OBSERVATIONS UPON THE BACTERIAL SPORE NUCLEUS

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(With Plate 11 and 3 Figures in the Text)

INTRODUCTION

Discrete, Feulgen-positive granules, attached to the periphery of bacterial spores, were described by Pietschmann & Rippel (1931), who considered them to be residual cytoplasmic structures; by Stille (1937), who was apparently in doubt as to their nature; and by others, including Robinow (1945), who at first described them as the natural appearance of the spore nucleus. Delaporte (1950) figured the spore nucleus as a less discrete, crescentic body lying against one side of the spore, and in a later study Robinow (1951) accepted this view, and presented evidence to prove that the appearance of the discrete body was an artefact, caused by the hydrolysis with HCl which was employed in staining. Robinow, in this later study, employed nitric acid instead of hydrochloric acid, and claimed that it gave a truer picture. Both Delaporte and Robinow observed a central, stainable body, which they considered to be cytoplasmic.

One of us has included, in a general study of the bacterial resting nucleus, a criticism of the original concept of a discrete, extra-cytoplasmic nucleus, and has suggested that the spore nucleus appears to be similar to that of other bacterial resting cells, a central, vesicular structure (Bisset, 1950*a*). The partially revised suggestion of Delaporte and of Robinow, of a crescentic, external nucleus, and an internal cytoplasmic body, appears equally unusual, with respect to the arrangement in cells of other types, and does not accord with our own observations upon the nuclei of bacterial spores.

In this paper, we will attempt to show that the bacterial spore, like the eubacterial and myxobacterial microcyst (Bisset, 1950*a*), possesses an internal, vesicular nucleus, which can be observed in the living spore, under suitable conditions, by phase-contrast microscopy, and that the discrete and the crescentic external nuclei are alike artefacts.

TECHNIQUE

Spores were stained after treatment with normal HCl at 60° C. or with normal nitric or perchloric acid, at room temperature. These methods were not significantly different in respect of their capacity to induce the various artefacts described in this paper. After acid treatment, preparations were stained with Giemsa, crystal violet, or tannic acid and crystal violet, for the simultaneous demonstration of the cell wall. Preparations were mounted in water for examination.

Living spores were examined by phase-contrast microscopy. Gold-shadowed electron micrographs were also made.

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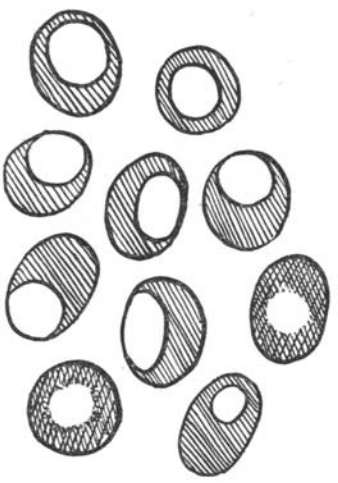
OBSERVATIONS

Spores of several species of *Bacillus* and *Clostridium* were examined. Different strains varied markedly in their reaction to acid hydrolysis. In extreme cases, after approximately 5 min., large protuberances appeared upon their surfaces. These protuberances stained deeply with Giemsa or crystal violet. If these preparations were first hydrolysed and then stained by the tannic-acid-violet technique it was apparent that they lay outside the spore coat (Pl. 11, fig. 1). At the other end of the scale, spores of certain strains showed the stained appearance of an undisturbed central nucleus (Pl. 11, fig. 2). In the spores which exhibited protuberances the position of the central nucleus was occupied by a less deeply stained central body (Pl. 11, figs. 1, 3 and 4).

Cl. welchii, which was examined with great thoroughness, showed all stages between these extremes, sometime sin a single preparation (Pl. 11, figs. 3, 4; Text-



Text-fig. 1.

Text-fig. 2.

Text-figs. 1 and 2. Spores of *Clostridium welchii*. Text-fig. 1. As seen by phase-contrast microscopy; central or slightly eccentric, vesicular nucleus. Text-fig. 2. Spores from the same culture, treated with N/1 nitric acid for 20 min., stained crystal violet. All stages seen, from normal condition of the nucleus to complete ejection.

fig. 2). Spores of strains which were very markedly affected by a brief exposure to acid sometimes disintegrated completely if the treatment was prolonged for more than a few minutes, but in these cases the stainable body retained its form (Pl. 11, fig. 6).

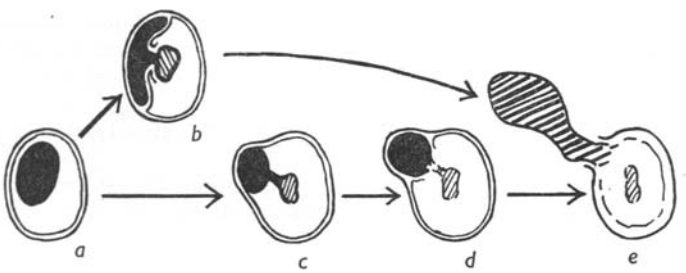
Electron micrographs were made of spores subjected to normal nitric acid. These confirmed that the protuberances were beyond the spore coat which appeared relatively undistorted (Pl. 11, fig. 7).

Suspensions of spores were mounted in normal nitric acid and examined by the phase-contrast microscope. After a few minutes the oval outline of the majority of spores was abruptly broken by the appearance of a bulge. In a short time, many had burst and disintegrated completely.

Slide cultures were made of spores of *Cl. welchii* upon nutrient agar containing

0·05 % sodium thioglycollate to initiate anaerobic conditions, and sealed under a cover-glass with paraffin wax. These were incubated at 37° C. and examined at intervals by the phase-contrast microscope. These spores took a considerable time to germinate, seldom less than 12 hr., often much more. Before germinating most of them became much less opaque, and in them a spherical or oval eccentric nucleus could readily be discerned, occupying the same position as the stained nuclei seen in the undisrupted spores (Text-figs. 1, 2). No signs were seen of any structure resembling the 'external nucleus'.

Stained preparations of the spores of *Cl. welchii* in process of maturation showed the nucleus in a central position, from which no amount of acid treatment was capable of moving it (Pl. 11, fig. 5).



Text-fig. 3. Artefacts produced by acid treatment of bacterial spores. *a*, natural appearance of the nucleus; *b*, 'crescentic nucleus' produced by nuclear material forced into a pool between the spore coat and the cytoplasm; *c*, *d*, 'discrete peripheral nucleus', when the spore coat is bulged outward; *e*, nuclear material ejected.

DISCUSSION

It appears from the foregoing observations that the nucleus of the bacterial spore is a central body resembling the nuclei of the resting cells of other types of bacteria. It can be demonstrated in stained preparations and can be observed by phase-contrast microscopy in the living spore. A short exposure to normal acid produces a whole series of artefacts in some strains but not in others. In the mature (but not in the immature) spore, the nuclear contents are apparently in a condition of great turgidity. This is an observation which accords well with what is known of the concentration of the spore proteins (Bisset, 1950*b*). When the spore coat is softened by acid attack, it may be bulged outwards to form a small discrete blister of nuclear material (Text-fig. 3, *a*, *c*, *d*), or if the spore coat holds relatively firm, or stretches more evenly, the nuclear material may form a pool between the spore coat and the body of the cytoplasm, giving the appearance of the crescentic nucleus of Delaporte (1950) (Text-fig. 3*b*). If the spore coat is ruptured, the nuclear material may be ejected (Text-fig. 3*e*). This fluidity of the nuclear material was remarked upon by Delaporte. The central body, less strongly stained, which appears in preparations showing one or other of these artefacts, is presumably a remnant of nuclear material, occupying the natural site.

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EXPLANATION OF PLATE 11

Fig. 1. Spores of *Bacillus* sp., treated with N/1 nitric acid for 5 min., stained Giemsa and restained tannic-acid-violet, to demonstrate that the ejected nuclear material is outside the spore coat. One spore has retained it within the spore coat, and is exhibiting the 'peripheral nucleus'. × 5000.

Fig. 2. Spores of *B. subtilis*, showing the spore nucleus in its natural condition. Acid-Giemsa. × 3000.

Figs. 3, 4. Spores of *Cl. welchii*. Nitric acid for 10 min., stained Crystal violet. All conditions seen: *a*, nucleus in natural condition; *b*, 'crescentic nucleus'; *c*, 'peripheral nucleus'. Other spores are in intermediate stages of change. × 3000.

Fig. 5. Maturing spore of *Cl. welchii*, as in Figs. 3 and 4, showing no displacement of nuclear material. × 3000.

Fig. 6. Spores of the same *Bacillus* as Fig. 1. Several hours after subjection to N/1 nitric acid for 5 min. The spore coat is disrupted, but the nuclear material retains its form. Stained Giemsa. × 3000.

Fig. 7. Spores of the same *Bacillus* as Fig. 1. Electron micrograph, gold-shadowed, made after treatment of the mount with N/1 nitric acid for 5 min. Showing ejected nuclear material. × 16,000.

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