FINE STRUCTURE OF *MYXOCOCCUS XANTHUS* DURING MORPHOGENESIS

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ABSTRACT

VOELZ, HERBERT (Indiana University Medical Center, Indianapolis) AND MARTIN DWORKIN. Fine structure of *Myxococcus xanthus* during morphogenesis. J. Bacteriol. **84**:943–952. 1962.—This investigation concerns the nature of the structural changes in *Myxococcus xanthus* during cellular morphogenesis. These changes have been investigated by means of electronmicrographs of thin sections of cells taken during various stages of the life cycle. The conversion of vegetative cells to microcysts involves the formation of a capsule but no drastic reorganization of the limiting cell membranes. Vacuoles appear in the cell during microcyst formation and germination. Microcyst germination involves a separation of the inner cell and the outer sheath, followed by the dissolution of a segment of the outer sheath and the emergence of the cell. Dense bodies within the cytoplasm and peripheral bodies between the two limiting membranes have been observed.

The fruiting myxobacteria undergo a series of developmental events involving both cellular and colonial morphogenesis. A clear understanding of the physiological bases of these changes would be facilitated by an unequivocal description of the events.

An earlier report (Dworkin and Voelz, 1962) dealt with the gross morphological changes occurring in living cells of *Myxococcus xanthus* during cellular morphogenesis. However, apart from the preliminary investigations of Mason and Powelson (1958) on *M. xanthus*, and Imshenetsky and Alferov (1962) on *Sorangium*, information on the fine structure of the fruiting myxobacteria is rather meager.

This report describes some of the fine morphological changes in *M. xanthus* during the conversion of vegetative cells to microcysts and the germination of microcysts. These changes have been investigated by means of electronmicrographs of thin sections of the cells.

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MATERIALS AND METHODS

Organism and media. The fruiting strain of *M. xanthus* (strain FB), which was used for these investigations, was obtained from the stock culture collection of the Department of Bacteriology of the University of California, Berkeley.

For vegetative growth, the cells were grown on CT medium (Casitone, 2%; $MgSO_4$, 0.1%; K_2HPO_4-KH_2PO_4, 0.01 M, pH 7.2). CA medium (coli agar) was used for the formation of fruiting bodies; this was prepared by autoclaving a suspension of *Escherichia coli* (about 5×10^8 bacteria/ml) and agar (2%) for 1.5 hr. Cultivation was at 28 C and has been described in detail elsewhere (Dworkin, 1962). Cells were harvested by centrifugation from liquid media or were scraped off the surface of solid media.

Cells at different stages of conversion to microcysts (a process lasting about 50 hr) were harvested at intervals from the surface of CA medium and were prepared for thin sectioning.

Microcysts will germinate on the surface of CT agar. Accordingly, mature fruiting bodies (more than 3 days old) consisting primarily of microcysts were scraped off the agar surface of CA medium and suspended in 0.01 M phosphate buffer (pH 7.2). The microcysts were washed twice with the phosphate buffer, then plated on CT agar and incubated at 28 C. During the germination period (about 8 hr), cells were harvested at intervals and were prepared for thin sectioning.

Electronmicroscopy. Cells were fixed without washing, so as to avoid the possibility of disturbing any structural organization. Among several fixatives, OsO_4 was found to be the most satisfactory. All of the specimens were prefixed in 0.1% OsO_4, and buffered with a Veronal-phosphate mixture (pH 7.2) containing calcium ions as described by Ryter and Kellenberger (1958).

This was followed by fixation in 1% OsO_4 for 4 more hr at room temperature. The cells were then treated with 0.5% uranyl acetate for 2 hr, followed by dehydration in a graded ethyl alcohol series.

Comparisons of the various embedding mixtures were made on specimens embedded in methacrylate, Vestopal W (M. Jaeger, Vésenaz, Geneva, Switzerland), Araldite (Ciba Products Corp., Fair Lawn, N.J.), and Epon (Shell Chemical Co., New York, N.Y.). The following mixture of Araldite and Epon (H. Mollenhauer, *personal communication*) was found to be the most effective: Araldite 502, 20 ml; Epon 812, 25 ml; dodecenylsuccinic anhydride, 60 ml.

After dehydration, the cells were impregnated with the plastic mixture by immersion in a graded series of propylene oxide-embedding material mixtures at ratios of 3:1, 1:1, and 1:3 for 3 hr each. Before the final embedding step, the specimens were treated with the embedding material without accelerator for 1 day at room temperature. For the final embedding step, 0.25 ml of DMP (tri-dimethylaminoethyl phenol; Rohm & Haas Co., Philadelphia, Pa.) was added as accelerator to 1 ml of the plastic mixture. This was held at 80 C until the specimen was sufficiently hardened (usually about 24 hr). The specimens were post-stained with 4% $KMnO_4$. Thin sections were cut with glass knives at an angle less than 45 deg on a Porter-Blum Ultramicrotome and were examined with an RCA model EMU-3E electronmicroscope.

RESULTS

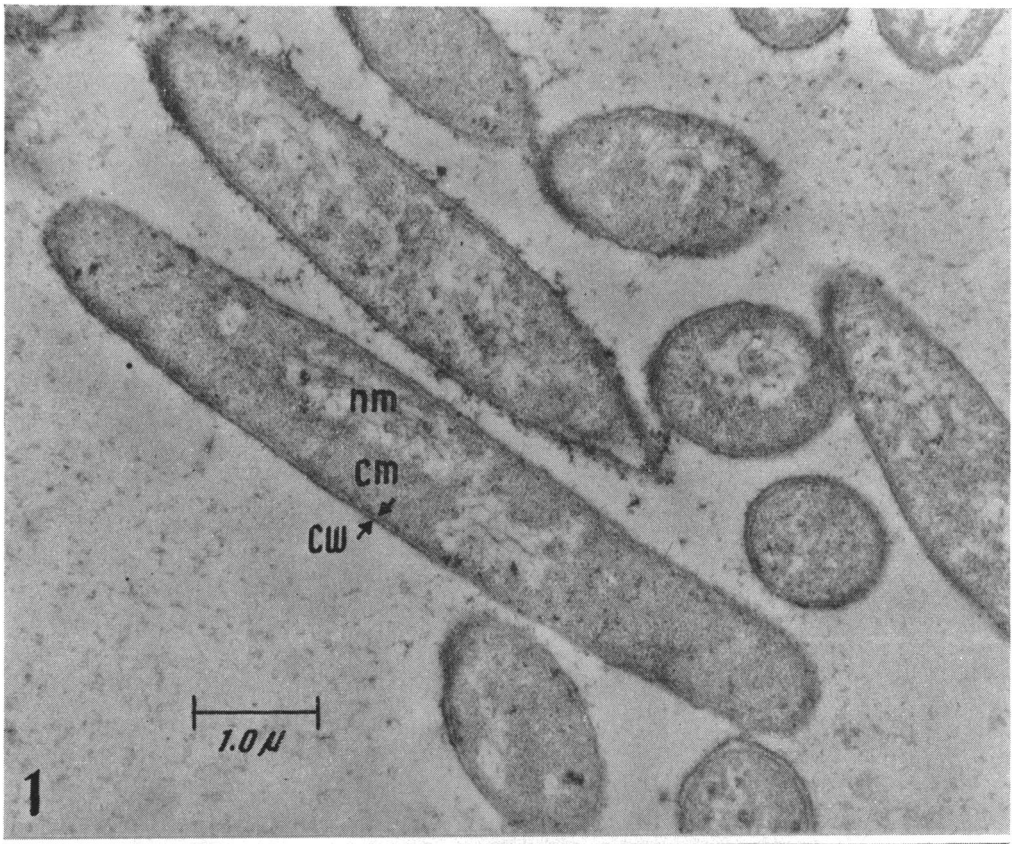
Conversion of vegetative cells to microcysts. The vegetative cells were long flexible rods whose dimensions were approximately 0.75 to 1 μ by 6 to 10 μ. These cells, when plated on a suitable fruiting medium, moved over the agar surface in an organized swarm. After about 30 hr, the cells aggregated, and distinct fruiting bodies were formed. This was the phase of colonial morphogenesis. Within the fruiting bodies, the vegetative rods underwent morphological changes, becoming converted to round refractile microcysts. This occurred by means of a gradual shortening and thickening of the cells (Dworkin and Voelz, 1962) and constituted the phase of cellular morphogenesis. The conversion from vegetative cells to microcysts required about 24 additional hr. A small number of vegetative cells remained morphologically unchanged.

The inner structure of the vegetative cell (Fig. 1) generally resembled that of other bacteria which have been investigated in a similar fashion. There appeared to be two double-layered limiting membranes. Without wishing to imply an understanding of their functions, we designated them as cell wall and cell membrane. The nucleoplasm was coagulated in large vacuoles which have been attributed by Ryter and Kellenberger (1958) to phosphate ions present in the growth medium or in the fixative. Although the vegetative cell produced slime, it was not evident as a discrete capsule or in any organized form. This has been confirmed by using negative staining techniques.

Figures 2, 3, and 4 show sections through cells during conversion to microcysts. As the cell was shortening and thickening, the cell membranes appeared to be morphologically unchanged. The two double-layered membranes occasionally became separated and round bodies could be seen to lie within the space between the membranes (Fig. 2, 3, and 4). These were referred to as peripheral bodies (pb). [The term peripheral body is used in a general sense, and does not imply a functional analogy with the peripheral bodies described by Chapman and Hillier (1953) or by Wyss, Neumann, and Socolofsky (1961).] Also, if one observed transverse sections through the ends of vegetative cells during conversion to microcysts, round bodies could be seen to be arranged peripherally about the cell within the cytoplasm (Fig. 11). Dispersed throughout the cell were a number of dense bodies (db) which appeared during the microcyst formation and also in newly germinated vegative cells (Fig. 2, 3, and 10).

Large vacuoles which seemed to be limited by monolayered membranes were formed within the cytoplasm (Fig. 2, 3, and 4). The nature of the material within the vacuoles has not yet been determined. Although no fat could be detected by Sudan Black staining of thin sections, it is quite possible that the fat may have been extracted during dehydration with alcohol.

As soon as the cells became spherical, the slime capsule began to be formed, and the process continued until the cyst was completed (Fig. 5). The laminated appearance of the slime suggested that the accretion may have occurred in waves.



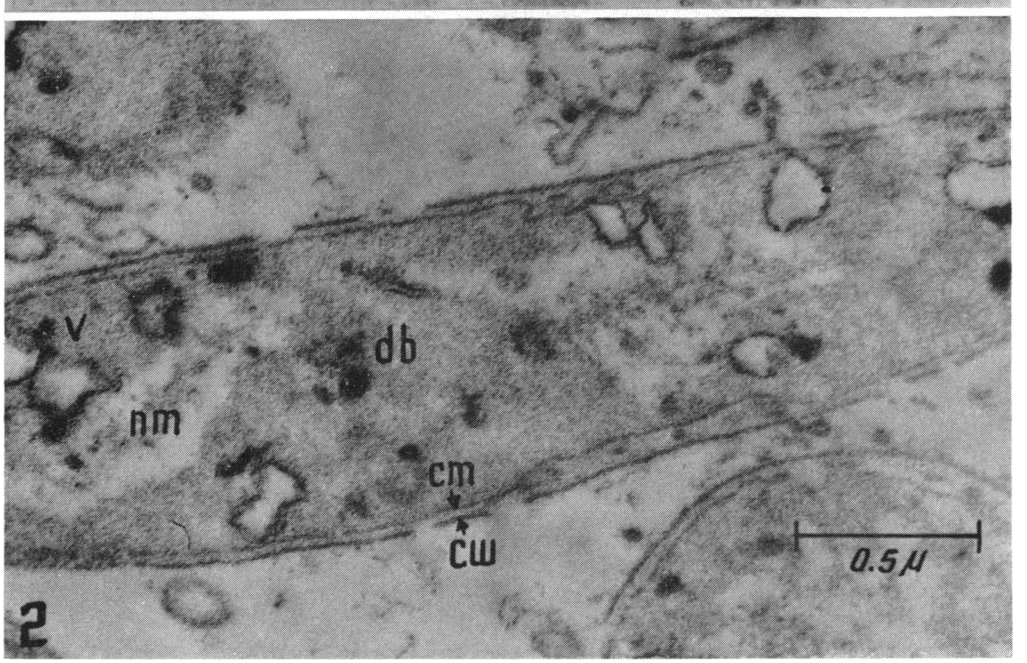


FIG. 1. *Vegetative cells 24 hr old, grown in CT broth. Explanation of abbreviations used in figures:* cw, *cell wall;* cm, *cytoplasmic membrane;* db, *dense body;* nm, *nuclear material;* pb, *peripheral body;* sc, *slime capsule;* v, *vacuole;* m, *membrane.*

FIG. 2. *Cell in conversion to microcyst; 30 hr after transfer to fruiting medium.*

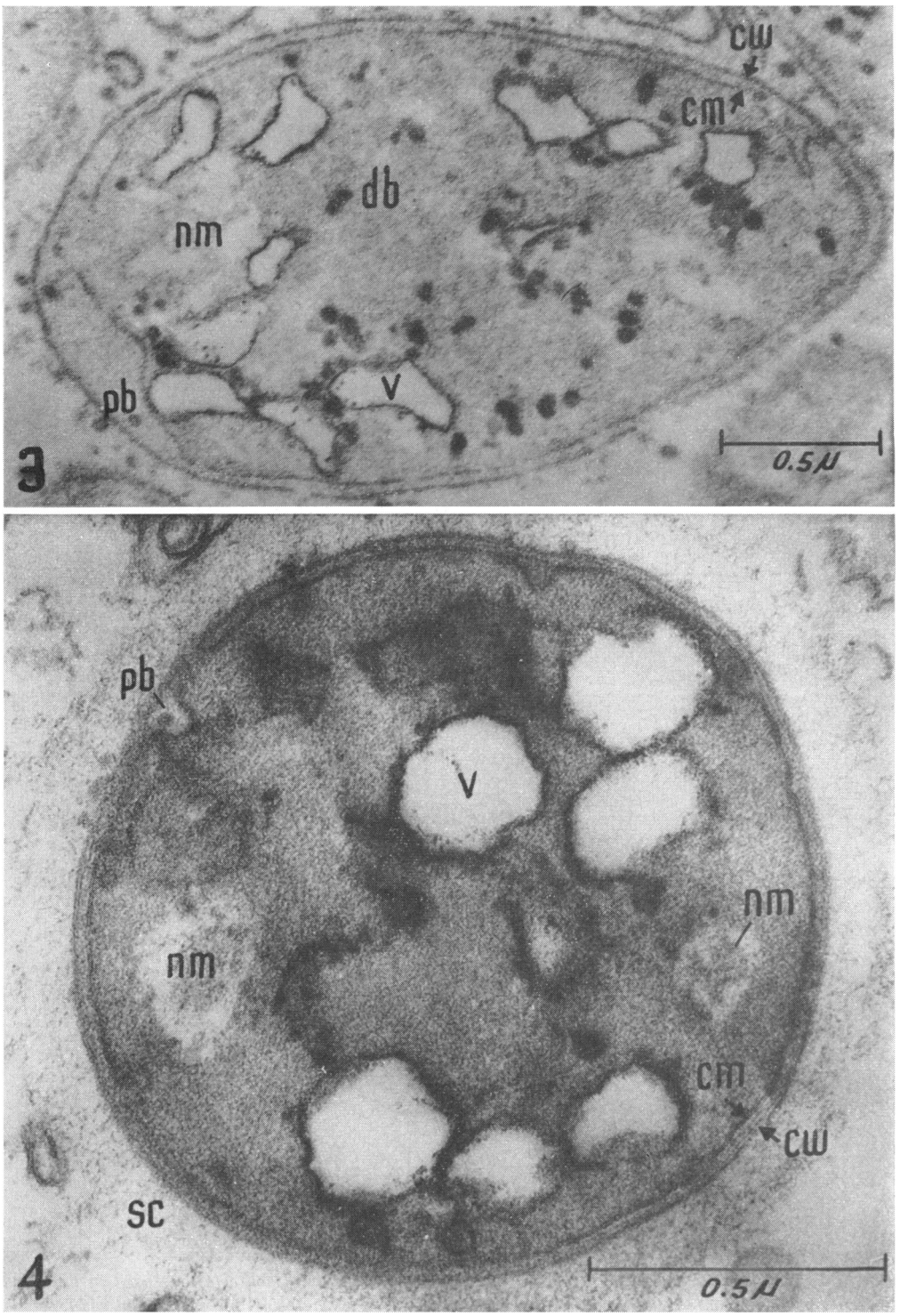


FIG. 3. *Cell in conversion to microcyst; 35 hr after transfer to fruiting medium.*
FIG. 4. *Cell in conversion to microcyst; 40 hr after transfer to fruiting medium.*

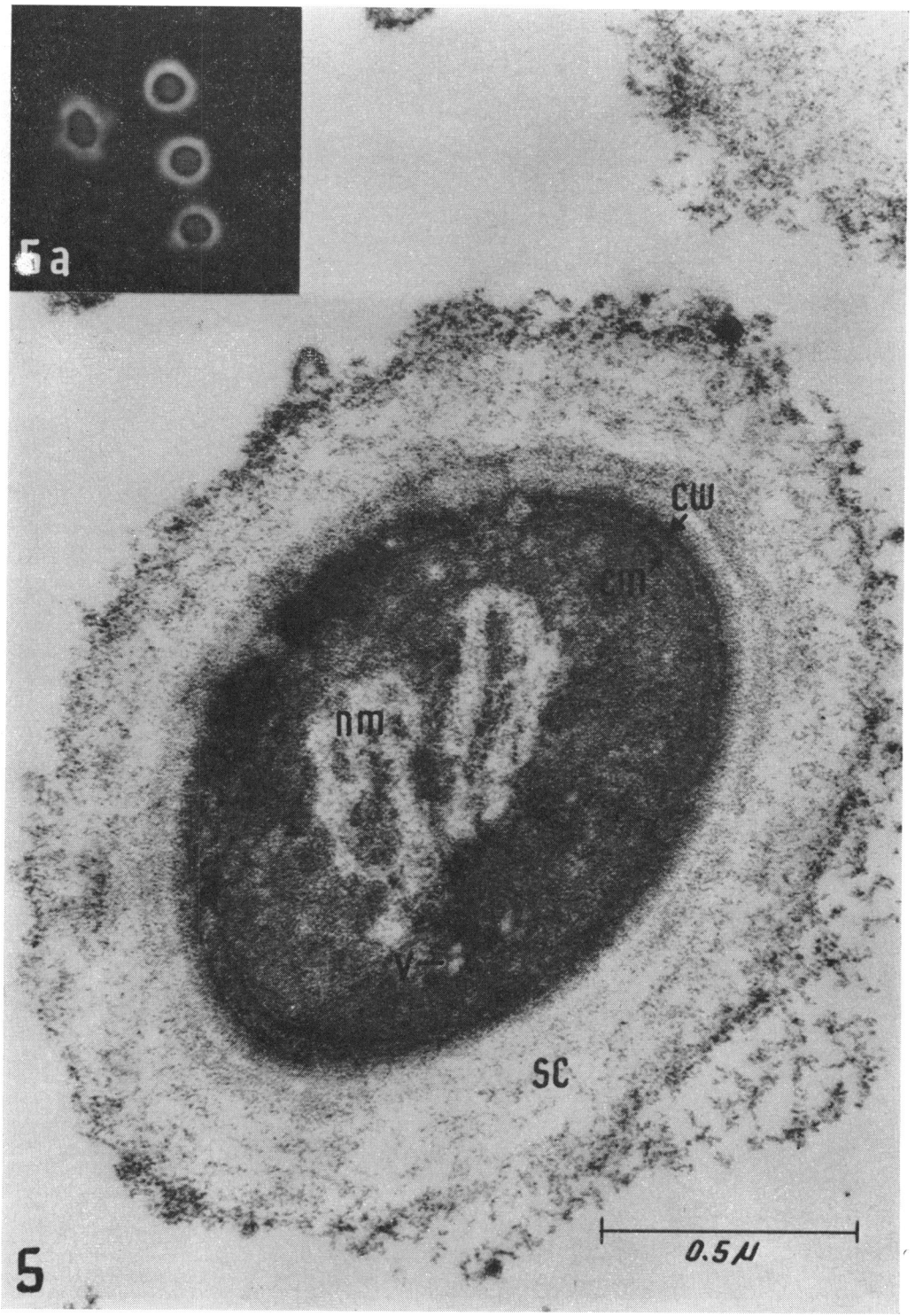
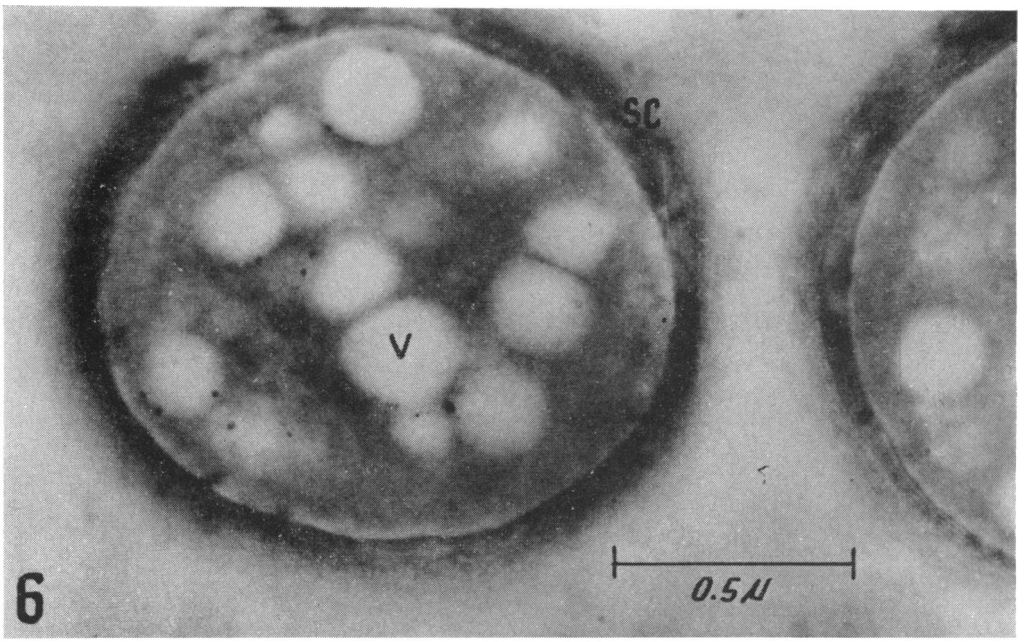


FIG. 5, 5a. *Four-week-old microcyst.* (a) *Microcysts, 1,825 ×; India ink negative stain.*



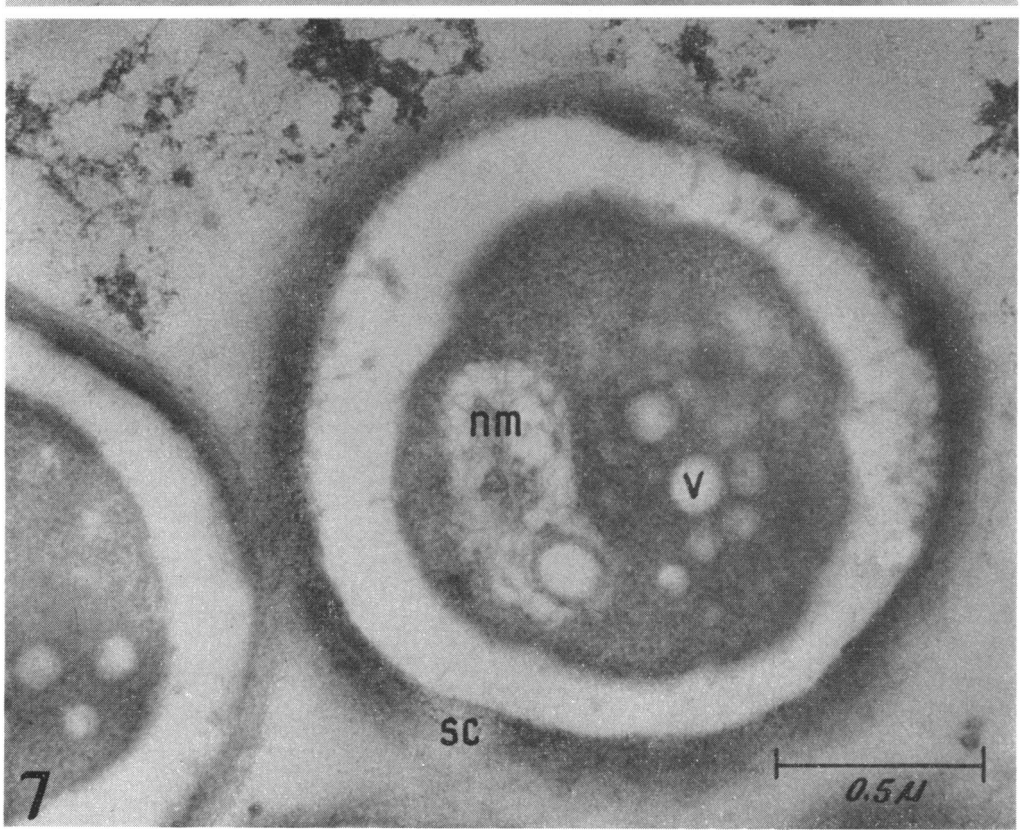


FIG. 6. *Germinating microcyst, 5 hr after transfer to germination medium.*
FIG. 7. *Germinating microcyst, about 6 hr after transfer to germination medium.*

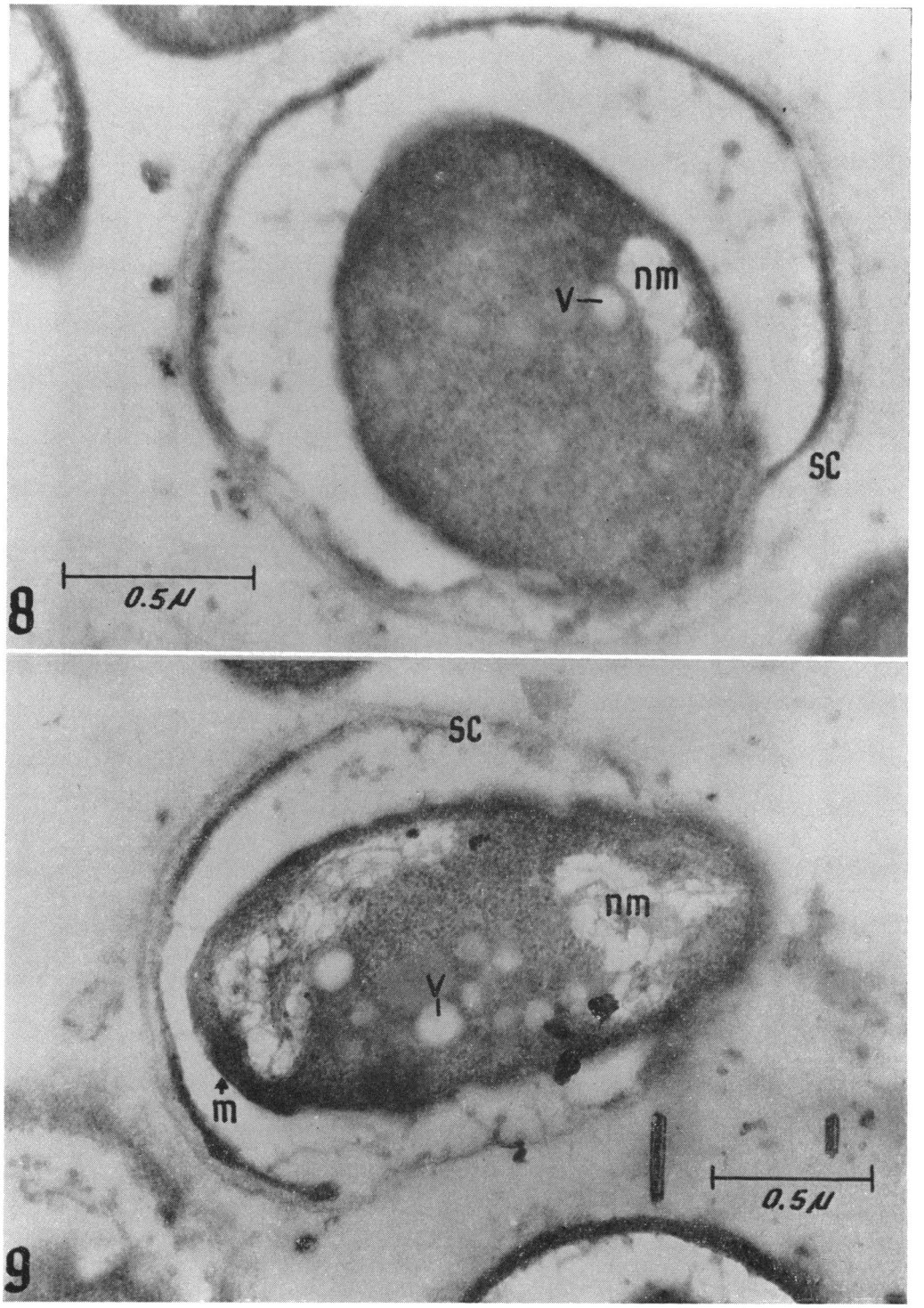
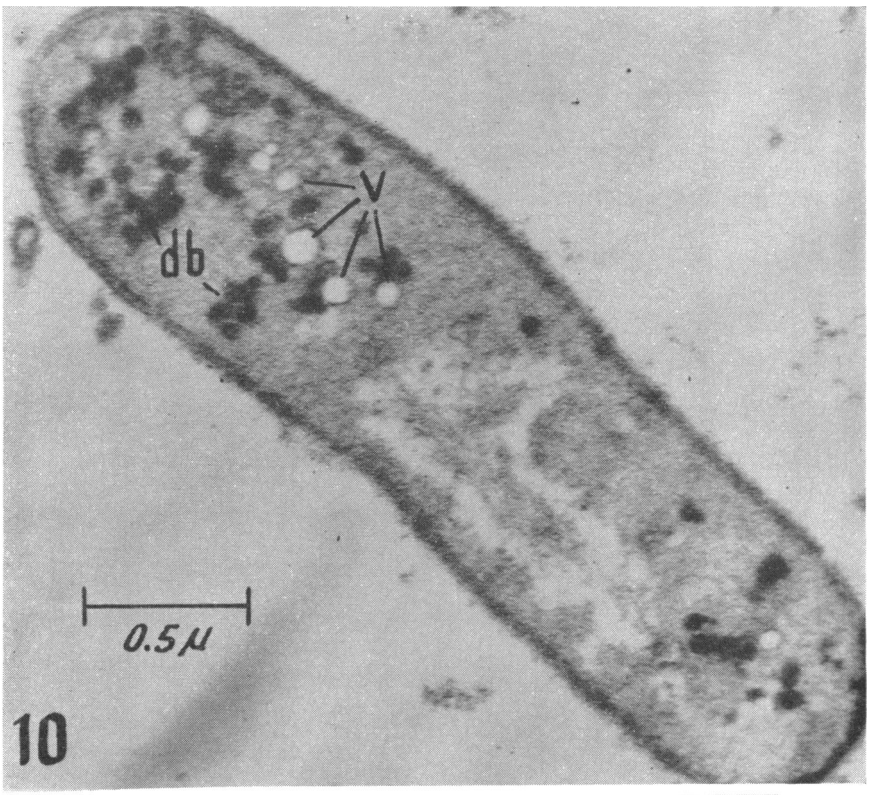


FIG. 8. *Germinating microcyst, about 7 hr after transfer to germination medium.*
FIG. 9. *Germinating microcyst, about 8 hr after transfer to germination medium.*



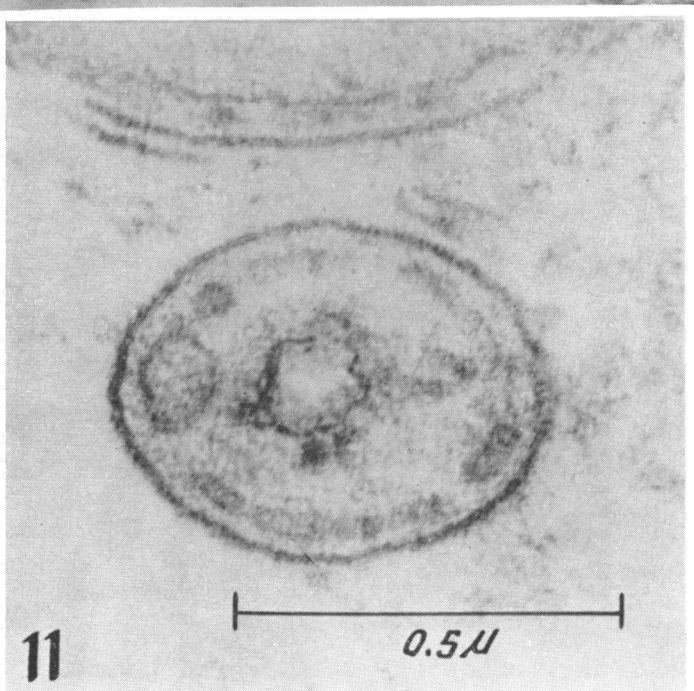


FIG. 10. *Newly formed vegetative cell, about 10 hr after transfer of microcysts to germination medium.*

FIG. 11. *Transverse section through the tip of a cell in conversion to a microcyst, showing peripherally arranged cytoplasmic bodies.*

Further evidence that the slime produced during the conversion process enclosed the microcyst as a capsule was provided by negative staining of the microcysts (Fig. 5a). The membranes of the mature microcyst remained intact and appeared still to be double layered. In mature microcysts (4 weeks old), the vacuoles were sharply reduced in size and the electron density of the protoplasm seemed to have increased (Fig. 5).

Germination of microcysts. Germination began about 5 hr after the transfer of the microcyst to the germination medium (CT agar) and was completed after about 8 hr. Germination involved the casting off of a sheath followed by gradual elongation and loss of refractility of the cell, as observed under the phase microscope (Dworkin and Voelz, 1962).

Figures 6 to 9 represent selected single stages during the germination of the microcyst. The cytoplasm seemed to contract (Fig. 6) and a space appeared between the sheath and the inner cell (Fig. 7). The inner cell emerged from its sheath, presumably as a result of enzymatic dissolution (Fig. 8, 9).

The fate of the limiting membranes, prior to the emergence of the cell from the sheath, is not clear. Although the inner cell seemed to be limited by an ill-defined membrane, as soon as the cell left the sheath both double-layered membranes reappeared. The vacuoles which were reduced in size in the mature microcyst stage (Fig. 5) became enlarged about 5 hr after the microcysts were transferred to a germination medium. During germination, their size was again reduced, and, though they were still present in the newly formed vegetative cell (Fig. 10), they gradually disappeared as the vegetative cell assumed its normal shape and size.

DISCUSSION

Figure 12 is a diagrammatic sketch of the over-all changes occurring during microcyst formation and germination.

In contrast with the drastic reorganizations of the cell walls and membranes which occur during the formation of bacterial endospores, the limiting membranes of *Myxococcus* seem to retain their integrity during the process of conversion of vegetative cells to microcysts. Both the vegetative cells and mature microcysts, as well as the intermediate forms, are bounded by two well-defined double-layered membranes. This is consistent with the observation that there is no obvious difference in the gram-staining properties of vegetative cells and microcysts. Unfortunately, the relative increase in the fragility of the cell during germination has made it difficult to follow the fate of the membranes during this process.

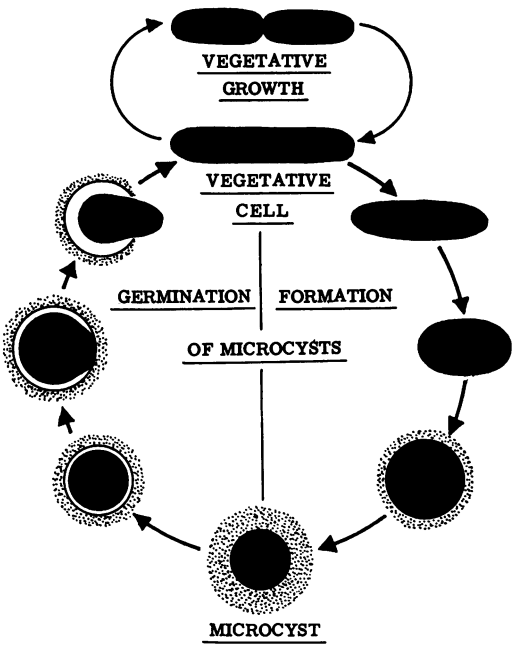


FIG. 12. *Diagrammatic sketch of the formation and germination of Myxococcus xanthus microcysts.*

Vegetative cells produce a complex slime which has been analyzed by Holt (1960). The slime does not, however, remain in contact with the vegetative cell in the form of a capsule. During microcyst formation, the slime begins to adhere to the cell and culminates as a thick capsule about the microcyst. Whether this reflects a change in the composition of the slime or in the nature of the cell surface, or both, is not clear. The presence of the slime capsule may, however, be responsible for the refractility of the microcyst as well as for its enhanced resistance to heat and desiccation.

The nature or function of the dense bodies is unknown. They appear distinctly within the immature microcysts and newly formed vegetative cells. The morphological similarity between these dense bodies and the immature phage particles in T2 phage-infected *Escherichia coli* (Kellenberger, Séchaud, and Ryter, 1959) is too striking to ignore. Although phage have

been reported for the myxobacterium *Chondrococcus columnaris* (Anacker and Ordal, 1955) we have, thus far, been unsuccessful in demonstrating phage for our strain of *M. xanthus*. Although peripheral bodies usually appear between the two double-layered membranes of the immature microcyst (Fig. 2, 3, 4), they have also been observed within the cytoplasm at the ends of vegetative cells during conversion to microcysts (Fig. 11). These may be different organelles. Another possibility is that the dense bodies are polymetaphosphate granules.

The presence of the large vacuoles seems associated with the processes of microcyst formation and microcyst germination. They do not appear in mature vegetative cells or mature microcysts. Their nature and function are unknown. However, since during the process of microcyst formation they appear to be limited by a membrane (Fig. 2, 3, 4), they may, during this process, be invaginations of the cell membrane rather than true vacuoles.

There is an interesting and striking similarity between microcyst formation and germination in *Myxococcus* and the process of cyst formation and germination in *Azotobacter vinelandii* as described by Wyss, Neumann, and Socolofsky (1961). The exine-intine complex is analogous to the slime capsule; similar dense bodies and peripheral bodies are observed; vacuoles appear during cyst formation; and germination takes place by means of the release of the inner cell from the ruptured outer sheath (slime capsule or exine-intine). Further, the process of cyst formation in both cases involves a continuous shortening and thickening of the vegetative cell followed by the deposition of a sheath.

Jahn (1924) and Stanier and van Niel (1941) suggested that the myxobacteria, on the basis of their morphology and peculiar gliding motility, are related to the blue-green algae. Wyss and Russell (1960), on the basis of antigenic and morphological similarities, suggested a similar relationship between species of *Azotobacter* and the blue-green algae. It is of interest, therefore, that the patterns of cell morphogenesis in *A. vinelandii* and *M. xanthus* are so closely similar.

It is hoped that current physiological studies will add an understanding of function to the above description of form.

ACKNOWLEDGMENT

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