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Metal Ion Content of *Escherichia coli* Versus Cell Age

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The potassium, calcium, magnesium, and zinc ion content of cells in exponential and synchronously growing cultures of *Escherichia coli* B/r was determined with an X-ray fluorescence spectrometer and an atomic absorption spectrophotometer. Cellular potassium, calcium, and magnesium content increased smoothly during the cell cycle, but cellular zinc showed a steplike increase about 10 to 15 min after cell division in a culture having a doubling time of 47 min. The possible role of cellular zinc in the control of cell division is discussed.

Studies of the control mechanism of cell division in the last decade were done mainly on the sequence of events such as the synthesis of macromolecules (4, 7-9, 16, 28, 37, 51) and the doubling of enzyme activities (1, 9) in the cell cycle. The initiation of deoxyribonucleic acid (DNA) synthesis has been considered to be one of the most important control processes in the cell cycle (16), but control of some small molecules and ions is also of great importance for cell growth. Metal ions, although constituting only about 4% of the dry mass of cells of *Escherichia coli* (24), play an important role in enzymatic functions and general cellular physiology. Potassium ion is the most abundant cellular cation under normal growth conditions. Epstein and Schultz (10) found that potassium ions act as osmotic regulators. K^+ (23) and Mg^{2+} are essential for cellular protein synthesis. Mg^{2+} is also well known for its role in the maintenance of the intact structure of ribosomes. Relatively little was known about the physiological role of Ca^{2+} in *E. coli* until the work of Silver et al. (43), who recently investigated calcium transport in *E. coli* and *Bacillus subtilis*. Zn^{2+} has been found in various metalloenzymes in *E. coli*; it is important for the assembly and activity of alkaline phosphatase (32, 47), and the regulatory activity of aspartate transcarbamylase (31). DNA polymerase I (44, 45) and DNA-dependent ribonucleic acid (RNA) polymerase (40) in *E. coli*, the DNA polymerase of phage T4 (45) and chicken embryos (36, 46), and the reverse transcriptase of RNA viruses (22) are zinc metalloenzymes.

Because of the importance of various cations in cell growth in protein synthesis, in the regulation of intracellular osmotic pressure, and in the function of important enzymes, we wished to determine if some class of cations might play a central role in the process of cell division and DNA replication. The content and transport systems of cellular cations including potassium (11, 38), magnesium (32, 41), calcium (35, 43), zinc (3), iron (14, 21), and manganese (42) in *E. coli* and some other microbial systems (5, 6, 43, 49) have been studied. But it is still unknown how cells double their ion content in the cell cycle. As an initial step, we determined the levels of K^+, Ca^{2+}, Mg^{2+}, and Zn^{2+} in synchronously growing cells of *E. coli* B/r. We used an X-ray fluorescence spectrometer and an atomic absorption spectrophotometer to measure these ions. In determining the pattern of increase of cellular ions during the cell cycle of *E. coli*, we found that levels of K^+, Ca^{2+}, and Mg^{2+} increased very smoothly during the cell cycle, but Zn^{2+} showed a steplike increase about 10 to 15 min after cell division.

MATERIALS AND METHODS

Bacterial strain. DG336, a histidine-requiring derivative of *E. coli* B/r HB60 (from H. Boyer), was used for all our experiments.

Medium and growth conditions. All cells were grown at 37 C on a minimal salts medium (solution K), which has been described (50); the pH was adjusted to 6.8 to improve synchrony. The medium contains 0.60 mM K^+, 0.60 mM Mg^{2+}, 0.14 mM Ca^{2+}, and 0.012 mM Zn^{2+}. Glucose and histidine were added to final concentrations of 2 g and 25 mg per liter of medium, respectively.

Synchrony. Cells were synchronized by the membrane elution method invented by Helmstetter and Cummings (15) and modified by Ward and Glaser (48). Cell number and volume were determined by a Coulter counter with 30-μm orifice (50). The cell volume distribution is displayed using a 256-channel pulse-height analyzer and recorded on magnetic tape for later analysis. Since the channel number is closely proportional to the cell volume, we multiplied each channel number by the number of bacte-

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rial pulses in the channel and took the sum of these products divided by the total number of cells in the channels used to compute the relative mean cell volume. We found that the time taken for the relative mean volume of cells to double was the same as the time required for the peak of the cell size distribution to move from the lower to the upper bounds of the distribution of channels. When the peak reaches the upper bound of its range, one-third to one-half of the cells have just divided. The rate of DNA replication was measured by the rate of incorporation of [*methyl*-^{3}H]thymidine (obtained from New England Nuclear Corp., Boston; 50 Ci/mmol). Synchronous cells were pulse-labeled for 4 min at 5 μCi/ml. A 0.05-ml portion of culture was then spread onto a trichloroacetic acid-impregnated sheet of Whatman 3M filter paper ruled in a 3-cm-square grid. The sheet was dried and washed twice in ice-cold 5% trichloroacetic acid, once with ice water and boiling water, and twice in 95% ethanol. After being dried, the filter paper was cut into 3-cm squares, and each square was placed in a scintillation vial with 17 ml of toluene and Permafluor (from Packard Instrumento., Inc., Downers Grove, Ill.) and counted in a scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.).

Ion determination. Aliquots of a synchronously growing culture (physiologically in log phase) were filtered onto a Nuclepore filter paper (pore size, 0.45 μm, obtained from Wallabs, San Rafael, Calif.) and washed with 4 ml of warm wash solution. The Nuclepore filters were used in preference to cellulose nitrate filters because the former filters have much less mass. Since the mass of the sample contributed to the background in the X-ray fluorescence measurements, a filter of smaller mass allows a higher sensitivity for ion determination. These filters also have a very low level of contaminating ions.

In earlier experiments, filtered cells were washed with sucrose solutions. Although washing with water alone results in leakage of ions, washing with 0.3 M sucrose prevents this loss. The solution was readily freed of contaminating ions by the same methods used for deionizing the water as described below. Control experiments showed that washing with a slightly hypertonic sucrose solution (compared with the calculated osmolality of growth medium) having neutral pH resulted in a constant and stable level of cations associated with filtered cells. The wash solution was made up of 0.3 M sucrose solution with tris(hydroxymethyl)aminomethane (Tris) and MOPS (morpholinopropane sulfonic acid) buffer at a total concentration of 0.03 M and pH 7.2, unless otherwise specified. In later experiments we switched to glucose wash solution to reduce background from retained wash. Washing with glucose gave the same results as washing with sucrose. Four milliliters of prewarmed wash was enough to achieve constant and stable results, usually in less than 30 s. Using larger volumes of wash, up to 10 ml (and more time), gave the same results. All water used had been treated either by passing it through an ion exchange cartridge (Scientific Products Inc., Evanston, Ill., ultrapure mixed resin) or a column of Chlex 100 (Bio-Rad Laboratories, Richmond, Calif., 100 to 200 mesh, sodium form).

The filter paper was transferred to a plastic holder to keep the filter flat when the X-ray fluorometer was used for measurements. The holder was then placed on an X-ray fluorescence spectrometer (12) capable of measuring levels of all elements with atomic number 19 (potassium) to 42 (molybdenum) in one 16-min measurement. The data, which were given as the number of counts for each energy level, was then used to calculate the mass of each element on the filters. Since this X-ray instrument cannot detect magnesium, and the amount of cellular zinc that could be put onto the filter paper is very close to the detection limit of this machine, we used an atomic absorption spectrophotometer for further measurements of Zn^{2+} and Mg^{2+} contents. For this purpose the filter paper was transferred into a polypropylene centrifuge tube. For magnesium determination, the filter papers were incubated in 1 N hydrochloric acid half-saturated with 8-hydroxyquinoline (Oxine, obtained from Fisher Scientific Co., Pittsburgh, Pa.) overnight at room temperature (a modification of the procedure of Lusk, Williams, and Kennedy [25]), and then centrifuged. The magnesium content of the supernatant was determined with an atomic absorption spectrophotometer (model 303; The Perkin-Elmer Corp., Norwalk, Conn.). Samples for zinc determinations were first digested in concentrated nitric acid overnight, and then diluted with deionized water. The zinc content of this liquid was determined by using a graphite furnace (Perkin-Elmer model HGA-2000) attached to the same model of atomic absorption spectrophotometer. We define the ions associated with cells on the filters as cellular ions regardless of their locations on or in the cell. The ion content can be expressed as the weight of ion per gram of dry cell mass. Just as the synchrony of cell division is graphed as the number of cells per 1 ml of culture versus cell age, the contents of ion in the cell cycle can also be graphed as the weight of ions in cells in 1 ml of culture versus cell age. The doubling times for approximately exponentially increasing variables plotted on semilog graph paper, derived either by least-mean-squares fit or by a visually determined best-fit straight line, were similar.

RESULTS

Cation contents in unsynchronized exponentially growing cells. The results of the determinations of K^+, Mg^{2+}, Ca^{2+}, and Zn^{2+} in unsynchronized exponentially growing cells are shown on Table 1. The values are similar to those previously reported (also shown), except for zinc. Different strains of bacteria and richer medium were used in the previous zinc measurements, and that probably accounts for the differences in measured values for zinc.

Ion content of synchronously growing cultures of *E. coli*. The synchrony of cell division and DNA replication was measured as described in Materials and Methods. Initiation of DNA replication occurred about 30 min after cell division (data not shown) in synchronously

TABLE 1. *Ion contents in Escherichia coli*[a]

Ion	mg/g (dry weight)			
	This work	Epstein and Schultz (10)	Lusk et al. (25)	Bucheder and Broda (3)
K^+	12	18	14[b]	
Ca^{2+}	2.3			
Mg^{2+}	2.8		2.4[b]	
Zn^{2+}	0.15			0.5

[a] We used *E. coli* B/r, whereas all other workers used *E. coli* K-12.

[b] Originally these values were expressed as concentrations (millimolar) based on the assumption that 1 mg (dry weight) can account for 4 μl of cell water.

growing cultures with a doubling time of 47 min. The cellular contents of K^+, Ca^{2+}, Mg^{2+}, and Zn^{2+} in cells in 1 ml of culture at successive stages of the cell cycle are shown in Fig. 1. Cellular potassium, calcium, and magnesium content increased smoothly with cell mass. Within the limits of the measurement, we could not distinguish between linear and exponential increases of these three cations. Over several experiments, the doubling time of K^+ ions was 49 ± 2 min; of Ca^{2+}, 48 ± 2 min; and of Mg^{2+}, 47 ± 1 min.

Epstein and Schultz (10) pointed out that the level of cellular K^+ was dependent on the osmolality of the medium. To see whether the cellular potassium level changes when cells are grown in a medium of higher osmolality, we added sucrose to the medium to increase its osmolality from 0.12 to 0.35. This caused the cells to increase the amount of cellular K^+ from 12 to 23 mg/g (dry weight). The Ca^{2+} level also increased slightly from 2.3 to 2.6 mg/g (dry weight). The cellular content of K^+ and Ca^{2+} ions in milligrams per gram (dry weight) remained constant with cell age throughout the cell cycle (data not shown).

The level of cellular Zn^{2+} increased by steps during the cell division cycle with a doubling of cellular Zn^{2+} occurring about 10 to 15 min after cell division, but the exact doubling time of Zn^{2+} content was difficult to determine because the experiment covered only one complete jump. After the second division, synchrony became much poorer, making the determination of a doubling time for Zn^{2+} more difficult. The doubling times were estimated between the two time points where the cellular Zn^{2+} per milliliter was halfway between the two levels (before and after the Zn^{2+} doubling). The doubling time determined by this method was 50 ± 3 min over several experiments.

We wished to determine the physical location of Zn^{2+} in the cell relative to the other cations. Table 2 shows the results of a wash experiment. A water wash osmotically shocks the cells, causing the loss of soluble ions or relatively small molecules such as amino acids (2). Such a wash removed half the K^+ and one-third of the Ca^{2+}, whereas all of the Mg^{2+} remained. The loss of Ca^{2+} suggests that not all of the Ca^{2+} is bound tightly to the wall (as is the case for nonsporulating *Bacillus* [43]), but that some of it is in soluble intracellular form, in spite of the possibility of the existence of active transport of Ca^{2+} out of the cells. The loss of Ca^{2+} might also be due to the flow of other cations leaving the cell, some of which could exchange with the Ca^{2+} on the wall. This would be analogous to the situation using an ionic wash solution, such as the NaCl wash (Table 2). Mg^{2+}, as has been found by others (41), is removed from the cells neither by water wash nor by ionic washes. The behavior of Zn^{2+} is similar to that of Ca^{2+}, but much less of the Zn^{2+} is removed with either an ionic wash or a water wash. This result suggests that some Zn^{2+} may be held tightly within the cell in a fashion similar to Mg^{2+}.

DISCUSSION

The content of cellular K^+ that we determined was close to that reported by Epstein and Schultz (10) and Lusk et al. (25). The small

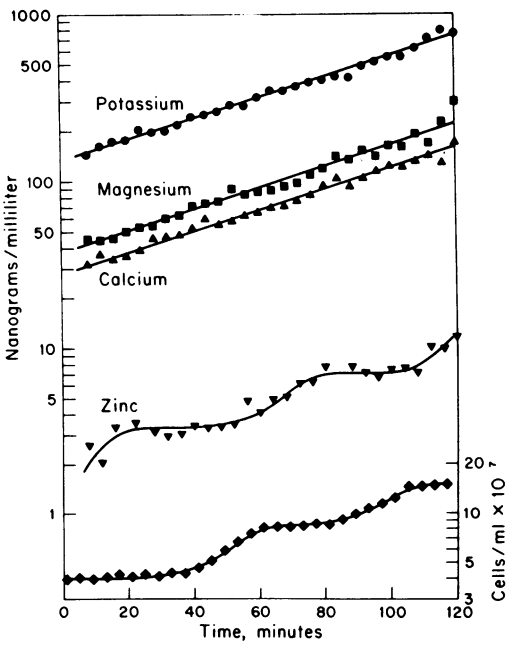

FIG. 1. *The increases of cell number (◆) and cellular potassium (●), magnesium (■), calcium (▲), and zinc (▼) in the cell cycle of Escherichia coli B/r grown in minimal glucose medium.*

TABLE 2. *Relative cellular ion contents after different washes*[a]

Ion	Wash		
	0.3 M sucrose (pH 7)	Water (pH 7.5)	0.3 M NaCl (pH 6.5)
K^+	100	54	93
Ca^{2+}	100	67	46
Mg^{2+}	100	100	100
Zn^{2+}	100	82	61

[a] All cellular ion contents have been normalized to 100 after washing with 4 ml of 0.3 M sucrose solution.

differences are probably the result of the different strains used and differences in the osmolality of growth media. It has been found (29, 30) that *E. coli* take up K^+ and excrete putrescine when the osmolality in the medium is raised. These two processes are found to be closely connected and may be mediated by the same transport system, which balances the electrochemical potential across the cell membrane. Kubitschek et al. (20) found that in a synchronous culture of *E. coli*, the rate of uptake of K^+ is constant during cell growth and doubles simultaneously with the doubling of cell number. The result of that change in rate would be a linearly increasing level of cellular K^+ during the life cycle, with a doubling in slope at the time of cell division. Our results are not sufficiently precise to enable us to distinguish linear from exponential uptake between cell divisions, but we find that the K^+ level remains approximately constant relative to cell mass. Even under conditions of higher osmolality in the medium, the K^+ level increases smoothly, suggesting that no leakage of ions occurs at cell division.

The Mg^{2+} level per cell mass in the cell cycle also remains roughly constant. Mg^{2+}, unlike K^+, has been shown to be tightly restricted within the cell, presumably bound to macromolecules. A water wash results in no loss of Mg^{2+}, although losses of K^+, amino acids have been shown (2, 34). Even washing with an 0.3 M NaCl solution does not remove cellular Mg^{2+}. Using a plasmolysis technique and measuring the high-frequency electrical conductivity of intact cells of *Streptococcus faecalis* and *Micrococcus lysodeikticus*, Marquis and Carstensen (27) found that most of the small ions in growing cells are free to move. These ions may contribute to cytoplasmic osmolality. With their technique, they could not tell which ions are not free to move. Although Hurwitz and Rosano (17) have reported that the freely soluble Mg^{2+} inside the cell is proportional to that outside the cell, their measurement was of the Mg^{2+} contained in the high-speed supernatant of the cells disrupted by passage through French pressure cells. Clearly, they could be measuring Mg^{2+}, bound to those protein and nucleic acids that are not part of ribosomes. Hurwitz and Rosano found that a prolonged wash of cells with Tris-EDTA (ethylenediaminetetraacetic acid) solution after the cells were washed for 5 min in cold Tris solution removed 30% of cellular Mg^{2+}. Apparently this Mg^{2+} is associated with high-molecular-weight substances from which it can slowly diffuse if some very strong chelating agents are present. They also found that the ribosome-bound Mg^{2+} was proportional to the Mg^{2+} concentration in the medium and that the ribosome-bound spermidine level varied inversely with extracellular Mg^{2+} concentration. It may be that no Mg^{2+} leaks out when washed by our sucrose solution containing no Mg^{2+} because the equilibrium between intra- and extracellular Mg^{2+} levels may take much more time than the wash process, or it may be that no cellular Mg^{2+}, either free or ribosome bound, can leave until some coupled reaction, such as the synthesis of spermidine, has been carried out.

Mg^{2+} is clearly required for cell growth and is associated with synthesis of DNA and protein. Marchesi and Kennell (18, 19, 26) showed that Mg^{2+} starvation limits functional ribosome content and DNA synthesis in *Aerobacter aerogenes*. Mg^{2+} content within the cell could vary during the cell cycle either independently or alternating with one of the polyamines. This hypothetical variation could be part of a control mechanism related to the length of the cell cycle, but since Mg^{2+} content appears to be quite constant during the cell cycle, fluctuating no more than K^+, it probably plays no important role in the control of cell division. Recent studies showed that the rates of synthesis of both ribosomal RNA (7) and proteins (8, 37) increase continuously and uniformly. Since ribosomes bind most of cellular Mg^{2+}, the mode of increase of Mg^{2+} in the cell cycle is very consistent with the synthesis of ribosome in cell cycle.

Both K^+ and Mg^{2+} play definite roles in the function of the cell, and both are actively transported into the cell. It has been suggested that Ca^{2+} is actively transported out of cells; Silver et al. (43) reported that extensive washing of cells on filter paper or by centrifugation will remove more than 99% of the Ca^{2+} initially associated with growing cells. Although several other studies (5, 49) have shown that most cellular Ca^{2+} may be cell surface bound, these were studies that involved disruption of cells so

that Ca^{2+} released by the opening of the cells could rebind to the new sites. As shown in Table 2, 4 ml of water wash can remove about 50% of cellular Ca^{2+} content, so that much of Ca^{2+} we measured may be internal. In this case, the level of Ca^{2+} we measured represents a sum of both cell wall-bound and intracellular Ca^{2+} pools.

We have also noticed a slight but repeatable increase in Ca^{2+} content when cells are grown in medium supplemented with extra sucrose to raise the osmolality. Since cells have a transport system for removing Ca^{2+} from the cells (43), it was possible that Ca^{2+} might play some role in division by flooding in at a crucial moment, and then being excreted until it reached a minimum level that would allow the triggering of a new cycle of division. That Ca^{2+} has no such role can be seen by the constant level maintained throughout the cell cycle.

The cellular Zn content we measured was only about one-third of that of Bucheder and Broda (3). Perhaps the difference is due to the medium and the strains used, but the Zn^{2+} content observed is also sensitive to low pH, water, or washing with a hypotonic or NaCl solution. These investigators used a water or diluted buffer wash for their cells.

The behavior of cellular Zn^{2+} is similar to that of Ca^{2+}. When cells are washed, much of Zn^{2+} behaves as if it is cell surface bound, but a substantial portion behaves as if it is internally bound. Although Chipley (5) and Webb (49) showed that about half of the Zn may be surface bound, their methods were very disruptive and could have resulted in a change of distribution, just as would be expected for Mg^{2+} and Ca^{2+}. Bucheder and Broda (3) showed that some of the cellular Zn can be quickly exchanged with Zn in the medium in the absence of metabolic energy, as if it were surface bound, but there is a substantial fraction that is slowly exchanged. The exchange of this fraction is energy dependent and represents about 85% of the Zn of the cell. They found that washing cells that had taken up ^{65}Zn with nonradioactive Zn removed much of the label. They did not try washing with slightly hypertonic solutions, but compared the Zn wash with 10 mM buffer wash. This might be similar to comparing the results of a NaCl wash to that of water wash in Table 2. The result shown in Table 2 was a 25% loss of Zn^{2+} in our hands, and a 15 to 20% loss in theirs. Thus, like Mg^{2+}, there is a fraction of internally bound Zn^{2+} that can be slowly exchanged with external pools for Zn^{2+} (or perhaps substituted with internal small molecules as shown for Mg^{2+} by Lusk et al. [25]).

The results in Fig. 1 show clearly that the increases in cellular Mg^{2+}, K^+, and Ca^{2+} content were continuous throughout the cell cycle. It is unlikely that these ions play a triggering role in the cell cycle, unless this triggering can be carried out by small changes in ion content interacting with a sensitive cellular-level detector. The cellular content of Zn^{2+} increases stepwise during the cell cycle, unlike any of the other three cations examined. The jump can be detected by using either an X-ray fluorometer or atomic absorption spectrophotometer. There is little indication of a nonzero slope during jumps, although our methods might not pick out easily the 10 to 20% increase through the flat part of the cycle representing an increase in cell-wall-bound Zn^{2+}. Apparently the rise is due mostly to the internal Zn, that portion transported into the cell. The nature of the Zn-related step remains obscure. This rise ends about 10 min before the initiation of a new round of DNA synthesis. A number of proteins related to DNA synthesis, including some of the polymerases and nucleotide synthesizing enzymes, have Zn^{2+} as cofactors, and may be affected by the Zn^{2+} jump in a way that is related to the initiation of DNA replication. Certainly there are other possibilities. Some murein hydrolases (13, 39) act on the cell sacculus during the septation process. Since some Zn^{2+} is associated with the cell surface, the action of murein hydrolases may release some Zn^{2+}, but somehow not Ca^{2+}, from its binding sites. This effect will change the pattern of a continuously increasing Zn^{2+} content to the stepwise-like pattern. Whether in fact the rise in Zn^{2+} is a cause, a result, or an epiphenomenon is not clear, but it is clearly associated with the timing of cell division.

We are currently studying the kinetics of Zn^{2+} uptake in synchronously growing culture. This study may shed some light on the nature and mechanism of the Zn^{2+} jump in the cell cycle.

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LITERATURE CITED

1. **Bellino, F. L.** 1973. Continuous synthesis of partially depressed aspartate transcarbamylase during the division cycle of *Escherichia coli* B/r. J. Mol. Biol. 74:223-238.
2. **Britten, R. J., and F. T. McClure.** 1962. The amino acid pool in *Escherichia coli*. Bacteriol. Rev. 26:292-335.
3. **Bucheder, F., and E. Broda.** 1974. Energy depend-

ent zinc transport by *Escherichia coli*. Eur. J. Biochem. 45:555-559.
4. **Chandler, M. G., and R. H. Pritchard.** 1975. The effect of gene concentration and relative gene dosage on gene output in *Escherichia coli*. Mol. Gen. Genet. 138:127-141.
5. **Chipley, J. R.** 1972. Cation uptake and exchange in *Salmonella enteritidis*. Can. J. Microbiol. 18:509-513.
6. **Coleman, G.** 1974. Nature of major inorganic ions concentrated during growth of *Bacillus amyloliquefaciens*. J. Gen Microbiol. 84:297-302.
7. **Dennis, P. P.** 1972. Stable ribonucleic acid synthesis during the cell division cycle in slowly growing *Escherichia coli* B/r. J. Biol. Chem. 247:204-208.
8. **Dennis, P. P., and R. F. Young.** 1975. Regulation of ribosomal protein synthesis in *Escherichia coli* B/r. J. Bacteriol. 121:994-999.
9. **Donachie, W. D., N. C. Jones, and R. Teather.** 1973. The bacterial cell cycle. Symp. Gen. Microbiol. 23:9-44.
10. **Epstein, W., and S. G. Schultz.** 1965. Cation transport in *Escherichia coli*. V. Regulation of cation content. J. Gen. Physiol. 49:221-234.
11. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. 108:639-644.
12. **Giauque, R. D., F. S. Goulding, J. M. Jaklevic, and R. H. Pehl.** 1973. Trace elements determination with semiconductor detector x-ray spectrometers. Anal. Chem. 45:671-681.
13. **Hartmann, R., S. B. Bock-Hennig, and U. Schwarz.** 1974. Murein hydrolases in the envelope of *Escherichia coli*. Properties *in situ* and solubilization from the envelope. Eur. J. Biochem. 41:203-208.
14. **Haydon, A. H., W. B. Davis, J. E. L. Arceneaux, and B. R. Byers.** 1973. Hydroxamate recognition during iron transport from hydroxymate-iron chelates. J. Bacteriol. 115:912-918.
15. **Helmstetter, C. E., and D. J. Cummings.** 1963. Bacterial synchronization by selection of cells at division. Proc. Natl. Acad. Sci. U.S.A. 50:767-774.
16. **Helmstetter, C., S. Cooper, O. Pierucci, and E. Revelas.** 1968. On the bacterial life sequence. Cold Spring Harbor Symp. Quant. Biol. 33:809-822.
17. **Hurwitz, C., and C. L. Rosano.** 1967. The intracellular concentrations of bound and unbound magnesium ions in *Escherichia coli*. J. Biol. Chem. 242:3719-3722.
18. **Kennell, D., and A. Kotoulas.** 1967. Magnesium starvation of *Aerobacter aerogenes*. I. Changes in nucleic acid composition. J. Bacteriol. 93:334-344.
19. **Kennell, D., and A. Kotoulas.** 1967. Magnesium starvation of *Aerobacter aerogenes*. II. Rates of nucleic acid synthesis and methods for their measurements. J. Bacteriol. 93:345-356.
20. **Kubitschek, H. E., M. L. Freeman, and S. Silver.** 1971. Potassium uptake in synchronous and synchronized cultures of *Escherichia coli*. Biophys. J. 11:787-797.
21. **Langman, L., I. G. Young, G. E. Frost, H. Rosenberg, and F. Gibson.** 1972. Enterochelin system of iron transport in *Escherichia coli*: mutations affecting ferric-enterochelin esterase. J. Bacteriol. 112:1142-1149.
22. **Levinson, W., A. Faras, B. Woodson, J. Jackson, and J. M. Bishop.** 1973. Inhibition of RNA-dependent DNA polymerase of Rous sarcoma virus by Thiosemicarbazones and several cations. Proc. Natl. Acad. Sci. U.S.A. 70:164-168.
23. **Lubin, M., and H. L. Ennis.** 1964. On the role of intracellular potassium in protein synthesis. Biochim. Biophys. Acta 80:624-631.
24. **Luria, S. E.** 1960. The bacterial protoplasm: composition and organization, p. 1-34. *In* I. C. Gunstalus and R. Y. Stanier (ed.), The bacteria, vol. I. Academic Press Inc., New York.
25. **Lusk, J. E., R. J. P. Williams, and E. P. Kennedy.** 1968. Magnesium and the growth of *Escherichia coli*. J. Biol. Chem. 243:2618-2624.
26. **Marchesi, S., and D. Kennell.** 1967. Magnesium starvation of *Aerobacter aerogenes*. III. Protein metabolism. J. Bacteriol. 93:357-366.
27. **Marquis, R. E., and E. L. Carstensen.** 1973. Electric conductivity and internal osmolality of intact bacterial cells. J. Bacteriol. 113:1198-1206.
28. **Matzura, H., B. S. Hansen, and J. Zeuthen.** 1973. Biosynthesis of the β and β' subunits of RNA polymerase in *Escherichia coli*. J. Mol. Biol. 74:9-20.
29. **Munro, G. F., K. Hercules, J. Morgan, and W. Sauerbier.** 1972. Dependence of the putrescine content of *Escherichia coli* on the osmotic strength of the medium. J. Biol. Chem. 247:1272-1280.
30. **Munro, G. F., and W. Sauerbier.** 1973. Osmotically induced excretion of putrescine by mutants of *Escherichia coli* defective in potassium transport. J. Bacteriol. 116:488-490.
31. **Nelbach, M., V. Pigiet, J. Gerhart, and H. K. Schachman.** 1972. A role for zinc in the quaternary structure of aspartate transcarbamylase from *Escherichia coli*. Biochemistry 11:315-327.
32. **Nelson, D. L., and E. D. Kennedy.** 1972. Transport of magnesium by a repressible and nonrepressible system in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 69:1091.
33. **Reid, T. W., and I. B. Wilson.** 1971. *E. coli* alkaline phosphatase. *In* P. D. Boyer (ed.), The enzymes, vol. IV, 3rd ed. Academic Press Inc., New York.
34. **Roberts, R. B., P. H. Abelson, D. B. Lowie, E. T. Bolton, and R. J. Britten.** 1957. Studies of biosynthesis in *Escherichia coli*. Carnegie Inst. of Washington, Publ. 607.
35. **Rosen, B. P., and J. S. McCless.** 1974. Active transport of calcium in inverted membrane vesicles of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 71:5042-5046.
36. **Rubin, H.** 1972. Inhibition of DNA synthesis in animal cells by ethylene diamine tetraacetate, and its reversal by zinc. Proc. Natl. Acad. Sci. U.S.A. 69:712-716.
37. **Schleif, R.** 1967. Control of the production of ribosomal protein. J. Mol. Biol. 27:41-55.
38. **Schultz, S. G., and A. K. Solomon.** 1961. Cation transport in *Escherichia coli*. I. Intracellular Na and K concentrations and net cation movement. J. Gen. Physiol. 45:355-369.
39. **Schwarz, U., A. Asmus, and H. Frank.** 1969. Autolytic enzymes and cell division of *Escherichia coli*. J. Mol. Biol. 41:419-429.
40. **Scrutton, M. C., C. W. Wu, and D. A. Goldthwait.** 1971. The presence and possible role of zinc in RNA polymerase obtained from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 68:2497-2501.
41. **Silver, S., and D. Clark.** 1971. Magnesium transport in *Escherichia coli*. Interference by manganese with magnesium metabolism. J. Biol. Chem. 246:569-576.
42. **Silver, S., P. Johnseine, and K. King.** 1970. Manganese active transport in *Escherichia coli*. J. Bacteriol. 104:1299-1306.
43. **Silver, S., K. Toth, and H. Scribner.** 1975. Facilitated transport of calcium by cells and subcellular membranes of *Bacillus subtilis* and *Escherichia coli*. J. Bacteriol. 122:880-885.
44. **Slater, J. P., A. S. Mildvan, and L. A. Loeb.** 1971. Zinc in DNA polymerases. Biochem. Biophys. Res. Commun. 44:37-43.
45. **Springgate, C. F., A. S. Mildvan, R. Abramson, J. L. Engle, and L. A. Loeb.** 1973. *Escherichia coli* deoxyribonucleic acid polymerase I. A zinc metalloenzyme. J. Biol. Chem. 248:5987-5993.
46. **Stavrianpoloulos, J. G., J. D. Karkas, and G. Chargaff.**

1972. DNA polymerase of chicken embryo: purification and properties. Proc. Natl. Acad. Sci. U.S.A. 69:1781-1785.
47. **Torriani, A.** 1968. Alkaline phosphatase subunits and their dimerization in vivo. J. Bacteriol. 96:1200-1207.
48. **Ward, C., and D. A. Glaser.** 1969. Origin and direction of DNA synthesis in *E. coli* B/r. Proc. Natl. Acad. Sci. U.S.A. 62:881-886.
49. **Webb, M.** 1970. Interrelations between the utilization of magnesium and the uptake of other bivalent cations by bacteria. Biochem. Biophys. Acta 222:428-439.
50. **Wehr, C. T., L. Waskell, and D. A. Glaser.** 1975. Characteristics of cold-sensitive mutants of *Escherichia coli* K-12 defective in deoxyribonucleic acid replication. J. Bacteriol. 121:99-107.