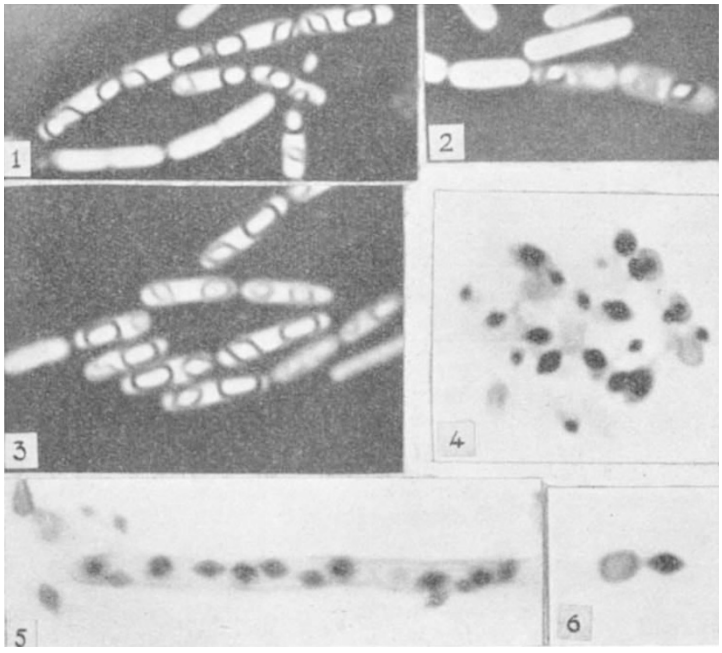
Crystalline Inclusions in Aerobic Spore-forming Bacteria

B. thuringiensis Berliner[1] is an aerobic spore-forming bacillus, classified by Smith, Gordon and Clark[2] as *Bacillus cereus* var. *thuringiensis*, because apart from its pathogenicity for certain insects and the tendency of the spores to lie obliquely in the cell, the organism is indistinguishable from *B. cereus*.



(1) *B. thuringiensis*. Air-mounted nigrosin film. Unstained. Robinow's technique (ref. 5). × 2,400
(2) Crystal-bearing cell of *B. thuringiensis* in which the spores have lysed before reaching maturity. × 2,400
(3) Crystals and spores in Bacillus No. 1. A constituent of 'Sporeine' powder which is pathogenic for *Ephestia kühniella* Zell. Jacobs (ref. 6). × 2,400
(4) Free crystals and spores of *B. thuringiensis*. Hydrolysed at room temperature in *N*/3 nitric acic containing 0·1 per cent potassium permanganate (Method 2. Robinow, ref. 7). Stained with crystal violet and photographed mounted in water. × 2,400
(5) Crystals alternating with spores in a chain of *B. thuringiensis*. Hydrolysed in hydrochloric acid at 60° C. for 15 min. and stained with crystal violet. Photographed mounted in water. × 2,400
(6) Crystal attached to free spore. Stained with crystal violet and photographed mounted in water. × 3,600

During a study of spore formation in this organism, it was noted that the spores were invariably accompanied by what appeared to be diamond-shaped crystals (Fig. 1). Each cell contained only one crystal, and the order in a chain of cells was random. The crystals vary a great deal in size (Fig. 4) but are always the same shape, and are set free with the spores and persist indefinitely. Occasionally a crystal may be firmly attached to a spore (Fig. 6). Lysis may occur in the early stages of spore formation so that a cell may contain a crystal but no spore (Fig. 2).

Cultures of spore-forming insect pathogens (variants of *B. cereus*) from other sources have been examined and it has been found that the majority form crystals on sporulation (Fig. 3). All strains of *B. cereus* that were obtained from other sources and which were not known to be pathogenic for insects, together with strains isolated from soil and foodstuffs, failed to form crystals on sporulation.

The crystals stain readily with Giemsa but more intensely with basic fuchsin and crystal violet, and most cleanly with Victoria blue. With the acid dye ponceau de xylidine dissolved in *N*/10 hydrochloric acid they stain a pale orange-red. After hydrolysis in *N* hydrochloric acid at 60° for 15 min. or in *N*/3 nitric acid with 0·1 per cent potassium permanganate, the crystals both free and within the cells are unaltered in size and shape and take up the stain more easily than before hydrolysis (Figs. 4 and 5).

The crystals are readily soluble in dilute alkali but are insoluble in water, physiological saline, ethanol, methanol, chloroform, ether, benzene and acetone. When dilute alkali is run under an air-dried coverslip preparation of crystals and spores, the crystals, while still retaining their shape, greatly increase in size, then lose their refractility and finally disappear, leaving behind a thin shell or membrane.

A biological phenomenon in which crystalline inclusions have only been found in strains of bacteria pathogenic for insects must inevitably, in the absence of additional observations, lead to some speculation as to the possible association of inclusions with pathogenicity. The crystals have certain features in common with the nuclear polyhedral crystalline inclusion bodies as elucidated by Bergold[3]; but their ability to take up stains is similar to the cytoplasmic inclusion bodies of Smith and Xeros[4]. They differ, however, in that so far as has been observed, only one crystal is formed in each cell. At the moment nothing is known about their composition, whether they contain a virus or phage, or whether the crystal formation is a genetical characteristic of the organism which is in some way connected with the formation of a toxic substance encouraging septicæmia of the insect larvæ.

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Acetylcholine in Blowflies

A study of two species of blowfly, *Calliphora erythrocephala* and *Lucilia sericata*, has shown that a rapid synthesis of an acetylcholine-like substance can take place in extracts prepared from these insects. This synthesis, which occurs in the absence of additional substrate, may account for some of the high concentrations of acetylcholine which have been previously reported in insects[1–3].

Flies were immobilized by cooling at 0° C., weighed, and extracts prepared according to one of the following methods: (*A*) grinding in a Potter–Elvehjem homogenizer[4] with Ringer solution containing 0·001