JOURNAL OF BACTERIOLOGY, Oct. 1973, p. 257-262

Vol. 116, No. 1
Printed in U.S.A.

Effects of External Osmolarity on Phospholipid Metabolism in *Escherichia coli* B[1]

GEORGE F. MUNRO AND CAROL A. BELL

Division of Research, National Jewish Hospital and Research Center, Denver, Colorado 80206, and Department of Biophysics and Genetics, University of Colorado Medical Center, Denver, Colorado 80220

Received for publication 7 May 1973

The turnover of total [^{32}P]phospholipids in *Escherichia coli* B is shown to be inversely related to the osmolarity of the medium and a reflection of the rates of turnover of the major phospholipid classes, phosphatidylglycerol and phosphatidylethanolamine. External osmolarity also affects the phosphatidylglycerol content of the culture. These results suggest that alterations in the metabolism of membrane phospholipids may be part of the process of adaptation to the external osmotic environment.

Osmotic adaptation may be defined as the process by which cells are able to survive and grow in media of different osmolarities. Epstein and Schultz have proposed that regulation of the internal osmolarity is an important part of the adaptive response in *Escherichia coli* and that this regulation is brought about, at least in part, through changes in the internal concentration of K^+ (8, 9). Polyamines may also play a role in osmotic adaptation. The cellular putrescine content is substantially elevated in cultures which have been adapted to media of low osmotic strength (18). Sudden addition of solutes to the medium results in rapid loss of intracellular putrescine, the final putrescine content being extremely low and characteristic of cells fully adapted to high external osmolarities. Under these conditions, loss of putrescine specifically requires simultaneous uptake of K^+ from the medium.

The metabolism of the cell membrane might be affected by the osmolarity of the external environment since the membrane is in contact with the environment and is distorted by rapid changes in external osmolarity (10). A convenient way to study membrane metabolism is to examine the metabolism of phospholipids since most, if not all, the cellular phospholipid is associated with the plasma membrane and cell wall (16, 19). Also, phospholipids are a reasonably homogeneous class of molecules which contributes less than 10% to the total cell mass (5). Approximately 70 to 80% of this phospholipid is phosphatidylethanolamine (PE), with phosphatidylglycerol (PG) being only 15 to 20% of the total (1, 2, 5). PE is usually said to be metabolically stable; the phosphate group of PG turns over with a half-life of approximately one generation (14, 15). Evidence presented here suggests that PE does turn over in vivo and that phospholipid metabolism is involved in the process of osmotic adaptation.

[1] This paper is publication 536 of the Department of Biophysics and Genetics, Univ. of Colorado Medical Center, Denver.

MATERIALS AND METHODS

Bacteria, media, and estimation of growth. Cultures of *E. coli* B were routinely grown with shaking at 37 C in minimal medium (MM) or low-osmolarity minimal medium (LOMM). MM (220 mosM) contains the basal salt mixture of Davis and Mingioli (6), supplemented with 4 mg of glucose per ml. LOMM (90 mosM) is similar to MM except that phosphate and citrate are present at one-sixth the levels found in MM. LOMM media of high osmolarity were prepared by substituting concentrated solutions of NaCl or glucose for some of the added water. Growth was monitored by following the optical density at 600 nm (OD_{600}) of the culture with a Beckman DB spectrophotometer.

Measurement of total phospholipid turnover. Cultures were grown for at least 10 generations in each medium to insure that they were completely adapted to that medium. Each culture in LOMM medium (10.6 mM phosphate) was then diluted to an initial OD of 0.05 and incubated for 2 h with 225 μCi of [^{32}P]orthophosphate per ml. Cultures in MM medium (63.6 mM phosphate) were incubated with 2,000 μCi of isotope per ml. Cells were then collected on Schleicher and Schuell B-6 membrane filters, washed, and resuspended in the same medium lacking isotope (OD, approximately 0.020). Duplicate samples were withdrawn immediately, and additional single samples were withdrawn at later times for estimation of [^{32}P]phospholipid by the Bligh-Dyer-Ames procedure (see below).

Extraction of phospholipids. For extraction by the method of Bligh and Dyer (3) as modified by Ames (1), culture samples of 0.8 ml were rapidly mixed with 3 ml of methanol-chloroform (2:1). After addition of 1 ml of chloroform and 1 ml of water, the resultant two-phase system was vortexed vigorously and allowed to stand for 3 h at 4 C. After centrifugation, the lower chloroform layers were removed and evaporated to dryness at room temperature in scintillation vials in a hood; this requires approximately 1 to 2 h. Dry residues were then dissolved by adding 0.8 ml of water and 3 ml of methanol-chloroform (2:1) and solutions were then pipetted into test tubes; this method removed more than 90% of the total radioactivity in the vials. Chloroform and water, 1 ml each, were then added to the solutions, and after vortexing, the chloroform layers were removed. Radioactivity in each sample was then determined by using a Nuclear Chicago Unilux II-A counter with no scintillation solution present. The samples were once again evaporated to dryness. As shown by thin-layer chromatography, the second extract contains all of the radioactive PG present in the first extract and at least 80% of the PE. High concentrations of NaCl and glucose in some cultures do not affect the recovery of phospholipids. This was shown by adding NaCl or glucose to methanol-chloroform-LOMM mixtures to simulate the concentrations of these solutes in media of high osmolarity.

For extraction by the method of Kanfer and Kennedy, 0.8-ml samples from radioactive cultures were mixed with 10 ml of a similar, nonradioactive culture and extracted exactly as described by those authors (15).

Thin-layer chromatography. For thin-layer separation, dried extracts were dissolved in 0.3 ml of methanol-chloroform (2:1); the vials were then rinsed with an additional 0.2 ml of the same solution. PG was found to be more readily soluble in this mixture than in chloroform; at least 75% of the total ^{32}P was removed by the mixture. The extracts were spotted on plates of Silica Gel F-254, used as supplied by the manufacturer (E. Merck, Darmstadt), and developed in chloroform-methanol-glacial acetic acid (65:25:8, vol/vol/vol; references 1, 20). With this solvent system and a similar one described by White and Tucker (25), a mixture of phosphatidylserine (PS), cardiolipin (CL), PG, and PE is resolved into four distinct spots. In the solvent described above, PS, PE, PG, phosphatidic acid (PA), and CL have R_f values of 0.16, 0.28, 0.45, 0.59, and 0.61, respectively. PA is not well separated from CL, but fortunately PA and CL represent very minor fractions of total phospholipid in *E. coli* (1) and were not included in the present studies. Developed plates containing radioactive samples were dried and autoradiograms were prepared by exposing Dupont Cronex film for approximately 16 h.

The two prominent areas of radioactivity were identified as either PG or PE by the following criteria: (i) presence of radioactivity; (ii) identity of migration rates on thin-layer chromatography with known standards (Supelco, Inc., Bellefonte, Pa.) which were visualized with iodine vapor; and (iii) mild alkaline hydrolysis to yield either glycerolphosphorylglycerol or glycerolphosphorylethanolamine, subsequently identified by paper chromatography (1, 7). By this last technique, both the PE and PG spots were shown to be free of contamination by the other. Areas containing labeled phospholipids were then scraped into scintillation vials, and radioactivity was determined after addition to each vial of 2 ml of methanol-chloroform-water (2:1:0.8).

Phospholipid composition. *E. coli* B was grown in LOMM medium in the presence of 50 μCi of [^{32}P]orthophosphate per ml or in MM medium containing 300 μCi of isotope per ml. After five generations of growth, culture turbidities were estimated with a Klett-Summerson colorimeter (green no. 54 filter) and five samples from each culture were removed for phospholipid extraction and thin-layer chromatography. Identical cultures were grown in the absence of isotope; after determining the turbidity, the protein content of these cultures was estimated by the method of Lowry et al. (17) by using bovine serum albumin as standard (Sigma Chemical Co.). For determination of the specific activity of the medium, samples were removed from the cultures immediately after adding isotope, diluted with water to appropriate levels of radioactivity, and dried in scintillation vials. Radioactivity was determined after addition of 2 ml of methanol-chloroform-water (2:1:0.8). By combining these data, one may then calculate the molar content of specific phospholipid classes and normalize these values to the protein contents of the cultures.

RESULTS

Turnover of total phospholipids in E. coli. To follow turnover of total phospholipids, logarithmically growing cultures of *E. coli* B were incubated for 2 h in the presence of [^{32}P]orthophosphate, and then the cells were collected on filters and resuspended in fresh medium of the same composition. As shown in Fig. 1, cultures grown for many generations in LOMM (approximately 90 mosM) lose radioactivity from the phospholipid fraction very rapidly; more than 80% of the label is lost after 4 h. Cultures permanently adapted to media containing various concentrations of NaCl lose radioactivity less rapidly; the higher the external NaCl concentration, the slower the rate of loss. Identical curves may be generated by following the loss of ^{32}P from cultures containing high concentrations of glucose; for example, a culture containing 0.3 M glucose loses ^{32}P at a rate intermediate between that of cultures with 0.1 M and 0.2 M NaCl. Turnover of total phospholipid thus appears to be dependent on the osmolarity of the medium.

A trivial explanation for these results would be that high concentrations of NaCl or glucose reduce the growth rates of the cultures; slowly growing cultures would then turn over phospholipids at a reduced rate. Actually, of the five cultures shown in Fig. 1, the culture containing

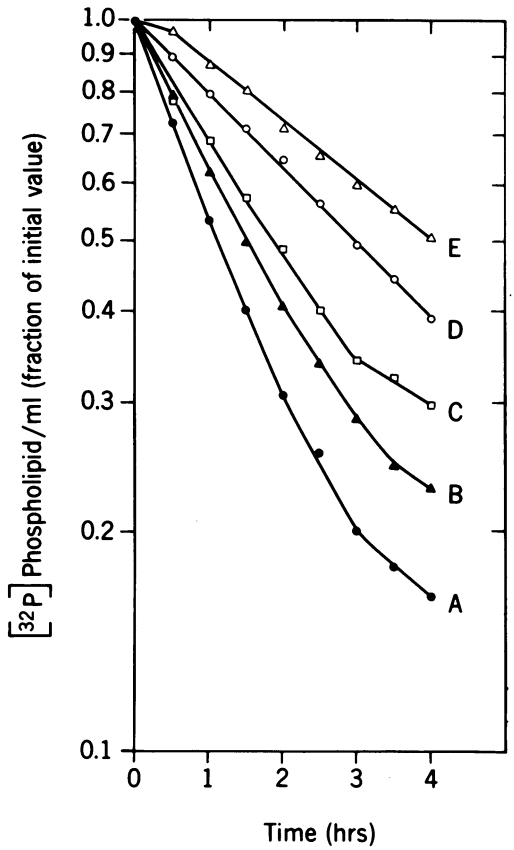

FIG. 1. *Effect of external osmolarity on turnover of total [^{32}P]phospholipids in E. coli B. Cultures of E. coli B were grown for at least 10 generations in LOMM medium (A; ●), LOMM medium containing 0.10 M NaCl (B; ▲), 0.2 M NaCl (C; □), 0.3 M NaCl (D; ○), or 0.35 M NaCl (E; △). Each culture was then incubated with [^{32}P]orthophosphate, washed, and resuspended in fresh medium as described in Materials and Methods. Samples were withdrawn at later times for estimation of [^{32}P]phospholipid by the Bligh-Dyer-Ames procedure (1, 3).*

0.1 M NaCl (curve B) grows most rapidly; its doubling time of 40 min may be compared with doubling times of 50 and 57 min for cultures in LOMM medium and LOMM medium with 0.3 M NaCl. Cultures in LOMM medium or LOMM medium with 0.3 M glucose have identical doubling times of 50 min and yet lose radioactivity at quite different rates. Cultures in media of high osmolarity (0.6 M glucose or 0.3 M NaCl) lose radioactivity at identical rates even though the doubling times are 75 and 57 min, respectively. Thus, the turnover of total phospholipid is not simply a reflection of the growth rate of the culture.

Since LOMM medium contains only 10.6 mM phosphate, it is also possible that rapid turnover is a result of partial phosphate depletion. This is not the case since cultures in MM medium (63.6 mM in phosphate; 220 mosM) lose radioactivity from the phospholipid fraction at approximately the same rate as LOMM cultures with glucose added to a total milliosmolarity of approximately 220.

The extensive degradation of radioactive phospholipids in LOMM medium could not be predicted from previous studies. Since PE is said to be entirely stable metabolically and to comprise 70 to 80% of the total phospholipid (1, 2, 5, 14, 15), no more than 20 to 30% of total radioactivity should ever be lost from the phospholipid fraction. Our results might be explained by an artifact of extraction, an alteration in the phospholipid composition of *E. coli*, and/or an alteration in the rate of PE turnover. These possibilities are discussed in the following sections.

Phospholipid composition of E. coli B. The data shown in Fig. 1 were obtained by extracting culture samples twice by using the method of Bligh and Dyer (3) as modified by Ames (1) and described in Materials and Methods. To assay the quality of the preparation, extracts were developed on thin-layer plates and autoradiograms were prepared. Samples extracted only once by this procedure contain a dark spot at the origin (probably [^{32}P]orthophosphate) and a generally high background of radioactivity from the origin to the solvent front (Fig. 2). A second extraction substantially reduces the spot at the origin and the diffuse background. It thus appears that performing a second extraction allows one to measure total [^{32}P]phospholipids in the absence of substantial levels of contaminants. (This autoradiogram reveals a higher background than usually observed for samples extracted twice; it is included to show the maximum level of contamination encountered.) Other culture samples were extracted by the method of Kanfer and Kennedy (15); this preparation lacks significant background radioactivity (Fig. 2).

For quantitative measurement of phospholipids, cultures of *E. coli* B were grown for five generations in the presence of [^{32}P]orthophosphate, and five samples of each were withdrawn for analysis. Since the protein content of the cultures and the specific activity of [^{32}P]orthophosphate were also determined, the phospholipid recoveries can be reported in moles and normalized to the protein content (see Materials and Methods). In our hands the method of Kanfer and Kennedy (15) recovers approximately half as much PE and PG as the Bligh-

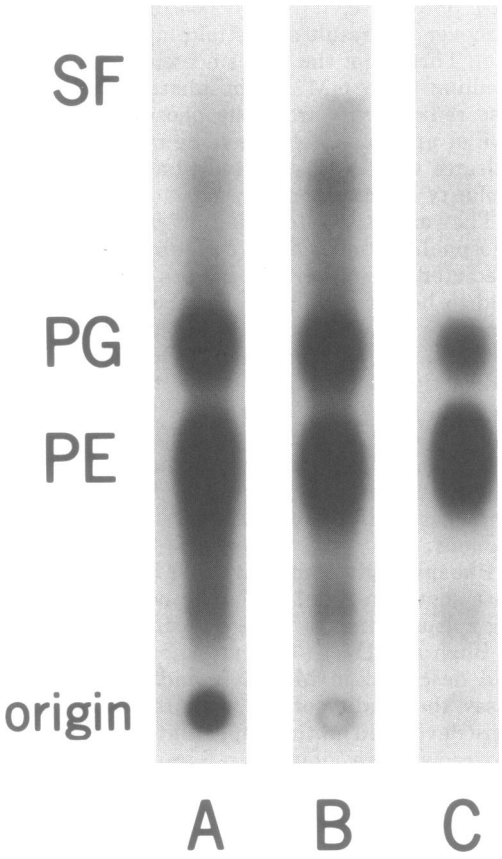

FIG. 2. *Autoradiograms of [^{32}P]phospholipids separated by thin-layer chromatography. Cultures of E. coli B in LOMM medium were labeled with [^{32}P]orthophosphate, and phospholipids were extracted as described in Materials and Methods. Sample A was extracted once and sample B was extracted twice by the Bligh-Dyer-Ames method (1, 3). Sample C was extracted by the method of Kanfer and Kennedy (15). Abbreviations: PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SF, solvent front.*

Dyer-Ames procedure (1, 3). By using the latter technique, approximately 88 μmol of PE and 25 μmol of PG/g of protein were recovered from a culture in LOMM medium. Cultures in MM medium or LOMM medium with 0.6 M glucose contained similar quantities of PE, compared with the LOMM culture, and approximately 40 μmol of PG/g of protein. These two cultures of higher osmolarity thus contain a somewhat elevated PG content. Differences in total phosphate concentration (63.6 mM for MM and 10.6 mM for LOMM with 0.6 M glucose) do not appear to affect the quantities of phospholipids recovered. It is clear that growth in media of different osmolarities does not have an effect on phospholipid composition sufficient to account for the observed rates of total phospholipid turnover. The reduction in turnover rate in media of high osmolarity must be a reflection of reduced turnover of at least some individual phospholipid species.

Turnover of PG and PE. The results of two studies of PE and PG turnover are shown in Fig. 3 and Table 1. In contrast to previous studies (2, 14, 15), both the major phospholipid components of *E. coli* B were found to undergo turnover. The rate of PE turnover is clearly dependent on the osmolarity of the medium and inversely related to it. The turnover rate of PG is less strictly dependent on the osmolarity of the medium; however, the rate of loss is consistently slower in LOMM medium than in LOMM medium containing either 0.3 or 0.6 M glucose. Under the present conditions, turnover of PE and PG is thus a function of the external osmolarity.

DISCUSSION

Results presented here indicate that the turnover of total phospholipid in *E. coli* B is affected by the external osmolarity and is especially rapid in media of low osmolarity. These rates of turnover do not arise from an artifact of phospholipid extraction or a gross alteration in the composition of the cell, and are reflected in the rates of turnover of the major phospholipid fractions of *E. coli* B. The relatively minor effects of external osmolarity on PG content should be included with other factors which affect the phospholipid composition of *E. coli*. These include temperature (21) and stage of growth of the culture (4, 22).

Since PE has been reported not to turn over in *E. coli* B, it is important to compare the present results with previous experiments. Kanemasa et al. (14) and Kanfer and Kennedy (15) have reported no loss of ^{32}P from PE over periods up to 3 h. In these studies, cultures of *E. coli* B were exposed to ^{32}P for 10 min in media of very low total phosphate concentration and osmolarity. The cultures were then either centrifuged and resuspended in high-phosphate, high-osmolarity medium (14) or diluted directly into such a medium (15). Over the subsequent "chase" period, the amount of radioactivity in the total phospholipid extract and in PE, PG, and CL fractions actually increased. For PE, the total radioactivity increased by more than 60% during the first hour of the "chase" (14).

Clearly, these studies differ in many technical respects from ours. Since in the previous studies ^{32}P continued to be incorporated into PE for a prolonged period of the "chase," such synthesis could reduce the apparent rate of turnover of

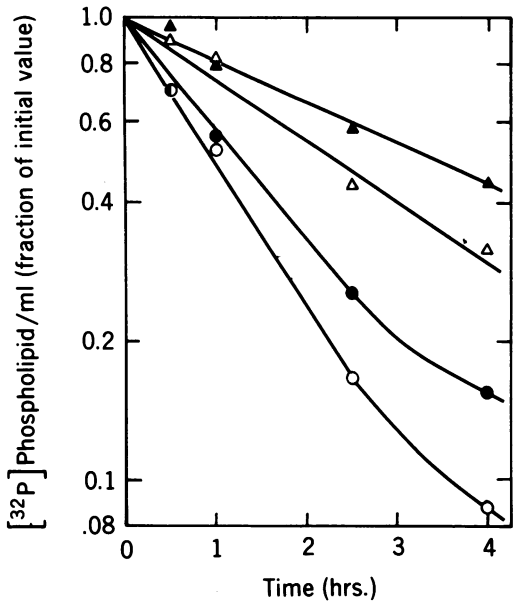

FIG. 3. *Turnover of ^{32}P-labeled PE and PG in media of low and high osmolarity. Cultures of E. coli B in LOMM medium or LOMM with 0.6 M glucose were incubated with [^{32}P]orthophosphate, washed, and resuspended in unlabeled medium as described in Materials and Methods. Samples were then extracted twice by the Bligh-Dyer-Ames procedure (1, 3) and separated on thin-layer chromatograms. Areas of radioactivity were located by autoradiography and scraped into scintillation vials. Symbols: PG from LOMM culture, O; PE from LOMM culture, Δ; PG from culture with 0.6 M glucose, ●; PE from same culture, ▲.*

TABLE 1. *Turnover of [^{32}P]phospholipids in E. coli B*

Culture medium	Osmolarity (mosM/liter)	Doubling time of culture (h)	Half-lives of [^{32}P] phospholipids (h)[a]		
			Total phospholipids	PE	PG
LOMM	90	0.83	1.2	1.7	0.92
MM	220	0.66	1.8	2.4	0.85
LOMM with 0.3 M glucose	390	0.83	2.0	2.6	1.2
LOMM with 0.6 M glucose	690	1.2	2.7	3.1	1.2

[a] Experimental details are similar to those of Fig. 3. Half-lives apply to the first 2 h of the chase period.

the PE fraction. Perhaps increasing the external osmolarity or phosphate concentration, or both, blocks exchange of intracellular and extracellular phosphate pools and thus inhibits removal of radioactivity within the intracellular pool. In our studies, both the total phospholipid extract and the PE and PG fractions lost radioactivity immediately after removal of the isotope, consistent with the conclusion that very little ^{32}P may have entered the phospholipid pools during the chase period. Certainly a general criticism of any turnover experiment is that isotope from degraded molecules may be recycled into the synthesis of new molecules of the species being studied; the measured turnover rate would then be lower than the actual value by the amount of recycling. With this in mind, we feel that the actual rate of turnover is probably at least as rapid as shown in the present experiments.

Ballesta and Schaechter reported that PE labeled with [^{3}H]glycerol did not undergo turnover for periods up to 75 min (2). Their medium is similar to MM medium, except that the osmolarity is higher than MM by approximately 200 mosM and it contains an amino acid supplement. It is possible that these differences in composition affect the turnover rate of PE; however, our results for cells grown in LOMM with 0.6 M glucose (690 mosM) demonstrated definite turnover of PE. It is more likely that the turnover rate for glycerol in PE is different from the rate for turnover of phosphate. In *Hemophilus parainfluenzae* the phosphate group of PE is turned over, whereas the glycerol moiety is not lost in four bacterial doublings (25). Ballesta and Schaechter did find "turnover" of PE under conditions of incipient lysis (2). Incipient lysis is not a plausible explanation for PE turnover in our experiments since PE was degraded in cultures in standard MM medium.

PE has been reported to turn over under two other conditions. Golden and Powell have reported instability of ^{32}P-labeled phosphatidylethanolamine during amino acid starvation of a relaxed strain of *E. coli* (12). (PE labeled with [^{14}C]acetate was much more stable, again consistent with the concept that different parts of the molecule may turn over at different rates.) Furrow and Pizer also noted a slight stimulation of PE turnover after T4 infection of *E. coli* B (11). In the latter studies, uninfected cells were shown to lose 20% of the ^{32}P from the phospholipid fraction in 80 min; this degree of turnover is consistent with our results.

Since most if not all phospholipids are located in the membrane (16, 19), the alterations in phospholipid metabolism, described here, may be part of a more general modification of the cell membrane in response to the composition of the medium. Our results may be related to observations on temperature-sensitive mutants defective in cell division. Certain of these mutants grow normally at the nonpermissive temperature if high concentrations of salts and

sugars are present (23). Other temperature-sensitive mutants, defective in deoxyribonucleic acid (DNA) synthesis, may be rescued by addition of NaCl to the medium (23). When grown at the nonpermissive temperature in the absence of NaCl, one such mutant contains very small quantities of a particular envelope protein; cultures grown at the nonpermissive temperature in the presence of 1% NaCl contain normal levels of this protein and synthesize DNA (13). For other mutants in DNA synthesis, NaCl has been shown to reverse the phenotype without reverting the membrane protein patterns to normal (24). It is not clear at present whether the composition of membrane proteins in wild-type strains is affected by the external osmolarity or ionic strength. Such alterations, in addition to changes in membrane phospholipids, could be part of the process of adaptation to the external osmotic environment.

ACKNOWLEDGMENTS

We appreciate the thoughtful comments of J. Munro and M. Pato during the course of this work and the preparation of the manuscript.

This research was supported by grant GB-36895 from the National Science Foundation.

LITERATURE CITED

1. Ames, G. F. 1968. Lipids of *Salmonella typhimurium* and *Escherichia coli*: structure and metabolism. J. Bacteriol. **95**:833-843.
2. Ballesta, J. P. G., and M. Schaechter. 1971. Effect of shift-down and growth inhibition on phospholipid metabolism of *Escherichia coli*. J. Bacteriol. **107**:251-258.
3. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911-917.
4. Cronan, J. E., Jr. 1968. Phospholipid alterations during growth of *Escherichia coli*. J. Bacteriol. **95**:2054-2061.
5. Cronan, J. E., Jr., and P. R. Vagelos. 1972. Metabolism and function of the membrane phospholipids of *Escherichia coli*. Biochim. Biophys. Acta **265**:25-60.
6. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17-28.
7. Dawson, R. M. C. 1960. A hydrolytic procedure for the identification and estimation of individual phospholipids in biological samples. Biochem. J. **75**:45-53.
8. Epstein, W., and S. G. Schultz. 1965. Cation transport in *Escherichia coli*. V. Regulation of cation content. J. Gen. Physiol. **49**:221-234.
9. Epstein, W., and S. G. Schultz. 1968. Ion transport and osmoregulation in bacteria, p. 186-193. *In* L. B. Guze (ed.), Microbial protoplasts, spheroplasts, and L-forms. Williams & Wilkins, Baltimore.
10. Fischer, A. 1903. Vorlesungen über Bakterien, p. 20-31. Fischer Verlag, Jena, Germany.
11. Furrow, M. H., and L. I. Pizer. 1968. Phospholipid synthesis in *Escherichia coli* infected with T4 bacteriophage. J. Virol. **2**:594-605.
12. Golden, N. G., and G. L. Powell. 1972. Stringent and relaxed control of phospholipid metabolism in *Escherichia coli*. J. Biol. Chem. **247**:6651-6658.
13. Inouye, M. 1972. Reversal by sodium chloride of envelope protein changes related to DNA replication and cell division of *Escherichia coli*. J. Mol. Biol. **63**:597-600.
14. Kanemasa, Y., Y. Akamatsu, and S. Nojima. 1967. Composition and turnover of the phospholipids in *Escherichia coli*. Biochim. Biophys. Acta **144**:382-390.
15. Kanfer, J., and E. P. Kennedy. 1963. Metabolism and function of bacterial lipids. I. Metabolism of phospholipids in *Escherichia coli* B. J. Biol. Chem. **238**:2919-2922.
16. Lennarz, W. 1967. Lipid metabolism in the bacteria. Advan. Lipid Res. **4**:175-225.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. **193**:265-275.
18. 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.
19. Nagata, Y., I. Shibuya, and B. Maruo. 1967. Preparation and properties of an active membrane system from *Escherichia coli*. J. Biochem. Tokyo **61**:623-632.
20. Nichols, B. W. 1963. Separation of the lipids of photosynthetic tissues: improvement in analysis by thin layer chromatography. Biochim. Biophys. Acta **70**:417-422.
21. Okuyama, H. 1969. Phospholipid metabolism in *Escherichia coli* after a shift in temperature. Biochim. Biophys. Acta **176**:125-134.
22. Randle, C. L., P. W. Albro, and J. C. Dittmer. 1969. The phosphoglyceride composition of gram-negative bacteria and the changes in composition during growth. Biochim. Biophys. Acta **187**:214-220.
23. Ricard, M., and Y. Hirota. 1969. Effet des sels sur le processus de division cellulaire d'*E. coli*. C. R. Acad. Sci. **268**:1335-1338.
24. Siccardi, A. G., B. M. Shapiro, Y. Hirota, and F. Jacob. 1971. On the process of cellular division in *Escherichia coli*. IV. Altered protein composition and turnover of the membranes of thermosensitive mutants defective in chromosomal replication. J. Mol. Biol. **56**:475-490.
25. White, D. C., and A. N. Tucker. 1969. Phospholipid metabolism during bacterial growth. J. Lipid Res. **10**:220-233.