FINE STRUCTURE OF THE NUCLEOID AND INTERNAL MEMBRANE SYSTEMS OF STREPTOMYCES

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Recently, there have been significant advances in our knowledge of the biochemical and genetic characteristics of the streptomycetes. However, cytologic studies do not fully support the genetic mechanisms which have been advanced, and the familiar complaint of inadequate cytologic information has been reiterated (Bradley, 1958).

The variable structure of the streptomycetes has been intensively studied, especially for taxonomic purposes and mutant selection. The work of several investigators clearly proves the existence of genetic recombinations (Sermonti and Spada-Sermonti, 1956; Braendle and Szybalski, 1957; Bradley, 1958). Early morphologic studies supporting a two-phase life cycle have been reviewed (Jones, 1954). More recent investigations (McGregor, 1954; Dickenson and Macdonald, 1955) favor the theory of diploidization by hyphal fusion (Klieneberger-Nobel, 1947). Nuclear fusion, mediated by anastomosis between hyphae arising from the same and different spores, has been demonstrated in light micrographs (Gregory, 1956) and hyphal bridges have also been seen in electron micrographs (Baldacci *et al.*, 1956). In no case has the continuity of nuclear material between hyphae of heterogeneous origin been demonstrated. Nuclear division and centrosome-like terminal bodies in the ungerminated spore have been described as resembling mitosis of certain higher organisms (McGregor, 1954). The formation of spores within the hyphal wall and the structure of the spore sheath have been shown in electron micrographs (Vernon, 1955). More recently a study based on the electron microscopy of *Streptomyces coelicolor* provided a more detailed description of the internal nuclear and cytoplasmic structure of the genus (Hopwood and Glauert, 1958).

Streptomyces noursei was isolated in this laboratory (Hazen and Brown, 1950). The present study was undertaken to delineate the internal structures of this microorganism and provide comparative material for a study of the origin and function of cellular inclusions and membrane systems. This first report is concerned with the variable structure of the nucleoid and the internal membrane systems of the cells of the substratum and degenerating sporophore.

METHODS

Cultures of *S. noursei* were maintained in glucose-tryptone broth with 0.1 per cent agar at 22 C. Within 3 to 5 days these cultures develop a typical mycelium of dense vegetative hyphae surmounted by a grayish-white mass of aerial sporophores. A few square millimeters of this growth (5 and 21 days old) were prepared for electron microscopy. Fixation in 2 per cent osmium tetroxide (in Veronal-acetate buffer, pH 7.4) for 7 hr was followed by washing in water and dehydration within ½ hr in graded alcohol series. Small pieces of the dehydrated mycelium were embedded in a mixture of 90 per cent butyl and 10 per cent methyl methacrylate monomers plus 1 per cent Luperco catalyst. The embedding capsules were evacuated for 15 min and capped in a nitrogen atmosphere prior to polymerization at 45 C. Sections were cut on a Porter-Blum ultramicrotome with a diamond knife and mounted on Formvar-covered copper grids. Electron micrographs were taken at initial magnifications of 5,000 and 10,000 in the Siemens Elmiskop I.

RESULTS

Vegetative hyphae. The long, branching hyphae in the substrate growth are 0.3 to 0.5 μ in diameter. Figure 1 shows the upper limit of the dense vegetative mycelia. The compact filaments are bound by a low-density cementing-substance, probably of cell wall material, which reduces the clarity of cell walls and is visible as strands between unapposed hyphal sections. The filamentous network of the nucleoid (*N*) and cytoplasmic filaments (*CF*) are evident. Figure 1 (*arrows*) also illustrates the septal separation of the denser vegetative protoplast from the membranous aerial segment of the same hypha.

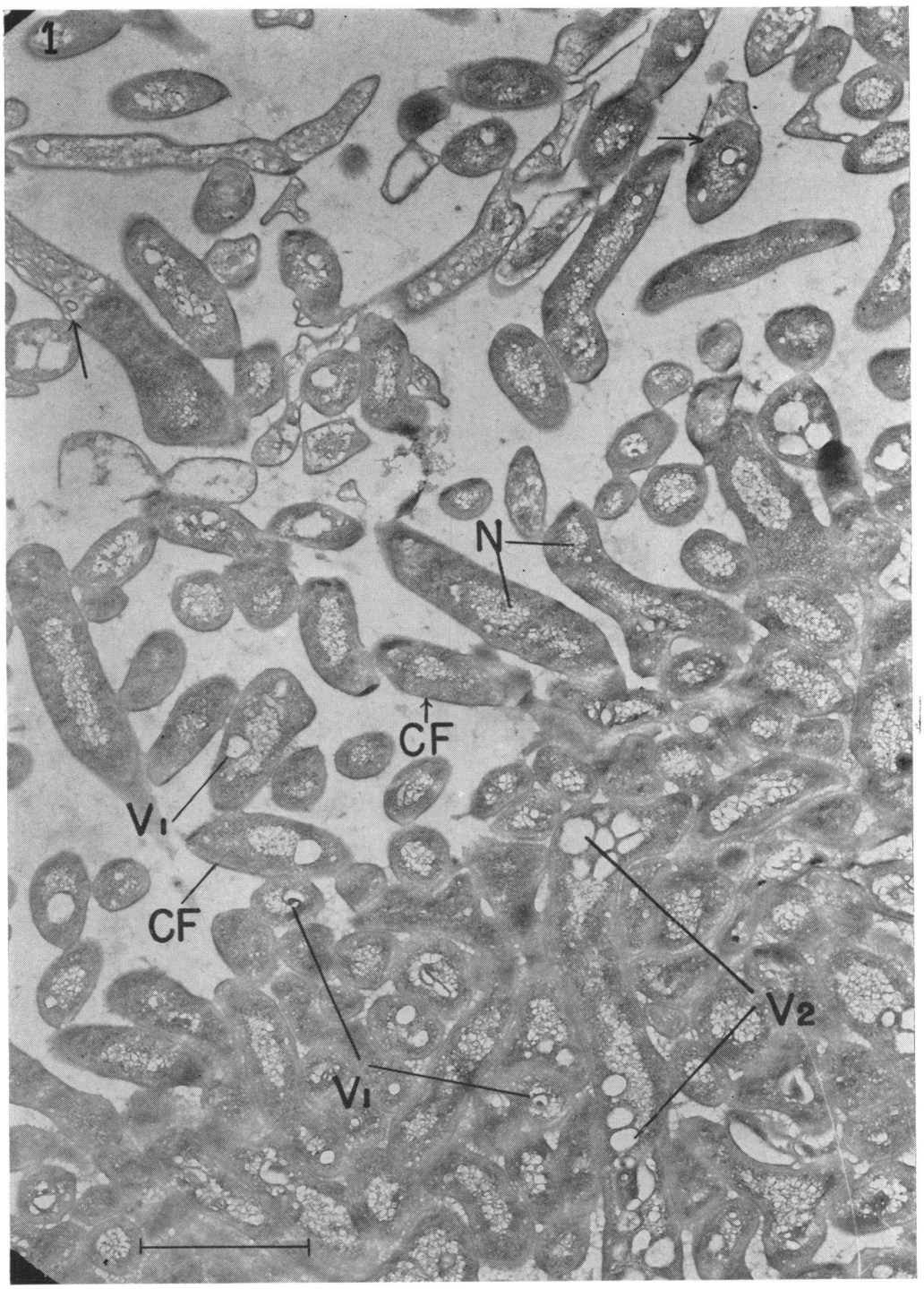


Figure 1. Mycelium of *Streptomyces noursei* at border between aerial and vegetative growth (× 24,000).

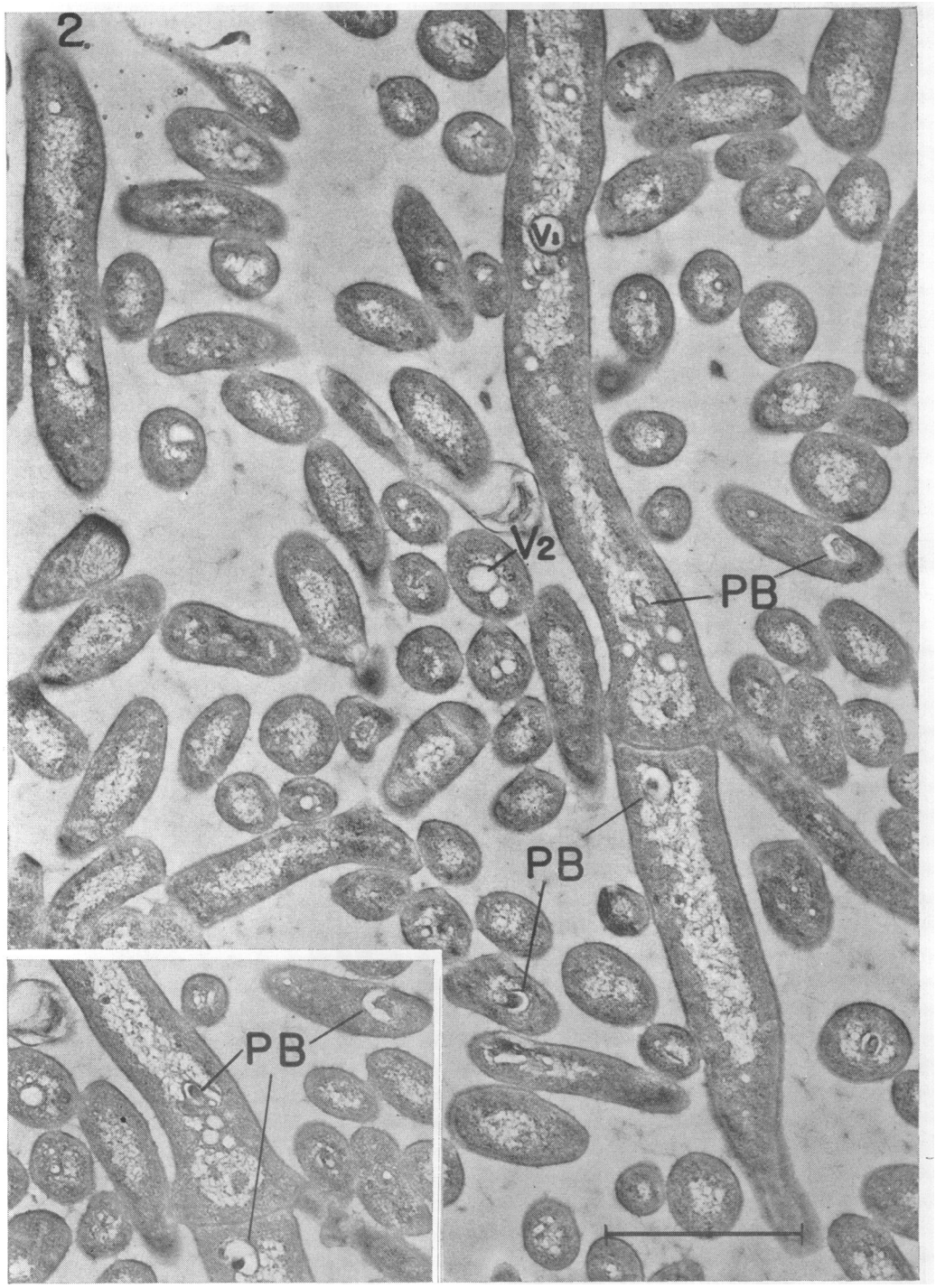


Figure 2. Sections of vegetative hyphae of *Streptomyces noursei* with branching hypha, septum, and "peripheral bodies" (× 28,500). *Insert.* Parallel section showing continuity of "peripheral body" and cytoplasmic membranes (× 28,500).

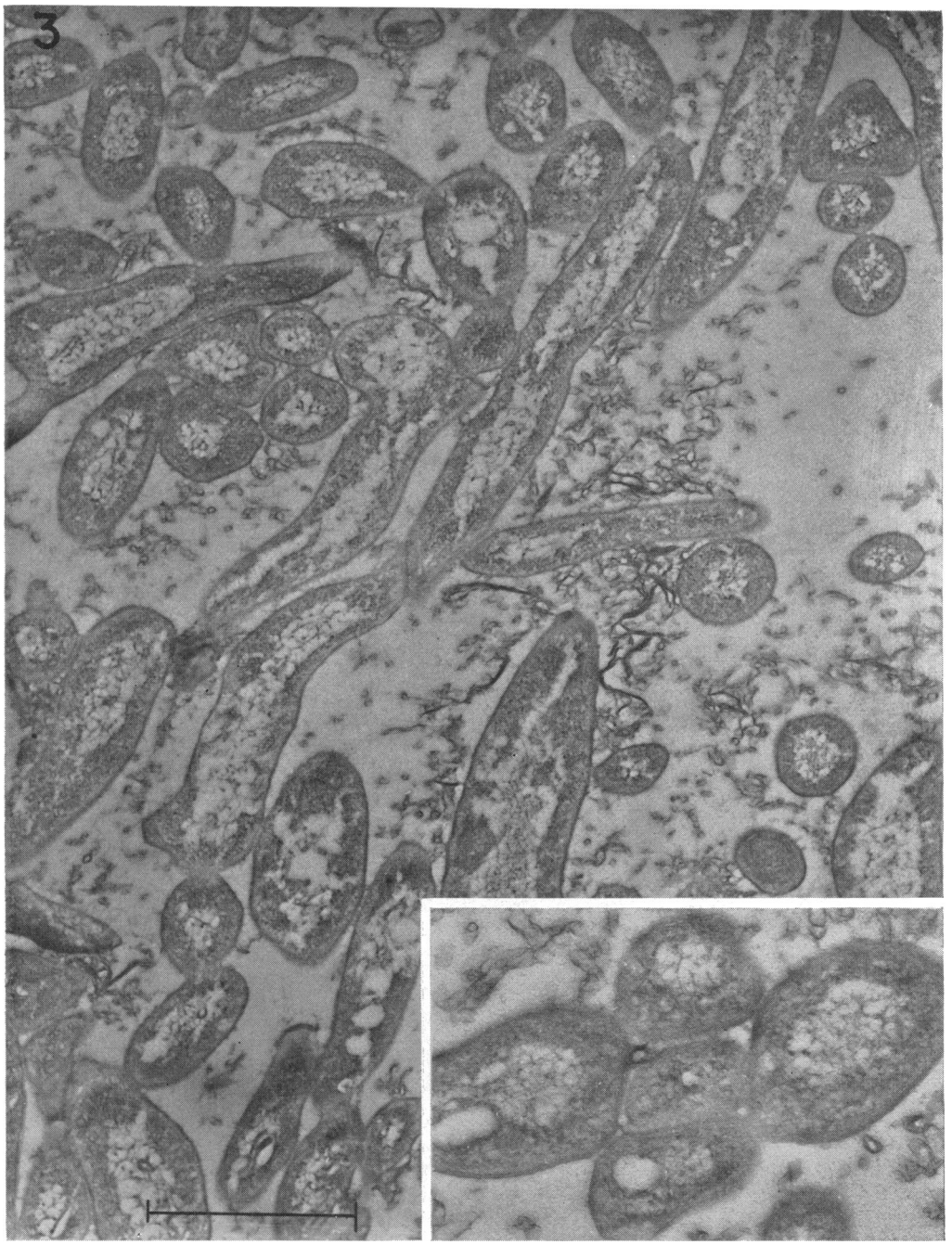
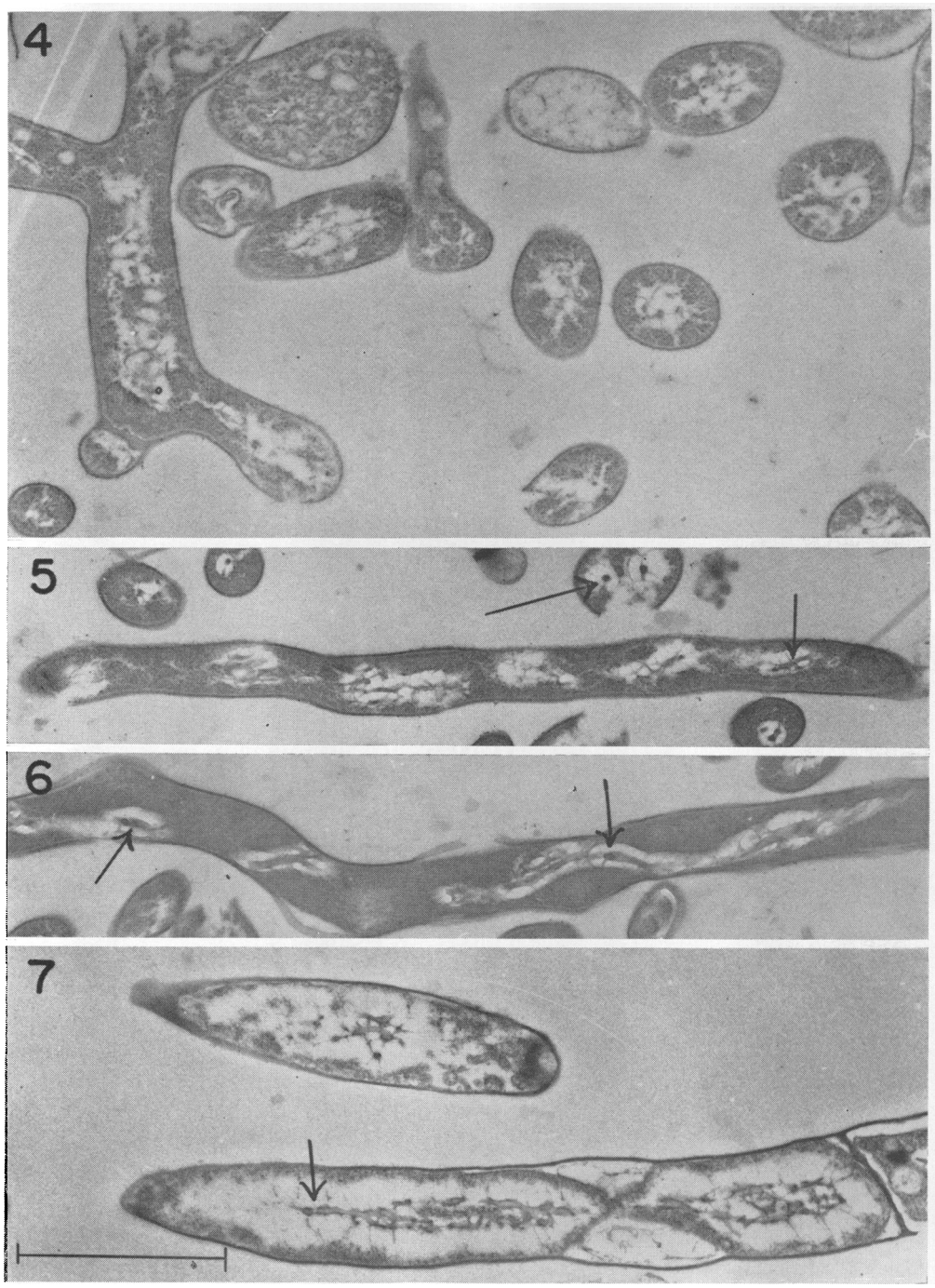


Figure 3. Sections of vegetative hyphae of *Streptomyces noursei* (× 28,500). *Insert.* Enlargement showing components of cell wall, plasma membrane and nucleoid (× 57,000).

Walls of dispersed hyphae are well-defined (12 mμ in thickness) and consist of double lamellae separated by a less dense interspace (figure 3, insert). The lobulated plasma membrane (4 mμ) (figure 3, insert) is most clearly visible in cross sections of hyphae. Septa are seen infrequently. The single septum shown in figure 2 is a double-walled structure formed by the centripetal infold-



Figures 4 to 6. Filaments of *Streptomyces noursei* showing organized structure of tubular nucleoid (× 28,500).

Figure 7. Initial stage of protoplast division and sporogenesis (× 28,500).

ing of the plasma membrane. Numerous longitudinal sections with no more than one septum indicate a minimal length of 4 μ for the interseptal segment or cell. Monopodial branching usually occurs at segment poles, adjacent to the septum (figure 2).

The protoplast consists of dense peripheral cytoplasm and a relatively empty core of nucleoplasm. The nucleo-cytoplasmic ratio varies considerably. The cytoplasm contains a filamentous component and low-density granules (figure 3). Oblique sections through hyphae show that cytoplasmic filaments are organized into an imperfect reticulum (figure 3 and insert) which is more dense at the nucleoplasmic border and around vacuoles. The occurrence of vacuoles in vegetative hyphae is quite variable, and two types are distinguishable. Isolated vacuoles (figures 1 and 2, V_1), surrounded by a distinct membrane, may contain a smaller dense peripheral body, which is not always visible in the section. Serial sections show the peripheral body as a spheroid formed by the circumflexion and condensation of several parallel cytoplasmic membranes (figure 2, insert). The neck of the membrane system becomes pinched off, leaving the spheroid free within the vacuole. There is some evidence from $KMnO_4$-fixed and sectioned mycelia that a continuity of the vacuolar membrane and cell wall breaks down, freeing the peripheral body from the hypha. A second type of vacuole (figures 1 and 2, V_2) frequently occurs in clusters (figure 1) and is also surrounded by a limiting membrane.

The central core of diffuse or organized nucleoplasm extends throughout the length of the hyphal segment. The nuclear membrane is poorly defined. Presumably an equivalent membranous structure is the local condensation of the cytoplasmic reticulum, which appears as an interrupted border of dense granules and short filaments circumscribing the nucleoid. In cross sections, radial extensions of the core frequently subdivide the peripheral cytoplasm (figure 4). In vegetative hyphae the nuclear core consists of a network of fine filaments which merge imperceptibly with those of the enveloping reticular condensation (figures 1 to 4). Within the network are numerous, discrete, dense granules which appear to be the focal point of filament distribution. Although the diffuse nucleoid structure is not shown to vary within hyphal segments of the substratum, variations do occur in the nucleoid of the developing conidiophore.

Aerial hyphae. In 3-week-old cultures, the predominant structure within the nuclear core of immature conidiophores consists of a few filaments and an organized nucleoid consisting of one or more tubular structures which contain a less dense material (figures 5 to 7, *arrows*). The granules of the diffuse, vegetative nucleoid and the tubular structures of the conidiophore nucleoid are thought to be identical. In cross sections the filaments frequently radiate from the dense granules and tubules (figures 4 and 5). Longitudinal sections clearly delineate the tubular structures suspended within the central core by the filaments. The tubule may follow a spiral course through the hypha (figures 5 and 6). The initial stage of spore formation is evidenced by a girdling fissure bounded by the centripetal invagination of the primitive plasma membrane (figure 7).

The greater part of the sporogenous area of older mycelia consists of degenerate sporophores or hyphal "ghosts" (figure 8, *HG*), whose interstices are filled with a compact mat of short tubules (figure 8, *M*). The straight or smoothly curved tubules, approximately 40 mμ in diameter, are usually oriented at random within the mat. Individual components of the mat are shown clearly as tubules (figure 8, insert), though this unique structure is obscured in the denser portions of the mat. Within the hyphal "ghost" degenerate hyphal walls (figure 8, *W*) with inner membranes are evident. It is presumed that the sequence of sporophore maturation and degeneration results in the deposit of the tubular mat around immature aerial filaments, whose further development does not alter the mat pattern. This is evidenced by the tenuous connections between the degenerate hyphal walls and the mat. The diameter of the "ghost" (0.6 to 0.8 μ) gives an approximation of the sporophore diameter. The outline of the hyphal "ghost" is usually broken by intruding portions of the tubular mat (figure 8, *T*), which, on the other hand, forms an unbroken outline around spores (figure 8, *S*).

Figures 9 to 11 show successive stages in the formation of the tubular mat from the sporophores. Degenerate segments of the sporophore are readily distinguished from vegetative hyphae by the lack of cytoplasmic substance and nuclear material, thus rendering their membrane systems

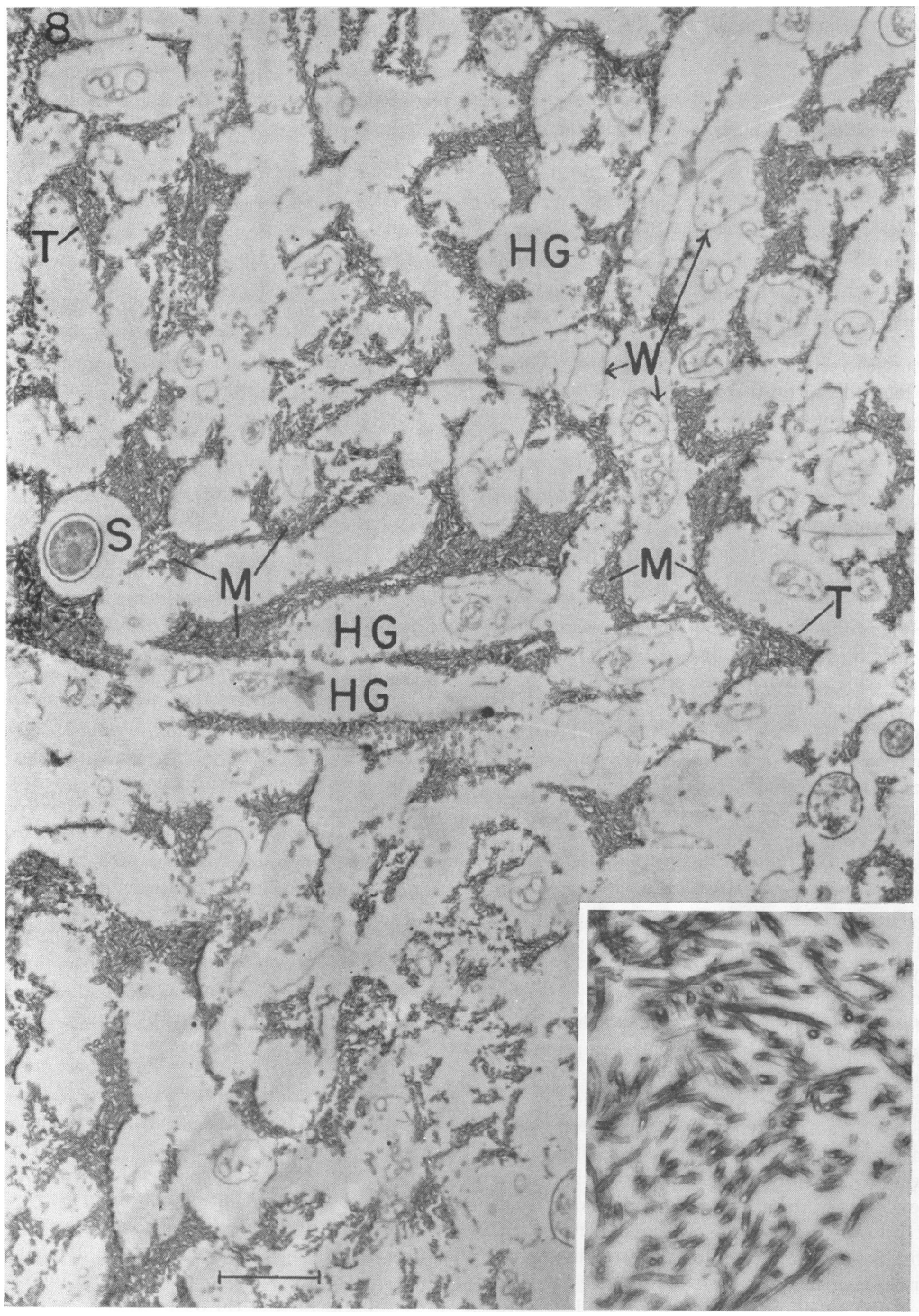


Figure 8. Hyphal "ghosts" of *Streptomyces noursei* outlined by "tubular mat" and enclosing degenerate hyphal walls (× 14,025). *Insert.* Illustrates tubular structure of mat component (× 28,500).

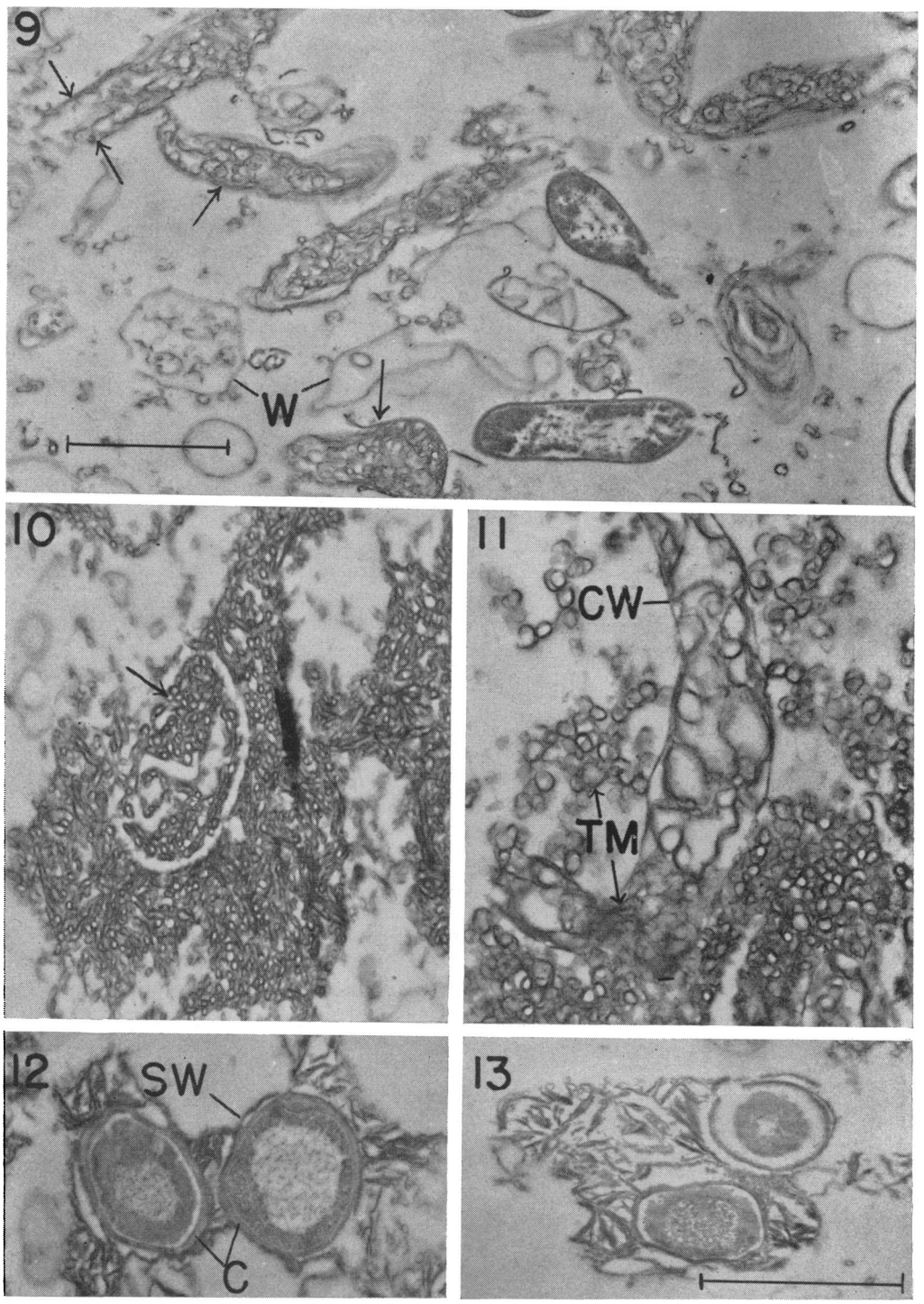


Figure 9. Membranous degenerate sporophores (× 24,000).
Figure 10. Tubular forms within sporophores (× 24,000).
Figure 11. Final stage of sporophore degeneration (× 28,500).
Figures 12 and 13. Mature spores of *Streptomyces noursei* (× 28,500).

more distinct. It is presumed that the nucleoid is concentrated within spores at this stage. The aerial protoplast is initially reduced to a mass of concentric membranes which are frequently paired and retain their continuity with one another and with the plasma membrane (figures 1 and 9, *arrows*). The orderly orientation of the membranes undergoes successive infolding (figure 9, *arrow*) which gives rise to a large number of parallel tubular structures (figure 10, *arrow*). The final stage of hyphal degeneration is the rupture of the cell wall (*CW*) and release of the tubular membranes (*TM*) (figure 11). Sporophore degeneration does not result in the transformation of all membranous components into the uniform tubular system. The fate of the cell wall is uncertain. It may remain intact (figure 11) or be absent (figure 10), though usually the two degenerate wall layers persist (figures 8 and 9, *W*) as a thickened membrane of low electron density. The latter is also noted in figure 6.

Relatively few mature spores were visible in the sections of aerial mycelia observed in this study. The spores are approximately 0.6 μ in diameter (figures 12 and 13). The double layer of the spore coat consists of an inner, less dense component (*C*), which is considerably thicker than the outer spore wall layer (*SW*) and is not always distinguishable from it. The nucleoplasm of the spore consists of a network of fine filaments and dense granules quite similar to the nucleoid of the young vegetative hypha. Contraction of spore material usually leaves a clear zone of varying width between the cytoplasm and spore wall. Numerous spines project from the dense spore wall. The spines vary in size and shape and form a tangled mass with those of adjacent spores. The relationship between the walls of the sporophore and spore and the mechanism of spine formation have not been determined.

DISCUSSION

Hyphal fusion with the formation of initial cells from which the secondary or aerial hyphae develop (Klieneberger-Nobel, 1947; McGregor, 1954; Dickenson and Macdonald, 1955) and direct interhyphal connections by anastomosis (Gregory, 1956) or bridges (Baldacci *et al.*, 1956) have been observed and in some cases presented as evidence of nuclear interactions. However, these structural findings may not yet warrant the genetic interpretations attributed to them. Exchange of nuclear material between fused hyphae has not been demonstrated by direct microscopic evidence. The parallel alignment of hyphae perhaps can be accounted for by surface forces active during the drying of specimens, preparatory to light and electron microscopy. A mucoid cementing-substance between substratal hyphae is observed in figure 1 and appears adequate to cause filament binding. The cell walls of a *Streptomyces* species probably consist of a lipozyme-sensitive mucopolysaccharide (Romano and Nickerson, 1956). In no instances was an indication of hyphal fusion seen in several hundred electron micrographs, but factors are recognized which invalidate the significance of this negative finding. Fusion may be more frequent at a developmental stage other than those observed and ultrathin sectioning greatly reduces the chance of observing an infrequent and randomly distributed entity.

Hyphal anastomosis (Gregory, 1956) is a more likely mechanism for heterokaryon formation although the absence of anastomosis among the streptomycetes has been emphasized (Erikson, 1949). The occurrence and significance of these connections must be clarified by further cytologic studies.

There is no evidence in this study for the existence of multinucleate cells or segments. In all vegetative hyphae, the nuclear core is visualized as a diffuse network of filaments and discrete granules, which are not subdivided within the cell. There was no evidence of sporogenesis within the substratum. In other segments, the nucleoid is organized into one or more long, tubular spirals supported by filaments within the central core. It was especially noted that this structure is frequently paired. Subsequent development of the conidiophore nucleoid, leading to spore formation, was not observed. There is no completely satisfactory explanation for the difference between these findings and the discrete nuclear structures observed in light micrographs (Gregory, 1956; Bradley, 1958). Further investigation of the sequence of nucleoid changes may provide a structural basis for the explanation of genetic phenomena.

The relatively poor delineation of the plasma and nuclear membranes of this streptomycete, as compared with those of higher fungi, may indicate the more primitive nature of the genus and may be of evolutional and taxonomic sig-

nificance. The membranes of the degenerating conidiophore and the membranous precursors of the peripheral bodies and septa clearly demonstrate the potential of the hyphae for membrane formation. It is possible that the membranes of the vegetative protoplast are made less distinct by the dense cytoplasmic matrix. The plasma membrane forms the septa and is continuous with the membranous reticulum of the cytoplasm. This network, which appears as interrupted filaments, is distributed throughout the protoplast, but its density is demonstrably increased at the borders of the nucleus, where it forms a primitive nuclear membrane. The continuity of primitive endomembrane systems has been shown in a species of Deuteromycetes (McAlear and Edwards, 1959). The membranes of the degenerate conidiophore, which give rise to the tubular mat, were first demonstrated as an array of narrow, pointed plates in electron micrographs of metal-shadowed specimens (Vernon, 1955). The significance of the tubular mat and its relation to sporulation is uncertain. Some evidence was observed for the coiling of conidiophores, which is usual among the streptomycetes.

ACKNOWLEDGMENT

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SUMMARY

The fine structure of the substratum and aerial mycelium of a *Streptomyces* species, as seen in ultrathin sections, was studied by means of electron microscopy. Septa formed by the plasma membrane, dense peripheral bodies formed by the condensation of cytoplasmic membranes, and a primitive cytoplasmic reticulum which forms poorly defined membranes around the nucleus and vacuoles, were observed in the vegetative protoplast. Variations of the nucleoid, from a filamentous and granular structure in hyphae of the substratum to a spiral, tubular form in the developing conidiophore, are demonstrated. The origin of the tubular mat of the aerial mycelium is traced to the endomembranes of the degenerating conidiophore. No evidence of hyphal fusion or multiple nuclei was found.

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