OBSERVATIONS ON THE CHROMATINIC BODIES OF *Streptomyces coelicolor*

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ABSTRACT

Colonies of *Streptomyces coelicolor* growing on cellophane and impression preparations from sporing colonies were stained for chromatin by the methods of Feulgen, DeLamater (1951), and Piéchaud (1954). The chromatinic bodies of the substrate hyphae have a great variety of configurations. 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.

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INTRODUCTION

During a study of genetic recombination in a strain of *Streptomyces coelicolor* (Hopwood, 1959) we investigated the nuclear material of the organism, with the light and electron microscopes, in the hope that genetical and cytological data may eventually be integrated to give a more complete picture of the structure and behaviour of the hereditary material. This paper describes observations made with the light microscope on the chromatinic material of *S. coelicolor*. Three methods of staining were used: the Feulgen method, which is specific for DNA; DeLamater's method, a modification of the Feulgen method which results in preparations whose details are more easily recorded photographically; and Piéchaud's method, which provides an interesting comparison with the other two techniques since it avoids the necessity for preliminary hydrolysis with acid. The different appearances of the chromatinic bodies in different parts of the colony were related to one another by reference to the cycle of development of the organism as seen in the phase-contrast microscope (Hopwood, 1960). This investigation of the chromatinic bodies provides a background for the interpretation of the appearance of the nuclear material in electron micrographs of thin sections (Hopwood and Glauert, 1960), and also helps to resolve some of the confusion in the literature over certain aspects of the behaviour of the chromatinic bodies of streptomycetes (Badian, 1936; Klieneberger-Nobel, 1947; Saito and Ikeda, 1958).

MATERIALS AND METHODS

The chromatinic bodies of the substrate mycelium and young aerial hyphae were studied in young colonies growing on cellophane. Squares of cellophane were laid on the surface of "minimal" agar medium (Hopwood, 1960), or on minimal medium with glucose omitted, and inoculated with a spore suspension. The cellophane squares were removed from the medium after various periods of incubation at 30°C., and the whole colonies were fixed, stained, and examined with little disturbance of the arrangement of the hyphae. Strain A3(2) of *Streptomyces coelicolor* can utilise agar as a carbon source and grows sparsely on minimal medium with glucose omitted. The interrelations of the hyphae in the colony can thus be seen more easily than on a medium

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containing glucose, on which growth is more luxuriant (Erikson, 1955; Hopwood, 1960). Stages in the delimitation of spores in the aerial mycelium were examined in impression preparations made by placing coverslips in contact with sporing colonies. These preparations consisted largely of isolated spores and chains of spores in different stages of development, with a few aerial hyphae.

The organisms were fixed in the vapour of 2 per cent osmic acid for 4 minutes and then immersed in distilled water. The nuclear material was stained by the Feulgen, DeLamater (1951), or Piéchaud (1954) methods. For the Feulgen and DeLamater methods, the preparations were treated with N-HCl at 60°C. for 8 minutes and then rinsed in distilled water. *Feulgen:* the organisms were stained for 3 to 16 hours in leuco-basic fuchsin, rinsed in two changes of sulphurous acid solution, and mounted in acetocarmine (McIntosh, 1954; Robinow, 1956). *DeLamater:* the organisms were stained for 3 to 10 hours in azure A-SO_2 or thionin-SO_2 (prepared by the addition of thionyl chloride to 0.25 per cent aqueous solutions of the dyes) and then rinsed and mounted in distilled water. *Piéchaud:* the stain was prepared by adding 10 to 16 drops of 0.05 per cent eosin Y to 10 ml. of tap water, pH 7.2, and mixing this solution with 20 drops of G. T. Gurr's "R66" Giemsa stain just before use. A higher proportion of eosin was required to differentiate the young aerial hyphae and immature spores than the mature spores. The fixed material was rinsed in tap water, immersed for 10 minutes in the stain, and then mounted in tap water.

Observations were made with a Zeiss microscope, with a ribbon-filament lamp, Köhler illumination, and × 90 1.4 N.A. apochromatic objective. Photographs were taken on Ilford microneg 35 mm. film at a magnification of about 300, with a Wratten No. 59 green filter and a × 10 periplanatic eyepiece.

RESULTS

1. The Substrate Mycelium

The chromatinic bodies of the substrate mycelium appear essentially the same whether stained by the Feulgen or DeLamater methods; well differentiated preparations were not obtained by Piéchaud's method. The chromatinic bodies of the substrate hyphae have a great variety of configurations (Figs. 1, 2). In the radially growing major hyphae of the colony, the bodies appear to be nearly as wide as the hyphae, and differ considerably in length. The smallest units have

EXPLANATION OF FIGURES

FIGURES 1 to 19. Photomicrographs of *Streptomyces coelicolor* strain A3(2), fixed in the vapour of 2 per cent osmic acid, except where otherwise stated. Magnification, 3,000. The scale mark represents 10 microns.

Parts of colonies grown on cellophane on minimal agar medium, with glucose omitted, for 44 hours. Stained with thionin-SO_2 (DeLamater, 1951).

FIGURES 1 and 2

Major hyphae of the substrate mycelium with narrow side branches. The chromatinic bodies of the major hyphae have a variety of configurations: small circular profiles (*A*), annuli (*B*), multiple structures, apparently composed of two or three subunits (*C*), and complex lobed rods (*E*). Septa are visible at *S*. The chromatinic bodies of the narrower branch hyphae are elongated (*F*), and some are continuous with chromatinic bodies in the major hyphae (*X*).

FIGURE 3

A young aerial hypha. A basal segment contains a dense rod of chromatinic material (*X*). In more distal segments, the elongated chromatinic bodies are narrower and beaded (*Y*). Substrate hyphae are visible in a lower focal plane (*H*).

FIGURE 4

Two aerial hyphae in which spores are forming. The basal segment of the lower hypha contains a dense rod of chromatinic material (*X*). In the distal segments, rows of small chromatinic bodies are present; some are dumb-bell-shaped and others have circular profiles. Septa are visible between adjacent chromatinic bodies.

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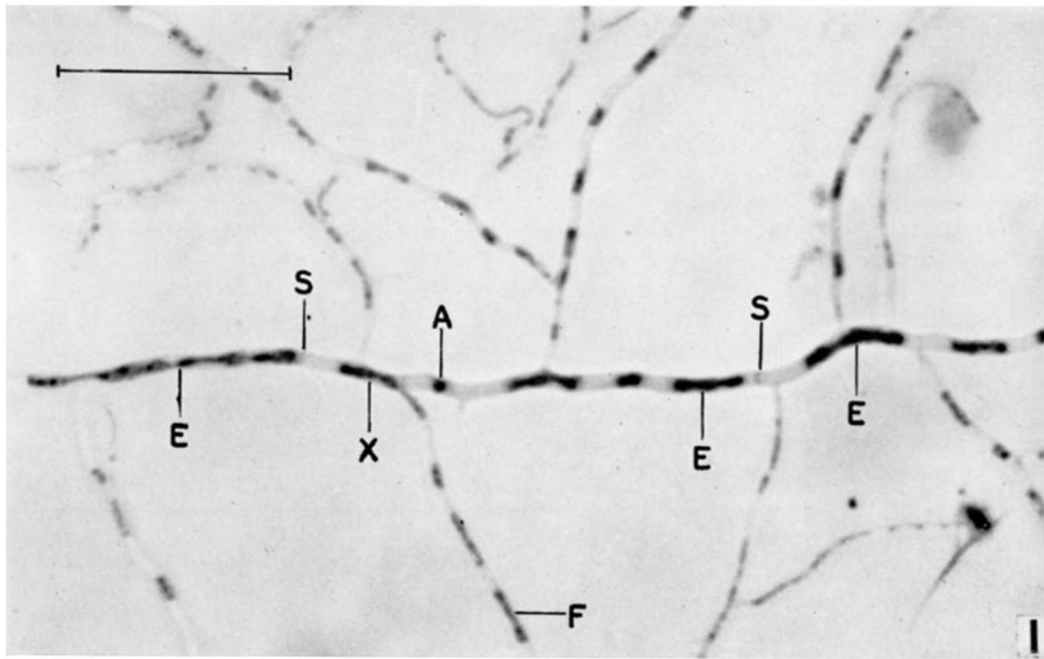

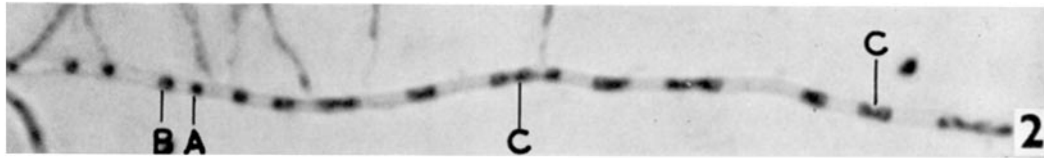

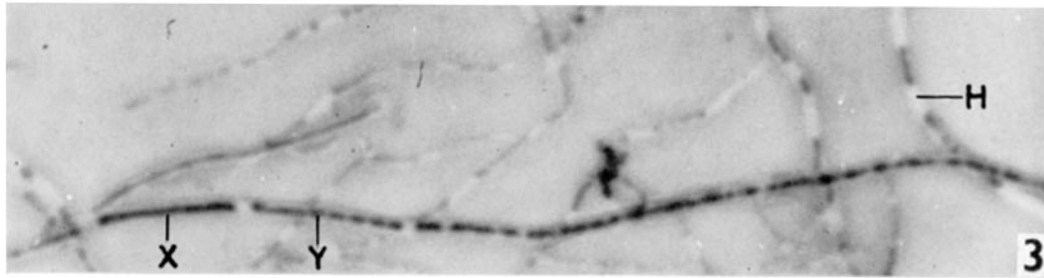

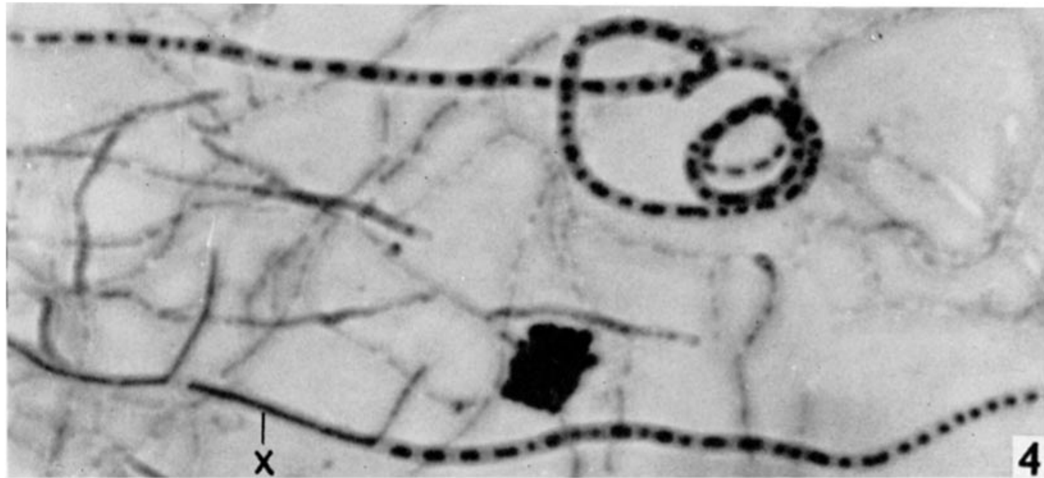

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circular profiles (Figs. 1, 2, *A*), and sometimes appear as an annulus with a light centre (Fig. 2, *B*). Larger structures are apparently composed of two or three such units joined by narrow bridges of chromatin (Fig. 2, *C*), or are long irregularly shaped bodies which are often made up of a number of lobes joined by narrow necks (Fig. 1, *E*). These bodies are not uniformly dense, so that probably they have a complex structure. The cross-walls cannot always be seen in preparations stained for chromatinic material, but it appears that a compartment of a hypha between two septa may contain a single long chromatinic body or several smaller ones. The chromatinic bodies of the narrower hyphae of the substrate mycelium also vary considerably in length, and most of them are elongated (Fig. 1, *F*). Sometimes a chromatinic body in a major hypha is continuous with a narrower body in a branch hypha (Fig. 1, *X*). Throughout the substrate mycelium, the chromatinic bodies are separated from one another by spaces apparently free from chromatin, which make up approximately half the total length of the hyphae.

2. *The Aerial Hyphae and the Spores*

The chromatinic material of the young aerial mycelium stained by DeLamater's method, occupies a much larger proportion of the length of the hyphae than in the substrate mycelium. In the

Impression preparations from sporing colonies, 48 to 84 hours old.

FIGURES 5 and 6

Stained with thionin-SO_2 (DeLamater, 1951).

FIGURE 5

Part of an aerial hypha. The elongated segments of the hypha contain a double chromatinic body and each segment will eventually form two spores. More mature spores are spherical and contain a single dot-like chromatinic body.

FIGURE 6

An elongated segment of an aerial hypha subdividing into two spores. Fixed in the fixative of Kellenberger, Ryter, and Séchaud (1958).

FIGURES 7 and 8

Stained with azure A-SO_2 (DeLamater, 1951).

FIGURE 7

Chains of spores which are nearly mature. In one chain many of the chromatinic bodies are eccentric.

FIGURE 8

The final stage in spore delimitation. Adjacent spores are separating from one another.

FIGURES 9 to 12

Stained by the Feulgen method.

FIGURE 9

Spores in different stages of maturation, and the tip of an aerial hypha containing a rod of chromatin.

FIGURE 10

An aerial hypha in which chromatinic rods are subdividing into smaller units.

FIGURE 11

Spores in different stages of maturation. Some contain ring-shaped chromatinic bodies (arrows).

FIGURE 12

Spores fixed in the fixative of Kellenberger, Ryter, and Séchaud (1958). The chromatin is stained as intensely as after a brief fixation in osmic acid vapour, and similar configurations of the chromatinic bodies are seen.

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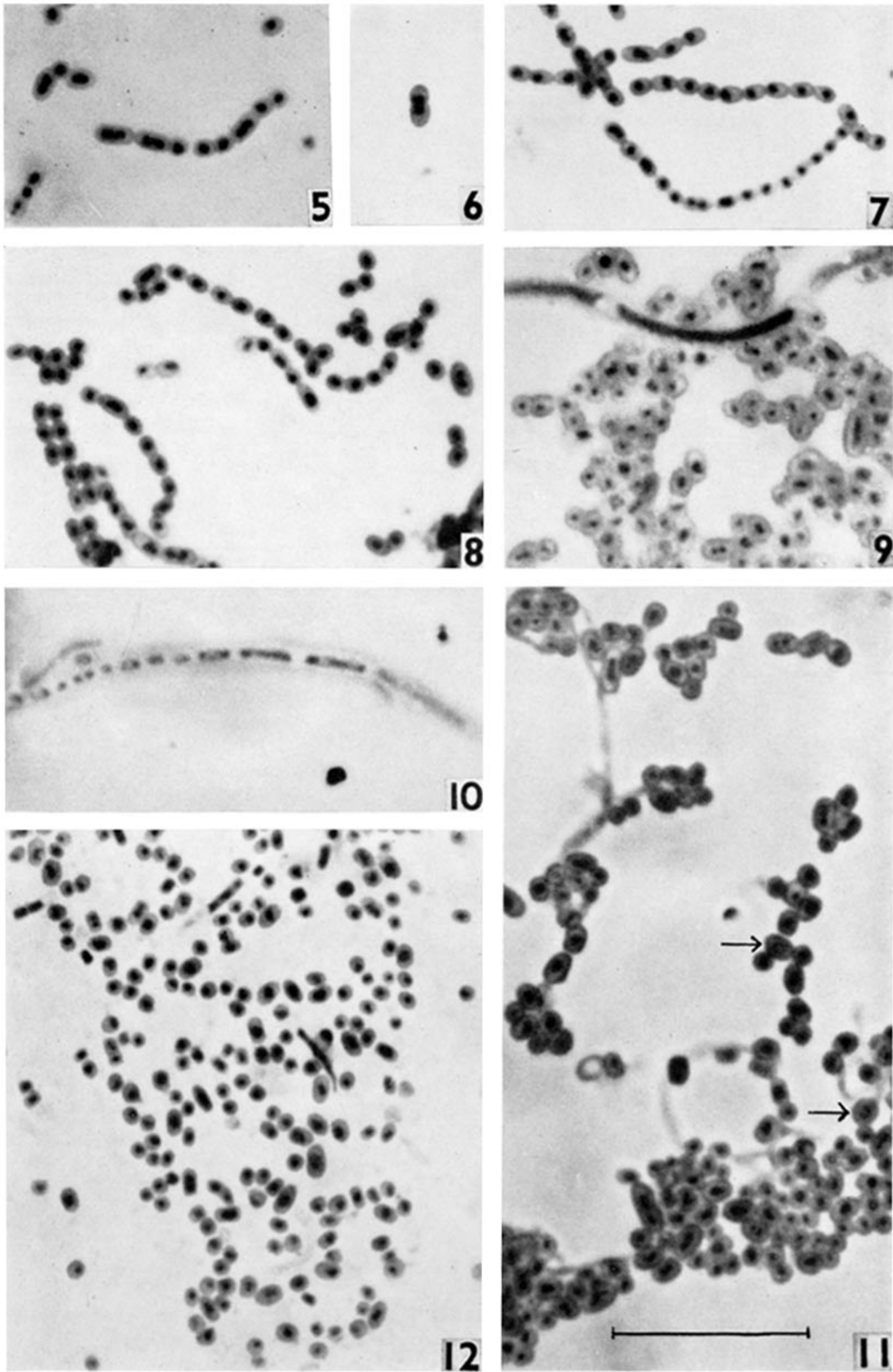

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youngest hyphae, nearly the whole of the space between two adjacent septa is filled by a dense rod of chromatinic material. The same appearance is observed in the basal segments of older hyphae, which are probably in an earlier developmental stage than the distal segments (Figs. 3, 4, *X*), where the elongated chromatinic bodies are narrower and beaded (Fig. 3, *Y*). In older hyphae, in which closely spaced septa are beginning to delimit chains of spores, rows of small chromatinic bodies are present (Fig. 4). A septum is usually visible between adjacent chromatinic bodies, which are of two sizes; the smaller bodies have circular profiles, while the larger are elongated and often appear as dumb-bell–shaped structures consisting of two small bodies joined by a narrow bridge (Fig. 4). In hyphae in which adjacent spores are beginning to separate from one another, many spores contain a single round chromatinic body. Some segments of the hyphae are approximately twice as long as these spores, and contain elongated, dumb-bell–shaped chromatinic bodies (Fig. 5). Such segments occasionally have a chromatinic body on either side of a median constriction (Fig. 6). In more mature spore chains, in which adjacent spores are joined by only a narrow neck, each spore contains a single small chromatinic body (Fig. 8), which is sometimes eccentric (Fig. 7).

In preparations stained by the Feulgen method (Figs. 9 to 12), the chromatinic bodies are less densely stained, but have similar configurations to those stained by DeLamater's method. Elongated "spores" with bipartite chromatinic bodies and isodiametric spores with single round chromatinic bodies are seen (Fig. 11), and spores with a ring-shaped chromatinic body are frequent (Fig. 11, arrows). The chromatin is stained as

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Impression preparations from sporing colonies, 48 hours old. Stained by Piéchaud's method (1954).

FIGURE 13

A young aerial hypha containing dense rods of chromatinic material (*X*).

FIGURE 14

A slightly older aerial hypha. The basal segment contains a dense rod of chromatin (*X*). In the more distal segments the chromatinic bodies consist of a series of subunits of varying length, joined by narrow bridges of chromatin (*Y*).

FIGURE 15

The upper hypha is in the same stage of development as the one in Fig. 14, and contains a similar complex chromatinic body (*Y*). In the lower hypha, the chromatinic material has separated into smaller units.

FIGURE 16

An aerial hypha in the same stage of development as the lower one in Fig. 15. Long and short chromatinic bodies are seen, and two short bodies are occasionally joined by a thread of chromatin (arrow).

FIGURE 17

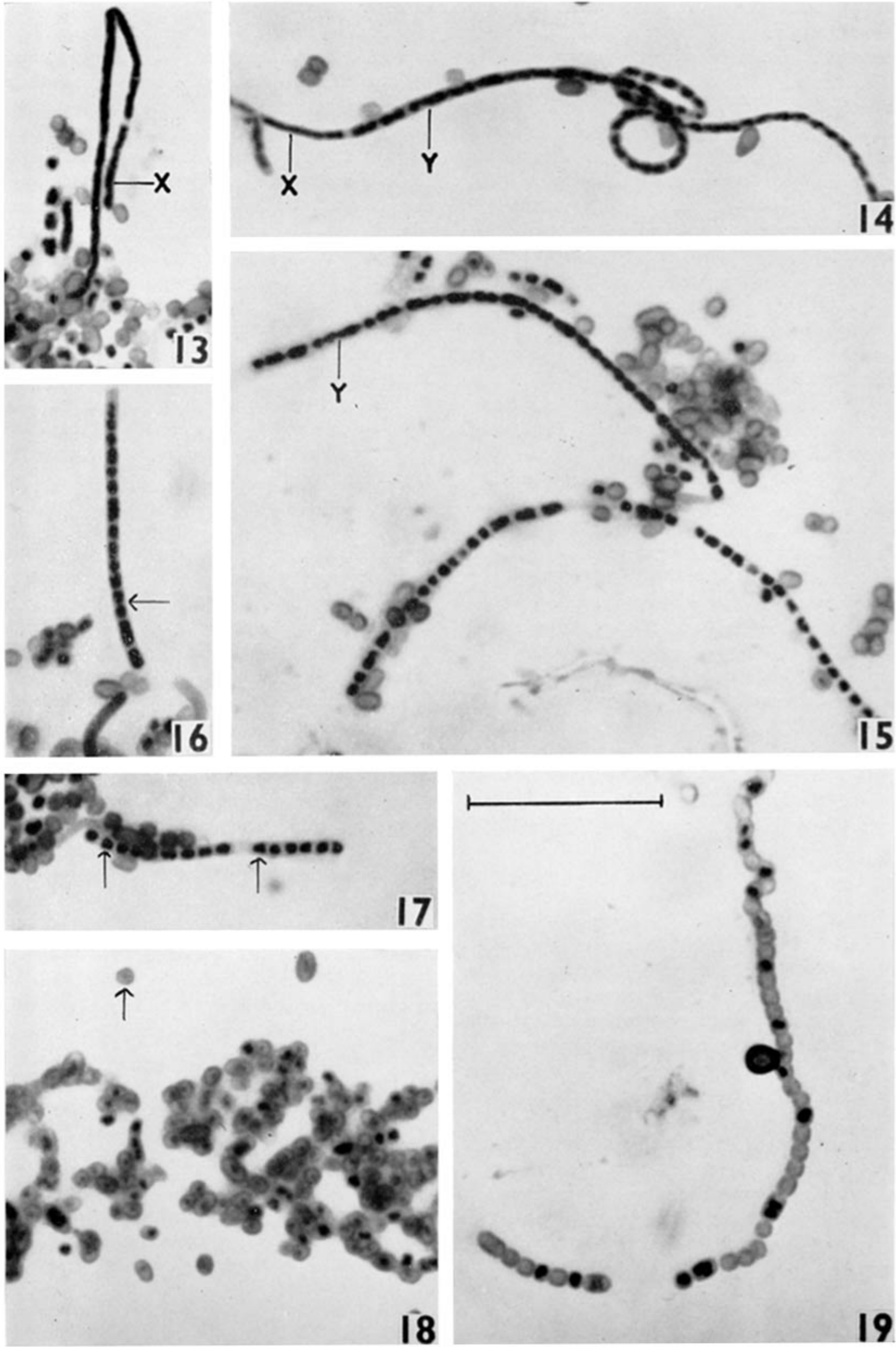
At a slightly later stage in the development of the hyphae the short chromatinic bodies predominate, and some of these are apparently made up of two or three subunits (arrows).

FIGURE 18

Spores in different stages of maturation. The mature spores have a single round chromatinic body (arrow).

FIGURE 19

A chain of spores stained with a proportion of eosin which is too high to allow staining of the chromatin of the mature spores. Only the immature segments of the hypha have stained, and two of these contain bipartite chromatinic bodies.



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intensely after fixation with the fixative of Kellenberger, Ryter, and Séchaud (1958) (Fig. 12) as after brief fixation in osmic acid vapour.

In preparations stained by Piéchaud's method, the chromatinic material has the same distribution in the hyphae as in preparations stained by the Feulgen and DeLamater methods, but the outlines of the chromatinic bodies are less diffuse (Figs. 13 to 19). The youngest aerial hyphae and the basal segments of older hyphae are largely filled by elongated chromatinic bodies, which show some variation in density along their length (Figs. 13, 14, *X*). In older hyphae, the long beaded chromatinic bodies appear to consist of a series of subunits of varying length, joined by narrow bridges of chromatin (Figs. 14, 15, *Y*). At a later stage, long and short chromatinic bodies occur in rows in the hyphae, two short bodies occasionally being joined by a thread of chromatin (Fig. 16, arrow). The short bodies, instead of appearing simply as dots, as in preparations stained by DeLamater's method (Fig. 4), often have more complex profiles, as if they were made up of two or three subunits (Fig. 17, arrows). Chains of nearly mature spores, in most of which the chromatin is barely discernible, contain scattered spores with well stained chromatin (Fig. 19). Some of these spores are round, with a single chromatinic body; others are elongated and their chromatinic body consists of two parts apparently joined by a narrow bridge. In preparations stained with a lower proportion of eosin, the chromatin of the mature spores is seen as a single small round body (Fig. 18, arrow).

DISCUSSION

Piéchaud (1954) devised a method of staining bacterial chromatin without preliminary hydrolysis with acid. In the present study, the distribution of chromatin in the long axis of the aerial hyphae of *Streptomyces coelicolor* is the same when stained by the Feulgen, DeLamater, or Piéchaud methods, but hydrolysis seems to alter slightly the outlines of the chromatinic bodies since they appear sharper when stained by Piéchaud's method. The chromatinic bodies of the mature spores seem to be modified least by the staining procedure and appear as dots when stained by all methods. Possibly this is a reflection of a more condensed state of the chromatin in the mature spore. That the configurations of the chromatin, even after treatment with acid, are a good indication of the distribution of the nuclear material in the living organism is suggested by the correspondence between these configurations and the outlines of the nuclear regions in electron micrographs of thin sections (Hopwood and Glauert, 1960) of material fixed by the method of Kellenberger, Ryter, and Séchaud (1958). These regions are often surrounded by a cytoplasm which seems to be well preserved, since it contains complex and apparently organised membranous systems (Glauert and Hopwood, 1960), so that the form of the nucleocytoplasmic boundaries have probably been little distorted.

A striking feature of the chromatinic bodies of *Streptomyces coelicolor* is their variability in size and shape. The large chromatinic bodies of the substrate mycelium often appear to be multiples of small round or annular units and are probably stages in the production of new chromatinic bodies which supply developing branch hyphae. The rod-like bodies in the aerial hyphae appear to subdivide in a series of stages, giving rise to units of varying length and complexity, and finally small spherical bodies are formed, one of which is included in each spore. It is probable that each rod-like body contains a number of genomes and that these separate from one another to form about ten spore nuclei, with no increase in the amount of chromatinic material. Two chromatinic bodies or a single dumb-bell-shaped body are seen in elongated segments of the aerial hyphae; these segments seem to be immature since they occur most commonly in spore chains in which adjacent spores are still connected by a broad attachment (Fig. 5), and their chromatin is well stained by Piéchaud's method with a concentration of eosin that is suitable for the aerial hyphae but leaves the chromatin of mature spores almost unstained (Fig. 19). These segments appear to undergo maturation by subdividing into two spores each containing a single dot-like chromatinic body, as suggested by the occurrence of segments of hyphae containing a chromatinic body on either side of a median constriction (Fig. 6). This agrees with phase-contrast observations (Hopwood, 1960) that units of an aerial hypha may become sharply delimited from their neighbours before their final subdivision into spores is completed. In contrast, Klieneberger-Nobel (1947) and Saito and Ikeda (1958, 1959)

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interpreted dumb-bell-shaped chromatinic bodies in immature spores as stages in the fusion of two elements of chromatin to produce the single nucleus of the mature spore. The two-hit x-ray survival curve of a spore suspension of *S. griseoflavus* (Saito and Ikeda, 1959) was taken as evidence for the presence of two genetic units in the spore; in contrast, *S. coelicolor* strain A3(2) has a one-hit curve (Hopwood, unpublished). Badian (1936) interpreted the chromatinic rods of the aerial hyphae as stages in a process of "autogamy" during which rows of chromatinic bodies fused in pairs or in groups to form structures which later broke up into the chromatinic bodies of spores, but he produced no objective evidence for the existence of autogamy.

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