Sequential Changes in Cell Volume Distribution During Vitamin B₁₂ Starvation of Euglena gracilis

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During vitamin B₁₂ starvation of Euglena, a new peak appears in the cell volume distribution. Some cells are inhibited at a unique point in the cell cycle between the initiation of DNA synthesis and nuclear division. The mechanism of inhibition of other cells differs.

The absolute requirement of Euglena gracilis for submicrogram quantities of vitamin B₁₂ is well known. Unlike other organisms, the need of Euglena for vitamin B₁₂ is not spared by end products of its biosynthetic reactions (3). It has been shown that B₁₂ deficiency in E. gracilis results in cell gigantism and prolongation of generation time; depletion also causes an increase in protein and RNA synthesis without concomitant DNA replication and mitosis. There have been no reports of the effects of B₁₂ depletion on the distribution of cell volumes in the population for lack of accurate methods.

Figure 1 shows that in a culture starved of vitamin B₁₂ the cell numbers increased initially with a doubling time twice that of the controls, but reached a plateau after 90 to 100 h of starvation as did the total cell volume (i.e., counts × mean cell volume). In the control experiments, the total volume of cellular material increased exponentially at the same rate as cell number.

Figure 2 shows the cell volume distributions during B₁₂ starvation. The top distribution (0-h starvation) is representative of steady-state cultures of E. gracilis in minimal medium (4, 6). During starvation there was a marked increase in the average size of cells and the progressive formation of two size classes. By 100 h, a secondary mode of larger cells had developed, while the number of cells (Fig. 1) was still increasing. The bimodal distribution is typical of late stages in B₁₂ starvation.

In control cultures, the amounts of protein, RNA, DNA, and cell number per milliliter (5) increased at the same absolute exponential rate; the mean volume remained constant, showing steady-state growth. In vitamin-starved cultures, protein increased more rapidly than did RNA, DNA, or cell number and attained 10 times the original concentration (milligrams per milliliter of culture) after 240 h of starvation. This corresponds to about a threefold increase per cell. RNA increased about eightfold, corresponding to a doubling of cell concentration. DNA increased about fivefold, approaching, but not reaching, a doubling per cell.

Microscopic observations were made of Euglena cells that had been starved of vitamin B₁₂ for at least 100 h. Hematoxylin-stained cells as well as living cells viewed with Nomarski optics showed less than 1% binucleate cells.

Like many microorganisms, the normal life cycle of a single Euglena is very simple. Small cells grow into large cells, which then divide into two new small cells. Changes in cell volume during the cycle can therefore be correlated with growth and age. The growth of an entire population, on the other hand, involves increases in both numbers and sizes of individual cells. For the special case of a culture in balanced growth, the distribution of cell sizes (and therefore the mean) is invariant, so growth is proportional to the rate of increase in cell number. When the steady state is perturbed, the change in mean cell volume must be considered as well. In a vitamin B₁₂-starved culture, the increase in total volume of cellular material (Fig. 1), which indicates the overall synthetic capability of the cells, is much faster than the rate of increase in cell number.

It was reported previously (4) that there was a dramatic decrease in the mean cell volume of E. gracilis during glutamic acid starvation. Analysis of the cell volume distribution (E. S. Kempner and A. G. Marr, unpublished data) indicates that all cells, regardless of age, suffered a proportional volume decrease. These results were obtained under culture conditions in which glutamic acid supplied all carbon, nitrogen, and energy. Such conditions of general starvation would be expected to have a universal effect on all biochemical pathways rather than the specific action of a micronutrient in the present study.

The sequential changes in cell volume distribution brought about by vitamin B₁₂ starvation
shows the appearance of a second peak due to the accumulation of larger-than-average cells. The simplest explanation consistent with this observation is that growth of a subpopulation of cells is inhibited at a unique point in the life cycle. The remaining cells, although also inhibited, are not arrested at a specific locus that is identifiable by these procedures. With extended starvation the cells achieve a partial phasing at some control step before division. One possible reaction is the replication of DNA. During B\textsubscript{12} starvation, DNA synthesis should stop or nuclear division would lead to the accumulation of binucleate cells. Although the new population reached as much as one-third of the total (Fig. 2), there was no corresponding accumulation of binucleate cells. It is therefore unlikely that cellular division per se is blocked.

In Euglena, B\textsubscript{12} starvation causes significant alterations in macromolecular synthesis (1, 2). The differential inhibition of DNA synthesis supports the hypothesis that vitamin B\textsubscript{12} is involved in a late stage of DNA replication, which would eventually inhibit division.

Bulk measurements of macromolecules or total cell volume in a known number of cells have been used to establish average cellular values. Measurement of individual cells not only allows a determination of the average, but also gives the distribution among the population. In the present case, it appears that vitamin B\textsubscript{12} starvation does not affect all cells identically; in
some cells there is an inhibition of growth at a single point in the cell cycle, and this interference is kinetically localized between the initiation of DNA synthesis and nuclear division.

The precise biochemical role of vitamin $B_{12}$ in *Euglena* has not been established. The implicit assumption that a single limiting process caused previously observed effects may not be correct in view of this new evidence that *Euglena* cells can respond in at least two different ways to vitamin $B_{12}$ starvation.

**LITERATURE CITED**