MORPHOLOGY OF THE GENUS ACTINOMYCES. I[1]

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While the genus *Actinomyces* has received a large measure of attention in its relations to soil biology and to human, animal, and plant pathology, the natural affinities of the congeneric organisms that it has been customary to include in the group have been the subject of diverse opinions. Under a variety of synonyms, among which *Cladothrix*, *Nocardia*, *Discomyces*, and *Oospora* have been used nearly as frequently as *Actinomyces*, the group has been placed with the bacteria, with the Hyphomycetes, or assigned to an intermediate position. In the earlier publications on the ray fungus, including the papers by Bostroem (1)[2], and by Wolff and Israel (24), this organism was referred to the pleomorphic bacteria. The belief was seriously entertained that cocci, bacteria, and spirilla were produced by the plant, and in such regular succession that a number of investigators were led to draw up detailed ontogenetic schemes of considerable complexity. It is frequently not easy to determine the exact nature of the structures that were interpreted as pleomorphic stages. There are plenty of indications that contaminating bacteria were often present as secondary invaders; but more frequently aërial spores, segments of spiral or sinuous hyphae, and degenerative bodies of metachromatic substance were mistaken for schizomycetous types of nearly every description.

More recently *Actinomyces* has been frequently associated with the tubercle and diphtheria organisms on the assumption that they may represent a transition between the Hyphomycetes and the true Schizomycetes. A family of Actinomycetes has thus been erected as a natural group from these diverse components, united chiefly by resemblances in their staining reactions, a usual or an occasional filamentous habit, and the development of clavate elements in the animal body. It has been supposed by adherents of such a taxonomic disposition that either a progressive phylogeny

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[2] The bibliography will appear at the end of Part II, which will be printed in February.

has occurred in the family, with *Actinomyces* at the head of a transition series developing increasingly well marked fungoid characteristics, or else that *Actinomyces* is the probable progenitor of the groups *Corynebacterium* and *Mycobacterium* by degenerative reduction.

The view that the ray fungus represents an organism with hyphomycetous affinities was advanced early by HARZ (7) and DEBARY (2). These authors regarded as conidia the clavate elements of the actinomycotic lesion, which BOSTROEM's studies later properly degraded to the rank of degenerative structures. SAUVAGEAU and RADAIS (20), DOMEC (3), THAXTER (22), GASPERINI (4), and others placed a number of congeneric forms among the Hyphomycetes on account of their production of aërial spores. It may be mentioned in this connection that an examination of a considerable number of species has convinced the writer that this disposition is the only one which is in harmony with the morphological conditions represented in the genus.

The material used in these studies, with the exception of authentic cultures of the species described by WAKSMAN and CURTIS (23), and of a number of organisms isolated by H. J. CONN from soil collected near Geneva, New York, was largely obtained from soil collected in Cambridge, Massachusetts. By the use of the dilution method more than 1000 plants belonging to the genus were isolated from this source; and of these about 300, representing probably more than 100 species, were selected for morphological examination. Approximately 400 additional individuals were derived from soil collected in Porto Rico, Cuba, Panama, Montana, Wisconsin, and Kansas, as well as from outdoor air, tap water, horse manure, and gross cultures of dung, dead leaves, and other vegetable matter. The potato scab organism was obtained from Mr. M. SHAPAVALOV, who had isolated it from a diseased tuber, and experimentally established its pathogenicity.

The morphology of the vegetative thallus of *Actinomyces*, apart from its astonishing minuteness, the diameter of the filaments ranging commonly from 0.5 to 1.2 μ, presents no features unusual among the fungi. In most species the mycelium is generally sparsely and irregularly septate; and although in other forms transverse walls may appear with somewhat greater frequency, there are none in which septation approaches any pronounced degree of regularity or closeness. Ramifications are abundant, and the branching is altogether of the "true" type. MACÉ (15), who first carefully observed the formation of branches, found it to proceed by the elongation of lateral buds arising some distance back from the growing point of an axial filament, the branch thus produced giving rise similarly to secondary branches by lateral proliferation. LACHNER-SANDOVAL (12) confirmed MACÉ, designating the process as monopodial and denying the existence of true dichotomy in the genus, which had been affirmed by previous investigators. Later NEUKIRCH (18) reported that the branching in *Actinomyces ochroleuceus* was occasionally of the nature of a true dichotomy. From an examination of very young mycelium (fig. 3)[3] it is apparent that, at least in stages following the germination of the spore, filaments are not infrequently terminated by two elements too closely similar in size and angular relationships to be distinguished as bud and axial tip. The branching in such cases must be regarded as dichotomous, although all gradations toward the prevailing well defined monopody may be found. It seems reasonable to suppose, however, that the distinction is one of convenience, not implying any fundamental difference in manner of development.

The cytological structure of *Actinomyces* is equally devoid of bacterial characteristics. The branches forming the periphery of the actively growing pellicle, or the young sporogenous branches attached at intervals to the superficial mycelium, are filled with dense protoplasm, which, with haematoxylin, takes a deep homogeneous stain. Further toward the origin of the hyphae the contents become more attenuated, and vacuoles appear, increasing in number and size until they occupy the larger portion of the filaments. When individual vacuoles become excessively large and extend through a considerable length of filament, the cytoplasm is in a large measure confined to a peripheral layer, a condition which led NEUKIRCH to distinguish a thin, strongly refringent "Aussenplasma" and a less refringent "Innenplasma."

[3] The plates will appear in connection with the second part, to be published in the following number.

The presence of large vacuoles is commonly associated with local distentions of the filament wall. These may occur with such regularity in the degenerate mycelium of some species as to suggest the appearance of *Leptomitus*, each swollen segment being largely occupied by a single elliptical vacuole, separated from the vacuole in the adjacent distention by a protoplasmic partition at the constriction (figs. 47, 48, 106). In other species and quite generally in the nutritive mycelium of all forms, that is, the mycelium immersed in the substratum, no marked regularity in the alternation of inflated portions and constrictions is observable; but pronounced deviations in the diameter of filaments may occur with more or less variable frequency.

A great deal of importance has been attributed by some writers to a variety of abnormalities and products of degenerative changes occurring in the thallus of *Actinomyces*. In the earlier literature on the ray fungus, especially in the publications of ISRAEL (**8**), JOHNE (**9**), and MACFADYEAN (**16**), bodies described as "micrococci," "cocci," or "coccus-like granules" were given minute attention, and assigned an important rôle in the complex ontogeny ascribed to the parasite, then supposed to belong to the pleomorphic bacteria. WOLFF and ISRAEL (**24**), whose photomicrographs of these bodies leave no reasonable doubt about their identity with structures very frequently observed by the writer (figs. 15, 31, 32, 42, 91), confused them with the spores reported by other authors; and as the structures did not possess the heat resistance common to spores of bacteria, these investigators were inclined to question the production of spores by *Actinomyces*. Since the organism used by WOLFF and ISRAEL was constantly sterile, their conclusion concerning it was undoubtedly correct. BOSTROEM, who experimented with a sporiferous form, did not succeed in avoiding the same confusion, and refers indiscriminately to the unicellular products formed from aërial hyphae, and to the spherical endogenous granules, as "spores."

Round granules, deeply stained in the living filament by very dilute methylene blue, were studied later by NEUKIRCH. He noted in them a variable size, a method of multiplication, and an orientation related to the regions of growth in the thallus. These observations led him to believe that the structures represented nuclei. SCHÜTZE (**21**), who applied NEUKIRCH's method of staining, designated the bodies as metachromatic granules. After an examination of their occurrence in the aërial mycelium of a considerable number of species, such an interpretation seems, in the opinion of the writer, to offer the greater degree of plausibility.

The metachromatic material is easily distinguished by a powerful affinity for most of the stains ordinarily employed in laboratories. In material fixed in alcohol, and treated with Delafield's hematoxylin, it retains a nearly opaque stain after all other structures have been completely decolorized. Indications of its presence in the tips of growing filaments, or in sporogenous branches, in general are very infrequent. Some distance toward the origin of the hyphae, associated with a more attenuated or vacuolated protoplasm, the material makes its appearance in the form of rather minute granules widely separated from one another. As the filament is followed still farther back, the granules increase in size and frequency; often their arrangement is one of much regularity, the individual spherical bodies being of nearly equal size, exactly filling the lumen of the filament, and separated by nearly equal spaces (fig. 42). In other cases the granules seem to coalesce and occupy entire segments of hyphae (fig. 32); and in a few species extensive portions of mycelium were frequently found entirely filled with long unbroken masses of metachromatic substance. It is this property of coalescence of smaller granules, to form incomparably larger masses, bearing out the similarity in appearance to a homogeneous liquid with a relatively high surface tension, that makes it difficult to believe that we are dealing here with anything relating to spores or to nuclei.

The function of the metachromatic material in the *Actinomyces* thallus cannot be ascertained with certainty. A number of views have been advanced regarding the rôle of metachromatic substance in the cell, none of which has gained universal acceptance. The best explanation, in the opinion of the writer, seems to be that it represents an occluded waste product. While its presence in small or moderate quantities in the sterile hyphae bearing the sporogenous branchlets is probably more or less normal, its abundant occurrence here, as everywhere else, is an indication of advanced degeneration. In the more mature mycelium of *Actinomyces* VIII (fig. 47) metachromatic granules are usually very conspicuous, often occupying most of the space in the narrowed constrictions between the large vacuoles in the highly inflated mycelial segments. The appearance of such a thallus is not in the least suggestive of the structure of bacteria, and indicates that the resemblance between *Actinomyces* and the true Schizomycetes in the consistency of protoplasm, emphasized by some writers as an important phylogenetic connection, has been unduly overestimated.

While the sterile filaments in the nutrient and in the aërial mycelium are relatively uniform in structure throughout the genus, the sporogenous apparatus of many species exhibits a large degree of morphological individuality. This diversity has not usually been recognized by writers, and has undoubtedly been responsible for a portion of the controversy that has arisen, particularly with regard to the method of spore formation. LACHNER-SANDOVAL (**12**), from a study of *Actinomyces albido-flava*, distinguished two kinds of propagative bodies: (1) fragmentation spores appearing as spherical to cylindrical segments in old hyphae, formed by a contraction of the protoplasm; and (2) segmentation spores developed by a septation of the tips of aërial filaments. Segmentation was usually found to involve only lateral branches coming from aërial hyphae, but in submerged growths it frequently extended also to the main filament, leading to the development of a dendroidic system of spore chains. LACHNER-SANDOVAL'S figures of these formations, however, are much less striking than might be expected from the description in the text, and do not convey the impression of ramifications approaching treelike proportions.

NEUKIRCH identified the segmentation of LACHNER-SANDOVAL with oidium-spore formation among the fungi, and abandoned the use of a specific term. He vigorously disputed the development of aërial spores by a septation of the mycelium. According to his account the spores are formed as the result of successive contractions of protoplasm until approximately isodiametric portions are separated by regularly alternating empty spaces. This process he identified with the fragmentation of MACÉ, localizing it in a different region from LACHNER-SANDOVAL, and properly relegating the fragmentation of the latter to the category of degenerative changes.

GILBERT (5) found some lateral branches to begin the process of forming spores by becoming differentiated into highly refractive and weakly refractive portions. Constrictions later appear, unassociated with visible changes inside the filament, and soon the spores are completely cut off. GILBERT designated the process as segmentation, following LACHNER-SANDOVAL, who, however, had actually observed septa appearing more or less simultaneously with the constrictions, their appearance being followed by the enlargement and rounding up of the segments to form spores.

MIEHE (17), in his study of *Actinomyces thermophilus*, only incidentally examined the mode of sporulation. He believed spores were produced singly on very short stalks attached laterally to the main hyphae, or possibly by successive contractions in chains. In either case conidia were produced, not by the segmentation of a completed filament, but by the development of a structure which at no time constituted a cylindrical, continuous hypha. This account, in general, bears strong resemblances to the later description by SCHÜTZE (21) of *Actinomyces monosporus*, a form in which the spores are borne singly on delicate stalks in racemose arrangement on a thicker axial filament. It might well be questioned, however, whether forms like this, which depart so widely from the main morphological trend of *Actinomyces*, are properly to be assigned to this genus, even if allowance is made for much liberality in the definition of hyphomycetous form-genera.

The same criticism, however, cannot be extended to the condition described by SCHÜTZE in his strain of *Actinomyces thermophilus*. In his account of this species its author strongly defended NEUKIRCH's position that the mode of sporulation was one of fragmentation. However, while NEUKIRCH found long filaments converted into spore chains by successions of protoplasmic contractions, the long portions finally becoming resolved into ultimate spores, SCHÜTZE found that only short terminal portions or short lateral branches yielding about 5 spores were involved. According to NEUKIRCH, the slightly refractive spaces between the masses of protoplasm that later develop into spores are entirely empty, and the intervening portions of filament wall merely collapse as the spores mature. SCHÜTZE believed that these intervals were filled with attenuated protoplasm, and that by their constriction the spores were delimited without the evacuation of portions of hyphal wall. The spore of NEUKIRCH, consequently, is a structure possessing its own spore wall, enveloped, except at its ends, by the remnants of the old filament wall; that of SCHÜTZE, on the other hand, is without a separate spore wall, the filament wall constituting the only membrane present, and forming a spherical shell everywhere inclosing the protoplasm.

NEUKIRCH gave much attention to certain structures he designated as oidium-spores. They developed in submerged growths, the transformation of the filament consisting only in more or less close septation, followed by a slight swelling of the segments. Under suitable conditions filaments grew out from them, an occurrence NEUKIRCH regarded as germination. "Aussenplasma" and "Innenplasma," in his opinion, were sharply defined, but a spore wall was absent. The elements did not exceed the filaments in resistance either to heat or desiccation. NEUKIRCH believed their function to be the dissemination of the fungus in liquid media.

LACHNER-SANDOVAL seems to have seen the same structures and regarded them as segmentation spores that had developed in the submerged condition. GILBERT, SCHÜTZE, and KRAINSKY (10) record their failure to find these bodies without, however, denying their existence. According to SCHÜTZE and GASPERINI, sporulation may occur in hyphae which are not truly air hyphae.

It seems questionable whether any desirable end is served by calling NEUKIRCH's elements spores at all. To apply the term to structures with so little individuality, even though a sort of promiscuous viability may be attributed to them, is approaching very close to the point where all bodies not filaments of uniform thickness are to be regarded as spores. Certainly the distended elements in old mycelium of *Actinomyces* VIII (figs. 47, 48), which represent enlargements of axial filaments developed gradually in the course of time at the junctures with moderately complex systems of sporogenous hyphae, frequently have an equal or greater resemblance to reproductive bodies; and the behavior, under similar conditions, of forms like the smuts, *Mucor*, and *Penicillium*, would make advisable a larger measure of caution in dealing with fungi growing irregularly in a submerged condition.

Although various details associated with the sporulation of *Actinomyces* have thus been dealt with in the literature, the opinion still seems to prevail widely that the process is of an irregular and miscellaneous nature. LUTMAN and CUNNINGHAM (14) in recent years have denied the character of spores to the "gonidia" produced by the potato-scab organism; the elements are believed simply to "serve as a segment of the mycelium, which, by increasing the number of segments, may increase the chances for spread and continuous existence." This view seems, in the opinion of the writer, very much at variance with the distinctiveness of the well characterized sporogenous apparatus found in *Actinomyces*.

In pursuing the present studies a method of mounting material was employed which, in view of the exceptional fragility of all species of *Actinomyces*, and the great difficulty ordinarily encountered in attempting to stain undisturbed sporulating conditions, gave exceptionally good results. The plants were grown on a suitable substratum, usually potato or glucose agar. Growth on potato agar, as a general rule, is more prompt and productive of mycelium; but as its use, especially with species exerting a strong tyrosinase reaction, stimulates to excessive guttation and disruption of the sporophores by the extruded droplets, a medium not possessing this property is often found to be advantageous. After the cultures had attained a proper degree of maturity, the whole growth was cut from the agar and removed from the tube as carefully as possible. A slide smeared with albumen fixative was now brought into firm contact with the mycelium and then separated from it, precautions being taken to avoid altogether any sliding of the two surfaces on each other. If the growth is not too young, this procedure will leave the upper portions of the aërial mycelium adhering to the slide without serious disarrangement, and killing and fixation may be at once effected by the use, for example, of strong alcohol. The material was subsequently stained and mounted in balsam. The quality of preparations in which the spore chains have commenced to disintegrate in large numbers is impaired by the presence of large masses of free spores, which retain their staining properties for some time after maturing. Later the spore walls seem to become entirely impervious to stains, and as a result when the secondary mycelium develops beyond a slight clouding effect no difficulty is encountered from this source. The best results are obtained when the print is made soon after the mycelium begins to adhere readily to the smeared slide.

The nature of the killing agent employed was found to have no noticeable effect on the preparation. Flemming's mixtures, both weaker and stronger, picro-formol, picro-acetic, Carnoy's fluid, and 95 per cent alcohol were tried with apparently the same results. Owing to the small diameter of the filaments, the penetration is probably so nearly instantaneous that plasmolysis is effectively prevented by nearly any toxic agent capable of readily wetting the material. In order to obviate the necessity of washing, strong alcohol was used almost exclusively.

Much more depends on the proper choice of a stain. Saffranin, gentian violet, Bismarck brown, and eosin usually fail to bring about a sufficiently deep coloration. Carbol-fuchsin acts powerfully and rapidly, but is poor for purposes of differentiation. Haidenhain's iron-alum haematoxylin is good for protoplasmic structures. The most satisfactory results were obtained with Delafield's haematoxylin, which if allowed to act for 24 hours, with the proper degree of decolorization, yields deeply stained, clear preparations, showing vacuoles, metachromatic, and nuclear structures, as well as septa, with remarkable distinctness.

The spores of all species of *Actinomyces* are developed by a transformation of more or less specialized hyphal branches distinguishable from the sterile hyphae of the aërial mycelium at an early stage in their development. In general, with the exception of such inflated hyphae as are shown in figs. 47, 48, and 106, the diameter of any portion of sterile mycelium is attained at the time it arises through the elongation of the growing filament tip, subsequent increase in thickness being very slight. The sporogenous branches, however, are in the beginning conspicuously thinner than the axial hyphae from which they are derived. Later, when their final linear extension has been nearly attained, increase in thickness generally follows. This increase may be slight, as in some species in which the mature sporogenous hyphae are still somewhat thinner than the vegetative hyphae (fig. 46), or more considerable, as in forms in which they conspicuously exceed the latter in thickness (figs. 4–6). The very simple type represented by *Actinomyces* XIII, in which the aërial mycelium is represented by very long filaments, rarely branching and apparently sporogenous almost to their point of origin in the nutritive mycelium, constitutes the only exception, since in this instance there is no indication of thickening in the young fertile hyphae, nor indeed any variation in the diameter of its vegetative filaments.

In a majority of the species the maturation of the sporogenous hyphae is associated with a peculiarity in growth by which they become coiled in more or less characteristic spirals. The tendency toward the coiled condition is usually clearly manifested before the branch has grown to half its final length through the open flexuous habit of the young filament (figs. 5*c2*, 107). As elongation continues, the turns become increasingly definite, but the contraction leading to the final condition, which ranges from that illustrated by *Actinomyces* XIII with its open, barely perceptible turns, to one in which the spirals are so strongly compressed that its adjacent turns are in continuous contact (figs. 44, 51, 57) in a fashion resembling that of the spores of the hyphomycetous genus *Helicoön*, is usually delayed until the later growth in thickness of the filament. Specific differences may not only be indicated by the obliquity of the spiral, but involve also the number and diameter of its turns, and its construction with reference to the dextrorse or sinistrorse condition. The range in different species extends from the 2 or 3 turns exhibited in forms like *Actinomyces* II and XVI, to over 20 turns in others; but the range in a particular species is always considerably smaller. The writer once observed a spiral with 24 turns, but this probably approximates the extreme maximum; spirals with 14–16 turns (figs. 23, 57, 94*c*) are by no means abundant, and probably no species produces many in which there are more than 12 turns.

The diameter of the spirals as a whole is more or less in inverse ratio to the number of turns characteristic of the species. This correlation is very evident in a comparison of types like *Actinomyces* II and XVI showing spiral sporogenous hyphae with a few wide turns, and types like IV, V, and XVII illustrating spirals of many narrow turns.

Rotation in the formation of spirals is specifically sinistrorse or dextrorse in different species of the genus; and it is interesting to note that here, as in the vegetable world in general, sinistrorse are much more abundant than dextrorse species. Of the 17 species with spiral sporogenous branches figured in the present paper, which have been selected as representative of a much larger number, 5 are dextrorse, 11 are sinistrorse, while the condition of the remaining one could not be ascertained with certainty. In general, the proportion appears throughout the entire genus. As a morphological feature, the absolute constancy with which a species adheres to one kind of rotation is noteworthy, particularly in view of the extremely minute dimensions of the structures concerned.

An examination and comparison of the relation of the sporogenous branches to each other, and to the axial filaments, enables one to recognize several tendencies, the distinguishing characteristics of which are correlated with differences in the sequence of proliferation. Two main types may thus be recognized, approaching each other in apparently intermediate forms, but moderately distinct at the extremes: (1) an erect dendroidic type in which the sequence of development of the sporogenous hyphae is successive; and (2) a prostrate, racemose type in which the development is more nearly simultaneous.

In the erect type, well exemplified in *Actinomyces* I, the development of the fructification starts from a single erect hypha with a spiral termination. Sporogenesis commences at the tip by the insertion of regularly spaced septa, and proceeds downward toward the base of the filament. Usually before much of the hypha has been involved, a single septum will appear well toward its base, and immediately below it the bud anlage of a new sporogenous branch appears. As the latter is attaining its growth in length and thickness, and its spiral disposition, the basipetal septation in the axial filament proceeds to the septum above the insertion of this first branch, the young spores thus delimited undergo maturation processes, the spiral becomes relaxed, and the chain of spores subject to disruption. The branch now passes through the same course of development as the axial filament and in turn gives rise to a sporogenous branch below a septum a little above its own insertion. The number of sporogenous branches developed below a single septum is generally increased to several by proliferations subsequent to the first; and as the initiation and development of successive orders may be indefinitely repeated, complex fructifications are frequently developed, in which a succession of the processes described are simultaneously taking place at many points.

In the second type there is no such clearly defined relation between younger and more mature sporogenous hyphae. Development of a fructification is initiated by the proliferation of branches at irregular intervals on the distal portion of a prostrate axial filament which often exceeds 1 mm. in length. The branches may either stop their more extensive development after forming a spiral, or themselves proliferate a secondary branch a short distance above their own insertion; and this in turn may form a spiral and give rise to a tertiary branch (fig. 43). By a repetition of this process each lateral element may become branched several times, the whole apparatus as well as its insertion on the axial filament being characterized by an absence of septa. Sporulation, instead of beginning in any individual spiral as soon as it is formed, is usually delayed until the branching and growth of spiral hyphae in the same lateral process have come to an end (figs. 42–44, 46), when it will often proceed rapidly and almost simultaneously in all the spirals (fig. 41). The termination of the axial filament itself develops into a spiral, and behaves essentially like a primary lateral branch.

Occasionally the axis of one of these racemose arrangements may be comparatively short, resulting in a rather intricate structure in which the spirals of one lateral branch may be entangled with those of another (fig. 44). The tendencies characteristic of the type, however, are maintained: the absence of a septum above the insertion of branches, and the delay in sporulation in the spirals first formed, until the growth of the last order of sporogenous branches is more or less complete.

In addition, however, to species in which these two types are clearly distinguished, a still larger number of species present a combination of the two features. Frequently the open racemose arrangement of the lateral branches on the main axial filament is associated with a successive order of development in the further ramifications of the branches (figs. 63, 79). The presence of a septum above the insertion of a branch is characteristic of more species than is its absence (figs. 2, 5*c*); and in some species both conditions prevail (figs. 53, 79, 81). In other forms a fructification with successive development may terminate a long prostrate filament.

In a few species, particularly *Actinomyces* X and XVIII, there are formed, in addition to the more regular fructifications, others of a more miscellaneous tendency. The branching axial filaments are relatively thick, densely filled with protoplasm, and bear at very close and irregular intervals a short, thick, unbranched sporogenous hypha with little or no spiral modification (fig. 103). It seems quite probable that this type of development is associated with the excessively rapid growth that characterizes the two forms in which it was most frequently observed.

The degree of completeness to which the aërial mycelium of *Actinomyces* is converted into spores has generally been overestimated. On the contrary, sporulation is quite strictly confined to terminal elements, never as a rule passing beyond the first junction with another element. The proliferation of the branch nearest the end of the axial filament limits spore production in this filament to the portion beyond the insertion of the branch; and in the same manner the proliferation of a secondary from a primary lateral branch results in a sterilization of the portion of hypha below the insertion of the new branch. In one species, *Actinomyces* V, sporulation is even further restricted by the apparent abortion of a number of potential spores at the proximal end of the unbranched lateral branches. The hyphal portion involved first develops as usual, but when the characteristic septation associated with the delimitation of spores in this species appears in the spiral, it is not extended to the base of the branch, although indications of regularly spaced membranes may usually be distinguished (figs. 21, 24, 25). Later the unsegmented portion is gradually evacuated and converted into a sterile stalk devoid of protoplasm (figs. 29, 30). It is interesting to note that the basal septum, which in an allied and very similar form, *Actinomyces* VI, delimits the lowest spore from the axial filament, here also is present as a well developed cross-wall.

The delimitation of the ultimate cells in the process of sporulation occurs usually as the growth in thickness and the contraction of the spiral (where this is present) are approaching a stage of completion. It has usually been believed by investigators that the details connected with spore formation are uniform throughout the genus. This belief, which the writer was at first inclined to share, must be considerably modified in view of the diversity of conditions actually found. In most species the sporogenous hyphae become divided into regular cylindrical cells separated by septa; the latter generally stain deeply with Delafield's haematoxylin, probably as the result of an association with metachromatic or possibly nuclear material. The species which are thus characterized by clearly defined septation may be assigned to three different categories, separated by differences in the disposition of their septa and in the development of their spores.

In the first category, represented by *Actinomyces* I, the cross-walls in the sporogenous hyphae remain without any very pronounced change, continuing to separate the adjacent cells until these have developed into a chain of mature contiguous spores. The insertion of these septa progresses from the tip toward the base, and does not break the physiological continuity of the hyphae; for food material apparently is readily transported through them to the young spores at the termination, since these subsequently increase in size, and may deposit a wall of measurable thickness.

In the second category the septa apparently split into halves, which are then drawn apart by the longitudinal contraction of the individual protoplasts (figs. 5*c1*, 8*a–f*, 59). In *Actinomyces* II the very pronounced growth in thickness of the sporogenous hyphae, following the insertion of septa, indicates that in this species also septation brings about no impediment in the transfer of food material. This is particularly remarkable on account of the extraordinary thickness of the septa characterizing this species. *Actinomyces* XVII, however, while less striking, probably represents more nearly the usual condition prevailing in the second category. The segment of the filament wall evacuated by the contraction of each two successive spores undergoes no change until fractured by the disruption of the chain of mature spores.

In the third category (*Actinomyces* IV, V, VI, VII, and XII) the cross-walls first undergo a deep constriction, which by involving the ends of the young cylindrical spores gives to the latter an elongated ellipsoidal shape (fig. 70*a–d*). The constricted septum now gradually loses its staining properties, and appears to become slightly drawn out in a longitudinal direction (fig. 70*e*). A preparation stained with Delafield's haematoxylin usually shows many old spore chains in which the individual spores are thus connected by hyaline isthmuses. Occasionally an isthmus may be found with a remnant of the old deep staining septum still unchanged in its center (figs. 16, 70*e*).

Beyond these three types of sporulation another must at least be provisionally recognized, in which septa are either absent from the developing sporogenous hyphae, or are not demonstrated by the use of ordinary stains. The protoplast appears to contract at regular intervals, yielding a series of non-contiguous spores, held together for a time by the connecting segments of evacuated filament wall (fig. 73). It is this type of sporulation which NEUKIRCH and his followers, in opposition to LACHNER-SANDOVAL, believed to prevail throughout the genus. NEUKIRCH's conclusion that septa are never involved in the sporogenesis of *Actinomyces* certainly cannot be extended to the large majority of species; and its application to any forms whatsoever is associated with some reasonable doubt. The writer is inclined to believe that cross-walls appear in the development of the sporogenous hyphae probably throughout the genus, but in some members are too thin to be recognized as distinct septa. Such an interpretation is suggested by the wide range in the thickness of septa found to occur, from the very massive structures of *Actinomyces* II, through those of moderate thickness in *Actinomyces* I, XII, and XVI, to the condition prevailing in

Actinomyces III and VIII, where cross-walls can only rarely be distinctly perceived.

All investigators, with the exception of SCHÜTZE, agree in attributing to the peripheral wall of the filament of *Actinomyces* an extreme thinness. Indeed, KRUSE (11) and others have urged the single contoured character of the membrane as an evidence of the bacterial affinity of the genus. It is only necessary to examine fungus forms like *Chlorosplenium* or *Phoma*, to convince one's self that the single contoured wall is generally characteristic of minute cells, whatever their taxonomic connections may be. Yet while the phylogenetic inference may safely be rejected, it still remains true that the peripheral wall of every species of *Actinomyces*, except possibly those of some old enlarged hyphae, cannot be made out as a distinct structure with double contour. In evacuated portions its location is indicated by only the faintest indication of its outline. Nor is this surprising when we consider that the maximum resolving power of any combination of lenses employing visible light is approximately 0.17 μ. As this magnitude barely equals the widths of the thinnest cross-walls observed, it is not difficult to suppose that, in the type of sporogenous hyphae represented in *Actinomyces* XIII, the dimensions of the partitions, like the filament wall generally, fall below the limit of visibility.

It is pertinent in this connection to emphasize the peculiarity in the nature of the cross-walls, the appearance of which in many species of *Actinomyces* initiates the development of the individual spores. Their unusual relative thickness, even in species in which they can be distinguished only with difficulty, but where nevertheless their thickness must exceed 0.17 μ, in filaments with a diameter of only 0.9 μ, is indicative of a composition essentially different from that of the peripheral wall. This indication is strengthened by the strong affinity for dyes characteristic of the septa, the evident ease with which they permit of the passage of food material, and their apparent plasticity of behavior, resulting in a median split in some species, and in others in a gradual constriction followed by a slow transformation into an attenuated isthmus.

The disappearance of the deep staining derivatives of the septa from the ends of the young spores is in some species accompanied by the appearance of one or several deep staining granules within the spore. Whether the latter represent nuclei or bodies of metachromatic material cannot definitely be determined. It seems not at all improbable, however, that some of the structures that can be differentiated within the more mature spores, particularly those characterized by uniform size and moderate staining properties (figs. 1, 2, 33, 35) are nuclei. In *Actinomyces* IV and XII they may frequently be distinguished comparatively early, before the septa, with which they alternate in regular succession, show any perceptible constriction, indicating that their existence is not related to the subsequent disposition of the partitions (fig. 67). When two of these bodies occur in the same spore they uniformly occupy opposite or diagonal positions (fig. 2, *a*, *d2*). The question arises why these bodies, if they actually represent nuclei and not structures originating *de novo*, cannot be distinguished in the young continuous sporogenous hyphae. The only explanation that can be advanced is that the protoplasm in the earlier stages is too dense to make possible any conspicuous contact between cytoplasm and nucleus. Later, with the attenuation and vacuolization of the cytoplasm that occur with the maturation of the spore, apparently as the result of the deposition of a special wall, the nucleus becomes increasingly distinct, and in some species it constitutes the only spore structure clearly visible in the stained preparation (fig. 41).

It cannot be denied, however, that granules having more the appearance of the metachromatic granules found in degenerate sterile filaments occur in the spores of some species, either alone or together with a nucleus-like body. They differ from the latter in taking a deeper stain; in having an absolutely smooth contour; in offering considerable variability in size; and when present in numbers assuming no definite orientation with reference to each other. They have been noted in those species in which the septa associated with the delimitation of spores is particularly massive; and in *Actinomyces* II (fig. 8*f*) their derivation from the excessive wall material seems reasonably well established. After the septa have separated along a median plane, the deep staining substance at each end may contract, yielding a number of spherical bodies inside of the spore. This process is probably of a more or less pathological nature, since in the usual type of development the wall material is gradually distributed through the inclosed protoplasm, causing the normal mature spore, except for the presence of a vacuole, to take an almost homogeneous stain.

Another indication of the similarity in nature existing between metachromatic material and the deep staining transverse septa of *Actinomyces* is found in the occurrence of both within peculiar large spherical structures. These structures appear generally to occupy nearly the entire lumen of the filament, and not infrequently are related to local enlargements. Occasionally, however, their diameter is considerably smaller than that of the hyphae (fig. 103). In any case they may contain either one or several peripherally located metachromatic granules, or a uniformly thick, well defined, deep staining, transverse septum, exactly median in position. It is interesting to note that whenever granules occur their surfaces in contact with the periphery of the structure represent portions of convex spherical surfaces conforming accurately to the confining surface; and whenever a septum is found traversing one of these structures considerably smaller in diameter than the filament, it does not extend into the protoplasm, but remains in its finished state as a curious partial partition.

The germination of the spores of *Actinomyces* takes place readily in dilute nutrient solutions, such as 1 per cent glucose solution, or nearly any vegetable decoction. During the first few hours of incubation at a moderate temperature they increase considerably in volume by swelling. From 1 to 4 germ tubes are then produced, apparently more or less successfully, the approximate number being, in a measure, characteristic of the species. Specific characteristics are expressed also in the diameter of the hyphae, and in the frequency of branching.

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MORPHOLOGY OF THE GENUS ACTINOMYCES. II

CHARLES DRECHSLER

(WITH PLATES II–IX)

Taxonomy of species

The obscurity that has surrounded the morphology of *Actinomyces*, besides involving the genus in improbable speculations concerning its phylogenetic relationship to the bacteria, has brought about also a most unfortunate condition in the taxonomy of the many described species. Most of the work on the genus has been done by investigators with bacteriological inclinations, and even where this has not been true, the prevalent view of the nature of these minute plants has lead to the adoption of methods scarcely applicable to mycological research. A discussion of characteristics like the occurrence of endospores, flagella, capsules, sheaths, and involution forms, cannot be regarded as constituting a morphological treatment more satisfactory for species of *Actinomyces* than for species of *Mucor* or *Boletus*.

The dependence of certain biochemical processes, particularly chromogenesis, on definite conditions of nutrition, and the conspicuous differences resulting from comparatively slight changes in the substratum, have long been noted by students of *Actinomyces*, yet descriptions of new species have continued to appear, based largely and often quite exclusively, on these variable activities. Very frequently writers have not compared their organisms with others reported by previous investigators; and in recent years there has been a tendency to disregard altogether the taxonomic contributions of the preceding decades. Moreover, while identities have frequently not been recognized where they existed, in other cases organisms have been supposed to be identical on extremely slight evidence. One of these cases that has led to an unusual measure of confusion is that of GASPERINI'S *Actinomyces chromogenus*. This species was identified by both GASPERINI and ROSSI-DORIA (19) with an organism isolated from the air by the latter and designated as *Streptothrix nigra*. In culture it was characterized by a dark brown or black discoloration of certain kinds of substrata, a reaction easily obtained on potato agar, for example, and ascribed by LEHMAN and SANO (13) to the production of tyrosinase. Until recently it has been the custom among writers to refer nearly every member of the genus showing a tyrosinase reaction to *Actinomyces chromogenus*, LUTMAN and CUNNINGHAM (14) going so far as to identify this species with the potato scab organism. This practice, which would unite forms so different in appearance and method of development as, for example, *Actinomyces* I and III, regardless of pronounced differences in size and in dextrorse or sinistrorse condition, is not defensible on morphological grounds. KRAINSKY resolved the "chromogenus" complex into 4 species; while WAKSMAN and CURTIS increased the number of derivatives to 8. Of the 17 morphologically distinct saprophytic species figured in this paper, 11 exhibit a tyrosinase reaction; and these represent less than one-fifth of the number of similarly active species which the writer had occasion to examine.

The genus awaits the attention of an investigator in a position to make a comprehensive study involving at least the larger proportion of species existing within wide geographical ranges. The summaries given later, of the more important facts about each species selected for morphological treatment, are not to be regarded as descriptions intended for taxonomic purposes.

ACTINOMYCES I

CULTURAL CHARACTERS.—On glucose agar (0.5 g. peptone, 10.0 g. glucose, 20.0 g. agar, 1000 cc. tap water) nutritive mycelium of individuals smooth, opalescent, more or less confluent; sporulation moderately slow and commencing as a light creamy zone near the periphery; no diffusible stain. On potato agar (decoction of 200 g. peeled potatoes, 2.0 g. glucose, 20.0 g. agar, 1000 cc. water) nutritive mycelium light olivaceous; sporulation moderately abundant, the raised areas where the yellowish gray fructifications are to appear being previously distinguishable by a deep brownish green coloration; guttation never copious, often absent; tyrosinase reaction moderate, but distinct.

MORPHOLOGY.—The development of the erect sporogenous hyphae of this species is strictly successive, and may be followed in the branch *d* in fig. 2, the younger of any 2 hyphae being distinguishable by its attenuated attachment. The partly disrupted chain of spores *d1* here represents the original prolongation of branch *d;* the chain *d2* represents a secondary branch, the spores here being mature but still retaining their spiral disposition without showing indications of disruption; while *d3* represents a tertiary branch, in which septation has not commenced. A similar sequence is illustrated in the succession of derivatives *b1*, *b2*, *b3*, and *b4* from the branch *b*, as well as in the 5 elements *c1–c5* associated with the branch *c*.

A more complex system of fertile hyphae is shown in fig. 1, but the larger fructifications are probably 10 or even 15 times more extensive, and bear many thousands of spores. The species is characterized by close sinistrorse spirals, of 2–6 turns, and 3–4 μ in diameter, which during the later stages of maturation are relaxed, although indications of them may persist in the flexuous or sinuous course of the mature chains of spores. The mature spores are ellipsoidal, 1–2-nucleated, with a distinctly visible wall and a central vacuole of varying size. They measure $1.2–1.4 \times 1.4–20\ \mu$, and upon germinating produce 2–4 germ tubes, which early proliferate numerous branches, and show at intervals some dark staining granules.

Isolated 5 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES II

CULTURAL CHARACTERS.—On glucose agar, growth moderate; nutritive mycelium colorless, early covered with a cretaceous or downy aërial mycelium; pigment absent. On potato agar, development of nutritive mycelium moderately rapid; aërial mycelium appearing in scattered areas, first white, later becoming slightly discolored; substratum stained yellow by a soluble pigment; tyrosinase reaction absent.

MORPHOLOGY.—The most conspicuous feature of this species is the extraordinary thickness of the septa ($0.3–0.35\ \mu$) associated with spore production, and their insertion at distensions in the sporogenous hyphae. A comparison of branch *c1* with the younger branch *c2* (fig. 5) corresponding to the conditions shown diagrammatically in figs. 8*b* and 8*c* respectively, shows that the growth in thickness of the hyphae takes place subsequent to the appearance of the septa. After the filament has attained its growth, the septa split along a median plane (figs. 5*a*, 5*c1*, 8*d*), and the 2 halves are drawn apart by the contraction of the delimited protoplasts. Further maturation occurs in the distribution of the deeply staining wall substance, in the strengthening of the peripheral wall, and by an enlargement of the latter, the elimination of the median, hourglass-like constriction of the spore, resulting finally in an approximately cylindrical structure measuring $0.7–0.9 \times 0.8–1.1\ \mu$.

The terminal and the basal spores of each chain retain a somewhat asymmetrical shape, owing to the absence of the massive septum at one of their ends. By an apparently abnormal development, metachromatic granules may be formed in the spores derived from some hyphae, resulting in a condition illustrated in the lowest spore in fig. 8*f*.

The axial filaments are represented by long prostrate hyphae, branching at irregular, long intervals. Septation is confined to the fertile branches. The sterile hyphal portions below the sporogenous terminations taper gradually toward their attenuated attachments. Development and sporogenesis near the axial terminations are successive, and involve the formation of close sinistrorse spirals of 1–5 turns, $3.5–6.0\ \mu$ in diameter.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES III

A. lavendulae Waksman and Curtis

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium slightly yellow on reverse side, central area completely covered with velvety aërial mycelium, first white but gradually assuming a beautiful lavender shade; no soluble stain. On potato agar, growth very profuse; mycelium abundant, changing from white to lavender; guttation moderate to profuse; tyrosinase reaction vigorous.

Identity with *A. lavendulae* established by comparison of cultural and morphological characteristics.

MORPHOLOGY.—The mycelium consists of long prostrate axial filaments, branching rarely except at the end. Sporulation is usually initiated at the tip of the filament, and proceeds basipetally by the insertion and transformation of almost invisible septa, to the point of attachment of the first sporogenous branch (figs. 10, 11). The sporogenous branches are rarely crowded, although at the base of the sporogenous axial termination an opposite arrangement is not uncommon. Secondary branching occurs frequently; septa are entirely absent, except when associated with the progressive basipetal delimitation of spores.

The sporogenous hyphae terminate in dextrorse, moderately compact spirals of 4–12 turns, 2.0–3.8 μ in diameter. The spores are ellipsoidal, with nuclei not readily demonstrable. Metachromatic material occurs abundantly in many old hyphae (fig. 15).

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES IV

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless; aërial mycelium moderately profuse, velvety in appearance, changing from white to smoky blue; no guttation. On potato agar, tyrosinase reaction vigorous; aërial mycelium first produced white, not subsequently much discolored, becoming matted to the substratum as a result of excessive guttation, and later completely overgrown by a loose growth of smoky blue secondary mycelium.

MORPHOLOGY.—The sporogenous branches with dextrorse spirals of 2–12 open turns, 1.5–2.5 μ in diameter, are attached to the long axial filaments usually at wide intervals, in a loose racemose arrangement. Secondary branching, although rare, occurs occasionally, and is then associated with simultaneous sporulation (fig. 18). Development of the 1–2-nucleated spores, 0.7–0.8 $\times$ 0.9–1.1 μ, proceeds by the insertion of conspicuous septa, followed by their constriction and subsequent conversion to hyaline isthmuses (figs. 70*a–e*). Two germ tubes are usually produced, of a more or less uniform diameter, and proliferating branches at relatively wide intervals.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES V

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium on reverse side slightly yellowish; the surface completely covered with a luxuriant velvety or cottony weft of pinkish-yellow aërial mycelium; guttation slight. On potato agar, nutritive mycelium chocolate-colored, firm lichenoid, crimped around margin; aërial mycelium, as on glucose agar, but less profuse; tyrosinase reaction vigorous.

MORPHOLOGY.—The fertile hyphae, which are attached to prostrate axial filaments at long intervals, are terminated by relatively close sinistrorse spirals of 4–12 turns 2.0–4.0 μ in diameter, developing spores (0.6–0.8×0.9–1.1 μ), like *Actinomyces* IV, by the insertion of conspicuous septa, followed by their constriction and conversion. A peculiar characteristic is found in the sterilization of the basal portion of the fertile hyphae, by an apparent abortion of its lower potential spores.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VI

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, completely covered with a felty aërial mycelium, first white, later assuming a deep smoky tinge. On potato agar, nutritive mycelium excessively wrinkled, partially covered with a slightly discolored aërial mycelium; tyrosinase reaction vigorous.

MORPHOLOGY.—The species appears closely allied to *Actinomyces* V, differing from the latter chiefly in the absence of any evidence of sterilization, and in the shorter length of its sinistrorse sporogenous spirals, which consist of only 2–6 turns, 2.0–4.0 μ in diameter. The spores are uninucleated, measure 0.7–0.8×0.9–1.1 μ, and are developed by the insertion and transformation of conspicuous septa. Fertile hyphae are attached to the axial filaments with considerably greater frequency, and secondary branching, characterized by the successive type of development, is common.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, early developing an aërial mycelium from the center outward, the latter changing from white to light gray with increasing age. On potato agar, nutritive mycelium luxuriant, developing rapidly; aërial mycelium represented by a slight cretaceous development toward the top of the slant; tyrosinase reaction vigorous.

MORPHOLOGY.—This species departs from the main trend of the 3 preceding forms in the relatively close arrangement of its branches on the axial filament, and in the elaboration of these branches by further ramifications in a typically successive order. Nearly spherical to ellipsoidal spores, $0.6–0.8 \times 0.7–1.0\ \mu$, are produced from moderately close sinistrorse spirals of 3–8 turns $2.0–3.0\ \mu$ in diameter, by the development represented in fig. 70*a–e*, but the septa are relatively thin, and occasionally fall below the limit of clear visibility.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VIII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium nearly colorless, secreting a diffusible yellow pigment; aërial mycelium moderately profuse, velvety, first white, later changing to a light bluish color. On potato agar, growth similar, but soluble pigment absent; no tyrosinase reaction.

MORPHOLOGY.—The fertile hyphae of this species may be attached to the axial filaments in a diffuse racemose arrangement (fig. 46), or crowded in a compact capitate system. The swellings in the axial filaments in figs. 43, 44, 45, and 46 at the bases of sporogenous branches indicate the mode of origin of the *Leptomitus*-like distensions shown in figs. 47 and 48.

The small, ellipsoidal, uninucleated spores, 0.5–0.6×0.6–0.8 μ, are formed from close, sinistrorse spirals of 2–10 turns 1.2–2.5 μ in diameter. Indications of septa can be seen only rarely. The mature spore chains upon collapsing cohere in irregular zooglœa-like masses, a peculiarity of behavior dependent probably on a gelatinization of wall material. Upon germination, 1 or 2 tubes are produced, relatively thick and abundantly branching.

Isolated 6 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES IX

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, forming no soluble pigment; aërial mycelium at first white, becoming light smoky blue in the course of a few days. On potato agar, cultural characters similar, but growth more profuse, guttation moderate, discoloration of aërial mycelium more rapid; tyrosinase reaction absent.

MORPHOLOGY.—The most characteristic feature of this species is the greater thickness of the fertile hyphae below the second turn of the spiral. The latter are sinistrorse, usually with very close turns, varying in number from 1 to 16, and measuring 1.5–2.0 μ in diameter. They give rise to ellipsoidal, uninucleated spores, 0.5–0.7×0.6–1.0 μ, without the appearance of clearly visible septa. It seems highly probable that cross-walls nevertheless occur, since occasionally a median partition may be differentiated in the hyaline attentuated connections between two spores (fig. 51), suggesting a development similar to that indicated in fig. 70*a–e*.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES X

Streptothrix alba Rossi-Doria; *Actinomyces griseus* Krainsky (?)

CULTURAL CHARACTERS.—On glucose agar, growth poor and not characteristic. On potato agar, growth excessively rapid, nutritive mycelium colorless; aërial mycelium firm, white, changing rapidly to a yellowish gray; secondary growth occurring in the formation of numerous successive rings of sporodochia, or in the development of cottony white masses of mycelium from below the thick crust of old mature spores; tyrosinase reaction absent; substratum stained a faint greenish yellow in old cultures.

MORPHOLOGY.—According to WAKSMAN and CURTIS, the aërial filaments of this species possess only a slight tendency to branch. The writer was led to a somewhat different conclusion, as the axial hyphae are usually found to proliferate fertile branches at moderately close intervals. Occasionally, as in fig. 58, indications of a successive sequence may be observed, but more frequently the development of the different elements of a ramifying system occurs without any recognizable interrelation. The short, cylindrical spores, $0.7 \times 0.7-1.0\ \mu$, are formed, as in *Actinomyces* XVI, by a septation of the fertile hyphae, followed by splitting of the partitions along a median plane, but the septa are usually less conspicuous, and often not clearly visible, and the fertile hyphae show no indication of a spiral condition.

A striking dimorphism characterizes the mycelium of this species, as well as that of a number of other forms observed by the writer. The deeper sterile aërial hyphae below the sporogenous layer typically are extremely minute, with a diameter frequently not exceeding $0.3\ \mu$; their protoplasmic contents show little affinity for stains; and the contours of their walls are uniformly smooth. The more superficial hyphae, which usually attain a thickness of $1.0\ \mu$, and are distinguishable by markedly irregular contours, contain dense deep staining protoplasm; and when septa are present, they are sometimes associated, as in *Actinomyces* XVII and XVIII, with spherical structures. The thicker filaments bear the sporogenous branches, and, in general, appear to constitute the expanded prolongations of the minute hyphae (fig. 59).

Isolated twice from soil collected in Cambridge, Massachusetts; once from tap water; very frequently from outdoor air; several times from gross cultures of dead leaves; 4 times from horse dung undergoing fermentation at 50–60° C.

SYNONOMY.—In his description of *Streptothrix alba*, ROSSI-DORIA records two characteristics that establish its identity beyond much danger of confusion: a conspicuous preponderance in number over any of its congeners on plates exposed to the air, and a tendency toward the formation of concentric rings more pronounced than that of any other species. ROSSI-DORIA attributed this preponderance in the air to its omnivorous character, enabling it to develop on a large variety of substrata, "Questa *Streptothrix* cresce, si può dire, dappertutto, tanto su terreni di natura vegetale quanto su terreni di natura animale. E per ciò nonchè la grande sua produzione di spore, che essa si trova cosi diffusa nell'aria ed altrove. Pare che essa possa svilupparsi anche nel terreno." In spite of this fortunate and quite distinctive characterization, the specific term "albus" subsequently came to be used in a manner as miscellaneous as "chromogenus," being applied generally to any type with a light mycelium showing no tyrosinase reaction.

The same species was treated in the publication of WAKSMAN and CURTIS as *Actinomyces griseus* Krainsky. I have not been able to satisfy myself fully about the identity of KRAINSKY'S organism; nor would it seem possible to reach any definite conclusion without an examination of authentic material.

ACTINOMYCES XI

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium first colorless, becoming slightly reddened with increasing age; aërial mycelium first white, rapidly changing to a bluish violet. On potato agar, nutritive mycelium gradually becomes deep red by the slow accumulation of a slightly diffusible pigment; tyrosinase reaction absent.

MORPHOLOGY.—More or less erect fructifications are developed along the distal portions of long prostrate filaments. Branching is abundant and only occasionally shows indications of a successive sequence. The aërial hyphae in the dendroidic structures (figs. 64, 66) are often conspicuously vacuolate, especially in the inflated distensions from which a number of fertile branches arise. The latter terminate in sinistrorse spirals of 4–6 turns, 2.0–$3.0\ \mu$ in diameter, from which, by the insertion of conspicuous septa and their subsequent transformation to hyaline isthmuses, spores 0.5–0.7×1.0–$1.2\ \mu$ are produced.

Isolated once from soil collected in Cambridge, Massachusetts; identical with an organism isolated by Mr. H. J. CONN from soil collected near Geneva, New York.

ACTINOMYCES XII

A. aureus Waksman and Curtis

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium yellowish on reverse side; aërial mycelium changing from white to pale yellowish gray; soluble stain absent. On potato agar, nutritive mycelium darker on reverse side; aërial mycelium more profuse, forming a somewhat more deeply colored felty layer; tyrosinase reaction moderate. Identity with *Actinomyces aureus* established by comparison with authentic material of the latter.

MORPHOLOGY.—In this species long prostrate filaments terminate in more or less erect fructifications. Secondary branches are proliferated from the lateral elements, generally in successive sequence. A more or less pronounced cuneate thickening of the hyphae below the insertion of a branch is characteristic of the species. The ellipsoidal, uninucleated spores, $0.5–0.7 \times 0.8–1.2\ \mu$, are formed by the insertion of conspicuous septa in open, sinistrorse spirals of 2–7 turns, $3.0–4.0\ \mu$ in diameter.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XIII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium light orange-brown, the separate individuals fused into a massive pellicle with a depressed, crimped margin. On potato agar, nutritive mycelium dark chocolate-brown, wrinkled, lichenoid, secreting a diffusible red pigment; tyrosinase reaction absent. Aërial mycelium on both substrata loose, cottony; developing slowly, first white, later changing to a dull bluish tint.

MORPHOLOGY.—The aërial mycelium consists of extremely long filaments, which rarely show any evidence of branching (figs. 74 75), and toward their terminations follow an undulating or slightly spiral course. Sporulation occurs as the result of protoplasmic contractions without the appearance of visible septa, the chains of cylindrical spores, $0.4 \times 1.2–1.6\ \mu$, being held together for some time by the evacuated portions of hyphal wall, that seem to undergo no apparent constriction.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XIV

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium usually colorless, but frequently becoming deep brown or black; aërial mycelium consisting of a dense velvety weft, first white, later changing to a creamy yellow. On potato agar, growth similar; tyrosinase reaction absent.

MORPHOLOGY.—This species is characterized by the production of extensive prostrate fructifications through the proliferation of numerous lateral branching processes from long axial filaments (figs. 76, 79, 81). A septum is occasionally present immediately above the attachment of a branch, but more frequently is absent. Secondary ramifications, resulting in more or less complex elements, take place without reference to the stage of sporogenesis in the proliferating branch. The ellipsoidal uninucleated spores, $0.5-0.7 \times 0.8-1.2\ \mu$, are derived from sinistrorse spiral hyphae of 1–8 turns, $2.0-4.0\ \mu$ in diameter, by the insertion and transformation of relatively thin septa, or without the appearance of demonstrable septa.

Isolated 4 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XV

CULTURAL CHARACTERS.—On glucose or potato agar, nutritive mycelium opalescent; aërial mycelium first white, becoming only slightly discolored with age; tyrosinase reaction moderate.

MORPHOLOGY.—Microscopically this species closely resembles *Actinomyces* IV, differing from the latter chiefly in the abundant proliferation of branches of the second or of a higher order. The lateral elements thus formed follow the successive type of development (figs. 82, 83). The uninucleated spores, $0.7 \times 0.9-1.0\ \mu$, are formed from dextrorse spiral hyphae of 3–12 turns, $1.8-2.5\ \mu$ in diameter, by the constriction of conspicuous septa, and their transformation into hyaline isthmuses.

Isolated twice from soil collected in Manhattan, Kansas.

ACTINOMYCES XVI

CULTURAL CHARACTERS.—On glucose agar, growth very meager; never producing an aërial mycelium. On potato agar, development rapid; nutritive mycelium dark brown or greenish brown; aërial mycelium profuse, changing from white to violet or pinkish gray; guttation profuse; tyrosinase reaction moderate.

MORPHOLOGY.—In this species the characteristic development consists in the proliferation of a number of long branches in an irregular whorl from a long and somewhat thickened axial filament. Secondary branching is common, but usually more or less remote. Vacuoles associated with hyphal distensions are found in the axial filaments and in the main branches, and metachromatic granules occur abundantly in many of the older sterile hyphae (fig. 91). The long cylindrical spores, $0.6–0.7 \times 1.0–2.0\ \mu$, are formed by the septation of sporogenous hyphae that terminate in open, sinistrorse spirals of 2–3 turns, $4.0–5.5\ \mu$ in diameter, followed by the splitting of the septa along a median plane, and the separation of the two halves by a contraction of the delimited protoplasts. The progress of sporogenesis is usually basipetal, but not infrequently the first divisions may result in a number of segments of varying lengths, which by subsequent divisions are reduced to the magnitude of the ultimate spores.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XVII

A. scabies (Thaxter) Güssow (6)

MORPHOLOGY.—The aërial mycelium of this species, which is one of the largest of dextrorse forms, consists of long prostrate filaments on which lateral branches are inserted at short intervals. Secondary branching is abundant and usually associated with a successive order of development (figs. 93*a1–a3*). The more or less cylindrical spores, $0.8–0.9 \times 1.3–1.5\ \mu$, are developed from dextrorse spiral hyphae of 3–14 turns, $2.0–3.5\ \mu$ in diameter, by the insertion of conspicuous septa and their subsequent splitting along a median plane. In many hyphae the septa before their division can be seen to occupy a transverse equatorial position in the peculiar spherical structures to which reference has been made elsewhere, and which here occupy slight but perceptible hyphal distensions (figs. 92, 93*h1*, 93*h2*, 101*cy*). Whenever the spherical structures are absent, the fertile hyphae are uniformly isodiametric. It is not certain whether these structures appear in all sporogenous branches at some time preceding the contraction of the delimited protoplasts, or are more or less accidental in their occurrence. They also are found associated with septa in the sterile axial filaments, and here similarly occupy local hyphal distensions. After the individual spores have become separated, the connecting segments of evacuated hyphal wall contract slightly to form somewhat narrowed isthmuses, which persist until the mature spore chains are disrupted. In germinating, the spore usually produces 1 or 2 germ tubes.

The preparation from which figs. 92–101 were drawn was derived from one of 5 organisms communicated by Mr. M. SHAPAVALOV, who writes that "all were tested in inoculation experiments in 1912–1913, and proved to be pathogenic." Three of the other organisms were found to be identical morphologically with the one figured in plate VIII, while the fifth did not produce an aërial mycelium sufficiently profuse to permit of a satisfactory microscopic examination, although the general appearance of the culture indicated that it also is identical with *Actinomyces* XVI.

ACTINOMYCES XVIII

CULTURAL CHARACTERS.—On glucose agar, growth meager; nutritive mycelium colorless; aërial mycelium slow to develop, first white, later showing slight discoloration; diffusible pigment absent. On potato agar, development very rapid; nutritive mycelium dark; aërial mycelium profuse, felty, bluish gray; guttation moderate; tyrosinase reaction vigorous.

MORPHOLOGY.—This species is characterized by an unusual degree of variability in its fructifications. In figs. 102 and 107 is represented a relationship between axial filament and sporogenous branches common to many members of the genus. Fig. 108 shows a slight departure from this type in the thickening of the subterminal portion of the axial filament bearing the spiral branches. Further departures are expressed in the tufted grouping of the spiral hyphae in fig. 104, and in the distended and extremely vacuolated condition of the axial filament in fig. 106. A strikingly aberrant type is seen in fig. 103, the fertile branches being short, inserted at close, irregular intervals, and showing no spiral tendency; while the axial filament is thick and abounding in spherical structures containing either deposits of metachromatic material or a partial equatorial septum.

In the dextrorse spiral hyphae of 1–8 open turns, 2.0–3.0 μ in diameter, the ellipsoidal spores, 0.8–0.9 $\times$ 1.0–1.6 μ, are produced by the insertion of conspicuous septa, sometimes in association with spherical structures. The presence of the latter (fig. 106), however, is not here indicated by local distensions. Subsequently the cross-walls undergo constriction and conversion to narrow connecting isthmuses. In the aberrant fertile hyphae (those without any spiral tendency), sporogenesis appears to take place in a more miscellaneous manner. Definite septa can rarely be distinguished, the spores seeming to result from protoplasmic contractions.

Isolated once from soil collected in Cambridge, Massachusetts.

Summary

1. The vegetative thallus of *Actinomyces* consists of a mycelium composed of profusely branching hyphae, the terminal growing portions of which are densely filled with protoplasm. Toward the center of the thallus the vacuoles increase in size and may be associated with the presence of metachromatic granules, the latter having nothing in common with bacterial endospores or "micrococci," for which they were mistaken by early observers.

2. The vegetative mycelium attains an extent incomparably greater than the branching figures recorded for bacteria of the acid-fast group, and the hyphae lack the uniformity in diameter generally characteristic of the Schizomycetes.

3. The aërial mycelium produced on suitable substrata by most species occurs usually in the form of a mat of discrete fructifications; but in other species these fructifications are frequently combined to form numerous and peculiar erect Isarioid sporodochia.

4. In any case each individual fructification represents a well characterized sporogenous apparatus, consisting of a sterile axial filament bearing branches in an open racemose or dense capitate arrangement. The primary branches may function directly as

sporogenous hyphae, or may proliferate branches of the second and of higher orders, sporogenesis in the latter case being confined to the terminal elements, the hyphal portions below the points of attachment of branches remaining sterile.

5. Two tendencies in the development of fructifications are recognizable: one leading to an erect drendroidal type, in which successively proliferated fertile elements undergo processes of sporogenesis in continuous sequence; and the other leading to a prostrate racemose type, in which sporogenesis is delayed in the older branches until the younger branches have also attained their final extension. The majority of species show these tendencies combined in different ways.

6. The sporogenous hyphae of most species are coiled in peculiar spirals, sometimes resembling the spores of the hyphomycetous genus *Helicoön*. These spirals exhibit pronounced specific characteristics in the number, diameter, and obliquity of their turns, and especially in the direction of rotation (whether dextrorse or sinistrorse).

7. Sporogenesis, where it can be followed, begins at the tips of the fertile branches and proceeds basipetally. In the larger number of species the process involves the insertion of septa which, in certain cases, are relatively very massive, and in others so thin as to be barely discernible. The disposition of these septa, while the delimited spores undergo maturation processes, varies with the species: (1) they may remain more or less unaltered; (2) they may suffer a median split, the two resulting halves being then separated as the result of the longitudinal contraction of the young spores, leaving alternate portions of hyphal walls completely evacuated; or (3) they may first become considerably constricted and subsequently converted into non-stainable isthmuses connecting the mature spores. The apparent absence of septa in the sporogenous hyphae of other forms is perhaps attributable to optical difficulties.

8. Granules are readily differentiated in the spores of many species which possess the staining properties and uniformity of size characteristic of nuclei; they generally occur singly, but in the larger spores of a few forms two are often found occupying diagonally opposite positions.

9. As in the vegetative thallus, metachromatic granules occur in the aërial mycelium, being very rarely found in the spores or sporogenous hyphae, but becoming very abundant in degenerate sterile hyphae.

10. The older axial filaments of some species show marked distensions which, in extreme cases, result in figures simulating *Leptomitus*. These arise as local distensions at the points of attachment of the more extensive lateral sporogenous processes. Cuneate modifications of the sterile axial filaments below the origins of branches also occur.

11. Curious spherical structures appear regularly in some forms, both in the sterile axial hyphae, where they may contain either a median septum or a number of peripheral metachromatic granules, and in the sporogenous hyphae, where they are associated with the regularly spaced septa.

12. The spores germinate readily in suitable solutions, producing 1–4 germ tubes, the approximate number being more or less characteristic of the species.

13. Owing to the absence of any well defined bacterial characteristics, the writer is of the opinion that the view that *Actinomyces* represents a transition between the Hyphomycetes and the Schizomycetes, as well as the phylogenetic corollary based upon it, may safely be abandoned. If mere size is to be regarded as important, it would appear to be equally profitable to look for bacterial affinities in some ascomycetous and sphaeropsideaceous forms, the hyphae of which are similarly very minute. It is doubtful whether far-reaching taxonomic generalizations can be based on the "acid-fast" staining reaction, especially as this reaction has not played a very important rôle in mycological research. There seems to be no adequate reason why the genus should not be classed in an unqualified manner with the Hyphomycetes, as a mucedineous group with tendencies toward an erect Isarioid habit.

The writer wishes to acknowledge his indebtedness to Professor R. THAXTER, under whose direction this work was done; to Professor W. G. FARLOW for the use of books; and to Professor B. FINK for samples of soil collected on the islands of Porto Rico and

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EXPLANATION OF PLATES II–IX

All except figs. 7, 8, 70, and 101, which are semidiagrammatical representations with a magnification of approximately 8000, were drawn with the aid of a camera lucida with a magnification of 2750.

PLATE II

Actinomyces I

FIG. 1.—Moderately well developed fructification.

FIG. 2.—Somewhat smaller fructification showing successive order of development: *a*, chain of spores, largely disrupted, developed from termination of axial hypha; *b*, *c*, *d*, secondary branches that have given rise respectively to series of elements *b1–b4*, *c1–c5*, and *d1–d3*.

FIG. 3.—Spore germinating with 4 germ tubes.

Actinomyces II

FIGS. 4–6.—Portions of aërial mycelium, showing conspicuous septa in fertile branches, and relation of latter to axial filaments: *a*, *b*, *c*, branches proliferated successively from same filament; *b1–b3*, *c1–c3*, elements proliferated successively from branches *b* and *c* respectively.

FIG. 7.—Portion of branch *c* (fig. 5) showing attachment of successively formed spiral elements.

FIG. 8*a–f*.—Successive stages in development of fertile hypha.

Actinomyces III

FIGS. 9–14.—Portions of aërial mycelium.

FIG. 15.—Portion of degenerate hypha containing abundance of metachromatic material.

PLATE III

Actinomyces IV

FIG. 16.—Short chain of spores showing nuclei, and 2 deep staining remnants of constricted septa in hyaline isthmuses between spores.

FIGS. 17–20.—Portions of aërial mycelium.

FIG. 28.—Spore germinating with 2 germ tubes.

Actinomyces V

FIG. 21.—Aërial hypha with spiral termination and 2 fertile branches, more mature elements showing failure of spore to develop in proximal portion.

FIG. 22.—Aërial hypha with 2 spiral elements.

FIG. 23.—Young spiral branch of 15 turns attached to axial hypha containing metachromatic granules.

FIGS. 24–26.—Spiral branches soon after insertion of septa, showing cross-walls absent from portion above basal septum.

FIG. 27.—Young spiral branch.

FIGS. 29, 30.—Spiral branches with spores mature, and non-septate portion completely evacuated.

FIGS. 31, 32.—Degenerate filaments containing much metachromatic material.

Actinomyces VI

FIG. 33.—Portion of aërial mycelium showing 2 spiral elements with nuclei in mature spores of one; septum in axial filament associated with basal septum in branch.

FIG. 34.—Similar to fig. 33, but without visible nuclei.

FIGS. 35–37.—Other portions of aërial mycelium.

Actinomyces VII

FIGS. 38–40.—Portions of aërial mycelium with sporogenous branches in various stages of development.

PLATE IV

Actinomyces VIII

FIG. 41.—Sporogenous apparatus with mature spores cohering in zoogloea-like masses.

FIG. 42.—Prostrate hypha containing numerous metachromatic granules and bearing a branch with many crowded spiral ramifications.

FIG. 43.—More open type of sporogenous apparatus with lateral elements attached to axial hypha at intervals.

FIG. 44.—Young sporogenous apparatus with spiral branches more or less crowded.

FIG. 45.—Somewhat older system of spiral hyphae, some of which have become converted into zoogloea-like masses of spores.

FIG. 46.—Lateral element bearing 8 spiral branches.

FIGS. 47, 48.—Portions of degenerate mycelium showing *Leptomitus*-like enlargements occupied by vacuoles, and metachromatic granules in constrictions.

FIGS. 49, 50.—Spores germinating with 1 germ tube.

Actinomyces IX

FIG. 51.—Portion of aërial mycelium showing spiral termination converted into chain of uninucleated spores, and presence of remnants of septa in hyaline isthmuses.

FIG. 52.—Similar to fig. 51, but without indications of septa between spores.

FIG. 53.—Portion of aërial mycelium showing septa in axial filament above insertions of some sporogenous branches.

FIG. 54.—Sporogenous branches with portion below second turn of spiral conspicuously thickened.

FIG. 55.—Sporogenous branch of 11 turns.

FIG. 56.—Spore germinating with 1 germ tube.

FIG. 57.—Sporogenous branch of 15 close spiral turns.

Actinomyces X

FIGS. 58–60.—Portions of aërial mycelium.

FIGS. 61, 62.—Spores germinating with 1 and 2 germ tubes respectively.

PLATE V

Actinomyces XI

FIGS. 63, 64.—More or less erect fructifications terminating long prostrate filaments, showing origin of groups of sporogenous branches from local hyphal distensions occupied by conspicuous vacuole.

FIGS. 65, 66.—Intermediate portions of aërial mycelium.

FIG. 113.—Spore germinating with 1 germ tube.

Actinomyces XII

FIGS. 67, 68.—Erect fructifications terminating long prostrate aërial filaments, exhibiting a pronounced tendency toward successive type of development, and showing cuneate hyphal enlargements below insertions of branches.

FIG. 69.—Intermediate portion of aërial mycelium.

FIG. 70*a–e*.—Progressive stages in development of sporogenous hypha, occurring in this and numerous other species.

FIG. 71.—Spore germinating with 3 germ tubes.

PLATE VI

Actinomyces XIII

FIG. 72.—Two portions, *a* terminal, *b* subterminal, of one long, unbranched, continuous sporogenous hypha showing very slight spiral tendency.

FIG. 73.—Chain of spores with deep staining polar granules.

FIGS. 74, 75.—Portions of aërial mycelium showing branching.

Actinomyces XIV

FIGS. 76–81.—Portions of aërial mycelium showing arrangement of sporogenous branches on hyphae, and method of sporulation.

PLATE VII

Actinomyces XV

FIG. 82.—Portion of aërial mycelium: elements *a1–a3* and *b1–b3* successively proliferated from branches *a* and *b* respectively.

FIG. 83.—Sporogenous branch *a* with 2 secondary branches; younger (*a3*) associated with a septum above insertion (successive type); older (*a2*) not set off by septum.

FIG. 84.—Axial filament with 3 branches, bearing successively poliferated elements *a1–a3*, as well as branch *ax*, latter not associated with septum in primary branch above point of attachment.

FIG. 85.—Fructification developed entirely in successive sequence, with 2 chains of uninucleated spores.

FIGS. 86–88.—Spores germinating with 2 germ tubes.

Actinomyces XVI

FIG. 89.—Large fructification consisting of axial filament *a–a1*, with whorl of 5 primary branches *a2–a6*, each bearing 1 or more secondary branches.

FIG. 90.—Spiral termination of sporogenous branch.

FIG. 91.—Old filament containing many metachromatic granules.

PLATE VIII

Actinomyces XVII

FIG. 92.—Portion of aërial mycelium showing spherical structures associated with septa in local distensions of sporogenous branch.

FIGS. 93, 94.—Portions of aërial mycelium, some lateral elements bearing secondary branches (indicated by numerals above 1) developed successively; 94*c*, unusually long fertile branch.

FIG. 95.—Portion of aërial mycelium similar to one shown in fig. 92.

FIGS. 96–100.—Spores germinating with 1 or 2 germ tubes.

FIG. 101*a–e*.—Successive stages in development of sporogenous branch, *cx* and *cy* representing either alternative or probably successive stages.

PLATE IX

Actinomyces XVIII

FIG. 102.—Sporogenous branch of usual type soon after appearance of septa.

FIG. 103.—Portion of fructification bearing aberrant fertile branches without spiral terminations.

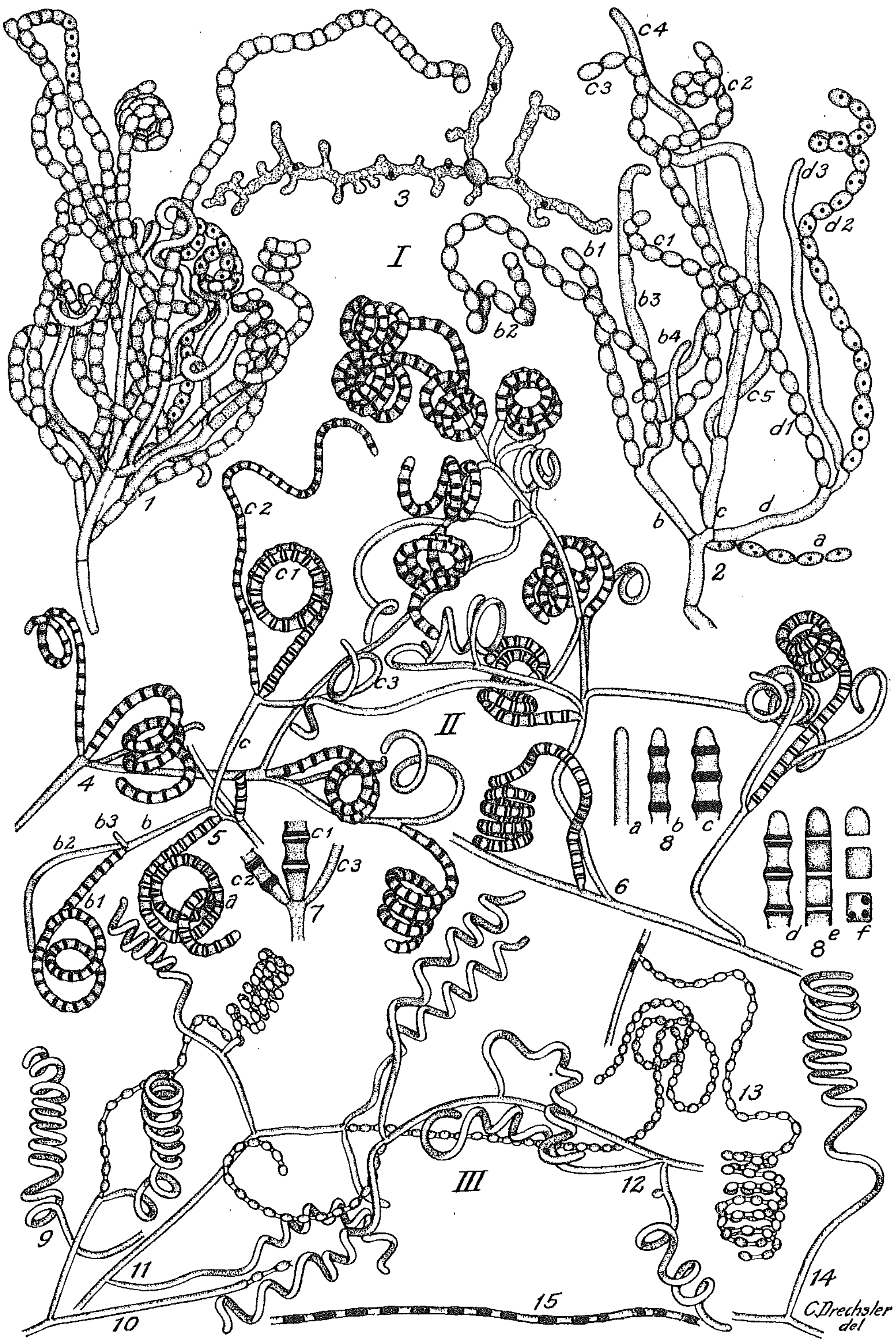
FIG. 104.—Aërial filament with several spiral branches borne terminally.

FIG. 105.—Chain of mature spores developed from branch of spiral type.

FIG. 106.—Degenerate axial filament containing large vacuoles and spherical structures and bearing a fertile spiral branch.

FIGS. 107, 108.—Portions of aërial mycelium showing fertile hyphae of spiral type.

FIGS. 109–112.—Spores germinating with 1 or 2 germ tubes.



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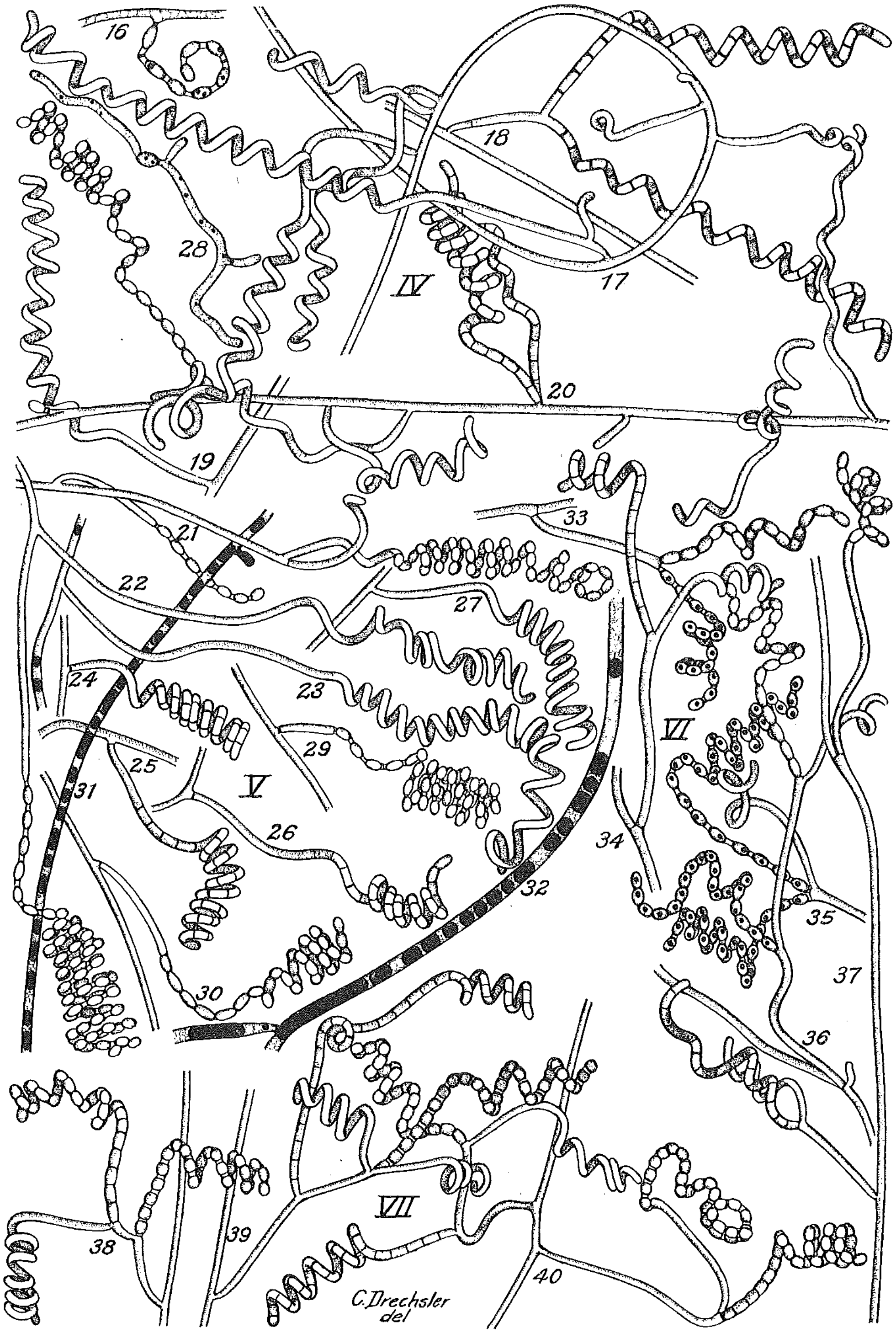

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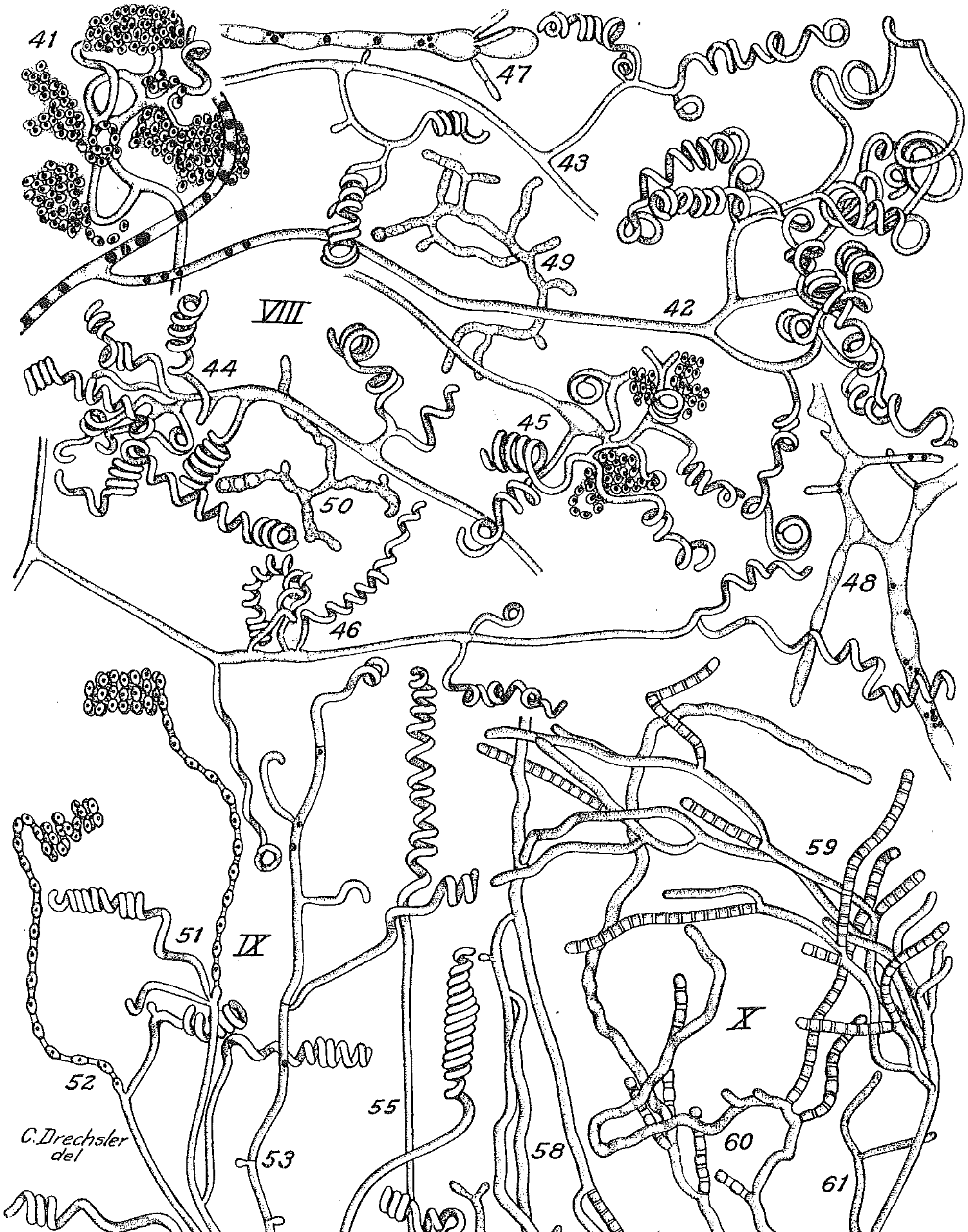

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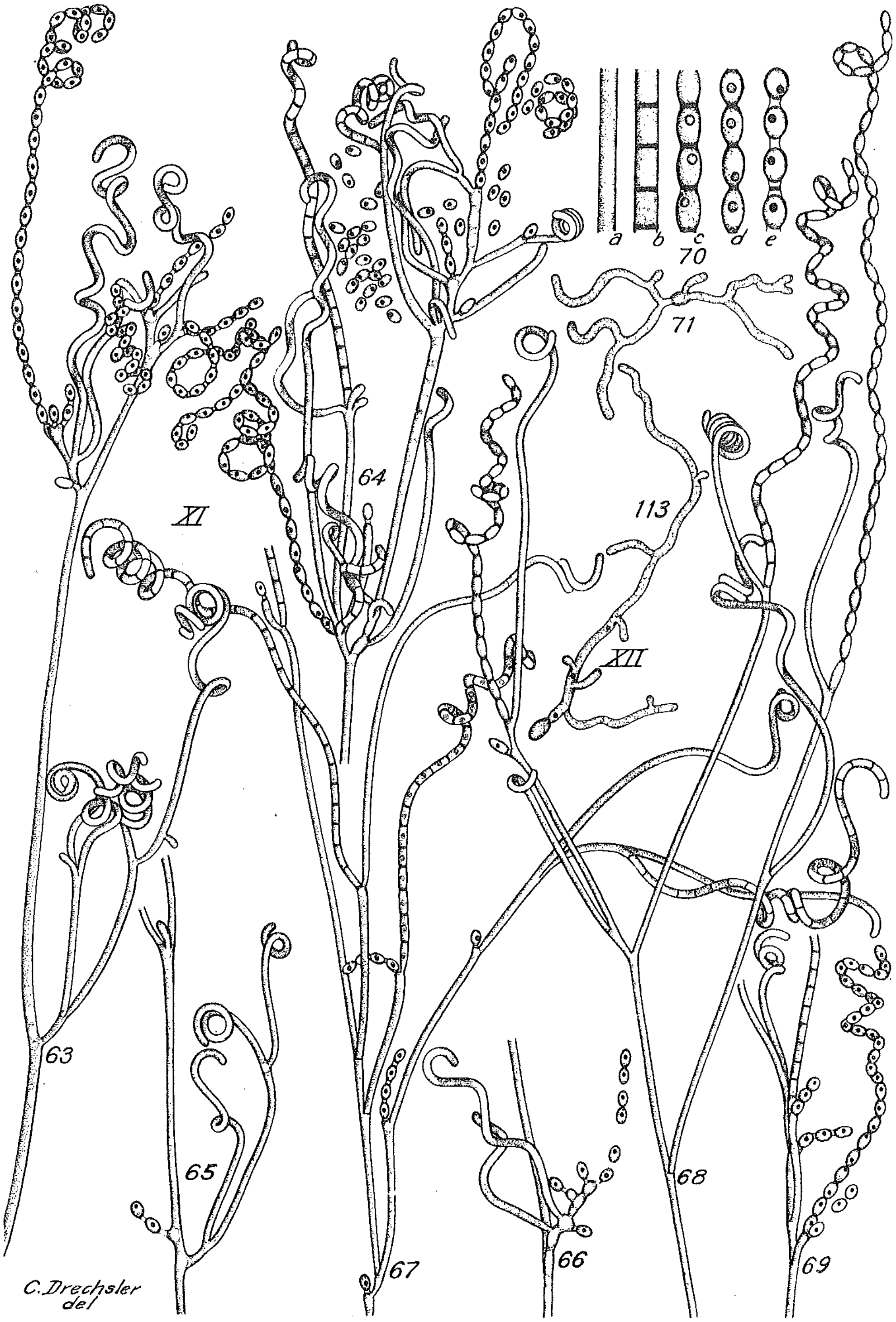

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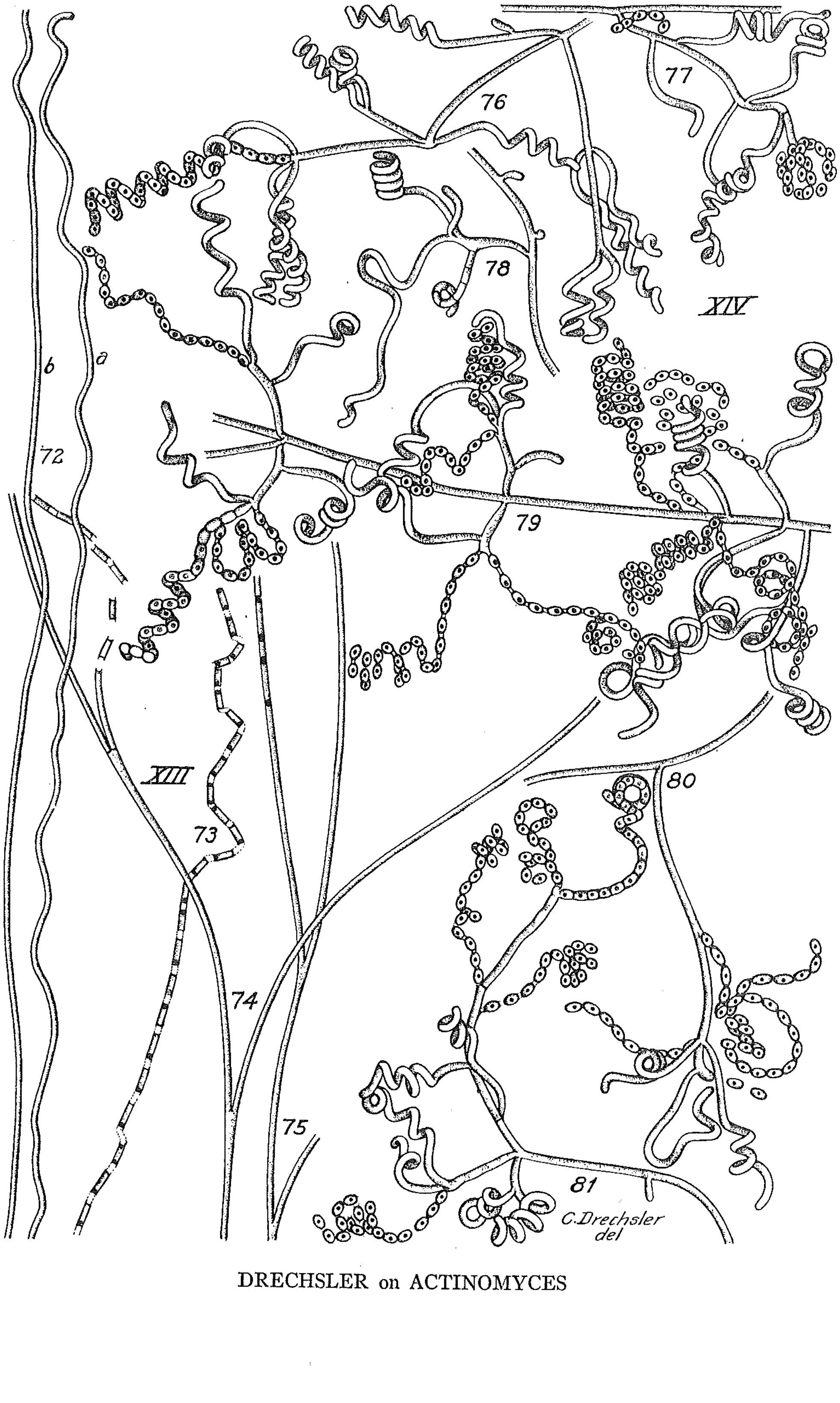

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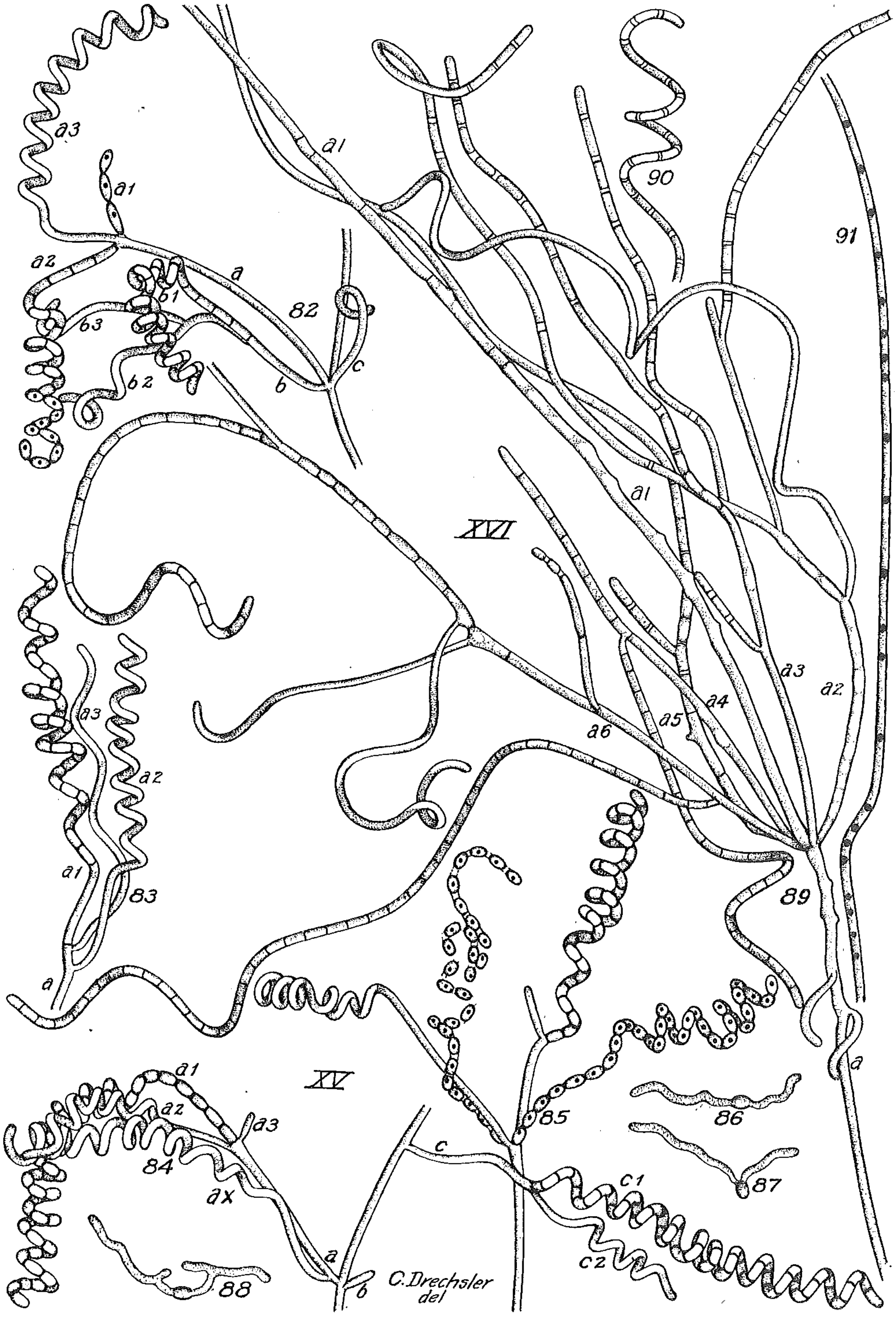

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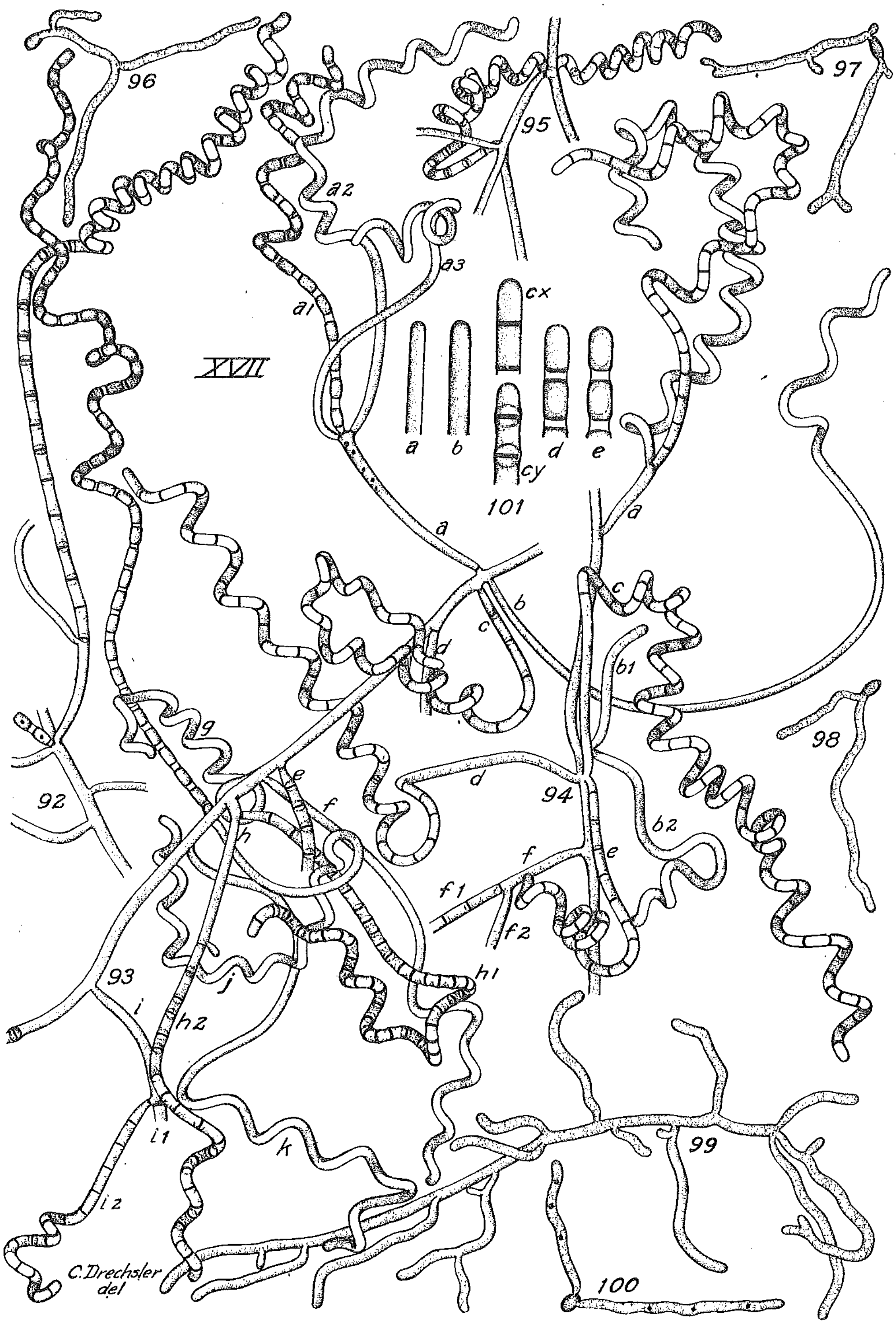

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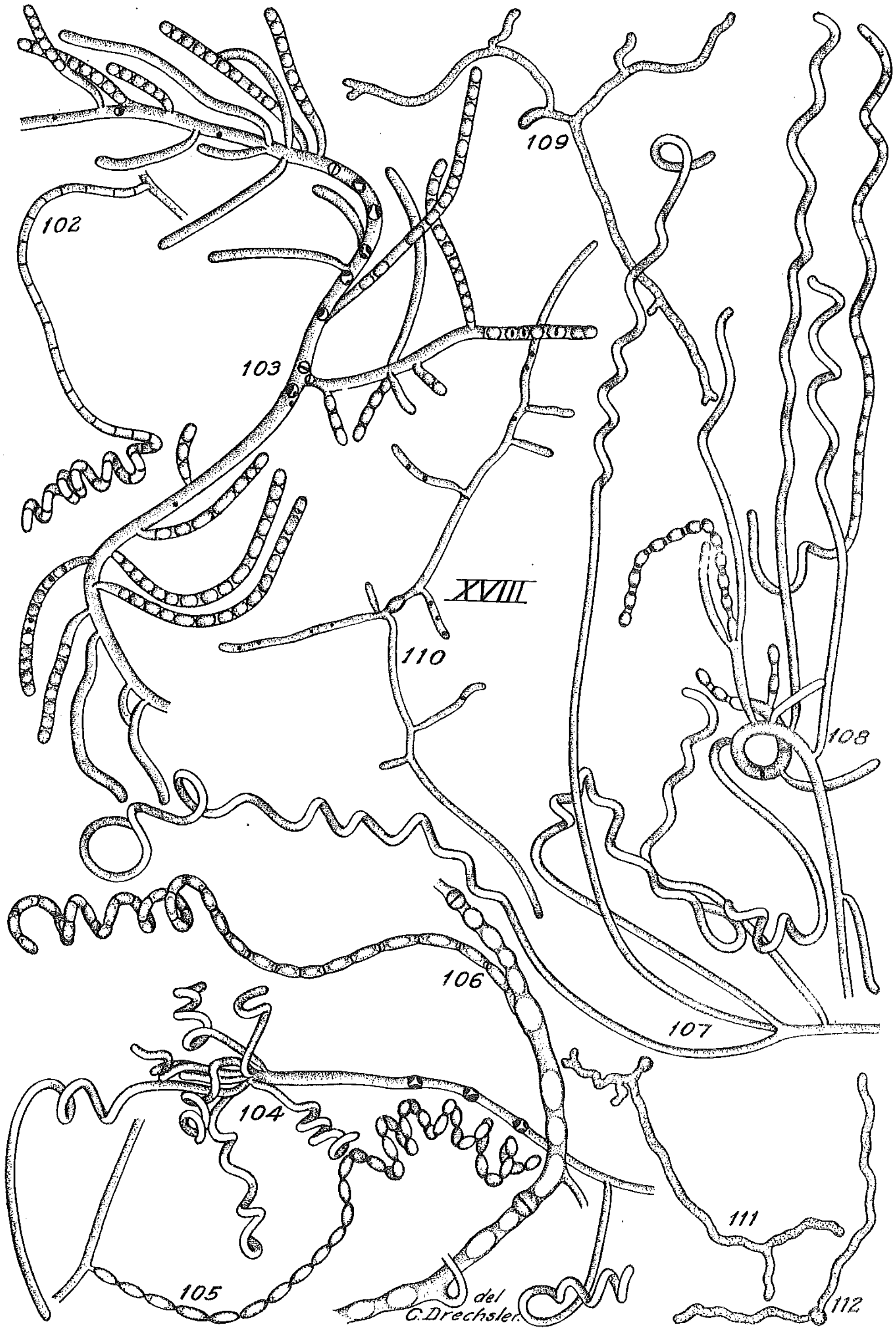

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