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Aberrant germination and accumulation of calcium by spores of the genus *Bacillus*

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One of the germination characteristics of spores is their loss of refringency as judged for instance by phase-contrast microscopy: resting spores of bright appearance turn to dark germinated spores.

A reverse process has been studied by which great numbers of initially germinated spores revert to a less intense though still highly refractive state similar to that of resting spores. These cells, however, show an even stronger swelling than germinated spores, and appear to be non-viable. The phenomenon was tentatively called retrogression of germinated spores.

Like germination, retrogression of spore suspensions could be followed by turbidimetric methods. It was found to depend on the simultaneous presence of phosphate and calcium ions. Taking into account the relative concentrations of these ions and an established pH-range from 6.5 to 7.1 for optimal retrogression, one is tempted to see the phenomenon as resulting from a precipitation of a calcium-phosphate salt on or in the germinated spore. In view of the swelling a precipitation within the spores seems to be more probable.

Attempts to analyse the refractive cells were hampered by the fact that these cells lost much of their refringency on washing. However, retrogression was found to be stimulated as well as stabilized by increased temperature. When, following on germination at 35 C, the temperature was raised to 55 C, retrogression was greatly enhanced and the cells lost practically none of their refringency on washing. Under these conditions cells of advanced stages of germination and even rods also became refractive, though in lesser numbers. No retrogression occurred with vegetative cells of later generations.

Determination of the ash content of these "heat-treated" refractive cells showed a striking increase from 10–12% for resting spores to 50–60% for retrogressive spores. This increase could be readily accounted for by the amounts of calcium and phosphate found on analysis of the ash. The molar ratio Ca/P appeared to be about 1.6, which may indicate the deposition of hydroxyapatite in the refractive cells.

The accumulation of calcium as described has been observed with all strains so far studied of four *Bacillus* species and may be a general property of germinated spores. Though the conditions for retrogression were identical in principle, some strains displayed the phenomenon more readily than others. Whether this is of simple physico-chemical or of physiological nature is not clear. Some features of the process call to mind the energy-requiring accumulation of calcium recently described for mitochondria of higher cells.