Bi 1x

Talking points: "Constant obsession with dN/dt" by F. Neidhardt

1. Be able to describe in words that the equation $\mathrm{d}N/\mathrm{d}t = kN$ means. This is a big deal. Being able to connect mathematics to concepts is essential for interpretation of measurement.
2. There are several terms in this commentary that are worth looking up to understand what they mean.
   (a) What does "diauxic" mean? What does "auxotrophic" mean? What is the significance of choosing auxotrophic bacteria when studying growth curves?
   (b) What is "mutably specific autocatalysis?"
   (c) What are replication, transcription, and translation? What molecules are responsible for them? Why do their rates show little variation?
   (d) What are regulons and operons?
3. Neidhardt uses the term "performing life." What does he mean by this? Fundamentally, how do you think life is performed?
4. Neidhardt includes a long quote from Jacques Monod in which he explains that relatively simple laws describe bacterial growth despite the enormous complexity of the underlying processes. Are we neglecting important complexity? What is the merit, if any, of simple laws?
5. It seems contradictory that Neidhardt says that "the first-order rate constant k is most conveniently expressed in minutes or hours for bacteria rather than in days, months, or years (as for most eucaryotes)," yet then says, "k for bacteria is very large, probably the largest for all Earth creatures." Is there a contradiction here?
6. Neidhardt highlights one of the challenges to maintenance of balanced growth to be "increasingly inadequate rate of gas exchange." How do we combat this in our bulk growth experiment this week?
7. Neidhardt talks about differential gene expression in response to environmental cues. We will learn more about how this works later on in the course. For now, try to dream up mechanisms by which this may occur.
8. Be ready to comment on the statement, "No description of the average bacterial cell of a given species is possible unless one specifies the growth rate." As an aside, what is meant by "the average cell"? What is meant by "the average American"?

9. The synthesis rate of DNA by DNA polymerase in *E. coli* is about 750 nucleotides per second. The genome in *E. coli* is about 4.6 million base pairs. This means that the entire genome can be replicated by a polymerase in about 100 minutes. The maximal doubling time of *E. coli*, as we will measure in the lab, is about 20 minutes. Can you reconcile this apparent contradiction?
10. Why must an investigator be an integrationist when studying bacterial growth?
11. What does Neidhardt mean when he says "We must solve the cell."? Do you think we must do this? What challenges do you think will arise as we go about solving the cell?

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GUEST COMMENTARY

Bacterial Growth: Constant Obsession with *dN/dt*

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One of life's inevitable disappointments—one felt often by scientists and artists, but not only by them—comes from expecting others to share the particularities of one's own sense of awe and wonder. This truth came home to me recently when I picked up Michael Guillen's fine book *Five Equations That Changed the World* (4) and discovered that my equation—the one that shaped my scientific career—was not considered one of the five.

I met this equation in the winter of 1952–1953 when Emanuel Suter, the bacteriology and immunology instructor in the integrated Medical Sciences program at Harvard Medical School, brought three very young colleagues to help teach the instructional laboratory of this innovative course. In this way we 20 privileged students met Boris Magasanik, Marcus Brooke, and H. Edwin Umbarger and were plunged into bacterial physiology.

They had designed a laboratory experience to introduce us to contemporary issues and cutting-edge techniques in 1950s bacterial physiology. As I remember, one objective was to study diauxic growth by varying the limiting amount of glucose added to a minimal medium containing a secondary carbon source and inoculated with an enteric bacterium. A second objective was to construct the steps in a biosynthetic pathway by examining the abilities of various compounds to satisfy the nutritional needs of auxotrophic mutants. Both experiments required measuring the growth of bacteria, the former as a kinetic process.

For me, encountering the bacterial growth curve was a transforming experience. As my partner and I took samples of the culture at intervals to measure optical density and plotted the results on semilogarithmic paper, we saw, after the lag period, a straight line developing. . .beautiful in precision and remarkable in speed. As the line extended itself straight-edge true, I imagined what was happening in the flask—living protoplasm being made from glucose and salts as the initial cells (*Klebsiella aerogenes*, they were called then) grew and divided. The liquid in the flask progressed from having a barely discernible haze to a milky whiteness thick with the stuff of life, all within a very brief Boston winter afternoon. Mutably specific autocatalysis, the physicist Erwin Schrödinger had declared a few years earlier (28), was the defining characteristic of living systems, and I had just witnessed the working out of the mathematical statement of that property, $dN/dt = kN$ (where N is the number of cells or any extensive property thereof, t is time, and k is the first-order rate constant [in reciprocal time units]).

I had never before seen such a clear display of autocatalysis. Its mathematical elegance and simplicity—but more importantly, its invitation to explore—affected me profoundly. The first-order rate constant k in the growth equation seemed to me the ideal tool by which to assess the state of a culture of cells, i.e., the rate at which they were performing life, as it were. I elected to pursue my Ph.D. studies with Boris Magasanik, studying the molecular basis of diauxic growth. Over the ensuing half-century, close analysis of growth curves was to be a central feature of my work, as I followed my intense curiosity (read obsession) about the processes that form living matter from salts and sugar. Catabolite repression, the growth rate-related regulation of stable RNA synthesis, the isolation and use of temperature-sensitive mutants in essential functions (particularly aminoacyl-tRNA synthetases), and the molecular responses of bacteria to heat and other stresses—all these studies depended on inferences and deductions from the growth behavior of bacterial cultures.

For anyone interested in the synthesis of protoplasm, bacteria are the system to study (reviewed in reference 17). With four billion years of practice they have perfected the art of growing in many environments, and they outclass all other known forms of life in their rate of metabolism geared for autocatalysis. The first-order rate constant k is most conveniently expressed in minutes or hours for bacteria rather than in days, months, or years (as for most eucaryotes). Little matter that k is not a constant for long during batch cultivation of bacteria in the laboratory or fermentation vat or that its value may vary continuously in any given natural population. Suffice it that k for bacteria is very large, probably the largest for all Earth creatures.

Of course, these organisms are appropriately studied for properties other than growth. Bacteria have evolved a dazzling array of capabilities along with rapid growth. As a group, they utilize almost any chemical source to harness energy for growth and maintenance and have mastered photosynthesis as well. They assess their chemical and physical environment with great sensitivity. They move with purpose. They communicate with each other. They employ devilishly clever strategies for colonizing eucaryotic hosts, both plant and animal. (It is through this adeptness that most humans have come to know and respect bacteria.) The spore-formers in particular are famous for enduring long periods of nongrowth under conditions hostile to life (high or low temperature, dryness, and atmospheric pressures from a vacuum to many bars of hydrostatic pressure). Even non-spore-forming bacteria differentiate from a growing form to a form remarkably able to survive prolonged periods inimical to growth.

Each of these properties is the focus of contemporary studies, intensified in many cases by useful hints supplied by knowledge of a score or more of completely sequenced bacterial genomes. In particular, the quest to understand the molecular details of the conversion of bacterial cells from growth to stationary phase has attracted the attention of scores of bacterial physiologists, as Roberto Kolter has highlighted in his

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essay in this series of Guest Commentaries (8). The multiple, coordinated processes by which bacteria achieve a nongrowing state are proving a rich ground for molecular genetic, biochemical, and ultrastructural analyses.

There is no question, however, where emphasis was placed by bacterial physiologists during much of the past half century. Rapid growth and the ease with which it can be measured in bacteria provided both the subject matter and the background for most studies in bacterial physiology from 1950 onward. Many talented bacteriologists contributed to this interest, but it was probably Jacques Monod's seminal studies in the 1940s (e.g., references 12 and 13) that inspired modern studies on bacterial growth. Development of the theory of continuous culture by Aaron Novick and Leo Szilard (19), the stimulating musings of Arthur Koch (e.g., reference 6), and detailed studies by many others can be traced to Monod's precise mathematical formulation of the growth of bacterial cultures. Roberto Kolter (8) is correct when he credits Monod with fostering growth studies. There was no question about Monod's view. Hear him, for example, in 1949 (13): "The study of the growth of bacterial cultures does not constitute a specialized subject or branch of research: *it is the basic method of Microbiology*." (Emphasis supplied.) His own classic analysis had convinced him that ". . .the growth of bacterial cultures, despite the immense complexity of the phenomena to which it testifies, generally obeys relatively simple laws, which make it possible to define certain quantitative characteristics of the growth cycle. . .The accuracy, the ease, the reproducibility of bacterial growth constant determinations is remarkable and probably unparalleled, so far as quantitative biological characteristics are concerned."

If Monod founded what we might call the Paris school of growth physiology (with scores of postdoctoral and sabbatical fellows from the United States and around the world), the Danish microbiologist Ole Maaløe was not far behind in organizing a Copenhagen school, similarly peopled by many Americans and other foreigners (reviewed in reference 10). There are few outcomes of research on bacterial growth in the second half of the 20th century that do not derive directly or indirectly from the inspired leadership that flowed from these two cities.

Did Monod's views divert attention from the stationary phase and delay serious study of the developmental processes preparing cells for survival during nongrowth? I doubt it. I suggest the opposite is true—that the attention given by Monod to the usefulness of growth analysis in bacterial physiology and biochemistry hastened the day when nongrowth could be meaningfully studied. To recognize the right questions and pursue them required an understanding and an information base gained from growth studies. The physiology of nongrowth is not the absence of the physiology of growth. Understanding both the phenotypic and genotypic processes involved in stationary-phase physiology involves building on what we understand about growth physiology.

What do growth physiologists study, and what have they learned by peering into growth flasks and chemostats the past 50 years? Much of the work has been done with *Escherichia coli* and related enteric bacteria (summarized in reference 18), but many of the findings have been found to hold generally among procaryotes.

(i) Differential gene expression in response to environmental signals leads to a cellular enzyme mélange unique to each particular environment. When growing at constant temperature with surplus nutrient, bacterial cells synthesize all of their protoplasmic constituents at near-constant differential rates and divide at a particular cell mass. Growth under constant conditions results in each cellular component increasing by the same proportion in each interval of time—the state called balanced growth (1). Often this state is maintained only transitorily because of rapid utilization of nutrients, accumulation of metabolic by-products, and increasingly inadequate rate of gas exchange—all of which induce changes in the differential rates of expression of many genes. In most instances, detrimental changes in the environment are met with changes in gene expression and hence, enzymic makeup, that permit continued growth, if only at a reduced rate. These adaptive patterns of unbalanced growth are responsible for the famous capacity of bacteria to grow under a variety of ambient conditions. It was only the advent of two-dimensional polyacrylamide gel electrophoresis that opened the eyes of bacteriologists to the magnitude of changes in the cell's protein complement (see references 5, 9, 21, 29, 30).

(ii) Certain major phenotypic characteristics are coordinated with the absolute growth rate and are completely independent of the chemical nature of the medium. Cell size and gross chemical composition (protein, RNA, DNA, carbohydrate, and lipid) are dramatically monotonic functions of the steady-state rate of growth at any given temperature. Except for the very lowest of growth rates, the faster the growth rate, the larger the cells, the richer they are in ribosomes and tRNA, and the greater their level of transcription and translation factors, including aminoacyl-tRNA synthetases (15, 17, 26). (Throughout the lower quarter of the growth rate range of *E. coli*, an irreducible minimum number of ribosomes is maintained [6]). No description of the average bacterial cell of a given species is possible unless one specifies the growth rate (17).

(iii) Over a range of growth rates at a given temperature, the rates of chain elongation of proteins, DNA, and RNA vary little, as does the time required between replication termination and cell division. From these observations, one can infer that the cell's enzymatic machinery for replication, transcription, and translation usually operate at near saturation (10, 20). Combined with the fact that the cellular levels of ribosomes and associated enzymes vary directly with growth rate, the somewhat constant rate of ribosome function makes great biological sense. Bacterial cells do not generally vary the rate of protein synthesis by changing how fast ribosomes work but by adjusting the number of ribosomes to the task at hand. Given the fact that the protein-synthesizing machinery makes up half or more of the cell's mass during fast growth, the economy of being able to reduce the rate of making this machinery during slow growth is quite evident (discussed in reference 17). At very low growth rates, the cells maintain a complement of nonfunctioning ribosomes that spring into action upon enrichment of the environment.

(iv) When environmental conditions change, forcing a reduction or permitting an increase in growth rate, the pattern of macromolecule synthesis follows a consistent pattern. On enrichment, the order of increase in rates of synthesis is RNA, then protein, and finally DNA. Frequency of cell division is the last parameter to increase. In this fashion, the cell rather quickly adopts the size and macromolecule composition characteristic of a rapidly growing cell. Upon nutrient restriction, the order of decrease in rates of synthesis is the same, with the net accumulation of RNA ceasing almost instantly, followed by declining rates of protein and DNA synthesis and of cell division. Again, the cell responds in such a way as to assume the phenotype of a small, slow-growing cell as rapidly as possible (14, 16, 24).

(v) Specific molecular mechanisms coordinate the expression of large sets of unlinked genes during growth. The number of these regulons (sets of unlinked operons controlled by a

single regulator) and modulons (sets of operons and regulons controlled by a common regulator) is very large. Prominent among those related to growth rate per se are catabolite repression (23) and the stringent response (2), both of which contribute to the economy of metabolism during growth. Catabolite repression determines by a variety of mechanisms (including, but not restricted to, cyclic AMP and its regulatory protein, cyclic AMP receptor protein) the extent to which genes encoding catabolic enzymes are expressed in the presence of their inducing substrates. It is a device that in effect enables the cell to optimize use of available carbon and energy sources. The stringent response—the granddaddy of global-control systems—directly or indirectly has dominion over a large portion of the cell's genome, restricting the synthesis of the entire translation machinery and other major cellular components during periods of limiting charged tRNA or constrained energy supply. The stringent response, whatever else it accomplishes, contributes to the ability of the cell to negotiate the transition between fast and slow growth.

Study of growth cultivates in the investigator an integrationist perspective. Analysis of problems relating to growth rate involves experimental approaches as reductionist as any in molecular biology, genetics, or biochemistry. Yet, when growth is the ultimate interest, one cannot long delve into single enzymes and genes, or even individual pathways and mechanisms, without at some point returning to the whole cell and asking about the coordinated operation of processes. One must ask not just how something works but how it works in context, i.e., in the context of all the other components and processes of the cell.

Thus, growth physiologists typically ask such disparate questions as the following: what limits the maximum growth rate, and can it be mutationally increased; can one predict accurately the effect of nutrition on the synthesis of β-galactosidase; is the level of ppGpp a sufficient predictor of the rate of ribosomal RNA synthesis during different states of balanced growth; what differentiates functioning from resting ribosomes in cells growing slowly; why are the phenotypes of many mutants (not affected in any biosynthetic reaction) different when grown on minimal and rich media; is the temperature characteristic of ribosomes what determines the Arrhenius-like response of bacterial growth to temperature; how much of the genome can be deleted without adversely affecting growth in batch liquid culture; can growth be halted without triggering stationary-phase development; can the synthesis of heterologous protein be improved by designing a cell with properties optimal for commercial fermentation processes; and so on.

Finally, the overarching context for the growth physiologist must be the ecological one: the cell in its natural environment. The revelation that *E. coli* has evolved in a nutritionally rich, well-mixed, somewhat aerobic environment without extensive surface colonization (7, 25) provides an important rationale for the elaborate measures developed by these facultative cells to achieve both speed and economy during respiratory as well as fermentative growth.

As the growth physiologist moves more and more toward integrationist studies, the goal becomes clear. We must solve the cell. That is, we must do our best to design a computer-based model that can predict overall cell behavior for steady states of growth and for transitions between steady states. The model will at first be crude, inaccurate, and a complete failure at some tasks. With increasing refinement based on additional experimental data, the model should gradually improve. Importantly, the model will guide experimental inquiry by indicating areas of inadequate, insufficient, or incorrect information. Vitally, it is only through such modeling of whole-system behavior—that is, of growth—that one will learn how near and how far our knowledge takes us toward understanding the living cell.

Securing the enormous amount of quantitative information needed for this project is daunting. The lacunae in our knowledge are only now being appreciated. Fortunately, the advent of genomics and proteomics raises expectations that, at least in the biochemical, metabolic, and genetic aspects of cell biology, there will be a wealth of data and information that will contribute the necessary real data for model building. (In fact, isn't this challenge what gives meaning and significance to the whole-genome project?) The most recent addition to the armamentarium of bacterial physiology enables monitoring of the transcriptional pattern of the whole cell through the use of cDNA hybridization to spot blots on nylon membranes or to glass microarrays (3, 22). Monitoring the complete translational pattern is not yet so facilely done, though refinement and enhancement of two-dimensional gel technology (through improved solubilization procedures for proteins and the added analytical capacity of mass spectrometry) hold promise for the future (30). With these two whole-cell monitoring techniques, one will quickly be able to apply various techniques of systems analysis to the integrated operation of the living cell. Of course, the critical cytological and topological issues around cell growth and division will continue to be a great challenge (see reference 27), but even these efforts should benefit from the materials and procedures spun off from the genome projects.

My mentor, Boris Magasanik, has described how bacterial biochemistry became molecular biology at midcentury (11). I would suggest that we are witnessing at the end of the century a transformation of similar magnitude in bacterial physiology. It is not so clear that a new name is necessary for the new field, but perhaps some descriptor to signify the change in emphasis from reductionism to synthesis might be helpful. (Suggestions, anyone?)

Returning to the start of our discussion, on reflection, the failure of Guillen, who is the science editor of a popular television program (ABC's *Good Morning America*) and Harvard instructor of physics and mathematics, to elect to write the story of $dN/dt = kN$ holds no real surprise for me. Some of my closest scientific colleagues—geneticists, many of them—have never constructed a microbial growth curve. Nor, for that matter, have many microbial biochemists, ecologists, structural biologists, and even some physiologists. I would hope, however, that current students will soon recognize the usefulness that growth measurements can play in the coming era of functional genomics and proteomics. And they may then understand what Moselio Schaechter declares about the special source of satisfaction and inspiration available to bacterial physiologists: when we meet a dry time, we can always go into the lab and construct a growth curve.

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