OBSERVATIONS UPON THE BACTERIAL NUCLEUS

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(With Plate 9 and 7 Figures in the Text)

This paper, which consists mainly of new observations, is also intended to correlate, expand and revise some of the material contained in the author's previous studies upon bacterial cytology.

TECHNIQUE

The majority of the observations were made upon water-mounted preparations stained by the acid-Giemsa technique (Robinow, 1942). This method employs acid hydrolysis to reduce the affinity for stain of the cell membranes and cytoplasm. Myxobacteria, which lack the cell wall of the Eubacteria, stain readily with Giemsa alone, whereas the Gram-negative cocci resist these methods and reveal their nuclear structures best when stained with methylene blue and differentiated with eosin, according to the technique of Badian (1933). This method is not easy; but it produces preparations of great beauty, with blue cytoplasm and red nuclei.

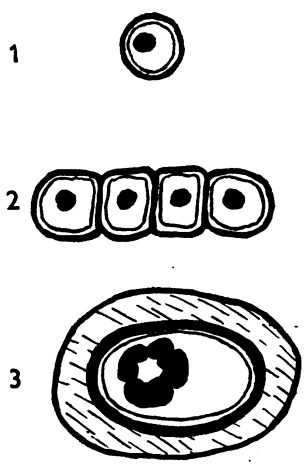
OBSERVATIONS

Gram-negative cocci

The strains examined were derived from the respiratory passages and faeces of man and laboratory animals, and cultivated upon routine media. Like the staphylococci (Bisset, 1948 *a*), the Gram-negative cocci may be unicellular or divided into two cells by a transverse membrane. The latter, two-celled cocci are the typical *Neisseria*, and their morphology is clearly visible by simple staining techniques. In this they differ from the Gram-positive cocci which appear as undifferentiated spheres when unsuitably stained. The great majority of Gram-negative cocci which were studied, however, were unicellular, and possessed a spherical nucleus, usually slightly eccentric in position (Pl. 9, fig. 1; Text-fig. 1). They divided by simple constriction (Pl. 9, fig. 2), and dividing cells contained two or more nuclei. Occasional short filaments were seen, containing as many as five nuclei (Pl. 9, figs. 3, 4). Although this form of nucleus is apparently simple in morphology, it is near the limit of optical resolution and cannot be examined critically. Some evidence as to its nature may be obtained, at least by analogy, from the nucleus of *Azotobacter*, which is described in the next section.

Azotobacter

Azotobacter chroococcum was grown upon Petri dishes of moistened soil. This bacterium, despite its size, has cytological characters resembling those of the unicellular cocci. It divides by simple constriction (Pl. 9, fig. 5), and possesses a spherical, slightly eccentric nucleus (Pl. 9, figs. 6, 7). The cells are frequently coccal, and appear to be capable of



Text-figures 1–3. Bacteria possessing a discrete nucleus. 1, *Coccus*; 2, *Corynebacterium*; 3, *Azotobacter*. Thick line represents cell wall; thin line, cell membrane. Capsule of *Azotobacter* is shown hatched.

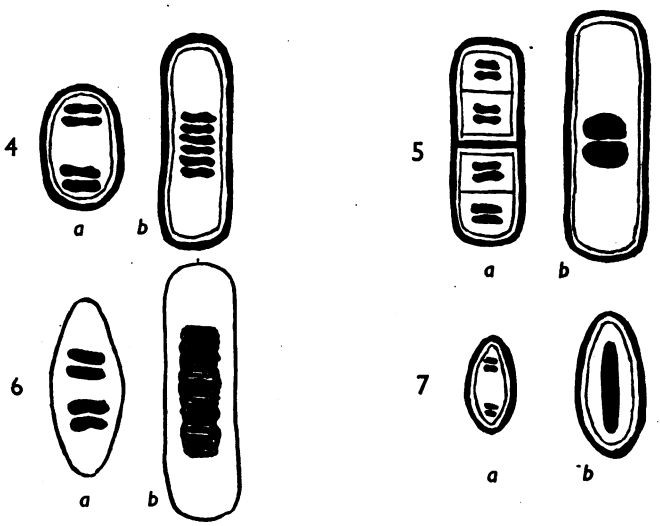
dividing at right angles to the previous plane of division, which true bacilli are not. This can be deduced from the arrangement of cells within the common, mucous capsule (Pl. 9, fig. 8). The nucleus resembles that of the Gram-negative cocci in that it can readily be stained by the methylene-blue-eosin technique, but resists acid-Giemsa. When fully differentiated the nucleus appears as a vacuole, surrounded and partly obscured by chromatinic granules, and closely resembling the nucleus of certain yeasts (Pl. 9, fig. 7; Text-fig. 3). It is not impossible that the smaller spherical nuclei of the Gram-negative cocci also have this type of structure.

Other bacteria possessing spherical nuclei

Certain other bacteria possess spherical nuclei, or appear to do so. Like the staphylococci, the streptococci vary in this respect. The lanceolate, short-chained streptococci possess a structure analogous to that of some rod-shaped bacteria, and will be discussed in the next section. Other types resemble the staphylococci more closely (Bisset, 1948*a*) (Pl. 9, fig. 9). The Corynebacteria are markedly multicellular, and the component cells, which often are almost spherical in shape, possess central nuclei (Pl. 9, fig. 10; Text-fig. 2). These nuclei are perfectly distinct from the 'metachromatic granules' which are artefacts. This question is the subject of a separate study.

bacillus contains two pairs of chromosomes, whose division precedes that of the cell, exactly as is the case with plant or animal cells (Pl. 9, fig. 11; Text-fig. 4*a*). The short-chained streptococci possess a similar morphology to the smooth bacilli, which they resemble in other respects (Bisset, 1948*a*) (Text-fig. 7*a*).

The bacteria of this type, whose nuclei are most frequently found in the chromosomal condition, possess an alternative nuclear pattern. The chromosomes appear to fuse in the centre of the bacillus, forming a nucleus, the pattern of which varies somewhat in the different genera (Pl. 9, figs. 12, 13, 15; Text-figs. 4*b*, 5*b*, 6*b*, 7*b*). The chromatinic material is redistributed throughout the elongating bacillus, which then divides into several daughter



Text-figures 4–7. Types of bacteria possessing chromosomes. *a*, chromosomal condition; *b*, fusion nucleus. 4, Rod-shaped *Eubacteria*, smooth morphology; 5, Rod-shaped *Eubacteria*, rough morphology; 6, *Myxobacteria*; 7, Short-chained *Streptococci*. Thick line represents cell wall (absent in *Myxobacteria*), thin line cell membrane.

Rod-shaped Eubacteria

The great majority of rod-shaped Eubacteria differ from the genera described in the preceding paragraphs in that their nuclei are most frequently observed in a condition in which they consist of a number of paired chromosomes. These are the 'dumb-bell-shaped chromatinic bodies' described by Robinow (1942) and other workers. The small size of the chromosomes and the difficulty of resolving them optically has resulted in unresolved pairs being described as single bodies. The author has previously described the four-celled, rough type of bacillus as having a single chromatinic body in each cell, but more recent observations make it appear that this body represents a pair of chromosomes (Bisset, 1947) (Text-fig. 5*a*). The unicellular, smooth type of cells (Bisset, 1948*b*, 1948*c*). The details of the process probably vary considerably, but in coliform bacteria of smooth morphology a single division of the chromosomes occurs within the nucleus, and a second division before fragmentation into daughter bacilli. Types of fusion nuclei are shown in Text-figs. 4*b*, 5*b*, 7*b*. This process of nuclear reorganization has so far been observed only in non-sporing genera, and is quite distinct from the various cytological processes connected with sporulation. These have recently been reviewed by Knaysi (1948).

Myxobacteria

In addition to the processes concerned with microcyst formation, Myxobacteria give evidence of undergoing a nuclear cycle similar to that described in the rod-shaped Eubacteria. The vegetative cells

composing the mature swarm possess two pairs of chromosomes, similar to those of a smooth *Eubacterium* but lying together near the centre of the cell (Pl. 9, fig. 14; Text-fig. 6*a*). In the early stages of the swarm, however, a high proportion of cells contain fusion nuclei, and many are filamentous (Pl. 9, fig. 15; Text-fig. 6*b*). These forms closely resemble those described in the previous section, and are not concerned, as far as can be determined, with microcyst formation or germination.

(I am indebted to Miss J. B. Grace for the material from which the observations of Myxobacteria were made.)

SUMMARY AND CONCLUSIONS

Two different types of nucleus are found to occur in bacteria. The first is a spherical, central or eccentric body occurring in some types of cocci and in Corynebacteria. By analogy with a similar structure in *Azotobacter*, it is considered that the nucleus may be of vesicular form, surrounded by chromatinic granules, and resembling the nuclear vacuole of certain yeasts.

The second type of nucleus consists of paired chromosomes, usually either one or two pairs. These chromosomes form fusion nuclei within which chromosome divisions take place; they are then redistributed to a number of daughter cells.

The majority of rod-shaped Eubacteria, including the lanceolate-celled streptococci, have this type of nucleus, which is also found in Myxobacteria. The nuclear cycle is quite distinct from the cytological processes which accompany spore and microcyst formation and germination.

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EXPLANATION OF PLATE 9

Fig. 1. A Gram-negative coccus isolated from faeces. Methylene-blue-eosin. × 3000.
Fig. 2. A Gram-negative coccus isolated from faeces. Tannic-acid-violet. × 3000.
Figs. 3, 4. A Gram-negative coccus isolated from faeces. Methylene-blue-eosin. × 3000.
Fig. 5. *Azotobacter chroococcum*. Methylene blue. × 3000.
Figs. 6, 7. *Azotobacter chroococcum*. Methylene-blue-eosin. × 3000.
Fig. 8. *Azotobacter chroococcum*. Basic fuchsin. × 3000.
Fig. 9. *Streptococcus pyogenes*. Acid-Giemsa. × 3000.
Fig. 10. *Corynebacterium* sp. of vaginal origin. Acid-Giemsa. × 3000.
Fig. 11. *Bact. dysenteriae* (Flexner). Composite photograph. Acid-Giemsa. × 3000.
Fig. 12. *Streptococcus faecalis*. Fusion nuclei and filament. Acid-Giemsa. × 3000.
Fig. 13. *Lactobacillus* sp. Fusion nucleus. Acid-Giemsa. × 3000.
Fig. 14. *Chondrococcus exiguus*. Vegetative cells. Giemsa. × 3000.
Fig. 15. *Chondrococcus exiguus*. Fusion nuclei. Giemsa. × 3000.

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