NUCLEAR REORGANIZATION IN NON-SPORING BACTERIA

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(With Plate 7 and 11 Text-Figures)

INTRODUCTION

The fusion nuclei, first described by Robinow (1944) in *Proteus* and *Bact. coli*, were later shown by Bisset (1948) to be stages in a cycle of nuclear reorganization in non-sporing Eubacteria, having points of resemblance with the cycle described in spore-bearing genera by Klieneberger-Nobel (1945). In cultures of *Lactobacillus* and *Streptococcus* the chromosomes fused into a central nuclear body. The cell containing the fusion nucleus then grew considerably in length without dividing, and the nuclear material was redistributed throughout the resulting filament, which eventually returned, by fragmentation, to the bacillary or coccal form. The bacteria studied were small in size, and the behaviour of the individual chromosomes could not be followed throughout the process. The present study is an attempt to remedy this defect, the bacteria employed being the slightly larger Gram-negative intestinal genera, in some of which the individual chromosomes may be observed with reasonable clarity.

TECHNIQUE

Robinow's modification of the Feulgen reaction for nuclear apparatus, and the same author's tannic-acid-violet stain for cell walls, were used throughout. Preparations were always examined in the wet condition.

OBSERVATIONS

A large number of preparations were made of Gram-negative intestinal bacteria. In young cultures fusion nuclei were regularly found (Pl. 7, figs. 1–4). Some of the cells which contained them were distinctly enlarged (Pl. 7, fig. 2), but more frequently they were similar in breadth to a normal bacillus, and about twice the length. Pl. 7, fig. 1, which shows a fusion nucleus, together with a single cell, and a dividing bacillus, in which the transverse septum, derived from the cell membrane, is clearly seen, enables a comparison to be made. The resting cell contains two pairs of chromosomes and the dividing cell four pairs. The term 'pairs of chromosomes' is employed because it is the author's experience that, where they are large enough to be resolved by the microscope, bacterial chromosomes, like those of other living organisms, are found to be in pairs. Previous descriptions of single chromosomes, by the author and by others, probably refer to optically unresolved pairs. Indications can be found that even the minute and apparently spherical chromatinic bodies of a streptococcus are in fact paired structures.

Staining with tannic-acid-violet revealed occasional elongated cells with a thickening of the cell wall corresponding in position and size to the fusion nucleus. These cells were internally undivided, and showed no sign of the constriction of the cell wall which accompanies division in smooth cultures. (Pl. 7, fig. 3). This constriction of the cell wall should not be confused with the production of a transverse septum, derived from the cell membrane, which is referred to above.

In many of the fusion nuclei, it was possible to estimate the number of chromosomes taking part in its composition; this usually appeared to be six (Text-figs. 1, 2). In the latter stages of the fusion

Text-figs. 1 and 2. Tracings of fusion nuclei in Plate 7 figs. 1 and 4, showing combination of six chromosomes. Fig 1. corresponds to Plate 7, fig. 4, and Fig. 2 to Plate 7, fig. 1.

process, when the nucleus had commenced to expand, the number of chromosomes was seen to have increased (Pl. 7, figs. 5–7). When the chromosomes had moved out still farther along the growing filament, it was observable that their number increased to approximately double the original three pairs, indicating a single nuclear division (Pl. 7, figs. 8, 9). Further growth of the filament, and another doubling of the chromosomes was followed by fragmentation and a return to the bacillary condition (Pl. 7, figs. 10–12).

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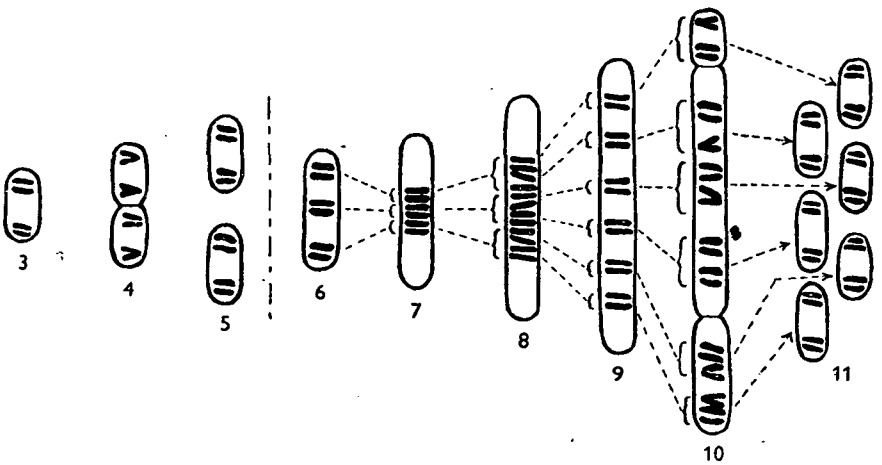
These observations exactly parallel those described for *Lactobacillus* and *Streptococcus* (Bisset, 1948), but, in addition, it has been found possible to follow the behaviour of the individual chromosomes throughout much of the process. The regular occurrence of three chromosome pairs in the fusion nuclei makes it necessary to postulate the existence of a pre-fusion stage, containing three pairs of chromosomes instead of the usual two, and, in fact, such bacilli are found (Pl. 7, figs. 13, 14). They are not at all common in their occurrence, but the complete absence of any other form containing an odd number of pairs of chromosomes, makes it reasonable to suppose that they represent the immediate precursor of the fusion nucleus. In the case of the *Lactobacillus*, previously described, the organism was of multicellular, rough morphology, and there was no occasion to presuppose the existence of such a form. Preparations were also made in the present case from a number of cultures of varying degrees of roughness, but in these no fusion nuclei were observed. Most rough cultures contained filaments, but these merely repeated the nuclear pattern of the individual bacilli (Pl. 7, fig. 15). When stained by tannic-acid-violet these filaments appeared as chains of bacilli.



Text-figs. 3–11. Chromosome behaviour in a bacterium (diagrammatic). 3–5. Normal cell division, 6. Trinucleate cell (autogamy and reduction?). 7–8. First fusion division. 9–10. Second fusion division. 10–11. Fragmentation.

DISCUSSION

The foregoing observations indicate that the previously described nuclear fusion and redistribution in non-sporing bacteria entails one chromosome division within the fusion nucleus, and a second division during the redistribution of the chromosomes throughout the elongating filament (Text-figs. 7–10). Thus each chromosome of the trinucleate cell, which precedes the fusion nucleus (Text-fig. 6), may give rise to a single bacterium, after fragmentation of the filament has taken place. This, however, is problematical, until it can be proved that reduction of nuclear material does not take place at the fragmentation stage, or, alternatively, that new nuclear material is not derived from the cytoplasm. The mode of formation of the trinucleate bacillus is not easy to understand. The production of an uneven number of chromosome pairs may conceivably indicate some process of reduction at this stage.

The obvious pairing of the chromosomes provides additional evidence that the nuclear apparatus of bacteria is not *sui generis*, as has been supposed, but closely resembles that of other organisms.

Recent observations by Klieneberger-Nobel (1947) appear to indicate that the occurrence of fusion nuclei is more frequent in bacteria which have been grown at low temperatures, but no explanation of this can, at present, be suggested.

SUMMARY

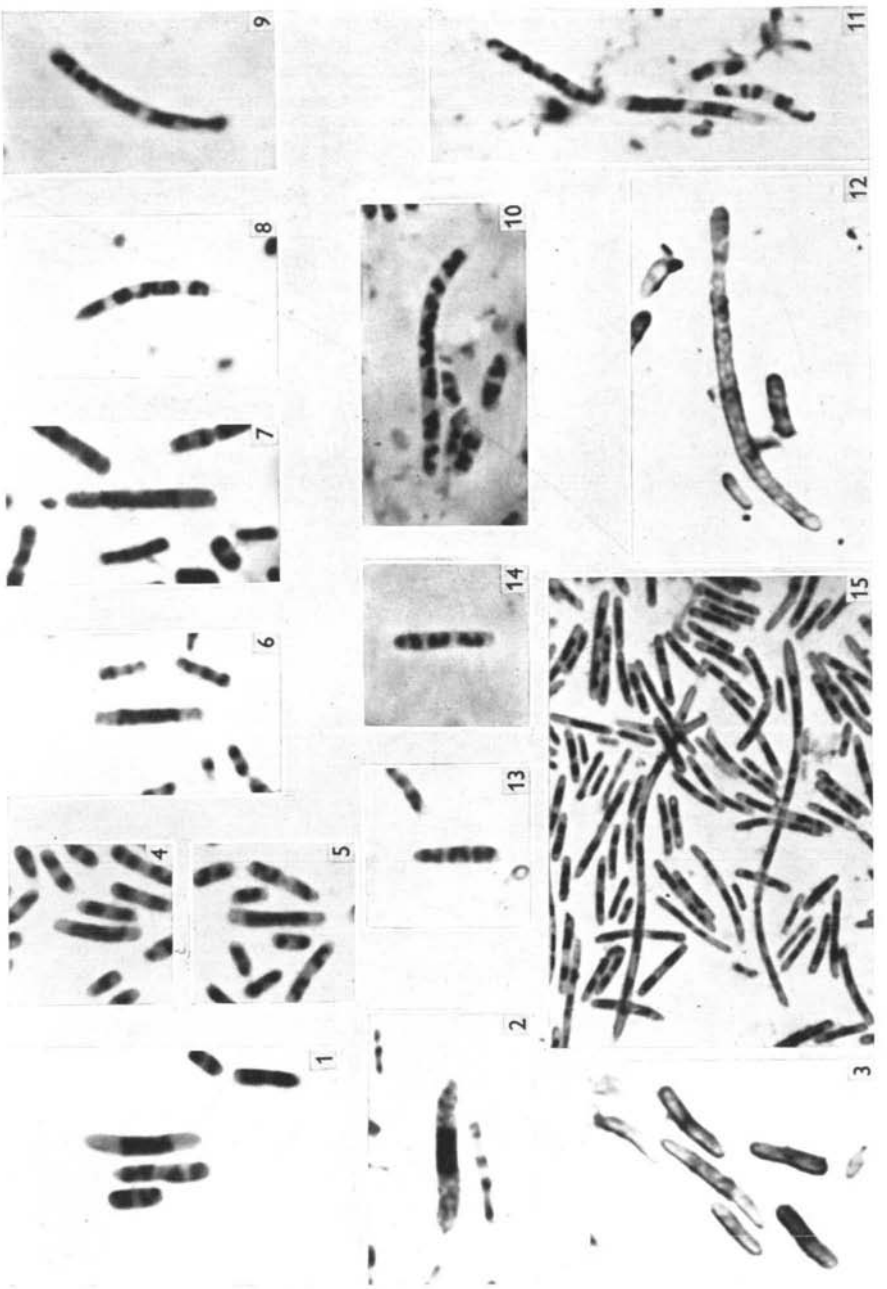
1. The process of nuclear fusion and reorganization as it occurs in members of the Bacteriaceae is described.

2. The chromosomes behave as pairs at all times, the normal bacillus, of smooth morphology, contains two pairs.

3. The fusion nucleus contains three pairs and is preceded by a corresponding trinucleate bacillus.

4. One division of the chromosomes takes place in the fusion nucleus, and another during the process of redistribution of the chromosomes. The second division is followed by fragmentation, and return to the bacillary condition.

PLATE 7



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REFERENCES

BISSET, K. A. (1948). *J. Gen. Microbiol.* (In Press.)
KLIENEBERGER-NOBEL, E. (1945). *J. Hyg., Camb.*, **44**, 99.
KLIENEBERGER-NOBEL, E. (1947). *J. Hyg., Camb.*, **45**, 410.
ROBINOW, C. F. (1944). *J. Hyg., Camb.*, **43**, 413.

EXPLANATION OF PLATE 7

Fig. 1. *Bact. dysenteriae* Flexner, normal cell, dividing cell and fusion nucleus. (× 2000.)
Fig. 2. *Bact. dysenteriae* Flexner, fusion nucleus. (× 2000.)
Fig. 3. *Bact. dysenteriae* Flexner, cell walls of dividing bacilli, showing constriction, and of cell containing fusion nucleus. (× 2000.)
Figs. 4, 5. *Bact. coli*, fusion nuclei, normal and dividing bacilli. (× 2000.)
Figs. 6, 7. *Bact. dysenteriae* Flexner, expanding fusion nuclei. (× 2000.)
Figs. 8, 9. *Bact. dysenteriae* Flexner, growing filaments, showing redistribution of chromosomes after first division. (× 2000.)
Figs. 10–12. *Bact. dysenteriae* Flexner, fragmenting filaments; figs. 10 and 11 stained to demonstrate chromosomes, fig. 12 to demonstrate cell wall. (× 2000.)
Figs. 13, 14. *Bact. dysenteriae* Flexner, trinucleate bacilli. (× 2000.)
Fig. 15. *Bact. coli*, rough culture showing filaments. (× 1000.)

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