COMPARISON OF THE DEVELOPMENTAL CYCLES OF SOME MEMBERS OF THE GENUS *NOCARDIA*[1]

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ABSTRACT

ADAMS, JAMES N. (University of Georgia, Athens) AND NORVEL M. McCLUNG. Comparison of the developmental cycles of some members of the genus *Nocardia*. J. Bacteriol. **84**:206–216. 1962.—A staining technique specific for cell walls was used to determine the characteristic development of *Nocardia rubra*, *N. erythropolis*, *N. asteroides*, and *N. canicruria* comb. nov. When inoculated on a fresh medium, these organisms were observed to germinate, with the production of one or more germ tubes from coccoid, ovoid, and short bacillary cells. Upon continued incubation, the germ tubes elongated to form filaments from which branches could be formed. Two other mechanisms of branching were noted. One was a result of multiple germination of cells and the other a result of single or multiple germination of short chains of cells. Both of these mechanisms could be readily differentiated from true branching. Mycelial formation varied in extent among the species studied and was governed, in part, by the types of branching which an organism exhibited. After the phase of branching development has been completed, fragmentation of the filaments and branches occurred as a result of the separation of the corresponding filament fragments following the random deposition of septa along the lengths of the cells. As the culture age increased, the separating filament fragments became shorter until they ultimately reached sizes like those of cells found in the inoculum. The observed developmental cycles of the genus *Nocardia* were compared to those which have been described for the genera *Mycobacterium* and *Streptomyces*, and the taxonomic implications of the differences between these developmental cycles was discussed.

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Cytological studies of fragmenting actinomycetes have been made by several investigators. Bisset and Moore (1949), for example, studied several not fully identified *Nocardia* species, while attempting to devise a classification scheme for the actinomycetes based on septational differences between groups. McClung (1949, 1950, 1955) studied various aspects of the cytology of *N. rubra* and developmental cycles of *Nocardia* species. Morris (1951) reported on the nuclear behavior of *Nocardia* species; Webb and Clark (1957) and Hagedorn (1959*a*,*b*) made similar studies with *N. corallina*. Not all of these authors observed the organisms throughout complete developmental cycles nor did all select a representative sample of the group with which to work.

The present investigations were initiated to supplement the knowledge of these organisms by observing representative members of the groups designated by McClung (1949, 1954) throughout their developmental cycles by means of standard cytological techniques. We feel that such an approach to the study of these organisms will permit a further evaluation of many of the varied observations which have been reported concerning the fragmenting actinomycetes and permit an estimation of the variation which may be encountered within the group. As a consequence, the fragmenting actinomycetes may be more readily differentiated from *Mycobacterium* and *Streptomyces*, genera with which the genus *Nocardia* has been consolidated by some investigators (Gordon and Mihm, 1957, 1958, 1959; Bradley, 1959; Hesseltine, 1960; Bradley, Anderson, and Jones, 1961).

MATERIALS AND METHODS

The organisms used in these studies were *N. erythropolis*, *Jensenia canicruria*, *N. rubra*, and *N. asteroides* (Table 1). These organisms were selected as typical representatives of morphological

TABLE I. *List of organisms studied*

Organism	Received as	Source
Nocardia rubra	*Proactinomyces ruber*	Centraalbureau voor Schimmelcultures, Baarn
N. erythropolis	*N. erythropolis*	Waksman 3407
N. asteroides	*N. asteroides*	Emmons 9973
Jensenia canicruria	*Jensenia canicruria*	F. A. Clark, who obtained the culture from K. A. Bisset's laboratory

groups I, II, and III of McClung (1949, 1954). Cultures of these organisms were routinely maintained on Difco nutrient agar slants and transferred to appropriate media before carrying out further manipulations.

The surface of a nutrient agar plate was inoculated with a distilled water suspension, prepared from a 3-day-old culture, of the organism to be studied. Inoculated plates were incubated for intervals from 1 to 72 hr at 28 C. Blocks of agar approximately 1 cm^2 were cut from these plates and heat-fixed impression smears prepared. Some studies of *N. asteroides* were carried out by placing clean, sterile slides or cover slips on the surface of the inoculated medium and removing them after appropriate intervals of incubation. The organisms adhering to the slides or coverslips were then heat fixed. All smears were stained with the cell-wall stain of Webb (1954) as used by Adams and McClung (1960, 1962).

The microscope employed for observation and photomicrography was equipped with a Bausch and Lomb 2-mm, na 1.30 apochromatic oil-immersion objective; 12.5 × compensating oculars; a variable focus condenser, na 1.40; and a Bausch and Lomb 35-mm photomicrographic camera and accessories. A green filter was used to increase specimen contrast for photography. Kodak High Contrast Copy 35-mm film was utilized as the negative material, and magnification at the negative plane was 900 ×.

RESULTS

Developmental cycle of N. rubra. At the outset, three types of cells, coccoid, ovoid, and bacillary, were found in cultures (3 days or older) used as inocula (Fig. 1). These occurred singly, or in short chains consisting of the three primary cell forms or their combination. During the first 12 hr of incubation, growth was initiated by the formation of germ tubes. A small protrusion originated at any point on the periphery of the coccoid cells, but such protrusions were usually formed at a hypothetical junction of the longer peripheral axis with the shorter axis in bacillary and ovoid cells (Fig. 2). After the germ tube protrusions were formed, their continued growth, up to 15 to 20 hr after inoculation, produced filamentous cells. The resultant germ tubes frequently had less affinity for the cell-wall stain than the originating cells so that each young filamentous cell typically appeared as a filament with a darker staining knob at a polar position (Fig. 2 and 4).

Two- to seven-celled chains were found in cultures up to 10 hr old, and cells composing these chains (Fig. 3) were capable of germination. Multiple germination of cells was also encountered, which was similar to that observed when single germ tubes formed (Fig. 4). The parent cells formed a single germ tube during the first 6 to 8 hr of incubation; in the period of 8 to 12 hr, a second germ tube, readily differentiated from the first by its shorter length (Fig. 4), was produced at the end of the cell opposite the first. The production of a third or fourth germ tube was observed infrequently. In the second phase of the developmental cycle, mycelium was formed. Branches were produced by different mechanisms from about the first 8 hr of incubation onwards. However, the greatest abundance of branches was observed in cultures older than 12 hr. Branches forming basally near the originating cell from filamentous germ tubes were frequently observed and continued to elongate upon continued culture (Fig. 5). They were also produced from long filamentous cells (Fig. 6) at any point on the hyphae in a random manner (Fig. 5 and 6).

Studies of the developmental cycle of *N. rubra* during the phases of germination and mycelial development indicate that three types of branching resulting in mycelium formation may be differentiated. These are: (i) true branching (Fig. 5 and 6), wherein branches are formed from a previously unbranched filament; (ii) multiple

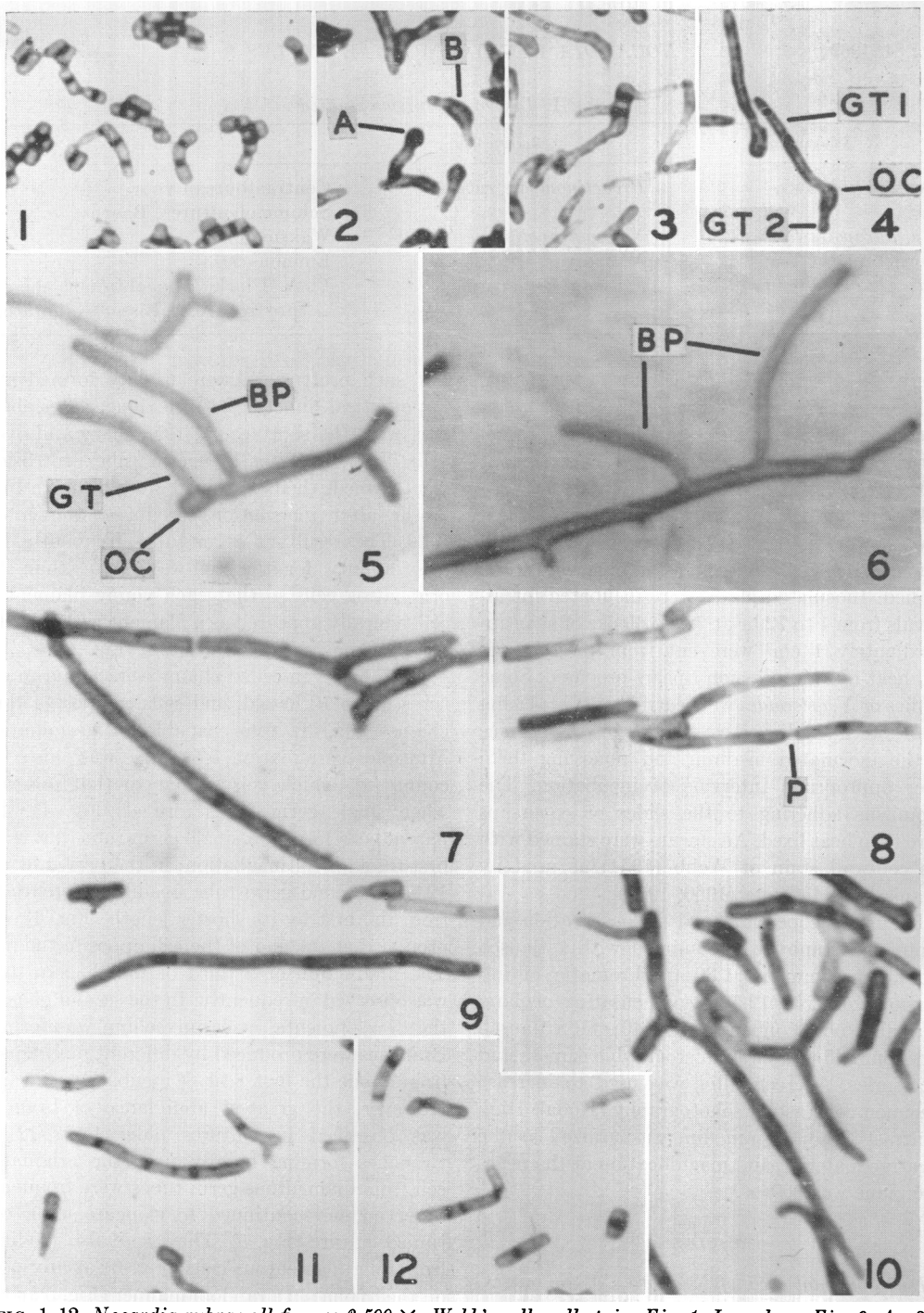

FIG. 1–12. *Nocardia rubra; all figures 2,500 ×; Webb's cell-wall stain. Fig. 1. Inoculum. Fig. 2. An 8-hr culture. Germinating cells. Cell with one germ tube at* A; *multi-germinating cell at* B. *Fig. 3. A 10-hr culture. Short chain of cells germinating with the production of one germ tube per cell. "False" branching. Fig. 4. A 12-hr culture. Uni- and multi-germinating cells.* OC, *originating cell;* GT1, *first germ tube;* GT2, *second germ tube. Fig. 5. A 13-hr culture. Branching cell.* OC, *originating cell;* GT, *germ tube;* BP, *basally occurring primary branch. Fig. 6. A 13-hr culture. Branching cell showing true branching.* BP, *primary branch. Fig. 7. A 17-hr culture. Early fragmentation. Fig. 8. A 28-hr culture. Fragmenting cells.* P, *plasmodesma-like structure at site of septum formation. Fig. 9. A 28-hr culture. Fragmenting filaments. Fig. 10. A 28-hr culture. Fragmenting branched filament. Fig. 11. A 32-hr culture. Shortening of filament length as a result of the random deposition of septa. Fig. 12. A 34-hr culture. Completion of fragmentation.*

germ tube production (Fig. 2, 4, and 5), which, after a suitable period of incubation, resembles true branching; and (iii) "false" branching, the result of single or multiple germination of cells composing short chains (Fig. 3). Of course, a combination of these three branching mechanisms was observed during the period of mycelium formation (12 to 24 hr). However, when employing simple (not specific for cell walls) stains, it was not possible to differentiate between these mechanisms of branching. The location of septa was the only means by which they could be differentiated.

Although septa occurred sparsely in filaments as young as 10 to 18 hr (Fig. 7), the rapid and general onset of fragmentation, the third phase of the developmental cycle, began only after periods of incubation beyond 24 hr (Fig. 9, 10, 11, and 12). That fragmentation occurred at the site of septum formation is indicated in Fig. 7 and 8. Occasionally, "plasmodesma-like" structures were found between segments of separating filaments at the site of septum formation (Fig. 8). In general, fragmentation was preceded by the random production of septa in filaments (Fig. 9) and branching filaments (Fig. 10). Septation increased with the aging of the cultures and resulted in divided filaments, as shown in Fig. 11 and 12. As the number of septa per cell or filament increased, the individual filament fragments cut off by the septa became shorter. Approximately 36 hr after inoculation (Fig. 12), fragment lengths approached those of cells composing the initial inoculum (compare Fig. 12 with Fig. 1). Multiple septation (Fig. 11 and 12) not followed by immediate separation of the individual cells accounted for the presence of short chains of cells frequently found in the inoculum (Fig. 1).

Developmental cycle of J. canicruria. The de-

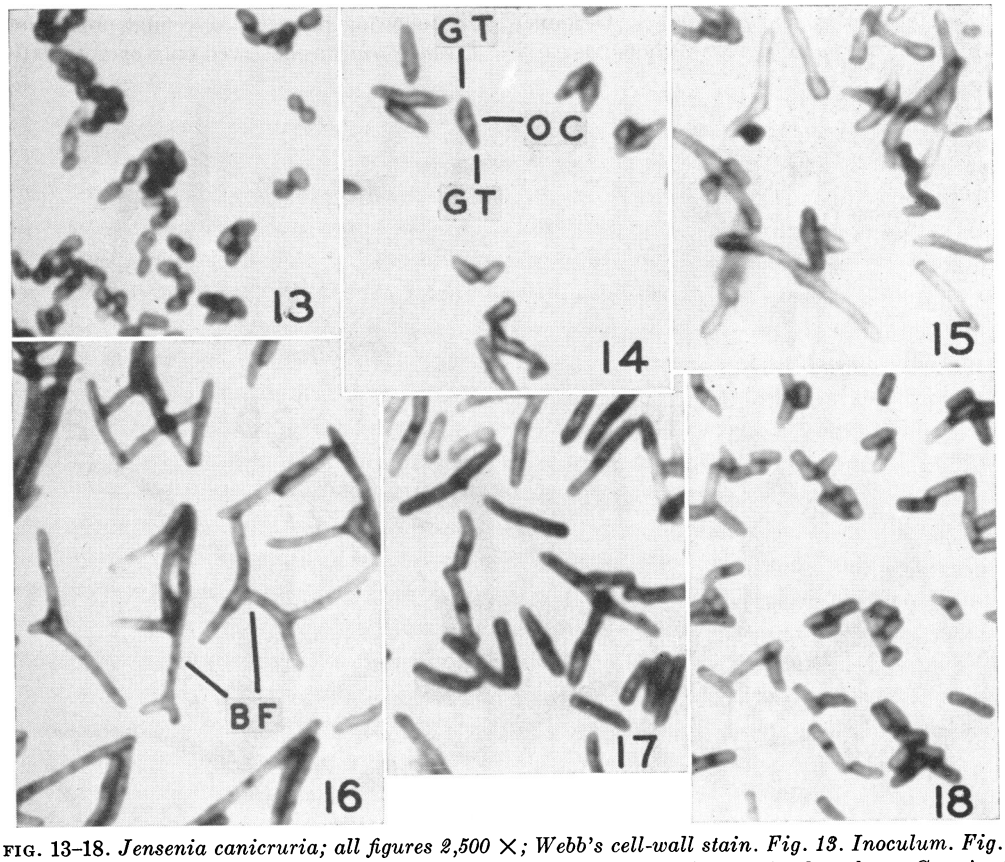

FIG. 13–18. *Jensenia canicruria; all figures 2,500 ×; Webb's cell-wall stain. Fig. 13. Inoculum. Fig. 14. A 6-hr culture. Germinating cells.* OC, *originating cell;* GT, *germ tube. Fig. 15. A 9-hr culture. Germinating cells, and filament formation. Fig. 16. A 15-hr culture. Filaments exhibiting true branching.* BF, *branched filament. Fig. 17. A 21-hr culture. Fragmentation. Increase in septation. Fig. 18. A 24-hr culture. Completion of fragmentation.*

velopmental cycle of *J. canicruria*, although somewhat abbreviated, was similar to that observed for *N. rubra*. Coccoid, ovoid, and bacillary cells composing the 3-day-old culture used as the inoculum (Fig. 13) germinated when placed on fresh medium (Fig. 14 and 15). These cells germinated with the production of one or more germ tubes (Fig. 14). Elongation of the germ tubes preceded the brief phase of mycelial development (12 to 18 hr). Mycelial development was apparent only as a transitional phase between germination and fragmentation, and during this period true branching was observed (Fig. 16). Increasing septum formation (Fig. 17) presaged the period of fragmentation (18 to 24 hr), during which cells like those of the inoculum appeared in the culture (Fig. 18).

Developmental cycle of N. erythropolis. In the present investigations, *N. erythropolis* was found to exhibit a developmental cycle similar to that described for *N. rubra* and *J. canicruria*. Germination of the inoculum (Fig. 19) was noted as early as 3 hr after inoculation (Fig. 20) and continued rapidly during the first 12 hr. Multiple germination (Fig. 20), as well as germination of individual cells of short chains, was observed. Multiple germination of individual cells was a more common occurrence in *N. erythropolis* than in the two previously described organisms; triple germination was relatively frequent, and upon several occasions single germinating cells produced four germ tubes (Fig. 21). Mycelial development began from 12 to 18 hr after inoculation but was never extensive, and septa were produced almost as rapidly as branches were formed (Fig. 22). Such primitive mycelium formation, followed by immediate septation, was observed over the incubation period of 12 to 30 hr. Although fragmentation was usually completed by 33 hr, long fragmenting filaments were often observed in cultures of this age (Fig. 23). *N. erythropolis* differed then from *J. canicruria* in the following respects. Mycelium production of *J. canicruria* was observed only over a relatively

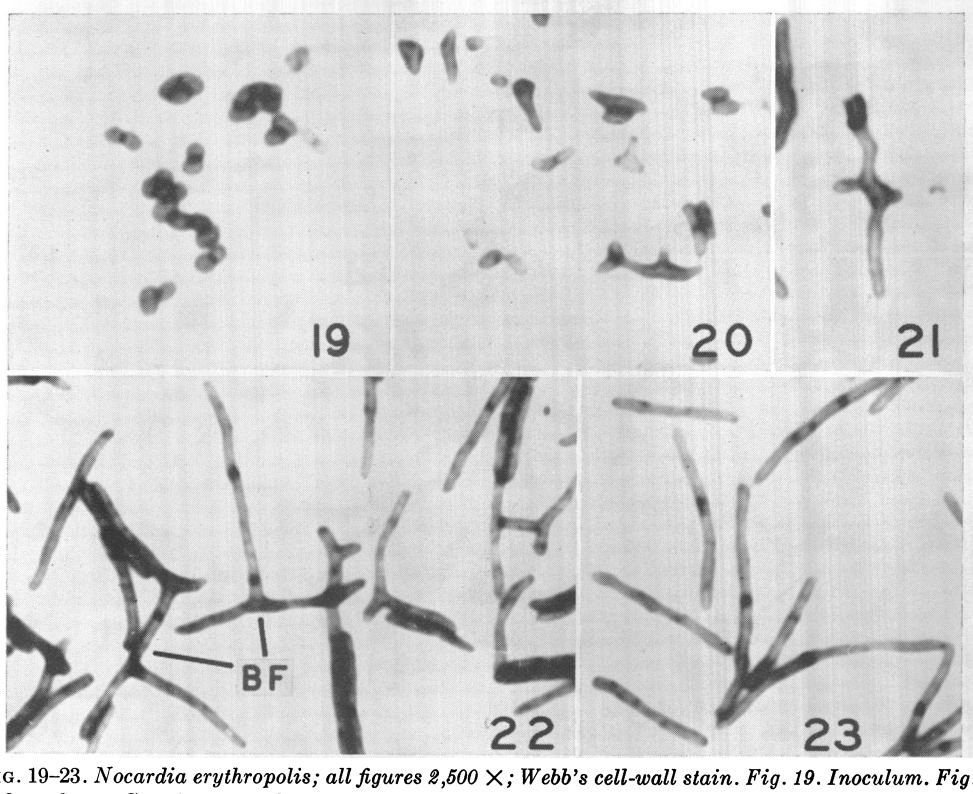

FIG. 19–23. *Nocardia erythropolis; all figures 2,500 ×; Webb's cell-wall stain. Fig. 19. Inoculum. Fig. 20. A 6-hr culture. Germinating cells. Fig. 21. A 9-hr culture. Multi-germinating cell producing four germ tubes. Fig. 22. An 18-hr culture. Filaments exhibiting true branching and septum deposition after branching.* BF, *branching filament. Fig. 23. A 21-hr culture. Early fragmentation.*

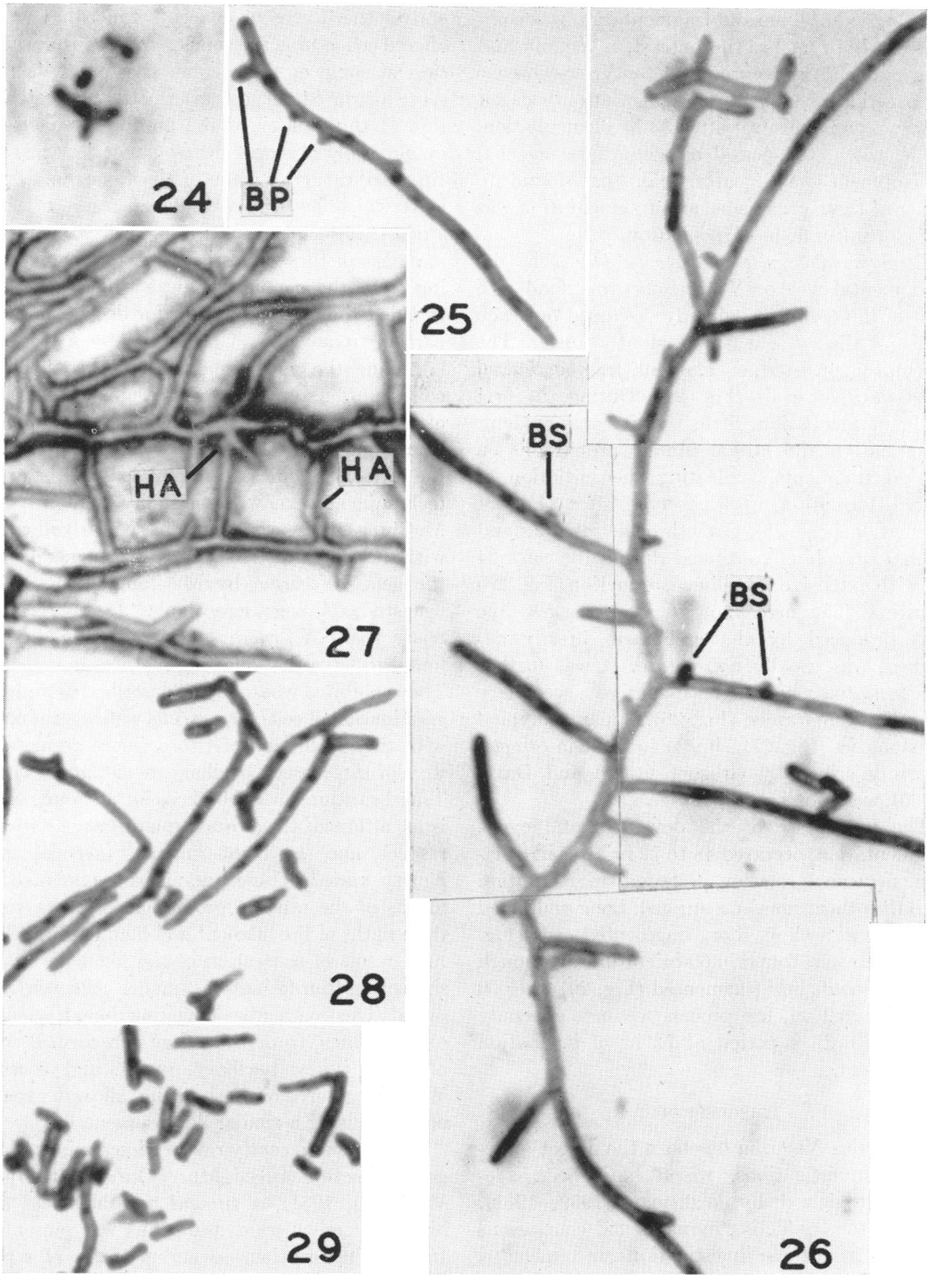

FIG. 24–29. *Nocardia asteroides; all figures 2,500 ×; Webb's cell-wall stain. Fig. 24. Inoculum. Fig. 25. A 24-hr culture grown in contact with slide.* BP, *primary branch protrusions. Fig. 26. A 48-hr culture grown in contact with slide. Mycelium formation.* BS, *secondary branches. Fig. 27. A 48-hr culture grown in contact with slide.* HA, *points at which apparent hyphal anastomoses taking place. Fig. 28. A 60-hr culture. Fragmenting filaments. Fig. 29. A 60-hr culture. Fragmenting filaments.*

short period of 6 hr, and fragmentation was completed within 24 hr. In contrast, mycelium and long filaments were produced by *N. erythropolis* for a period of 18 hr but fragmentation was not usually complete until after 33 hr of incubation. In *N. rubra*, the period of nonseptate mycelial development was greater than that found in either of these organisms, and fragmentation was complete after 36 hr of incubation.

Developmental cycle of N. asteroides. The developmental cycle of *N. asteroides* was similar to that of the organisms already discussed but each phase of the cycle was consistently longer. The inoculum consisted of filament fragments and cocco-bacillary cells (Fig. 24). During the first 24 hr of incubation, little activity was evident. Germination and lateral branch protrusions on hyphal fragments, indicating the initiation of branch formation, were observed after 24 hr of incubation (Fig. 25). Growth of germ tubes and hyphal branching continued during the next 24 hr, with extensive mycelium production (Fig. 26) resulting. The mycelium of *N. asteroides*, like that produced by the organisms already described, was usually nonseptate. It was further differentiated in that extensive secondary branches were formed (Fig. 26). Apparent hyphal anastomoses (Fig. 27), similar to those in *Streptomyces* described by Gregory (1956) and Davis (1960), were observed.

The last phase of the developmental cycle, fragmentation, occurred 48 to 72 hr after inoculation. Septum formation preceded fragmentation as in the other organisms studied. Long undivided filaments as well as short fragmented cells (Fig. 28 and 29) were found in 60-hr cultures. Although most filaments had fragmented (Fig. 29) after 60 hr of incubation, the process was not generally complete until a period of 72 hr of incubation had elapsed.

DISCUSSION

The genus *Nocardia* has been the object of extensive investigations, which have been adequately reviewed by Waksman (1959, 1961). Some of the conflicting opinions and conclusions resulting from these investigations were included in his reviews. We feel that the results of the present and earlier studies (McClung, 1949, 1954, 1955; Webb, Clark, and Chance, 1954; Webb and Clark, 1957; Hagedorn, 1959*a*,*b*) offer sufficient evidence to allow a critical re-evaluation of several of the divergent concepts which have been offered concerning the genus *Nocardia*. Re-evaluation of some of these conflicting concepts may serve a twofold purpose: (i) a better characterization of the biology of the group, based on the cytologically observed developmental cycles, and (ii) clarification of some of the taxonomic problems which have been a direct result of these studies. If such purposes were realized as a consequence of these investigations, the stigma arising from the conflicting ideas which have surrounded the genus *Nocardia* for decades may be partially removed.

Among the most important of the biological considerations are those concerning descriptions of the genus from a morphological standpoint. The kinds of developmental cycles elucidated in the present studies, using a cell-wall staining technique of known specificity (Adams and McClung, 1962), were found to be nearly identical with those described for many diverse members of the genus *Nocardia* by McClung (1949, 1955), who studied living microcolonies. *J. canicruria*, *N. erythropolis*, *N. rubra*, and *N. asteroides* were found to have the following features in common. They exhibited what may be described as multiple germination of cells. These cells which germinated with the production of two or more germ tubes were of three types, bacillary, ovoid, and coccoid. True branching was observed in all four organisms, although they varied quantitatively in this respect, and, as a consequence, mycelial complexity varied. These organisms fragmented by means of the random production of septa along the lengths of the filament and filament branches. As a result of separation of the filaments at the sites of septum formation, smaller cells were produced. The fragments, depending upon the length of the filament from which they were formed, were of three types: bacillary, ovoid, and coccoid. When inoculated on fresh media, all were capable of reproducing a similar developmental cycle.

In the most recently revised descriptions of the genus *Nocardia* (Breed, Murray, and Smith, 1957; Waksman, 1961), a general morphological life-history of members of the genus was presented. In these descriptions several patterns of reproduction are mentioned whose existence was not confirmed in the present studies or by McClung (1949, 1955), Webb, et al. (1954), and Hagedorn (1959*a*,*b*) in previous investigations.

Budding has been described (Breed et al., 1957;

Waksman, 1961) as a method of reproduction in members of the genus *Nocardia*. It was reported to occur in the following manner: ". . . the buds are formed on the lateral surface of the cells; when they have reached a certain size, they fall off and develop into rod-shaped cells or filaments. . . ." In Fig. 25, lateral protrusions may be seen in *N. asteroides*. However, as culture age increases (Fig. 26) such lateral "buds" become branches. In older cultures, for example of *N. rubra* (Fig. 11), fragmenting cells have frequently been observed with small "buds" present on the lateral surface of the cells. Since the cultures used in the present investigations were not synchronous, it was always possible to find cells in a smear which were in earlier or later stages in the developmental cycle than the representative majority of cells. If the cell in Fig. 11, showing lateral "buds," had been observed in a culture whose previous developmental history had not been determined, the casual observer might conclude that the coccoid and bacillary cells also present in Fig. 11 arose as a result of these lateral protrusions. Furthermore, since unfragmenting filaments do appear in old cultures, the results of the present investigations indicate that they arise from cells which have germinated and are in the process of re-entering the first phase of the developmental cycle. Similarly, McClung (1949, 1955), observing microcolonies of *Nocardia* species, reported that lateral protrusions invariably formed filament branches; Hagedorn (1959*a*) reported that germination, a phase in which "buds" might be encountered, resulted in the production of filamentous hyphae. It is the observation of germinating cells of *Nocardia* species in old cultures which probably account for the erroneous accounts of yeastlike budding which have been described.

Chlamydospore production has been described for *Nocardia* species (Breed et al., 1957; Waksman, 1961) in the following manner: ". . .plasma inside the filaments of the mycelium condenses into elongated portions." Chlamydospore production was not observed in the present investigations, nor has such a process been reported in the careful cytological studies of McClung (1949, 1955) and Webb et al. (1954) of various members of the genus *Nocardia*. Chlamydospore production as previously reported may be a process similar to that of nonuniform fragmentation, a result of the random deposition of septa described in the present investigation. However, Michaels (1961) reported the presence, in older cultures of *N. rubra*, of small coccoid bodies which he termed microcysts. The microcysts did not appear to differ morphologically from the small coccoid cells observed in the present studies or the microcysts described by Morris (1951). Physiologically, the microcysts were found to be more resistant to a temperature of 80 C for 10 min than were bacillary fragments (Michaels, 1961). Further studies will be required to determine whether such microcysts are produced by a mechanism other than nonuniform fragmentation, which resembles the chlamydospore production previously described.

Gordon and Mihm (1958) and Bradley (1959) reported the production by *N. asteroides* of spores similar to those observed in the streptomycetes. Gordon and Mihm (1958) presented electron micrographs which showed chains of cells of uneven lengths remaining attached to each other by means of structures similar to the plasmodesma we have observed (Fig. 8). These spores appear similar in shape and length to the unseparated, fragmenting filaments which we have observed in stained cell-wall preparations (Fig. 12, 18, and 29). The spore production reported by Bradley (1959) also resembles nonuniform fragmentation of filaments. However, the lack of clear photomicrographs and the nonspecificity of the staining techniques used by Bradley made his work exceedingly difficult to interpret in light of the present investigation. Typical streptomycete-like sporulation such as that reported by Hopwood (1960) in *Streptomyces coelicolor* and Gregory (1956) in *S. scabies* was not observed in the present studies of *N. asteroides* nor in the other organisms studied.

Morris (1951) and Clark and Frady (1957) suggested that coccoid and ovoid cells may reproduce by means of binary fission in older cultures of *Nocardia*. The results of the present investigation indicated that coccoid and ovoid cells of the four strains tested were capable of germination (Fig. 2, 3, 15, 16, and 17). If binary fission is a mechanism of division, chains of cells should be produced by binary fission in a manner similar to those produced by members of the *Eubacteriales*. However, we observed that "chains" of cells were a result of incomplete separation of fragmenting filaments (Fig. 11, 12, 18 and 28). Since bacillary, ovoid, and coccoid cells were the predominant

forms in older fragmenting cultures, and since septum formation increased rapidly in such cultures, it would hardly appear justifiable to call their production binary fission; this is simply the completion of fragmentation.

In microcolonies of *N. asteroides*, processes similar to the hyphal anastomoses (Fig. 27) described by Gregory (1956) and Davis (1960) in *Streptomyces* spp. were observed. Evidence indicating nest production, as reported for the streptomycetes (Klieneberger-Noble, 1947), was not obtained. Careful genetic analysis will be required before the significance of these filament fusions can be determined.

In the previous discussion it has been pointed out that there are several discrepancies between the morphological description of the genus *Nocardia* presently given in *Bergey's Manual* (Breed et al., 1957) and by Waksman (1961) and data presented in this paper and by other workers (McClung, 1949, 1954, 1955; Webb et al., 1954; Webb and Clark, 1957; Hagedorn, 1959*a*,*b*). Perhaps as a consequence of these discrepancies, several recent investigators have attempted to subdivide the genus *Nocardia* and to suggest distribution of the products of such a division between the genera *Mycobacterium* and *Streptomyces* (Gordon and Mihm, 1957, 1958, 1959, 1961; Bradley, 1959; Hesseltine, 1960). Gordon and Mihm (1958) and Bradley (1959) reported that sporulation can be found in *N. asteroides*, and Hesseltine (1960) has stated that these data "... prove that sporulation in *Nocardia* occurs in the same fashion as that in *Streptomyces* ..." and "... the distinction between the genus *Nocardia* and *Streptomyces* is not real." Although we have been unable to confirm the presence of such streptomycete-like sporulation in *N. asteroides*, we have been able to demonstrate a basic integrity of all of the representative fragmenting actinomycetes studied, namely, similar developmental cycles involving reproduction by means of fragmentation. These results are in agreement with those of McClung (1949, 1955), who demonstrated differences in degree of mycelial complexity exhibited among diverse members of the genus *Nocardia*, on the basis of development of individual cells. Webb et al. (1954) reported similar likenesses between two *Nocardia* species which they studied. In the present investigation, it appears that the genus *Nocardia* can readily be differentiated from the genus *Streptomyces* in the following respects. The genus *Nocardia* reproduces by means of fragmentation of the mycelium but does not produce conidia borne on sporophores, although ovoid and coccoid fragments or microcysts (Michaels, 1961) may sometimes be mistaken for conidia. The genus *Streptomyces*, on the other hand, is typified by a mycelium which normally remains undivided and produces conidia borne on definite sporophores (*Bergey's Manual*).

It would appear that the definition (*Bergey's Manual*) of the genus *Mycobacterium* as usually acid-fast, rod-shaped cells that do not branch under ordinary cultural conditions, if strictly interpreted, would be sufficient to provide separation of the genus *Mycobacterium* from the genus *Nocardia* on the basis of the data discussed above and those presented in earlier studies (McClung, 1949, 1954, 1955; Webb et al., 1954; Webb and Clark, 1957). It is unfortunate that Gordon and Mihm (1957, 1959, 1961) attempted to place several organisms, among them the strains which are designated in this report as *N. rubra* and *N. erythropolis* (Gordon and Mihm, 1957) and *J. canicruria* (Gordon and Mihm, 1961) in the genus *Mycobacterium* on physiological and gross colonial morphological grounds, without regard to what Bisset (1950) refers to as a more basic phenomenon: the structure and growth of the individual cells. The *Bergey's Manual* definition of the genus *Mycobacterium* explicitly implicates consideration of such cellular morphology. It is difficult to understand why these authors (Gordon and Mihm, 1957) should suggest that an organism such as *N. rubra*, with the characteristic and extensive branching shown in the present and earlier studies (McClung, 1949, 1954, 1955), should be placed in the genus *Mycobacterium* as currently defined. However, it is clear how an organism which is less complex, e.g., *N. erythropolis*, in which mycelial development is transitional, might be mistakenly alloted to the genus *Mycobacterium* on the basis of colonial morphology (Gordon and Mihm, 1957).

A similar fate has befallen the organism *J. canicruria*. The genus *Jensenia* was proposed by Bisset and Moore (1949, 1950) to separate unicellular soil diphtheroids from the reportedly multicellular genus *Nocardia*. In the present studies, the frequency of septation resulting in unicellular or multicellular forms was found to be dependent on the stage of the developmental

cycle of the organisms (*Nocardia* spp. on the one hand, and *J. canicruria* on the other), in agreement with our previous findings (Adams and McClung, 1960). Consequently, uni- versus multi-cellularity would appear to be an inadequate criterion for differentiating the genera *Jensenia* and *Nocardia*. However, on the basis of our previous data (Adams and McClung, 1960), in which we suggested similarity of cellular morphology of members of the genera, Gordon and Mihm (1961) placed *J. canicruria* in the genus *Mycobacterium* on physiological and gross colonial morphological grounds. The present studies indicate that this organism, like *N. erythropolis*, does indeed have a phase of mycelial development, albeit short and transitional. The existence of branching is definite (Fig 16) and this must exclude this organism from the genus *Mycobacterium* as currently defined. It is suggested that *Nocardia canicruria* comb. nov. should be the name applied to the organism described by Bisset and Moore (1950).

Lieske (1921) first recognized the transitional nature of the fragmenting actinomycetes and hypothesized that they may be progenitors, on the evolutionary scale, of both the bacteria and fungi. This concept has been more recently discussed (Davis and Freer, 1960), and, while the present data do not suggest a solution to the evolutionary problem, the present studies, like those of others (Lieske, 1921; Ørskov, 1923; McClung, 1949, 1955), serve to show that even a "transitional" group of bacteria have certain integrity when studied in detail. The current investigation substantiated the existence of forms which may be classified in the genus *Nocardia*, and which range in degree of mycelial development from those that are quite bacteria-like (*N. canicruria* and *N. erythropolis*) to those whose mycelium is as extensive as that of the streptomycetes, as described by McClung (1949). It appears that morphological subgrouping of the genus (McClung, 1949) may conveniently serve for subdivisions of the genus, if we remember that intermediate forms do exist between the morphological groups which he proposed.

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