THE NUCLEAR CYCLE IN BACTERIA

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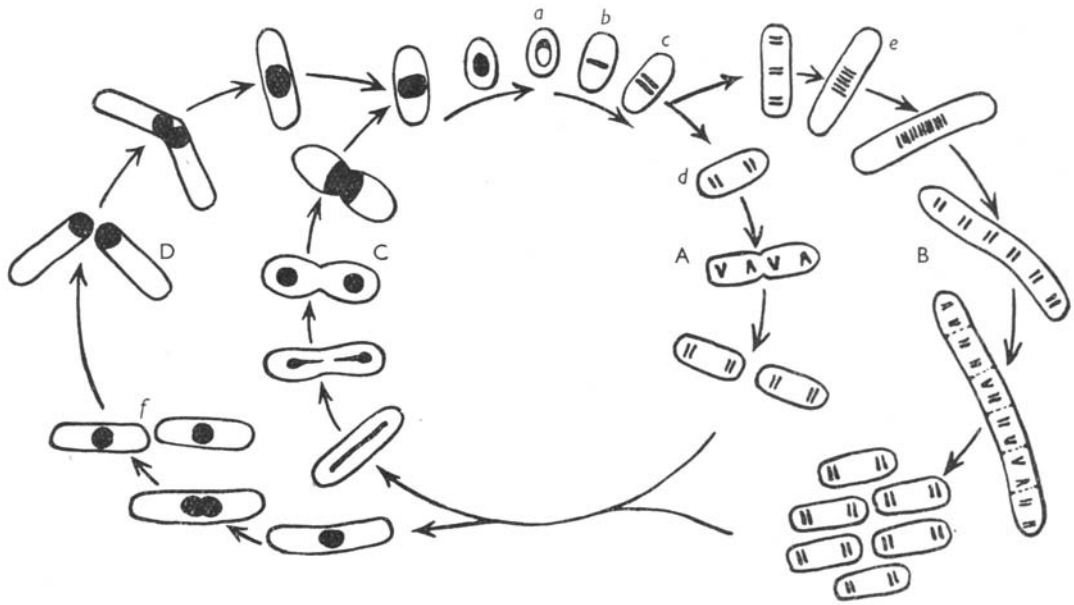
(With Plates 5 and 6 and 9 Figures in the Text)

The occurrence in Myxobacteria, including Cytophagas, of a complex cycle of nuclear changes, is well known (Krzemieniewski, 1928; Badian, 1930, 1933*a*; Beebe, 1941). A recent description by Klieneberger-Nobel (1947) has clarified the evidence of the existence of an autogamous or sexual nuclear fusion preceding microcyst formation in Myxobacteria. This process is clearly seen in Cytophagas also (Krzemieniewska, 1930; J. B. Grace, personal communication). An analogous process, resulting in the production of a nucleated 'coccus' from a phytopathogenic bacterium, was described by Stoughton (1929, 1932). Stoughton also described, in ageing cultures of the same organism, a dumb-bell-shaped nucleus, which divided with the cell. This body was not, however, demonstrable in young cultures, and the chromatinic bodies which were so strikingly demonstrated by Robinow (1946), in the cells of bacteria in young cultures, differed notably from the structures which were described by Stoughton. Robinow stated that, in cultures older than a few hours, the chromatinic bodies could not be defined microscopically.

The behaviour of the chromatinic bodies, in non-sporing bacteria, has been more fully investigated by the author (Bisset, 1948*a*, *b*), and they were shown to behave in a manner analogous to that of the chromosomes of plants and animals, being paired structures, and dividing by longitudinal fission (Text-fig. 1*A*). They were also shown to take part in an alternative method of reproduction, occurring in young cultures, in which three pairs of bodies, contained in an enlarged cell, became fused at the centre of the cell, and there underwent two nuclear divisions. The resulting chromatinic material was then redistributed throughout the growing, filamentous cell,



Text-fig. 1. A, simple vegetative reproduction (primary nuclear phase); B, sexual vegetative reproduction (primary nuclear phase); C, direct microcyst formation; D, microcyst formation from secondary nuclear phase. *a*, Microcyst; *b*, *c*, germination of microcyst; *d*, vegetative cell, primary nucleus phase; *e*, vegetative fusion cell; *f*, vegetative cells, secondary nuclear phase.

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which afterwards fragmented into bacilli (Text-fig. 1B). This process was found to occur also in Myxobacteria (Bisset, 1948*c*).

It is a matter of common experience that, in cultures more than 12–24 hr. old, the nuclear structures are difficult to demonstrate, and accordingly a large number of strains were examined in an attempt to overcome this difficulty. It was found that strains of Bacteriaceae were occasionally encountered which stained well at all ages, and which showed evidence of the existence of a life cycle closely resembling that of the Myxobacteria. This cycle also showed certain points of resemblance to the system of nuclear changes described in sporulation in the Bacillaceae (Badian, 1933*b*; Klieneberger-Nobel, 1945; Flewett, 1948), where an autogamous fusion has also been described. Some points in connexion with the nuclear cycle in morphological variants of sporing and non-sporing genera were also investigated, and observations were made upon the primary and secondary nuclear phases, described by Piekarski (1937).

TECHNIQUE

Preparations were stained by the HCl-Giemsa technique of Robinow (1946), and were always mounted in water for examination.

Eight strains of *Bact. coli* and *Bact. aerogenes* and one of *Pseudomonas pyocyanea*, which had been found to stain well at all ages, were examined. All were freshly isolated. Three rough strains of *Bact. coli* were also examined, and some comparative observations were made upon spore-bearing bacilli, lactobacilli and cytophagas. The two last were cultivated upon glucose agar and filter-paper respectively, and the remainder upon meat-infusion agar, at 37° C. Ageing cultures were usually left at room temperature.

A large number of strains which were tested proved difficult or impossible to stain suitably, except in very young cultures. These were rejected. There did not appear to be any other difference between the stainable and unstainable cultures.

OBSERVATIONS

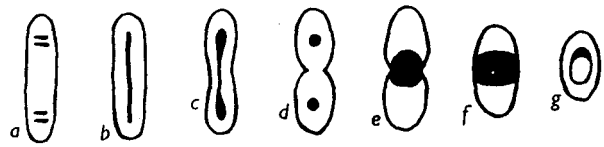
Young cultures. In cultures from approximately 2 until 24 hr. old the bacteria were large in size, and the paired chromatinic bodies were clearly visible, arranged in their characteristic mitotic figures (Pl. 5, fig. 1, Text-fig. 1A). At this stage the alternative method of reproduction (Bisset, 1948*a*), by nuclear fusion, growth and fragmentation, was also to be found (Pl. 5, figs. 2, 3; Text-fig. 1B). In cultures aged from 24 hr. to 4 or 5 days (the period differing with the individual strain) the nuclear material of the bacteria was irregularly scattered in the cell, or arranged in the form of a beaded rod, longitudinally disposed (Pl. 5, figs. 4, 5).

Formation of microcysts. In older cultures, up to 2 or 3 weeks, an increasing proportion of bacteria were observed to have assumed a spherical or oval shape, containing an eccentric chromatinic body. This body could sometimes be observed to lie at the edge of a large, unstained, central nucleus (Pl. 5, figs. 6, 7; Text-fig. 2*g*). In addition to these spherical forms, a number of characteristic conditions of the bacterial cell were observable in these ageing cultures. In some of these the central chromatinic rod was to be seen, but was usually constricted centrally, or was divided into two halves (Pl. 5, figs. 5, 8; Text-fig. 2*c*). In other cells the two halves of the rod had rounded off, and the cell was constricted centrally, as though in the process of division (Pl. 5, figs. 8, 9; Text-fig. 2*d*). In other forms these two nuclei had once more fused at the centre of the cell, which became, by degrees, transformed into a mature coccus or microcyst (Pl. 5, figs. 6, 9; Text-fig. 2*e*, *f*, *g*). These appearances were capable of interpretation as an autogamous or a sexual fusion, but it was not possible to determine which was, in fact, occurring. Most of the oval, nucleated bodies, which were always found in company with the conjugating cells (Pl. 5, figs. 6, 9) were almost certainly immature microcysts, but some may have been gametes produced by the division of the binucleate precursor cells, and subsequently conjugating sexually.

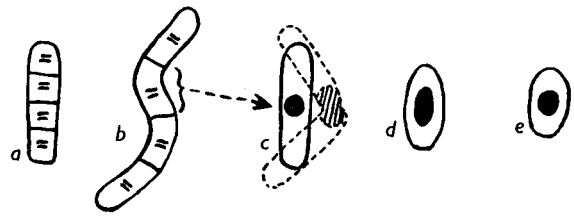
The entire picture bore a most striking resemblance to the process of microcyst formation in Myxobacteria (Text-figs. 5, 6) and differed from the 'coccus' formation, described by Stoughton (1929, 1932, Text-fig. 9), mainly in that the latter described the coccus as being extruded from the side of the bacterium. The size of the microcysts was variable; usually they were small by comparison with the vegetative cell, but some were almost as large (Pl. 5, figs. 6, 7, 10, 11).

The secondary nuclear cycle. The cycle in some strains included a stage in which the cells contained a single, central nuclear body, dividing with the cell (Pl. 6, fig. 12, Text-fig. 1D). This was observed, and described as the 'secondary' nucleus, by Piekarski (1937). The organism studied by Stoughton (1929, 1932) was also in this condition (Text-fig. 9), but could not be stained, by Stoughton's technique, in very young cultures, when it may be assumed to have been in the 'primary' condition, typical of young bacterial cultures.

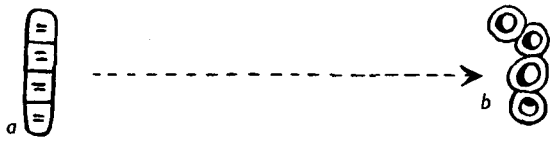
The cultures studied in the present investigation usually passed from the primary to the secondary phase after 24 or 36 hr., and thereafter the cells were gradually transformed into microcysts, without any of the obvious cytological changes which occurred when the microcystic stage was derived directly from cells in the primary nuclear phase, as described in the previous section (Text-fig. 1). The formation of the resting nucleus by this method was not, however,



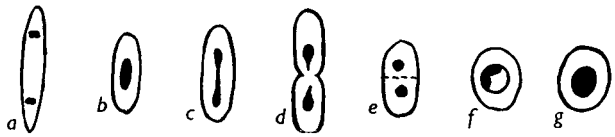
Text-fig. 2. Microcyst formation in *Bact. coli*, smooth variant.



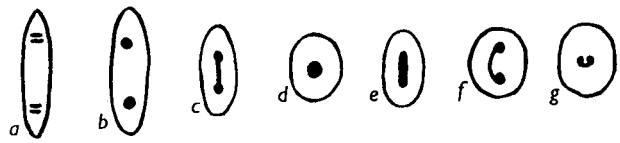
Text-fig. 3. Microcyst formation in *Bact. coli*, rough variant.



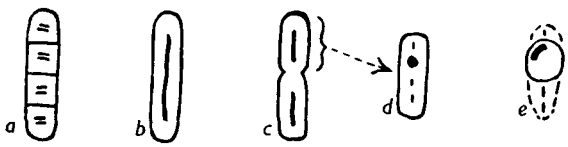
Text-fig. 4. Microcyst formation in *Lactobacillus* sp.



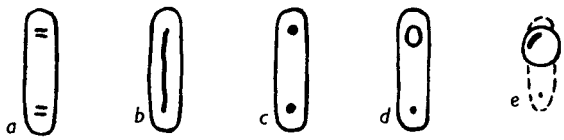
Text-fig. 5. Microcyst formation in *Cytophaga* sp., after Grace (unpublished).



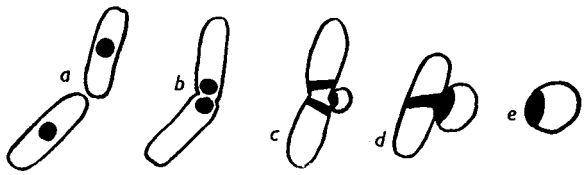
Text-fig. 6. Microcyst formation in *Mxyococcus* sp., after Klieneberger-Nobel (1947).



Text-fig. 7. Spore formation in *Bacillus* sp., rough morphology, partly after Klieneberger-Nobel (1945), Flewett (1948).



Text-fig. 8. Spore formation in *Bacillus* sp., smooth morphology (original).



Text-fig. 9. Microcyst formation in *Bact. malvacearum*, modified after Stoughton (1929, 1932).

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devoid of some suggestion of a sexual mechanism. Pairs of bacilli, apparently attached end-to-end, with their nuclear material concentrated at the point of contact, were often seen (Pl. 6, figs. 12, 13; Text-fig. 3*c*), and as exactly similar appearances, preceding 'coccus' formation, were interpreted by Stoughton (1932) as indicative of conjugation, the suggestion cannot be disregarded (Text-fig. 9).

The rough strains of *Bact. coli* which were examined appeared invariably to adopt this mode of microcyst formation. The young, multicellular bacterium, each cell of which contained a single pair of chromosomes (Bisset, 1947), developed, apparently by simple growth and fragmentation, into individual 'secondary' bacilli, which were transformed into microcysts (Pl. 6, figs. 12, 13; Text-fig. 3). Lactobacilli, which possess the rough morphology, tended to retain the four-celled structure throughout the cycle. Each cell became a microcyst while remaining in contact with its three neighbours (Pl. 6, fig. 14; Text-fig. 4).

Germination of microcysts. When the microcysts were transplanted upon fresh medium they rapidly regained the typical morphology of a young culture. Usually the transformation was complete in 2 hr. The germination commenced as an increase in the size of the microcyst and the transformation of the nucleus into a single, transverse bar (Pl. 6, figs. 15, 16*a*, *b*; Text-fig. 1*b*). The bacterium then further increased in length, and the single bar divided into two (Pl. 6, figs. 15, 16*c*). The two bars migrated towards the poles of the cell and were transformed into the paired chromosomes of the 'primary' nucleus. In rough variants the process was similar, but proceeded to the formation of a multicellular bacterium (Pl. 6, fig. 17).

Some of the stages of this process are figured by Robinow (1946), as types of cells found in young cultures. One of the cells figured is a mature microcyst, as yet ungerminated.

Sporulation in bacilli of smooth morphology. The mode of spore formation in rough, multicellular bacilli is well known (Badian, 1933*b*; Klieneberger-Nobel, 1945; Flewett, 1948), although the multicellular structure of the organisms described has not always been made clear; mainly because the cell membranes and nuclear structures are difficult to demonstrate at the same time. This process differs markedly from that of microcyst formation in the non-sporing genera of similar morphology, described in a previous section, in that the nuclear units of three out of the four cells of the bacillus are rejected, and only one is included in the spore (Text-fig. 7). A small number of observations was made upon the sporulation of three strains of smooth, unicellular bacilli (Text-fig. 8). It was seen that a rod-shaped fusion nucleus was formed, as in the case of rough strains (Pl. 6, fig. 18), and thereafter two nucleoids were formed, of which one was included in the spore, and one rejected (Pl. 6, fig. 19). Thus a reduction process appears to occur in the sporulation of both morphological types of spore-bearing bacillus.

DISCUSSION

The conception of a vegetative phase, succeeded by a phase of conjugation, in bacterial cultures, is not new. Dubos (1946), from a discussion of theoretical considerations, has concluded that this normally occurs. Autogamous or sexual processes preceding the formation of the spore in Bacillaceae and the microcyst in Myxobacteria are now fully established. The present work serves to bring the Bacteriaceae into line with other bacterial groups, but as the life cycle described in this paper is based upon a subjective interpretation of the observed changes in the appearance of the bacteria, at various ages, it is necessary to discuss the logical arguments in favour of the adoption of this interpretation, however much the same objection may be held against almost all previous cytological studies.

The nuclear structures and their behaviour during the period of active reproduction have already been fully reported. Their description in this paper is intended only to give a picture of the entire life cycle, and to place the initial stages of the culture in perspective as a period of microcyst germination. This may be of importance in explaining the phenomena associated with the lag phase of a culture, and the absence of lag when inoculation is made from the active phase of the parent culture. In the latter case the bacteria are already in the nuclear condition associated with active reproduction, and no delay occurs. The mode of division involving simple fission of the chromatinic bodies, and that which involves the more complex processes of fusion and redistribution, are probably merely alternative modes of vegetative reproduction, and the possibility that the latter includes a sexual process is perfectly compatible with this view.

The general arrangement, in time, of the various stages in the culture, is readily observable. Very young cells (Pl. 6, figs. 15, 16) precede mature, reproductive forms (Text-fig. 1A), and these are followed after a period of hours or days, by cells with apparently disorganized nuclear material, or containing a central, chromatinic bar. Cultures of increasing age contain an increasingly high proportion of mature microcysts, so that the logical problem is simple to arrange the intervening stages in their probable order, and here a complete series of stages, representing the division of the central bar and its refusion as a spherical nucleus, is available. The alternative explanation, that division is complete, and the subsequent recombination sexual, rather

than autogamous, is equally well admitted by the observed facts.

In addition to the difficulty of arranging these stages in any other logical order, the resemblance between this type of process and the known nuclear cycle in Myxobacteria and Cytophagas is so great that to ignore it would be impossible. The author has studied the cytological appearances of these organisms also, and considers that the identity of the two cycles is complete. The further observation (Bisset, 1948*c*) that the alternative method of reproduction, by nuclear fusion and redistribution (Text-fig. 1B) occurs also in Myxobacteria, indicates that the complete, nuclear cycle of Myxobacteria and non-sporing Eubacteria is the same, except that Eubacteria, like Cytophagas, fail to form complex fruiting bodies. The processes described by Stoughton (1929, 1932) appear to be no more than a rather unusual version of the same life cycle, as it arises from the secondary nuclear phase (Text-figs. 1D, 3, 9). It is probable that the early stages of the cultures which he examined would have proved to be in the primary nuclear phase, had they been stained by a technique capable of overcoming the masking effect of the strongly staining cytoplasm and cell membranes in the young bacteria, which he describes as staining uniformly. The author has accepted Stoughton's interpretation of the, apparently, conjugating cells which are found in the secondary nuclear phase, when it occurs. It appears entirely logical to do so, because it renders the two methods of microcyst formation essentially similar.

The relationship of the mature, resting nucleus, as found in the microcyst, to the paired chromosome-like bodies of the primary phase, vegetative cells is problematical. It seems possible that these structures are chromosome complexes, such as occur in the mitosis of yeasts (Lindegren, 1946). The nuclei of the microcysts resemble the vesicular nuclei of yeast cells, and also resemble that of *Azotobacter* and of certain types of coccus (Bisset, 1948*c*). If the microcyst nucleus represents the resting nucleus of the bacterium, and the 'chromatinic bodies' are mitotic complexes, we are led to the remarkable, but not inconceivable conclusion, that these bacteria remain permanently in the mitotic condition throughout the period of active cell division.

The exact form of the chromosome complexes is not easy to determine. As they have a marked tendency to present a long axis to the observer, it might be postulated that they are disk-shaped structures, resembling the nuclei of *Caryophanon* (Peshkoff, 1940). In other preparations they give the impression of ribbons wound around the axis of the cell. The chromosomes proper, in their dispersed condition, may be too small to be resolved, or may be represented by the beading upon the central, chromatinic rod (Pl. 5, figs. 4, 5). The occurrence of a rod of this type is a feature of both spore and microcyst formation, and was first observed by Schaudinn (1902–1903). In a recent review, Lewis (1941) attempted to explain the numerous, recorded observations of such rod-like nuclear structures by the suggestion that they represent the stainable contents of the cell compressed centrally by an accumulation of fat globules. But there seems little doubt that it is a regular stage in nuclear development.

Spore and microcyst formation differ from one another in that a reduction process is obvious in the former, where half or three-quarters of the nuclear material is excluded from the spore, and apparently lacking in the latter. This difference is especially striking in the case of rough variants. In the spore-forming genera, one bacillus forms a single spore, whereas each cell of the non-sporing bacillus goes to form one microcyst; whether, as in the case of the lactobacilli, they remain attached in fours, or whether, as in the case of the rough variants of *Bact. coli*, the cells first separate into individual, secondary phase bacilli (Text-fig. 3). The general pattern of microcyst or spore formation is the same in smooth or rough variants, as far as can be determined. The formation of the secondary nuclear phase may be indicative of a reduction process, and Allen, Appleby & Wolf (1939) described a process which they considered indicative of meiosis, preceding sporulation in a large *Bacillus*, but the appearances which they figure do not appear to be commonly observable in other bacteria. It appears axiomatic, however, that where conjugation of any kind occurs, reduction must also take place. Demerec & Latarjet (1946) have produced evidence of increased rate of segregation of heterozygotes during late bacterial generations. This may be considered as evidence of the occurrence of reduction, and also of a sexual process in ageing cultures.

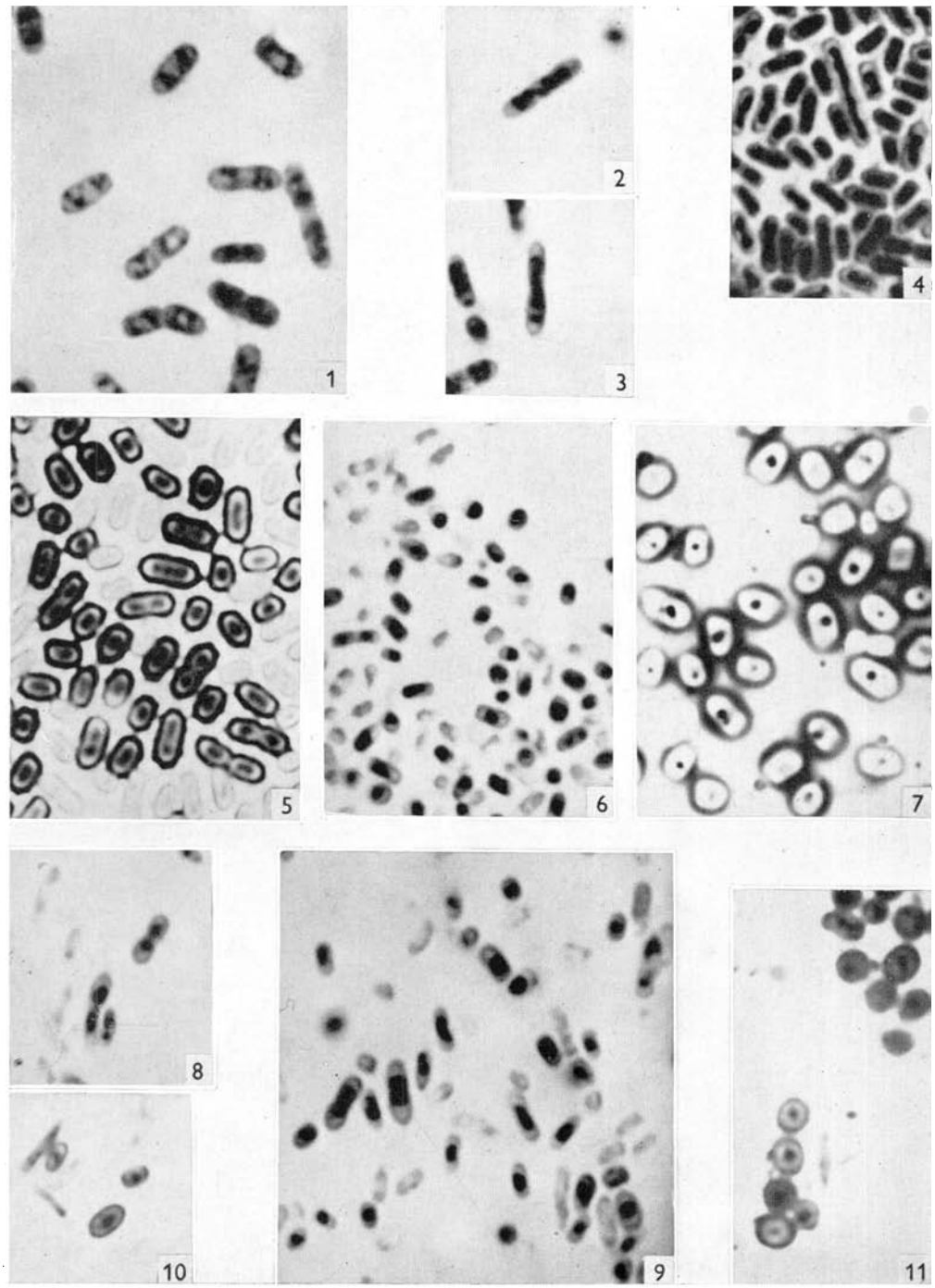
SUMMARY

1. Strains of *Bact. coli* and related bacteria possess a life cycle resembling that of Myxobacteria. The vesicular, resting nucleus is contained in a microcyst, which is formed by a process suggestive of sexual conjugation.

2. The microcyst germinates by the transformation of the resting nucleus into the chromosome-like bodies typical of active, vegetative cultures. These may be analogous to the chromosome complexes of yeasts. The period of germination of microcysts corresponds to the lag phase of cultures.

3. The nucleus remains permanently in the mitotic condition during the active, vegetative phase of growth, and reproduces by an asexual and a sexual method.

4. Older cultures may be transformed directly into microcysts or may first adopt a secondary,



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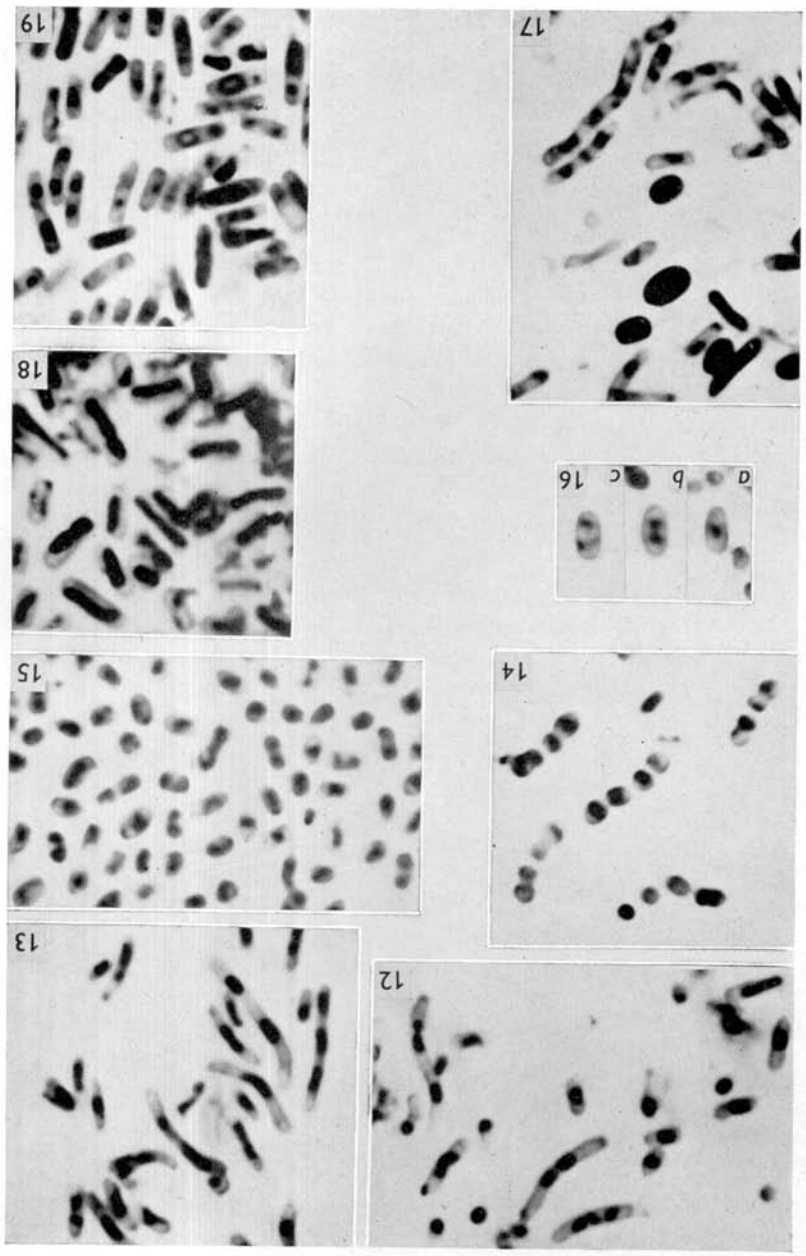

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vegetative phase, in which the nucleus is in the form of a single, central body.

5. Microcyst formation differs from spore formation in that it lacks the obvious reduction processes associated with spore formation, upon which a few original observations are included.

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EXPLANATION OF PLATES 5 AND 6

All the preparations are stained by acid-Giemsa, and the magnification is × 3000 in all cases.

PLATE 5

Fig. 1. *Bact. coli*, young vegetative cells.
Figs. 2, 3. *Bact. coli*, vegetative fusion cells.
Fig. 4. *Bact. coli*, cells with central nuclear rods.
Fig. 5. *Bact. aerogenes*, cells with central nuclear rods.
Fig. 6. *Bact. coli*, microcysts.
Fig. 7. *Bact. aerogenes*, microcysts.
Figs. 8, 9. *Bact. coli*, stages in microcyst formation.
Fig. 10. *Bact. coli*, rough variant, microcysts.
Fig. 11. *Cytophaga* sp., microcysts.

PLATE 6

Figs. 12, 13. *Bact. coli*, rough variant, secondary nuclear phase.
Fig. 14. *Lactobacillus* sp., microcysts, retaining arrangement in fours.
Figs. 15, 16. *Bact. coli*, germinating microcysts.
Fig. 17. *Bact. coli*, rough variant, germinating microcysts.
Fig. 18. *Bacillus* sp., smooth morphology, showing nuclear rods, early stage of sporulation.
Fig. 19. *Bacillus* sp., smooth morphology, later stage of sporulation than fig. 18, showing rejection of one nucleoid.

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