USE OF MICROORGANISMS FOR STUDIES OF GROWTH AND MORPHOGENESIS

W. R. LOCKHART

Department of Bacteriology, Iowa State College, Ames, Iowa

CONTENTS

I. INTRODUCTION

Many students of microbial physiology are dedicated to the idea that a cell is a cell is a cell. They hope that what is learned of a bacterium will be applicable, with appropriate reservations, to other cells. This approach has been fruitful in some instances, notably in the study of intermediary metabolism. It remains to be seen whether such concepts can be extended with profit and any degree of impunity to discovery of the fundamental relationships between structure, form, and function in living cells. Beginnings have been made; microbial genetics, for example, has added significantly (if sometimes confusingly) to our information on the mechanisms of hereditary transmission and control of the life-process. It now seems legitimate to inquire whether studies with bacteria and other microorganisms may not similarly cast some light on the most complex, though fundamental, of all biological phenomena: growth and morphogenesis. It is not the purpose of this review to attempt an inclusive cataloguing of all the past experiments with protists having possible significance in this context, but to call attention to some illustrative and provocative data which may indicate those areas of current general hypothesis most susceptible to analysis by the microbiologist.

II. A COMPARATIVE PHYSIOLOGY OF GROWTH

Microbes are at least superficially parallel to more complex organisms in their growth behavior. Microorganisms in culture produce distinct growth patterns, divisible into recognizable phases on the basis of changes in proliferation rates and physiological activity of the cells (58). The sigmoid curve obtained when this growth-time relationship is plotted is of course not unique, but is characteristic of biological growth in general. Furthermore, patterns of culture growth essentially similar to those of microorganisms are shown by isolated mammalian cells when they are cultivated *in vitro* under like experimental conditions (56). Such patterns occur also when mammalian cells are grown *in vivo* (29). Observations of the effects of inoculum size on growth patterns in cultures of microorganisms (85) are paralleled by reports of similar effects with *in vivo* implantation of sarcoma cells (29). The requirement for cell division of a high concentration of available sulfhydryl groups, and the general effects of —SH in growth and morphogenesis of the higher metazoans (6), have analogies in reports that —SH is required for cell division in yeast (63, 71) and that both cellular reducing activity (42) and —SH content (60) are greatest during

periods of active proliferation in cultures of bacteria.

Experiments with the slime molds (9, 36, 84) have illustrated mechanisms of environmental and genetic control of morphogenesis, providing another analogy. Anomalies in genetic control of morphogenesis provide most of the markers used in genetic studies with plants and animals, and environmental—especially chemical—interference with morphogenesis is a classic technique of experimental embryology. Slime molds are considered particularly favorable for such studies because of a fortuitous dissociation in these organisms between growth and morphogenesis, so that it is often possible to analyze one process without complications being introduced by the presence of the other.

Another such dissociation, this time between growth and cell division, has been exploited in numerous studies with bacteria and yeasts. A variety of agents (53) are capable of selectively inhibiting cell division in microorganisms; growth continues virtually at unaltered rates, with the formation of elongated, filamentous cells. Such filaments have been compared with normally dividing cells (38, 63, 64, 67, 80) by workers in search of clues as to the mechanism of cell division.

It is perhaps unfortunate that much of the study of growth and morphogenesis in microorganisms has been based upon the thesis that some of the complex, interrelated phenomena involved could thus be studied in isolation from the rest. It is sometimes stated, for example, that bacteria provide a good test system for the study of growth because in these simple forms of life the growth process is uncomplicated by the superimposition of differentiation and morphogenesis. Yet phenomena at least resembling morphogenesis do occur among bacteria. The fruiting bodies of myxobacteria constitute a primitive morphogenetic structure, and all bacteria when grown on the surface of solidified culture media produce colonies which are distinctive, recognizable morphological entities. Even in liquid cultures, as Henrici (33) pointed out long ago, bacteria show phases of cytological and physiological development analogous to those occurring in cells of more complex organisms.

III. MECHANISMS OF GROWTH CONTROL

A. Growth vs. Differentiation

Progress from embryonic to differentiated cell states in complex organisms apparently involves the development of new metabolic patterns directed toward elaboration of specialized products rather than simply toward quantitative increase of protoplasmic mass. Possibly this process includes changes in the quantitative contributions of fermentation and respiration to energy metabolism (57, 87). After proliferation has ceased in cultures of bacteria, substrates continue to be utilized and the cells show changes in composition and physiological response which might be attributed to further growth unaccompanied by cell division (50). But there is no significant change in protoplasmic mass during this period (2), indicating that any alterations in cell behavior must result from the functioning of metabolic pathways which utilize nutrient materials for processes other than growth and cell division. At the end of active proliferation in cultures of *Escherichia coli*, there is in fact a shift in carbohydrate dissimilation from apparent predominance of a hexose-monophosphate pathway to greater quantitative importance of the Embden-Myerhoff pathway (2). Such shifts are noted also in yeasts and molds at a comparable period in culture development (7, 32). Selective inhibition of cell division with 5-diazouracil does not precipitate a similar metabolic change (2).

The "morphogenesis" induced in yeast or bacteria by interference with cell division is filamentation, not true morphogenesis in the sense of the development of differentiated metabolic patterns and structures. Filamentous bacteria are metabolically like normally dividing cells in most respects (38, 63, 64, 67). Differentiation thus appears not to be simply an altered ratio between rates of growth and cell division, which ordinarily are nearly equivalent. Even though normal growth and division may alternate in some cyclic fashion (15, 73, 82), inhibition of the division phase of this cycle neither interferes substantially with growth nor triggers a metabolic shift to a more differentiated state. Although they are not totally incompatible, there doubtless is a certain amount of antagonism between differentiation and morphogenesis on one hand and growth and cell

division on the other, probably because of competition for energy between the alternative metabolic pathways involved. These pathways may share some common intermediates; possibly both are functional at all times and chemical transitions from "growth" to "differentiation" are largely quantitative in nature.

B. Factors Limiting Growth or Promoting Differentiation

1. Nutrient availability. Without at the moment inquiring further into details of the chemical nature of these postulated alternative metabolic routes, we may now seek a clue as to the mechanisms by which readjustments in their quantitative use by the cell might be initiated. A number of factors have been implicated as having controlling influences on growth; nutrient availability is one of these. Thus, exhaustion of a limiting nutrient has been suggested as the cause for cessation of growth in cultures of bacteria (11, 47) and protozoa (14), and as an initiating factor for morphogenesis in yeasts and slime molds (64, 84). Sussman (84) has reported, in fact, that myxamoebae from any stage of the growth cycle may be induced to begin aggregation and morphogenesis simply by subjecting them to nutrient deficiency. The same sort of nutrient deficiency could easily occur in tissue cells of more complex organisms, for example by a cytoplasmic screening which controls the nutrient concentration available to the nucleus (25) or by changes in permeability of cell membranes. The latter could be a consequence of new molecular configurations at the cell surface, resulting from stereochemical interactions with molecules on adjacent cells (89) or from the presence in the cell cortex of substances which, accumulating with age, eventually interfere with transport of materials across the cell membrane (43).

Probably the deprivation of nutrient need not be complete in any case; it has been suggested that the ability of bacteria to continue growth is a function of the *per cell* concentrations of limiting nutrilites (22, 34). The further utilization, in bacterial cultures, of amounts of a limiting nutrient which were insufficient to support continued proliferation is accompanied by a spectrum of cellular changes in physiological response, suggesting that growth and certain other processes (differentiation?) are subject to different chemical equilibria (50). Growth may thus cease (or slow) and differentiation commence (or increase) as a result of new equilibrium states brought about by limitations in the number of available molecules of nutrient. These equilibria may be sensitive to changes in relative as well as absolute concentrations of certain substrates. Both the degree of physiological change and the extent of shift in glucose dissimilative pathways in cultures of bacteria upon cessation of growth are more marked when growth is limited by the concentration of carbon substrate than when nitrogen is limiting (2, 50). In *Neurospora* (35) and in yeast (63), morphogenesis is reported to proceed more readily if the nitrogen supply is low in comparison to carbon.

The specific metabolic steps subject to these postulated equilibria are of course not known, but such controlling processes have been observed experimentally in bacteria; it is reported (83), for example, that when the supply of two-carbon units available for oxidation is drastically reduced, there occurs an accumulation of oxalacetate which prevents the conversion of succinate to fumarate, blocking the tricarboxylic acid cycle and conserving these metabolites against future need. At any rate, once such equilibria had been established as a result of nutrilite limitations brought about either by the influence of external environment or by cortical changes in the cells themselves, there would be an opportunity for development of new enzymatic patterns subject to different equilibria and leading to differentiation rather than growth. Spiegelman (79) and Stanier (81) have presented models, based upon studies of enzyme induction in microorganisms, showing that changes in the available nutrient substrates may induce new enzyme systems in genetically unaltered cells, culminating through sequential induction in an entirely new metabolic pattern.

These relationships can be quite complex. Not only do relative concentrations of the substrates and products of key reactions influence equilibria and the elaboration of new enzyme systems through induction, but accumulation of "normal" metabolites may have more direct effects. In microbial systems, products of certain metabolic sequences sometimes act as competitive antagonists of earlier metabolites in the

same pathway, effectively lowering enzyme activity and exerting a "negative feedback" effect on metabolism (24*a*, 93). Furthermore, the end products of some metabolic pathways actually inhibit *formation* of enzymes required to complete earlier steps in the reaction sequence. This phenomenon has been observed both with bacteria (24*a*, 93) and with cultured mammalian cells (24*b*).

2. Availability of hydrogen acceptors. Oxygen tension (or the availability of hydrogen acceptors) appears also to affect both microbial and mammalian cells in similar fashion. Many workers have suggested that oxidation-reduction effects exert a controlling influence on growth and morphogenesis, with high potentials selectively favoring differentiation whereas more reduced conditions are most favorable for growth (57, 87). High oxygen tensions are apparently inhibitory to growth of bacteria, especially during early culture growth (3, 24, 41) or when nutriments are in short supply (17, 91). Even under normal conditions, growth of bacteria (48, 61) and protozoa (13) is optimum at intermediate rather than high aeration levels. The optimal oxygen tension appears to be characteristic for a particular cell clone, with various species and strains of bacteria showing widely different patterns of response to variations in rate of oxygen supply (48). The selective action of changing oxygen availability on mixtures of cell types will be discussed later.

Shifts in metabolic patterns or cell composition as a result of changes in oxygen tension are fairly commonplace among microorganisms (*e.g.*, 62, 72, 75). It is not difficult to imagine that characteristic differentiated patterns could result from local concentration gradients of oxygen or other hydrogen acceptors occurring in tissue masses of more complex cell communities (57). Similar concentration gradients of metabolically produced carbon dioxide have been suggested as a controlling factor by Loomis (52) on the basis of the demonstrated effects of this substance on growth and differentiation in *Hydra* and other organisms. A possibly similar effect of carbon dioxide on differentiation in the mold *Blastocladiella* has been studied by Cantino (19), who believes that metabolic accumulation of carbon dioxide prevents decarboxylation in the citric acid cycle, halting growth and causing accumulation of ketoglutarate which then serves as a hydrogen acceptor in a new series of reactions leading to formation of melanin, a characteristic product in differentiated cells. It is interesting, though not necessarily pertinent, to note at this point that a large-colony variant (*i.e.*, presumably one with an increased growth potential) of the pneumococcus has reduced respiratory capacity and produces endogenous CO_2 only in the presence of added glucose (27).

3. Autospecific cell products. There are indications also that cells may produce more or less autospecific inhibitory or stimulatory substances which control their own growth. The literature abounds with reports of possible growth-limiting factors being produced in cultures of bacteria (*e.g.*, 23, 37, 92) and protozoa (78), in mammals during wound healing (5), and perhaps even in plant tissue cultures (20). Although the information in these reports has been somewhat contradictory, the general consensus of opinion is that such materials are dialyzable, diffusible, extremely thermolabile, nonfilterable or only partially filterable, and are destroyed by most organic solvents. They appear to inhibit the growth of the cells which produce them, and to inhibit other (often related) cells to varying degrees. Unfortunately no one has yet isolated or characterized chemically such an autoinhibitory agent, and some workers (*cf.* 49) are not convinced that they even exist. Nevertheless it is not possible entirely to dismiss them.

Other investigators have reported the production of two diffusible, antagonistic substances—one inhibitory and one stimulatory—by protozoa (39) and by echinoderm larvae (70), whereas still others (1, 51) believe that bacteria and yeasts produce a diffusible inhibitory material and an antagonistic, intracellular, stimulatory substance which is released only upon disruption or injury of the cell. These observations are strikingly similar to Weiss's general theory of growth control (90) based upon the presumed existence of specific, intracellular growth "templates" and diffusible, antagonistic "antitemplates." A single cell product which is stimulatory at low concentrations and inhibitory at high concentrations has been reported (54) to control the characteristic pattern of growth in *Chilomonas*, whereas a single substance produced by yeast cells (86) perhaps either stimulates or inhibits growth depending upon a dynamic equilibrium involving the cells and the culture medium.

4. Complex interactions. This last notion suggests that it may not be necessary to decide *which* of the factors mentioned thus far controls growth, but rather to determine the nature of the interaction among some or all of them. There is evidence that such interactions do exist. Reports that the effects of oxygen tension on growth are altered under conditions of low availability of nutrients were mentioned earlier; this effect is manifested during carbon deficiency but not when the cells are starved for nitrogen (17), indicating again the importance of carbon to nitrogen ratios as well as absolute concentrations. Failure of bacteria to grow further in "staled" cultures, whether attributed to exhaustion of nutrient (11) or to the presence of inhibitory cell products (92), is reported to be more marked on agar media than in broth. This could easily be a result of the greater availability of oxygen on an agar surface, similar to the situation wherein rough mutants of *Brucella* become established in broth cultures but not on agar where lack of available oxygen does not become a selective factor (4). Staled agar media will support further surface growth, in fact, if incubated under conditions of lowered oxygen availability (49) though not if incubated anaerobically. Oxygen tension appears to mediate the inhibitory effects of a number of agents; for example, the toxic effect of copper ions on enzyme activity in rat heart homogenates is intensified at high oxygen tensions (31), carcinogenic chemicals are more toxic for bacteria under anaerobic than aerobic conditions (12), and bacteria under conditions of low nutrient availability require lowered oxygen tensions for aerobic growth under environmental stresses such as above-optimal temperatures or exposure to sunlight (17). It is likely that the growth process depends upon a quite complex equilibrium involving any or all of the agencies suggested above, and may be controlled in particular cases by manipulation of any of a number of seemingly unrelated factors.

IV. ABNORMAL GROWTH

A. Implications for the Study of Normal Growth

It is not fashionable to omit from a discussion of growth phenomena some mention of the Cancer Problem. Hirschberg (36) has reviewed some of the contributions of microbiology to cancer research. Much of this work has been limited to the use of microorganisms for screening of potentially carcinostatic agents or for working out biochemical details of the mode of action of these agents and the mechanism of cellular resistance to their toxic effects. But he points out that indirect benefits may accrue from other, more fundamental studies such as the investigations of morphogenesis in slime molds. Although it may not be necessary to explain life before we can understand the cause of neoplasia (88), it is perhaps not wise to invert the statement. Study of the unique properties of tumor cells can contribute much to our understanding of normal growth.

Neoplastic cells do possess unique patterns of metabolism (30, 87). Such patterns could be the result of a new response to the complex equilibria mentioned above, making the cell less likely to shift from "growth" to "differentiation" pathways and thus endowing it with an increased growth potential and lessened capacity for differentiation. The neoplastic change could consist of blocking of one of the alternative pathways, as by respiratory impairment (87); or quantitative overemphasis of one pathway, as by the acquisition of nutritional autonomy *via* increased ability to synthesize a critical metabolite available exogenously only in rate-limiting quantities (40); or metabolic by-passing of a process especially sensitive to one of the controlling variables, such as carbon dioxide (52). None of these needs be the exclusive means by which normal cells acquire neoplastic properties; it would be possible for different tumors to arise in different ways, or for more than one of these mechanisms to be involved in the origin of a single tumor. All these changes, however, could produce strikingly similar metabolic consequences, so that one might study "THE metabolism of THE tumor cell" (16) without regard for possible diversity of initial causes.

B. Origin of Tumor Cells

As to the origin of such altered cells, one popular hypothesis is that they arise by a somatic mutation. Studies with microorganisms have provided examples of the sort of changes which may occur. The mutagenicity of known carcinogenic agents applies also to bacteria (77), and in at least one case bacteria which had survived treatment with potent chemical car-

cinogens were shown (12) to possess altered growth characteristics. Tumors are presumed to develop after the original mutational event by a selective process, in which the tissue environment—be it deficient in critical nutrilites (40), deficient in oxygen (87), oversaturated with carbon dioxide (52), or simply unsuitable for maturation in a general sense (8)—confers an advantage on the few mutant cells present and permits them eventually to predominate. Selection of mutant types in heterogeneous cell populations of bacteria has been widely studied. Lederberg has demonstrated (44) the selective advantages accruing to nutritionally autonomous cells by analogy with selection of prototrophic mutants in cultures of bacterial auxotrophs, and has provided a possible link from such phenomena to the virus theory of tumor etiology with his suggestion (46) that virus-induced genetic modification may constitute the original alteration. The availability of oxygen or other hydrogen acceptors is also an important selective factor in mixed bacterial cultures (4, 21, 28). Furthermore, both bacteria in pure culture (55) and free sarcoma cells growing in the mouse peritoneal cavity (29) appear spontaneously to produce cells with an increased growth potential in the prevailing environment; these cell types soon become predominant in the culture. The appearance *in vitro* of apparently malignant cells among populations of cultured mammalian cells is commonplace (76). In bacteria, the change in growth potential was shown to be under genetic control, with separate loci governing growth rate and the total attainable population density (55).

V. CONCLUSIONS

From the foregoing it is possible to indicate some areas of investigation which show promise for future research. Many lines of experimentation already in progress will continue to yield useful data. Studies with filamentous cells were cited earlier; such investigations are providing important information regarding the chemical mechanisms of cell division in yeast (65, 66, 68). Techniques for achieving synchrony of division in cultures of microorganisms are being constantly improved (18) and may be expected to contribute to our knowledge of the growth process, as will the work being done with microorganisms in continuous culture (69). Study of the genetic control of morphogenesis, and its physical and chemical mechanisms, is progressing rapidly in work with the amoeboid slime molds (9, 84) and with water molds (19, 26). Undoubtedly similar attention will be devoted to the slime bacteria and to colonies of eubacteria (10). Since morphogenesis and tumor development are essentially problems in cellular ecology, regardless of whether individual differences originate with genetic change or with less fundamental metabolic modifications, we may anticipate further significant contributions from investigations, similar to those cited above, which deal with cell-cell interactions among bacteria in a selective environment, with direct or virus-mediated genetic exchange, and with the plasticity of metabolic response in genetically unaltered cells under the influence of a changing environment.

Some important questions—concerning the nature of complex equilibria such as those postulated earlier, the biochemical details of subsequent shifts in metabolism, and the influence on these of genetic and environmental factors—seem especially susceptible to analysis with the techniques of microbiology. But, since the most obvious advantage of working with microorganisms is the greater degree of experimental control which can be exercised by the investigator, an attractive alternative is to apply microbiological methodology to the study of other kinds of cells. Such applications have in fact been made in work with ascites tumors (40, 45), in growing mammalian cells in submerged culture (56), and in the ingenious experiments of Puck (74) with clonal isolates of cultured mammalian cells. This type of approach is still in an early stage of development, however, and suffers sometimes from a lack of strict definition of experimental conditions, especially with regard to control of nutritional environment (59). The fact that it has been easier to achieve something approaching full control of the experimental material in microbial systems suggests that microorganisms themselves are less complex, with the growth process stripped of superficial trappings and reduced to only its more fundamental manifestations. Growth of bacteria might thus be limited and controlled by the same factors, and "differentiation" may proceed along essentially similar lines, but to a less chemically elaborate final

result than in the development of, say, a chick embryo.

It has been said that biological research methods consist essentially of substituting easy, unimportant problems for difficult, important ones. We must accept this risk; perhaps there exist no "blueprints" for life, perhaps the process of growth with its associated phenomena is not quantitatively but qualitatively different in protists and in the complex, higher metazoa. Subject to this note of caution, the possibilities for comparative studies are obvious, and clearly a need is indicated for continuing liaison among those who investigate growth in the various forms of life. The healthy scepticism (or suspicion) with which each of us tends to regard practitioners in other disciplines can be relied upon to impose a decent circumspection on any temptation to progress prematurely toward the eventual goal of a unified concept of biology.

VI. REFERENCES

1. ADELSTEIN, S. J., HERSHEY, F. B., LOOFBOUROW, J. R., AND SIZER, I. W. 1953 The stimulation of yeast growth and respiration by compounds produced by yeast cells irradiated with ultraviolet light. J. Cellular Comp. Physiol., **40**, 269–278.
2. ALLEN, S. H. G. AND POWELSON, D. M. 1958 Pathways of glucose oxidation in dividing and nondividing cells of *Escherichia coli*. J. Bacteriol., **75**, 184–189.
3. ALLYN, W. P. AND BALDWIN, I. L. 1930 The effect of the oxidation-reduction character of the medium on the growth of an aerobic form of bacteria. J. Bacteriol., **20**, 417–437.
4. ALTENBERN, R. A., WILLIAMS, D. R., KELSH, J. M., AND MAUZY, W. L. 1957 Metabolism and population changes in *Brucella abortus*. II. Terminal oxidation and oxygen tension in population changes. J. Bacteriol., **73**, 697–702.
5. AUERBACH, E. 1952 An inhibiting effect observed in the course of normal wound healing. Proc. Soc. Exptl. Biol. Med., **81**, 294–296.
6. BARRON, E. S. G. 1953 The importance of sulfhydryl groups in biology and medicine. Texas Repts. Biol. and Med., **11**, 653–670.
7. BEEVERS, H. AND GIBBS, M. 1954 Participation of the oxidative pathway in yeast respiration. Nature, **173**, 640–641.
8. BERENBLUM, I. 1954 A speculative review: The probable nature of promoting action and its significance in the understanding of the mechanism of carcinogenesis. Cancer Research, **14**, 471–477.
9. BONNER, J. T. 1957 A theory of the control of differentiation in the cellular slime molds. Quart. Rev. Biol., **32**, 232–246.
10. BORNSIDE, G. H. AND RICHARDSON, R. L. 1958 Giant colony patterns exhibited by a pseudomonad. J. Bacteriol., **75**, 89–97.
11. BROOM, J. C. 1929 The exhaustion of media in bacterial culture. Brit. J. Exptl. Pathol., **10**, 71–83.
12. BROWN, A. 1951 The action of some water-soluble carcinogens on bacteria. J. Infectious Diseases, **89**, 59–75.
13. BROWNING, I., BERGENDAHL, J. C., AND BRITTAIN, M. S. 1952 Cellular reproduction efficiency in various oxygen concentrations. Texas Repts. Biol. and Med., **10**, 790–793.
14. BROWNING, I., BRITTAIN, M. S., AND BERGENDAHL, J. C. 1952 Synchronous and rhythmic reproduction of protozoa following inoculation. Texas Repts. Biol. and Med., **10**, 794–802.
15. BRUCE, V. G., LARK, K. G., AND MAALØE, O. 1955 Turbidimetric measurement on synchronized *Salmonella typhimurium* cultures. Nature, **176**, 563–564.
16. BURKE, D. AND SCHADE, A. L. 1956 On respiratory impairment in cancer cells. Science, **124**, 270–272.
17. CAHN-BRONNER, C. E. 1940 Oxygen requirement of pathogenic bacteria under starving conditions. Proc. Soc. Exptl. Biol. Med., **45**, 454–456.
18. CAMPBELL, A. 1957 Synchronization of cell division. Bacteriol. Revs., **21**, 263–272.
19. CANTINO, E. C. 1956 The relation between cellular metabolism and morphogenesis in *Blastocladiella*. Mycologia, **48**, 225–240.
20. CAPLIN, S. M. AND STEWARD, F. C. 1952 Investigations on the growth and metabolism of plant cells. II. Variables affecting the growth of tissue explants and the development of a quantitative method using carrot root. Ann. Botany (London), **16**, 219–234.
21. CHARLTON, G. 1955 Direct antagonism in mixed bacterial populations. J. Bacteriol., **70**, 56–59.
22. CLEARY, J. P., BEARD, P. J., AND CLIFTON, C. E. 1935 Studies of certain factors influencing the size of bacterial populations. J. Bacteriol., **29**, 205–213.
23. COBLENTZ, J. M. AND LEVINE, M. 1947 The effect of metabolites of *Escherichia coli* on the growth of coli-aerogenes bacteria. J. Bacteriol., **53**, 455–461.

24. Dagley, S., Dawes, E. A., and Morrison, G. A. 1951 The effect of aeration on the growth of *Aerobacter aerogenes* and *Escherichia coli*, with reference to the Pasteur mechanism. J. Bacteriol., **61**, 433–441.

24*a*. Davis, B. D. 1955 Competitive inhibition of microbial growth by normal metabolites. In *Antimetabolites and cancer*, pp. 19–32. Am. Assoc. Advance Sci., Washington, D. C.

24*b*. Demars, R. 1958 The inhibition by glutamine of glutamyl transferase formation in human cells. Biochim. et Biophys. Acta, **27**, 435–436.

25. Dounce, A. L. 1950 Cytochemical foundations of enzyme chemistry. In *The enzymes*, vol. I, pp. 264–266. Edited by J. B. Summer and K. Myrbäck. Academic Press, Inc., New York.

26. Emerson, R. 1955 The biology of the water molds. In *Aspects of synthesis and order in growth*, pp. 171–208. Princeton Univ. Press, Princeton, N. J.

27. Ephrussi-Taylor, H. 1954 A new transforming agent determining the pattern of metabolism of glucose and lactic acid in Pneumococcus. Exptl. Cell Research, **6**, 94–116.

28. Fukui, G. M., Ogg, J. E., Wessman, G. E., and Surgalla, M. J. 1957 Studies on the relation of cultural conditions and virulence of *Pasteurella pestis*. J. Bacteriol., **74**, 714–717.

29. Goldie, H. and Felix, M. D. 1951 Growth characteristics of free tumor cells transferred serially in the peritoneal fluid of the mouse. Cancer Research, **11**, 73–80.

30. Greenstein, J. P. 1954 *Biochemistry of cancer*, 2nd ed. Academic Press, Inc., New York.

31. Haugaard, N., Hess, M. E., and Itskovitz, H. 1957 The toxic action of oxygen on glucose and pyruvate oxidation in rat heart homogenates. J. Biol. Chem., **227**, 605–616.

32. Heath, E. C. and Koffler, H. 1956 Biochemistry of filamentous fungi. II. The quantitative significance of an oxidative pathway during growth of *Penicillium chrysogenum*. J. Bacteriol., **71**, 174–181.

33. Henrici, A. T. 1928 *Morphologic variation and the rate of growth of bacteria*, 194 pp. Charles C Thomas, Springfield.

34. Hershey, A. D. and Bronfenbrenner, J. 1937 On factors limiting bacterial growth. Proc. Soc. Exptl. Biol. Med., **36**, 556–561.

35. Hirsch, H. M. 1954 Melanogenesis and the differentiation of protoperithecia in *Neurospora crassa*. Bacteriol. Proc., **1954**, 88. (Abstract)

36. Hirschberg, E. 1955 Some contributions of microbiology to cancer research. Bacteriol. Revs., **19**, 65–78.

37. Jandasek, L. 1950 Vznik Produktu vlastniho metabolismu mikrobu v jednoduchych pudach. Biol. listy, **31**, 72–75.

38. Katchman, B. J., Spoerl, E., and Smith, H. E. 1955 Effects of cell division inhibition on phosphorus metabolism of *Escherichia coli*. Science, **121**, 97–98.

39. Kidder, G. W. 1941 Growth studies on ciliates. V. The acceleration and inhibition of ciliate growth in biologically conditioned medium. Physiol. Zoöl., **14**, 209–226.

40. Klein, E. 1955 Gradual transformation of solid into ascites tumors. Evidence favoring the mutation-selection theory. Exptl. Cell Research, **8**, 188–212.

41. Knox, R., Lister, A. J., and Thomas, C. G. A. 1957 The effect of oxygen on growth of *Mycobacterium tuberculosis* in semi-solid agar. J. Gen. Microbiol., **17**, ix–x. (Abstract)

42. Kopper, P. H. 1952 Studies on bacterial reducing activity in relation to age of culture. J. Bacteriol., **63**, 639–645.

43. Lansing, A. I. 1951 Calcium as an indicator and regulator of cell activities. Science, **114**, 685.

44. Lederberg, J. 1946 A nutritional concept of cancer. Science, **104**, 428.

45. Lederberg, J. 1956 Prospects for a genetics of somatic and tumor cells. Ann. N. Y. Acad. Sci., **63**, 662–665.

46. Lederberg, J. 1957 Viruses, genes and cells. Bacteriol. Revs., **21**, 133–139.

47. Levine, S. 1953 Staling in membrane filter cultures. J. Bacteriol., **66**, 624–625.

48. Lockhart, W. R. and Ecker, R. E. 1958 Apparatus for independent control of aeration rates in a series of cultures. Applied Microbiol., **6**, 93–96.

49. Lockhart, W. R. and Powelson, D. M. 1953 Staling in bacterial cultures. J. Bacteriol., **65**, 293–296.

50. Lockhart, W. R. and Powelson, D. M. 1954 Control of cell division in bacterial cultures. J. Cellular Comp. Physiol., **44**, 177–189.

51. Loofbourow, J. R. and Morgan, M. N. 1940 Investigation of the production of growth-promoting and growth-inhibiting factors by ultra-violet irradiated microorganisms. J. Bacteriol., **39**, 437–453.

52. Loomis, W. F. 1957 Sexual differentiation in *Hydra*. Science, **126**, 735–739.

53. Loveless, L. E., Spoerl, E., and Weisman, T. H. 1954 A survey of effects of chemicals

on division and growth of yeast and *Escherichia coli*. J. Bacteriol., **68**, 637–644.

54. MAST, S. O. AND PACE, D. M. 1938 The effect of substances produced by *Chilomonas paramecium* on rate of reproduction. Physiol. Zoöl., **11**, 359–382.
55. MCDONALD, D. J. 1955 Segregation of the selective advantage obtained through ortho-selection in *Escherichia coli*. Genetics, **40**, 937–950.
56. MCLIMANS, W. F., GIARDINELLO, F. E., DAVIS, E. V., KUCERA, C. J., AND RAKE, G. W. 1957 Submerged culture of mammalian cells: the five liter fermentor. J. Bacteriol., **74**, 768–774.
57. MOMENT, G. B. 1952 A theory of differentiation. Growth, **16**, 231–242.
58. MONOD, J. 1949 The growth of bacterial cultures. Ann. Rev. Microbiol., **3**, 371–394.
59. MORGAN, J. F. 1958 Tissue culture nutrition. Bacteriol. Revs., **22**, 20–45.
60. MORTENSON, L. E. AND BEINERT, H. 1953 Appearance of sulfhydryl groups during growth of *Bacillus globigii*. J. Bacteriol., **66**, 101–104.
61. MOSS, F. 1952 The influence of oxygen tension on respiration and cytochrome a_2 formation in *Escherichia coli*. Australian J. Exptl. Biol. Med. Sci., **30**, 531–540.
62. MOSS, F. 1956 Adaptation of the cytochromes of *Aerobacter aerogenes* in response to environmental oxygen tension. Australian J. Exptl. Biol. Med. Sci., **34**, 395–406.
63. NICKERSON, W. J. 1950 Role of nutrition in the morphogenesis of yeasts. Intern. Congr. Microbiol., Abstracts of Papers, 5th Congr., Rio de Janeiro, 130–131.
64. NICKERSON, W. J. 1954 An enzymatic locus participating in cellular division of a yeast. J. Gen. Physiol., **37**, 483–494.
65. NICKERSON, W. J. AND FALCONE, G. 1956 Enzymatic reduction of disulfide bonds in cell wall protein of baker's yeast. Science, **124**, 318–319.
66. NICKERSON, W. J. AND FALCONE, G. 1956 Identification of protein disulfide reductase as a cellular division enzyme in yeasts. Science, **124**, 722–723.
67. NICKERSON, W. J. AND SHERMAN, F. G. 1952 Metabolic aspects of bacterial growth in the absence of cell division. II. Respiration of normal and filamentous cells of *Bacillus cereus*. J. Bacteriol., **64**, 667–678.
68. NICKERSON, W. J., TABER, W. A., AND FALCONE, G. 1956 Physiological bases of morphogenesis in fungi. 5. Effect of selenite and tellurite on cellular division of yeastlike fungi. Can. J. Microbiol., **2**, 575–584.
69. NOVICK, A. 1955 Growth of bacteria. Ann. Rev. Microbiol., **9**, 97–110.
70. PEEBLES, F. 1929 Growth-regulating substances in echinoderm larvae. Biol. Bull., **57**, 176–187.
71. PINE, L. 1954 Studies on the growth of *Histoplasma capsulatum*. I. Growth of the yeast phase in liquid media. J. Bacteriol., **68**, 671–679.
72. PIRT, S. J. 1957 The oxygen requirement of growing cultures of an *Aerobacter* species determined by means of the continuous culture technique. J. Gen. Microbiol., **16**, 59–75.
73. PRESCOTT, D. M. 1957 Relations between cell growth and cell division. In *Rhythmic and synthetic processes in growth*, pp. 59–74. Princeton Univ. Press, Princeton, N. J.
74. PUCK, T. T. 1957 The mammalian cell as microorganism. In *Rhythmic and synthetic processes in growth*, pp. 3–17. Princeton Univ. Press, Princeton, N. J.
75. ROLINSON, G. N. AND LUMB, M. 1953 The effect of aeration on the utilisation of respiratory substrates by *Penicillium chrysogenum* in submerged culture. J. Gen. Microbiol., **8**, 265–272.
76. SANFORD, K. K., LIKELY, G. D., AND EARLE, W. R. 1954 The development of variations in transplantability and morphology within a clone of mouse fibroblasts transformed to sarcoma-producing cells *in vitro*. J. Natl. Cancer Inst., **15**, 215–238.
77. SCHERR, G. H., FISHMAN, M., AND WEAVER, R. H. 1954 The mutagenicity of some carcinogenic compounds for *Escherichia coli*. Genetics, **39**, 141–149.
78. SENECA, H. AND HENDERSON, E. 1951 Growth of hemoflagellates in dialyzing sacs and the production of growth inhibitory factor. Am. J. Hyg., **53**, 17–21.
79. SPIEGELMAN, S. 1948 Differentiation as the controlled production of unique enzymatic patterns. Symposia Soc. Exptl. Biol. (England), **2**, 286–325.
80. SPOERL, E. AND CARLETON, R. 1954 Studies on cell division. Nitrogen compound changes in yeast accompanying an inhibition of cell division. J. Biol. Chem., **210**, 521–529.
81. STANIER, R. Y. 1955 The plasticity of enzymatic patterns in microbial cells. In *Aspects of synthesis and order in growth*, pp. 43–67. Princeton Univ. Press, Princeton, N. J.
82. STEARNS, R. N. 1953 Respiration, rhodanase, and growth in *Escherichia coli*. J. Cellular Comp. Physiol., **41**, 163–170.

83. STONE, R. W. AND WILSON, P. W. 1952 The effect of oxalacetate on the oxidation of succinate by *Azotobacter* extracts. J. Bacteriol., **63**, 619–622.
84. SUSSMAN, M. 1956 On the relation between growth and morphogenesis in the slime mold, *Dictyostelium discoideum* Biol. Bull., **110**, 91–95.
85. TABER, W. A. 1957 The influence of the inoculum on variability in comparative nutritional experiments with fungi. Can. J. Microbiol., **3**, 803–812.
86. TAYLOR, H. E. 1954 The lag phase in the growth of yeast cultures. Rev. can. biol., **13**, 144–169.
87. WARBURG, O. 1956 On the origin of cancer cells. Science, **123**, 309–314.
88. WARBURG, O. 1956 On respiratory impairment in cancer cells. Science, **124**, 269–270.
89. WEISS, P. 1949 Differential growth. In *The chemistry and physiology of growth*, Chap. VII. Princeton Univ. Press, Princeton, N. J.
90. WEISS, P. 1955 Specificity in growth control. In *Biological specificity and growth*, pp. 195–206. Princeton Univ. Press, Princeton, N. J.
91. WILLIAMS, J. W. 1939 Growth of microorganisms in shake cultures under increased oxygen and carbon dioxide tensions. Growth, **3**, 21–33.
92. WYNNE, E. S. AND NORMAN, J. O. 1953 On the concept of "direct antagonism" in bacteria. J. Infectious Diseases, **93**, 243–246.
93. YATES, R. A. AND PARDEE, A. B. 1957 Control by uracil of formation of enzymes required for orotate synthesis. J. Biol. Chem., **227**, 677–692.