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## Nuclear Morphology of *Bacillus cereus* grown on Partially Defined Media

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**SUMMARY:** When *Bacillus cereus* was grown on a medium solidified with agar but of otherwise known composition (salts, glucose, urea) impression preparations made after 2½ hr. of growth at 30° showed simple round nuclei by the osmic acid-hydrochloric acid-Giemsa technique. Similarly treated preparations made after 24 hr. of incubation showed well-marked unstained refractile inclusions. These inclusions, whose lipid nature was indicated by staining with Sudan Black, were closely associated with nuclear material, often appearing to be surrounded by an unbroken ring, a granular band, or a horse-shoe shape of nuclear material. These observations support the suggestion of Delaporte (1950) that lipid inclusions may distort nuclear shapes. These distortions might be misinterpreted as mitotic figures.

The subject of the structure and mode of division of the bacterial nucleus is still controversial. In an attempt to bring a different approach to this problem, a study has been made of the way in which various nuclear patterns can be produced regularly in response to altered nutritional conditions.

### METHODS

The organism used throughout was a strain of *Bacillus cereus* (NCTC 8035). Stock cultures were maintained on nutrient agar. A washed suspension (0.1 ml.) of organisms grown for 18 hr. in meat-extract broth at 30° was spread over the surface of the following media solidified with agar: basal medium; basal medium + 1 % (w/v) glucose, or + 1 % (w/v) urea, or + 1 % (w/v) glucose and 1 % (w/v) urea; nutrient agar. The basal medium (modified from Meiklejohn, 1950) had the following composition: dipotassium hydrogen phosphate ( $K_2HPO_4$ ) 1 g., sodium chloride 2 g.,  $MgSO_4 \cdot 7H_2O$  0.5 g., ferric chloride, trace, calcium carbonate 10 g., agar 20 g., tap water 1000 ml. The final pH value of this medium was 7.6; it was sterilized at 15 lb./sq.in. for 15 min.

Impression preparations, made from the growths on all these solid media after 2½ and after 24 hr. of incubation at 30°, were treated by the osmic acid-hydrochloric acid-Giemsa technique (HCl-Giemsa) (Robinow, 1944) for the demonstration of nuclear material. Preparations 24 hr. old were also stained by Sudan Black for the demonstration of lipid material. Wet preparations were made and examined by the phase-contrast microscope. Preparations made after 34 hr. of growth on basal medium + urea + glucose were stained by HCl-Giemsa and also examined by phase-contrast microscopy.

Photographs were taken by a 'Laboratory' photomicrographic camera (W. Watson and Sons Ltd.) on Ilford rapid process panchromatic plates.

## RESULTS

When the organism was grown on basal medium + glucose + urea at 30° for 2½ hr. the nuclear material demonstrated by the HCl-Giemsa method had the form of a simple round structure, generally in the centre of the cell (Pl. 1, fig. 1*a*). The division of the nuclear material in this actively growing culture appeared to be simple, consisting of a constriction of the nucleus into two daughter nuclei (Pl. 1, fig. 1*b*). Occasionally there was elongation of the nucleus as well as constriction (Pl. 1, fig. 1*c*).

After 24 hr. of incubation, the arrangement of the nuclear material was considerably changed. There were a few simple nuclei but the majority of the organisms contained nuclei with a round unstained refractile area in their centres (Pl. 1, fig. 2*a*). These structures, i.e. the central unstained area and the surrounding nuclear material, were named X-structures. There was a range in size of these unstained areas from small to large; they also showed variety in distribution, some being single, others in groups of two (Pl. 1, fig. 2*b*) or three. The surrounding band of nuclear material also showed differences in shape; in some organisms it was a complete band (Pl. 1, fig. 2*c*), in some it was horse-shoe shaped (Pl. 1, fig. 2*d*) and in others it was granular (Pl. 1, fig. 2*e*).

Staining with Sudan Black revealed that visible lipid (Pl. 1, fig. 3) was present in the organisms grown on the basal medium + urea + glucose. These lipid structures showed a range in size from small to large; they also showed variety in their distribution, some being single, others in groups of two or three. In short, they were similar in size, shape and distribution to the unstained areas of the X-structures. In a given set of fields counts were made of the number of X-structures demonstrated by the HCl-Giemsa method and of the number of inclusions in the Sudan Black preparations. The two counts were very close: 41·2 X-structures and 42·7 lipid granules/100 organisms.

Wet preparations of the same 24 hr. growth examined by phase-contrast microscopy revealed dark round granules (Pl. 2, fig. 4). These granules had a range in size from small to large, and they also showed variety in their distribution, some being single, others in pairs, and others in groups of 3.

Phase-contrast microscopy of cultures grown on basal medium agar + urea + glucose for 34 hr. at 30° revealed that developing endospores had a characteristic appearance when examined by this method (Pl. 2, fig. 5). They were oval and very refractile and could not be mistaken for the dark granules illustrated in Pl. 2, fig. 4. Developing endospores also gave a characteristic appearance when impression preparations taken after 34 hr. growth were stained by the HCl-Giemsa method (Pl. 2, fig. 6). They were darkly staining oval structures without internal differentiation, and could not be mistaken for X-structures.

Neither lipid inclusions nor X-structures were seen when the organism was grown on: basal medium alone; basal medium + glucose; basal medium + urea; nutrient agar after 24 hr. growth at 30°.

## DISCUSSION

The observations of this paper show that the nuclear material of *Bacillus cereus* (NCTC 8035) can be distorted into bizarre and unusual shapes by lipid inclusions and that these distorted nuclear forms can be produced by growing the organisms on a suitable medium. These observations support the suggestion of Delaporte (1939, 1950) that lipid and other inclusions may alter the shape of the bacterial nucleus, producing structures which in Delaporte's words are 'not determinate ones but altered passive ones'. The possibility of taking nutritional factors into consideration in the interpretation of results may well deserve more attention from bacterial cytologists. Perhaps the mitotic figures described by DeLamater & Mudd (1951) in *Bacillus megaterium* were no more than simple nuclei distorted by inclusions composed of lipid. The present findings emphasize the need for more and different approaches to the study of the bacterial nucleus. Not only must techniques for the demonstration of nuclear material be correlated with studies of living unstained and unfixed organisms, but the results need integration with the demonstration of cell inclusions, especially lipid inclusions.

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## EXPLANATION OF PLATES

*Bacillus cereus* NCTC 8035 grown on basal medium agar + 1% glucose + 1% urea at 30°.

## PLATE 1

- Fig. 1. Actively growing culture (2½ hr. old) stained by the HCl-Giemsa method showing: (a) round nucleus; (b) constriction of dividing nucleus; (c) elongation and constriction of nucleus. (× 3000)
- Fig. 2. X-structures. 24 hr. culture stained by the HCl-Giemsa method showing many nuclei with clear central areas as in (a). These clear areas may be single or in groups of two as in (b). Nuclear material is sometimes a complete band (c), horse-shoe shaped (d) or granular (e). (× 3000)
- Fig. 3. 24 hr. culture stained by Sudan Black, showing lipid inclusions which show the same variety in size and distribution as the clear areas of the X-structures in fig. 2. (× 2500)

## PLATE 2

- Fig. 4. 24 hr. culture examined by phase-contrast microscopy, showing round dark granules of different sizes and with a varied distribution. ( $\times 2500$ )
- Fig. 5. Developing endospores as demonstrated by phase-contrast microscopy in a 34 hr. culture. ( $\times 2000$ )
- Fig. 6. Darkly-staining developing endospores demonstrated by the HCl-Giemsa method in a 34 hr. culture. ( $\times 2000$ )

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