# THE FINE STRUCTURE OF *Streptomyces coelicolor*

## II. The Nuclear Material

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ABSTRACT

Colonies and spore suspensions of *Streptomyces coelicolor* were fixed for electron microscopy by the method of Kellenberger, Ryter, and Séchaud (1958). In thin sections the nuclear regions have a lower average density than the cytoplasm and the outlines of these regions correspond well with the profiles of the chromatinic bodies observed with the light microscope. The nuclear regions contain fibrils, about 5 mμ in diameter. In contrast, after fixation by the method of Palade (1952) the nuclear material is coagulated into irregular dense masses and tubular structures about 20 mμ in diameter, lying in a nuclear "vacuole." The significance of these observations is discussed in relation to the observations of other workers on the fine structure of the nuclear material of other bacteria and the chromosomes of higher cells.

### INTRODUCTION

Observations on stained preparations of *Streptomyces coelicolor* with the light microscope (Hopwood and Glauert, 1960) indicated that there is a characteristic sequence of changes in the configurations of the chromatinic material during the development of the spores. Elongated chromatinic structures in the young aerial hyphae separate into a number of subunits and a single round chromatinic body is included in each spore. It was therefore of interest to study the nuclear material in thin sections in the electron microscope to see if there were any corresponding alterations in fine structure.

Kellenberger and his coworkers (Kellenberger, Ryter, and Séchaud, 1958; Ryter and Kellenberger, 1958) have developed a method of fixation for electron microscopy specifically designed to preserve the nuclear structure of eubacteria. In this study we have applied this method to *Streptomyces coelicolor* and have compared the results with those obtained with the conventional Palade technique (Palade, 1952).

### MATERIALS AND METHODS

The methods of growth of the organism and the preparation of thin sections for examination in the electron microscope have been described in detail in the first paper of this series (Glauert and Hopwood, 1960).

The methods of staining the organism and taking the photomicrographs are described in the preceding paper (Hopwood and Glauert, 1960).

### RESULTS

The general organisation of the hyphae of *Streptomyces coelicolor* as seen in thin sections of material

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fixed by the method of Kellenberger, Ryter, and Séchaud (1958) has already been described (Glauert and Hopwood, 1960). The nuclear material occupies the central regions of the hyphae; it has a somewhat lower average density than the granular and membranous material of the cytoplasm (Fig. 1), so that the boundaries of the nuclear regions are seen very clearly in photographs at magnifications of 70,000 or less (Figs. 1, 9, 10). These profiles correspond well with those of the chromatinic bodies in stained preparations viewed in the light microscope. Thus, the outline of the nuclear region in a thin section of a major hypha of the substrate mycelium (Fig. 1) closely resembles that of the irregularly lobed chromatinic bodies in stained preparations (Fig. 2). Compare also the nuclear region of Fig. 5, which occupies a large proportion of the area of a section of an aerial hypha, with the rod-shaped chromatinic bodies of a young aerial hypha in Fig. 6, and the nuclear regions of nearly mature spores in Fig. 7 with the eccentric chromatinic bodies of spores in Fig. 8.

Although the configurations of the nuclear regions vary characteristically in the different parts of the mycelium and in the spores, the fine structure of the nuclear material is very similar at all stages of development. The commonest images in the nuclear regions are very dense circular profiles, approximately 5 mμ in diameter, and less dense, elongated, irregularly curved profiles, approximately 5 mμ wide and of varying length and density (Figs. 9*a*, 10*a*). Material of low density occupies the space between the dense

## EXPLANATION OF FIGURES

FIGURES 1 to 15, except FIGURES 2, 6, 8, and 11. Electron micrographs of thin sections of *Streptomyces coelicolor* strain A3(2), fixed by the method of Kellenberger, Ryter, and Séchaud (1958), except where otherwise stated, and embedded in methacrylate. The scale mark represents 0.1 micron.

FIGURES 2, 6, 8, and 11. Light microscope photographs of organisms fixed in the vapour of 2 per cent osmic acid and stained for chromatin. (See Hopwood and Glauert, 1960). × 3,000.

FIGURE 1

A longitudinal section of a major hypha of the substrate mycelium from a 48-hour colony on minimal agar medium. The nuclear region (*N*) has a lower average density than the cytoplasm and has the outline of an irregularly lobed rod. Compare this outline with the profiles of the elongated chromatinic bodies in Fig. 2. The base of a side branch is visible at the left hand side, and the nuclear material extends in the direction of the branch. The cytoplasm of the hypha is mainly granular, but pockets of membranous material (*P*) are present at the periphery, and a more complex membranous structure (*CM*) is associated with a cross-wall at the top of the picture. × 68,000.

FIGURE 2

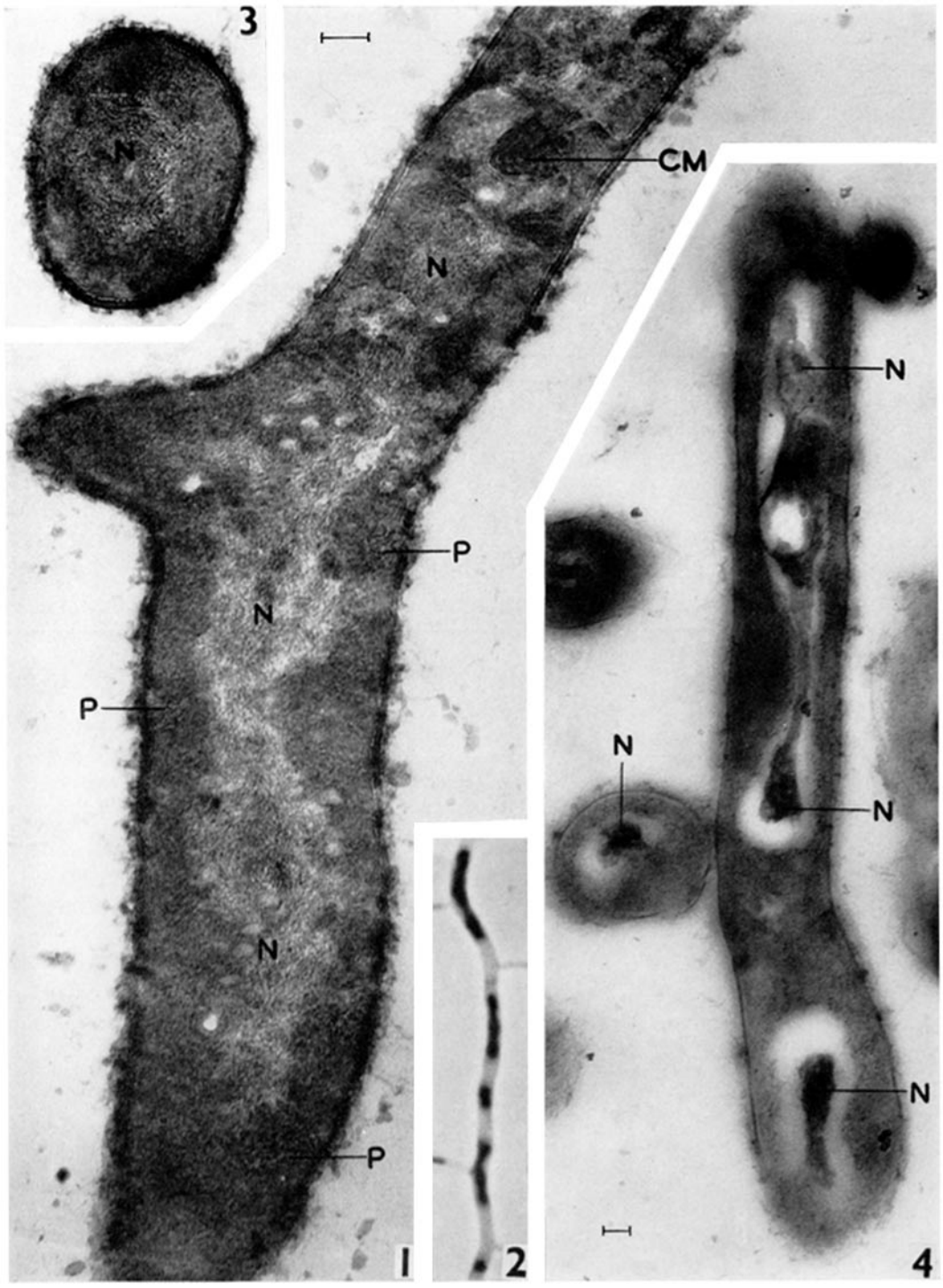
A major hypha of the substrate mycelium containing irregularly lobed rods of chromatin. Stained with thionin-$SO_2$. × 3,000.

FIGURE 3

A transverse section of a similar hypha. The nuclear material (*N*) occupies the central region of the hypha. × 68,000.

FIGURE 4

A longitudinal section and a transverse section of hyphae from a 4-day colony on minimal agar medium. Fixation by the method of Palade (1952). The dense material within the nuclear region (*N*) is not uniformly distributed as in Figs. 1 and 3, but has coagulated into a dense central structure, leaving a nuclear "vacuole." The outline of this vacuole corresponds with the profiles of the lobed rods of chromatinic material in Fig. 2. × 34, 000.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N
CM
N
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structures. These observations suggest that the nucleoplasm contains fibrils of about 5 mμ diameter, which run in different directions within a matrix of low density. Sectioning of the fibrils in different planes gives rise to profiles of varying length and density. The amount of dense material in different nuclear regions varies more widely than would be expected from variations in the thickness of the sections. In particular, the nuclear regions of the spores have a low electron density compared with those of some hyphae (compare Figs. 9 and 10). In many nuclear regions, the individual fibrils appear to have a random orientation with respect to one another and to the axes of the hypha (Figs. 1, 3, 5, 9), but in other regions they have an ordered arrangement. Thus, in a section of the elongated nuclear region of a young aerial hypha (Fig. 12), places in the nucleoplasm in which most of the fibrils are cut transversely (*T*) are separated by areas in which most of them run longitudinally (*L*).

Other structures are sometimes observed among the fine nuclear fibrils; small electron-opaque bodies are found (Fig. 13, *D*), and vacuoles, occurring singly or in groups, are seen in mature and nearly mature spores (Fig. 15, *V*). Elements of the intracytoplasmic membrane system are often closely associated with or embedded in the nuclear regions of hyphae (Fig. 13, *CM*; and Glauert and Hopwood, 1959), and an apparently discrete membraneous body is very often embedded in the nuclear region of developing spores (Figs. 10, 15, *CM*; and Glauert and Hopwood, 1960); this may explain the appearance of ring-shaped chromatinic bodies in the light microscope (Fig. 11), since a spherical nuclear region with a core of Feulgen-negative material would appear as an annulus.

The fine structure of organisms fixed by Palade's method (1952) differs greatly from that of organisms fixed by the method of Kellenberger, Ryter, and Séchaud (1958); in the former the cytoplasm is coarsely granular and shows only traces of poorly preserved elements of the intracytoplasmic membrane system (Glauert and Hopwood, 1960) and the nuclear regions of many hyphae appear as "vacuoles" containing irregular masses of dense material (Fig. 4). The

FIGURE 5

Part of a young aerial hypha from a 48-hour colony on minimal agar medium. The nuclear region (N) occupies a large proportion of the cross-section of the hypha, and is uniformly filled with fine fibrillar material. Compare the outline of the nuclear region with the profile of a rod-shaped chromatinic body of a young aerial hypha in Fig. 6. × 100,000.

FIGURE 6

Part of a young aerial hypha. The basal segment (uppermost in the figure) contains a dense rod of chromatinic material. Stained with thionin-$SO_2$. × 3,000.

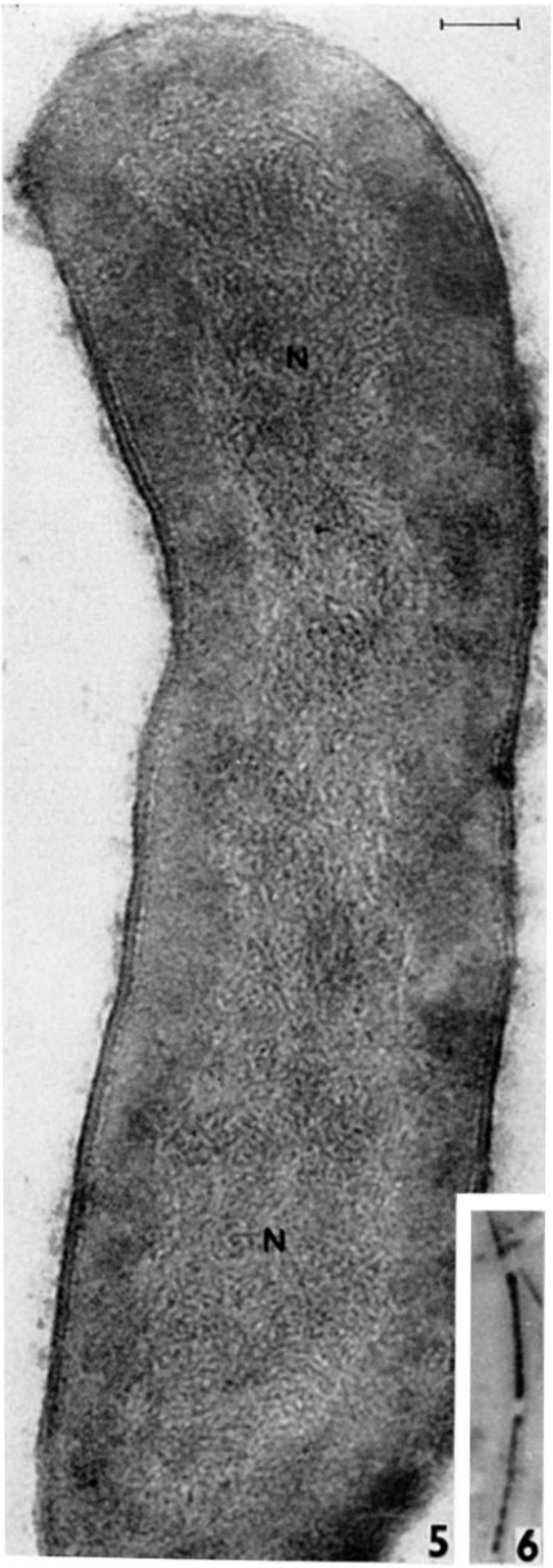
FIGURE 7

A chain of spores which are nearly mature, from a 6-day colony on minimal agar medium. Each spore has a single nuclear region (*N*). In the central spore the nuclear region is eccentric and can be compared with the eccentric chromatinic bodies in a chain of spores in Fig. 8. A membranous body is present at *CM*. Small vacuoles (*V*) of unknown nature are present in the nuclear regions. × 100,000.

FIGURE 8

A chain of spores which are nearly mature. In several of the spores the chromatinic body is eccentric. Stained with azure A-$SO_2$. × 3,000.

A comparison of Figs. 5 and 7 indicates the different distribution of the nuclear material in the aerial hyphae, before and after spore delimitation. An intermediate stage may be represented by the hypha in Fig. 12; the corresponding appearance in the light microscope is probably the beaded chromatinic rod in the lower segment of the hypha in Fig. 6.

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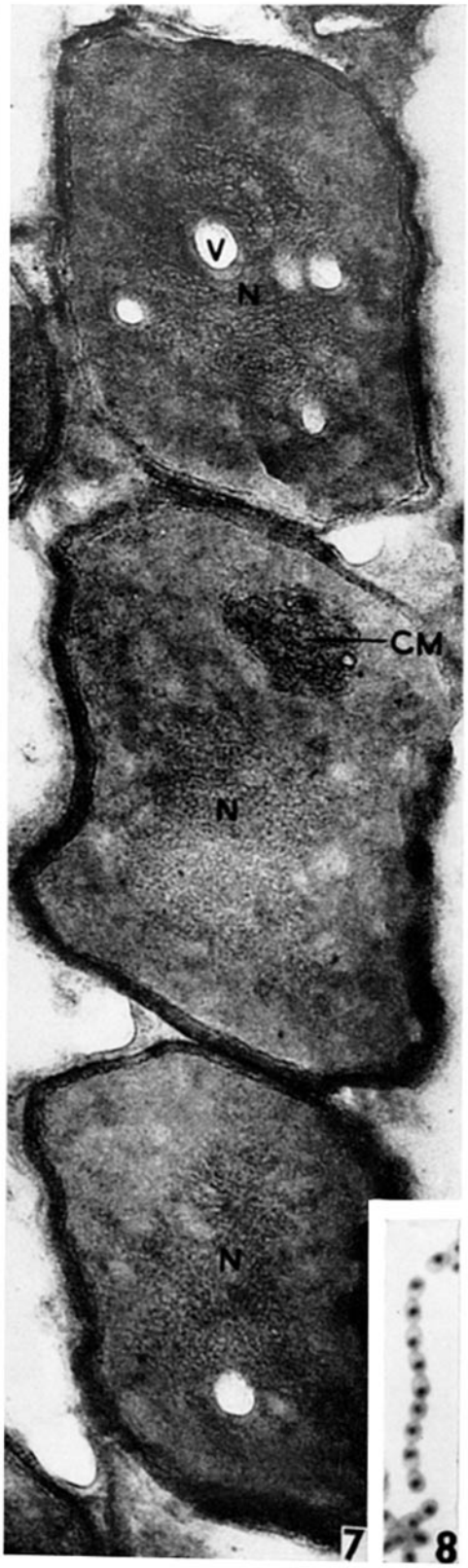


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nuclear regions of spores sometimes contain more regular configurations of dense material, which may appear as a series of tubules approximately 20 mμ in diameter (Fig. 14). The outlines of the vacuoles, rather than those of the dense masses, correspond to the profiles of the chromatinic bodies in stained preparations.

## DISCUSSION

Ryter and Kellenberger (1958) have shown that the fine structure of the nuclear material of eubacteria varies widely with the method of fixation, and have given reasons for the view that the appearance produced by their "standard" fixation is the nearest approximation so far achieved to the true structure of the nuclear material. This method of fixation applied to *Streptomyces coelicolor* results in a finely fibrillar nucleoplasm similar to that of eubacteria. In our preparations the nuclear regions contain a greater concentration of dense material than in most of the pictures of Ryter and Kellenberger (1958) and of Kellenberger, Ryter, and Séchaud (1958) and also the material is more uniformly distributed within the nuclear regions. Whether this reflects a real difference between organisms or is due to a difference in technique cannot be decided at present. The fibrils in the nuclear regions of *S. coelicolor* usually have a random arrangement. Occasionally, they appear to have a parallel arrangement (Fig. 12), which may reflect a movement and segregation of the fibrils into certain regions during the subdivision of the nucleoid. In addition to fibrils of approximately 5 mμ diameter, granules of about the same diameter may well be present in the nuclear regions; indeed, the excess of circular over elongated profiles suggests that they are. Moreover, the boundary between the cytoplasm and the nuclear regions, well defined as it seems to be at low magnification (Figs. 9, 10), appears very diffuse at higher magnification (Figs. 9*a*, 10*a*). This possibly reflects an interchange of granular material between the nuclear regions and the cytoplasm, which may well occur during the synthesis of the cytoplasmic RNA and proteins. It is possible that the difference in density between the nuclear regions of hyphae and of spores is due to a greater amount of non-genetic material in the former than in the latter, which

FIGURE 9

Part of an aerial hypha from a spore suspension. The nuclear region occupies a large proportion of the cross-section of the hypha. × 70,000.

FIGURE 9*a*

An enlargement of part of Fig. 9. The nuclear material of the hypha appears to consist of fine fibrils and granules, about 5 mμ in diameter, and there is no clear boundary between the nucleoplasm and the cytoplasm. × 140,000.

FIGURE 10

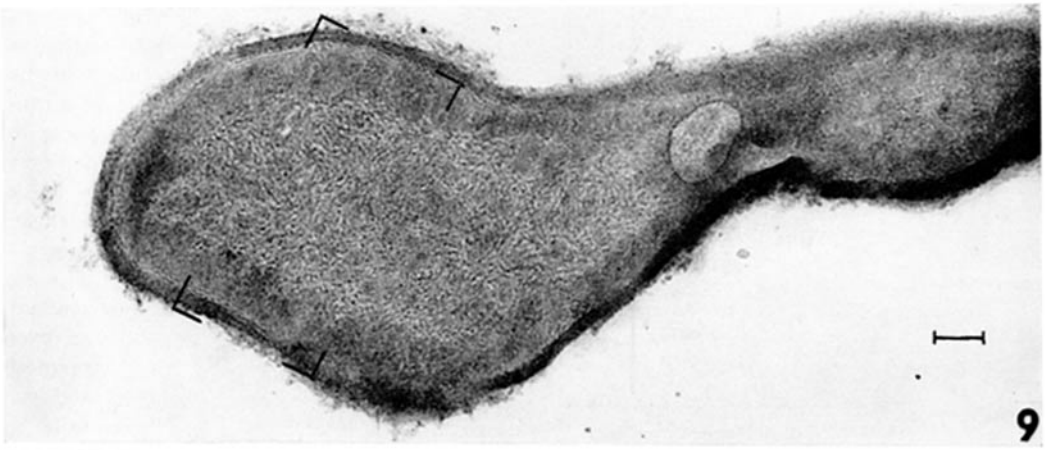
A developing spore from a spore suspension. A body composed of interconnected membranous elements (*CM*) is embedded in the nuclear region and is apparently continuous with the main part of the cytoplasm. Such elements may be responsible for the Feulgen-negative core to the chromatinic bodies observed in the light microscope (see Fig. 11, arrow). × 70,000.

FIGURE 10*a*

An enlargement of part of Fig. 10. The nuclear material appears to consist of fine fibrils and granules, about 5 mμ in diameter. The nuclear region of the spore has a lower average density than the nuclear region of the hypha in Fig. 9. Again there is no clear boundary between the nucleoplasm and the cytoplasm. × 300,000.

FIGURE 11

Spores stained by the Feulgen method. One spore contains a ring-shaped chromatinic body (arrow). × 3,000.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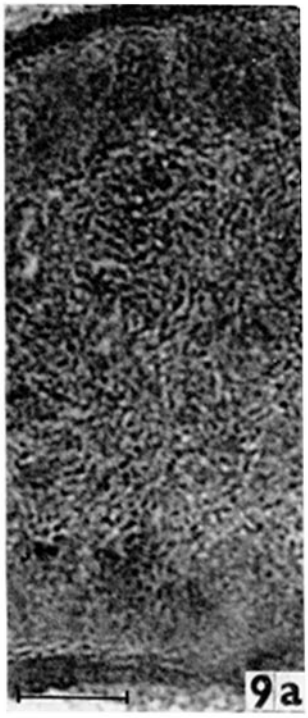
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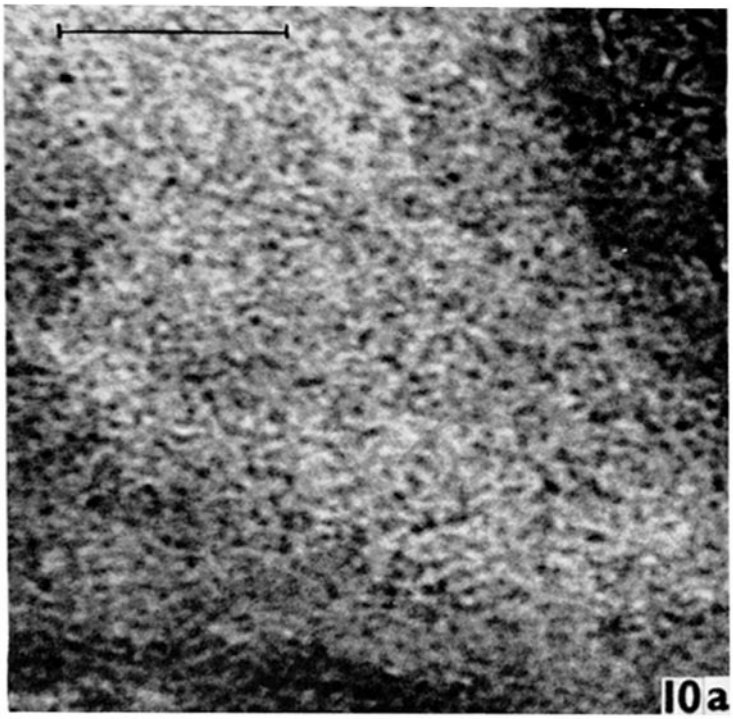
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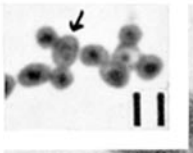
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are in a "resting" condition. If this were so, it might account for the difference in staining reaction to Piéchaud's method shown by the hyphae and spores (Hopwood and Glauert, 1960).

After fixation by the method of Kellenberger, Ryter, and Séchaud (1958), there is no evidence of organised structures larger than the individual fibrils within the nuclear regions. In contrast, after fixation by the method of Palade (1952), which was developed for animal tissues, various thread-like and tubular structures are seen suspended in a nuclear "vacuole" (Figs. 4, 14; Hopwood and Glauert, 1958; Stuart, 1959), as in many of the earlier micrographs of eubacteria (Birch-Andersen, Maaløe, and Sjöstrand, 1953; Maaløe and Birch-Andersen, 1956; Chapman and Kroll, 1957). Such structures in *Bacillus megaterium* have been interpreted as chromosomes by Giesbrecht (1958, 1959). Since it is probable that dense structures within a nuclear "vacuole" are produced by the coagulation of a finely fibrillar nucleoplasm during imperfect fixation (Ryter and Kellenberger, 1958), the configurations assumed by this material may not mean very much, and attempts to deduce the structure of bacterial nuclei from organisms fixed in this way may be misleading. Chapman (1959) suggested that fibrils and coarser threads may be alternative forms of the chromatin, but whether it can exist in more than one form during the life of the organism remains to be seen. In view of the fact that tubular structures 12.5 mμ (Kaufmann and De, 1956; Kaufmann and McDonald, 1956) or 20 mμ (Ris, 1956) in diameter have been described as the smallest unit of the chromosomes of the cells of higher organisms fixed by methods resembling that of Palade (1952), it is significant that this method reveals tubules approximately 20 mμ in diameter in the nuclear regions of the spores of *S. coelicolor* (Fig. 14). If such tubules are fixation artefacts in this organism, the same may be true for higher cells, although, as pointed out by Kellenberger, Ryter, and Séchaud (1958), the possibility remains that differences in chemical composition between the DNA complexes of bacteria and higher cells (Zubay and Watson, 1959; Wilkins and Zubay, 1959) may be reflected in a different structural organisation. It is interesting, however, that fibrils of diameter 3 to 8 mμ have been described in the chromosomes of the grasshopper *Laplatacris dispar* (De Robertis, 1956) and of the dinoflagellate *Amphidinium elegans* (Grell and Wohlfarth-Bottermann, 1957) when fixed by methods having some features in common with that of Kellenberger and his coworkers. Also in more recent work, Ris (1958) has described 4 mμ fibrils of nucleohistone in calf-thymus chromosomes, but considers them to be associated in pairs with non-histone protein to form larger fibrils 10 mμ in diameter; in sperm nuclei the non-histone protein is absent and the 4 mμ fibrils fill the whole nucleus.

The nature of the small dense granules in the nuclear regions of some hyphae (Fig. 13) is unknown. Possibly they are analagous with the nucleoli of higher cells and owe their electron density to an accumulation of RNA, or they may be metaphosphate granules comparable to those of mycobacteria (Glauert and Brieger, 1955). Similar granules are present in the nuclear region of an unidentified bacterium studied by Chapman (1959). The vacuoles in the nuclear regions of some spores (Fig. 15) may be artefacts caused by imperfect fixation or embedding of the spores which are more resistant to penetration than the hyphae, or alternatively they may indicate the sites of material that has been extracted.

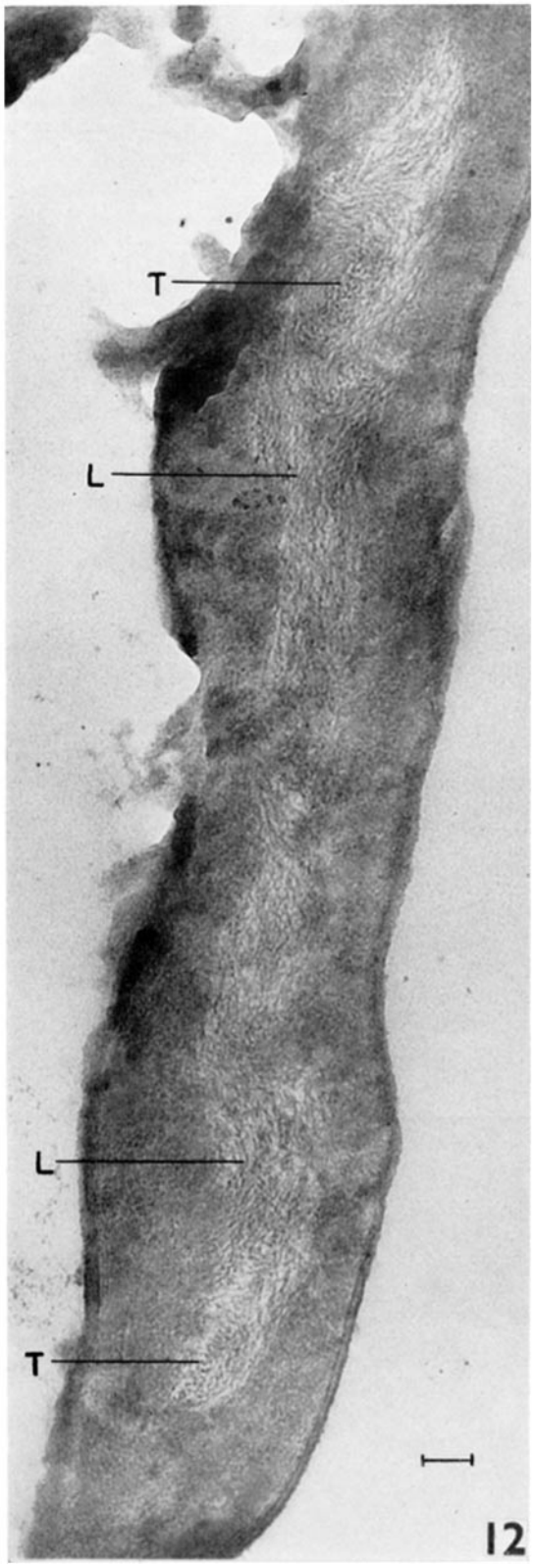
An apparent association between the nuclear material and elements of the intracytoplasmic membrane system (Glauert and Hopwood, 1960) seems to be characteristic of the spores of *Strepto-*

FIGURE 12

Part of an aerial hypha from a spore suspension. In places the fine fibrils of the nuclear region are orientated in a direction parallel to the long axis of the hypha (*L*). Elsewhere the fibrils are cut transversely (*T*). × 69,000.

FIGURE 13

Part of a hypha from the substrate mycelium of a 48-hour colony on minimal agar medium. Dense granules (*D*) are present within the nuclear region (*N*). Elements of the intracytoplasmic membrane system are visible at *CM*. × 69,000.

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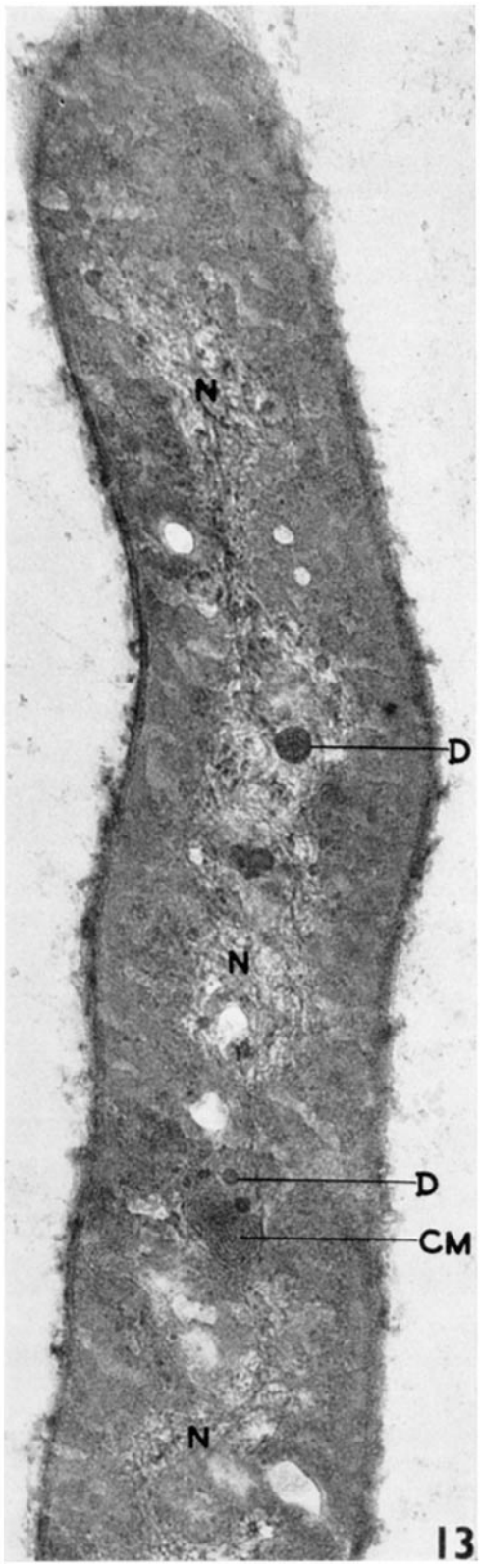


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*myces coelicolor*, many of which contain a membranous body (Figs. 10, 15). A connection between this body and the main part of the cytoplasm is sometimes seen (Fig. 10), so that, apart from showing a highly organised membranous structure, these bodies are comparable with the cytoplasmic extensions into the nuclear region that have been observed in many bacteria. These extensions may be responsible for the appearance of an achromatic core in some chromatinic bodies (Fig. 11; Murray, 1953). The significance of the association of the membranous bodies with the nuclear material will not be understood until more is known about the function of the intracytoplasmic membrane system as a whole.

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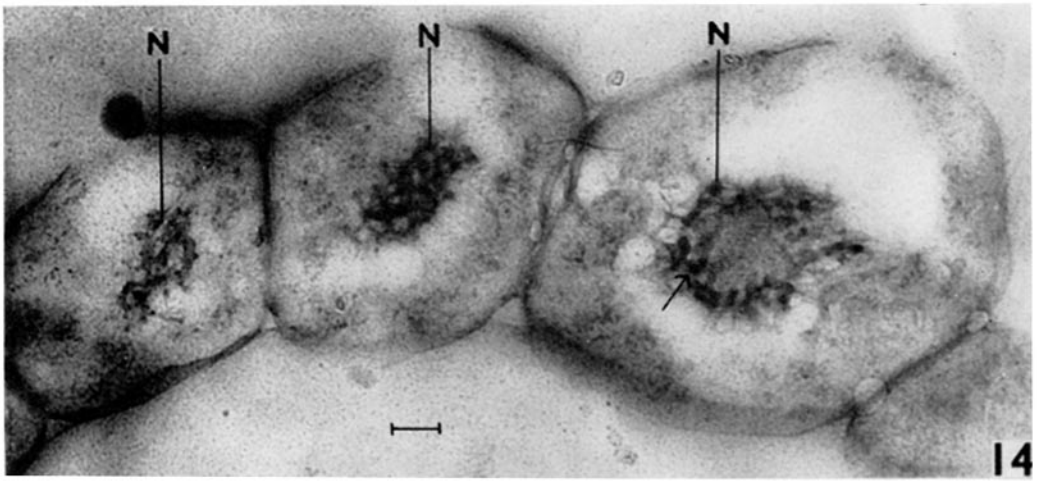
FIGURE 14

A chain of spores which are nearly mature, from a 4-day colony on minimal agar medium. Fixation by the method of Palade (1952). The nuclear region (*N*) contains a number of tubules about 20 mμ in diameter (arrow), within a nuclear "vacuole." × 60,000.

FIGURE 15

A similar chain of spores, from a spore suspension. After fixation by the method of Kellenberger, Ryter, and Séchaud (1958), the nuclear region (*N*) contains fine fibrils, about 5 mμ in diameter. A number of vacuoles (*V*) of unknown nature, are present in the nuclear regions. Condensed elements of the intracytoplasmic membrane system are present at *CM*. × 100,000.

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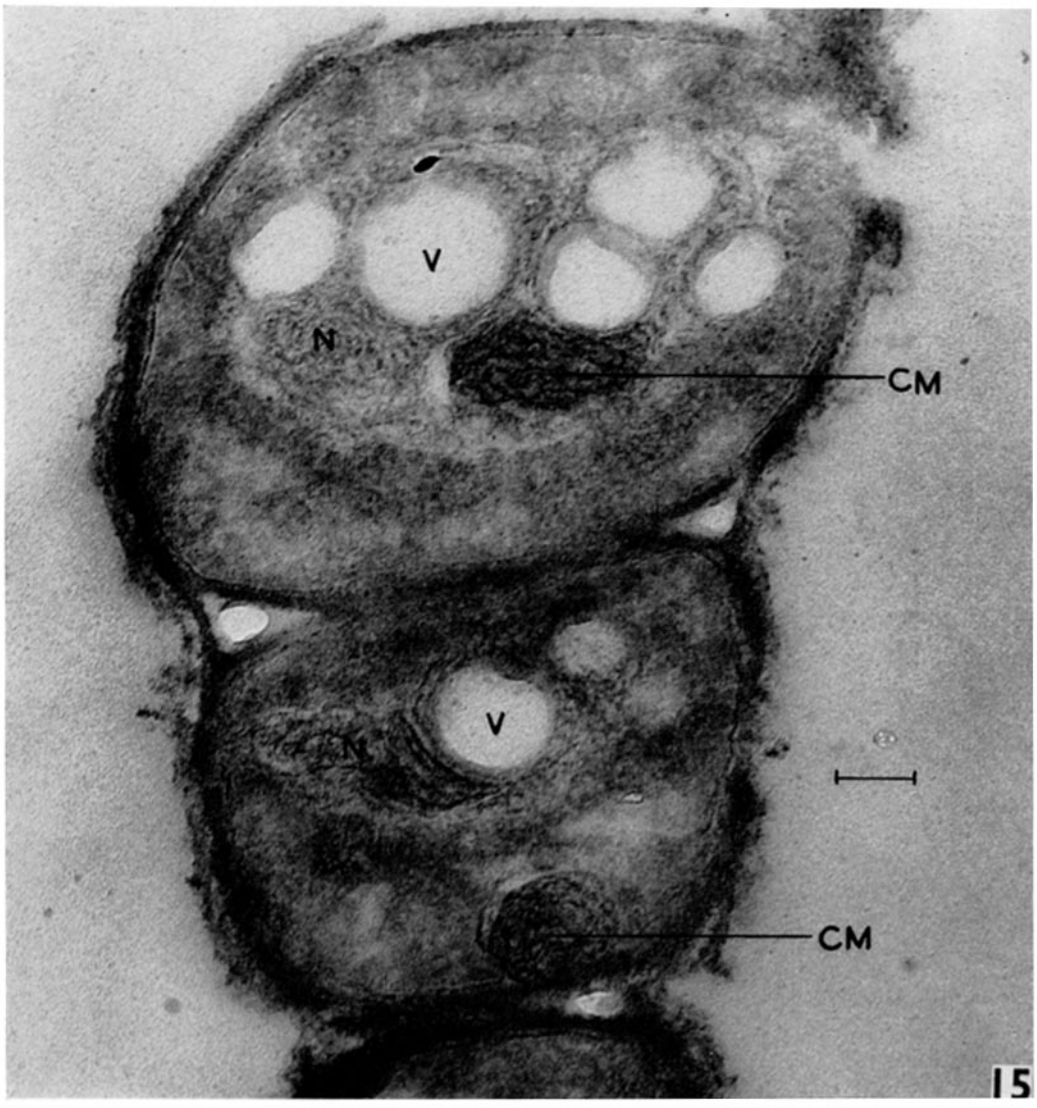


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