Molecular Microbiology (1995) 15(5), 789–794

MicroReview

On microbial states of growth†

Itzhak Fishov,[1]* Arieh Zaritsky[1] and N. B. Grover[2]
[1]*Department of Life Sciences, Ben-Gurion University of the Negev, PO Box 653, Be'er-Sheva 84 105, Israel.*
[2]*Hubert H. Humphrey Center for Experimental Medicine and Cancer Research, The Hebrew University Faculty of Medicine, PO Box 12 272, Jerusalem 91 120, Israel.*

Summary

It is crucial to the reproducibility of results and their proper interpretation that the conditions under which experiments are carried out be defined with rigour and consistency. In this review we attempt to clarify the differences and interrelationships among steady, balanced and exponential states of culture growth. Basic thermodynamic concepts are used to introduce the idea of steady-state growth in open, biological systems. The classical, sometimes conflicting, definitions of steady-state and balanced growth are presented, and a consistent terminology is proposed. The conditions under which a culture in balanced growth is also in exponential growth and in steady-state growth are indicated. It is pointed out that steady-state growth always implies both balanced and exponential growth, and examples in which the converse does not hold are described. More complex situations are then characterized and the terminology extended accordingly. This leads to the notion of normal growth and growth that can be synchronous or otherwise unbalanced but still reproducible, and to the condition of approximate steady state manifested by growth in batch culture and by asymmetrically dividing cells, which is analysed in some detail.

Introduction

Bacterial growth physiology as a rigorous discipline was born in Denmark nearly 40 years ago. It was there that a group of young scientists, later to be known as the Copenhagen School, came to realize that the absence of systematic and consistent definitions of the conditions under which cells are grown was in large measure responsible for the poor understanding (Henrici, 1928) of the mechanisms governing bacterial growth (Kjeldgaard *et al.*, 1958; Schaechter *et al.*, 1958; and see Cooper, 1993). They established rigid criteria that a batch culture had to fulfil before it could be considered suitable for meaningful studies. The following decade, the sixties, can truly be called the golden age of bacterial physiology, during which such concepts as *steady state* and *balanced growth* were defined operationally and unambiguously in the context of growing cell cultures (Campbell, 1957; Marr *et al.*, 1969; Painter and Marr, 1968). It has often been stated since that a physiological experiment performed with a poorly characterized culture is all but useless (e.g. Ingraham *et al.*, 1983; Neidhardt *et al.*, 1990), yet the requirements laid down then are rarely fulfilled in practice today. Considering the high esteem (Schaechter, 1985), almost reverence (Friesen, 1985), in which the founder of the Copenhagen School and its central figure for many years, the late Ole Maaløe, is universally held, it is surprising that despite his counsel that 'every effort must be made to achieve [true steady-state growth] before commencing serious measurements' (Ingraham *et al.*, 1983), proper specification of the state of growth has become a practice more honoured in the breach than the observance. Indeed, there is considerable confusion, particularly in the recent literature (Cooper, 1991; Neidhardt *et al.*, 1990), as to what is really meant by 'steady-state growth' and the relationship it has with both 'balanced growth' and 'exponential growth'. It is the purpose of this *MicroReview* to clarify this issue.

We begin with a few definitions from thermodynamics, extend them to living systems, and provide some examples. We also describe more complex situations that cannot be readily categorized by the current classification scheme and refine the definitions accordingly.

Basic definitions

Physical chemistry

In classical thermodynamics, *equilibrium* is a static and time-invariant state of a system where no spontaneous processes take place and all macroscopic quantities remain unchanged; *steady state* is the corresponding time-invariant condition of a system that is open to its environment (Denbigh, 1951). This latter state differs

Received 19 September, 1994; revised 17 November, 1994; accepted 21 November, 1994. *For correspondence. E-mail: fishov@bgumail.bgu.ac.il; Tel. (7) 461368; Fax (7) 276201. †Dedicated to the late Ole Maaløe, 1914–1988.

from equilibrium in that flows continue to occur and, consequently, entropy is produced (Katchalsky and Curran, 1965). Indeed, the German term for steady state, *Fliessgleichgewicht*, means, literally, flow equilibrium.

In order to apply these concepts to living systems, it is necessary to distinguish between intensive and extensive properties, again thermodynamic terms: an *extensive* variable is one whose value is proportional to the mass of a system, and an *intensive* variable is independent of the mass (Denbigh, 1951). As an example, consider a system in equilibrium divided into two parts of equal mass: the extensive properties (volume, entropy) are halved, but the intensive ones (temperature, density) remain unchanged (Denbigh, 1951). Thus, a system is in steady state when all its properties, both extensive and intensive, are independent of time.

Biology

Biological systems are almost invariably open to their environment, with flows of metabolites, energy and information; they are separated from it by envelopes that preserve the internal conditions, so different from those of the external world, that are necessary for survival. This applies to both unicellular and multicellular organisms and to viruses, but our concern here is with populations of unicellular organisms suspended in liquid medium, particularly bacterial cultures.

A living system can be in true equilibrium only when it is dormant, as in the case of suspensions of spores or bacteriophages; resting cells, prokaryotic in stationary phase or eukaryotic in G_0, are more properly classified as being in steady state, but what about growing cultures, prokaryotes in exponential phase or eukaryotes beyond G_0? Clearly, such cells cannot be included in these categories because their extensive variables increase continuously.

Microbiology

A culture of single cells can be described by both its extensive and its intensive properties. Thus attributes of the whole population, such as volume, biomass, protein and total DNA, are extensive, whereas those of the individual cells, such as size, age, lifespan and content of a specific molecular species, are intensive and can be averaged or used to construct frequency distributions (Kell *et al.*, 1991; Powell, 1956); the ratio between any two extensive variables is always intensive.

In their rigorous treatment of the mathematics of microbial populations, Painter and Marr (1968) defined *steady-state growth* as a situation in which 'the distribution of each intensive random variable' is time-invariant. The term itself was not new, having been used before, though not always consistently (e.g. Dean and Hinshelwood, 1966; Northrop, 1954; Novick, 1955; and see later). Thus, Dean and Hinshelwood (1966) considered steady-state growth to have been reached when 'the proportions of the various constituents have settled down to constant values', a condition much less restrictive than the above but identical to Campbell's (1957) *balanced growth* (a state in which 'every extensive property of the growing system increases by the same factor over a time interval'). It is clear from the context of this definition, a review of cell-division synchronization, that cell number is not included. Here we follow the usage of Painter and Marr and of Campbell.

A culture in balanced growth is also in exponential growth if the factor defined by Campbell is constant over time, and it is in steady-state growth if that same factor applies to cell number as well. These issues are treated in more detail below.

Theoretical considerations

Exponential growth

In steady-state growth, the frequency distributions (Kell *et al.*, 1991; Powell, 1956) of the various components that make up the cell are all time-invariant, by definition, implying that all components increase at the same relative rate; in other words, steady-state growth implies balanced growth. Since this includes cell mass, it also implies exponential growth. These relationships were pointed out a quarter of a century ago by Painter and Marr (1968) and widely accepted at the time (Kubitschek, 1971), but more recently seem to have been forgotten (Cooper, 1991; Neidhardt *et al.*, 1990).

Steady-state growth implies balanced growth, but the converse is not true. This can easily be seen by considering conditions that specifically block cell division without affecting growth, such as low penicillin concentrations (Donachie and Begg, 1970; Hadas *et al.*, 1995) or temperature-sensitive division mutants at restrictive temperatures (Hirota *et al.*, 1968). Whereas steady-state growth clearly implies exponential growth, again the converse does not hold — exponentially growing cultures can even be unbalanced. This is perhaps best exemplified by an experiment in which thymine-requiring cells in steady-state growth are supplied a higher concentration of thymine, the so-called 'thymine step-up regime' (Pritchard, 1974; Pritchard and Zaritsky, 1970). During the transition period between the two steady states, total cell mass continues to grow exponentially, and at the same rate, but total DNA increases more rapidly, so that the DNA/mass ratio climbs monotonically from its initial, pre-step level to its final steady-state level. Such an exponentially growing culture is therefore neither balanced, nor in steady state *a fortiori*, since the latter is actually a special case of the former.

'Steady-state growth' is thus a complete description of a culture and is not the same as 'balanced growth' or 'exponential growth', nor can it be replaced by the commonly used term 'balanced exponential growth'.

Linear growth

When the accumulation of protein-synthesizing machinery is blocked specifically (Takebe *et al.*, 1985), the rate of translation from mRNA to protein no longer increases with time. This results ultimately in a constant absolute rate of mass growth. Such a culture cannot be balanced, of course, because the various macromolecules are synthesized differentially.

Synchronous growth

If individual cells increase in mass exponentially (Cooper, 1991), then a synchronous culture also grows exponentially, but it is certainly not in steady-state growth (cells divide synchronously), and not even in balanced growth (DNA, for example, is synthesized discontinuously). Does this mean that synchronous cultures are not reproducible and so cannot form the basis of a serious study? This is not necessarily the case. Consider the situation in which all extensive variables double over a fixed time interval, τ. If, in addition, the distribution of every intensive variable is reproduced at the end of this same period, then we have what might be termed *quasi steady state*, a condition analogous to true steady-state growth but with the sampling interval restricted to τ min (Campbell, 1957; Cooper, 1991). Synchronous cultures in quasi steady-state growth are amenable to rigorous analysis in the same way as are true steady-state cultures and, in addition, can address questions unapproachable by other means.

Synchronous versus synchronized cultures

All synchronizing methods, whether physical or chemical, are bound to perturb the cellular metabolism in some way; synchronous cultures, on the other hand, because they are obtained by selection, usually provide a much better approximation of true quasi steady-state growth as defined above (Maaløe, 1962; Ingraham *et al.*, 1983; Neidhardt *et al.*, 1990).

Asymmetrically dividing cells

Not every experimental design is consistent with strict steady-state growth as defined above. Consider, for example, a budding yeast growing in batch culture. Every time a bud separates from its parent, it leaves behind a scar. These bud scars persist and accumulate, one per cell division. If we classify these cells according to the number of bud scars they carry (Lord and Wheals, 1980; Woldringh *et al.*, 1993), then it is clear that a new class is created every generation and so a steady state can never be attained, at least in principle. We distinguish three practical situations. In the first, there is a limit to the resolution of the experimental system. Formally, this is stated as follows: any cell with $s+k$ scars is identical to, and indistinguishable from, a cell with s scars for all $k>0$. We term this *experimental steady state*, and it is attained after about s generations (starting from a single cell without scars), when the oldest cell gets its s^{th} scar. Bacteria that divide by binary fission into identical daughter cells can be considered a special case of experimental steady state with $s=0$.

The second situation involves cell death, and is more difficult to handle; formally, it is expressed by the statement that cells with d scars grow slowly, if at all, but do not divide (Jazwinski, 1993). In order to distinguish it from the previous case, d must be less than the resolution of the system s. We have simulated cell growth and division for this case, beginning with a single newborn cell, for various values of d. The results are presented in Fig. 1. It is clear, and not unexpected, that the difference

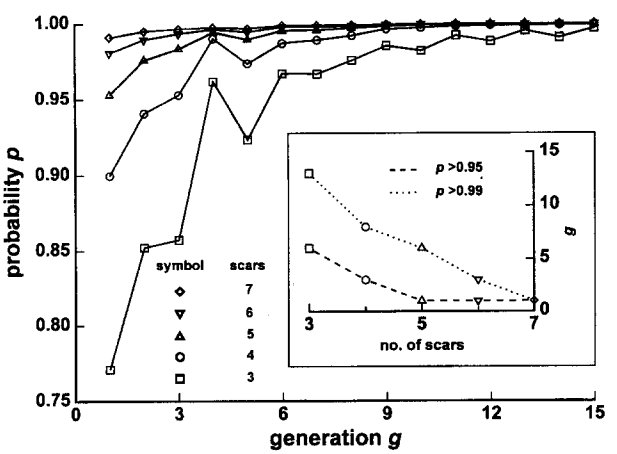

Fig. 1. Approach to the statistical steady state in budding yeast. Cell growth and division was simulated for a case in which the daughter:parent doubling-time ratio is 1.5 (Hartwell and Unger, 1977). Scar distribution was computed as function of time following senescence in the oldest cells, and compared with asymptotic distribution (attained after a very large number of generations, when it becomes essentially invariant) using a one-sample Kolmogorov–Smirnov test (Conover, 1980); two-tail probability, p, that distributions are from same underlying populations (that differences between them are due to chance, or that the simulated distribution is statistically indistinguishable from its steady-state form) is plotted against number of generations, g, following onset of senescence (solid lines), for several values of the parameter d, the number of bud scars that a parent cell accumulates before ceasing to divide. Inset: levels of g at which p first exceeds and remains above 95% (broken line) and 99% (dotted line) for these same values of d. (Note that g is in units of parental doubling time and is an integer — the various lines are meant to serve as visual aids only.)

between the scar distribution in a simulated sample and that in the corresponding asymptotic population decreases rapidly and quickly becomes statistically insignificant as the number of (parental) generations increases beyond the point when the oldest cells reach senescence. This situation can be called *statistical steady state*.

The last case we consider is in a sense the complement of the other two: the scars are distinguishable *ad infinitum* and the cells are immortal. Formally $s,\ d \rightarrow \infty$. Here, steady state is approached asymptotically, the proportion of new cells being 2^{-g}, where g represents the number of cell doublings since the inoculation of the culture from a single cell and is approximately equal to the number of bud scars on the oldest cell: this is *asymptotic steady state*.

The above discussion has been presented in terms appropriate to budding yeast, because that is the context in which it first arose (Grover and Woldringh, 1995), but it applies to any growing system containing identifiable entities that reflect genealogical age, such as the DNA in semi-conservative replication or the inert poles of bacilliform bacteria.

Microbial growth in practice

In what follows, we illustrate some of the definitions and concepts presented above. Our treatment is by no means exhaustive; it is intended to supplement rather than supersede the standard laboratory manuals on the subject.

The growth cycle in batch

Inoculating a sample of unicellular microorganisms into fresh medium results in a *batch culture* that passes through three distinct phases: lag, exponential (more commonly, but less appropriately, termed logarithmic) and stationary, and ends in the final phase, death. A batch culture is in steady-state growth only if all its intensive properties remain constant. In practice, depending on the nature of the intended experiment, this is taken to obtain when the rates of cell division and mass doubling become equal (Maaløe and Kjeldgaard, 1966) or, more rigorously, when the frequency distribution of at least one variable becomes time-invariant (Steen, 1990; Woldringh *et al.*, 1980). Steady-state growth can be attained only during the exponential phase of a culture and only when the composition of the growth medium is constant. Current procedures employ a low initial inoculum (Cooper, 1991; Dean and Hinshelwood, 1966; Ingraham *et al.*, 1983; Maaløe and Kjeldgaard, 1966; Neidhardt *et al.*, 1990; Schaechter, 1985) followed by successive dilutions in fresh, prewarmed, identical medium. In this way, an extended exponential phase is achieved with maximum growth rate. In principle, such a regime provides merely an approximation to true steady-state growth, but it is a very good one, because whatever changes do occur in the intensive properties of the culture lie beyond experimental resolution.

Continuous cultures

Chemostats and turbidostats (Kubitschek, 1971; Monod, 1950; Novick and Szilard, 1950a) permit a degree of flexibility and control not available with the earlier methods. In the chemostat, the rate of cell growth is regulated by limiting the supply of a growth factor, so that growth control is external, whereas in the turbidostat, all nutrients are provided in excess, allowing the cells to approach their inherent maximum rate of division, control thus being essentially internal (Kubitschek, 1971). This latter type of growth is also termed *unrestricted* (Schaechter *et al.*, 1958), and has been found to fulfil the conditions required for steady state. (The state of the whole system, including cells, nutrients and waste products, was originally defined as 'stationary' (Novick and Szilard, 1950b) but later renamed 'steady state' (Novick, 1955) to avoid confusion among microbiologists; in the physical sciences, the two terms are interchangeable (Katchalsky and Curran, 1965).) Whether a chemostat culture, with its restricted growth, ever attains a steady state, is still under debate (Cooper, 1991; Kell *et al.*, 1991; Novick and Szilard, 1950b; Schaechter *et al.*, 1958).

Accumulation of mutants in a chemostat confirming the Neo-Darwinian view of the spontaneous origin of bacterial mutation (Luria and Delbrück, 1943) was one of the first applications of this device (Novick and Szilard, 1950b). The existence of spontaneous mutation, it should be noted, precludes the possibility, at least in principle, of ever achieving a true long-term steady state, owing to the slow but inevitable increase over time of the mutant fraction.

Balanced, normal, and steady-state growth

Mass growth in the absence of cell division, such as occurs at low penicillin concentrations (Donachie and Begg, 1970; Hadas *et al.*, 1995), is an extreme case of balanced growth without steady state. A more subtle situation exists, namely that of a balanced culture which, although dividing exponentially, is still not in steady state. This was discovered in fast-growing *thy* strains limited by the exogenous thymine concentration (Pritchard, 1974; Pritchard and Zaritsky, 1970; Zaritsky and Pritchard, 1971). Under such conditions, there is a slight perturbation in cell division that causes the rate of increase in cell number to be somewhat less than that of total mass, resulting in a continuous enlargement in average cell size (Zaritsky and Pritchard, 1973). The difference in rates depends on

the thymine concentration and can be eliminated in *drm* mutants by the addition of deoxyguanosine (Pritchard, 1974; Zaritsky and Pritchard, 1971). This phenomenon precludes the possibility of obtaining meaningful estimates of average cell mass or genome content, but the DNA doubling time in such cultures is the same as that of mass, so the DNA/mass ratio does stay constant. This led to the concept of *normal growth*, defined (Zaritsky and Pritchard, 1971) as growth in which (i) mass increases exponentially at a fixed rate that does not change when thymine concentration is varied, (ii) the DNA/mass ratio remains fixed at a level that depends on the external thymine concentration (indicating that mass growth is not limited by DNA concentration), and (iii) the ratio between particles and colony-forming units is close to unity (implying that the viability is unimpaired). These criteria were found necessary in order to distinguish between normal growth under *thymine limitation*, and *thymine starvation*. The latter is a totally different physiological state: during thymine starvation of *thy* mutants, the cells continue to accumulate protein and RNA at the pre-starvation rate for a time, but DNA synthesis is specifically inhibited (Barner and Cohen, 1956; Campbell, 1957; Cohen and Barner, 1954). As a consequence, the DNA concentration decreases continuously (Pritchard, 1974; Zaritsky and Pritchard, 1971; 1973) and cells lose their capacity to form colonies on agar plates (Cohen and Barner, 1954) — a clear case of *unbalanced growth*. The discovery of thymine starvation raised the need to define a state of balanced growth (Campbell, 1957) so that a distinction could be made between it and the case of specific inhibition of cell division (at low penicillin concentrations (Donachie and Begg, 1970; Hadas *et al.*, 1995), say, or at restrictive temperatures (Hirota *et al.*, 1968) in *fts* mutants).

Unbalanced growth

This concept was originally used (Barner and Cohen, 1956; Cohen and Barner, 1954) to describe situations in which the normal ratio of nuclear to cytoplasmic components is disturbed (Campbell, 1957). More generally, it includes all cases in which the rate of synthesis of at least one cellular component differs from that of the others, as for example during the transition from exponential to stationary phase.

Are unbalanced growth conditions reproducible and properly defined? Starting from a well-defined state of growth (steady or balanced) and perturbing the culture by any established means ensures reproducibility. There are many ways by which unbalanced growth is obtained, generally involving specific inhibition of macromolecular synthesis. Other, secondary, effects, however, begin to occur soon after the onset of such inhibition. The primary act of nalidixic acid, for instance, is to block DNA synthesis specifically (Goss *et al.*, 1965), thereby mimicking thymine starvation (Zaritsky, 1975), but the effect on the balanced synthesis of RNA and protein is concentration-dependent (Goss *et al.*, 1965). Blocking of RNA synthesis by antibiotics (rifampicin for initiation of transcription, for example, or streptolydigin for elongation) is quickly followed by the inhibition of protein synthesis, then by a gradual reduction of DNA synthesis up to a complete halt within an hour (Lark, 1972). Inhibition of protein synthesis by starvation for one or more required amino acids is associated with an immediate inhibition of RNA synthesis in wild-type strains and a differential block of mRNA in *rel* mutants (Lazzarini and Winslow, 1970), followed by a slow deceleration of DNA synthesis (Goss *et al.*, 1965). Indeed, it is impossible to obtain a clear specific effect because of the multitude of interactions among the various pathways in a bacterial cell.

A more refined state of unbalanced growth occurs when a particular small-molecular-weight building block is added to a culture in well-defined growth. Here, the total rate of macromolecular synthesis is infinitesimally increased and practically indistinguishable from that in the unperturbed culture. The instantaneous change in the degree of repression of the particular pathway involved, however, will result in an asymptotic approach of the system to a new steady-state level (Maaløe and Kjeldgaard, 1966).

Concluding remarks

Traditionally, microbiology is not included among the exact sciences; nevertheless, modern microbiology is fast becoming less descriptive and more quantitative. This process requires clear and unambiguous terminology as well as meaningful results obtained from well-designed experiments performed reproducibly. The rapid progress now being made in molecular biology and its obvious attraction to biologists in all fields and of all ages, although certainly justified, has led to the neglect of classical bacterial physiology. It is not uncommon to read in the current literature of a culture in 'late log', 'mid exponential', 'early stationary' and the like in the description of an experiment requiring isolation of an enzyme or a plasmid. One should be aware of the fact that certain plasmids are amplified during the stationary phase of their host and that patterns of gene expression can change with the physiological state of the cell, as in the case of sporulation in Gram-positive bacteria.

This *MicroReview* was aimed at clarifying the differences and inter-relationships among steady, balanced, and exponential states of culture growth. These basic terms were defined decades ago, but their original meanings and distinctions have become blurred with time. In addition, several new situations have arisen over the

years that require further and more refined definitions. We trust that this *MicroReview* will help promote the rigour of our experiments and clarify the conclusions drawn from them.

Acknowledgements

We wish to thank Conrad L. Woldringh, Robert H. Pritchard, Charles E. Helmstetter and Stephen Cooper for fruitful discussions, the last of these also for inadvertently making us aware of the problem, and Mario Baras for technical help. This work was partially supported by a Guastalla Fellowship of the Fondation Raschi (to I.F.) and by Grant No. 91-00190/2 from the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel (to A.Z.).

References

Barner, H.D., and Cohen, S.S. (1956) *J Bacteriol* **72:** 115–123.

Campbell, A. (1957) *Bacteriol Rev* **21:** 263–272.

Cohen, S.S., and Barner, H.D. (1954) *Proc Natl Acad Sci USA* **40:** 885–893.

Conover, W.J. (1980) In *Practical Nonparametric Statistics*, 2nd edn. New York: Wiley, p. 344.

Cooper, S. (1991) In *Bacterial Growth and Division: Biochemistry and Regulation of the Division Cycle of Prokaryotes and Eukaryotes.* San Diego: Academic Press, pp. 7, 8, 27, 150, 375.

Cooper, S. (1993) *J Gen Microbiol* **139:** 1117–1124.

Dean, A.C.R., and Hinshelwood, C. (1966) *Growth, Function and Regulation in Bacterial Cells.* Oxford: Clarendon Press, p. 106.

Denbigh, K.G. (1951) *The Thermodynamics of the Steady State.* London: Methuen, p. 1.

Donachie, W.D., and Begg, K.J. (1970) *Nature* **227:** 1220–1224.

Friesen, J.D. (1985) In *The Molecular Biology of Bacterial Growth.* Schaechter, M., Neidhardt, F.C., Ingraham J.L., and Kjeldgaard, N.O. (eds). Boston: Jones and Bartlett, pp. 373–376.

Goss, W.A., Deitz, W.H., and Cook, T.M. (1965) *J Bacteriol* **89:** 1068–1074.

Grover, N.B., and Woldringh, C.L. (1995) *J Theor Biol,* in press.

Hadas, H., Einav, M., Fishov, I., and Zaritsky, A. (1995) *Microbiology*, in press.

Hartwell, L.H., and Unger, M.W. (1977) *J Cell Biol* **75:** 422–435.

Henrici, A.T. (1928) *Morphologic Variation and Rate of Growth of Bacteria.* Microbiology Monographs. London: Baillière, Tindall and Cox.

Hirota, Y., Ryter, A., and Jacob, F. (1968) *Cold Spring Harbor Symp Quant Biol* **33:** 677–693.

Ingraham, J.L., Maaløe, O., and Neidhardt, F.C. (1983) *Growth of the Bacterial Cell.* Sunderland: Sinauer Associates, pp. 5, 248.

Jazwinski, S.M. (1993) *ASM News* **59:** 172–178.

Katchalsky, A., and Curran, P.F. (1965) *Nonequilibrium Thermodynamics in Biophysics.* Cambridge: Harvard University Press, p. 66.

Kell, D.B., Ryder, H.M., Kaprelyants, A.S., and Westerhoff, H.V. (1991) *Antonie van Leeuwenhoek* **60:** 145–158.

Kjeldgaard, N.O., Maaløe, O., and Schaechter, M. (1958) *J Gen Microbiol* **19:** 607–616.

Kubitschek, H.E. (1971) *Introduction to Research with Continuous Cultures.* Englewood Cliffs: Prentice-Hall, p. 1.

Lark, K.G. (1972) *J Mol Biol* **64:** 47–60.

Lazzarini, R.A., and Winslow, R.M. (1970) *Cold Spring Harbor Symp Quant Biol* **35:** 383–390.

Lord, P.G., and Wheals, A.E. (1980) *J Bacteriol* **142:** 808–818.

Luria, S.E., and Delbrück, M. (1943) *Genetics* **28:** 491–511.

Maaløe, O. (1962) In *The Bacteria.* Gunsalus, I.C., and Stanier, R.Y. (eds). *Vol. 4: The Physiology of Growth.* New York: Academic Press, pp. 1–32.

Maaløe, O., and Kjeldgaard, N.O. (1966) *Control of Macromolecular Synthesis: A Study of DNA, RNA, and Protein Synthesis in Bacteria.* New York: Benjamin, p. 57.

Marr, A.G., Painter, P.R., and Nilson, E.H. (1969) *Symp Soc Gen Microbiol* **19:** 237–261.

Monod, J. (1950) *Ann Inst Pasteur* **79:** 390–410.

Neidhardt, F.C., Ingraham, J.L., and Schaechter, M. (1990) *Physiology of the Bacterial Cell: A Molecular Approach.* Sunderland: Sinauer Associates, pp. 3, 6, 199, 216, 394.

Northrop, J.H. (1954) *J Gen Physiol* **38:** 105–115.

Novick, A. (1955) *Annu Rev Microbiol* **9:** 97–110.

Novick, A., and Szilard, L. (1950a) *Science* **112:** 715–716.

Novick, A., and Szilard, L. (1950b) *Proc Natl Acad Sci USA* **36:** 708–719.

Painter, P.R., and Marr, A.G. (1968) *Annu Rev Microbiol* **22:** 519–548.

Powell, E.O. (1956) *J Gen Microbiol* **15:** 492–511.

Pritchard, R.H. (1974) *Proc Roy Soc* **B267**: 303–336.

Pritchard, R.H., and Zaritsky, A. (1970) *Nature* **226:** 126–131.

Schaechter, M. (1985) In *The Molecular Biology of Bacterial Growth.* Schaechter, M., Neidhardt, F.C., Ingraham, J.L., and Kjeldgaard N.O. (eds). Boston: Jones and Bartlett, pp. 370–372.

Schaechter, M., Maaløe, O., and Kjeldgaard, N.O. (1958) *J Gen Microbiol* **19:** 592–606.

Steen, H.B. (1990) In *Flow Cytometry and Sorting.* New York: Wiley–Liss, pp. 605–622.

Takebe, Y., Miura, A., Bedwell, D.M., Tam, M., and Nomura, M. (1985) *J Mol Biol* **184:** 23–30.

Woldringh, C.L., Grover, N.B., Rosenberger, R.F., and Zaritsky, A. (1980) *J Theor Biol* **86:** 441–454.

Woldringh, C.L., Huls, P.G., and Vischer, N.O.E. (1993) *J Bacteriol* **175:** 3174–3181.

Zaritsky, A. (1975) *J Bacteriol* **122:** 841–846.

Zaritsky, A., and Pritchard, R.H. (1971) *J Mol Biol* **60:** 65–74.

Zaritsky, A., and Pritchard, R.H. (1973) *J Bacteriol* **114:** 824–837.