A Cytological Study of Myxococci

BY EMMY KLIENEBERGER-NOBEL

From the Bacteriological Department, Lister Institute, London

SUMMARY: Myxococci are distinguished from other bacteria by the complete lack of a cell membrane as well as of transverse septa. Even the microcyst is enclosed by an outer layer which differs from a bacterial cell wall. In cytological character the young stages in the life cycle resemble other bacteria in so far as they contain two to four small nuclear structures or 'chromosomes' arranged transversely in the cell and dividing longitudinally. Older organisms about to form microcysts differ; they contain two fairly large nuclear structures which fuse to form a round chromatinic body. At the same time the cell shortens until a round organism containing one large, round, darkly staining nuclear body is formed. This 'fusion cell' can be compared to a zygote though it is not yet the resting cell of the species. The fusion cell becomes oval, its nucleus divides into two, and its outer layer becomes tough and dense. Thus the microcyst is formed. When it germinates its outer layer disappears, and the cell is transformed by elongation into the young vegetative organism.

A study has been made of the life cycle of myxococci by methods used in recent years for the fixing and staining of nuclear structures, cytoplasm and membranes in bacteria (Klieneberger-Nobel, 1945, 1947; Robinow, 1942, 1944, 1945). The organisms investigated were Myxococcus fulvus, M. virescens and Chondrococcus exigus. They were kindly provided by Dr Singh of the Rothamsted Experimental Station.

METHODS

The methods employed are similar to those used in earlier work (Klieneberger-Nobel, 1945, 1947). The nuclear structures of young cells could be demonstrated by staining fixed preparations with Giemsa's solution. The more advanced stages, particularly the mature microcysts, needed the acid treatment before staining. The best growths for examination were obtained in the moist zone on the coverslip round the piece of agar. Here the cultures developed freely and were not disturbed when the agar was removed. A water agar enriched by a heat-killed suspension of Bacterium coli in saline served as the medium (Singh, 1946, 1947). Seitz-filtered glucose solution (Stanier, 1942a,b) and small amounts of dung extract were frequently added to promote growth. Softness of the agar was advantageous. The incubation temperatures varied between 25° and 34°. For the study of the germination of the microcysts it was necessary to incubate at temperatures slightly above 30° in order to ensure a quick and simultaneous development.

THE LIFE CYCLE

(a) The germination of microcysts

The mature, almost round microcyst occurs in the fruiting bodies of cultures 2–4 weeks old. It possesses a thick, dense outer layer which simulates a membrane and stains deeply. Two nuclear structures, which are usually in
close contact, are demonstrable in mature microcysts after treatment with HCl (Text-fig. 15).

In old cultures varying numbers of disrupted cysts are frequently found. They appear to arise through an extrusion of the contents of the microcyst (Pl. 1, figs. 9, 10). The two nuclear structures or granules are distinguishable in the extruded material. When cultures on fresh media are examined at short intervals it is found that the disrupted cysts show no further development and that their number does not increase. They must therefore be regarded as dead. They might easily be mistaken for germinating cells if the mode of microcyst development was not known (see Beebe, 1941; Badian, 1930). Other forms in old cultures which show no further development are organisms which have not been able to complete their cycle by forming microcysts.

If mature microcysts are spread on agar and incubated so that they develop more or less simultaneously, germination can be observed clearly. At first the microcyst swells slightly and gradually acquires a transparency until its staining qualities are comparable to those of the young vegetative cell. At the same time its definite contours gradually become less distinct; its nuclear structures, which now appear bigger, more deeply stainable and conspicuous than in the resting cyst, move further apart (Text-figs. 1, 2). Up to this point the actual shape of the cell may not have changed noticeably, but from now onwards the cell begins to elongate (Text-fig. 2; Pl. 1, figs. 1–4), or occasionally to develop a finger-like protuberance (Pl. 1, figs. 3, 4). Thus the microcyst slowly transforms itself into the young bacillary form which possesses no membrane and contains two darkly staining nuclear structures (Text-fig. 3; Pl. 1, figs. 2, 7). On occasions a subdivision of the nuclear structures has already started in this very young stage and the bacilli show two pairs of nuclear structures (Text-fig. 4; Pl. 1, fig. 5, a). The mode of germination is not sudden; there is no bursting of a 'shell'; nothing is left behind when the young bacillus is formed. The whole process is a gradual one: the tough outer layer disappears, the cytoplasm becomes transparent, the nuclear structures swell and move apart and the cell elongates and takes on the bacillary shape. The first bacillary elements have been observed after 4–6 hr. of incubation and after 10–15 hr. the majority of microcysts have been transformed into rods.

(b) The development of the young vegetative cells

The young cells are slender organisms and do not possess a cell membrane proper or any transverse septa. These elements, which are so characteristic of the Eubacteriales, were never demonstrated in myxococci by means of Robinow's (1944) method for the staining of membranes, although tests were carried out repeatedly during the course of this study. The cells gradually divide into two by constriction (Text-fig. 6). When division has been completed the pointed ends of the resulting cells round off. Before a cell divides its two nuclear structures divide lengthwise into four bodies (Text-figs. 5, 7), which are often connected by a skein of chromatinic material until the constriction sets in. This deeply staining skein sometimes resembles a continuous nuclear band, but close scrutiny of delicately stained preparations reveals the fact that the young
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vegetative organisms contain two to four single chromatinic structures arranged more or less transversely to the axis of the cell (Pl. 1, figs. 5–8). In their arrangement and mode of division they closely resemble the so-called ‘chromosomes’ of other bacteria.

(c) The formation of ‘fusion cells’

When a culture is well established and the young vegetative organisms have spread over the medium many cells start on a new development. The two nuclear structures of each cell increase in size (to about double) as if preparing for longitudinal division, yet they do not split but remain ‘double-sized bodies’ (Text-fig. 8). The cell now becomes shorter and the ‘double-sized bodies’ approach each other (Text-fig. 9; Pl. 1, figs. 11, 12; Pl. 2, fig. 13). Eventually they fuse and form a rod-like and later a round and conspicuous chromatinic structure (Text-figs. 10, 11; Pl. 2, figs. 14, 15). The cell shortens until finally a roundish cell with a round or ring-like nuclear body results (Text-figs. 10, 11). This cell, which I have called a ‘fusion cell’, might perhaps be regarded as the end stage of the cycle; yet it is not the resting stage, which arises from it by a further development. The fusion process occurs particularly in the centre of swarms of myxococcal cells that are collecting together in order to form the fruiting bodies.

(d) The formation of the microcysts

The nuclear body of the ‘fusion cell’ (Text-fig. 11) soon stretches and grows into a rod- or dumbbell-like structure (Text-figs. 12, 13a; Pl. 2, figs. 16, 17, 19). At the same time the whole cell enlarges and stretches to the oval form.
As development proceeds its nuclear structure divides into two round bodies which usually, but not always, remain connected by a string of chromatinic material (Text-fig. 14). At this stage the cell has acquired a greater affinity for dyes and at the same time formed a tough, dense outer layer. Thus the microcyst is formed (Pl. 2, figs. 16–18). With increasing age the microcyst becomes more round and smaller; its nuclear structures move more closely together and the whole cyst's affinity for dyes is now very pronounced (Text-fig. 15). In *Chondrococcus exiguus* the rearrangement of nuclear material that occurs during the maturation of the fusion cell suggests that a reduction of chromatin may take place. The cyst-forming cells often show a subdivision of their nuclear material into four structures instead of only two (Text-fig. 13b).

It was, however, not possible to determine whether two of these four structures were finally eliminated, or if they joined up again to form the two chromatinic bodies found invariably in the mature microcyst, or if these cells were involution forms. The development of the fusion cell may be regarded as a step towards the development of an embryonic vegetative cell; for the nuclear apparatus of the young vegetative cell is already fully developed in the resting microcyst, just as the embryo is already present in the mature seeds of higher plants. In contrast to the vegetative forms the microcyst is resistant against drying and ageing. When it eventually germinates on a fresh medium the young vegetative form arises as described under (a).

**DISCUSSION**

There are only a few papers dealing with the cell morphology of myxococci. The first of these was published in 1910 by Vahle. He used osmic acid fixation and stained with methylene blue. He describes and illustrates the two nuclear structures of the vegetative cell and their subdivision into four in dividing cells. He has also seen two structures in the 'spores', or 'microcysts' as they are called now. As knowledge regarding nuclear structures in bacteria was in an uncertain state at that time it was not possible to link up Vahle's observations with findings in other organisms and his valuable contribution to the cytology of micro-organisms was lost. Four recent workers, the Krzemieniewski (1928), Badian (1930, 1933) and Beebe (1941), have taken up the study of the cytology and development of myxococci. Badian certainly observed and illustrated some of the obvious appearances of the myxococcal cell with its conspicuous nuclear structures, but he suggests a complicated scheme of transformations of the chromatinic material during the life cycle of the organism which have not been confirmed in the experiments here described. Beebe, as well as Badian, observed genuine nuclear structures in *Myxococcus xanthus*, but his scheme of the development appears obscure, and from his evidence (drawings and photographs) no definite and clear idea of the cycle can be formed. It seems certain that both Badian's and Beebe's descriptions and drawings concerning the germination of microcysts are at fault. The observations here reported show that the developmental cycle in myxococci follows a much simpler course than was conceived by these workers. The most thorough investigation in the morphology of myxococci so far made is that of the two
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Krzemieniewski. Their observations are in excellent agreement with mine but were not worked out so as to suggest an outline of the life cycle of the organisms.

The present observations, together with others already reported, have brought to light a number of morphological characters in which endospore-forming bacteria, spore-forming actinomycetes and microcyst-forming myxococci exhibit both similarities and distinctions. It has been shown that the three groups of organisms have certain characteristics in common such as the chromatin distribution at some stage and the formation of a ‘fusion cell’, preceding the development of a resting stage.

REFERENCES

EXPLANATION OF PLATES

(Magnification × 3000)

PLATE 1

Fig. 1. *Myxococcus fulvus*. Germinating microcysts.

Fig. 2. *Chondrococcus exiguus*. Germinating microcysts. The cysts are stretching to form the young bacillary forms.

Fig. 3. *Myxococcus fulvus*. Germinating microcysts. Note the chromatinic bodies that have moved apart and the forms that have stretched.

Fig. 4. *M. fulvus*. Germinating microcysts. Note the finger-like protuberances.

Figs. 5, 6. *M. fulvus*. Young bacillary stage showing two to four chromosomes. Note the organism at a.

Fig. 7. *Chondrococcus exiguus*. Young bacillary stage. The couple of chromosomes are dividing into four.

Fig. 8. *Myxococcus fulvus*. Young bacillary stage. The organisms are dividing rapidly and each round nuclear body corresponds to two chromosomes which can only occasionally be resolved.

Figs. 9, 10. *M. fulvus*. Burst microcysts.

Fig. 11. *M. virescens*. Older culture. The organisms are collecting in some places in order to form microcysts. Each organism contains two ‘double-sized’ nuclear structures.

Fig. 12. *M. fulvus*. Older culture. The organisms are slightly shortened and the two ‘double-sized’ chromosomes are approaching each other.

PLATE 2

Fig. 13. *Myxococcus virescens*. Older culture. Some organisms are very short and their ‘double-sized’ chromosomes have almost fused.

Fig. 14. *M. virescens*. Older outgrowth showing ‘fusion cells’; some are almost round.

Fig. 15. *M. fulvus*. Older growth showing various stages of chromatin fusion.

Fig. 16. *M. fulvus*. Microcyst formation. Notice some small round ‘fusion cells’ with one nuclear body and others that have grown into the oval form and show two nuclear bodies.

Fig. 17. *M. fulvus*. Microcyst formation, showing various stages in the development of the small round fusion cell with one chromatinic body to the oval mature microcyst with two chromatinic structures.

Fig. 18. *M. fulvus*. Part of fruiting body containing many microcysts.

Fig. 19. *M. fulvus*. Microcyst formation. Similar stages to those in Figs. 16 and 17. Note at a and b the stretched nuclear body which has not yet divided.

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Figs. 13–19

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