CELL DIVISION IN MICROCOCCUS PYOGENES VAR. AUREUS[1]

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Recent progress in bacterial cytology has left no doubt as to the existence of a discrete nuclear body in bacterial cells. Numerous publications have appeared describing the appearance of apparent nuclear bodies in coccus forms, and descriptions of the activity of these bodies have been meticulously recorded. The literature on the nuclei of the cocci has been covered aptly by DeLamater and Woodburn (1952). The development of a new nuclear stain involving mild treatment of the cells (Chance, 1952) suggested that a reinvestigation of the cocci might be profitable.

MATERIALS AND METHODS

The organism used in these studies was *Micrococcus pyogenes* var. *aureus*, strain FDA 209. Stock cultures were maintained on nutrient agar and on nutrient agar containing one per cent glucose. Cultures of various ages were used for microscopic examination, and nuclear stains were made by a modification of the crystal violet technique of Chance (1952). In this modification air dried impression smears of the organism were stained with 1 per cent crystal violet, pH 7.5, for 4 to 5 minutes. The slides then were washed with tap water and mordanted in $HgCl_2$. The cells grown on glucose agar were mordanted for 15 seconds in 0.5 per cent $HgCl_2$, and the cells grown on nutrient agar were placed in 1.5 per cent $HgCl_2$ for 30 to 45 seconds. After again washing with tap water, a film of 8 per cent nigrosin, pH 3.5, was spread over the smear to decolorize the cytoplasm. The freezing-dehydration technique of Blank *et al.* (1951) was used on some of the stained slides, but no increase in observable detail resulted. Cell wall stains were made by the technique of Chance (1953c).

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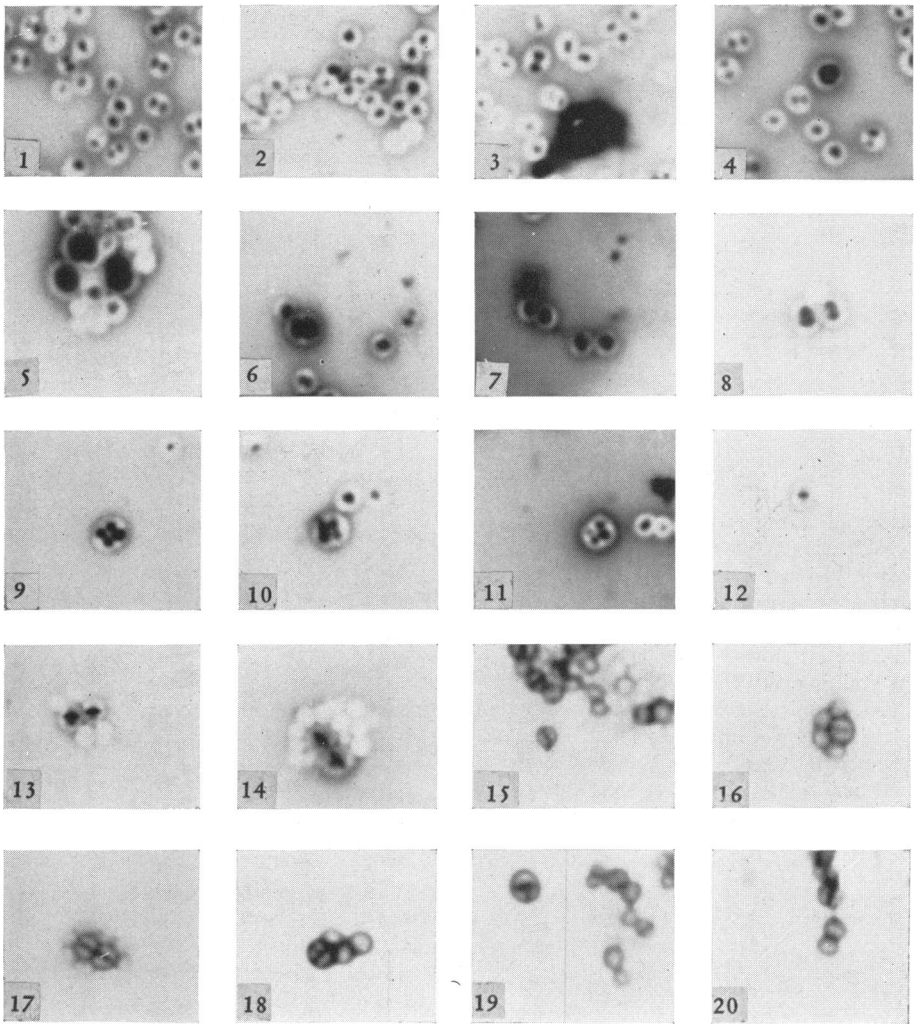
RESULTS AND DISCUSSION

It is evident that a cytological study of bacterial nuclei by itself is not sufficient evidence for forming broad conclusions as to the specific nature of the nucleus and its precise mechanism of division. A study of cytokinesis should parallel the nuclear study. Confirming genetical evidence also would be necessary to support any conclusions. We are presenting what appears to be the logical sequence of events based on many observations. All cytological forms reported here are seen routinely. Because of the inadequacies of staining procedures, we do not consider as significant any forms which appear in only a few microscopic fields. Such forms could easily be artifacts due to inconsistent slide composition or to uneven staining.

The crystal violet stain demonstrates the presence of one nuclear body in the mature cell. This body may appear rod shaped, ellipsoidal, or round. These are probably different views of a disc shaped nucleus since the random plane of division in this organism would enhance the possibility of random orientation of cells on the slide. The nucleus appears to swell to a spherical body just prior to division.

The division of the nuclear body can be interpreted to be amitotic since no mitotic figures were observed although mitosis-like figures have been reported in another *Micrococcus* species by DeLamater and Woodburn (1952). It does not appear probable that an amitotic division should occur since the genetic stability displayed by this organism indicates a regular reduplication and transference of genetic determinants to the daughter cells. On the basis of available evidence, we believe that mitotic division does occur, but that there is no disintegration of the nuclear membrane during the prometaphase. Such a nuclear division has been reported in some protozoa (Colwin, 1944) and in the ascomycetes (Guilliermond, 1911). All mitotic configurations could occur within an intact mem-



Cell division in *Micrococcus aureus*

Figures 1–3. Normal *M. aureus* cells in various stages of division.
Figures 4–5. Large forms of *M. aureus.*
Figure 6. Early mitotic division of large form.
Figure 7. Mitotic division of large form completed.
Figure 8. First meiotic division of large form.
Figures 9–11. Second meiotic division of large form.
Figures 12–13. Cell plate formation in large form.
Figure 14. Cell plate formation in large form.
Figure 15. Cell wall stain of normal cells. Note cross septa in some of the cells.
Figures 16–17. Cell wall stain of large forms. Note cross septa dividing these cells.
Figures 18–19. Cell wall stain of large forms. Cross septa in these cases divide cell into three sections. Second meiotic division is not complete in this stage.
Figure 20. Cell wall stain of large form. Cell divided into four segments by cross septa. (These forms show up poorly by this cell wall stain but are seen readily on slides.)
(Magnification of all pictures—33200 ×)

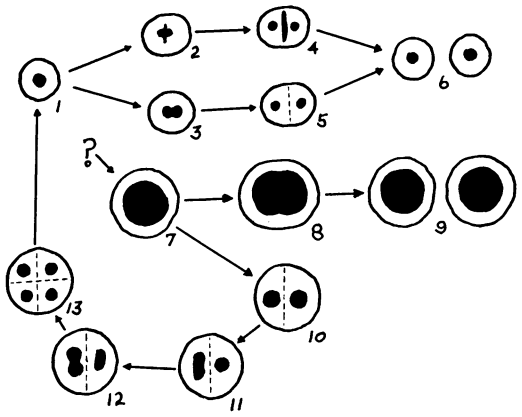


Figure 21. Proposed scheme of cell division in *Micrococcus aureus*. 1. Single cell (n?). 2. Initial nuclear division showing cell plate. 3. Initial nuclear division with cell plate unstained. 4. Completion of nuclear division showing cell plate; cytokinesis not yet complete. 5. Completion of nuclear division with cell plate not stained; cytokinesis not yet complete. 6. Karyokinesis and cytokinesis complete. 7. Large uninucleated cell (2 n?). 8. Initial mitotic nuclear division; cell plate not staining. 9. Completed mitotic division in large cell. 10. First meiotic division completed. 11. Beginning of second meiotic division. 12. More advanced stage of second meiotic division. 13. Second meiotic division complete; cytokinesis not complete. (Dotted lines in drawings represent location of cross septa as revealed by cell wall stain.)

brane which is deeply staining, and thus their presence be obscured.

The nucleus elongates during division and appears to constrict in the middle unless a cell plate is observed forming between the dividing nuclei. This staining procedure does not demonstrate uniformly cell plates on all stained preparations, but it has been used to demonstrate routinely cell plates in several genera of bacteria (Chance, 1953*a*, *b*). When the cell plate is observed, it appears to grow outward until it almost comes in contact with the cell wall. At this stage of development the plate is no longer demonstrated by the nuclear stain. It is probable that the young cell plate is composed of nuclear material; as the plate matures, cytoplasmic secretion deposits cell wall material on the plate so that it is no longer detectable by nuclear stains.

As the nucleus elongates, the vegetative cell also elongates slightly. After the cell plate has differentiated into cell wall material, the nucleus completes division, yielding two normal appearing nuclei which may be oriented at random at the poles of the cell. A cell wall stain reveals a septum between the two nuclei; however, at this stage of division a nuclear stain used by itself could give the impression of a binucleate cell. By utilizing cell wall studies in parallel with nuclear studies, it can be demonstrated that the apparent single cell actually consists of two individual cells. This type of uniform "multicellularity" in bacteria has been reported previously in the cocci by Knaysi (1942) and Bisset (1948).

Apparently as the cell plate matures into a cross septum of cell wall material, the outer cell wall constricts as the two cells begin to separate. This constriction is always noticed after nuclear division is complete and the nuclei have migrated to opposite poles. The constriction continues until two separate uninucleate cells are produced, after which the division cycle begins again.

A determination of the ploidy of a bacterial cell is impossible with the type of observations recorded here. Direct chromosome counts or a thorough genetical analysis would be necessary to establish the true nature of the nuclear material. However, the routine appearance of certain cytological forms leads to speculation as to the ploidy of the nucleus and the possibility of the presence of a life cycle. Forms averaging twice the size of the normal cell are seen routinely although in relatively small numbers on glucose agar. The number of these cells is increased when the cells are grown on nutrient agar in the absence of glucose. These forms appear primarily when transfers are made from an old culture to the moist surface of a fresh agar slant. The number of forms increases from the fourth to the tenth hour of growth, and by the eighteenth hour the forms become rare. The nucleus in these large forms occupies a major portion of the cytoplasmic area and is several times larger than the normal nucleus. It can be postulated that the normal nucleus is haploid (partly on the basis of genetic evidence) and that the large cell with large nucleus is diploid. The origin of this large cell is still obscure in spite of continued study of numerous preparations. The behavior of these cells has been determined, however. The cells undergo mitotic division and can remain in the diploid state for an undetermined number of divisions. The diploid cell apparently

also undergoes a meiotic division in which the first meiotic division yields two nuclei intermediate in size between the diploid and haploid stages. The second meiotic division results in a large cell containing four normal sized nuclear bodies (haploid). A cell wall stain shows the presence of septa dividing this round appearing cell into four individual cells. Cytokinesis continues until four normal haploid cells are formed. A meiotic division has been postulated previously in *Micrococcus ochraceous* (Lindegren, 1942). This cyclic process is presented schematically in figure 21.

It can be argued that the large nucleus is a polar view of a cell plate. However, we do not consider this to be valid. The cell containing the large nucleus is larger than the majority of cells showing cell plates. Also no cell plates are seen in a transverse view on slides showing the large nucleated cells. Cell plates are observed usually in cells that have elongated slightly so that a cell would have to be standing on end to present this polar view. It is also probable that the thinness of the cell plate would reveal it as a faint shadow from a polar view and not as a deep staining body. The strongest evidence in favor of the large bodies being nuclei instead of polar views of cell plates is in the observed nuclear-like division of these bodies.

The nuclear bodies revealed by the Chance crystal violet nuclear stain are very similar in appearance and behavior to those reported by Smith (1950), Robinow (1942), Knaysi (1942), and others. In general, however, the Chance technique demonstrates nuclei of more uniform size and shape which appear much more discrete. This crystal violet technique is advantageous because it utilizes aqueous dyes and does not involve harsh treatments such as acid hydrolysis, chemical fixation, or dyes dissolved in organic solvents. When dealing with cell structures whose size is near the limit of resolution of the microscope, it is evident that minor changes in appearance induced by stain techniques could lead to misinterpretation.

SUMMARY

The cytological sequences of cell division in *Micrococcus pyogenes* var. *aureus* were observed by using both nuclear and cell wall stains. The uninucleate cells appeared to follow a definite pattern of division, and no mitotic figures were seen. On the basis of the observations reported here, it is postulated that a haploid nucleus undergoes mitotic division within an intact cell membrane. An apparent diploid cell arises in the culture and undergoes meiotic division to form four haploid cells in addition to regular mitotic divisions. Cell wall studies revealed that the cells which appeared multinucleate were actually divided by septa to give a form of "multicellularity".

REFERENCES

BISSET, K. A. 1948 The cytology of the gram-positive cocci. J. Gen. Microbiol., **2**, 126–130.

BLANK, H., MCCARTHY, P. L., AND DELAMATER, E. D. 1951 A non-vacuum freezing-dehydrating technic for histology, autoradiography and microbial cytology. Stain Technol., **26**, 193–197.

CHANCE, H. L. 1952 Crystal violet as a nuclear stain for *Gaffkya tetragena* and other bacteria. Stain Technol., **27**, 253–258.

CHANCE, H. L. 1953*a* Cytokinesis in *Gaffkya tetragena*. J. Bact., **65**, 593–595.

CHANCE, H. L. 1953*b* The occurrence of "cell plates" in bacteria. J. Bact., **66**, 239–240.

CHANCE, H. L. 1953*c* A bacterial cell wall stain. Stain Technol., **28**, 205–207.

COLWIN, L. H. 1944 Binary fission and conjugation in *Urceolaria synaptae* type 11 (Protozoa, Ciliata) with special reference to the nuclear phenomena. J. Morphol., **75**, 203–249.

DELAMATER, E. D., AND WOODBURN, M. 1952 Evidence for the occurrence of mitosis in the micrococci. J. Bact., **64**, 793–803.

GUILLIERMOND, M. A. 1911 Aterçu sur l'évolution nucléaire des Ascomycètes. Rev. gén. botan. **23**, 89–120.

KNAYSI, G. 1942 The demonstration of a nucleus in the cell of a staphylococcus. J. Bact., **43**, 365–385.

LINDEGREN, C. C. 1942 Nuclear apparatus and sexual mechanism in a micrococcus. Iowa State Coll. J. Sci., **16**, 307–318.

ROBINOW, C. F. 1942 A study of the nuclear apparatus of bacteria. Proc. Roy. Soc. (London), B, **130**, 299–324.

SMITH, A. G. 1950 Electron and light microscopic studies of bacterial nuclei. II. Improved staining technique for the nuclear chromatin of bacterial cells. J. Bact., **59**, 575–587.