# POTASSIUM UPTAKE

# IN SYNCHRONOUS AND SYNCHRONIZED

# CULTURES OF *ESCHERICHIA COLI*

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ABSTRACT   Criteria are presented for distinguishing between synchronous and synchronized cultures (natural vs. forced synchrony) on the basis of characteristics of growth and division during a single generation. These criteria were applied in an examination of the uptake of potassium during the cell growth and division cycle in synchronous cultures and in a synchronized culture of *Escherichia coli*. In the synchronous cultures the uptake of $^{42}K$ doubled synchronously with cell number, corresponding to a constant rate of uptake per cell throughout the cell cycle. In the synchronized culture, uptake rates also remained constant during most of the cycle, but rates doubled abruptly well within the cycle. This constancy of $^{42}K$ uptake per cell supports an earlier interpretation for steady-state cultures that uptake is limited in each cell by a constant number of functional sites for binding, transport, or accumulation of compounds from the growth medium, and that the average number of such sites doubles late in each cell cycle. The abrupt doubling of the rate of uptake of potassium per cell in the synchronized culture appears because of partial uncoupling of cell division from activation or synthesis of these uptake sites.

## INTRODUCTION

Evidence was presented in an earlier report (1) that cells in steady-state cultures of *E. coli* grow at constant rates during most of each generation cycle (linear growth) Because a constant growth rate requires a constant rate of accumulation of matter, it was suggested that every major growth factor might enter the cell at a constant rate during the period of linear cell growth. This possibility was tested for several labeled compounds in steady-state cultures of *E. coli*, and it was found that the rate of influx (total uptake) of each was independent of cell size, and therefore of cell age, during most of the generation cycle (2).

These results suggested that rates of uptake of every material into the cell were limited by "uptake sites" involved in binding, transport, or accumulation of low

molecular weight compounds taken in by the cell. Certainly, if the number of uptake sites per cell remained constant during the cell cycle and if these limited uptake, then cells must grow at constant rates. It would also be required that the average number of uptake sites per cell double at some time during the cell cycle so that this number can remain constant from generation to generation. Some evidence for an abrupt increase in the number of sites near the end of the cycle was obtained in the experiments on rates of uptake of labeled compounds (2).

Those results, however, were open to several possible difficulties. First, although uptake rates were observed to increase near the end of the cell cycle, it was uncertain that the rates doubled, as predicted. Second, the data were obtained from experiments that had employed a relatively new selection technique in which cells were separated by size and cell ages were estimated from their volumes. Third, all of the labeled compounds used were macromolecular precursors, and it was possible that growth was controlled in some unknown way by macromolecular synthesis rather than by binding or transport systems. To avoid those difficulties, we studied potassium uptake in cultures in division synchrony. Potassium provides a more critical test because it is rapidly transported under variable growth conditions, acting mainly as a relatively freely exchangeable osmotic regulator (3, 4).

### THEORETICAL

Following the terminology of Abbo and Pardee (5), *synchronous* (unforced, natural [6]) cultures are, ideally, those in which cells are completely representative of normal, steady-state growth except for division synchrony. *Synchronized* cultures (forced or artificial synchrony) are those in which growth parameters or cell processes are entrained and fail to represent cell growth as it occurs under steady-state conditions. Since some perturbation probably occurs during application of any synchrony technique, these distinctions may appear to have limited utility. In practice, however, only a few synchrony methods appear to lead to minor or negligible perturbation; most lead to extensive, secular distortion of cell parameters. This is evident, for example, with one technique in which average values of cell protein in synchronized cultures of *Proteus vulgaris* decreased approximately fourfold after four divisions, and the average optical density per cell in synchronized cultures of *E. coli* decreased at least twofold during a corresponding period (7).

Such changes in average values of cell parameters over a number of cell cycles readily identify forced synchronization, but it is also possible to distinguish between the two kinds of synchrony on the basis of measurements over a single growth cycle. In this section we shall present two criteria for distinguishing between synchronized and (nearly) synchronous growth on the basis of measurements within a cycle.

The first criterion is one of cell growth. In a synchronous culture cell mass and volume must increase monotonically from the average size at birth to twice that size at division. In extension, modal cell sizes must also increase monotonically during the cell cycle. If cells grow at constant rates, as suggested earlier, then this

criterion can be sharpened: average and modal cell masses must increase at constant rates during most of the cycle. Synchronized cultures, however, can fail to meet even the less restrictive criterion, as will be demonstrated later in this paper.

The second criterion is based upon the pattern of cell division in the culture. The pattern of division for a perfectly synchronous culture can be calculated from the generation time distribution for the same cells in steady-state culture. Generation time distributions are regularly observed to be skewed toward long generation times, and have coefficients of variation of about 0.2 for enteric and related bacteria (8–11). The shape of the distribution appears to be reasonably well defined for *E. coli* B/r (9), with a coefficient of variation of 0.18 (Fig. 1 A). There is no evidence that the shape of the distribution changes with growth rate. On the contrary, very widely different kinds of cells appear to have generation time distributions of the same general shape (12).

The expected increase in cell numbers during the first cycle in a perfectly synchronous culture is the running integral over the generation time distribution. Integration of the curve in Fig. 1 A yields the population increase shown in Fig. 1 B. This figure shows that the time required for division to proceed from 10% of the population to 90% in a perfectly synchronous population of this kind would be slightly
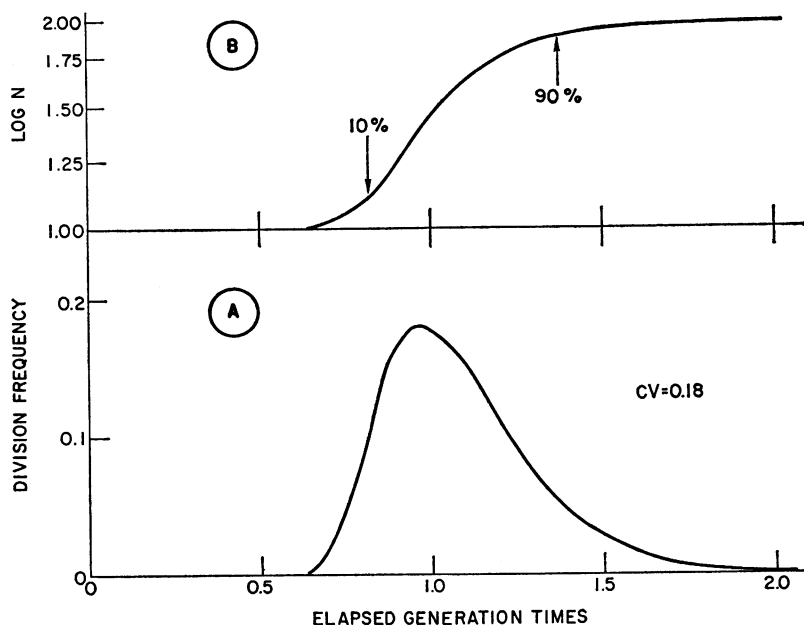


FIGURE 1 Generation time distribution and cell numbers increase for an ideally synchronous population. (A) The distribution of generation times for *E. coli* B/r as a function of the average number of elapsed generation times. The coefficient of variation (CV) of this distribution is 0.18. (B) Increase in cell numbers $N$ for the same culture if all cells were born at the same time. The arrows indicate the time at which 10% and 90% of the population have completed division.

more than 0.5 generations. Deviations from this pattern are evidence for synchronized growth.

As will be seen, these two criteria, growth during the cycle and duration of the division period, provide a practical means for identifying synchronous and synchronized cultures.

## MATERIALS AND METHODS

### Cell Culture

*E. coli* strains B/r and $B_{s-1}$ were grown in a low potassium medium (Na-M9), the standard M9 salts medium modified by replacement of potassium with sodium salts (3.18 g $Na_2HPO_4$, 3 g $NaH_2PO_4$, 1 g $NH_4Cl$, 0.5 g NaCl, and 0.2 g $MgSO_4 \cdot 7H_2O$ per liter deionized water) with 0.1% glucose or 0.1% sodium acetate as the carbon source. Sucrose (nonutilizable by these strains) was added to 0.5% to maintain steady-state growth conditions during later synchronous growth, as described previously (1). Nonradioactive potassium was added as $K_2CO_3$ to give a final $K^+$ concentration of $10^{-4}$ M. Within experimental errors, both carbon sources gave the same doubling time, 55 ±3 min (standard deviation).

Parental cultures (50 ml) were grown overnight on a rotary shaker at 37°C to final concentrations of between $10^7$ and $5 \times 10^7$ cells/ml. In our cultures, cell division rates and cell volume distributions remained invariant up to densities of about $5 \times 10^7$ cells/ml, but beyond this density both division rate and mean cell volume decreased with time.

### Synchrony

The method for producing synchronous cultures has been described earlier (1). Briefly, a parent culture is concentrated by filtration through a cellulose nitrate filter (Bact-T-Flex, 45 mm diameter, 0.45 μ pore size; Schliecher and Schuell Inc., Keene, N. H.), resuspended in about 0.25 ml of the filtrate, and layered upon a linear sucrose density gradient (5–15% sucrose in the Na-M9 salts at 25°C. After centrifuging briefly (1500 g, 1–2 min), the cells form a visible band with the smallest cells near the top. These are removed (usually in 0.25 ml) and reinoculated into the filtrate at 37°C, where they grow and divide in synchrony.

The synchronized culture was produced by the same gradient technique, differing only in that cells in the parent culture were allowed to reach slightly higher densities, about $6 \times 10^7$ cells/ml. More important, the parent culture was monitored for a discernible decrease in the mean cell volume, which is a more sensitive indication of departure from exponential growth than the corresponding small change in growth rate. The increased number of cells was layered on the gradient as before. That these relatively minor differences were sufficient to produce a synchronized rather than a synchronous culture may be because of some amplification of departure from steady-state growth by the gradient technique; however, we have not investigated the factors responsible for synchronization, and the explicit manner in which synchronization is established here and in other synchronization methods remains unknown.

### Isotope Exposure

$^{42}K$ was purchased from International Chemical and Nuclear Corporation (Burbank, Calif.) as $^{42}K_2CO_3$. Samples (1 ml) were removed at intervals from a culture in division synchrony and added to an equal volume of Na-M9 salts with added $^{42}K$, generally at 1 μCi/ml and $10^{-5}$–$2.5 \times 10^{-5}$ M. After 5 min incubation at 37°C, the samples were filtered through cellulose acetate membrane filters (Millipore HA, 25 mm diameter, 0.45 μ pore size; Millipore Corpora-

tion, Bedford, Mass.), and washed three times with 5 ml of nonradioactive growth medium. Control experiments with exponentially growing cultures determined that uptake of ⁴²K increased linearly for more than 5 min.

### Counting Procedures

Washed filters were placed in scintillation vials containing 10 ml distilled water, and counted by the emission of Cerenkov radiation (13) in a CPM-100 Liquid Scintillation System (Beckman Instruments, Inc., Fullerton, Calif.). Counting efficiency was not affected appreciably by changes in the volume of water in the vial, varying about 3% when volumes were altered 30%. The efficiency of counting ⁴²K by Cerenkov radiation was about 80% of the efficiency in more conventional scintillation cocktails.

### Cell Counts and Volumes

Samples (0.5 ml) were removed from cultures for determination of cell counts and volume distributions using the Coulter counter–pulse height analyzer (Coulter Electronics, Industrial Div., Hialeah, Fla.) described previously (1). At least 300 cells were counted and sized for each sample. Actual cell densities (cells/ml) were $2.7 \times 10^4$ times the Coulter count.

## RESULTS

Measurements on a synchronous culture of E. coli B/r growing in the salts-glucose medium are shown in Fig. 2. The data in this figure are representative of those obtained in other trials and indicate present limitations of the method, which arise mainly from the small numbers of cells that are available for each point.

Figs. 2 A and B show that this culture was essentially synchronous. There was little or no increase in cell numbers during the first 25 min (Fig. 2 B). After that time, cell numbers increased through two successive synchronous divisions. The first synchronous division was half complete at 43 min (as determined by the point at which cell numbers reached the geometric mean between the values at the first and second plateaus, an increase by a factor of approximately $2^{1/2}$); this time is indicated by the position of the first vertical dotted line. The period from midpoint to midpoint is 58 min, in good agreement with the doubling time of the parent culture. The excellence of numbers synchrony during the first division is shown by the agreement of the data with the theoretical curve (Fig. 1 B), reproduced in Fig. 2 B as the thick line curve.

The data of Fig. 2 A show that although synchrony was generally good it was not perfect. During the course of growth of this culture, cell volumes were distributed around a single peak, in the same manner as observed earlier (1). The peak values of the cell size distributions increased uniformly and at the same rate during the first and second cycles, but the line for this increase was displaced to the left by about 10 min during the second cycle. Thus during the second cycle cells were slightly larger than they were at corresponding times during the first cycle. After the second division, a lag in cell growth occurred, apparently correcting the phasing of cell growth during the third cycle.
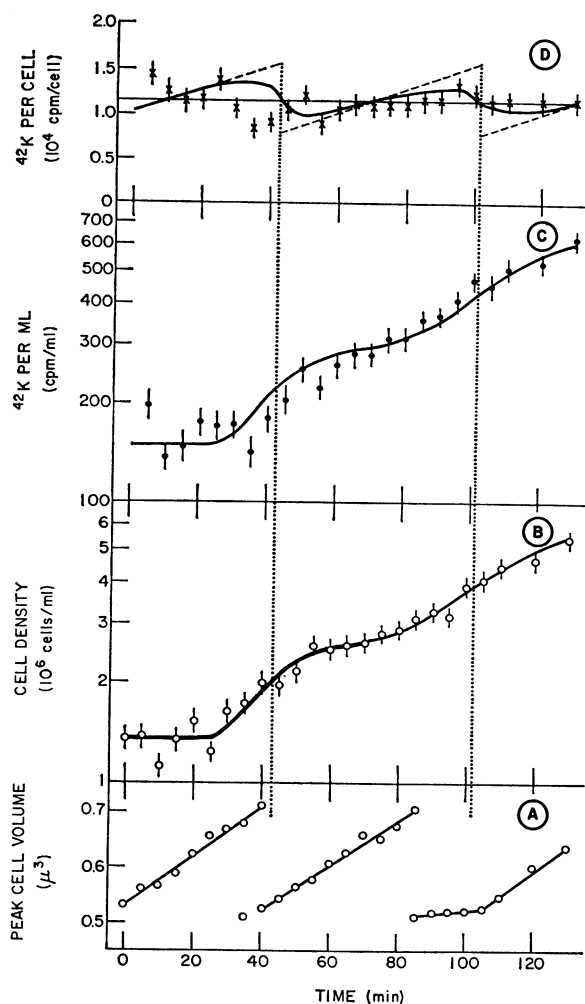
FIGURE 2 Potassium uptake by synchronously growing *E. coli* B/r in the salts-glucose medium. (A) Peak (modal) cell volume in cubic microns, as determined with a modified Coulter counter–analyzer system. (B) Cell density, determined with the counter. (C) Uptake of $^{42}$K/5 min per ml of culture. (D) Net uptake of $^{42}$K/cell in 5 min. The dotted vertical lines indicate the midpoints of the division phases shown in Fig. 2 B, as determined by the time when the cell number increased from the plateau value by a factor of $2^{1/2}$, the geometric mean. The vertical bars above and below the data points indicate standard errors. The thick line drawn through the data from time 0 to 70 min in Fig. 2 B reproduces the curve shown in Fig. 1 B. The same curve is drawn again in Fig. 2 C to aid comparisons of the timing of increase in cell numbers and potassium uptake. The lines in Fig. 2 D are the theoretical expectations for a constant rate of uptake (horizontal line), for a linearly increasing rate of uptake throughout the cell cycle if cells had divided simultaneously (dashed line), and for a linearly increasing rate corrected for the observed division synchrony (undulatory line).

Values for potassium uptake are again compared with the theoretical curve for numbers increase (from Fig. 1 B) in the first half of Fig. 2 C. The experimental points follow the trend of cell numbers increase except for the interval from 40 to 55 min, where values were generally lower. This may be related to the perturbation already noted during the second cycle of this culture. The data for the rate of $^{42}K$ uptake per cell in Fig. 2 D permit a comparison with theoretical predictions from two extreme models: (a) a constant rate of increase of potassium throughout the cell cycle, and (b) a linearly increasing rate of potassium uptake. The constant rate of uptake is described by the horizontal line in Fig. 2 D. The dashed, sawtooth line would give the expectation for a linearly increasing rate of uptake if all cells divided simultaneously; the undulatory curve shows the expectation for this model for the degree of division synchrony actually observed (Fig. 1 B). The experimental data favor the model of a constant rate of uptake: the ratio of the variances of the experimental data from the hypothetical curves (var $b$/var $a$) is 2.28 ($N = 24$), providing significantly better agreement for model $a$ ($P < 5\%$). Furthermore, a comparison of the experimental results with the expectations of a constant rate of uptake per cell by chi-squared analysis gave no significant difference between the two.

Synchronous cultures of strain $B_{s-1}$ grown in salts-acetate medium gave similar results (not shown). Division synchrony was about the same as that for B/r, although growth synchrony was somewhat better, with evenly spaced, linear increases in peak cell volume. In a comparison of the two models for potassium uptake, the variance ratio was 3.26 ($N = 16$), again significantly favoring the model of a constant rate of $^{42}K$ uptake throughout the cell cycle.

In control experiments in which all cells were collected after gradient centrifugation, mixed, and a small fraction of the culture reinoculated into the filtrate, these cultures grew exponentially in numbers, and at the same doubling time as the parent cultures, 55 min. The average value of the rate of uptake of $^{42}K$ was also measured in one such culture for strain $B_{s-1}$. This value, $1.4_9 \times 10^{-4}$ counts/5 min per cell, was in excellent agreement with the average value for a corresponding synchronous culture, $1.5_2 \times 10^{-4}$. The rate of uptake of $^{42}K$ in synchronously dividing cells also is closely comparable to that in undisturbed exponentially growing cells (uncentrifuged). At the same potassium concentration, $10^{-4}$ M, the average potassium uptake in 5 min in the synchronous acetate cultures was $1.2 \times 10^{-10}$ $\mu$g K/cell, in good agreement with the corresponding amount in an exponential culture, $1.3 \times 10^{-10}$ $\mu$g K/cell.

Growth, division synchrony, and potassium uptake were markedly different in a synchronized culture (forced division synchrony) of E. coli B/r growing in the salts-glucose medium (Fig. 3). This culture was prepared from a parent culture that had reached a concentration of about $6 \times 10^7$ cells/ml. The cell volume distribution indicated a departure from steady-state conditions, with a shift toward a greater frequency of small cells. The data for both cell size and cell numbers clearly show that the culture was synchronized (Figs. 3 A and B) rather than synchronous. Peak

cell volumes (Fig. 3 A) leveled off about 20 min before the end of the first cycle and failed to decrease appreciably with division. No clearly defined secondary peaks of small cells were observed either at the first or the second cell division (Fig. 4), although the first division occurred in sharp numbers synchrony. In fact, during this division cell numbers increased more abruptly than is theoretically predicted for a synchronous culture of B/r, as may be seen by comparing the data with the thick line in Fig. 3 B. Thus both criteria support synchronized growth and division for this culture.

Potassium uptake also followed a different pattern from that in synchronous
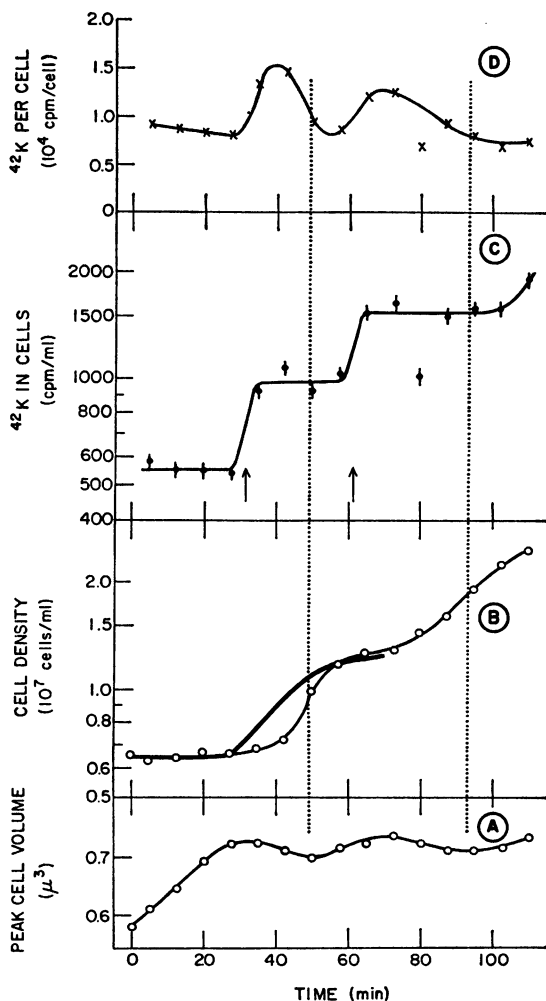


FIGURE 3   Potassium uptake in a synchronized culture of *E. coli* B/r in salts-glucose medium. Panels A, B, C, and D as in Fig. 2. The absence of growth synchrony in A and the unduly sharp numbers synchrony in B show that the culture was synchronized, rather than synchronous. The thick line in B again reproduces the theoretical curve shown in Fig. 1 B.
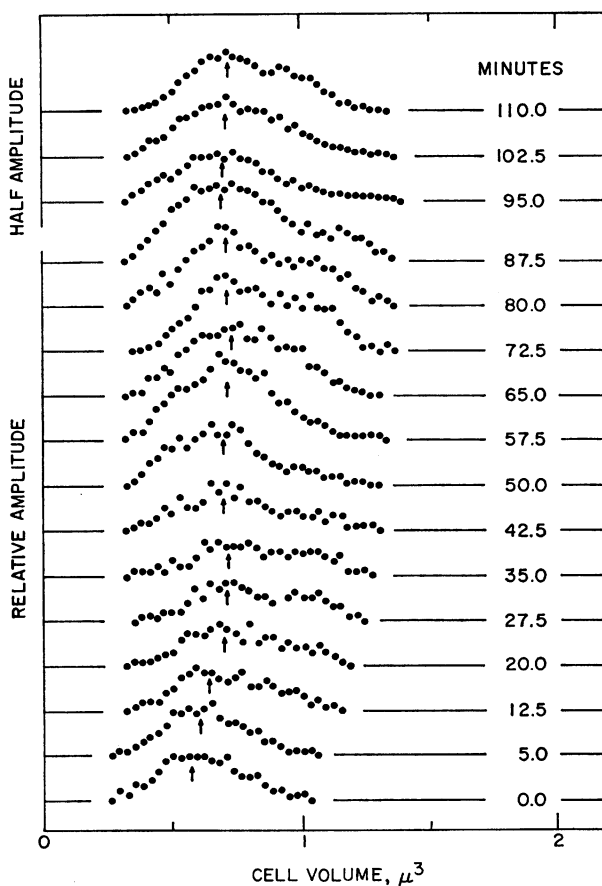
FIGURE 4   Cell volume distributions for the synchronized culture of Fig. 3. Numbers of cells are plotted on a relative scale against cell volume in cubic microns. The arrows indicate the positions of the peak cell volumes in each distribution.

cultures. The rate of uptake in the culture increased in a sharp, steplike manner (Fig. 3 C). These stepwise increases were more abrupt than those for cell numbers, and were displaced from them; the rate of uptake of $^{42}$K during the first cycle increased about 17 min before cell division (arrows in Fig. 3 C), almost doubling. Correspondingly, $^{42}$K uptake per cell (Fig. 3 D) reached a peak value and decreased to its original value as the cells went through their first division. A second peak, although broader and of decreased amplitude, was observed again in the second cycle.

## DISCUSSION

The primary question to which our experiments were addressed is, How does the rate of transport change during the lifetime of the cell? To answer this question, we examined the rate of uptake of $^{42}$K in cultures of E. coli in division synchrony.

Because there is very little loss or turnover of potassium during exponential growth at the concentration we used, $10^{-4}$ M (S. Silver, unpublished results), corrections for these processes should be negligible in synchronous or steady-state cultures.

In synchronous cultures we observed a doubling of the rate of potassium uptake in correspondence with the increase in cell numbers during the cycle (see Fig. 2). These results are consistent with the constant rate of uptake of nutrients observed in exponential cultures (2).

The rate of uptake of potassium in the synchronized culture also supports constancy (Fig. 3), at least for large parts of the cycle. Uptake was constant for at least the first 28 min, and then increased in abrupt steps from plateau to plateau. During the first cycle the rate of potassium uptake per cell increased by almost a factor of two. This rapid doubling of the rate of uptake per cell is in agreement with the concept that the actual number of functional transport sites doubles abruptly near the end of the cycle. The subsequent decrease with cell division in the rate of potassium uptake per cell to its original value supports the suggestion that the number of functional sites must double during normal cell growth in order to maintain constant the average number of uptake sites in daughter cells. In this synchronized culture, however, the doubling of uptake sites was uncoupled from cell division, occurring earlier in the cycle and at different times during the first and second cycle.

At least three different models may be advanced to account for these results. First, assuming that only functional uptake sites are synthesized, doubling of the rate of uptake might be due to doubling the total number of sites during a particular time in the cell cycle. Second, sites might be synthesized continually during the cell cycle but remain nonfunctional until activated or derepressed. Then the abrupt doubling in uptake rate would reflect this activation of newly formed sites. If applied to the synchronized culture, this model of continual synthesis would not account for the doubling in potassium uptake observed near the middle of the cycle in the synchronized culture (Fig. 3 C) because newly formed sites could have increased only by approximately 60%; however, since growth was forced in this culture, it is possible that all uptake sites were synthesized by this earlier time. Third, uptake sites for potassium might be synthesized continually and be under a feedback control system that maintains a constant rate of uptake by decreasing the transport efficiency of individual sites as their numbers increase; however, it is difficult to account for an abrupt doubling in rate of uptake with a feedback system of this kind because the control level itself would then be required to change abruptly. Thus this model would require an additional control system, and appears to be the least economical.

Our results for relatively constant mean cell volumes in the synchronized culture (Figs. 3 A and 4) are similar to those observed earlier for *Alcaligenes faecalis* by Lark and Lark (14). They interpreted this constancy as reflecting an abrupt increase in cell volume just before division. Although that interpretation may be

oversimplified, their results indicate that a carrier for transporting methionine into the cell was duplicated synchronously with division or immediately before, as they pointed out.

In summary, our results for potassium uptake from synchronous and synchronized cultures are consistent with the earlier interpretation that uptake of most or all compounds into the cell during steady-state growth are limited by the presence of a constant number of uptake sites. In exponential and in synchronous cultures the average number of functional sites doubles near the end of the cell cycle. In the synchronized culture, the duplication of functional sites was uncoupled from cell division and occurred much earlier in the cell cycle.

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