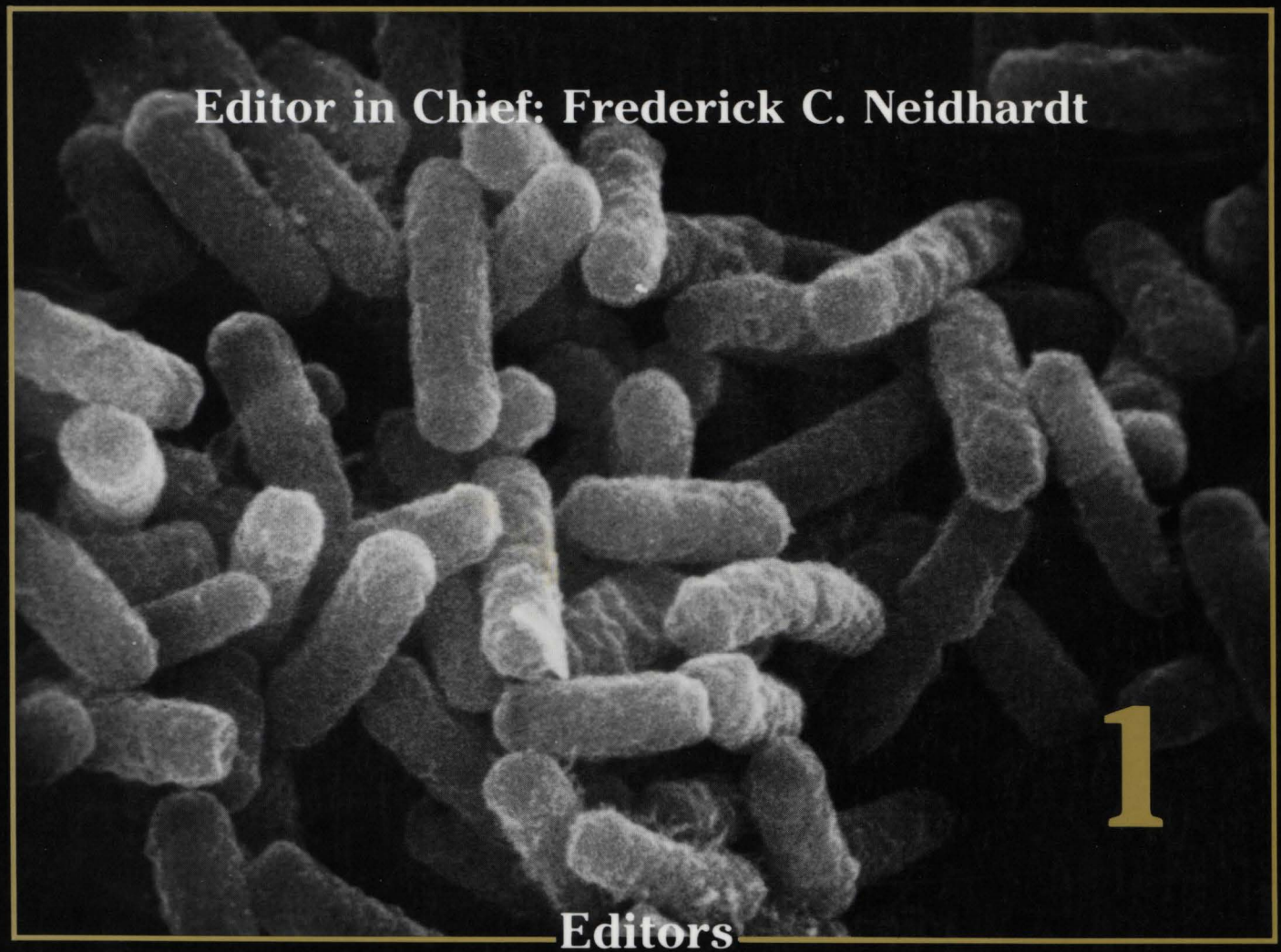
ESCHERICHIA COLI AND SALMONELLA TYPHIMURIUM

CELLULAR AND MOLECULAR BIOLOGY



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1. Introduction

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Escherichia coli and *Salmonella typhimurium* are gram-negative rods of the family *Enterobacteriaceae*. They resemble each other in most ways but differ in some essential details. Like many of the eubacteria, neither species is well delineated. *E. coli* represents a wide cluster of biotypes, whereas *S. typhimurium* is more circumscribed. Both species have been known since the early days of bacteriology, *E. coli* as a common member of the intestinal flora and *S. typhimurium* as a frequent agent of gastroenteritis.

These organisms have been preeminent in research laboratories for nearly a century. At first glance this may seem surprising because neither is a particularly good model for studying some of the more exciting phenomena in cell biology. They do not differentiate (like *Caulobacter*), sporulate (like *Bacillus*), fix nitrogen (like the closely related *Klebsiella*), photosynthesize (like *Rhodospirillum*), chemosynthesize (like *Nitrosomonas*), excrete large amounts of protein (like *Bacillus*), or grow in exotic environments (like *Thermus* or *Thiobacillus*).

The reason for the popularity of these two species in microbiological research is not quite lost in antiquity, but is somewhat complex. Since the early days, bacterial physiologists chose organisms that were easily accessible, were not highly virulent, and grew readily on defined media. *E. coli* emerged only gradually as the undisputed winner. By the late 1930s, it was the organism of choice, thanks to the work done on its bacteriophages by investigators like the Wollmans and Bronfenbrenner. This was followed by the crucial research of Monod on growth physiology and enzymatic adaptation and that of Delbruck and Luria on phage genetics. In the late 1940s its fate was sealed by the discoveries of conjugation by Lederberg and of transduction by Zinder and Lederberg. *S. typhimurium* became the popular organism for genetic and biochemical experiments that relied on transduction. Genetic analysis of cellular function became possible and was used to take full advantage of other convenient properties of these species. These include relative ease of preparing enzymatically active cell extracts and the flexible ways with which both small and large molecules can be tagged with radioactive isotopes. Labeling with different precursors was carried out extensively by a group of investigators at the Department of Terrestrial Magnetism of the Carnegie Institution of Washington. They published the earliest "*E. coli* bible" (1).

With these major advantages it is no wonder that *E. coli*, *S. typhimurium*, and their bacteriophages led to major triumphs of biology such as the elucidation of most biosynthetic pathways, the refinement of the concept of the gene, the solution of the genetic code, the discovery of molecular mechanisms of gene regulation, and the molecular portrayal of viral morphogenesis.

Today, *E. coli* and *S. typhimurium* are special to us because more is known about them than about any other cellular form of life. To give an idea of the current extent of this knowledge, about 1/3 of the gene products of *E. coli* have been studied in some biochemical detail, and their genes have been identified; of the order of 10% of the *E. coli* genome has been sequenced; and map positions have been determined for approximately half its genes. The rest are not far behind. We know perhaps 80% of the organism's metabolic pathways. We have a remarkably detailed picture of how its macromolecules are made, and we can use purified cell components to construct reasonable facsimiles of these molecules in the test tube. Less complete is our knowledge of how gene expression is regulated, for this turns out to be quite a rococo business. Nonetheless the main features of regulation can be described for many operons, and the list of control mechanisms that has been described is impressive, if somewhat daunting. Considerably less is known about how the component parts "talk" to each other to result in regulated growth or in rapid responses to changes in the environment.

Much of the work done with *E. coli* and *S. typhimurium* has been under defined laboratory conditions. Increasingly, we have become aware that this gives only a partial picture and that to understand many of the properties of these organisms we must take into account how they function in their natural environments. Alas, these environments are very complex, and it is difficult to assess the properties that are relevant from the eye view of the organism. In broad strokes, we know that strains of *E. coli* are found habitually in the large intestine of vertebrates, usually as minority members of the normal flora. They can cause diseases in other body sites, and they spread between individual hosts, often after a relatively short extracorporeal residence. Almost never do they find themselves in a constant, unchanging environment. Rather, they feed only intermittently in the large intestine and undergo partial desiccation when voided from their host. If deposited in a body of water they face the problems of nutrition at low concentrations of foodstuff. Between hosts they undergo shifts in temperature, some subtle, some drastic. These circumstances, plus some new ones, are sometimes faced by these organisms when they are grown in laboratory

media and intermittently saved in Bijou bottles, dunked in glycerol, and placed in a freezer, or lyophilized.

Given such a varied existence, it can be expected that *E. coli* and *S. typhimurium* are highly adaptive. This is, in fact, the case. These species and many of their relatives can synthesize the preponderance of their constituents, often from a single organic compound and a few minerals. Their repertoire of nutritional abilities is quite impressive. If anything, it is surprising that they have not yet learned to live on Tris buffer. They respond to changes in temperature and available nutrients by making rapid adjustments in the synthesis of regulatory molecules. As a consequence, they can vary considerably in size and chemical composition. Unamuno's dictum, "Yo soy yo y mi circunstancia" ("I am I and my circumstance"), applies to them very well.

As expected from organisms that grow in competition with others, *E. coli* and *S. typhimurium* are also highly efficient in the way they husband their energy resources. When presented with different kinds of nutrients, they grow at different rates. The richer the array of nutrients provided, the faster they grow, sparing themselves the task of synthesizing many of the compounds provided exogenously. Under conditions of active growth they synthesize the quantities of small and large molecules they require and very little extra. Only under conditions of starvation or other stresses do they accumulate constituents that they cannot use immediately. Under such conditions, a certain amount of energy is expended "inefficiently" for the sake of adaptation. For instance, nongrowing cells contain a small but significant number of ribosomes that are not engaged in protein synthesis. Although the making of these ribosomes is at a cost, their presence at the time of refeeding allows the cells to resume growth faster.

The grand strategy that allows these organisms to be both highly adaptive and very efficient is difficult to perceive. Unlike cells of metazoans, bacteria and other normally unicellular organisms have evolved an extraordinarily sensitive and complex set of controlling mechanisms that sense both the external and the internal milieux. A common characteristic of these sensing and controlling mechanisms is that they operate very fast. Thus, *E. coli* or *S. typhimurium* can go from a nongrowth state to one of active growth within seconds of the addition of nutrients to their culture. This is just what is expected from organisms that must compete with others for their food.

These organisms must often cope with strong environmental stresses, some life-threatening. They have evolved a series of "alarm reactions" which permit them to repair damaged DNA, shut off the synthesis of unwanted RNA, or protect their membranes. Most of these responses lead to the selective shut-off or turn-on of specific macromolecules. The number of regulatory devices used is very large and possibly beyond our current comprehension. Shut-off of gene expression at transcription or translation take on a bewildering number of versions. Even after proteins are made, their activity or stability can be modified. We know that our knowledge in this area is incomplete and that we have much to learn.

The impression gained is that the demands from selective pressure for efficiency and adaptability have resulted in a complex network of regulatory interactions. The constraints of space and speed do not permit the physical separation of different metabolic activities that is seen in higher cells. "Spaceship *E. coli*" is not a large space station with many compartments and with the luxury of many redundant parts and back-up systems. It must make do with fewer parts and make multiple use of many of them. If this is so, individual macromolecules can be expected to function in several ways. We have a few examples that suggest this: many proteins have not only an enzymatic or controlling function but they also regulate their own synthesis (autorepression); some polypeptides act as subunits of different enzymes or structural proteins. If this turns out to be true on a large scale, it will make it difficult to ascribe the regulatory properties of a given protein or nucleic acid to their proper place in the overall scheme. Pleiotropy reigns supreme! For this reason alone the task ahead is not trivial, although in our opinion it can be faced with optimism.

A central goal in biology is the achievement of a cellular paradigm. Until we understand thoroughly the growth of one cell, we shall be handicapped in imagining the nature of our understanding of any cell. There is little question that *E. coli*/*S. typhimurium* offer the best opportunity of achieving that paradigm. We propose two reasons: these organisms have a seemingly insuperable head start, and they can be studied by unmatched genetic and molecular techniques. Thus, they occupy a central role in current biology. Not everyone is mindful of it, but all cell biologists have two cells of interest: the one they are studying, and *Escherichia coli*!

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PART I. MOLECULAR ARCHITECTURE AND ASSEMBLY OF CELL PARTS

2. Chemical Composition of *Escherichia coli*

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INTRODUCTION

The need for determining the chemical composition of bacterial cells, especially that of *Escherichia coli*, has long been recognized. In the modern era, notable measurements of the total composition of this organism were made in 1946 by Taylor (15) and in the early 1950s by a group of biophysicists at the Department of Terrestrial Magnetism of the Carnegie Institution of Washington (12). The latter group, making full use of then newly available radioisotopes, mounted a monumental study of the composition of *E. coli*, its metabolism, and its control of biosynthetic pathways. Their report (12) has been used extensively for the past three decades. It has been such a valuable sourcebook and guide that it is often called the *E. coli* bible.

The possibility of an accurate inventory of the molecules of the cell is greater today than it was in the past. The chemical structures of the more complex components of the cell are closer to being known (parts I and IV). Advances have been made in both the isolation of cellular structures (part IV) and the resolution of complex mixtures of cellular molecules by gas chromatography, high-pressure liquid chromatography, and two-dimensional gel electrophoresis.

The value of an inventory is greater now as well. Most of the fueling and biosynthetic pathways of the cell have been described (part I, sections A and B), and the general mechanisms for the synthesis of the major macromolecules are reasonably well understood (part I, section C). Therefore, it is possible to calculate flow through pathways and to estimate growth requirements for energy, reducing power, and metabolites under defined conditions (see references 7 and 16). Such calculations help uncover areas in which knowledge is skimpy (energy costs for assembly, processing, and proofreading, for example) and can turn up discrepancies that otherwise would lay hidden. The single most important value of a chemical inventory of the cell, however, is that it frequently provides the basis for in vivo tests of theories of cell growth and regulation (see references 3 and 7).

PROBLEMS IN SPECIFYING CELL SIZE AND COMPOSITION

Aside from technical difficulties inherent in various analytical procedures, and there are many, there are some problems uniquely related to the nature of bacteria. Specifically, these include their size and compositional variation and the heterogeneity of growing cell populations.

It is obviously meaningless to talk about the size or composition of a bacterial cell without specifying the strain, the growth conditions, and the phase of growth. The stationary phase is not a unique state that can be reproducibly achieved in the laboratory, nor are the culture phases that are transitional between different stages in growth and between different states of balanced growth. Therefore, measurements intended to be compared with those made by others are ideally made on cultures in steady-state, exponential growth. At the risk of pedantry, it should be pointed out that phrases such as early-log-phase culture or late-log-phase culture are simply confessions that the investigator has not followed with care the prescriptions necessary to achieve steady-state growth and therefore to prepare an experimentally reproducible culture (e.g., see p. 267–270 in reference 7).

Since the size and the composition of the *E. coli* cell are such sensitive functions of growth rate (see chapter 6 in reference 7), it is recommended that the growth rate of a culture always be specified. The following items are suggested specifications of a particular culture used to measure a parameter of interest: (i) the organism identified by strain and source (e.g., *E. coli* B/r, obtained from S. Cooper), (ii) the medium (e.g., glucose-morpholinepropanesulfonic acid [MOPS] minimal medium as described by [cite reference]), (iii) the aeration (e.g., grown aerobically, with shaking [200 rpm, a 50-ml culture in a 250-ml Erlenmeyer flask with a Morton closure]), (iv) the temperature (e.g., 37°C), (v) the growth phase (e.g., balanced exponential growth, achieved by serial subcultivation through five mass doublings), and (vi) the growth rate (e.g., generation time = 42 min).

TABLE 1. Composition of an average *E. coli* B/r cell[a]

Component(s)	% Total dry wt[b]	Amt (g, 10^{15})/cell[c]	Mol wt	Molecules/cell	No. of different kinds of molecules[d]
Protein	55.0	156	4.0×10^4	2,350,000	1,850
RNA	20.5	58			
23 S rRNA		31.0	1.0×10^6	18,700	1
16 S rRNA		15.5	5.0×10^5	18,700	1
5 S rRNA		1.2	3.9×10^4	18,700	1
Transfer		8.2	2.5×10^4	198,000	60
Messenger		2.3	1.0×10^6	1,380	600
DNA	3.1	8.8	2.5×10^9	2.1	1
Lipid	9.1	25.9	705	22,000,000	
Lipopolysaccharide	3.4	9.7	4,070	1,430,000	1
Peptidoglycan	2.5	7.1	$(904)n$	1	1
Glycogen	2.5	7.1	1.0×10^6	4,300	1
Polyamines	0.4	1.1			
Putrescine		0.83	88	5,600,000	1
Spermidine		0.27	145	1,100,000	1
Metabolites, cofactors, ions	3.5	9.9			800+

[a] Calculated for an average cell in a population of *E. coli* B/r in balanced growth at 37°C in aerobic glucose minimal medium with a mass doubling time of 40 min. The cell is defined by dividing the total biomass, or the amount of any of its measured components, by the total number of cells in the population. This average cell, therefore, is approximately 44% through its division cycle (see reference 10 for the function describing the distribution of cell ages in a population), and, if increase in cell mass is exponential, is approximately 33% larger than when it was born. This table is modified from data in reference 7, Table 1.

[b] Relative amounts of the major components based on information in references 3, 12, and 16 and on unpublished experiments of F. C. Neidhardt (see the text). In some cases, data from strains other than B/r, from growth conditions other than the reference one, or from both had to be used (see references concerning glycogen [4], polyamine [9], and lipid [15]).

[c] Based on measurements of the total dry mass and the number of cells measured in portions of a reference culture (unpublished observations). The wet weight is calculated from the assumption that 70% of *E. coli* protoplasm is water. The total dry weight per cell is 2.8×10^{-13} g; the water content (assuming that 70% of the cell is water) is 6.7×10^{-13} g; the total weight of one cell is 9.5×10^{-13} g.

[d] Based on the following components: protein, examination of two-dimensional O'Farrell gels (T. A. Phillips and F. C. Neidhardt, unpublished observations); stable RNA, chapter 85; mRNA, assuming three genes per average transcriptional unit; lipid, an indeterminant number of species because of the variety of fatty acids associated with the following four major types of phospholipids exclusive of lipopolysaccharide: 76% phosphatidylethanolamine, 20% phosphatidylglycerol, and small amounts of cardiolipin and unidentified species (1, 11); and metabolites, cofactors, and ions, roughly estimated as described in reference 7, Table 3.

COMPOSITION OF AN AVERAGE *E. COLI* B/r CELL

The B and B/r strains of *E. coli* have been the subject of extensive biochemical and metabolic studies, more so than even the K-12 strains popular with geneticists. (Rapid growth in minimal medium, serving as host to T-even and other phages, and tight variance of cell division are some of the reasons that B and B/r strains have been favored by physiologists.) The information in Table 1 has been compiled from several sources listed in the footnotes to the table. The compilation was guided by that of Umbarger (16), and began with the overall percent composition data of Roberts et al. (12) for macromolecules. These data were then adjusted to match other information on glycogen (4), lipid (15), polyamines (9), and stable RNA/protein/DNA ratios (3). The dry weight per cell was determined in my laboratory by weighing the dried cells removed by filtration from samples of a culture which had also been assayed for total cell count with the aid of an electronic particle counter. The calculated weight per average cell was found to be consistent with the size of the DNA genome and the number of copies of DNA predicted for a cell of average age. The water content was assumed from the value commonly cited in textbooks.

RESIDUE COMPOSITION OF E. COLI B/r PROTOPLASM

The information in Table 2 is derived heavily from the primary data of Roberts et al. (12) (cited in reference 16), though the amino acid analysis has been replaced by similar results from measurements in my laboratory.

The footnotes to the table contain the sources of the other analytical data or assumptions used in the compilation or both.

FUTURE MOLECULAR INVENTORY

The tables in this chapter contain current information useful for computing various parameters for *E. coli*. It is hoped, however, that the main function served by them will be to highlight the softness and incompleteness of the information and the need for a more detailed, as well as more accurate, inventory. Many powerful tools are now available to construct an accurate inventory. In the context of ongoing efforts to identify the individual genes and proteins of this organism (see chapters 53 and 55), an explicit program to refine the overall chemical analysis of the cell makes great sense. Current interest in the assembly reactions of the cell, in the process of cell division, and

TABLE 2. Residue composition of *E. coli* B/r protoplasm[a]

Residues	Amt (μmol/g of dried cells)	Residues	Amt (μmol/g of dried cells)
Protein amino acids[b]		Lipid components[e]	
Alanine	488	Glycerol	161
Arginine	281	Ethanolamine	97
Asparagine	229	$C_{16:0}$ fatty acid (43%)	
Aspartate	229	$C_{16:1}$ fatty acid (33%)	
Cysteine	87	$C_{18:1}$ fatty acid (24%)	
Glutamate	250	Average fatty acid	258
Glutamine	250		
Glycine	582	LPS components[f]	
Histidine	90	Glucose	16.8
Isoleucine	276	Glucosamine	16.8
Leucine	428	Ethanolamine	25.2
Lysine	326	Rhamnose	8.4
Methionine	146	Heptose	25.2
Phenylalanine	176	KDO	25.2
Proline	210	Hydroxymyristic acid	33.6
Serine	205	Fatty acid ($C_{14:0}$)	16.8
Threonine	241		
Tryptophan	54		
Tyrosine	131	Peptidoglycan components[g]	
Valine	402	*N*-Acetylglucosamine	27.6
		N-Acetylmuramic acid	27.6
RNA nucleotides[c]		Alanine	55.2
AMP	165	Diaminopimelate	27.6
GMP	203	Glutamate	27.6
CMP	126		
UMP	136		
		Glycogen components (glucose)[h]	154
DNA nucleotides[d]			
dAMP	24.6		
dGMP	25.4	Polyamines[i]	
dCMP	25.4	Putrescine	34.1
dTMP	24.6	Spermidine	7.0

[a] Compiled and calculated for *E. coli* B/r in balanced growth at 37°C in aerobic glucose minimal medium, mass doubling time 40 min. This table is modified from data in reference 6, Table 10.

[b] There is 550 mg of total protein per g of dried cells (Table 1). With an average residue molecular weight of 108, there is a total of 5,081 μmol of amino acid residues. The amino acid composition is based on an analysis of *E. coli* B/r protein by T. A. Phillips, except that cysteine and tryptophan values are from reference 12, and no distinction is made between glutamate and glutamine and between aspartate and asparagine. The data have been corrected to exclude peptidoglycan (murein) amino acids. An alternative analysis is given in reference 12 and cited in reference 16.

[c] There is 205 mg of total RNA per g of dried cells (Table 1). This consists of 197 mg of stable RNA (167 mg of rRNA and 30 mg of tRNA) plus 8.3 mg of mRNA. With an average nucleotide residue molecular weight of 325, there is a total of 630 μmol of nucleotide residues. The A/G/C/U ratios are based on the analysis in reference 12, corrected for DNA.

[d] There is 31 mg of DNA per g of dried cells (Table 1). With an average residue molecular weight of 309, there is a total of 100 μmol of nucleotide residues. The ratios of individual residues are based on $(A + T)/(G + C) = 0.97$ (5, 12).

[e] There is 91 mg of total phospholipid exclusive of lipid A per g of dried cells (Table 1). With an average molecular weight of 705 (calculated as if all were phosphatidylethanolamine), there is 129 μmol of total phospholipid. The simplifying assumption is made that, of the total, 97 μmol is phosphatidylethanolamine and 32 μmol is phosphatidylglycerol, ignoring the small amounts of cardiolipin and minor, unidentified lipids (1, 11). The fatty acid composition is based on the analysis in reference 13, corrected for myristic and hydroxymyristic acid from lipid A.

[f] There is 34 mg of lipopolysaccharide per g of dried cells (Table 1). With an average molcular weight of 4,070, there is 8.4 μmol of lipopolysaccharide (LPS), assuming the following structure for the rough LPS of strain B/r: lipid A (4 hydroxymyristic acid residues, 2 saturated fatty acids assumed to be $C_{14:0}$, 2 glucosamines, 1 phosphoryl group, and 1 ethanolamine in pyrophosphate linkage); inner core (3 2-keto-3-deoxyoctulosonic acid [KDO], 1 rhamnose, 1 phosphoethanolamine, 3 heptose, 1 phosphoryl, and 1 ethanolamine in pyrophosphate linkage); and an outer core (2 glucose residues). This composition is compiled from information in several sources, including reference 2 (for lipid A) and reference 8 (for the polysaccharide portion). Different strains of *E. coli* differ slightly in LPS structure, particularly in the outer core. Many K-12 strains will have in the outer core 1 galactose, 1 glucose, and 1 acetylglucosamine residue in addition to the residues for B/r shown here (8).

[g] There is 25 mg of peptidoglycan per g of dried cells (Table 1). With an average molecular weight of 904 for a disaccharide subunit, there is 27 μmol of subunits consisting of 1 *N*-acetylglucosamine, 1 *N*-acetylmuramic acid, 1 D-glutamate, 1*m*-diaminopimelic acid, 1 D-alanine, and 1 L-alanine residue (for a review, see reference 6).

[h] There is 25 mg of glycogen per g of dried cells (Table 1). With a glucosyl molecular weight of 162, there is 154 μmol of glucosyl residues.

[i] There are 3 mg of putrescine (molecular weight, 88) and 1 mg of spermidine (molecular weight, 145) per g of dried cells (reference 9, Table 1).

in the operation of regulatory networks can benefit greatly from such an undertaking.

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