SOME ULTRAVIOLET PHOTOMICROGRAPHS OF B. SUBTILIS

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Plates 42 to 44

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In the course of some studies being made with the ultraviolet microscope we have prepared photographs of *B. subtilis* which yield useful information concerning the internal structure of this bacterium and the way spores arise within it.

Two bacterial strains were examined. One was non-sporulating and came from the Type Collection in Chicago; the other, which grew larger bacilli and readily produced spores in old cultures, was isolated in this laboratory. Before being photographed the living unstained cells were transferred to the surface of a thin layer of NaCl-agar spread upon a quartz slide. In this way they were fixed in position and kept stationary during exposure.

Technique

The microscope was a modification of the instrument designed by Barnard[1] and manufactured by Beçk. Its optical parts, as used in the present experiments, were, however, quartz objectives and eyepieces by Zeiss. As their design requires, these objectives have been employed with light of wave length 2750 A. Desired objects are found with the green line of the mercury arc; approximate focus in the ultraviolet is then achieved by applying a precise but arbitrarily determined correction. The focal plane is shallower with ultraviolet than with visible light and it is usually necessary to make a series of exposures at different depths through a preparation. This has been done by taking photographs upon motion picture film. The small Leica camera modified by removing its glass optical parts and giving it a very rigid support behind the eyepiece of the microscope is convenient for this purpose. Ordinary positive film gives excellent results—it is sufficiently

[1] Barnard, J. E., *Lancet*, 1925, **2**, 117.

rapid in the ultraviolet and its fine grain permits very satisfactory enlargements. The accompanying photographs have thus been made, the objective being the standard Zeiss 1.70 mm. glycerine immersion monochromat. Exposure times varied from $\frac{1}{2}$ to 3 seconds.

Ultraviolet microscopy has two outstanding advantages over that using visible light. One resides in the greater resolving power which is a consequence of the short wave length; the other arises from the fact that, since some proteins absorb in the ultraviolet more strongly than others, it is often possible to see detail in living and unstained cells. Both of these advantages are realized in the accompanying pictures. The first figure, of a 5 hour culture on agar, makes it clear that young and rapidly growing cells contain no observable structures. In older cultures of both the non-sporing and the sporing lines cells become granulated (Figs. 3–6). At first these granules are few in number and small. Very old bacilli, however, are often filled with inclusions of all sizes (Fig. 2). It is important to notice that in none of these photographs is there any indication of formed nuclei.

Especially interesting is the evidence bearing upon spore production. These bodies are intensely absorbing (Figs. 2–5) and many stages in their development can therefore be seen directly. They do not arise through the gradual accumulation and merging together of the refractile granules or of any preexisting cell bodies that have strong ultraviolet absorption. Instead, at the first appearance they have their final size and shape. In initial stages they are only faintly absorbing but as they develop this absorption becomes progressively greater (Fig. 4). Cells containing mature spores are approximately as opaque as other bacteria. This suggests that spores when fully formed are not a mere condensate of a bacterium's protoplasmic or chromatin content.

There are three frequently expressed views concerning the origin of spores.[2] According to one they arise after a nuclear division which has partitioned the chromatin between a spore-anlage and the cell nucleus. Other investigators have described them as resulting from the coalescence of chromatin granules which in more recent work are definitely associated with nuclei thought to be present. It is apparent

[2] See for example Gotschlich, E., in Kolle, W., Kraus, R., and Uhlenhuth, P., Handbuch der pathogenen Mikroorganismen, Jena, Gustav Fischer, and Berlin and Vienna, Urban and Schwarzenberg, 3rd edition, 1929, 1, 33.

that the spores of *B. subtilis* do not have the small beginnings required by this hypothesis. A third idea makes spores the result of a more or less gradual concentration of cell substance. Some have thought that this concentration was in the main a dehydration succeeded by the infiltration of lipoids. In its broad outlines such an hypothesis is not incompatible with our photographs. At present ultraviolet microscopy is obviously unable to interpret chemical changes that may take place but nevertheless pictures such as Fig. 4 seem to demand a mechanism more elaborate than mere dehydration.

EXPLANATION OF PLATES

PLATE 42

FIG. 1. A young culture of *B. subtilis* growing on agar, photographed 5 hours after inoculation and 10 minutes after being covered with quartz. 3300×.

FIG. 2. Cells from a 6 day agar culture transferred to a film of fresh nutrient agar just before being photographed. The large inclusions present in the degenerating cells can easily be seen. 2500 ×.

PLATE 43

FIG. 3. Cells of a 31 hour old culture of non-sporulating *B. subtilis* growing on agar. The inclusions present in nearly every cell are clearly visible. 3500×.

FIG. 4. Cells from a 20 hour culture of spore-forming *B. subtilis*. The bacteria were transferred to a film of pure agar immediately before being photographed. Different cells contain spores in all stages of development (and of corresponding opacity). 3500 ×.

PLATE 44

FIG. 5. Bacteria from a 24 hour culture of sporulating *B. subtilis* grown on agar. They were transferred before being photographed to a film of 2 per cent agar in 0.85 per cent NaCl on a quartz slide. It will be noticed in this and the preceding picture that, though spores do not grow from granules, cells containing mature or developing spores are relatively free from inclusions. 3500 ×.

FIG. 6. The same field as the preceding but with the focal plane slightly above that of the bacteria. 3500 ×.

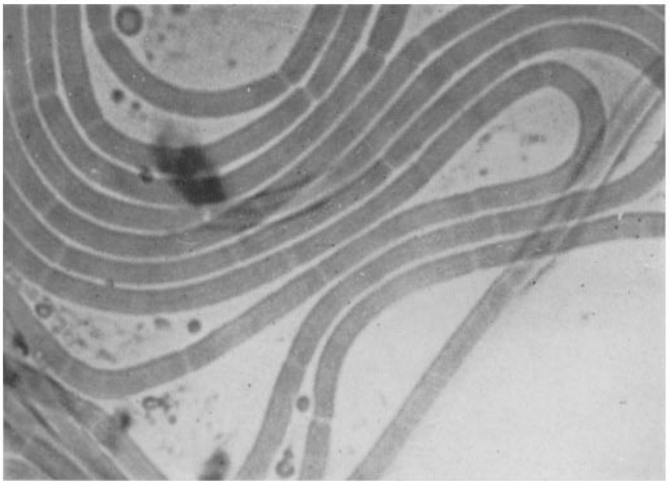

FIG. 1

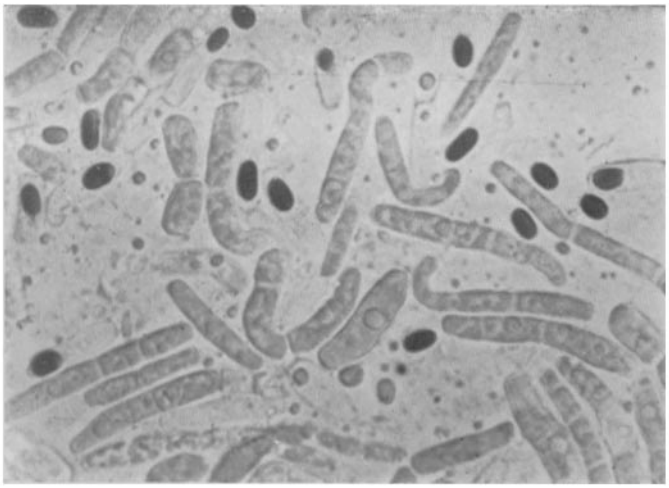

FIG. 2

(Wyckoff and Ter Louw: Ultraviolet photomicrographs of *B. subtilis*)

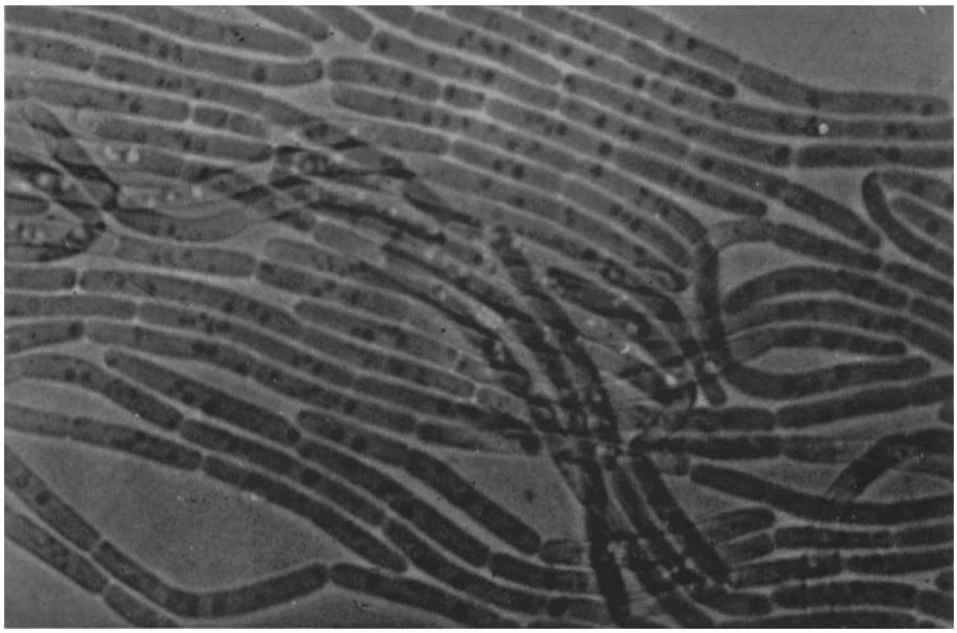

FIG. 3

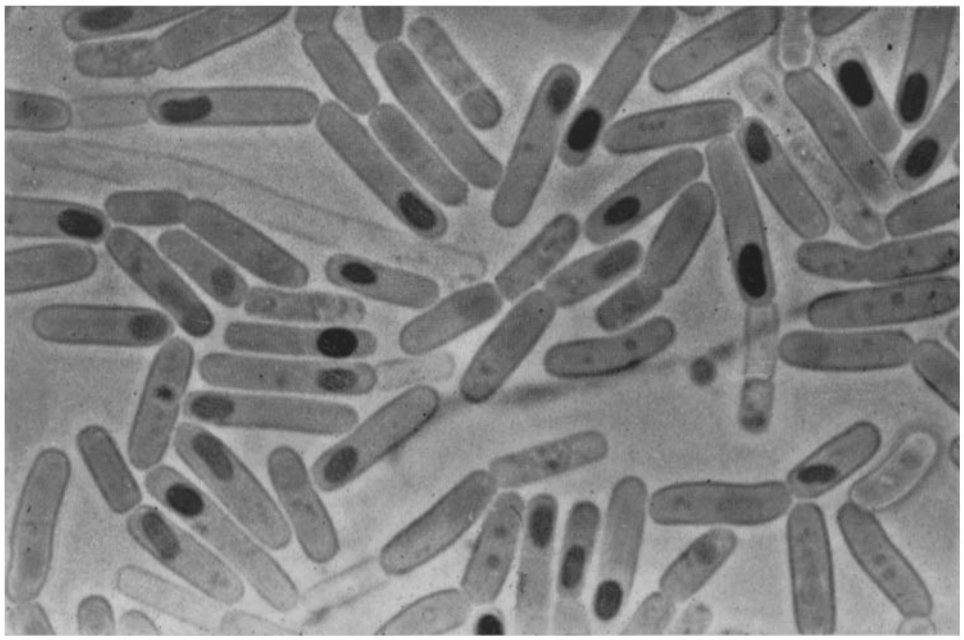

FIG. 4

(Wyckoff and Ter Louw: Ultraviolet photomicrographs of *B. subtilis*)

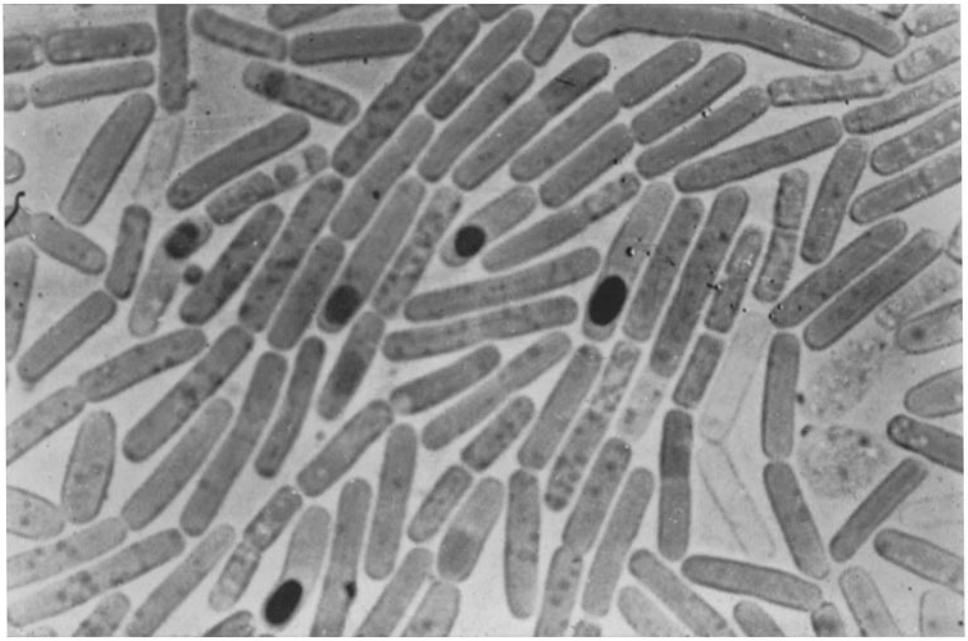

FIG. 5

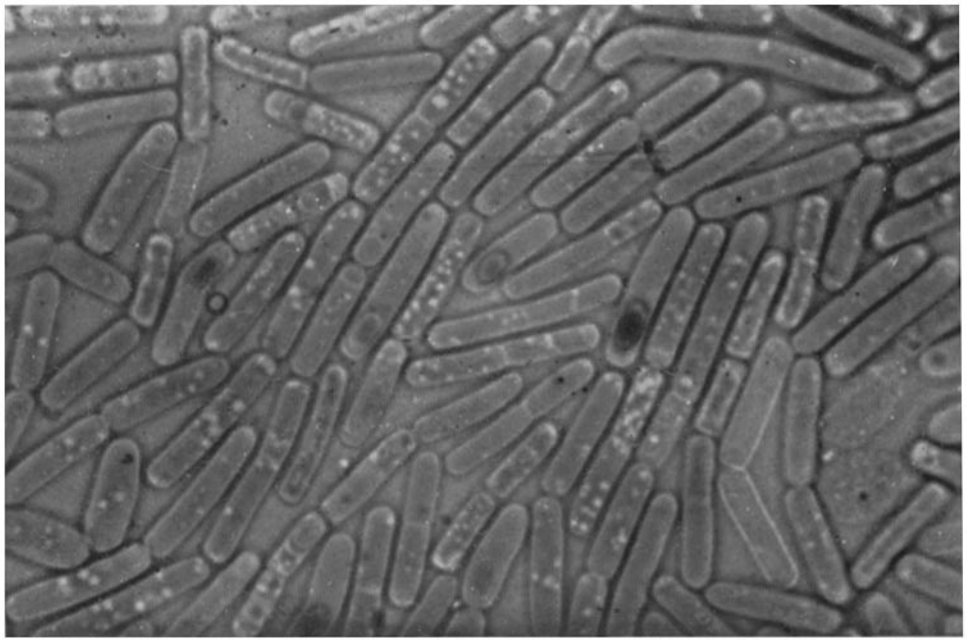

FIG. 6

(Wyckoff and Ter Louw: Ultraviolet photomicrographs of *B. subtilis*)