Adaptations to a Feast and Famine Existence in Protozoa

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Introduction

The bioenergetics of protozoa (and of other very small eukaryotes and of prokaryotes) is closely linked to growth and in growing cell populations by far the largest share of power generation is coupled to the synthesis of macromolecules. Motility, which in large organisms is responsible for a substantial part of power generation, requires considerably less than one percent of the energy metabolism of growing protozoa. This is because the power requirement for moving very small objects at a low velocity (typically < 1 mm sec^{-1}) through water is very low while on the other hand weight specific power generation of small organisms is very high. Other cell activities, such as osmoregulation, also require a very small fraction of the energy metabolism. Various considerations show that this must be so; the most convincing evidence derives from extended studies on protozoan growth in batch or continuous cultures which show that the growth efficiency is nearly invariant with growth rate, at around 60 % in aerobic, heterotrophic protozoa. Consequently, power input (as rate of oxygen consumption) is almost linearely proportional to the instantaneous growth rate (Calow, 1977; Curds and Cockburn, 1968, 1971; Fenchel, 1987; Fenchel and Finlay, 1983).

These considerations apply to balanced growth; that is, a state which cell populations approach given constant environmental conditions and which can be approximated in continuous cultures and, for a limited period, in batch cultures. During balanced growth, different measures of population size (cell numbers, biomass, organic carbon, DNA, rate of oxygen consumption, etc) all increase with the same exponential growth rate constant. Different levels of food concentrations yield different growth rate constants, but there is a minimum level of food resource availability below which balanced growth cannot be sustained. Cells will then enter another state often characterised by substantial phenotypical changes with respect to cell physiology, structure and behaviour (Trinci and Thurston, 1976), and usually including a rapid decrease in energy metabolism. Clearly, the concept of "basal metabolic rate", has no meaning in protozoa.

Practically all previous studies on protozoan bioenergetics and growth refer to balanced growth. However, this state probably never occurs in nature or if so then only periodically and for short periods (such as during the initial stages of "blooms"). Rather protozoa lead what Koch (1971) referred to as a "feast and famine existence". In contrast to the laboratory chemostat, natural populations experience a world which is patchy in space and time. The scale of this heterogeneity (which is conveniently measured in terms of generation time or the distance a protozoan can travel during a generation time) varies considerably and it may be more or less predictable. Many species of bacterivorous protozoa depend on local and ephemeral high densities of bacteria which develop in carrion or other organic debris. Protozoa which feed on other protozoa or on photosynthetic cells as food experience fluctuations over time or spatial patchiness with respect to prey density. Such fluctuations may be driven by the prey-predator interaction it-

self and lead to more or less regular oscillations or they may be driven by other biotic or abiotic factors and appear entirely unpredictable. Climatic factors directly or indirectly drive successional patterns of microorganisms which again affect their predators. Finally, parasitic protozoa have adapted to temporal heterogeneity (in terms of the life cycle of the host) and spatial heterogeneity (the problem of infecting another host individual). This paper discusses adaptations to such heterogeneity with respect to food resources and with special reference to energy metabolism.

Responses to Starvation in a Bacterivorous Flagellate

In a protozoon growing with a generation time of, say, four hours and with a net growth efficiency of 60 %, cell carbon will turn over every about six hours. If such cells are suddenly exposed to starvation they could not be expected to survive for much longer than one generation time unless they reduce their metabolic rate immediately. This has also proven to occur in most of the free-living species studied so far. An example is shown in Fig. 1. When exposed to starvation, the bacterivorous, 7–8 µm large, limnic flagellate, *Ochromonas* sp, rapidly undergoes a number of phenotypical changes. Some of these are less central to the discussion here. Thus the cells undergo one cell division withouth growth, producing cells half as large as the exponentially growing cells. This may be considered an adaptation to spatial heterogeneity in that it increases the probability that one of the daughter cells will find a new suitable patch. This increases the Darwinian fitness of the mother cell provided that the survival probabilities of the daughter cells are higher than half of that of a non-dividing starving cell. The starving cells also increase their motility. Simultanously, oxygen uptake decreases rapidly and eventually reaches a level of about 5 % of that of growing cells. This explains why

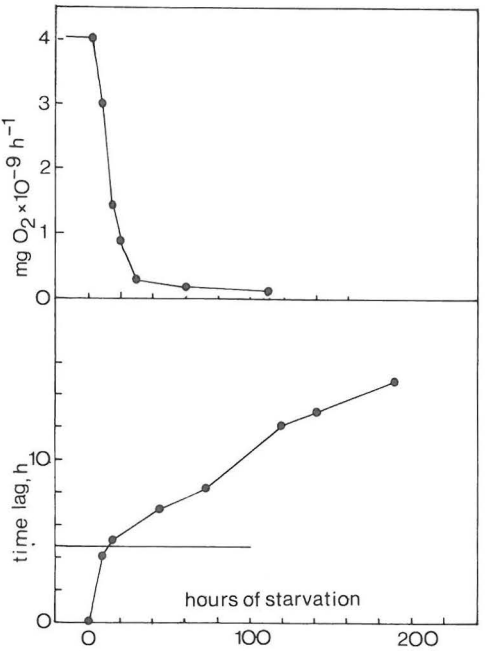

Fig. 1 Above: Oxygen consumption per cell of the flagellate *Ochromonas* sp as a function of time of starvation following exponential growth with* a generation time of about 4.5 h. Below: Time lag between re-feeding and initiation of cell divisions as a function of time of starvation. The generation time of the previous growth period is indicated by the horizontal line (Data from Fenchel, 1982).

starving cells may survive for nearly 200 hours corresponding to more than 40 generations during growth (Fenchel, 1982).

Electron microscopy shows a variety of changes in cell structure of the starved cells relative to growing ones. Among the most obvious features are vacuoles containing partly digested mitochondria. During about 80 hours of starvation the total mitochondrial volume per cell decreases by a factor of nearly ten (Fenchel, 1982). Finlay et al. (1983) found for the same organism that the content of dehydrogenases (assumed to be associated with respiration) decreases by a factor of 2-3. Thus neither the decrease in respiratory enzymes nor in mitochondrial

volume would seem sufficient to explain the measured reduction in oxygen uptake; rather it seems as if the organism maintains a certain potential for increasing the energy metabolism quickly, should food become available.

When a bacterial suspension is added to starving *Ochromonas* cells they immediately feed and increase in cell volume. The rate at which oxygen consumption increases, however, depends on the length of the starvation period. Cells starved for 25 hours reach the rate characteristic for growing cells after only one to two hours while this takes about six hours, or more than the duration of a generation, for cells starved for 100 hours. Similarly, the time lag between feeding and the first cell divisions increases with increasing duration of the starvation period. After 20 hours the time lag is about five hours (about one generation time). Longer time lags reflect that not only has the rate of enzyme synthesis been strongly reduced, but also that the machinery for macromolecular synthesis and for energy metabolism has in part been dismantled (Koch, 1971). After about 200 hours of starvation, time lag for cell division corresponds to the duration of about three generations, or 15 hours (Fenchel, 1982).

These observations may be interpreted as the outcome of an evolutionary compromise between survival during starvation and the ability to resume growth once food resources become available. One way to support this idea is to make comparative studies on the responses to fluctuating food resources for different species and, if possible, relate this to their ecology.

Diversity in Responses to Starvation in some Protozoa

While the literature contains observations on starvation induced changes in phenotype (including encystation) and on post starvation divisions, very few studies on the bioenergetics of starvation and non-balanced growth in protozoa exist. Most attention has been given to the ciliate, *Tetrahymena pyriformis*, which in many respects seems to resemble what has been described above including the formation of quickly swimming swarmer cells, a reduction in energy metabolism and autophagy of mitochondria following starvation (Cameron, 1973; Finlay et al., 1983; Nilsson, 1970).

I have recently studied three species of ciliates in this respect (unpublished observations); together they reflect some of the diversity amongst protozoa in coping with a heterogeneous world. *Uronema marinum* Dujardin is a small (ca. 30 µm long) holotrich ciliate. It occurs in marine environments, particularly on the surfaces of sediments and among detrital material and it is adapted to exploit dense patches of bacteria associated with decaying organic material. It is thus easily isolated from samples of marine material by adding a bacterial suspension or some decaying organic material. Under suitable conditions it will multiply with a generation time of about four hours at 20 °C. In nature, a single or a few cells guided by chemosensory behaviour, find patches of food. They grow for several generations until the food supply has been exhausted or the patch is destroyed for some other reason; the cells are now faced with starvation and will have to find a new patch.

When exponentially growing cells are suddenly starved, the otherwise sluggish ciliates immediately increase their motility and they undergo on the average two successive cell divisions to form small, but very lively swarmer cells ("theronts"). Simultanously, metabolic rate is strongly reduced like in *Ochromonas* and the swarmers will survive (and remain viable) for about 160 hours, corresponding to the duration of 40 generations. Clearly the organism is adapted to spatial heterogeneity and the absence of re-

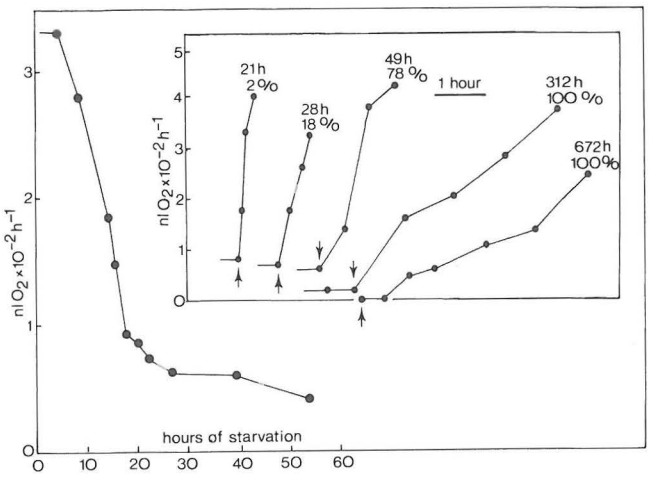

Fig. 2 Oxygen consumption per cell of *Pseudocohnilembus pusillus* during the first 55 hours of starvation following exponential growth with a generation time of 4 hours. Encystation takes place between 20 and 50 hours of starvation. Insert: Increase in oxygen uptake per cell following addition of heat-killed bacteria to starved cells (indicated by arrows). For each experiment the time of starvation and the percentage of cysts are indicated. Respiration was measured with an oxygen electrode in 20 ml suspensions with typically around 10^4 cells per ml (Fenchel, unpublished data).

sting cysts probably reflects the significance of dispersal for the colonisation of new patches.

The somewhat related and similarly sized species, *Pseudocohnilembus pusillus* (Quennerstedt) also depends on bacterial patches on or in sediments as a food resource. However, its response to starvation (and its life cycle) is different and more complicated than that of *Uronema*. When exposed to starvation it does not undergo additional cell divisions. Its respiratory rate is initially reduced by a factor of about four (Fig. 2). The cells also increase their motility. If fed within about 20 hours they can attain the respiratory rate characteristic of growing cells within 15-20 minutes and are capable of dividing within two to three hours. If they do not find food most cells will encyst during the following ten hours. (Whether a cell encysts seems to depend on the stage of the growth cycle at the moment the culture was starved. Initially small, and thus presumably newly divided cells generally fail to encyst and swim on for another 10-20 hours before succumbing to starvation; till then, however, they remain viable.) Young cysts have a respiratory rate which is 10-15 % of that of growing cells, decreasing gradually to about 2-4 % after 300 hours (Fig. 2). The oxygen uptake of 500 hours old cysts was not measurable. However, the cysts probably maintain a very low metabolic rate since their response to the presence of bacteria (or of certain dissolved organics) in the water changes over several months. When food is added, very young cysts excyst within 10-30 minutes and are capable of dividing after two to three hours. Thereafter the time taken to excyst as well as the time lag for cell division increases roughly linearly with age. Three month old cysts take about 24 hours after exposure to a bacterial suspension to excyst and another twenty hours of feeding before they are capable of dividing. The cysts retain a nearly 100 % viability for the first four months and at least some are capable of excysting and to found new clones even after nine months.

Pseudocohnilembus illustrates the opposing fitness components involved in responses to starvation. By postponing encystment and maintaining a relatively high metabolic rate for several hours the ciliates are able to exploit spatial heterogeneity and if successful to resume growth quickly. Since during this phase the ciliates will, on average, travel 20-30 cm this behaviour also serves to spread

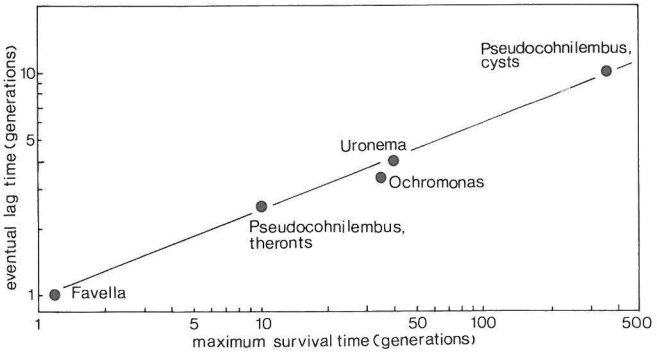

Fig. 3 The relation between the time of survival and the time lag for cell division following re-feeding, in four species of protozoa. Time is scaled by the minimum generation times of exponentially growing populations (Fenchel, unpublished data).

the cysts should they fail to find food. The cysts, which eventually have a very low metabolic rate and so may survive for a long time, represent an adaptation to temporal heterogeneity in the occurrence of food resources. Since the time lag for growth increases with time the fitness of the individual cyst gradually declines.

The planktonic tintinnid ciliate, *Favella ehrenbergi* (Claparede and Lachmann), represents another extreme. In laboratory cultures the ciliate will grow with a generation time of about 18 hours when fed with the dinoflagellate, *Heterocapsa*, at 20 °C. When suddenly starved, the cells survive at most for about 24 hours which is little more than the duration of one generation. During the starvation period the cells undergo some morphological changes, the most obvious one being that the volume decreases so that eventually the cells appear to consist of only a nucleus and the ciliary organelles used for swimming and filtering of food particles. However, when the starving cell are fed, the time lag for cell divisions never exceeds one generation time. Therefore, it seems that when starved, this organism maintains the entire synthetic machinery and presumably a relatively high metabolic rate. The cost of this is a short period of survival, but during this period the cells are capable of quickly resuming growth. Under some circumstances *Favella* may form resting cysts. These have been found in field samples (Fenchel, 1987) and probably enhance survival during the winter season when phytoplankton densities are low and induction of encystation requires special cues.

It is not known whether *Favella* is typical for other plankton protozoa. It may be speculated that turbulent mixing of the surface waters of the sea, which constitute the habitat of *Favella*, ensures the experience of a homogeneous environment. The adaptations described for the other species would therefore not confer any adaptive value on the planktonic ciliate.

Fig. 3 summarises the relationship between survival during starvation (the ability to rapidly reduce metabolic rate and to dismantle the metabolic and synthetic machinery of the cell) and the time lag required for resuming cell division. The graph is somewhat incomplete in that time lag for growth is age dependent. In the graph the time lag characteristic for a starvation age close to the maximum is shown, but at any time during starvation periods (measured in terms of generation times) the species which survive the longest also have the longest lag time for growth. The graph demonstrates in a general way the trade off between survival and competitive ability once food becomes available.

Perspectives

From an ecological point of view the study of the responses to environmental heterogeneity and their adaptive significance is primarily of interest because it contributes to the understanding of the diversity of species in nature. Predictable and unpredictable heterogeneity in time and space is part of the niche of organisms and different species may exploit or cope with this situation in different ways. In this context bioenergetics is, of course, only one among several aspects to consider and it cannot be isolated from aspects such as growth rate, behaviour, motility and the ability of dispersal.

From the point of view of the cell physiologist interesting questions relate to how the cells regulate energy metabolism and macromolecular synthesis and to the energetic costs of maintaining a potential for rapidly increasing these processes. It would seem that such studies may gain from the diversity of adaptations in different species in addition to the detailed study of a few cell models.

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