward drift of N_T and the downward drift of $\bar{v}_T$ lasted until about 600 h. After the initial increase, N_{E_2} exhibited a downward drift until about 500 h; N_{sp_2} also appeared to exhibit a very slow downward drift. $N_T\bar{v}_T$ exhibited apparent sustained oscillations with an amplitude of approximately 20% of the mean and a period of about 300 h.

The data on N_{E_1} , N_{sp_1} , $N_T\bar{v}_T$, $\bar{v}_T$ and N_T from cascade 6 compared well with those from cascade 5. The data on N_{E_2} and N_{sp_2} from the two cascades also compared well, except for the points around 300 h. Those differences, being only temporary, can be considered as of no particular significance.

At the times indicated by arrows f_1 and f_2 in Fig. 4, cascades 6 and 5 were terminated and sterile filtrates F6 and F5, respectively, were prepared. The amounts of *E. coli* growth supported by these filtrates are reported in Table 2.

Table 3 summarizes the mean rates (calculated by the least-squares method) of increase of N_T and decrease of $\bar{v}_T$ during the long-term drifts in the ciliate chemostats of cascades 2, 4 and 5. The time of a generation used to calculate the data was defined as $\ln 2/D$.

DISCUSSION

In all the experiments described above, the cultures in the ciliate chemostats exhibited long drifts but eventually attained steady states. Similarly, the population densities of viable bacteria and small particles in the upper chemostats become essentially constant after a long time period of slow downward drift. This section discusses the changes observed in the chemostats before the establishment of steady states, and discusses and compares the behaviour of the ciliate chemostats under steady-state conditions. Finally, it gives some speculations on the mechanisms that might be producing the observed results.

Fig. 4. Results obtained during cascade experiments 5 (open symbols) and 6 (filled symbols).

Long-term drifts

The downward drifts of bacterial density in the bacterial chemostats of cascades 1–6 lasted for at least 400 h (about 19 generations). These drifts were too small to affect significantly the dynamics of the ciliate chemostats, so they can be ignored in studies of the type reported here. The causes of the downward drifts are not clear, however. Perhaps BC medium deteriorates slowly with time, but a biological factor of the bacterial population may also be involved. Downward drifts of the density of *E. coli* EO2512 (a derivative of strain K12) were also observed by Glaser (1984) in chemostats operated at low dilution rates (about 0.04 h^{-1}). Those drifts were significantly more intense than those reported here, and it seems that their primary cause was the use of the iron-deficient medium of Watson *et al.* (1981), although Glaser attributed them to other factors. The initial increases of N_{E_1} and N_{sp_1} in the bacterial chemostats of cascades 5 and 6 were due either to the maintenance requirement of *E. coli*, to the oxygen limitation of the cultures when the first sets of data points were collected, or to both of those factors.

In the ciliate chemostats, the direction of drift of each measured quantity was the same in all experiments. Thus, N_T increased and $\bar{v}_T$ decreased with time, in agreement with the observations of Curds & Cockburn (1971) and Glaser (1984). In the chemostats operated at low dilution rates, N_T reached its steady-state value after an overshoot. The ciliate biovolume

Fig. 5. Relationship between *T. pyriformis* mean cell volume ($\bar{v}_T$) and the population density of viable *E. coli* (N_{E_2}) as expressed by the data in Figs 2–4. ○, Points from cascade experiment 5; ▲, points from cascade experiment 3; ■, points from cascade experiment 2. The straight line is the least-squares fit of the data from cascade 5.

concentration remained essentially constant, as was observed by Glaser (1984), but not by Curds & Cockburn (1971). In cascade experiments 3–6, N_{E_2} and N_{sp_2} decreased with time; the bacterial density also decreased in the experiments of Curds & Cockburn (1971), Ashby (1976) and Glaser (1984), but not in the experiments of Drake (1975). As seen from Figs 2–4 and Table 3, the rates of change during the drifts and the duration of the drifts depend on the dilution rate, but apparently not on the state of the cells in the ciliate inoculum (ciliates adapted or non-adapted to monoxenic growth). The drifts last longer but the rates of change become smaller as the dilution rate is increased. Thus, the ciliate chemostats of cascades 1–3 reached steady state within 500 h (25 generations) or less, whereas it took 800 h (125 generations) for the ciliate chemostat of cascade 5 to reach steady state. Filtrates of chemostat cultures from cascades 4 and 6, which were collected at times indicated by f_1 in Figs 3 and 4, supported roughly the same amounts of bacterial growth. Similarly, filtrates of chemostat cultures 3 and 5, which were collected at times indicated by f_2 in Figs 3 and 4, supported the same amounts of bacterial growth, but these were substantially less than the amounts supported by broths from cascades 4 and 6. Hence, the amount of bacterial growth supported by the abiotic phase of a ciliate chemostat decreases with time, and does not seem to depend on the dilution rate at which the chemostat is operated.

At a lower trophic level, the equivalent of the decrease in N_{E_2} would be that of a decrease in the concentration of the rate-limiting substrate in a chemostat containing a pure bacterial culture. Indeed, during the long-term continuous cultivation of *Cytophaga johnsonae*, Höfle (1983) observed stepwise decreases of the concentration of glucose (the rate-limiting nutrient). Unfortunately, Höfle did not measure any parameters of the bacterial population other than the concentration of its biomass, so it is not known whether that population underwent changes similar to the ones reported here for the ciliate population.

The results of the short-term experiments of Swift (1981) (some of which were also reported in Swift *et al.*, 1982) indicated the existence of a positive and in fact almost linear correlation between $\bar{v}_T$ and N_{E_2} . The data from the present experiments also indicate the existence of such a correlation (Fig. 5). The points which reveal the correlation best are from cascade experiment 5, whereas the points from the low-dilution-rate experiments 2 and 3 are clustered in the lower left corner of the diagram. What is cause and what is effect here is not clear, but the correlation is certainly consistent with the view that the ciliate population is a more effective scavenger of bacteria when its biomass is distributed among many small cells rather than among a few large cells (Fenchel, 1980).

The facts that (i) long-term drifts were reported by several investigators and for various ciliates and (ii) each measured quantity generally drifted in the same direction in the reported

Table 4. Values of the yield coefficient $X_N [= (N_T \bar{v}_T)/(N_{E_1} - N_{E_2})]$ calculated from the steady-state data of cascade experiments 2, 3 and 5

The reported values of $N_T \bar{v}_T$, N_{E_1} and N_{E_2} are the mean values of these quantities during the time periods considered.

Cascade expt	Dilution rate of ciliate chemostat (h^{-1})	Time period considered	$10^{-8} \times N_T \bar{v}_T$ ($\mu\text{m}^3 \text{ ml}^{-1}$)	$10^{-8} \times N_{E_1}$ (cells ml^{-1})	$10^{-6} \times N_{E_2}$ (cells ml^{-1})	X_N (μm^3 per cell)
2	0.033	650 h– ΔQ	1.603	7.951	3.605	0.20
3	0.037	480–600 h	1.785		2.798	
		450–900 h		9.117		0.20
5	0.116	700–1000 h	2.618		5.357	
		700–950 h		9.590		0.27

experiments, suggest that the long-term drifts are not experimental artifacts caused by such factors as slow deterioration of the medium fed to the cascade, by gradual accumulation of biologically active chemicals unknowingly added with the sparging air or leached from the chemostat vessels, or by slow changes in the conditions of the chemostat walls. Rather, we conclude that the drifts represent the ciliates' response to being placed in an environment with which they are not quite in biological equilibrium.

The observed changes in the ciliate population seem to be physiological rather than genetic in nature. In an attempt to investigate the type of change, Glaser (1984) conducted a number of test batch cultures of *T. pyriformis* on *E. coli* with ciliate and bacterial strains isolated from chemostats after 2230 h operation and with strains from stock cultures. The growth rates, yields, and maximum and minimum sizes reached in batch growth were not significantly different for the chemostat and stock strains, and the author concluded that the drifts he observed were probably not reflections of changes in the genotypes of the ciliates. The results shown in Fig. 2 of the present paper support this conclusion. Disturbance of the steady state by increase of the medium flow rate in cascade 2 led to a fairly rapid increase in the mean volume of the ciliate cells in response to the increased food availability that accompanied the disturbance. When this response was complete, the ciliate population characteristics resumed the drifts they had exhibited in the initial stage of the experiment: downward for $\bar{v}_T$ and upward for N_T .

Steady-state chemostats

At steady state, the densities of viable bacteria in the ciliate chemostats of cascades 1–6 were higher in the chemostats operated at the higher dilution rate. The ciliate density and mean cell volume and the density of small particles also increased with dilution rate but by smaller amounts. According to the short-term experiments of Swift (1981), $\bar{v}_T$, N_{E_2} and N_{sp_2} correlate positively but N_T negatively with D . The discord between the present results and those of Swift (1981) must be due to the fact that the latter data were collected before steady states were reached.

The yields of ciliate biovolume on bacterial numbers, X_N and on bacterial biovolume, X_V , are defined as follows:

$$X_N = \frac{N_T \bar{v}_T}{N_{E_1} - N_{E_2}} \text{ and } X_V = \frac{N_T \bar{v}_T}{(\bar{v}_E)(N_{E_1} - N_{E_2})}$$

where $\bar{v}_E$ is the mean volume of viable *E. coli* cells. The values of X_N obtained using the steady-state data from cascades 2, 3, and 5 are reported in Table 4. One sees that X_N increases with D , in agreement with the results of Swift *et al.* (1982). As explained in that paper, the positive correlation between X_N and D seems to be due to a higher water content of rapidly growing ciliate cells relative to slowly growing ones and not to a strong maintenance requirement of *T. pyriformis*. This view is also supported by the fact that X_V during experiment 5 was greater than 1, since the average volume of *E. coli* cells in the overflows from the bacterial chemostats was $0.19\text{--}0.22 \mu\text{m}^3$. Values of X_V well above 1 were also reported by Swift *et al.* (1982), especially at high dilution rates.

Speculations

The drift phenomena reported here may be a consequence of the peculiar genetic organization and mode of regulating cell cycle events of ciliates, which have recently been reviewed by Berger (1984). Almost all ciliate cell function (presumably all such function in the case of amiconucleate species like *T. pyriformis*) is controlled by a polygenomic macronucleus. Division of the macronucleus is amitotic, and therefore the distribution of macronuclear DNA between sister cells shows significant random deviations from an even split. Cell mass, like macronuclear DNA, is partitioned between sister cells with some randomness and is strongly correlated with macronuclear DNA content. Thus, variations in macronuclear DNA content and cell mass are introduced continually by divisions of ciliates, and one infers that these variations of phenotype should have some persistence from generation to generation. If such variations are correlated with variations in cell generation time, then the selective pressure of chemostat washout might well produce drifts in cell size of the type reported in this paper. For example, the low food availability in a chemostat might cause smaller cells to reproduce slightly more rapidly than large cells, resulting in selection of the smaller cells. The effects of chemostat dilution rate also seem explicable on such a basis. Decrease of chemostat dilution rate causes a decrease in food availability, and this in turn may be expected to increase the intensity of the selection pressure exerted by the chemostat.

Some results obtained with the ciliate *Didinium nasutum* suggest that the foregoing explanation of drifts may be too simple. Salt (1975) observed that the mean volume of cells in synchronously dividing clones of this organism drifted *upward* in experiments in which food (*Paramecium*) availability was maintained at high levels by periodic replenishments, and no *D. nasutum* cells were removed from the cultures. Clearly, selection cannot be invoked to explain such results, and it seems likely that the principal, even if not the only, effect producing the observed drift was that each cell more than doubled its size during its complete cell cycle. Did the *T. pyriformis* cells during the drifts reported here increase their size by less than a factor of two during their complete cell cycles? We cannot say. Evidently, experiments in which food availability can be held at high levels as well as low ones, and in which selection can be turned on or off, need to be done to resolve these questions.

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