CHANGES DURING THE DIVISION CYCLE IN BACTERIAL CELL WALL SYNTHESIS, VOLUME, AND ABILITY TO CONCENTRATE FREE AMINO ACIDS

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SUMMARY

Changes in cell volume, cell wall synthesis, and ability to concentrate reversibly an amino acid were studied in *Alcaligenes fecalis*. Cell volume and number were measured in a Coulter Counter; cell wall synthesis was followed by means of short-term incorporation of radioactive alanine and methionine; the ability of the cell to concentrate replaceable methionine served as a measure of the concentration of an amino acid. All of these parameters were studied in synchronously dividing cultures. In addition, cell volume and methionine concentration were studied in spheroplasts and in cells leaving the lag and entering the exponential phase of growth.

Whereas cell wall synthesis was found to occur at an increasing rate throughout the cell division cycle, cell volume remained constant, except for a rapid increase just before division. The maximum amount of methionine which could be concentrated also remained constant throughout most of the division cycle. In spheroplasts an increase in volume was not accompanied by an increase in ability to concentrate methionine. Cells leaving the lag phase of growth showed an increased ability to concentrate methionine not accompanied by a comparable increase in cell volume.

The results indicate that there is no direct relationship between cell volume, ability to concentrate methionine, and cell wall synthesis. However, cell volume and the concentration of replaceable methionine are regulated in conjunction with the division cycle.

INTRODUCTION

In bacteria, the cell wall is responsible for the shape of the cell and forms the barrier separating the interior of the cell from the environment. Little is known about the synthesis of cell wall material during the cell division cycle. Similarly, changes in those properties of the cell which may be expected to be related to cell wall synthesis, such as cell volume or the ability of the cell to concentrate medium metabolites reversibly, have not been studied with reference to division, except that SCOTT

* A portion of this work will be submitted by Cynthia Lark to the Graduate School of Saint Louis University in partial fulfillment of the degree of Doctor of Philosophy. This portion of the work is printed here by permission of the Graduate School of Saint Louis University.

AND CHU[1] have obtained results which suggest that the ability of *E. coli* to concentrate glucose reversibly may vary during the division cycle.

During the past two years methods for purification and analysis of bacterial cell wall material have been improved[2–4]. Recent advances in particle sizing[5,6] and in studies on metabolite transport in bacteria[7,8] have made it possible to study the variation in these cell parameters with greater accuracy and technical ease.

We have undertaken to determine when, during the division cycle, the bacterial cell wall is synthesized and whether synthesis of this material controls cell volume or the capacity of the cell to concentrate a medium metabolite.

The organism used in this study was *Alcaligenes fecalis*. A method for synchronizing the division cycles of the individual cells of a culture[9,10] has been described previously. The cell wall of this organism has been shown to consist of two fractions, one soluble in phenol, the other insoluble[11]. The latter fraction is responsible for the rigid rod-shape of the organism and is characterized by the presence of a few amino acids in extremely high concentration, the principal one being alanine.

MATERIALS AND METHODS

Growth and synchronization of A. fecalis *Strain LB*

The properties of Strain LB of *A. fecalis* and the materials and methods used for its growth and maintenance have been described[10,12,13].

Synchronization of cell division was carried out by a modification of a method described previously[10]. This modification was designed in collaboration with Dr. Y. MARUYAMA in connection with work presented elsewhere[14]. 7 l cultures of cells were grown at 37° in the medium in which cell division was to be studied (casamino acid medium or L amino acid medium[13] lacking methionine and alanine) to a titer of $2 \cdot 10^8$ cells/ml. The bacteria were sedimented in a Servall continuous flow centrifuge at 30° at a speed of 10,000 rev./min, the culture being passed through the centrifuge at the maximum rate, *i.e.*, 4 min/l. The sediment was resuspended in 15 ml of nitrogen-free medium at 37° and homogenized for 45 sec in a Waring blendor. The resulting suspension was adsorbed on to the top layer of a filter paper pile consisting of nine pieces of Eaton-Dikeman paper (No. 624–63) and clamped in a plastic funnel (internal diameter 15 cm). It was then rapidly washed through the filter paper pile with 300 ml of nitrogen-free medium under suction*. Synchronized growth was initiated by eluting the middle three papers within 1 min into 300–400 ml of complete medium to start the experiment.

Measurement of cell wall synthesis

Cell wall synthesis was measured by means of DL-[^{14}C]alanine and DL-[^{35}S]-methionine. 10 ml of a bacterial suspension growing in alanine- and methionine-free medium were added to 120 μg of radioactive alanine or methionine, and incubated with aeration for 5 min in a stainless steel centrifuge tube. The incorporation was

* We have found that the amount of liquid to be used in washing must be determined empirically and may vary with the type of funnel used. In addition, it is important to make sure (using ink or a dye) that organisms are adsorbed only to that area of the top layer which will filter through to the bottom layers. Organisms should be eluted only from that area of the paper directly in the main flow of the wash fluid.

stopped by the addition of 0.1 ml of saturated [^{12}C]alanine or [^{32}S]methionine, and the mixture frozen within 30 sec in a dry ice–alcohol bath.

Subsequently, samples were thawed to 0°, and centrifuged twice at 40,000 $\times$ g for 60 min to remove medium activity. Heat killed bacteria were added to facilitate the centrifugation and fractionation. Cell wall and cytoplasmic protein were then fractionated as described previously[11]. By this method, the rate of incorporation of alanine or methionine into cell wall was measured. Cells which were labeled with [^{14}C]alanine or [^{35}S]methionine showed no loss of radioactive label from the cell wall or cytoplasmic fractions on subsequent incubation and growth in radioactive free medium containing 0.01 M [^{12}C]alanine or [^{32}S]methionine. This indicated that the rate of incorporation of alanine or methionine represented a synthetic rate and was not due to a non-conservative exchange or turnover reaction.

Measurement of methionine concentrating ability

As the concentration of replaceable methionine (pool methionine) in exponential cultures of *A. fecalis* had been studied in detail previously[13], methionine was used again in these studies. Because determinations had to be carried out rapidly, certain modifications were made in the procedure for measuring methionine accumulation. 10 ml samples of cultures were added to chloramphenicol (500 μg/ml) and radioactive DL-[^{35}S]methionine (12 μg/ml). The purpose of adding chloramphenicol was to stop protein synthesis and thus preserve the cells' internal pool of free methionine. After exactly 3 min, a 5-ml aliquot (A) of this mixture was filtered through a Millipore filter, size DA, to collect the bacteria. These were immediately washed by passing 5 ml of methionine-free medium through the filter. The total time for filtration and washing was 30 sec. (Experiments had shown that a wash filtration by methionine-free medium did not remove measurable quantities of pool methionine.) This 5-ml aliquot measured the total methionine concentrated by the cell in all forms. Simultaneously with the time aliquot A was filtered, 5 mg of non-radioactive methionine was added to the remaining 5 ml of culture (B). This was incubated for 3 more minutes and then filtered and washed as the first. Sample B measured non-replaceable methionine within the cell. The difference between A and B measured the replaceable methionine concentrated during the first 3 min period of incubation.

Previous work[13] showed that amounts of L-methionine in excess of 3 μg/ml saturated the cells' methionine concentrating system. The present method yielded consistent results in that medium concentrations of 12 μg, 24 μg, or 6 μg/ml DL-methionine all yielded the same total amount of methionine replaceably accumulated by the cell.

Measurement of cell volume

Cell volume was measured by means of the Coulter Particle Counter, Model A (Manufactured by Coulter Electronics, Chicago, Ill. (U.S.A.)). The theory of operation of this machine, as well as a trial application to bacterial counting and sizing, have been described[5,6].

In the experiments to be reported, we used a 27-μ diameter orifice supplied by the Coulter Electronic Company. Maximum gain was used to obtain a maximum range on the sizing scale.

The counting medium was isotonic sodium chloride (Abbott Laboratories), which

gives a low background due to absence of countable particles. (This background = 1000–1200 particles of size greater than 0.13 μ^3 in 0.05 ml). Samples were diluted either directly in saline for counting, or following fixation in an 0.2% formalin solution. Formalin fixation preserved the samples for counting and split a large proportion of double cells. Samples for counting were usually diluted in a volume of 25 ml of saline at room temperature in such a manner as to give 20,000 particles in 0.05 ml of a size of 0.2 μ^3 or greater. (In this size range, the background from a comparable blank lacking only bacteria was approx. 700 particles/0.05 ml). At this bacterial concentration no appreciable coincidence effect was observed. This was checked by diluting the sample and ascertaining that the volume/count ratio was constant. This ratio would be increased by an appreciable coincidence effect.

Counts were carried out at a current setting of six on the machine, which yielded a maximum pulse height per particle without causing boiling in the orifice due to heating. The machine was calibrated with polystyrene latex particles (Dow Company) with a volume of 0.84 μ^3.

Particle volume was determined by counting the number of particles in a given size range, multiplying the number of particles in a given size range by the average volume of this range, and adding the products for the various size ranges. This sum gave the total cell volume in the 0.05 ml counted. Division by the total cell count yielded the average volume/cell.

The results obtained with the Coulter Counter were compared with those obtained by centrifuging the cell suspension in a hematocrit tube and visually calculating the volume. Values for bacteria obtained from a stationary phase culture of *A. fecalis* (presumably small cells) and from one leaving the lag phase (presumably larger cells) are shown in Table I. Despite the differences in absolute values, the volume ratios

TABLE I

RELATIVE VOLUME MEASUREMENT BY HEMATOCRIT AND BY COULTER COUNTER

	Volume/cell	
	Hematocrit	*Coulter counter*
(A) Stationary phase cells	0.744 μ^3	0.33 μ^3
(B) Late lag phase cells	1.05 μ^3	0.50 μ^3
A/B	0.708	0.660

of the two cell types agree well. The difference in absolute volume can be expected, since the Coulter Counter measures only the volume of electrolyte displaced by an isolated cell, whereas the hematocrit method includes intercellular water as well. (This latter factor can be quite large, since the rod-shaped bacteria will not necessarily pack into a closely packed parallel arrangement, but rather in a randomly oriented fashion.) As a result of this experiment, it seemed safe to conclude that measurements of relative cell volume could be made with the Coulter Counter.

RESULTS

The rate of cell wall synthesis during the division cycle of A. fecalis

Aliquots were removed at intervals from a synchronized culture and tested for rate of synthesis of cell wall as indicated by the incorporation of [^{14}C]alanine or

[^{35}S]methionine. As may be seen in Fig. 1, the rate of synthesis of both the phenol-soluble and phenol-insoluble cell wall components, as well as of cytoplasmic protein, increased steadily during the division and the inter-division periods. At no time was there a sufficiently large decrease in the synthetic rate to indicate a cessation of cell wall synthesis during the cell division cycle*.

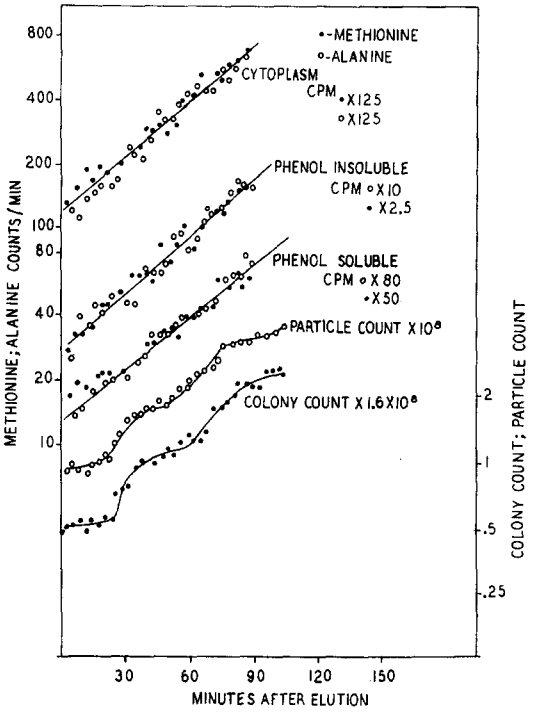

Fig. 1. Cell wall synthesis in synchronized cultures of *Alcaligenes fecalis*. Cell wall synthesis measured as counts/min of alanine or methionine incorporated into the cell. For the procedures used in carrying out these experiments see MATERIALS AND METHODS. The CPM of the various fractions have been normalized for convenience of presentation. To obtain the absolute values, they must be multiplied by the factors shown. The specific activity of the [^{14}C]alanine used was 4 mC/mmole, that of the [^{35}S]methionine, 30 mC/mmole.

The data in Fig. 1 afford a comparison of counts of the number of cells by means of colony count and by the particle counter. From these data, as well as those published previously[15], we have concluded that colony formers plate with an efficiency of about 70–80 % of the particle count.

Concentration of pool methionine during the cell division cycle

Fig. 2 shows the results of an experiment in which aliquots taken at intervals from a synchronized culture were tested for their methionine concentrating ability. As may be seen, the amount of pool methionine found at any time in the culture (under conditions of pool saturation), paralleled very closely the cell count. This amount was equal to that found in exponentially dividing cultures and corresponded

* Because of the scatter of the points, it was impossible to ascertain whether minor periodic variations in the rate of incorporation of [^{14}C]alanine into the cell wall fractions may, in fact, occur. However, if this were so, such variations would be less than $\pm$ 30 % of the average rate.

to about $5 \cdot 10^5$ molecules per cell*. The finding that the concentration of pool methionine per cell was constant during the cell division cycle implied that the duplication of the responsible mechanism might be closely synchronized with the separation of the cell into two daughter units.

Alternatively, some other attribute of the cell closely linked to cell division might limit the amount of pool methionine concentrated by the cell. The most obvious such parameter was cell volume.

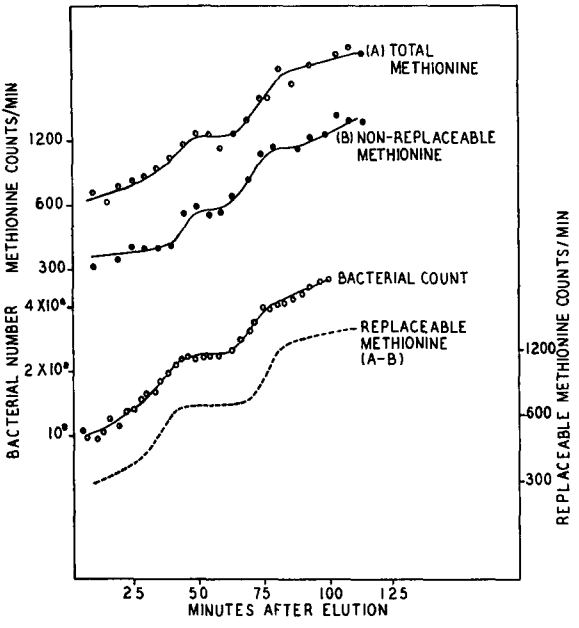

Fig. 2. Replaceable methionine concentration in synchronized cultures of *Alcaligenes fecalis*. Concentrated methionine is given in counts/min. For the conditions of the experiment see MATERIALS AND METHODS.

Changes in cell volume during the division cycle

Table II presents a volume analysis of cells taken at intervals from a synchronized culture. 0.1-ml samples were taken at the times indicated and added to 1 ml of 0.2 % formalin. During the 24-h period subsequent to the experiment, the samples were analyzed and sized. (Fixation in formalin was necessary because of the length of time required for the sizing analysis. Although it was possible that fixation itself might change cell volume, no further change in particle number, size distribution, or average cell volume occurred in cell suspensions during immersion in 0.2 % formalin for 24 or 48 h). Table II gives the number of cells at any time, the percent in any size range, and the average cell volume. A comparison of the average cell volume immediately before or after rapid increases in cell number, *i.e.*, at 20, 35, 60, and 70 min, revealed no major shift in cell volume or in distribution of cell size.

* This value is lower than that found previously[13] for L-methionine. This is due in part to the higher efficiency of the Coulter counter. In addition, D-methionine might also be concentrated in place of L but to a lesser extent. Finally, the shorter period in chloramphenicol (3 min instead of 20 min) might result in a saturated pool of smaller size.

TABLE II

SIZE ANALYSIS OF CELLS TAKEN FROM A SYNCHRONIZED CULTURE

Samples were taken from a synchronized culture as described in the text, and fixed and sized as described in MATERIALS AND METHODS. Results from an exponential culture are given for comparison.

Minutes after elution sample was taken	*20*	*25*	*30*	*35*	*40*	*45*	*50*	*60*	*65*	*70*	*Non-synchronized exponential culture*
Total number greater than 0.32 cubic microns	*12,840*	*13,223*	*16,174*	*18,700*	*18,000*	*19,386*	*22,168*	*23,508*	*28,300*	*39,021*	*20,745*
% in size range (cubic microns) =											
0.32–0.4	2.7	4.2	3.0	4.4	3.3	6.1	3.5	6.6	6.05	6.5	1.8
0.4 –0.48	10.4	10.2	21.4	22.8	20.0	18.0	17.0	20.0	18.4	20.7	19.7
0.48–0.56	15.5	19.1	17.5	18.5	19.1	16.0	19.6	21.3	18.5	18.4	15.6
0.56–0.64	14.9	12.8	17.8	16.8	16.9	17.0	14.3	9.9	14.6	14.8	16.0
0.64–0.72	13.2	13.2	9.1	10.8	11.0	12.3	14.0	14.3	9.7	11.9	13.9
0.72–0.80	9.3	7.9	10.5	4.9	8.6	7.05	7.4	3.8	9.6	6.1	8.1
0.80–0.88	8.6	7.8	8.9	7.6	3.9	6.8	10.6	9.5	5.5	6.7	5.3
0.88–0.96	4.2	4.7	5.3	3.8	5.7	3.5	1.3	3.84	6.0	2.86	4.6
0.96–1.04	4.7	7.1	5.25	3.7	3.06	4.6	2.94	3.2	2.83	3.5	2.8
1.04–1.12	2.9	2.8	2.95	1.5	2.76	1.26	3.0	2.26	2.4	2.3	4.3
1.12–1.20	2.5	2.5	2.3	2.1	1.45	2.0	1.4	1.68	2.5	2.26	2.4
1.20–1.28	1.68	2.4	1.6	0.96	2.46	1.95	1.85	0.85	1.35	1.24	2.4
1.28–1.36	1.67	0.69	1.3	1.01	0.27	1.37	0.82	1.08	0.72	1.09	1.1
1.36–1.44	1.25	0.69	0.204	0.53	0.86	—	1.25	0.36	0.19	0.43	0.3
1.44–1.52	2.9	1.66	1.5	—	0.24	0.75	0.53	0.85	1.14	0.57	0.5
1.52–1.60	0.195	0.23	—	0.73	0.68	0.94	0.75	0.32	0.36	2.7	0.34
1.60–1.68	0.47	0.88	0.55	0.19	—	—	—	0.18	—	0.615	0.86
1.68–1.76	—	0.144	0.25	0.01	0.23	—	—	—	0.164	0.0206	—
Average volume in cubic microns	0.72	0.72	0.76	0.65	0.67	0.66	0.68	0.65	0.66	0.68	0.71

The data indicated that, during the division cycle, volume was essentially constant per cell unit. To check the possibility that this might be the result of formalin fixation itself, an experiment was carried out in which samples were analyzed without formalin fixation. Each measurement had to be completed in 1 min, because the bacteria were growing. The analysis was, therefore, limited to a single size category in which variation would most sensitively reflect changes in cell volume. The population chosen was that of smaller cells of size 0.35 μ^3 to 0.5 μ^3. This cell population could be increased by division of larger into smaller cells. It could be decreased by an increase in cell volume, whereupon cells would leave this size category to enter a larger one.

Samples taken at intervals from a synchronous culture were diluted directly into saline and the number of particles of size greater than 0.35 μ^3 and 0.5 μ^3 were counted within 1 min.

The results from this experiment are shown in Fig. 3. As may be seen, the number of smaller cells in the population remained relatively constant, as did the total cell count until the onset of cell division. This indicated that the cell maintained a constant

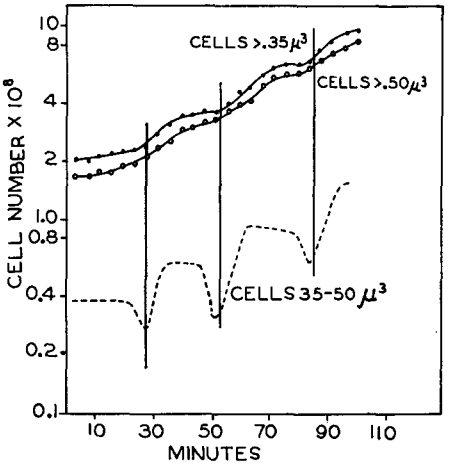

Fig. 3. Variation in the number of cells of volume 0.35 μ^3 to 0.5 μ^3 in synchronized cultures of *Alcaligenes fecalis*. The experimental procedures are described in the text and in MATERIALS AND METHODS. The percentage of cells in the 0.35–0.5 μ^3 size range is somewhat larger in cell suspension fixed in formalin (see Table II) due to a splitting of double cells by formalin. Cells were diluted at 45 and 90 min to maintain a constant density range. Dilutions have been corrected in plotting the results.

volume during the majority of the period between successive divisions. Just before cell division, cell volume rapidly increased—as was indicated by a loss of cells from the smaller cell category. This finding supported the detailed analysis already presented in Table II.

Relation of cell volume to pool methionine concentration

The experiments just described suggested that the maximum concentration of methionine which could be replaceably concentrated might be controlled by the cell volume. To test this, experiments were carried out in which cell volume was varied in dividing and non-dividing cells and the concentration of pool methionine was measured.

Cells which are treated with 50 units/ml penicillin enlarge into spheres (spheroplasts) whose size may increase greatly with time. In addition, they have lost most of the phenol-insoluble portions of their cell wall[11]. This increase in size parallels the

TABLE III

REPLACEABLE METHIONINE CONCENTRATION IN NORMAL CELLS AND SPHEROPLASTS

Cell number and volume measured with the Coulter Counter on formalin fixed cells. Replaceable methionine was measured as described in the MATERIALS AND METHODS.

	Normal cells	*Spheroplasts*
Cell number/5 ml	$4.05 \cdot 10^9$/ml	$1.35 \cdot 10^9$
Replaceable concentrated Methionine/5 ml	0.50 γ	0.16 γ
Average cell volume	0.69 μ^3	1.67 μ^3
Methionine/10^9 cells	0.125γ	0.119γ
Methionine/mm^3 cells	0.178γ	0.071γ

increase in protein per cell unit and spheroplasts may reach a volume of 10 times the normal cell volume[16].

An exponential culture of *A. fecalis* was divided into two aliquots, to one of which was added penicillin to a concentration of 50 units/ml. After 1 h the number of cells, cell volume, and maximum amount of methionine which could be replaceably concentrated, were measured in both aliquots. The results shown in Table III indicated that the increase in volume in the spheroplast was not accompanied by an increase in the amount of pool methionine accumulated. On the contrary, the amount of pool methionine remained constant per cell unit.

Another system for varying the cell volume independently of cell number is the transition of cells from the stationary, *via* the lag, to the exponential phase of growth. During the lag phase of growth, cells increase in mass, but not in number. A culture of *A. fecalis* was allowed to enter the stationary phase of growth by incubation at 37° in casamino acid medium with aeration for 36 h. The cells ($1.5 \cdot 10^{10}$/ml) were washed, diluted 1/50 into fresh L amino acid medium, and incubated with aeration at 37°. Samples were taken at intervals, and cell number, cell volume, and ability to concentrate pool methionine were measured. As can be seen in Fig. 4, there was a large initial increase in the cells' ability to concentrate pool methionine with no corresponding increase in either cell volume or cell number. This increase was extremely abrupt and amounted to almost a doubling of the pool size. It occurred about 45 min before the first increase in cell number. This length of time corresponds to the generation time of *A. fecalis* when it grows exponentially in this medium under these growth conditions.

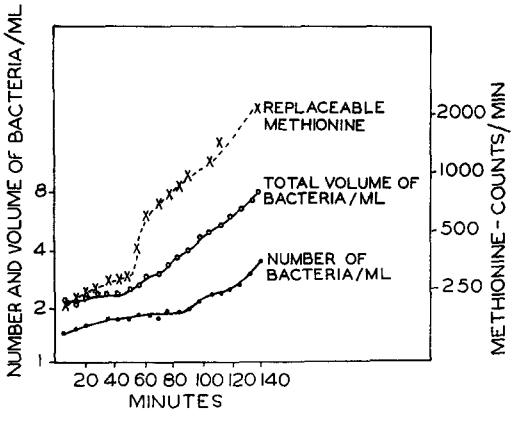

Fig. 4. Changes in replaceable methionine concentration and in cell volume of cells during the transition from the stationary to the exponential phase of growth. The methods used are identical with those used in the previous experiments. Relative cell volumes are of bacteria contained in 1 ml. Replaceable methionine was measured as in Fig. 2. Cells in the stationary phase of growth were diluted in to fresh medium at 0 min. Cell number and volume ordinate: Number of bacteria × ($2 \cdot 10^8$). Volume of bacteria × ($6.25 \cdot 10^{-2}$ mm^3).

From the experiments in Table III and Fig. 4, it is obvious that an increase in cell volume is neither a sufficient nor a necessary condition for an increase in the ability of the cell to concentrate pool methionine.

DISCUSSION

In the experiments described above we have studied the variation in three parameters of cell growth during the cell division cycle. Two of these, volume and ability to concentrate pool metabolites (pool methionine), remain constant per cell unit, because they increase simultaneously with cell division. The third, synthesis of cell wall material, proceeds continuously during the period between cell divisions as well as during the division period.

Microscopic examination during this interval between successive divisions shows that cells become elongated. Because they do not increase in volume, it must be assumed that they become thinner and that the surface/volume ratio must increase*. Thus, it is not necessary to assume that continued synthesis of cell wall material during the inter-division period leads to a thicker cell wall.

The simultaneous increase in cell volume and in pool metabolite capacity may balance each other and may thus lead to a constant intracellular metabolite concentration. This could eliminate sudden changes in internal osmotic pressure.

Previous work on spheroplasts has indicated that the presence of a bacterial cell wall is necessary to confine the cell volume. The present work would indicate that this structure does not completely control cell volume, though its presence is necessary for such control to be exercised. Changes in cell volume during division occur without any correspondingly radical change in rate of cell wall synthesis. This indicates that some other control over cell volume exists, which is closely related to cell division.

Similarly, the doubling of the cell's capacity to concentrate metabolites does not appear to be related to cell wall synthesis, but rather to cell division. Thus, in spheroplasts, the ability of each cell to concentrate methionine remains the same as in normal bacillary forms, although the volume of the latter is about 1/3 that of the former. Spheroplasts have been shown to contain a greatly reduced phenol-insoluble cell wall fraction as compared to the phenol-soluble fraction and the cytoplasmic protein[11].

In attempting to study the mechanism whereby the cell division cycle regulates increases in cell volume and metabolite concentrating ability, it is important to note that these two parameters are not causatively related; an increase in one does not necessitate an increase in the other. Independent control mechanisms may govern these parameters, because cells preparing to commence division—cells in the late lag phase of growth—increase their ability to concentrate metabolites before they increase in volume. Nevertheless, cells which have begun to divide have synchronized the control over these parameters.

The mechanism whereby bacteria replaceably concentrate metabolites has been

* It would be interesting to correlate these findings with volume determinations from electron microscopic examination of thin sections of bacteria. If volume remains constant, $(\text{width})^2 \times$ cell (length) should be equal for cells from the same culture. This should hold true for cells of different lengths and widths. A rough estimate by the authors using published data for *E. coli*[17] has indicated that this is so.

extensively studied[7,8]. Evidence for concentration of methionine in *A. fecalis*[13] and of other metabolites in other organisms[7,8], strongly implicates some carrier (pump) in the role of transporting the metabolite into the cell against a concentration gradient. The results obtained in this study suggest that duplication of this carrier takes place synchronously with division or immediately before. This recalls a previous finding suggesting that sites responsible for cell division and for directing cell wall synthesis were duplicated immediately before cell division[10]. These sites were penicillin sensitive. It is tempting to speculate that there may be a relationship between such sites and the metabolite carrier system (pump). Such a relationship is suggested by the lack of increase in methionine concentrating ability in penicillin-induced spheroplasts, which nevertheless retain the ability to synthesize protein and nucleic acids[16]. Similarly, staphylococci treated with penicillin show a striking decrease in their ability to concentrate glutamic acid[18].

No information is available as to the structural or chemical nature of the penicillin sensitive sites or of the metabolite carriers. If, indeed, some fraction of the cell wall is involved, it is too small to be observed in terms of a change in uptake of radioactive alanine or methionine.

About 70–80 % of the bacterial mass is water. From this it can be concluded that the change in cell volume will reflect a large intake of water. It is tempting to think that the cell operates a water pump in addition to metabolite pumps and that during cell division the number of such pumps is duplicated.

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