Ionic Germination of Spores of Bacillus megaterium QM B1551

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With 9 Figures in the Text

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Within the past few years the number of ways found to conduct the germination of bacterial spores has risen sharply. For one or another species the following have been reported to be effective in germination: L-alanine and adenosine (Hills 1949a, b) [subsequently, several other amino acids proved to be capable of substituting for alanine (Woese, Morowitz and Hutchinson 1958), and inosine for adenosine (Powell 1957)]; glucose (Levinson and Sevag 1953; Powell 1957); ethylenediamine tetraacetic acid (Brown 1957); ionic surfactants, especially long-chain primary amines (Rode and Foster 1960c, d; 1961a); calcium dipicolinate (Riemann and Ordal 1961); hydrogen peroxide (Falcone, Salvatore and Covelli 1959; Rode and Foster 1961b); pyridine carboxylic acids, salts of fatty acids, hydrazine, hydroxylamine and ammonium chloride (Rode and Foster 1961b); mechanical abrasion (Rode and Foster 1960b); and electrodialysis (Rode and Foster 1960a).

As pointed out elsewhere (Rode and Foster 1961b), it becomes increasingly difficult to rationalize the diverse means of germinating bacterial spores. Either a considerable number of different germination mechanisms exist for the various species, or some unifying mechanism remains to be elucidated.

This paper suggests that perhaps the diversity is not as great as it seems to be. An implication of ionic phenomena in the initiating event of germination is probable from the discovery that the bacterial strain studied here germinates readily in solutions of certain inorganic ions. Nothing else, including the glucose normally "required" by this strain, need be present. As impurities in glucose, these inorganic ions presumably have significance in the germinative properties of glucose. There is, however, clear evidence that glucose does have an augmentative influence on the germinative potentials of some inorganic ions.

Scattered data in the literature are suggestive of ionic germination, although no one has actually come to grips with it. Especially pertinent
are the instances of germination by means of various chelators (Brown 1957; Riemann and Ordal 1961; Rode and Foster 1961b). Also of significance in this regard is the germination induced by ionic but not non-ionic surfactants (Rode and Foster 1960c), by electrodialysis (Rode and Foster 1960a), and by some inorganic nitrogenous compounds (Rode and Foster 1961b). However, the general applicability of ionic germination and a cardinal function of ions in the initiating event of the germination process have not been hitherto realized.

Standing in the way of a serious consideration of ionic mechanisms of germination are the apparent requirements for organic compounds for the germination of all bacterial species. An amino acid (Hyatt and Levinson 1961), an amino acid and a riboside (Hills 1949a, b; Woese, Morowitz and Hutchinson 1958), or glucose (Powell 1957; Levinson and Sevag 1953; Hyatt and Levinson 1961) is essential. The elimination of an essential glucose function removes one of the perplexing aspects of germination. For these organisms at least, it re-directs attention to a mode of germination not dependent on exogeneous energy.

Levinson and collaborators (Levinson and Sevag 1953; Hyatt and Levinson 1961) examined the influence of various ions on the germination of *Bacillus megaterium* QM B 1551, an organism whose germination seemingly is initiated by glucose or mannose. Most of their tests also had organic germinative compounds in the germination liquid. A variety of monovalent anions induced germination, and a proper ionic balance was required (Levinson and Sevag 1953) even in the presence of the "essential" organic compounds. In a recent extension of those studies, Hyatt and Levinson (1961) observed that KNO₃ or KNO₂ alone would germinate the spores in an inorganic buffer system. It is clear that ionic germination had in fact been achieved though these authors concluded otherwise on the grounds that NaCl was inactive.

Brown's intriguing discovery (1957) that ethylenediamine tetraacetic acid germinates spores of the "putrefactive anaerobe" 3679 likewise points to an involvement of ions in germination. He concluded that various inhibitory inorganic cations normally on the spores were removed by the chelator. Riemann and Ordal (1961) described the germination of aerobic spores by calcium dipicolinate (1:1 molar ratio). The germinative properties of that compound have been confirmed for the Texas strain of *Bac. megaterium*, with the difference that inosine or L-alanine had to be present, and the compound specificity was not so marked (Rode and Foster 1961b). Germination of the Texas strain normally occurs promptly in a mixture of L-alanine and inosine. Significantly, the germinative organic compounds functioned better in phosphate buffer than in tris buffer, and very poorly in distilled water (Rode and Foster 1961b).