OBSERVATIONS OF THE FINE STRUCTURE AND MODES OF GROWTH OF A STREPTOMYCETE

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The actinomycetes have at various times been placed by various authors in close relationship to both the bacteria and the higher fungi. Bessey (1950), citing the observation of their possession of true nuclei (Drechsler, 1919; Newcomer and KenKnight, 1939) and on the basis of certain morphological characteristics, e. g., mycelioid colony habit, reproduction by exogenous conidia from conidiophores, and the lack of sexual reproduction, places the actinomycetes in the Family Moniliaceae of the Form-class Deuteromycetes (Division II of *Bergey's Manual of Determinative Bacteriology*, 7th ed.). Couch (1957), however, states that the cell structure of these organisms is like that of the bacteria and, referring to the report by Avery and Blank (1954) that the cell wall substance is neither chitin nor cellulose, notes that they differ thus from the condition present in the true fungi. Couch concludes that the actinomycetes bear a closer relationship to the bacteria than to the fungi. That conclusion is supported by the recent studies of the chemistry of the actinomycete cell wall by Cummins and Harris (1958) and by Sohler *et al.* (1958).

A streptomycete[3] has been encountered in this laboratory and has been examined in the electron microscope. It is believed that the observations throw fresh light on the problem of the phylogenetic relationship of the actinomycetes to the bacteria and fungi.

MATERIALS AND METHODS

For examination in the electron microscope, cultures of two types and ages were used. In each case, the substrate was yeast extract medium (Pridham *et al.*, 1956/57). Log-phase cultures were inoculated into yeast extract broth and incubated at 30 C in aerated flasks for about 4 days. Stable-phase cultures were grown on yeast extract agar (2 per cent) at room temperature for about 1 month. Log-phase cells were fixed for 17 hr and stable-phase cells for 12 hr at 10 C, in the following fixative: 2.5 ml acetate-Veronal (9.714 g sodium acetate and 14.714 g sodium-Veronal made up to 500 ml in distilled water), 1 ml 8.5 per cent NaCl, 3 drops 0.11 M $CaCl_2$, 2.75 ml distilled water, and 6.25 ml of a 2 per cent OsO_4 solution.

Following fixation, the samples were washed in a solution identical to the fixative except for the omission of the OsO_4. They were then dehydrated in an ethanol series and were infiltrated with a mixture of monomeric methacrylates (3 parts *N*-butyl methacrylate, 2 parts ethyl methacrylate) to which had been added 1.5 per cent catalyst (Luperco CDB). The specimens were dispensed into no. 1 gelatin capsules and polymerization was carried out at 70 C overnight. Ultrathin sections, about 250 A thick, were cut with a Porter-Blum ultramicrotome ultilizing glass knives prepared in the laboratory. The sections were floated off the cutting edge onto 40 per cent acetone in a collecting trough. They were lifted from the acetone surface on 200-mesh copper screen on which a thin collodion film had been deposited. Some sections were stained by being floated, sections down, on the surface of a saturated solution of uranyl acetate for 1 hr as described by Watson (1958). Electron microscopy was carried out with an RCA EMU-2D electron microscope which had been equipped with an externally centerable 0.015-inch condenser aperture (Canalco) and a 60-μ objective aperture in the standard objective pole piece.

RESULTS AND DISCUSSION

Figures 1 and 2 represent germinating conidia. In figure 1, no transverse cell wall, or even a stage

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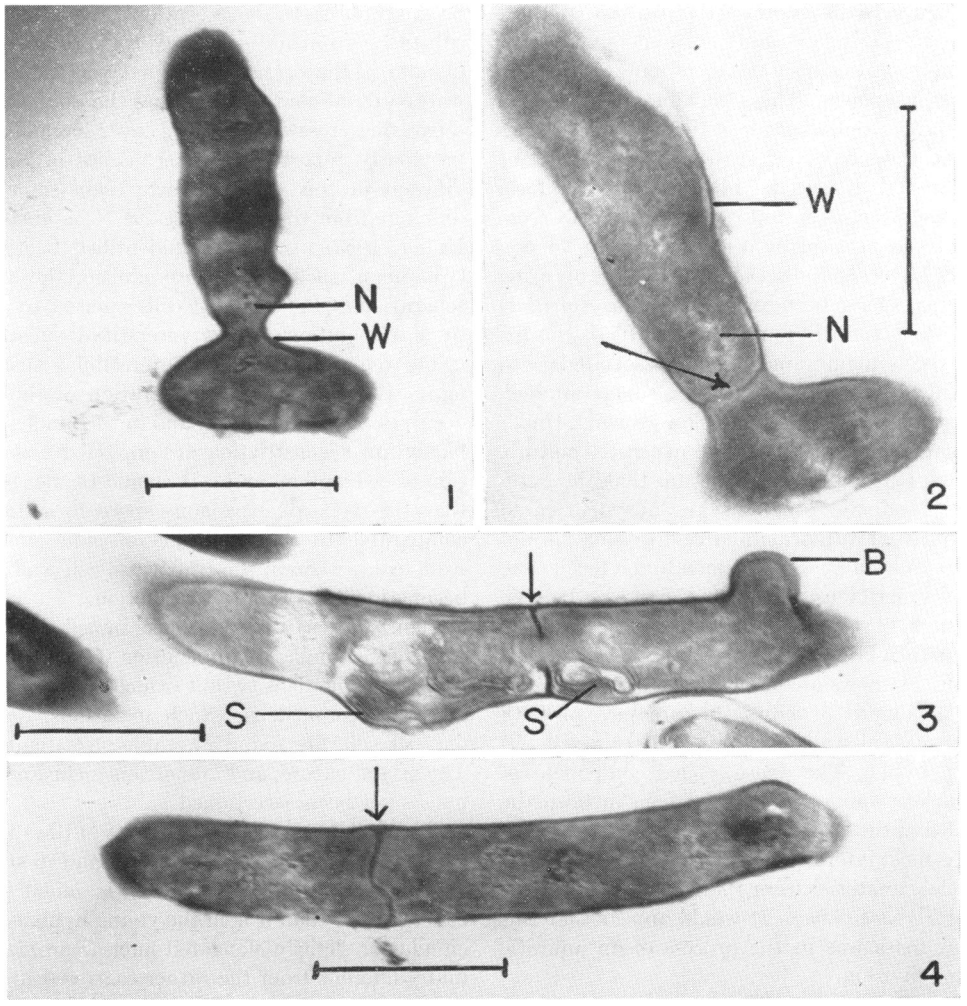
[3] The specific diagnosis and a table of selected characteristics of this streptomycete will be published later.

in its formation, is seen. In figure 2, a nearly complete transverse cell wall is seen. (The region of incompletion is designated by an arrow.) In these two figures, the cell wall (*W*) and nuclear material (*N*) may be identified. The cell wall appears homogeneously dense and the nuclear material appears of low density but contains several dense threads or granules. No nuclear membrane may be seen. The nuclear apparatus thus resembles grossly that of the eubacteria as described by Chapman and Hillier (1953). Chapman (1959*a*), Kellenberger and Ryter (1955), Piekarski and Giesbrecht (1956), and others. The fixation employed failed to reveal the fibrillar nucleoplasm demonstrated in *Escherichia coli* by Kellenberger *et al.* (1958) and considered by them to reflect optimum preservation of nuclear structure. It is notable that even the excellent fixation obtained by those authors produces either a parallel arrangement of the fibril



Figures 1 to 4. Electron micrographs of ultrathin sections of a streptomycete. Figures 1 and 2 represent germinating conidia. The arrow in figure 2 indicates a region of transverse cell wall incompleteness. *W*, cell wall; *N*, nuclear material. Figures 3 and 4 represent portions of hyphae. The unusual intracytoplasmic membranes are designated *S* in figure 3, which also shows a nearly complete transverse cell wall (*arrow*). *B*, branch initial. The arrow in figure 4 indicates a completed transverse cell wall. In all figures, the magnification mark equals 1 μ.

in the nucleoplasm or a network of the nuclear strands. It appears that the definitive work on nuclear fine structure remains to be done.

Figure 3 represents a portion of a hypha. A nearly completed partition, growing inward from the cell wall, is clearly visible in figure 3. It is interesting to note that this streptomycete forms its transverse hyphal walls in a manner practically identical to that shown by Chapman and Hillier (1953) for *Bacillus cereus*. The only obvious difference is the absence of peripheral bodies or comparable structures from the hyphae. This figure is also noteworthy because it illustrates the peculiar and not-understood phenomenon of superfluous membrane formation in the cytoplasm. These membranes (*S*) seem quite unlike endoplasmic reticulum membranes in that they have never been observed to be associated with vesicles, nor do they ever form cisternae. They seem to form spontaneously from the cytoplasm, as if by a condensation. No precursors have been observed. Similarly appearing membranes have been reported in *Rhodospirillum rubrum* by Niklowitz and Drews (1955) who believe these membranes to be associated with pigment. Unlike the eubacteria so far studied, the actinomycetes are rather slow growing. Under the stimulation of being grown in aerated culture, it seems reasonable to conjecture that the cytoplasmic anabolic processes are unsynchronized and that wall formation and cell division are unable to proceed apace with membrane formation. (These superfluous membranes may also be seen in figures 11 to 13.) Figure 3 also reveals the presence of a branch initial (*B*).

Figure 4 shows a complete transverse cell wall (*arrow*). Figure 5 shows the presence of both transverse cell walls (*arrows*) and a branch (*B*) from a hypha. The dense, coiled component of the nuclear apparatus may be seen in both the branch and the hypha. Figure 6 is similar to figure 5 but is most interesting because of the continuity of nuclear material from the hypha out into the branch (*double arrow*). It would appear that this nuclear mass was in the process of an amitotic type of division.

Figure 7 represents the maximum complexity of hypha-branch relationships observed in any field in the present study. The position at which a catenula was presumably attached to the hypha is designated (*C*). Two branches (*B*) may be seen. The transverse cell wall in the branch at the right in the figure should be noted. Superfluous membranes (*S*) may be seen.

Figure 8 is included to illustrate an aberration in the process of cross cell wall formation. In this type of aberration, a bifurcation in the transverse cell wall occurs. This aberration is quite different from the previously reported abnormality in transverse bacterial cell wall formation as reported by Chapman and Hillier (1953) and called by them supernumerary transverse cell walls.

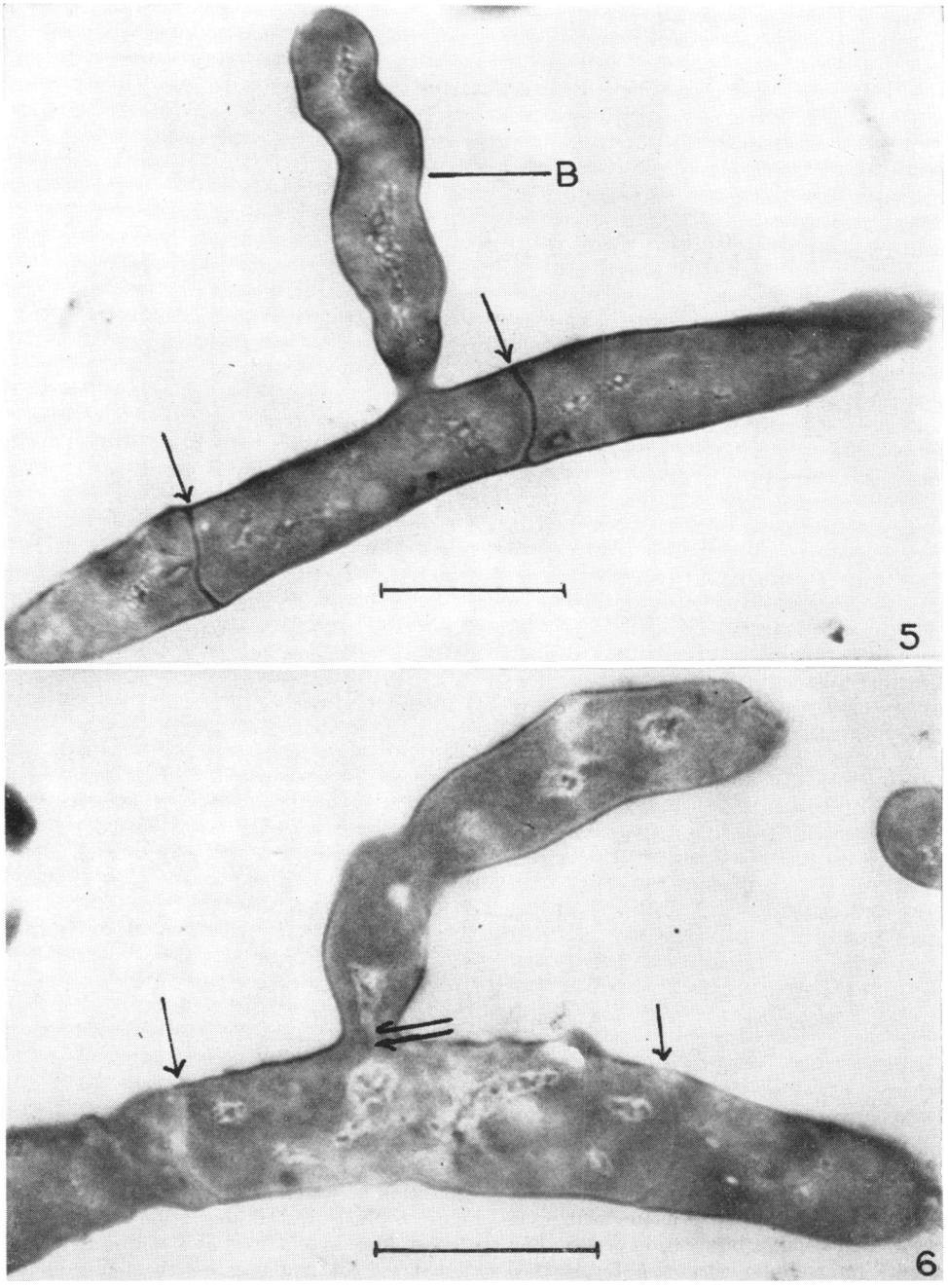
Figures 9 and 10 represent part of a coil of a sporogenous catenula from material in the stable-phase. It is to be noted that the crosswalls have become double. In some locations, an intercellular substance (cement?) (*I*) may be detected. Also of note is the occurrence of a layer (*L*) of cell wall material continuous across the doubled and separating crosswalls. Such a layer has not been previously reported. The mechanics of cellular division in this streptomycete seem somewhat different from those in *B. cereus* (Chapman and Hillier, 1953) and in an unidentified bacterium (Chapman, 1959*b*). In our organism, the actual separation of the adjacent cells appears to occur by a dissolution of the interstitial substance. (Note the pockets (*P*) of intercellular space in figure 12.) In *B. cereus*, separation of the cells occurred by a constriction and in the unidentified bacterium a constriction accompanied a deposition of cell wall material. It should be mentioned that this type of exogenous spore formation is comparable to the formation of oidia in some Eumycota, wherein the transverse cell walls also become double prior to disjunction.

Figure 9 reveals the presence, in cells of a sporogenous catenula, of an inclusion (*G*) which is initially low in density but which becomes progressively more dense. Such inclusions, usually one per cell, are found in stable-phase cultures. The origin, nature, and fate of this inclusion have not as yet been ascertained.

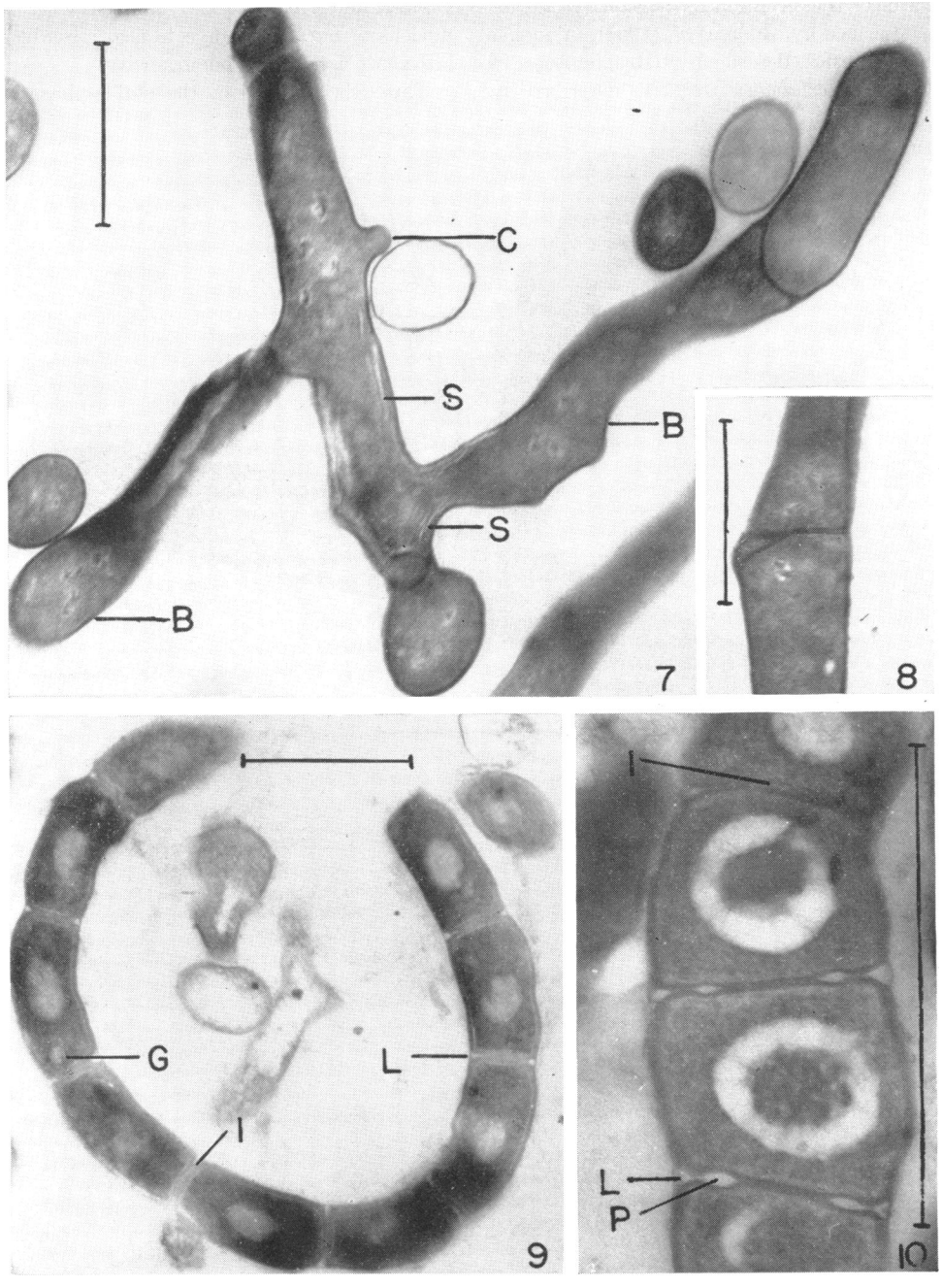
The nuclear material in the cells of the sporogenous catenula (figures 9 and 10) and in stable-phase hyphae (not illustrated) is much more concentrated than it is in the young hyphae. The circular or slightly elongated nuclear profiles are quite different from the rather scattered nuclear masses seen in the log-phase material. The occurrence of a central, dense mass in the nuclear zone of the cells in figure 10 is reminiscent of the figures of aureomycin-treated *E. coli* presented by Kellenberger and Ryter (1955). This configuration was rarely observed in this study and its significance is unknown. The localization of the nuclear material in a nearly spherical mass is

remindful of the situation in *Stilbum xacalloxanthum*, described by Moore *et al.* (1959); in *Saccharomyces cerevisiae*, described by Hashimoto *et al.* (1959); in *Coccidioides immitis*, described by O'Hern and Henry (1956); and elsewhere. The nucleus of our streptomycete also resembles the latter two types in its homogeneity.

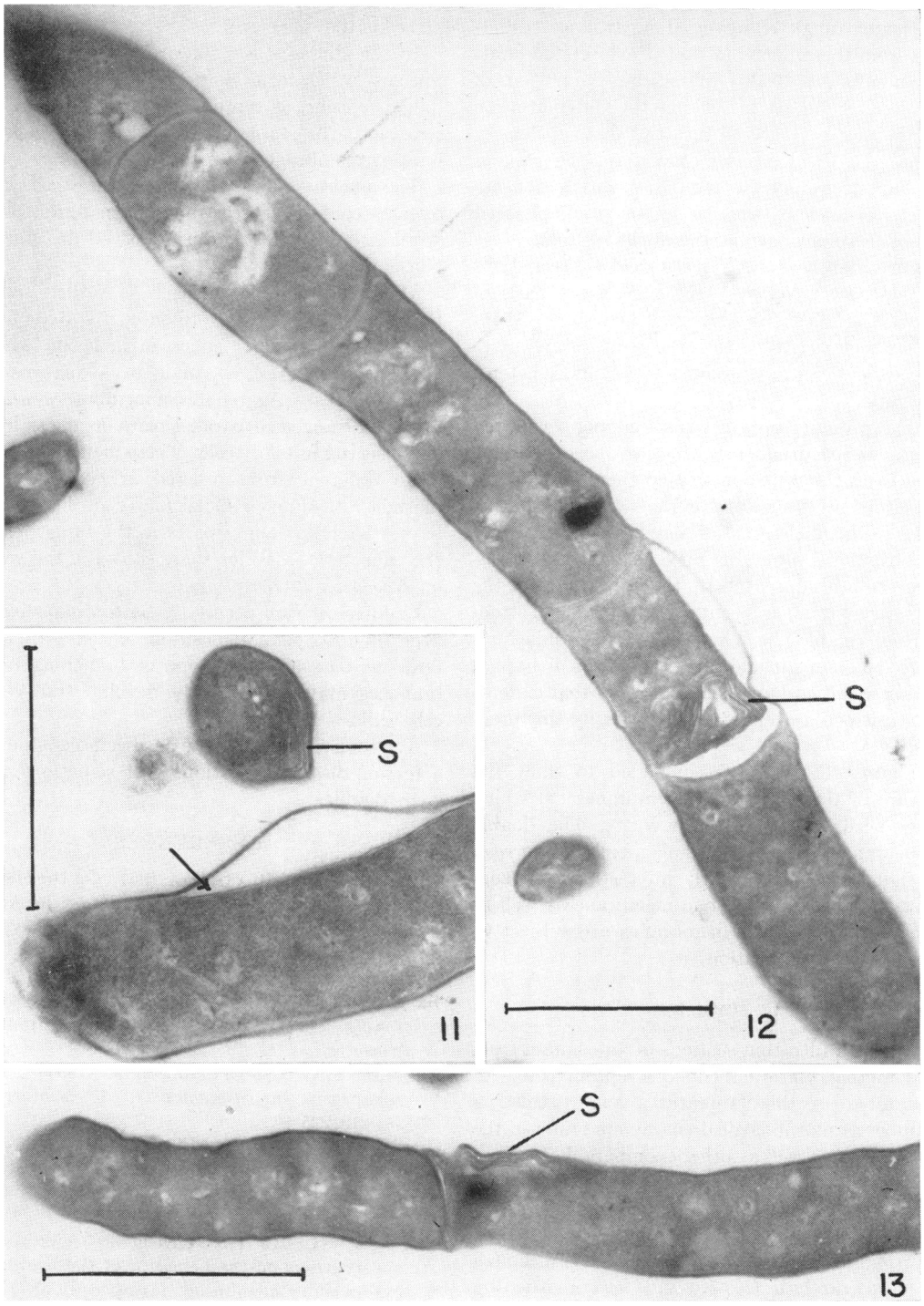
Thus, the nucleus of the stable-phase strep-



Figures 5 and 6. Electron micrographs of ultrathin sections of a streptomycete. Completed transverse cell walls are designated by arrows. *B*, branch from hypha. In figure 6, the double arrow indicates the connection between the nuclear mass in the hypha and that in the branch.



Figures 7 to 10. Electron micrographs of ultrathin sections of a streptomycete. Figure 7 indicates the complex hypha-branch relationships. *B*, branch; *C*, position at which a catenula was presumably attached to the hypha; *S*, superfluous membranes. Figure 8 illustrates an aberration in the process of cross wall formation; a bifurcation occurs. Figures 9 and 10 represent part of a coil of a sporogenous catenula. *I*, intercellular cement; *L*, layer of cell wall material continuous across the doubled and separating cross walls; *P*, pockets of intercellular space; *G*, inclusion.



Figures 11 to 13. Electron micrographs of ultrathin sections of a streptomycete. *S*, superfluous intracytoplasmic membranes; *arrow*, doubled cytoplasmic membrane.

tomycete does bear a slight resemblance to the nuclei of fungi. However, it is strikingly different from these nuclei in that it exhibits no limiting membrane. It also differs from the nucleus of *S. xacalloxanthum* in lacking the differentiation of the nuclear material into two distinct density zones. In the log-phase, therefore, the nucleus of our streptomycete resembles closely the eubacterial nucleus, whereas in the stable-phase it becomes somewhat like the nuclei of fungi.

On the basis of cytological observations (Klieneberger-Nobel, 1947) and on the basis of genetic studies (Bradley and Lederberg, 1956) it has been suggested that the genus *Streptomyces* is coenocytic, rarely produces septa, and is heterokaryotic.

The present study reveals that nonperforated septa occur frequently in log-phase hyphae. However, the cells demarcated thereby may still be either multinucleate or the scattered appearance of the nuclear material may be indicative of a continuous, ramifying nucleus. This latter configuration is supported by the appearance of the nuclear material as a centralized, ellipsoidal structure in stable-phase cultures. The studies reported herein include no observation of hyphal fusion. It therefore seems unlikely that heterokaryosis could occur in this species by this process.

Figures 11 to 13 are included to show the nature of the superfluous membranes (*S*) which may occur in this streptomycete. The double appearance of the cytoplasmic membrane (*arrow*) in figure 13 is particularly interesting as it suggests that the cytoplasmic membrane as well as the intracytoplasmic membranes are subject to disorganized duplication.

SUMMARY

Study of ultrathin sections of an osmium fixed and methacrylate embedded streptomycete, encountered in this laboratory, has revealed a number of morphological characters found in the eubacteria as well as others comparable to those found in the fungi.

This streptomycete, in the log-phase, possesses a typically bacterial nucleus which exhibits no limiting membrane. Nuclear division, although observed rarely in this study, is accomplished by a constriction and separation of the nuclear material into two distinct masses in a manner reminiscent of classical amitosis and commonly observed in the eubacteria.

In the stable-phase, the organism possesses a nucleus, which in its homogeneity resembles the fungal nucleus, as represented by *Saccharomyces cerevisiae*. The stable-phase nucleus also lacks a limiting membrane.

The nuclear zones of the log-phase cells frequently contain dense strands of material. Such strands have rarely been observed in stable-phase nuclei.

The cytoplasm of the cells is rather undistinguished save for the presence in stable-phase cultures of a distinct spherical inclusion which has been observed to undergo a progressive density increase. Aggregates of membranes, which may be arranged in parallel pairs or at random, are observed in many cells. These membranes are quite different from ordered arrays of endoplasmic reticulum or Golgi material. The membranes have no counterparts in the fungi nor in the eubacteria, save for those shown in the work on *Rhodospirillum rubrum*.

Cellular division occurs by both a eubacterial type of centripetal deposition of cell wall material and by a fungal type of branching. The branches may subsequently develop transverse cell walls.

A cytoplasmic membrane has been seen in only a few of these preparations, but sometimes appears double.

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