THE GENUS *Aspergillus*

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WITH A CHAPTER ON PATHOGENICITY

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CHAPTER

III

Description and Morphology

In the preparation of this book, and of those that preceded it (Thom and Church, 1926; Thom and Raper, 1945), thousands of cultures of *Aspergillus* have been studied. Many have been examined on natural substrata, but the great majority, including subcultures of all viable type strains obtainable, have been observed in comparative laboratory culture under defined growth conditions. These studies have been accompanied and supplemented by an analysis of published descriptions and by the examination of exsiccati from several large herbaria.

Type strains for some species have been maintained in culture since they were described. For many species, however, no type exists, and selection of a representative strain becomes a matter of critical judgment. As a rule, we have based our descriptions of such species upon several strains, rather than upon a single selected strain, in order to show the range of morphological or cultural characteristics encountered.

Interpretation of descriptions of Aspergilli, particularly those published in the older literature, requires that certain assumptions be made. Descriptions are assumed to have been based upon the organisms as encountered on their natural substrate unless the conditions of culture are cited. Colors described are assumed to apply to massed conidial heads unless otherwise

stated, although total colony color may result from a mixture of conidial structures, varying amounts of colored or uncolored vegetative hyphae and, in those species that produce them, cleistothecia or sclerotia in more or less abundance. Conidiophores and conidia are assumed to be colorless and smooth unless otherwise described or illustrated. Cleistothecia and sclerotia are assumed to be lacking unless their presence is specifically noted.

Great diversity in the use of descriptive terms is encountered in the literature. For our descriptions, in order to avoid additional and unnecessary duplication of terms, we have adopted the widely accepted standardized terminology of Thom and Church (1926) and Thom and Raper (1945), except for the use of *cleistothecia* as preferable to *perithecia* when referring to the ascocarps. In the following pages descriptive terms will be equated with others found in the literature and defined. A guide sheet of the type used by the authors for the compilation of information pertinent to the description or identification of an *Aspergillus* is presented on page 130.

THE ASPERGILLUS COLONY

Since relatively few of the Aspergilli produce cleistothecia and ascospores, a basis for identifying the majority of isolates must be found in the character of their colonies and in the details of morphology of their spore-bearing structures. To make the description of an Aspergillus colony meaningful to later investigators, the composition of the substratum, the temperature of incubation, the conditions of illumination, and the age of the culture must be specified. Under known and uniform conditions of culture, the following colony characteristics have been found to be significant in the identification or description of species of *Aspergillus*.

1. THE COLOR OF THE AERIAL PARTS of an Aspergillus colony, including the vegetative mycelium, conidial heads, and cleistothecia or sclerotia if present (Fig. 1—*C*, *D*), is usually its most striking character, and information regarding such pigmentation is universally used in the characterization of species. Generally speaking, there is a characteristic range of colors in the aerial parts for each group of Aspergilli, and a much narrower range for a particular species.

Color production was used by Bainier and Sartory (*ca.* 1910–1912) to separate members of the *A. glaucus* group by supplementing standardized observations of colony color with a routine series of solubility and precipitation tests. Blochwitz (1929–1935) followed by using solubility tests for all species producing bright colors but failed to coordinate his tests with conditions of culture. Later Gould and Raistrick (1934) and Raistrick, Robinson, and Todd (1937), studying the *A. glaucus* group, extracted and defined the pigments but failed to follow the successive color transformations exhibited by a single species during its development.

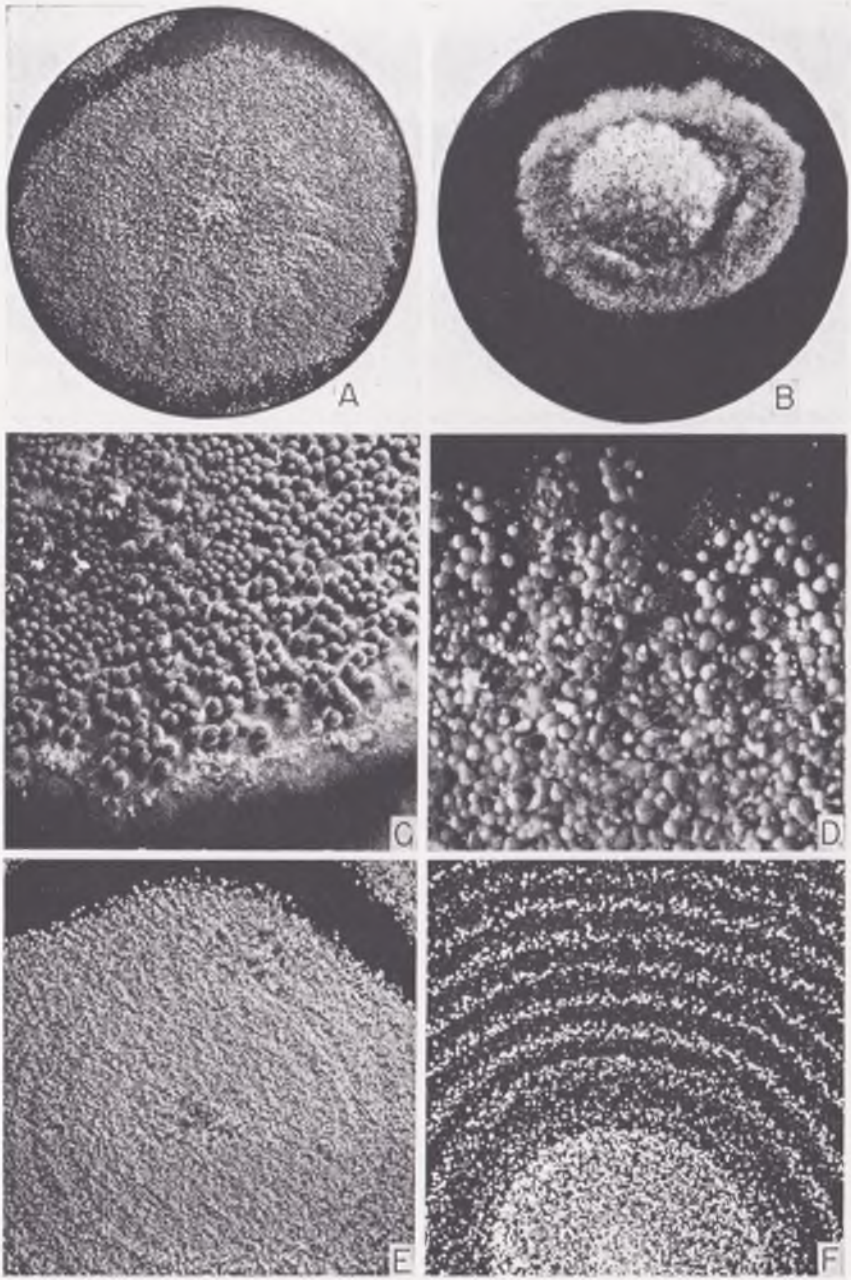

FIGURE 1. Colony types in *Aspergillus*. *A*, *Aspergillus parasiticus* WB 465, heavy-sporing, nonfloccose colony on Czapek's solution agar, room temperature, 10 days, × 2. *B*, *Aspergillus wentii* WB 375, light-sporing, floccose colony grown under the same conditions, × 2. *C*, *Aspergillus variecolor* WB 1954, characterized by the production of abundant, large cleistothecia, × 3. *D*, *Aspergillus auricomus* WB 391 characterized by abundant orange-colored sclerotia, × 5. *E*, *Aspergillus ochraceus* WB 408 on Czapek's solution agar, showing heavy-sporing, essentially azonate colony, × 2. *F*, The same on hay infusion agar, strongly zonate, × 2. (After Thom and Raper,

In the conidial structures, pigmentation may or may not occur in the conidia, the sterigmata, the vesicle, or the conidiophore. Pigmentation as it relates to the individual elements of the conidial apparatus or to sclerotia or cleistothecia will be discussed in the detailed considerations of these structures.

2. PIGMENTATION OF EITHER THE BASAL MYCELIUM OR THE UNDERLYING SUBSTRATUM, furnishes additional pertinent data, but since color in the substratum is the result of a particular *Aspergillus* growing under certain nutritional and environmental conditions, it is an unreliable criterion for characterizing a species. Such color reactions are confirmative, but not absolute, characters in separating species.

A major objection to the use of color as a diagnostic character rests in the fact that individuals differ in their interpretation of colors. In this study of the Aspergilli, Ridgway's *Color Standards and Nomenclature* (1912) has been used as a color reference, and his terminology is employed throughout. In the species descriptions, plates in Ridgway are referred to as, for example, "R., Plate XXII."

3. THE RATE OF COLONY GROWTH and the diameter attained within a specified time and on a specified medium differ from species to species and from group to group. Colonies may attain their maximum diameters, whether large or small, either quickly or slowly. Growth may cease abruptly within a short time, or it may continue over a period of weeks. Brancato and Golding (1953, 1954) found colony diameter a reliable measure of growth under standardized conditions of culture.

4. COLONY MARGINS may be heavy and sharply delimited or thin and diffuse, smooth and entire or irregularly lobed, submerged or aerial.

5. THE BASAL MYCELIUM may be thin or dense, fragile or tough or brittle, plane or buckled and furrowed.

6. THE TEXTURE OF THE SURFACE GROWTH of the colony is a fairly reliable diagnostic characteristic under uniform conditions of culture. Many Aspergilli produce colonies described as velvety (Fig. 1*A*) that consist of a submerged mycelium from which only fruiting structures rise above the surface, suggesting a turf (German: *Rasen*) or a field of ripening grain. Other species described as floccose produce a conspicuous aerial felt of branching and interlacing hyphae and conidiophores. This type of growth is normally one of the most striking characters of *A. wentii* under laboratory cultivation (Fig. 1*B*). Certain members of the *A. glaucus* group form long streamers of repent hyphae in laboratory culture on high protein substrates at low temperatures (*ca.* 15° C).

7. ZONATION may be expressed by the alternating production of conidial heads and sclerotia, or of conidial heads and cleistothecia, or as concentric rings of either sclerotia or cleistothecia alternating with rings of strictly

mycelial growth. In most species, however, it takes the form of fairly regular concentric rings of alternately crowded and sparse conidial structures (Fig. 1—*E*, *F*). The phenomenon appears to be a response to the periodic depletion of nutrients, to the periodic production or gradual accumulation of some metabolite, or to conditions of illumination. The literature on zonation in fungi was reviewed by Jerebzoff (1961).

In contrast to concentric zones, certain species characteristically develop conidial heads only in localized areas, usually as a response to drying and concentration of the medium.

8. Coremia, as specialized erect aggregations of conidiophores (*Stilbum*-like), have been reported and figured only for *A. vitellina* Ridley, which was described from its natural substratum. However, structures approaching true coremia are currently recognized in *A. clavato-flavus* n.sp.

9. Odor, while sometimes marked, is never a reliable diagnostic character and should be used only as corroborative evidence for species identification.

MORPHOLOGY

The Conidial Head

The color, shape, size, and arrangement of parts of the conidial heads are characteristic of the species and, to a lesser extent, of the groups to which they belong (Figs. 2 and 3). For example, the heads of *A. fumigatus* and *A. nidulans* are small, green, and compactly columnar (Fig. 2*C*); heads of *A. niger*, *A. ochraceus*, and *A. wentii* are large and radiate and black, ochraceous, and yellow-brown, respectively (Fig. 3*B*); heads in other species, notably *A. flavus* and *A. candidus* (Fig. 3*A*), usually show a characteristic variability in size and shape within the same colony and are yellow-green and white, respectively. Examination of many heads in colonies on several media is essential to a complete interpretation and description of their size and shape.

Wehmer (1901) roughly classified his Aspergilli into Microaspergilli and Macroaspergilli on the basis of the size of their fruiting structures. In a consideration of *all* the species of *Aspergillus*, certain of them fall readily into one or the other of these groups, but the distinction is not so easily made for others and hence is minimized in our treatment of the genus.

The Foot Cell

The first indication of the development of a conidial structure in the Aspergilli is the enlargement of certain cells in the mycelium and their subsequent development of a heavy wall. Each such cell, a foot cell, usually produces a single conidiophore as a branch perpendicular to the long axis of the cell (Fig. 4—*A*, *B*) and usually about midway of its length. Foot cells

FIGURE 2. Conidial heads characteristic of different groups in *Aspergillus*. *A*, *Aspergillus clavatus* group: conidial heads of *A. giganteus* WB 10, showing typical clavate form, × 15. *B*, *Aspergillus glaucus* group: heads of *A. nivco-glaucus* WB 127, showing characteristic radiate pattern, × 35. *C*, *Aspergillus nidulans* group: heads of *A. nidulans* showing typical short columnar form, × 35 (photograph by Edward Yuill). *D*, *Aspergillus flavipes* group: heads of *A. flavipes* WB 1959, typically barrel form, or loosely columnar as shown, × 18. *E*, *Aspergillus terreus* group: *A. terreus* WB 265, heads columnar, of uniform diameter throughout, often becoming quite long as shown, × 22. *F*, *Aspergillus ustus* group: *A. ustus* WB 1974, heads typically loose and radiate as shown, in other strains and under certain conditions approaching columnar. × 22. (After Thom and Raper, 1945, in major part.)

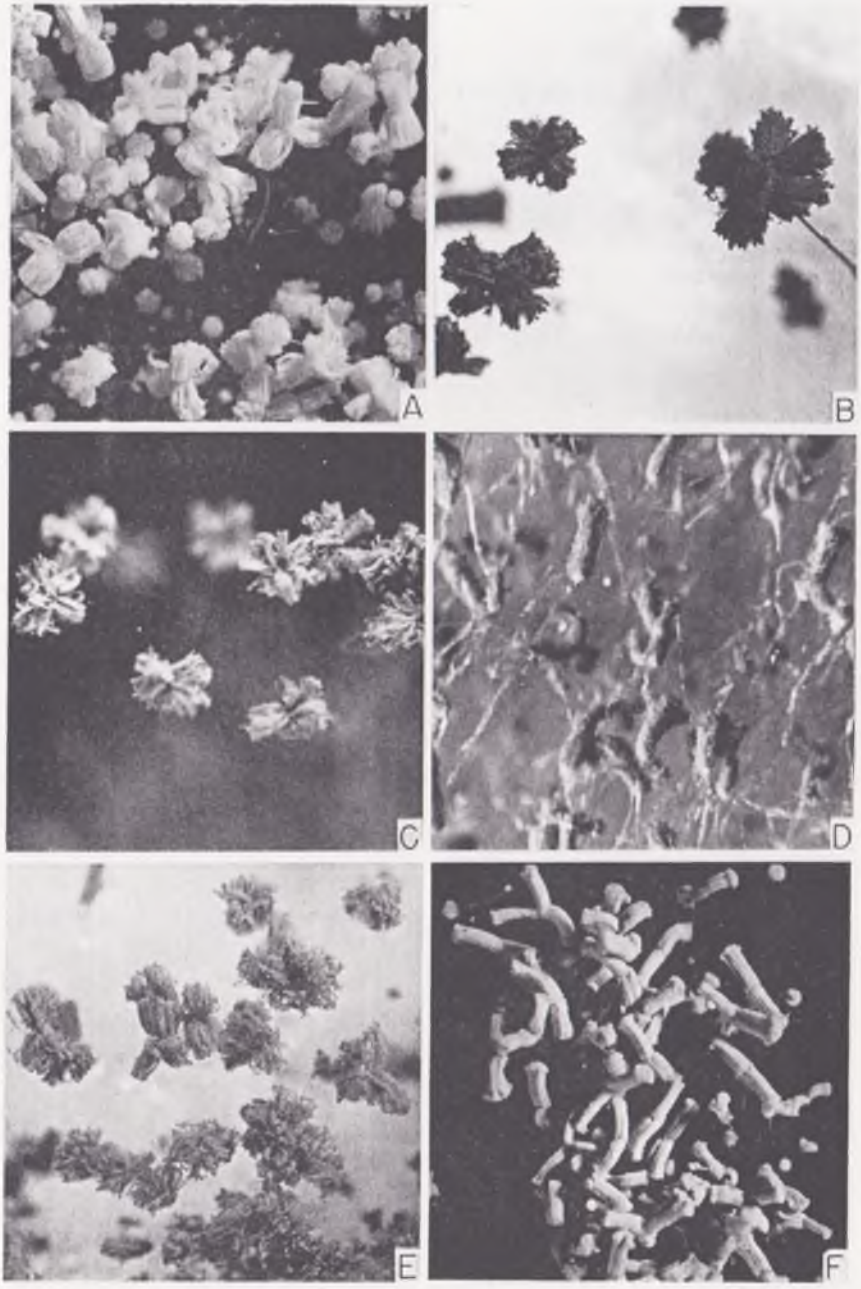

FIGURE 3. Conidial heads characteristic of different groups in *Aspergillus*. *A*, *Aspergillus candidus* group: *A. candidus* WB 308, showing characteristic mature heads of different dimensions, × 18. *B*, *Aspergillus niger* group: *A. carbonarius* WB 67, typical mature heads showing characteristic splitting into loose conidial columns, × 30; *C*, *Aspergillus sparsus* group: *A. biplanus* WB 5071, heads typically globose or becoming loosely radiate as shown, × 24. *D*, *Aspergillus ornatus* group: *A. paradoxus* WB 2162, heads loosely columnar, often radiate in other species, × 24. *E*, *Aspergillus flavus* group: *A. flavus* WB 1957, typical mature heads, × 18. *F*, *Aspergillus ochraceus*

may become curved and twisted in age, and their connection to vegetative hyphae may become inconspicuous and difficult to determine. They may be submerged in the substratum or arise from aerial hyphae. In *A. oryzae* var. *effusus*, an entire aerial hypha may be composed of a continuous series

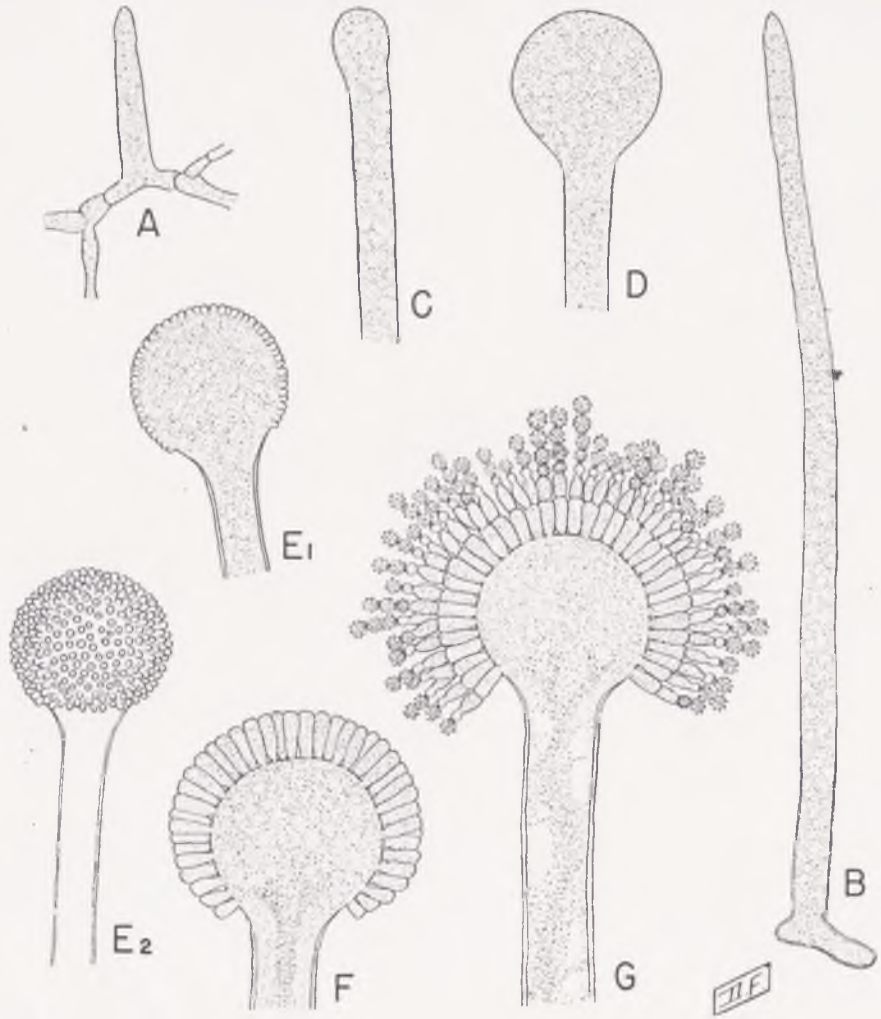

FIGURE 4. Development of the conidial apparatus in *Aspergillus niger*. *A*, Foot cell bearing young conidiophore as a vertical branch. *B*, Developing conidiophore, × 182. *C* and *D*, Development of the vesicle by swelling of the terminal portion of the conidiophore, × 280. E_1 and E_2, Vesicle in optical section and surface view showing early development of primary sterigmata, × 280. *F*, Later stage in development of primary sterigmata, × 280. *G*, Young fruiting head showing secondary sterigmata bearing chains of conidia, × 280. (After Thom and Raper, 1945.)

of foot cells, each bearing a short conidiophore. A detailed study of the development of the foot cell of *A. clavatus* was made by Klein (1944). Failure to recognize the foot cells as characteristic of the Aspergilli led Ferdinandsen and Winge (1910) to use these structures as the principal diagnostic character of *Sterigmatocystis dipus*. The presence of a foot cell may be taken as strong evidence that an isolate represents a species of *Aspergillus*, but its absence cannot be used to exclude an organism from the genus.

The Conidiophore or Stalk

The erect, perpendicular branch arising from the foot cell and ultimately producing the conidial head is known as the conidiophore or stalk (Figs. 4 and 5). In most species of *Aspergillus* conidiophores are unbranched, each bearing a single conidial head. However, a limited number of branched structures have been observed in certain species of the *A. cervinus*, *A. glaucus*, *A. wentii*, and *A. sparsus* groups. The length and diameter of the section of the conidiophore between the foot cell and the base of the vesicle is reported in describing species.

In most Aspergilli the unity of the conidiophore is accentuated by its continuous, characteristically thickened walls. The thickening may be fairly uniform throughout the length of the conidiophore, or it may be greater at the base and appreciably less toward the vesicle; more rarely, it is heavier below the vesicle. The conidiophore may be septate or nonseptate. Septa, if present, are usually comparatively thin and fragile. In some species, however, they may be quite conspicuous.

Two general sections may be readily distinguished by the character of the conidiophore wall as observed under high magnification. In the first, the outer surface of the wall is smooth; the wall appears homogeneous or nearly so under the microscope, it does not absorb the ordinary protoplasmic stains, and its structure is difficult to distinguish. The inside surface of the wall may show irregular clumps or uneven thickenings. When broken, many conidiophores show uneven or jagged ends like a broken glass tube or, as in the *A. niger* group, the ends splinter like bundles of lath. The structure and composition of the conidiophore walls of certain Aspergilli have been studied in detail by Castle (1945) and Farr (1954). In some species, particularly in the *A. ustus*, *A. versicolor*, *A. nidulans*, and *A. sparsus* groups, stalks otherwise completely smooth may show superficial, and usually more or less hemispherical, wartlike concretions of dried pigment on their outer surfaces. These may be fairly numerous or few and widely scattered. In the second section, the wall appears irregularly roughened, echinulate, or pitted. In the latter case, the secondary thickenings appear to have been laid down around protoplasmic areas which, for a time at least, maintain contact with the primary wall. True echinulations appear

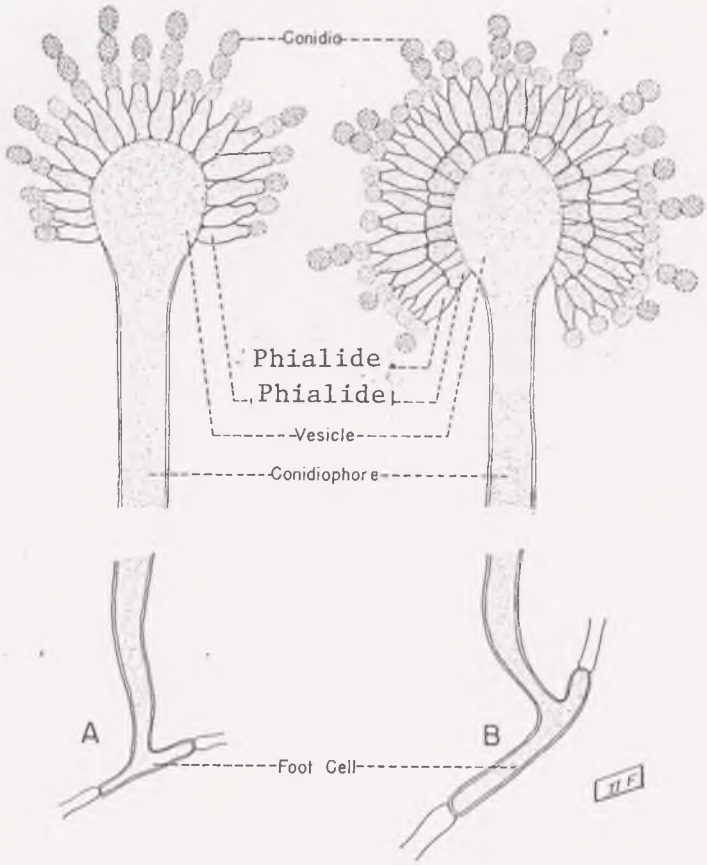

FIGURE 5. *A*, *Aspergillus niveo-glaucus*, WB 127, typical head showing only one series of sterigmata, × 515. *B*, *A. versicolor*, WB 239, typical head showing sterigmata in two series, × 108. (After Thom and Raper, 1945.)

to arise from the deposition of pigmented or non-pigmented crystalline material between the outer cell membrane and the inner wall. Irregular roughenings may be formed in a similar way or may result from the deposition of excreted substances during evaporation of the abundant droplets of liquid visible upon the young and growing conidiophores. Stalks which show this type of roughening are frequently completely smooth when young.

Conidiophores may be lightly or conspicuously colored throughout in some shade of green, yellow, or brown; or pigmentation may be confined to the wall or some portion of it. They may be colored over their entire length, or the pigmentation may be more concentrated at the base or at the apex. The degree, extent, and location of the pigmentation vary with the species

or strain and may represent an inherent characteristic of the organism, or they may reflect the absorption of pigment from some external source. In *A. ustus*, for example, pigment is elaborated in place and simultaneously with the development of the stalk; in *A. niger*, on the other hand, pigmentation of the conidiophore is progressive from the vesicle toward the base and results from the absorption of the soluble pigment, aspergilline (Linossier, 1891; Quilico, 1933; Quilico and Di Capua, 1933), from the conidia.

The Vesicle

The conidiophore enlarges at its apex to form a globose, hemispherical, elliptical, or long clavate structure known as the vesicle (German: *Blasen*), which furnishes an enlarged surface for the attachment of spore-bearing cells (Fig. 4). These cells may be either so thin walled that they collapse when mounted or relatively thick walled. In the latter case, perforations in the vesicle wall resulting from the development of sterigmata remain visible when the sterigmata are detached. In general, the lumen of the vesicle is continuous with that of the upper part of the conidiophore; only rarely is a septum formed near the base of the vesicle (*cf. A. mucoroides* Corda). Vesicles produced upright on the conidiophore in most Aspergilli are characteristically borne at an angle to its main axis in a few species in the *A. cervinus*, *A. restrictus*, and *A. fumigatus* groups. In these same groups, vesicles may also occasionally appear bifurcate. Vesicles may be hyaline or pigmented; pigmentation is more or less conspicuous than that of the conidiophores but always of the same shade. The shape and size of the vesicle are valuable diagnostic characters at the group level and should be included in all species descriptions. However, vesicle size has been shown to vary with the composition of the medium (Scurti, 1948), and care must be taken to specify the substrate. Vesicles may bear conidium-producing cells over their entire surface, or on some apical fraction thereof. This character usually influences the ultimate shape of the conidial head and should also be described.

Sterigmata

The fertile area of the vesicle gives rise to a layer of conidium-producing cells, the sterigmata, which usually develop simultaneously and approximately perpendicular to their point of origin. They may be hyaline or pigmented as the vesicle. Figure 5*A* shows a vesicle bearing a single layer or series (uniseriate) of sterigmata (phialides). In Figure 5*B* each of the first series of cells, or primary sterigmata (prophialides, metulae, basidia, pseudobasidia), bears two to several secondary sterigmata (branches, phialides, pseudosterigmata) in a crown or verticil at its apex and is biseriate. In the majority of species with biseriate sterigmata the primaries

develop first and, when these have achieved their maximum or near maximum growth, the secondaries are produced as buds from their apices. The secondaries appear more or less simultaneously on all primaries and before conidium production begins. Some species in the *A. niger* group differ in showing a marked increase in the length of the primaries after the secondary sterigmata are fully formed and producing conidia. In *A. niger*—and in *A. candidus*, which is morphologically similar to it—long primaries may also be septate. An even more unusual developmental pattern is observed in some members of the *A. cremeus* group. Sterigmata are initially uniseriate but subsequently become biseriate, by a process still incompletely understood which appears to function in the following manner. When the uniseriate sterigmata reach their ultimate proportions and sporulation has commenced, a septum is laid down near the vesicle; by continued basal growth of this cell, a structure appearing similar to a primary sterigma is formed and elongates considerably before additional secondary sterigmata are produced from its apex. Thus, the sterigmata of these species go through a transition from a uniseriate and nonseptate stage to a uniseriate, septate stage and finally to a biseriate stage.

Each conidium-producing cell bears a single unbranched chain of conidia. Sterigmata are usually characteristic in size and shape among closely related species. Primary sterigmata are essentially supporting cells which vary much more in form and dimensions than do the secondaries and, for this reason, are more useful in species diagnoses.

The Conidium or Spore

The actual spore-producing cell, or sterigma, ordinarily consists of an essentially cylindrical body which, after reaching a length more or less uniform for the species, narrows at the apex into a conidium-producing tube. Further elongation is confined to this apical tube.

In most species the multinucleate vesicle provides only the initial complement of one or more nuclei to each sterigma, and when sterigmata are biseriate each of the secondaries receives one or more nuclei resulting from nuclear divisions in the primaries (E. Yuill, 1950). Baker, on the other hand, contends that in *A. echinulatus* nuclei continue to pass from the vesicle to the phialide as conidia are formed (1945). In either case, the nucleus or nuclei in the sterigma divide, and one of each pair of daughter nuclei passes into the conidial tube, which is then separated from the sterigma by a septum. Parallel with repeated division of the sterigma nucleus (or nuclei) and migration of daughter nuclei into the conidial tube, new sections of the tip are cut off successively and the older cells pushed outward (Fig. 6). Each such chain of spores typically consists, then, of a series of up to several hundred equal sections cut from one tube or tip of a sterigma, and each

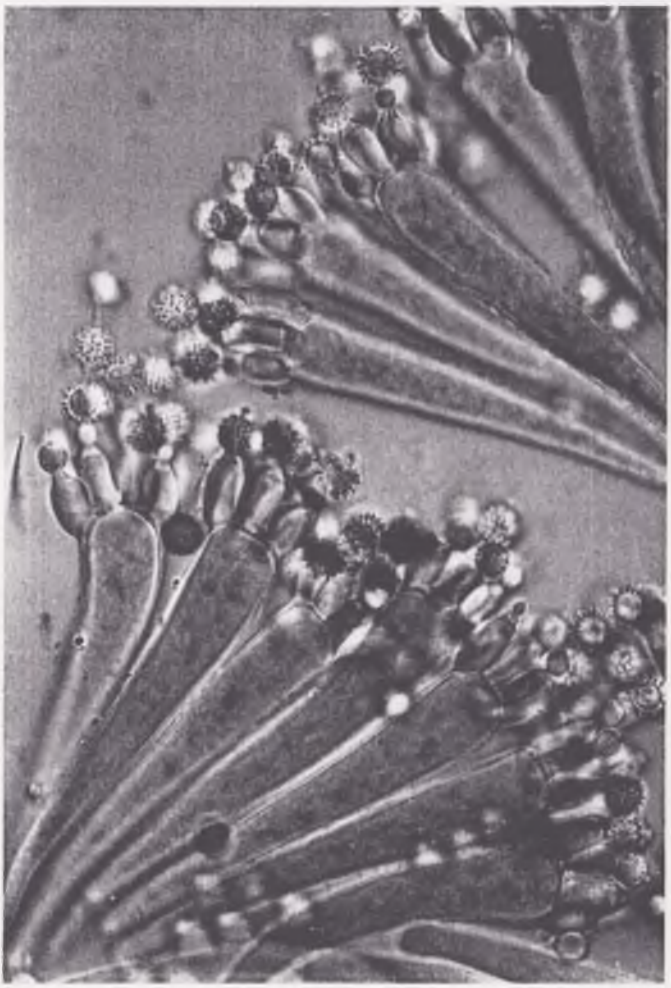

FIGURE 6. Detail of sterigmata and sporogenesis in *Aspergillus carbonarius*. Note how the very large club-shaped primary sterigmata bear several relatively small secondary sterigmata and how the conidia become strongly echinulate prior to maturity (photograph by Edward Yuill), × 740. Compare with mature spores illustrated in Figure 69A.

carries a daughter nucleus (or nuclei) derived directly from the parent sterigmatic cell.

In the majority of species of *Aspergillus* conidia are uninucleate. Nuclear number appears, however to be correlated with conidium size, and up to 12 nuclei have been observed in certain species of the *A. glaucus* group,

where conidia may reach 10μ or more in diameter. The mechanism by which multinucleate conidia are formed is not clearly understood: the entire number of nuclei may be provided to the sterigma by the vesicle; or nuclear multiplication may occur within the sterigma at either the primary or secondary level; or further nuclear divisions may occur in young conidia, as reported for *A. echinulatus* by Baker (1945) and for *A. repens* by E. Yuill (1950). Both of these species are members of the *A. glaucus* group, and there are no certain reports of such divisions in the conidia of member species of any other group. However, the appearance of young nuclei in conidia of *A. tamarii* suggests that they may also occur there (E. Yuill, 1950).

After the conidium is separated by a septum from the sterigma, it normally continues to draw nutrients from the parent cell* until it assumes the general size and shape characteristic of the species. It then lays down within its original, or primary, wall a secondary cell wall which separates it completely from its parent cell and assumes its characteristic color and markings. Exact uniformity is attained in neither size nor surface ornamentation, and according to Thielke (1959), the former may vary with environmental and nutritional conditions. For this reason, it is more important to report size ranges than exact measurements and the general pattern of surface markings than minute details. The character of conidial walls, as shown by the electron microscope, has been used by Iizuka (1955) to classify species of *Aspergillus*.

Color in the walls of conidia, which is significant because it is the determining factor in head color, may be present in diffuse form in smooth conidia or aggregated into spinules or bars between the outer and inner walls of rough conidia. This latter condition is most conspicuous in members of the *A. niger* group. A shift from olive-brown to red-brown in the pigmentation of maturing conidia is frequently observed in some members of the *A. niger* group and from bright green to brown shades in the *A. flavus* group. In the latter, K. Saito (1907*b*) and Thom and Church (1921, p. 115) found that bright green cultures subjected to ammonia vapor lost the green color and became somber yellow-brown; this reaction was reversed by exposure to acetic acid fumes. Reversible color changes were observed by Takagi and Sakaguchi (1957) and Takagi (1957) in yellow mutants of members of the *A. flavus* group in the presence of halogen or copper ions. Discoloration phenomena in the conidia of *A. flavus* and *A. oryzae* have also been observed by Kurosawa (1958).

In the chain, conidia remain attached to one another by a connective (disjunctor) bridge, which may be conspicuous or almost invisible. As

* Buller (*Researches on Fungi*, V, Ch. II, 1933) discusses the primary septum as having a central pore through which a protoplasmic connection is maintained.

developing conidia swell and assume their characteristic shape within the conidial tube, they may form their secondary wall in close and continuous contact with the primary wall (conidial tube) and the septa that separate them, or they may recede from the septa, leaving the original wall of the tube as a bridge across the open space (Fig. 69*A*). The presence or absence of connectives is not considered a significant character in species separation.

Cleistothecia

The existence of an ascigerous stage in *Aspergillus* was first reported and beautifully illustrated (Fig. 7) by De Bary (1854) in his now classic paper on the common origin of *Aspergillus glaucus* Link and *Eurotium herbariorum* (Wiggers) Link. In 1883, Eidam described and figured (Fig. 8) *A. nidulans*, which was not then recognized as closely related to *Emericella variecolor*, described by Berkeley and Broome in 1857 as a Gasteromycete or possibly a lichen. Since 1883 an ascosporic stage has been described for all but one

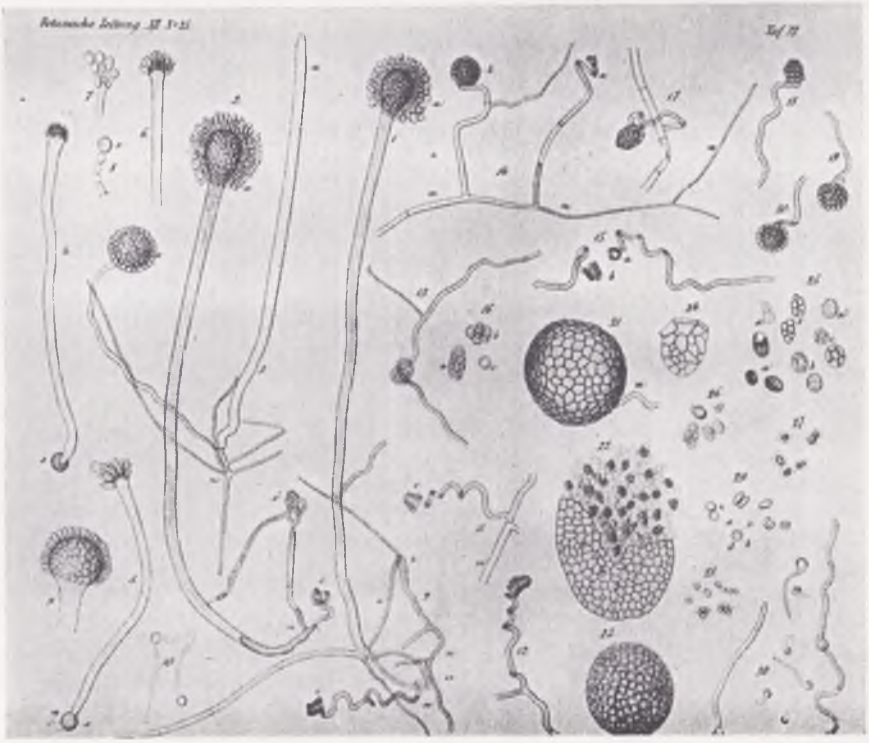

FIGURE 7. The development and relationship of *Aspergillus glaucus* and *Eurotium*, after De Bary (1854).

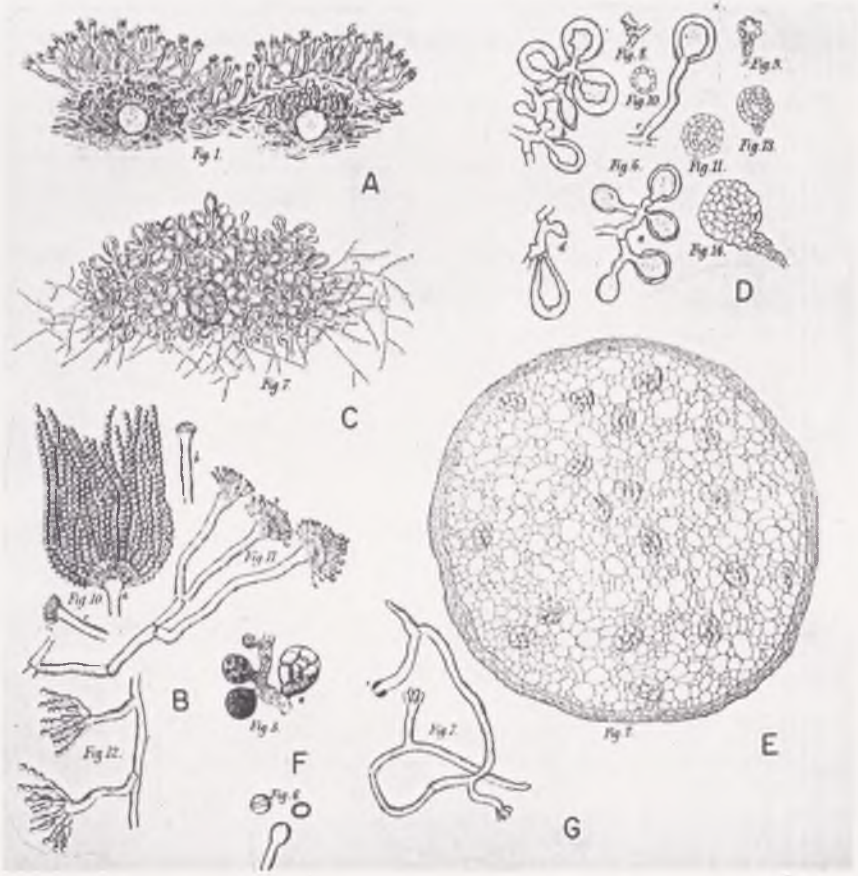

FIGURE 8. Developmental history of *Sterigmatocystis nidulans* as illustrated by Eidam, Plates XX, XXI, and XXII, in his description of this species (Cohn, Beitr. Biol. Pflanz. 3: 392–411 (1883)). *A*, Habit sketch showing relationship of conidial structures, hülle cells, and cleistothecia. *B*, Detail of conidial structures. *C*, Young cleistothecium with enveloping mantle of developing hülle cells. *D*, Early stages in the development of the cleistothecium and origin of hülle cells. *E*, Cleistothecium with some mature eight-spored asci. *F*, Asci in different stages of maturation and ascospores, one of which is germinating. *G*, Young mycelium derived from a germinated ascospore and bearing two diminutive conidial heads.

of the members of the *A. glaucus* group, for all of the members of the *A. fischeri* series of the *A. fumigatus* group, and for many species of the *A. nidulans* group. Additionally, cleistothecia are now known to occur in some species of the *A. ornatus*, *A. cremeus*, and *A. ochraceus* groups.

In all of these ascosporic Aspergilli, with the single exception of *A.*

alliaceus, in which cleistothecia arise within a heavy-walled sclerotium, cleistothecium formation has been found to be initiated by a terminally coiled ascogone resembling, but not consistently duplicating, that figured by De Bary for *Aspergillus glaucus*. The manner of coiling varies with the group and may be either in the same plane or at right angles to the uncoiled portion of the ascogone. An antheridium may or may not be present and may or may not show apparent fusion with the ascogone. Actual function in fertilization has never been demonstrated.

Detailed studies on the initiation and development of the ascocarps of members of the *A. glaucus* group have been made by Dangeard (1907), Frazer and Chambers (1907), Dale (1909), Gupta (1951), and Moreau and Moreau (1953). Dangeard (1907) and Olive (1944) described and illustrated these stages in the life cycle of *A. fischeri*. Both Dangeard and Benjamin (1955) found it impossible to determine the method of ascocarp initiation in *A. nidulans*, and Eidam's description remains the only detailed report. Dangeard (1907) also described and figured the development of ascocarps within the sclerotia of a strain identified as *A. flavus*. Although we have searched for ascospores in many strains of this species, Dangeard's report has not been verified. Discovery of the cleistothecia of *A. alliaceus* (Fennell and Warcup, 1959), however, lends support to Dangeard's findings and suggests that still other "sclerotial" species may be found to be cleistothecial.

The cleistothecia of all of the ascosporic species thus far recognized in *Aspergillus* appear to arise from basically similar initials and, except for *A. spinulosus*, show a marked degree of similarity in the structure of their walls, which at maturity consist of a thin layer of flattened cells; yet they differ markedly in gross appearance. In the *A. glaucus* group they are small, yellow, naked, borne on a single stalklike hypha, and loosely suspended in a network of pigment-encrusted mycelium; in the *A. nidulans* group cleistothecia are dark purple and completely enveloped by or associated with thick-walled hülle cells; in the *A. fischeri* series cleistothecia are white to cream and develop within a cottony mycelial matrix, fragments of which remain attached at maturity; in the *A. cremeus* group cleistothecia appear similar to those of the *A. fischeri* series but at maturity are covered with short, sterile hyphal projections, which in *A. chrysellus* often become brown; in *A. ornatus* and *A. citrisporus* cleistothecia become purple at maturity and are surrounded by a loose mantle of much branched hyphae but lack hülle cells; in *A. alliaceus* multiple cleistothecia develop within a dark-walled, persistently hard, sclerotium-like body.

Two species, *A. spinulosus* and *A. athecius*, fail to conform with any of the above groups. Characteristics of colonies, conidial heads, cleistothecial fundaments, and ascospores of *A. athecius* align it unmistakably with the

A. glaucus group, although it fails to develop walled cleistothecia. *A. spinulosus*, on the other hand, shows only limited structural similarities to any other presently known members of the genus.

With one very recent exception, all cleistocarpic species of *Aspergillus* are homothallic. Henrard (1934), working with 15 "species" of Aspergilli, reported that all were "sexually homothallic." He summarized the literature by saying that homothallism has been affirmed by Kniep (1928) for *A. repens;* by Schwartz (1928) for *A. ruber*, *A. repens*, four other strains of "*A. glaucus*," and four strains of *A. nidulans;* by Blochwitz (1932) for six strains in the *A. glaucus* group; and by Greene (1933) for *A. fischeri*. The single exception, *Aspergillus heterothallicus* Kwon *et al.*, isolated recently in our laboratory, has been found to occur in two mating types, *A* and *a*, which are allelic as confirmed by crosses between original isolates and between their progeny. Strains of this species currently are undergoing intensive study.

The Ascospore

In all of the cleistothecial Aspergilli which have been studied, asci arise from croziers and are nonlinear. With very rare exceptions, they have eight spores. Mature ascospores are commonly shaped as a double convex lens or as two symmetrical valves, as figured by De Bary (1854). The bivalve construction is inconspicuous in *A. alliaceus* and apparently lacking in *A. spinulosus*. Variations upon the basic pattern occur and characterize particular species (Figs. 34, 41, 58, 113). A conspicuous furrow may or may not mark the juncture between the two valves and may or may not be flanked by heavy ridges or crests of varying width and structure; the convex surfaces of the ascospore may be smooth or variously roughened, echinulate or ridged. Ascospores are hyaline in all species except those comprising the *A. nidulans* and *A. ornatus* groups. In the *A. nidulans* group color varies from red-brown through purple-red to violet; in *A. ornatus* ascospores are very lightly colored in red-brown shades when mature.

With few exceptions, the ascospores germinate in the manner illustrated by De Bary (1854): the spore swells, the valves separate at one edge, the germ tube appears through this opening, and the two valves then part completely and remain on opposite sides of the germ tube (Fig. 9). In *A. nidulans* the separated valves remain easily identifiable by their bright purple-red color.

Hülle Cells

Eidam described the cleistothecia of *A. nidulans* as having an external covering (Hülle) made up of a loose network of hyphae bearing large numbers of terminal or intercalary, ellipitical or globose, vesiculose cells with very heavy walls which almost obliterated the cell lumens (Fig. 8).

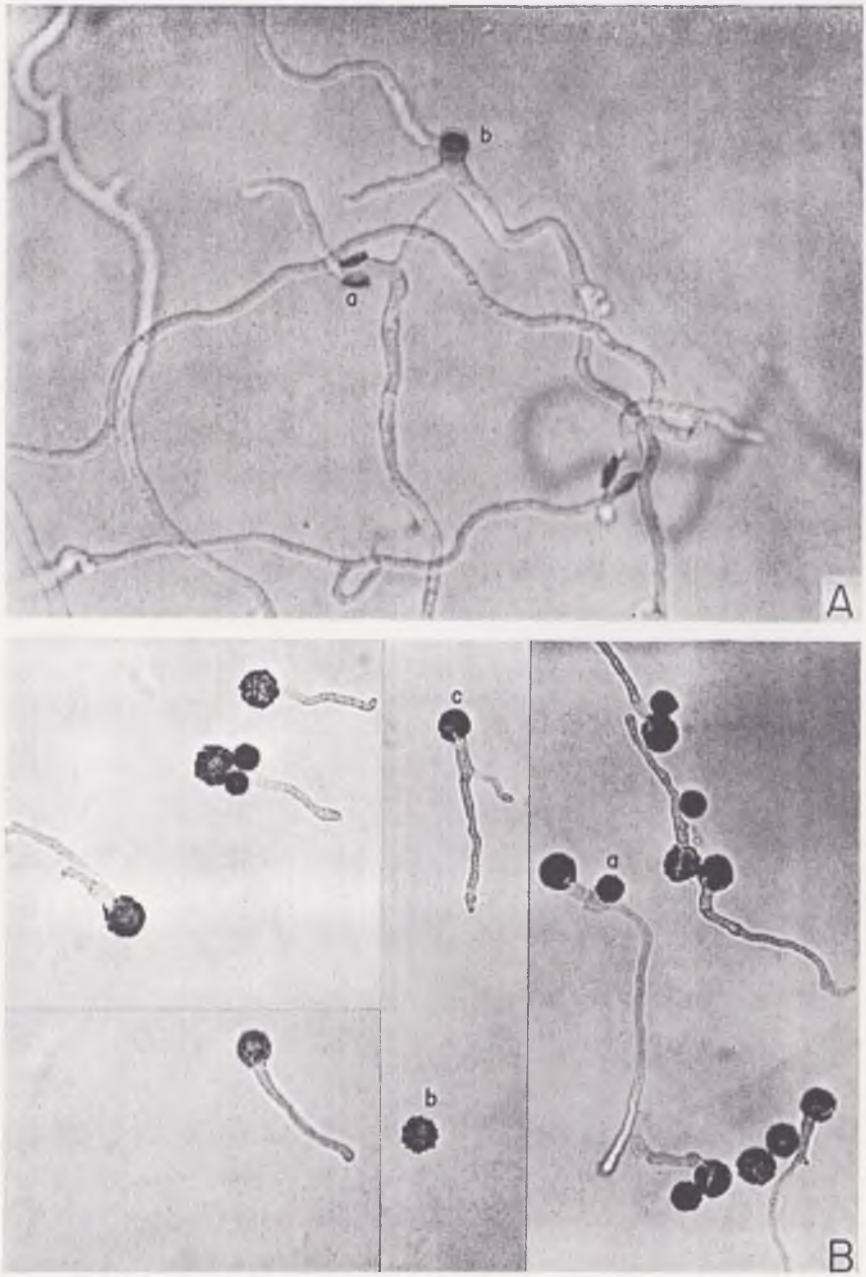

FIGURE 9. Spore germination. *A*, Germinated ascospores of *Aspergillus nidulans*, × 795: *a*, ascospore in profile showing how the two valves comprising the spore wall are pushed apart as the spore content enlarges and the sporeling develops; *b*, the same in face view. *B*, Germinating conidia of *Aspergillus niger*, × 635: *a*, ungerminated spore; *b*, spore in early stage of germination; *c*, germinated spore showing rapidly elongating sporeling. (After Thom and Raper, 1945.)

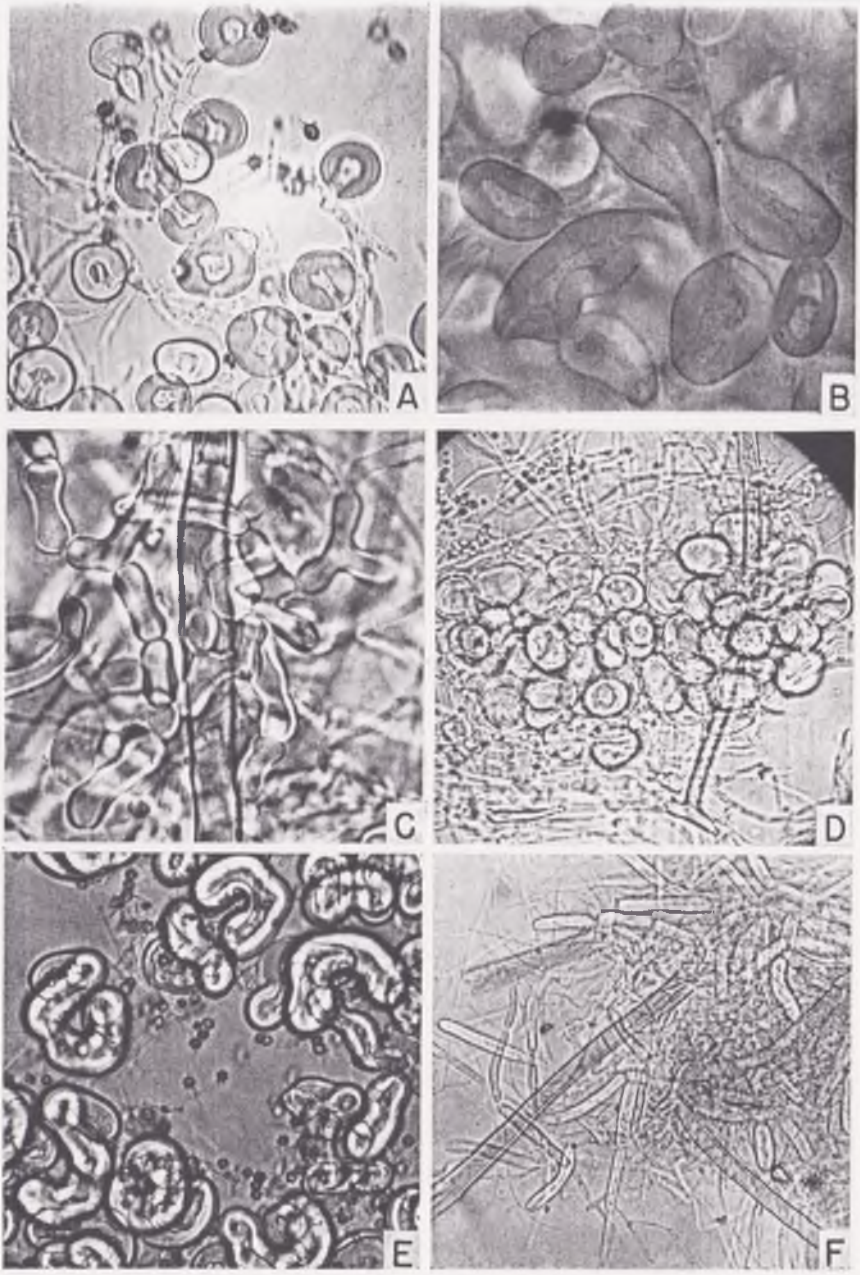

FIGURE 10. Hülle cells. *A*, Characteristic thick-walled, globose to subglobose hülle cells of the *Aspergillus nidulans* group: *A. variecolor* WB 1954, × 465. *B*, *A. heterothallicus* WB 5088, showing hülle cells of the same general type as *A. nidulans* and *A. variecolor*, but irregular in form, × 785. *C*, *Aspergillus flavipes* WB 287, showing characteristic irregular and often branched hülle cells, × 900. *D*, *Aspergillus granulosus* WB 1932, hülle cells globose to ovoid, but with walls somewhat thinner than in *A. nidulans* and *A. ustus*, × 280. *E*, *Aspergillus ustus* WB 280, characteristic hülle cells, elongate and much twisted, × 465. *F*, *Aspergillus conjunctus* WB 5080, elongate hülle cells, × 275. (After Thom and Raper, 1945, in part).

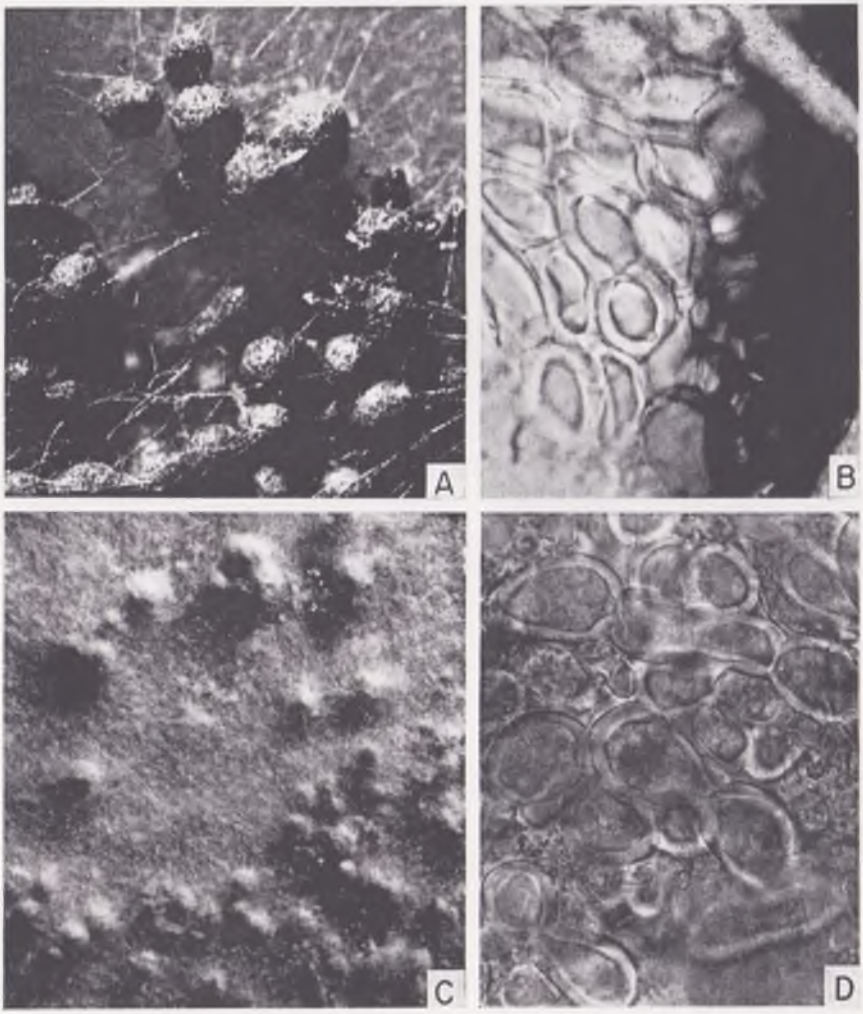

FIGURE 11. Sclerotia and sclerotium-like structures in *Aspergillus*. *A*, True sclerotia of *Aspergillus flavus* WB 500, illustrative of the very hard, typically globose to subglobose bodies found in the *A. flavus*, *A. niger*, *A. candidus*, and *A. ochraceus* groups, × 15. *B*, A thin section of the preceding showing the sclerenchyma-like tissue composed of heavy-walled cells that do not separate from each other upon maceration, × 925. *C*, Soft, pseudosclerotia of *Aspergillus malodoratus* WB 5083, × 17.5. *D*, A portion of the preceding showing that the constituent cells are easily dissociated upon compression, × 805.

These cells have since been referred to as chlamydospores, *Eidamsche blasen*, or hülle cells. The last designation has become generally accepted. Hülle cells (Fig. 10) are associated with the cleistothecia of all the ascosporic species of the *A. nidulans* group and occur in great abundance in the crustose, nonascigerous section of the group. Globose to subglobose hülle cells appear also in certain species of the *A. versicolor* group. Cells of the same general character but of markedly different size and shape appear in masses of hyphae in strains of the *A. ustus* group. Thick-walled, septate hyphae suggestive of hülle cells appear in the species comprising the *A. flavipes* group. Since the hülle cells of different groups assume characteristic shapes, they provide a valuable diagnostic group character.

Hülle cells are specialized structures of unknown function that normally occur in certain groups of the Aspergilli. Schwartz (1928) figured the hülle cells of *A. nidulans* as capable of germinating and thus acting as reproductive cells. This report has been corroborated by work in our laboratory. The close and consistent association of globose hülle cells with the cleistothecia of the *A. nidulans* group suggests that the presence of homologous structures in other groups may indicate a potential for cleistothecium formation.

Sclerotia

True sclerotia (Fig. 11)—discrete hard masses of characteristic shape, size, and color, and consisting of thick-walled, parenchyma-like cells—occur regularly in certain members of the *A. candidus*, *A. niger*, *A. flavus*, and *A. ochraceus* groups. In other members of these same groups grown under similar conditions, no such structures have been found. Coats (1959) and Rudolf (1962) studied the influence of environmental and physiological factors on the production of sclerotia in six species of *Aspergillus*. Sclerotium production was found to be favored by high nitrate and sucrose concentrations (but not glucose or lactose) at temperatures optimal or suboptimal for mycelial growth (20–25° C). Results of pH studies were not definitive but indicated that conditions most advantageous for vegetative growth were equally so for sclerotium formation. Continuous white light of low intensity had no influence on sclerotium production in the strains studied.

Pseudosclerotia—sclerotium-like masses of compacted hyphae or discrete but loosely organized masses of specialized cells (Fig. 11)—occur in certain species of the *A. versicolor*, *A. wentii*, and *A. terreus* groups. The significance of these structures, and the degree to which they are analogous to true sclerotia, has not been determined.

When true sclerotia or sclerotium-like bodies are present in a culture, their form and structure provide clues to group relationship. However, their sporadic development in obviously closely related strains, or in the same strain under the same or only slightly altered culture conditions, makes their presence or absence an unreliable diagnostic criterion.

CHAPTER

IV

Cultivation

An *Aspergillus* occurring upon a natural substratum may frequently be identified to group from the original material, but differences in the nature and composition of the substratum produce marked contrasts in growth character and coloration and in the dimensions and morphology of conidial heads. When such materials are dried as herbarium specimens, colors are often materially changed and the hyphal masses are rendered so fragile that the morphological data necessary for identification to species are often obliterated or made difficult to interpret. Accurate identification requires isolation in pure culture and examination on culture media of known composition. Classification within the genus has become so dependent upon observation in pure culture that the whole subject of laboratory cultivation needs to be considered.

CULTURE MEDIA

So-called natural media were utilized for most of the early culture work with Aspergilli. De Bary and Brefeld used decoctions of horse dung variously diluted and stiffened with gelatin. Much European work has been done with brewery wort; Mazé (personal communication to Thom, 1904) used extract of white beans; potato and carrot decoctions have been widely utilized; Bainier grew and described many of his molds upon pieces of