STRUCTURE IN THE BACTERIAL CELL-WALL DURING CELL DIVISION

by

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KNAYSI[1] and ROBINOW[2] described the changes in the bacterial cell during cell division as comprising three stages: first, the formation of a transverse septum derived from the cytoplasmic membrane; second, the formation of a transverse cell-wall septum which grows centripetally from the lateral cell-wall; and third, a constriction of the cell through the split cell-wall septum to complete the separation of the daughter cells. Rough and smooth strains of organisms were considered to differ only in the interval of time which elapsed between the second and third stages, the interval being long with rough and short with smooth strains. BISSET[3], on the other hand, considers that the processes of cell division are basically different in rough and smooth strains of bacteria. In his view, transverse cell-wall septa form only in rough strains of organisms, in which group he places the long-chained streptococci and most pathogenic staphylococci, whereas smooth strains, in which he includes the short-chained streptococci, divide by simple constriction of the cell-wall without preceding cell-wall septum formation. Moreover, BISSET differs from KNAYSI and from ROBINOW in believing that the transverse cell-wall septum does not grow centripetally but is secreted uniformly across the width of the cell.

Detailed understanding of the structural changes in the bacterial cell during cell division is necessarily difficult if intact cells are used in the investigations. If the light microscope is used the relatively low resolving power imposes a limit beyond which no real detail can be recognised and a realm is created in which speculation can be rife without much possibility of arriving at a definite solution. This state of affairs is exemplified by the current controversy concerning *Bacillus megatherium*, micrographs of which have been alternatively interpreted as showing the presence of either mitotic figures (DE LAMATER AND MUDD[4]) or cell-wall septa (BISSET[5]). Even with the electron microscope the problem is not without its difficulties since, although the necessary resolving power is present, the average bacterial cell is too thick to allow of sufficient electron penetration to show internal structure.

It occurred to us that, since it is readily possible to isolate the bacterial cell-wall (DAWSON[6]), which is ideally suited for examination in the electron microscope, a detailed examination of large numbers of cell-walls from an actively growing culture might be of value in elucidating the stages of formation of structures developed from and attached

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to the cell-wall during cell division. The present investigation is concerned with a study of *Staphylococcus aureus* and *Streptococcus faecalis*.

MATERIALS AND METHODS

Organisms

A coagulase-positive strain of *Staphylococcus aureus* and a non-haemolytic, short-chained *Streptococcus faecalis* were grown for 16 h in Hartley's digest broth at 37° C. The cells were collected by centrifugation and washed three times with distilled water.

Preparation of cell-walls

Washed cells were shaken in phosphate-citrate buffer with "ballotini" glass beads and the cell-walls separated by differential centrifugation and then washed with distilled water (HOTCHIN, DAWSON AND ELFORD[7]).

Treatment of intact cells with CTAB

The cells were suspended in 0.01% cetyl-trimethyl-ammonium bromide and incubated at 37° C for 24 h. The concentration of cells in the suspension was $8 \cdot 10^9$ cells per ml. The treated cells were washed twice with distilled water with the centrifuge before examination in the electron microscope. Detailed results of the treatment of *Staph. aureus* with CTAB were described in a previous paper (DAWSON, LOMINSKI AND STERN[8]).

Electron microscopy

Specimens were mounted on "formvar" films and shadowcast with palladium at an angle of 20° to the plane of the film. Examinations were carried out in the Philips' electron microscope.

RESULTS AND CONCLUSIONS

Observations on the isolated cell-walls of *Staph. aureus* and *Strept. faecalis* show that the structural changes follow the same general pattern in both organisms although the details are more readily obvious in *Strept. faecalis*. Fig. 1 shows a typical preparation of isolated cell-walls of *Strept. faecalis*. Some of the cells have a smooth surface but others show the presence of equatorial ridge and band-like structures. The following investigations indicate that these structures are not due to random folding in a spherical membrane which has collapsed flat on the supporting film but that they reflect the various stages of cell division.

Staph. aureus

Figs. 4 to 7 illustrate the various types of these structures in the isolated cell-walls of *Staph. aureus*. Fig. 4 shows a typical smooth cell-wall with no surface structure apart from the small fold visible. In Fig. 5 there is present a characteristic, double ridge of thickening across the diameter of the cell. In Fig. 6 the two ridges are quite separate from each other. Instead of a ridge the cell-wall surface in Fig. 7 shows a wide, band-like area of thickening.

A careful study of undisrupted staphylococci, both by the examination of intact cells in the electron microscope (Fig. 2) and by the use of surface replica techniques, failed to reveal the presence of similar structures on the surfaces of the intact organisms. However, if the cells are treated with the appropriate concentration of cetyl-trimethyl-ammonium bromide they assume the appearances shown in Fig. 3; the cells show obvious retraction of the cytoplasm from the cell-wall and many of them, marked X in the micrograph, can now be seen to consist of two hemispherical sections, this being indicated by a depression running across the equator of the cell. Often a partial sepa-

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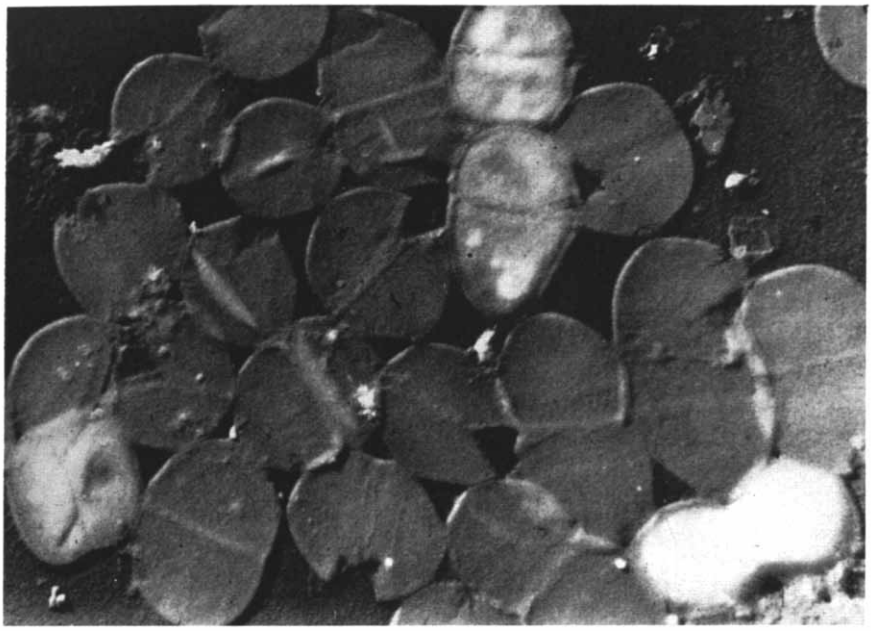
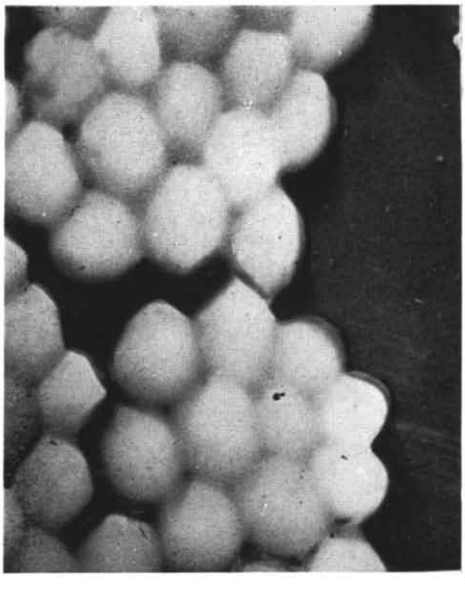


Fig. 1. Cell-walls of *Strept. faecalis* showing various equatorial structures (× 15,000)



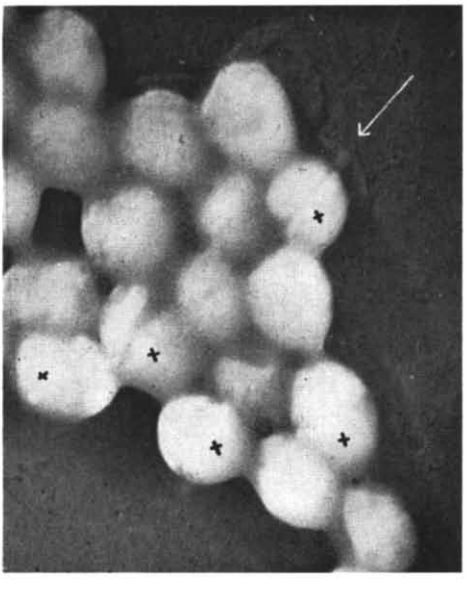
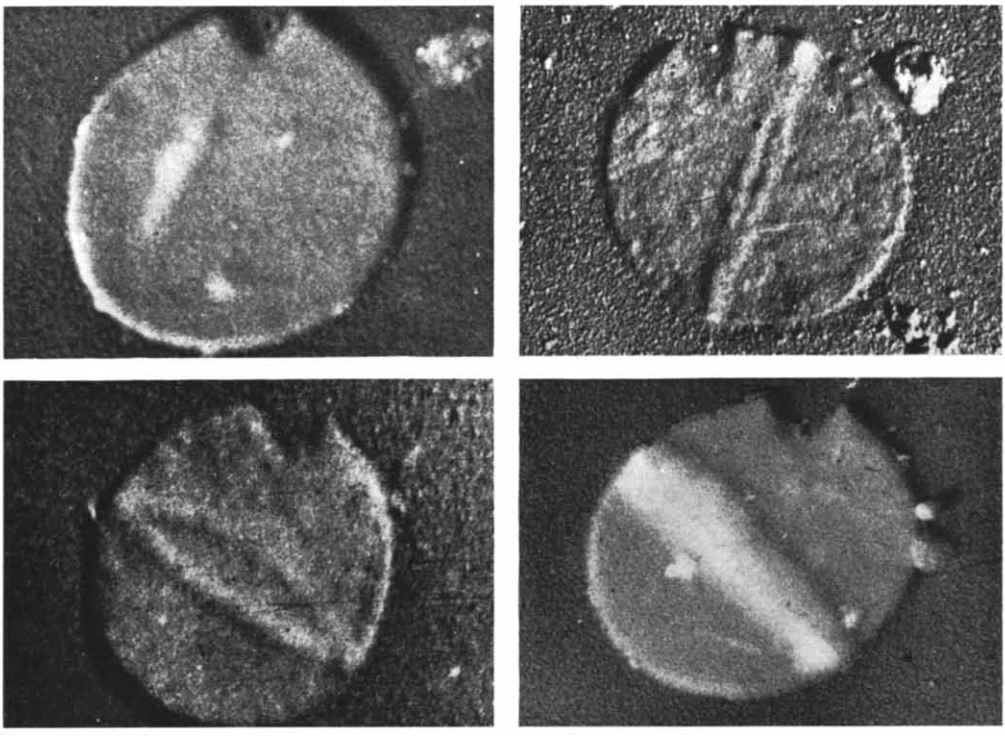


Fig. 2. Intact cells of *Staph. aureus*. The cell surface shows no obvious evidence of structure (× 15,000)
Fig. 3. Intact cells of *Staph. aureus* treated with CTAB. The letter X indicates the cells which appear to consist of two sections. The arrow indicates cell-wall ridging opposite the cytoplasmic constriction (× 15,000)

ration of the two halves of the cell takes place. Moreover, it can be seen that opposite the line of cytoplasmic division there is present on the flattened rim of cell-wall a distinct, raised ridge. These appearances we consider to represent a septum stretching across the centre of the intact cell. The septum, which is formed at an early stage of cell division, is not readily detected in the normal, intact cell; it becomes visible following treatment with CTAB which causes a retraction of the cytoplasm from the cell-wall and also from



Figs. 4 to 7. Cell-walls of *Staph. aureus* showing the successive stages of transverse cell-wall septum formation during cell division (× 34,000)

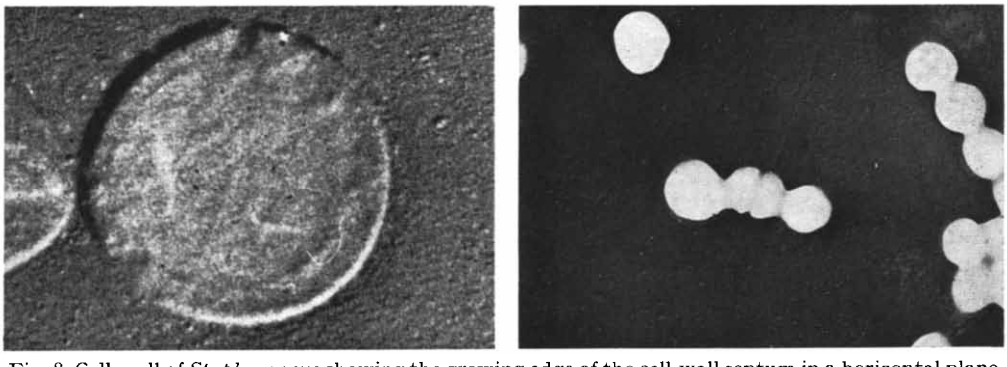


Fig. 8. Cell-wall of *Staph. aureus* showing the growing edge of the cell-wall septum in a horizontal plane (× 34,000)

Fig. 9. Dividing cells of *Staph. aureus* (× 9000)

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the central septum. In order to confirm that the appearances observed in the whole cells, treated with CTAB, and also the equatorial structures observed in the isolated cell-walls are related to cell division, counts of the proportions of cells and cell-walls, showing these appearances, in cultures at various stages of growth were carried out. It was shown that the younger and more actively growing a culture the higher the proportion of the cells and cell-walls described above. In a 6 hours' culture as many as 50 to 60% of the cells, after treatment with CTAB, showed the double structure of the cytoplasmic body and an even higher proportion of the isolated cell-walls the various forms of equatorial differentiation; after 48 hours as few as 10% of cells and cell-walls showed these appearances.

An attempt can now be made to reconstruct the stages in the formation of the cell-wall septum, using the structural appearances on the isolated, collapsed cell-walls. In Fig. 4 the septum has not yet begun to form. The first sign of its formation is the presence of a ridge of thickening on the cell-wall. The ridge, of course, encircles the originally spherical cell-wall and its part on the lower layer of the collapsed cell-wall will show through the upper layer, thus giving rise to the double-ridge appearance (Figs. 5 and 6); the degree of separation of the two ridges depends on the angle of the line of septum formation to the plane of the film at the moment of collapse of the originally spherical cell-wall. As the septum grows inwards to form a central membrane the collapse of the septum within the collapsed cell-wall will give rise to the band-like appearance shown in Fig. 7. Fig. 8 shows a cell-wall which has collapsed with the plane of the septum in the horizontal position. The growing edge of the septum can be seen as a concentric ridge within the periphery. Following the completion of growth of the septum an inward constriction of the cell-wall occurs through the line of the septum to separate the daughter cells. This final stage of constriction is the earliest visible stage in the electron microscope of cell division in whole cells (Fig. 9).

Strept. faecalis

In this organism the process of division is basically similar to that of the staphylococcus but the early structural changes, not readily observed in *Staph. aureus*, are more pronounced.

The first evidence of the process of division in the cell-wall is the appearance of parallel striations across the centre of the membrane (Fig. 10). These seem to coalesce to form a band of decreased density (Fig. 11). In the centre of this band there forms a line of increased density (Fig. 12) which becomes progressively more prominent to form a distinct ridge on the surface of the collapsed cell-wall (Fig. 13). Fig. 14 shows the double ridge appearance due to the ridge on the under layer of cell-wall showing through the upper. The inward growth of the septum then results in the band-like structure of Fig. 15. Fig. 16 suggests that the septum has now split to form a double septum with collapse of the two layers on either side of the mid-line. Finally, the cell-wall constricts through the line of the septum (Fig. 17). Figs. 18 to 20 illustrate the formation of septa in the daughter cells before the latter have themselves completed division. Mostly septum formation gives rise to two or four daughter cells (Fig. 21), but occasional exceptions are found in which three daughter cells are formed.

Thus, the usual method of division in the streptococcus is the formation of a transverse cell-wall septum followed by constriction of the cell-wall through the line of the septum. However, there are interesting but relatively uncommon exceptions. Fig. 22

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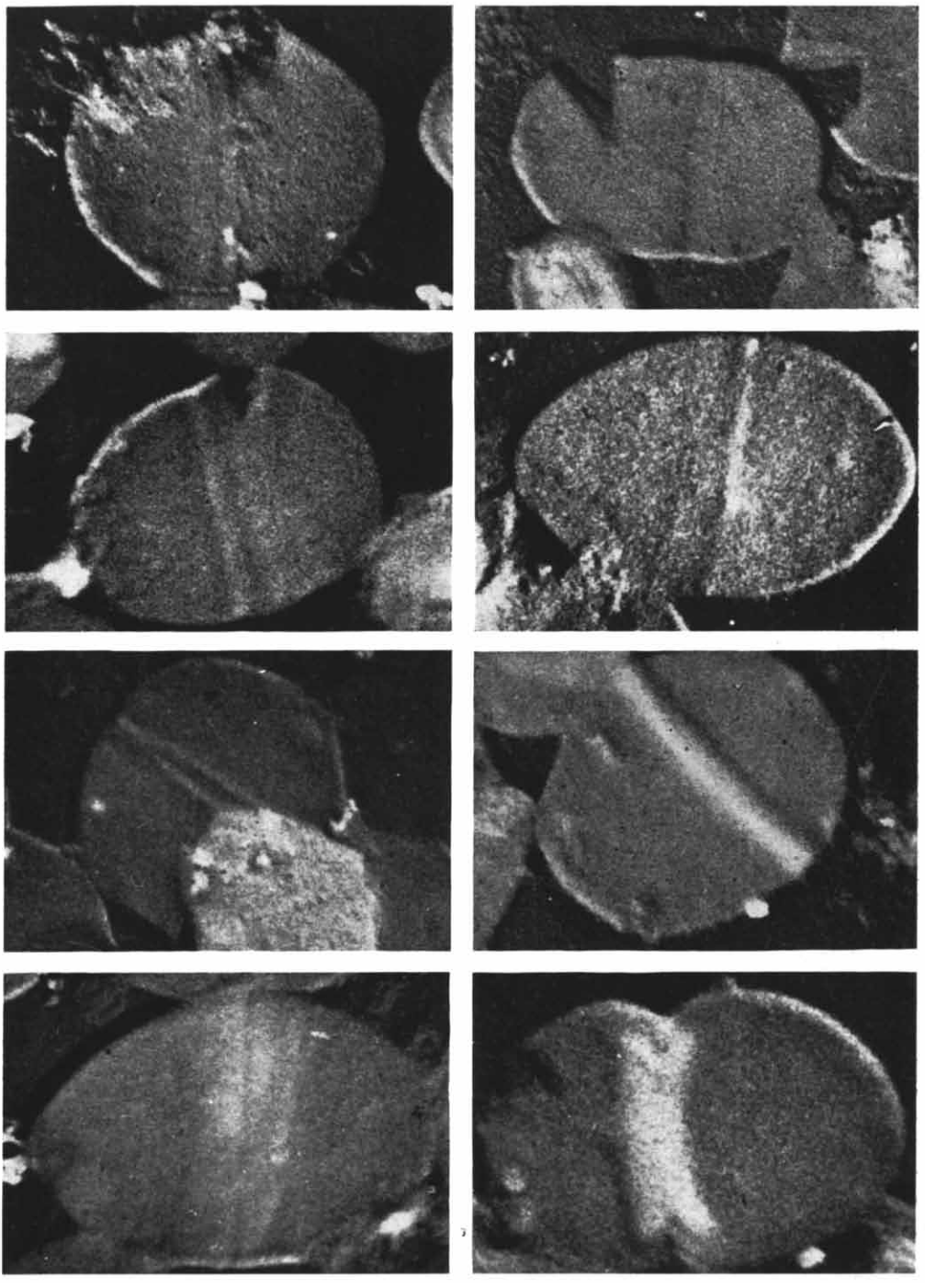
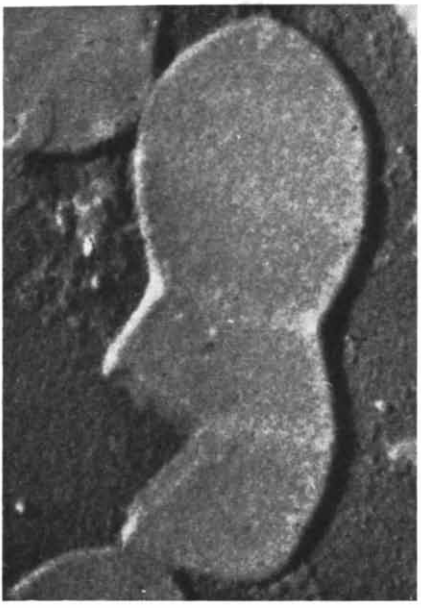


Figs. 10 to 17. Cell-walls of *Strept. faecalis* showing the successive stages of transverse cell-wall septum formation during cell division (× 34,000)



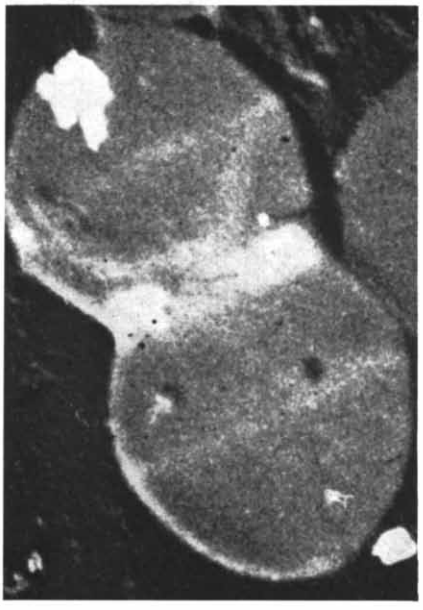
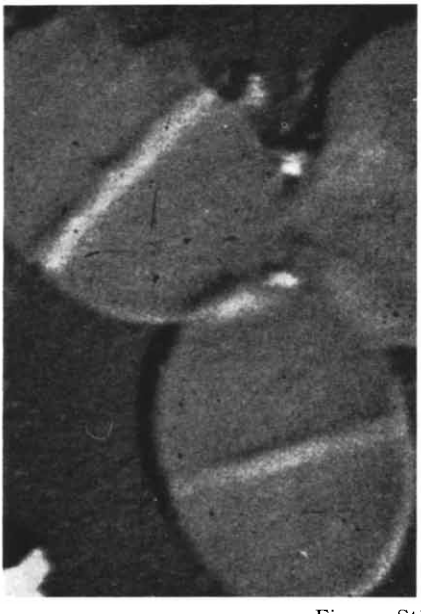


Fig. 18. Cell-wall of *Strept. faecalis* showing the formation of septa in the daughter cells before the latter have themselves completely separated (× 30,000)

Fig. 19. Later stage of Fig. 18



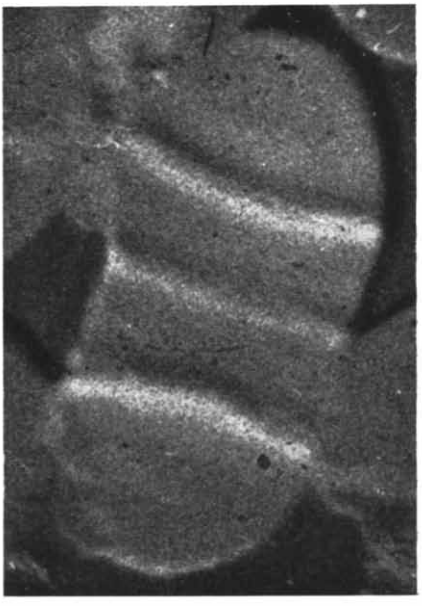
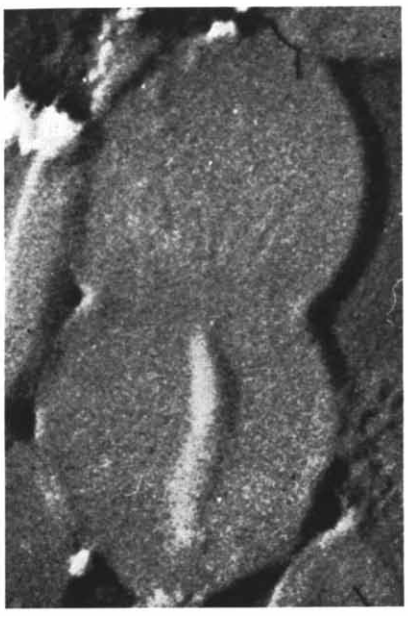


Fig. 20. Still later stage of Fig. 18

Fig. 21. Division of *Strept. faecalis* into 4 units (× 20,000)



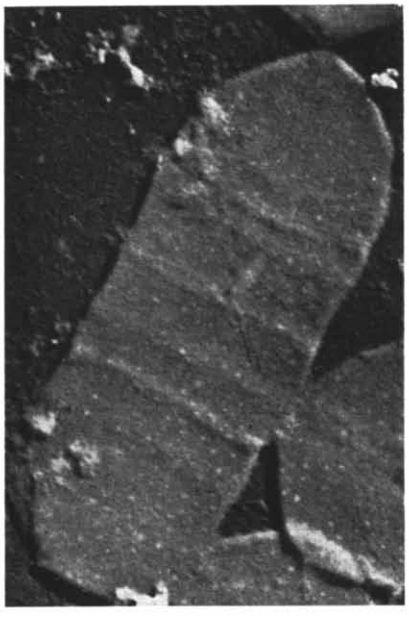
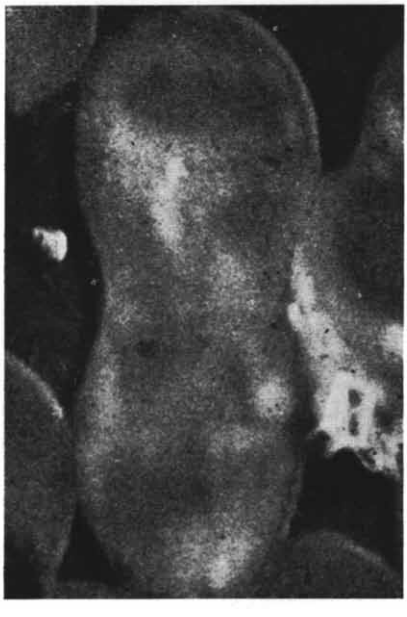


Fig. 22. Cell-wall of *Strept. faecalis* showing division by constriction without preceding septum formation (× 30,000)

Fig. 23. Cell-wall of *Strept. faecalis* with multiple cell-wall septum formation. The final dividing stage of constriction appears to have failed (× 25,000)



Figs. 24 and 25. Cell-walls of *Strept. faecalis* showing cytoplasmic membranes within them. Transverse septa originating from the cytoplasmic membrane are obvious (× 25,000)

shows cell division by simple constriction without preliminary septum formation. On the other hand, Fig. 23 would seem to illustrate multiple septum formation but with failure of the final process of constriction. As a result the cell elongates and forms a remarkable number of close-packed septa. We were unable to detect similar irregularities in the staphylococcus.

The cytoplasmic membrane

The presence of a cytoplasmic membrane in Gram-positive cocci has not hitherto been described. Both in a previous study (DAWSON, LOMINSKI AND STERN[8]) and in the present work we have been unable to detect such a membrane in *Staph. aureus*. However, Figs. 24 and 25 show appearances highly suggestive of a cytoplasmic membrane in *Strept. faecalis*. This membrane, moreover, shows evidence of transverse septum formation. These septa are invariably double in structure and the cell-wall transverse septum grows between the two layers of these transverse cytoplasmic membrane septa. We cannot yet say whether these septa are formed initially as double layers or as a single membrane which later splits.

DISCUSSION

These studies indicate the advantages of examination of the isolated cell-walls of bacteria in following the stages of cell division. Our results confirm, in general, the three-stage process of cell division, described by KNAYSI and by ROBINOW, in both *Staph. aureus* and *Strept. faecalis*. While we have not been able to detect a cytoplasmic membrane in *Staph. aureus* the possibility exists that a fragile membrane, lying within the tough cell-wall, may be unable to withstand the shaking process of cell-wall isolation. *Strept. faecalis* certainly seems to form cytoplasmic membrane septa as the primary stage of division.

The micrographs demonstrate that the normal mechanism of cell division in *Strept. faecalis*, a short-chained streptococcus, is by the formation of a transverse cell-wall septum, this septum arising after the formation of the cytoplasmic membrane septa. These observations directly conflict with the views put forward by BISSET. Moreover, the successive stages in the formation of the cell-wall septum indicate that these membranes originate from the cell-wall and grow inwards, a fact again in contradiction with BISSET's arguments that the bacterial cell-wall septum is secreted uniformly across the width of the cell.

A small but nevertheless significant proportion of the cells of *Strept. faecalis* were found to divide by constriction without preceding cell-wall septum formation. This would appear to support the observations of HENRICI[9] who stated that division by cell-wall septum formation and by simple constriction could occur in a single culture, the predominant method depending on various environmental conditions. The demonstration of multiple septum formation without subsequent constriction and division and of constriction without prior cell-wall septum formation indicates that the two processes, although obviously closely associated, are the result of separate and dissociable mechanisms. The nature of these mechanisms is obscure but by growing the organisms under various controlled conditions it might be possible to interfere with one or other of these processes and thus to elucidate the underlying physico-chemical principles.

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SUMMARY

The stages of bacterial cell division have been followed by electron microscope examinations of the isolated cell-walls of *Staph. aureus* and *Strept. faecalis*.

In both these organisms a transverse cell-wall septum is formed which originates in and grows centripetally from the cell-wall. The septum splits into two layers and division is completed by constriction of the cell-wall through the line of the double septum. Cell-wall septum formation and cell-wall constriction would appear to be dissociable phenomena.

Transverse cytoplasmic membrane septa, forming before the cell-wall septum, have been demonstrated in *Strept. faecalis*.

RÉSUMÉ

Les stades de la division des cellules bactériennes ont été suivis par examen au microscope électronique de parois cellulaires isolées de *Staph. aureus* et de *Strept. faecalis*.

Chez ces deux microorganismes, la paroi cellulaire donne naissance à un septum transversal qui s'étend peu à peu vers le centre de la cellule. Ce septum se divise en deux couches et la division de la cellule est réalisée par une constriction de la paroi cellulaire entre les deux couches du septum. La formation du septum et la constriction de la paroi semblent être des phénomènes distincts.

Chez *Strept. faecalis* un septum cytoplasmique transversal apparaît avant le septum issu de la paroi.

ZUSAMMENFASSUNG

Die Stadien der Bakterienzellteilung wurden an isolierten Zellwänden von *Staph. aureus* und *Strept. faecalis* durch Untersuchungen mit dem Elektronenmikroskop verfolgt.

In diesen beiden Organismen wird eine querlaufende Zellscheidewand gebildet, die an der Zellwand beginnt und von hieraus nach der Zellmitte zu wächst. Die Scheidewand trennt sich in zwei Schichten und die Teilung wird durch die Zusammenziehung der Zellwände der doppelten Scheidewand vervollständigt. Es könnte scheinen, dass die Bildung der Zellscheidewand und die Zusammenziehung der Zellwand getrennte Erscheinungen sind.

Querlaufende, sich vor der Zellscheidewand bildende cytoplasmatische Membranscheidewände wurden in *Strept. faecalis* gezeigt.

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