Effects of Magnesium on Cellular Division in Bacteria[1]

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IT HAS BECOME INCREASINGLY APPARENT during the last ten or fifteen years that the division of the bacterial cell follows a complex sequence, which, in many respects, resembles that occurring in the cellular reproduction of higher forms. It is now known, for example, that bacterial cell division entails division of the nuclear element, division of the cytoplasm, secretion of new cell wall material, and the separation of the daughter cells.

Some or all of the events of this sequence are readily thrown out of balance, or even completely inhibited. Thus bacteria, particularly the rod-shaped organisms, may be induced to elongate into filaments by various treatments which apparently inhibit cell division but which do not inhibit growth. Such an effect is produced by various chemical substances, by subbacteriostatic concentration of certain antibacterial agents, as, for example, methyl violet (*1*), sulfonamides (*2*), *m*-cresol (*3*), and penicillin (*4–6*), as well as by physical agents such as irradiation (*7–10*), or even higher temperatures of incubation (*11*).

These changes in morphology induced by chemical substances are usually temporary, since reversion to normal form occurs promptly when the filamentous bacteria are subcultured in the absence of the inhibitory agents. Irradiation, on the other hand, may give rise to a temporary (*7, 12*) or permanent (*9*) induction of filamentous cells.

From observations such as these the concept has arisen that bacterial growth, in the sense of an irreversible increase in cell substance or volume, and cell division may be considered to some extent as separate and independent processes; at least, in so far as growth may occur either with or without the operation of the cell division mechanism (*13–15*).

As pointed out by Nickerson (*16*), it is reasonable to suppose that the physiological processes resulting in the growth of microbial cells as elongated filaments are the same as the processes responsible for growth when the cells are also dividing. Thus it appears possible to investigate certain aspects of cell division by comparative studies of cells of normal morphology and the filamentous forms produced under conditions which are only partially or not at all inhibitory to growth. Such conditions as these may be realized very simply by growing bacteria in complex media deficient in ionic magnesium.

Previous studies by the present author (*17, 18*), which have been more than confirmed by the work of Nickerson (*16*), Hewitt (*19*), and Shankar and Bard (*20*), have shown that variations in the magnesium content of the culture medium may exert a marked effect upon the cell division of certain bacteria. Thus in a peptone medium rendered deficient in ionic magnesium, Gram positive[3] rod-shaped bacteria grow in the form of long filaments. These filaments, which, as shown by previously published photomicrographs (*17, 18*), frequently appear to possess diameters significantly less than those of the original rods, revert to cells of normal morphology when subcultured in the same medium supplemented with suitable amounts of magnesium (*17, 18*). Other metals, with the possible exception of divalent manganese, cannot overcome the adverse effects of magnesium deficiency on the cell division process (*21*). Indeed, the formation of filaments in a magnesium-deficient medium is enhanced by the addition of certain divalent metallic ions such as zinc and cobalt (*16, 21*). This may result either from competitive ion antagonism, as described by McLeod and Snell (*22*), between the zinc or cobalt ions and the residual magnesium present in the medium, or represent an additional inhibition of the cell division processes through the inactivation of essential sulfhydryl groups (*23*).

Inhibition of cell division in cultures of the Gram positive rod-shaped bacteria occurs not only in peptone media deficient in magnesium but also in these complex nutrient solutions when supplemented with excessive amounts of this ion. This latter effect has a parallel in the fact that certain enzymes which are activated by metallic ions at low concentrations are inhibited by the same ions at higher concentrations.

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[3] The terms "Gram positive" and "Gram negative" refer to the behavior of heat-fixed smears of bacterial cells to the conventional Gram-staining procedure. The so-called Gram positive bacteria retain the initial basic dye when mordanted and treated with neutral solvents such as ethanol or acetone, whereas the Gram negative organisms are decolorized under these conditions and are subsequently stained with a second dye of contrasting color.

Cytological examination of the filamentous cells, according to the procedures developed by Robinow (*24*) and others, shows that it is the final stages of cell division (i.e., the formation of transverse plasma membranes and/or cross cell walls) that fail to occur in bacteria grown in media deficient in, or containing excessive amounts of, magnesium, whereas division of the nuclear elements appears unaffected.

Cell division of Gram negative rod-shaped bacteria in peptone media, while inhibited by excessive amounts of magnesium, is unaffected by reduction in the magnesium concentration. One contributory factor to this marked difference in the behavior of the Gram positive and Gram negative rods is possibly related to the fact that the former accumulate magnesium during growth and incorporate it as an essential component in the structure of the "Gram complex" (*25*).

The difference between the amounts of magnesium necessary to support optimal growth of Gram positive and Gram negative bacteria is particularly apparent when the bacteria are cultivated in very simple chemically defined media composed only of inorganic salts and suitable single sources of carbon and nitrogen (*26*). In the absence of magnesium, these nutrient solutions are unable to support bacterial growth. With increasing concentrations of magnesium, the amount of growth increases to a maximum and then tends to decrease. Moreover, it is readily apparent from a study of the growth of a number of different bacterial species in such simple media that the concentration of magnesium necessary to support optimal growth of Gram positive bacteria, some 20–40 parts per million (ppm), is about ten times greater than that required by the Gram negative organisms under the same conditions.

In these simple chemically defined solutions, where, in contrast to the effects observed in peptone media, suboptimal amounts of magnesium predominantly inhibit bacterial growth, low concentrations of magnesium do not induce the formation of filaments in cultures of the Gram positive bacilli. In fact, under these conditions cell division is normal throughout the restricted populations that the media are able to maintain.

The concentration of magnesium necessary to support maximum growth in simple chemically defined media is reduced markedly by the addition of amino acids, and even for Gram positive bacilli may become as low as 2–3 ppm if complex mixtures of amino acids and growth factors are added (*27*).[4] Under these conditions, further increases in the magnesium concentration (to 100 ppm) are without effect upon the amount of growth.

The differences observed between the magnesium requirements of Gram positive and Gram negative bacteria for growth in the simple synthetic media are also apparent in these complex chemically defined solutions. Thus, although the relationship between growth and magnesium concentration is, of course, dependent upon the organic composition of the medium, in general Gram positive bacteria fail to grow when the magnesium content is less than .6 ppm, whereas this concentration is almost sufficient to maintain maximum growth of Gram negative species (*27*).

In these complex chemically defined solutions the magnesium requirements for cell division in cultures of the Gram positive rods are higher than for growth. Thus, in solutions containing low concentrations of magnesium (i.e., 1–6 ppm), as in peptone media, the cell division of the Gram positive rod-shaped bacteria is inhibited. The cells grow in the form of long filaments, which, when subcultured in the same medium containing increasing amounts of magnesium, change in appearance from filaments to chains and thence to isolated rods of normal morphology. However, under the same conditions, no significant changes are observed in the morphology of Gram negative rod-shaped bacteria. As it has been shown that a deficiency of ionic magnesium restricts the growth of other microorganisms such as yeasts (*16*), actinomycetes, *Polytomella,* and algae (*28*) to a greater or lesser degree, but has little or no effect upon their morphology, the inhibitory effects of magnesium deficiency upon cell division appear to be confined to the Gram positive rod-shaped bacteria. As it is unlikely that the Gram positive and Gram negative rod-shaped bacteria divide by essentially different mechanisms, or that magnesium is essential for the cell division of the former, but not of the latter, the difference in response of the Gram positive and Gram negative bacteria to reduced amounts of magnesium becomes of particular interest. From the foregoing considerations it appears that the formation of filaments in magnesium-deficient cultures of the Gram positive organisms is not due to a direct inhibition of the cell division mechanism, but is an indirect result of the inhibition of certain assimilatory or metabolic reactions. No evidence has been obtained throughout this work that the formation of filamentous cells in the magnesium-deficient cultures is due to the accumulation of products arising from partially inhibited, or altered metabolism, which are themselves inhibitory to cell division (*28*). However, through the application of the analytical techniques developed by Davidson and Leslie (*29*) in their biochemical studies on the growth and development of avian cells in tissue culture, it has been possible to show that the growth of Gram positive bacteria in magnesium-deficient media is accompanied by a decrease in the rates of amino acid assimilation and protein synthesis relative to the rates of synthesis of other cellular constituents.

The basis of the investigations of Davidson and Leslie is the experimental finding of a constant average value for the deoxyribonucleic acid content of the interphase cell nuclei in all somatic tissues of any one animal species (*30, 31*). This provides a chemical unit which can be used both as a measure of cell multiplication and as a standard of reference by which changes in the relative amounts of other cell constitu-

[4] Details of the procedures adopted to eliminate magnesium and other trace metals from the organic constituents of the complex chemically defined nutrient solutions are recorded in the paper cited in reference 27.

ents may be determined (*29*). Recent investigations have shown that the cells of a given bacterial species in the stationary (resting) state, in common with the interphase cells of plant and animal tissues contain a constant amount of deoxyribonucleic acid (*32–34*; see also Table 1). Furthermore, determinations of the average content of deoxyribonucleic acid per cell in cultures of *Escherichia coli* (*32*) and *Clostridium welchii* (Fig. 1) throughout the growth cycle show that the deoxyribonucleic acid content increases during the lag phase to approximately twice its initial value. This maximum is reached at the transition between the lag and log phases, just before the onset of cell division. Once active multiplication is established, the average deoxyribonucleic acid content of the cells from growing cultures does not vary greatly from that of the resting cells. Thus it is obviously possible to use the deoxyribonucleic acid content of the normal bacterial cell as a standard of reference in growth and metabolic studies.

The average deoxyribonucleic acid content of filamentous cells harvested at the end of growth from cultures of the Gram positive rod-shaped bacteria in peptone media deficient in magnesium shows a considerable variation when each filament is counted as a single cell (Table 1). The magnitude of this variation is dependent upon the mean length of the filaments. However, when each filament is counted as the equivalent number of normal cells, a value is obtained for the average deoxyribonucleic acid content of the "unit cell" which is independent of filament length. Furthermore, if allowance is made for inaccuracies associated with the visual estimation of the number of unit cells per filament, there is close agreement between the values found for the deoxyribonucleic acid content of the normal cells in the stationary state, and that of the unit cell of the filamentous forms (Table 1). This relationship, which has been shown to be of general application, is similar to that found by Caldwell and

TABLE 1

DEOXYRIBONUCLEIC ACID PHOSPHORUS (DNA P) CONTENT OF THE NORMAL AND FILAMENTOUS FORMS OF *Cl. welchii*

Normal cells mg DNA P/cell ($\times 10^{12}$)	Filaments*	
	mg DNA P/filament ($\times 10^{12}$)	mg DNA P/unit cell ($\times 10^{12}$)
2.29	6.60	2.24
2.21	6.26	2.05
2.14	15.0	2.73
2.50	21.4	2.60
2.27	28.4	2.69
2.40	7.5	2.06
2.44	26.7	2.40
2.30	7.10	2.11
Mean = 2.32	Mean = 2.36	

* The filamentous cells were harvested from 16–18 hr cultures incubated at 37° in a magnesium-deficient peptone medium, the cells of normal morphology from cultures of similar age in the same medium supplemented with 0.015% $MgSO_4 \cdot 7H_2O$.

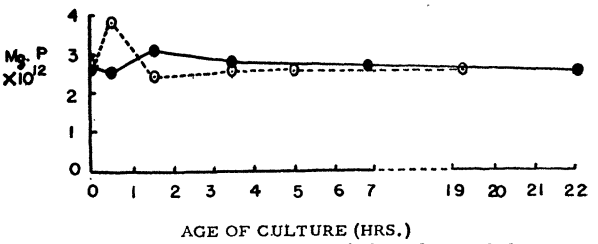

FIG. 1. Variation with the age of the culture of the average deoxypentose nucleic acid content per cell (or unit cell) of *Clostridium welchii*. Solid line, magnesium-deficient (filamentous) culture. Dotted line, control cultures containing 0.015% $MgSO_4 \cdot 7H_2O$.

Hinshelwood (*34*) for the filamentous cells induced in cultures of *Aerobacter aerogenes* by the presence of *m*-cresol. Moreover, on the assumption that all the deoxyribonucleic acid of the bacterial cell is located in the nucleus (or its equivalent), as suggested by Boivin (*35–37*), the results of the chemical analyses (Table 1) are in accordance with the cytological evidence which, as mentioned above, shows that the conditions of magnesium deficiency leading to an inhibition of cytoplasmic division are without effect upon the division of the nuclear structures. It must be emphasized, however, that without supplementary cytological evidence, it would be unjustified to claim that the values presented in Table 1 represent the average deoxyribonucleic acid content of the nuclear element of the resting *Cl. welchii* cell.

The curves (Fig. 1), showing the variation during growth of the average content of deoxyribonucleic acid in normal cells and the unit cells of the filamentous forms of *Cl. welchii*, are closely similar. Thus, both show an initial increase in deoxyribonucleic acid content to a maximum at the end of the lag phase. The fact that the deoxyribonucleic acid content of the unit cell in the filamentous cultures, where the lag phase is somewhat prolonged, does not increase to the same extent as does the deoxyribonucleic acid content of the normal cells is probably due to the decreased rate of growth (i.e., rate of increase in dry weight) of *Cl. welchii* in the magnesium-deficient medium.

With the constant deoxyribonucleic acid content of the resting cell or unit cell as a standard of reference, attempts have been made to determine changes in the relative amounts of other components of the bacterial cell as a result of growth in magnesium-deficient peptone media. In these experiments media of three different magnesium contents have been used. The first of these, the deficient medium (medium 1), was prepared from a concentrated aqueous solution of the peptone rendered deficient in magnesium by treatment with ammonium hydroxide in the presence of free phosphate ions in the normal way (*17*), whereas the second and third (media 2 and 3) consisted of medium 1 supplemented with 4 ppm and 15 ppm magnesium, respectively. Cells harvested at the end of growth in these nutrient solutions were submitted to extensive chemical analysis. The results of these determinations, full details of which will be published elsewhere, may be summarized as follows.

Relative to the deoxyribonucleic acid content, no uniform or significant changes are detected in the amounts of intracellular acid-soluble phosphorus, ribonucleic acid phosphorus, total carbohydrate, or total lipid and phospholipid phosphorus in either Gram positive or Gram negative bacteria as a result of growth in the magnesium-deficient media (media 1 and 2).

In Gram positive rod-shaped bacteria harvested at the end of growth in medium 2, where the concentration of magnesium is intermediate between that required for the growth of the organisms as long thin filaments and that necessary for the growth of cells of normal morphology and leads to the development of populations consisting mainly of chains of normal-sized cells together with some short filaments, the relative amounts per unit cell of both acid-soluble nitrogen and protein nitrogen are significantly less than the corresponding values found for cells grown in medium 3.

The relative amounts per unit cell of acid-soluble nitrogen and protein nitrogen of the abnormal, long, thin, filamentous forms of the Gram positive rods harvested from the magnesium-deficient medium (medium 1) are greater than those of cells grown in the medium containing intermediate concentrations of magnesium (medium 2) and may even approximate the values found for cells grown in the presence of adequate amounts of magnesium (medium 3). These results are in accordance with those of Nickerson and Sherman (*38*), who found little or no difference between the ribonucleic acid and protein nitrogen contents expressed as a percentage of the dry weight, of the normal and magnesium-deficient, filamentous forms of *B. cereus*.

With the Gram positive micrococci, where, in general, reduction in the magnesium content of the medium predominantly inhibits growth and has no effect upon the morphology of the cells, growth in medium 1 effects an even greater reduction in the relative amounts per cell of acid-soluble nitrogen and protein nitrogen than does growth in medium 2.

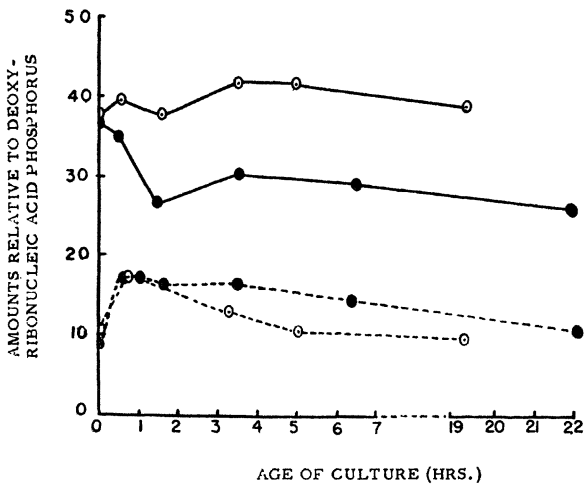

FIG. 2. Effect of magnesium on the variation with the age of the culture of the relative contents of protein nitrogen (solid line) and ribonucleic acid phosphorus (dotted line) in *Clostridium welchii* cells grown in a peptone medium deficient in magnesium. Shaded circles refer to culture grown in the absence of additional magnesium; open circles to cultures grown in the deficient medium supplemented with 0.015% $MgSO_4 \cdot 7H_2O$.

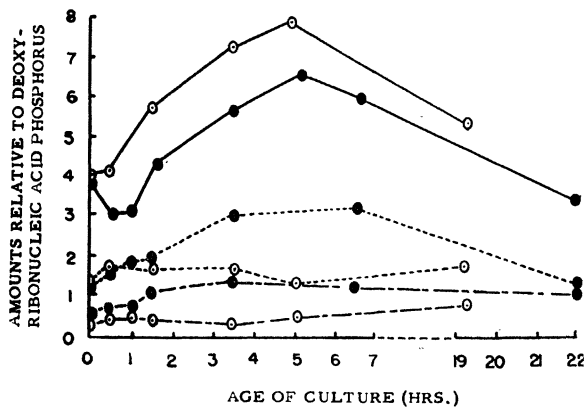

FIG. 3. Effect of magnesium on the variation with the age of the culture of the relative contents of acid-soluble nitrogen (solid line), phospholipid phosphorus (dotted line) and phosphoprotein phosphorus (dotted and dashed line) in *Clostridium welchii* cells grown in peptone medium deficient in magnesium. Shaded circles refer to cultures grown in the absence of additional magnesium; open circles to cultures grown in the deficient medium supplemented with 0.015% $MgSO_4 \cdot 7H_2O$.

With Gram negative bacteria no significant differences are found between the contents of acid-soluble and protein nitrogen in cells harvested from either medium 1, medium 2, or medium 3.

The results discussed above have been obtained with cells harvested at the end of growth, i.e., from cultures in the stationary state. However, throughout the growth of the Gram positive rods there is a marked reduction in the acid-soluble nitrogen and protein nitrogen contents of the cells as a result of growth in the magnesium-deficient medium. This is illustrated by the results presented in Figs. 2 and 3, which show the variation in relative amounts of certain cell constituents during the growth of heavy inocula of *Cl. welchii* (derived from an overnight culture in medium 3) in media 3 and 2.

There appears little doubt, therefore, that an inadequate supply of ionic magnesium in the culture medium adversely affects certain aspects of the nitrogen metabolism of Gram positive bacteria. Furthermore, the analytical results reveal not only that the changes induced in the relative contents of protein nitrogen and acid-soluble nitrogen of the Gram positive rods in magnesium-deficient media are diminished when growth occurs in the form of filaments, but also that there is an essential difference between the effects of magnesium deficiency on the chemical composition of Gram positive and Gram negative bacteria.

Thus, although the mechanism by which the cell division processes are held in check to permit growth in the form of filaments is still unknown, it now appears possible to advance certain tentative suggestions which may explain the formation of these abnormal cells in magnesium-deficient cultures. For ex-

ample, it has been shown by Gale and his colleagues that the Gram positive and Gram negative bacteria differ markedly in their amino acid metabolism (*39–41*). Thus, it has been established that Gram positive bacteria assimilate preformed amino acids from the external medium and concentrate them within the cell (*40*). Gram negative bacteria, however, do not effect this internal concentration. The amino acids which accumulate within the Gram positive cell form a reservoir for protein synthesis and for other metabolic processes. The assimilation of glutamic acid (and, presumably, certain other amino acids which enter the cell by an "activation" process) by Gram positive cells (*Staph. aureus*) requires a source of energy, which may be supplied by the exogenous metabolism of glucose, and either magnesium or manganese, or both (*42*). It is now known from the work of Nickerson and Sherman (*38*) that although no significant difference can be detected between the rates of endogenous respiration of the normal and magnesium-deficient filamentous forms of *B. cereus*, the oxidation of added substrate (e.g., glucose, pyruvate, alanine, or glutamate) by the filamentous cells proceeds at rates only 1/3 to 1/6 of those exhibited by the cells with uninhibited division mechanisms.

It seems possible, therefore, that a reduction in the magnesium content of complex nutrient solutions containing preformed amino acids may lead to a reduction in the rate of amino acid assimilation and a consequent decrease in concentration of amino acids within the cell. This decrease in the internal amino acid concentration, reflected in the analytical determinations by a reduction in the content of acid-soluble nitrogen, would result, in turn, in a decrease in the rate of protein synthesis. A decrease in the rate of protein synthesis, relative to the rates of synthesis of other cellular components, would lead to the reduction in the relative protein content of the cell shown by chemical analysis. The maintenance of certain proteins, at least, at a critical level must be essential for the growth of the cell. Thus, unless the bacterium can increase its rate of protein synthesis by increasing the rate of assimilation of amino acids, the growth of the culture is inhibited. With the Gram positive rod-shaped bacteria the formation of long filamentous cells appears in some way to provide a means of overcoming the effects of magnesium deficiency on amino acid assimilation and protein synthesis. Moreover, from such considerations it is possible to account not only for the production of the filamentous forms of the Gram positive rods and the inhibition of the growth of the Gram positive cocci in magnesium-deficient complex media, but also to offer an explanation for the fact that in simple chemically defined solutions, where the essential amino acid components of the cellular protein constituents are not assimilated directly, but are built up from the components of the medium, a deficiency of magnesium predominantly inhibits growth and is without effect upon the morphology of the Gram positive rods.

References

1. Ainley-Walker, B. W., and Murray, W. *Brit. Med. J.*, **2,** 16 (1904).
2. Tunnicliff, R. *J. Infectious Diseases,* **64,** 59 (1939).
3. Spray, G. H., and Lodge, R. M. *Trans. Faraday Soc.*, **39,** 424 (1943)
4. Gardner, A. D. *Nature* **146,** 837 (1940).
5. Pratt, R., and Dufrenoy, J. *Bact. Revs.*, **12,** 79 (1948).
6. Fleming, A., *et al. J. Gen. Microbiol.*, **4,** 257 (1950).
7. Gates, F. L. *Science,* **77,** 350 (1933).
8. Spenser, R. R. *U.S. Public Health Repts.*, **49,** 183 (1934) ; **50,** 1642 (1935).
9. Lea, D. E., Haines, R. B., and Coulson, C. A. *Proc. Roy. Soc. (London)* **B 120,** 47, (1936) ; **123,** 1, (1937).
10. Eisenstark, A., and Clark, G. L. *Science,* **105,** 553 (1947).
11. Hansen, E. C. *Compt. rend. Lab. Carlsberg,* **3,** 182 (1894).
12. Witkin, E. M. *Proc. Natl. Acad. Sci. U.S.*, **32,** 59 (1946).
13. Lodge, R. M., and Hinshelwood, C. N. *Trans. Faraday Soc.*, **39,** 420 (1943).
14. Nickerson, W. J. *Nature,* **162,** 241 (1948).
15. Webb, M. *J. Soc. Chem. Ind.*, **68,** 319 (1949).
16. Nickerson, W. J. *Abstr. Papers 5th Intern. Cong. Microbiol. Rio de Janeiro,* p. 33 (1950).
17. Webb, M. *J. Gen. Microbiol.*, **2,** 275 (1948).
18. ———. *Ibid.*, **3,** 410 (1949).
19. Hewitt, L. F. *Ibid.*, **5,** 278 (1951).
20. Shankar, K., and Bard, R. C. *J. Bacteriol.*, **63,** 279 (1952).
21. Webb, M. *J. Gen. Microbiol.*, **5,** 480 (1951).
22. McLeod, R. A., and Snell, E. E. *Ann. N. Y. Acad. Sci.*, **52,** 1249 (1950).
23. Nickerson, W. J., and Van Rij, N. J. W. *Biochim. Biophys. Acta* **3,** 461 (1949).
24. Robinow, C. F. *Proc. Roy. Soc. (London)*, **B 130**, 299, (1942) ; *J. Hyg. (Camb.)*, **43**, 413 (1944).
25. Henry, H., and Stacey, M. *Proc. Roy. Soc. (London)*, **B 133,** 391 (1946).
26. Webb, M. *J. Gen. Microbiol.*, **3,** 418 (1949).
27. ———. *Ibid.*, **5,** 485 (1951).
28. ———. Unpublished.
29. Davidson, J. N., and Leslie, I. *Nature,* **165,** 49 (1950) : *Cancer Research,* **10,** 587 (1950).
30. Vendrely, R., and Vendrely, C. *Experientia,* **4,** 434 (1948) ; **5,** 327 (1949).
31. Davidson, J. N. *The Biochemistry of Nucleic Acids.* London : Methuen. 1950.
32. Morse, M. L., and Carter, C. E. *J. Bacteriol.*, **58,** 317 (1949).
33. Hedén, C. G. *Acta Path. Microbiol. Scand. Suppl.*, **88** (1951).
34. Caldwell, P. C., and Hinshelwood, C. *J. Chem. Soc.*, 1415 (1950).
35. Boivin, A. *Rev. can. biol.*, **7,** 368 (1948).
36. ———. *Compt. rend. soc. biol.*, **142,** 1257 (1948).
37. Boivin, A., Vendrely, R., and Tulache, R. *Arch. sci. physiol.*, **1,** 35 (1947).
38. Nickerson, W. J., and Sherman, F. G. *J. Bacteriol.*, **64,** 667 (1952).
39. Gale, E. F. *Ann. Rev. Microbiol.*, **1,** 141 (1947) ; *J. Gen. Microbiol.*, **1,** 53, 327 (1947).
40. Gale, E. F., and Taylor, E. S. *J. Gen. Microbiol.*, **1,** 314 (1947).
41. Gale, E. F., and Mitchell, P. D. *Ibid.*, 299 (1947).
42. Gale, E. F. *Ibid.*, **3,** 369 (1949).