ATLAS OF MEDICAL MYCOLOGY

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Preface

This atlas is the natural outcome of a series of exhibits for medical conventions, demonstrations and lectures which were prepared as part of the training program for our residents in pathology, clinical residents, medical students and medical technologists.

There may be many who feel that we have oversimplified the clinical and laboratory diagnosis of fungous infections. We are not implying that either the diagnosis of mycoses or the identification of the etiologic agent is without difficulty. It has been our aim to present a concise basic plan for clinicians and clinical pathologists who are frequently presented with diagnostic problems dealing with fungous diseases.

The various wards, clinics and laboratories of the Charity Hospital of New Orleans have provided us with a wealth of clinical and laboratory material as well as unusual opportunities for observation and study of fungous disease. Cases of all of the mycotic infections have been observed by us with the exception of rhinosporidiosis and South American blastomycosis within the period of time this Atlas was in preparation.

The position that we have enjoyed in the diagnosis of fungous disease has been unique. We have been called in consultation on the wards and in the clinics; we have occasionally suggested the type of therapy and have been able to follow the results of therapy; we have advised biopsy and the specimens have passed under our microscopes for diagnosis; we have received specimens and have requested that specimens be submitted for examination for species of fungi; we have cultured material and studied characteristics of growth for identification; and we have been able to perform necropsies or to have access to necropsy material of proved and unproved cases of fungous infections.

In order to augment our own experiences we have freely consulted such authorities as Conant, Jacobson, Lewis and Hopper, Swartz and others. We have added considerable new material in the form of morphological studies on a new selective medium for the primary isolation of fungi, Littman oxgall agar. In our experience this medium often surpasses

Sabouraud glucose agar as an identifying medium when the growth characteristics of the fungi have become familiar.

Until the rather startling results of the histoplasmin skin test suggested the existence of much sub-clinical infection it was accepted that all infections produced by *Histoplasma capsulatum* were fatal; this is undoubtedly a false assumption. Apparently many infections heal spontaneously. Advances in chemotherapy offer a brighter prospect for cure in proved cases. Promin seems to have considerable promise.

Infection with *Coccidioides immitis* was also believed to be an invariably fatal infection. Extensive skin testing with coccidioidin with the detection of many patients with positive tests has proved this to be a fallacy. Fortunately, a relatively small number of infected individuals develop coccidioidal granulomas to which they ultimately succumb.

Definitive diagnosis in fungous disease is imperative and can be accomplished only in the laboratory. The signs and symptoms of fungous infections may be bizarre and mimic other chronic diseases to a marked degree. Many fungi produce lesions which are granulomatous, suppurative or a combination of the two. They can rarely be differentiated either clinically or histologically from tuberculosis or from other chronic diseases, except by isolation, cultivation and identification of the etiologic agent. This is also true for the vast number of cutaneous fungous infections. Although they are rarely fatal, they nevertheless cause great discomfort and morbidity.

Experiences during World War II with cutaneous and deep mycoses presented many diagnostic problems of serious magnitude. These stimulated new interest in fungous disease and gave added impetus to medical mycology.

It is our belief that definitive mycological diagnosis is no more formidable than definitive studies of bacteria. As a matter of fact, the requirements for identification of many species of fungi is much simpler. So ubiquitous a family as the fungi, capable of producing a variety of pathological lesions, places the problem of diagnosis and treatment of fungous diseases before clinicians and clinical pathologists alike.

ACKNOWLEDGEMENTS

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Dr. Charles A. Dunlap, Dr. Marion Hood, Dr. Edgar Hull and Dr. James K. Howles have also offered valuable suggestions. We offer our thanks to Miss Janis Smith for excellent technical assistance and Miss Sadina Bertucci, our secretary, for typing and retyping the manuscript with patience and precision.

Also we wish to acknowledge the assistance of the Department of Medical Illustration, Louisiana State University School of Medicine in preparing Chart 1, developing and printing our photomicrographs, taking some of the clinical photographs and assisting us in many ways with the illustrations.

We are indebted to our many friends and colleagues who afforded us the opportunity to see and study fungous diseases on the wards and in the clinics.

New Orleans, La.

EMMA S. MOSS, M.D.
ALBERT L. MCQUOWN, M.D.

Contents

NOTE: Illustrations follow each chapter.

1 *Classification of Fungi*

See Chart 1

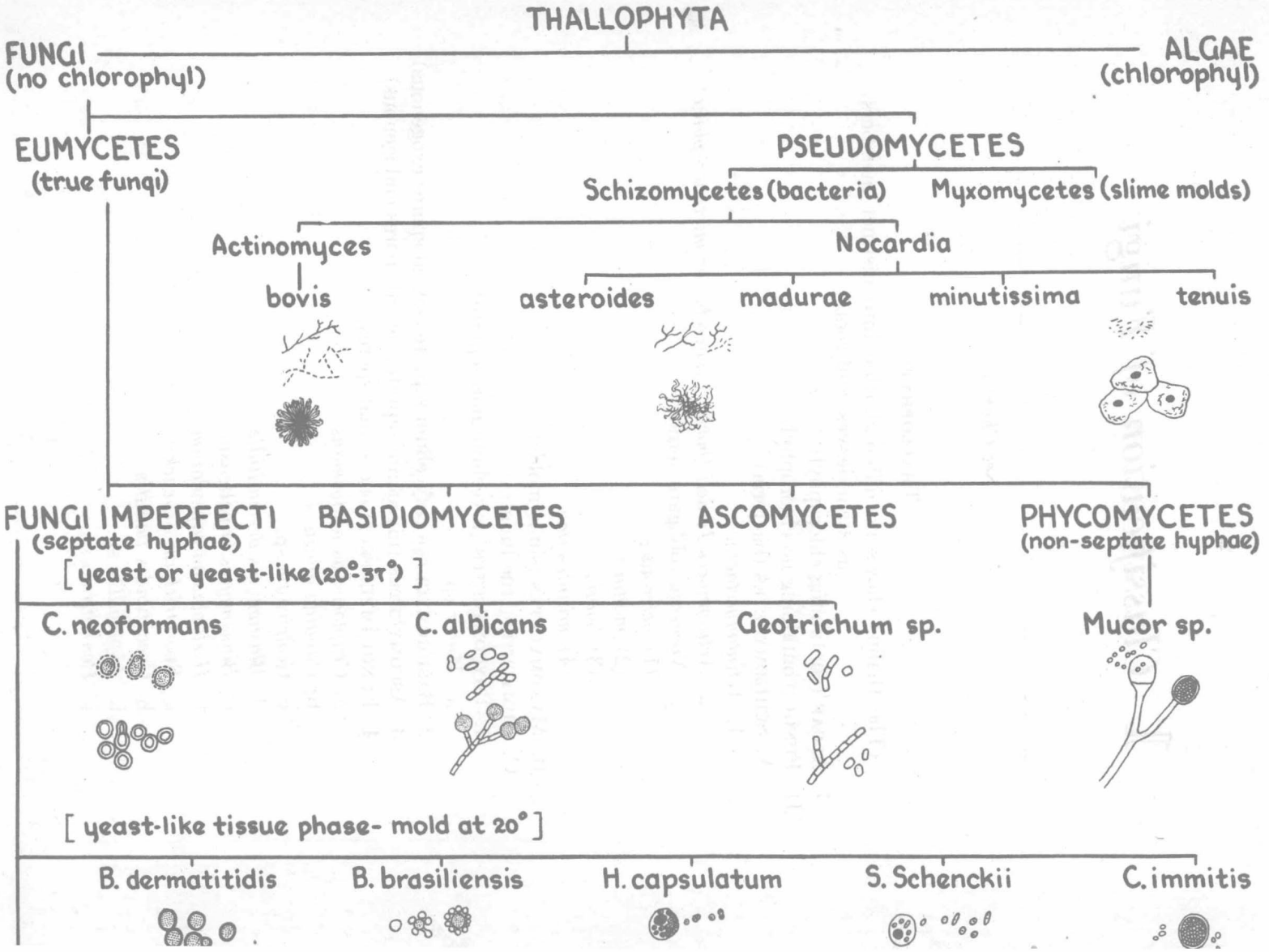
THALLOPHYTA

(The thallus shows no differentiation into distinct members as stem, leaves, and roots)

I. ALGAE (containing chlorophyl)

II. FUNGI (containing no chlorophyl)

- A. SCHIZOMYCETES (bacteria)
  - 1. *Actinomycetaceae*
    - a. *Actinomyces Israeli* (*bovis*) (*anaerobic or microaerophilic*)
    - b. *Nocardia* (obligate aerobic)
      - (1) *asteroides*
      - (2) *madurae*
      - (3) *tenuis*
      - (4) *minutissima*
- B. MYXOMYCETES (slime molds)
- C. EUMYCETES(true fungi)
  - 1. PHYCOMYCETES (mycelium non-septate)
    - a. *Mucor* sp.
  - 2. BASIDIOMYCETES (mycelium septate, sexual spores exogenous)
  - 3. ASCOMYCETES (mycelium septate, sexual spores endogenous)
  - 4. FUNGI IMPERFECTI (no sexual spores)
    - a. *Cryptococcus neoformans*
    - b. *Candida albicans*
    - c. *Geotrichum* sp.
    - d. *Blastomyces dermatitidis*
    - e. *Blastomyces brasiliensis*
    - f. *Histoplasma capsulatum*
    - g. *Sporotrichum Schenckii*
    - h. *Coccidioides immitis*
    - i. *Aspergillus* sp.
    - j. *Penicillium* sp.


THALLOPHYTA
FUNGI
(no chlorophyl)
ALGAE
(chlorophyl)
EUMYCETES
(true fungi)
PSEUDOMYCETES
Schizomycetes (bacteria)
Myxomycetes (slime molds)
Actinomyces
bovis
Nocardia
asteroides
madurae
minutissima
tenuis
FUNGI IMPERFECTI
(septate hyphae)
[yeast or yeast-like (20°-37°)]
BASIDIOMYCETES
ASCOMYCETES
PHYCOMYCETES
(non-septate hyphae)
C. neoformans
C. albicans
Geotrichum sp.
Mucor sp.
[yeast-like tissue phase- mold at 20°]
B. dermatitidis
B. brasiliensis
H. capsulatum
S. Schenckii
C. immitis

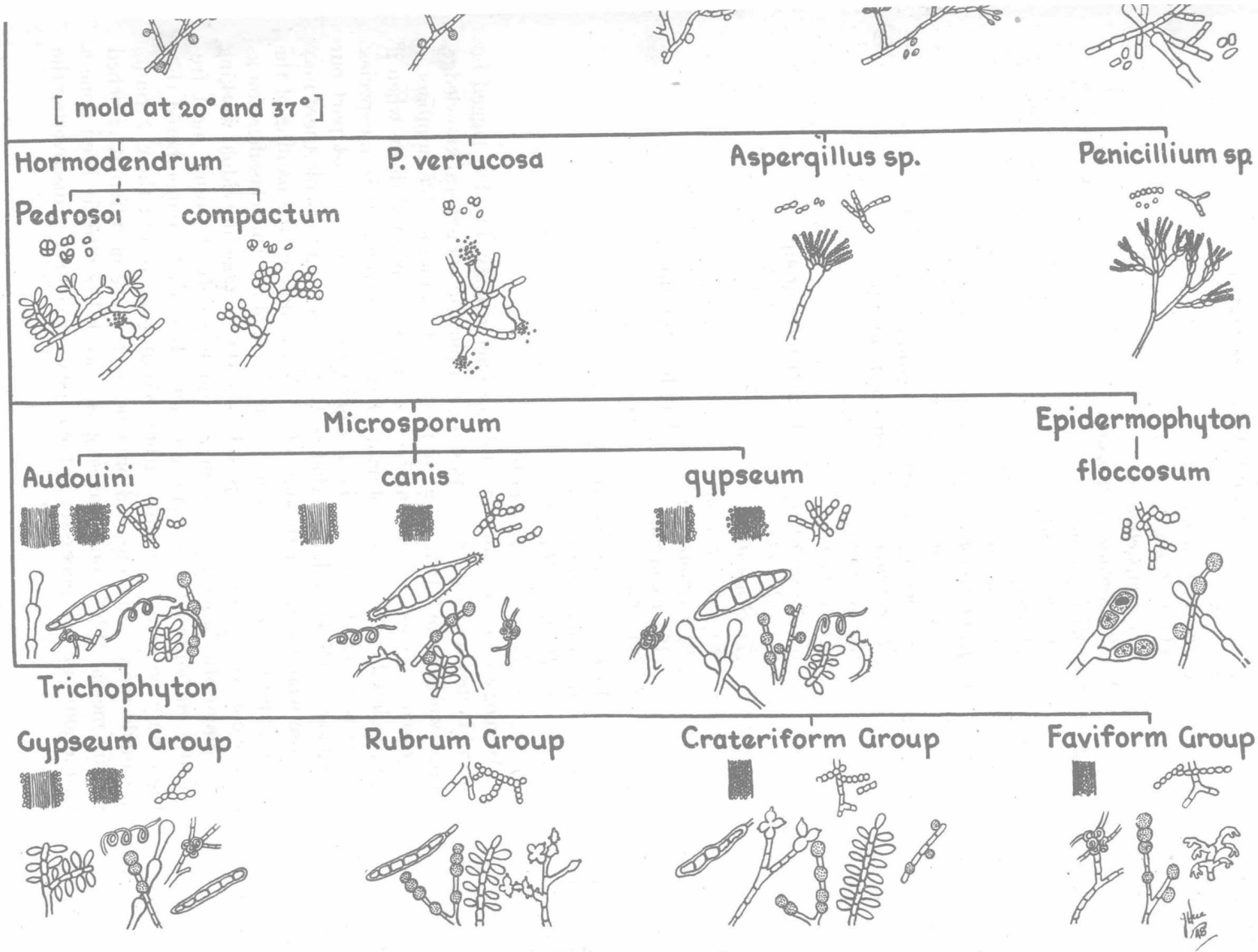


CHART 1. Classification—*Thallophyta*

k. *Hormodendrum*
   (1) *Pedrosoi*
   (2) *compactum*
l. *Phialophora verrucosa*
m. *Microsporum*
   (1) *Audouini*
   (2) *canis*
   (3) *gypseum*
n. *Epidermophyton floccosum*
o. *Trichophyton*
   (1) *mentagrophytes*—Gypseum group
   (2) *rubrum*—Rubrum group
   (3) *tonsurans*
   (4) *epilans*
   (5) *Sabouraudi* } Crateriform group
   (6) *sulfureum*
   (7) *Schoenleini*
   (8) *concentricum*
   (9) *ferrugineum* } Faviform group
   (10) *violaceum*
   (11) *faviforme*
p. *Rhinosporidium Seeberi*
q. *Malassezia furfur*
r. *Piedraia Hortai*
s. *Trichosporon Beigelii*

Mycology is older than the study of bacteriology, but has lagged far behind in its classification and practical application of general knowledge. Many workers find that what appears to be innumerable numbers of pathogenic species of fungi can be considerably narrowed down when it is appreciated that a variety of names have been given to the same species. This is particularly true of the dermatophytes. For the most part our classification closely followed that of Conant et al. and while there may be those who disagree with some of our selections for the naming of the species, and the omission of some others, we submit this classification as a basic one which will make it possible for the average individual working in a diagnostic laboratory to identify and classify the fungi most frequently encountered in human infections. It must be remembered that there may be many variants within certain strains and these variants are made more numerous and obvious by the use of different enriched culture media. While much investigative work has already been done a vast amount more is necessary in the hope of producing or discovering the

sexual stage of the organisms placed in the Fungi Imperfecti. When this occurs these organisms can be placed in their proper true classification.

We feel that the diagnostic laboratory and its reference laboratory manuals are not the place for a detailed discussion of taxomony. This can always be found in journals of investigation that are always available to those interested in advanced mycology. Thus the brief concise classification presented here is for the understanding, work and discussion of mycology in the hospital, laboratories and clinics.

Fungi reproduce by means of specialized cells called spores which on a suitable substrate germinate, producing one or more filamentous processes called *germ tubes*. These elongate into *filaments* which eventually branch. Each filament is called a *hypha* (pl. hyphae). At first the hypha is *non-septate*. When a hypha divides into chains of cells by transverse walls each wall is called a septum. They are laid down behind the growing point and the hyphae so divided are known as *septate hyphae*. Some fungi (*Phycomycetes*) do not develop septa and have *non-septate* hyphae which allow the protoplasm to flow uninterrupted through the hollow tube (coenocytic).

These filaments branch, rebranch and intertwine to form a mass called *mycelium*. That portion of the growth developing above the surface of the medium is known as the *aerial* mycelium and that below the surface for food collecting is known as the *vegetative* mycelium. The vegetative mycelium does not vary greatly in the different groups of fungi. The aerial mycelium is the reproductive portion from which the spores form. This type of plant structure is known as a *thallus* and such growths are called *Fungi* (*Mycetes*). They are placed in the phylum *Thallophyta* which contain these irregular plant masses that lack definite root, stem and leaf structures. The Thallophyta are divided into two main groups, the *Algae* and the *Fungi* with the *Lichens* forming a third division composed of both Algae and Fungi growing in symbiosis.

Algae are simple thallus plants but contain *chlorophyl* which allows them to produce their own food. Fungi do not contain chlorophyl and are either saprophytic or parasitic. The sub-groups of Fungi are *Pseudomycetes* (false fungi) and *Eumycetes* (true fungi). The Pseudomycetes are divided into (1) *Schizomycetes* or bacteria in which *Actinomycetes* and *Nocardia* species are classed and the (2) *Myxomycetes* (slime molds) which are non-pathogenic.

The *Eumycetes* contain four classes falling into two groups according to the characteristics of their mycelium and spores. The first group contains those fungi with non-septate hyphae—the *Phycomycetes*. Those with septate hyphae are *Basidiomycetes*, *Ascomycetes* and *Fungi Imperfecti*. The

majority of the pathogens are found in the group of Fungi Imperfecti and the individual members are identified and classified by the type of colony produced, presence or absence of mycelium, the type of mycelium, the method of spore development and the characteristics of the spores.

Spores may develop either *asexually* (division of a single cell without fusion with another) or *sexually* repeated division after fusion of nuclei from two similar or dissimilar cells).

Phycomycetes are the most primitive class of fungi and are differentiated from other classes of fungi by their (1) non-septate hyphae, (2) asexual spores and (3) sexual spores. The asexual spores are produced inside a swollen structure called a sporangium (pl. sporangia) on the end of branches or hyphae called *sporangiophores*. The spores are *sporangiospores* and when the sporangium ruptures the sporangiospores scatter and leave the thin-walled sporangium in place or it dissolves and the spores are dispersed. The sexual spores are either *Zygospores* produced by tips of approximating hyphae conjugating and resulting in large thick-walled bodies from fusion of the contents of the terminal portion or *Oospores* resulting from fertilization of a specialized female structure on a hypha by the sperm or nucleus of a male structure close by. The non-septate hypha or coenocytic hypha forms one large cell containing many nuclei. These fungi are characteristically non-pathogenic.

Basidiomycetes are characterized by (1) the exogenous development of sexual spores and (2) septate hyphae. Specialized mycelium develops which terminate in a club-shaped structure (*basidium*, pl., *basidia*) and it develops exogenous sexual spores known as *basidiospores*. These are typically four in number. The mycelium is organized in compact layers and the septate mycelium is binucleate. Mushrooms are of this family.

Ascomycetes are characterized by the production of (1) sexual endogenous spores, (2) asexual spores, and (3) the majority have septate hyphae. The sexual spores are produced *within* an enlarged cell by nuclear fusion. They usually number eight and are called *ascospores*. The outer wall or sac is called the *ascus*. The asexual spores arise from specialized branches called *conidiophores* which arise from the septate hyphae and the various shaped spores produced are called *conidia* (sing., conidium). The hyphae are septate and uninucleate.

The fungi belonging to the above classes characteristically produce their sexual and asexual spore stages and classification is not too difficult, but there remains a further group characterized by the formation of only asexual spores. These are placed in the *Fungi Imperfecti* as the perfect or sexual stage is unknown. The exact classification of all fungi in this group is difficult as the spore formation is varied by temperature, culture

medium and strain of the organism. Consequently this places them in a form where morphological simularities exist even though they arise from various genetic types. The hyphae of *Fungi Imperfecti* are septate and are uninucleate like the *Ascomycetes*.

The simplest type of *Fungi Imperfecti* are represented by *the true yeasts*. These organisms remain in the yeast form at both room (20°C.) and incubator (body) temperature (37°C.). They produce no mycelium and reproduce by budding. These buds break off from the parent and in turn propagate by budding. This spore type is called a *blastospore* and is asexual. The species of Cryptococci are true yeasts. A second type of development, and slightly more complex, is represented by *Candida* sp. which produce soft yeast-like colonies at room and incubator temperatures; however, in this case the aerial portion is yeast and the vegetative portion contains elongated buds remaining attached to the parent cell which on repeated division produces a chain of attached cells, somewhat constricted at their division point but resembling hyphae—these are called *pseudo-hyphae*. This colony is not a true yeast but is *yeast-like*. A third form of development is characteristic of the majority of the remaining pathogens. They develop both aerial and vegetative septate mycelium and produce asexual spores. An example of this form is the *Tricophyton* sp. A fourth method of development (a combination of the second and third form) is characterized by the formation of yeast like colonies at incubator temperature (37°C.) and development of a septate mycelium and spores at room temperature (20°C.). This can be illustrated by *Blastomyces* sp.

Asexual spores on which the classification of Fungi Imperfecti is based are of two major types, the *thallospores* and *conidiospores*. Thallospores which are formed by the thallus or mycelium are of three types. The first is the *blastospore* formed as described under yeasts—the budding of one cell from a larger parent cell as characterized by *Cryptococcus* sp. and *Candida* sp. The second type is the *chlamydospores* or large thick-walled resting spores developed from the hypha for existence during long periods of dormancy. These result from a concentration of the protoplasm of the hypha or pseudohypha as in the case of *Candida albicans*. They may be *intercalary* (interposed between the septae of hypha), *terminal* (at the end of the hypha), or *lateral* (adjacent to but on the side of the hypha). The third type is the *arthrospore* where the segmentation of the septate hypha by constriction results in rectangular or "pillow-shaped" thick-walled spores which tend to become ovoid after fragmentation of the hypha. This is typical of *Geotrichum* sp. and *Coccioides immitis*.

Conidiospores are produced by a specialized hypha (conidiophore) arising from the septate hypha or by abstriction at a point of attachment.

The spore produced (*conidium*, pl., *conidia*) varies as to size, shape, number, and number of septations. These are used in the description as well as in the differentiation of species. *Microconidia* are small single conidia which may be *spherical* (round), *elliptical* (ovoid), *pyriform* (pear-shaped), or *clavate* (club-like), or *muriform* (arranged like a course of bricks). The conidia may be found *sessile* (adjacent), *lateral*, *clustered* (en grappe), *pedunculated*, or in *chains*.

Occasionally the *conidiophore* is characteristic for identification as in the *Aspergilli* and *Penicilli* sp. The *Aspergilli* are characterized by a swollen end of the conidiophore called a vesicle and over its surface arise flask-shaped structures called *sterigma* (pl. *sterigmata*) from which the spores arise in chains from the tips of the sterigmata by the cutting off of successive conidia. They are said to be *catenate*. As the youngest spore is near the sterigma there is no branching of the chain and it is said to form *basipetally*. *Penicilli* species have sterigmata and produce spore chains similarly to the *Aspergilli* but lack the vesicle at the end of the conidiophore. In its place the conidiophore has numerous branches each with a sterigma that produces a chain of spores; thus the *Penicillium* sp. have a brush-like appearance.

If conidia are produced from the conidiophore by budding and the end bud in turn buds, a chain will be produced if they remain attached. The most proximal spore in this case is the oldest and chains developed by this method are formed *acropetally*. A cluster of spores may be produced if several buds give rise to branching chains. These types of chains and clusters are produced by the *Hormodendrum* sp.

2 *Actinomycosis*

ETIOLOGY: *Actinomyces Israeli* (*bovis*), Harz, 1877, (Anaerobic or microaerophilic).

DEFINITION: Actinomycosis is a chronic disease characterized by superficial or visceral granulomatous lesions which break down, form abscesses and produce multiple draining sinuses (Figs. 1, 2 and 3). The characteristic "sulfur granules" may be found in the exudate, the sinus walls or in the deep lesions.

DISTRIBUTION: World wide. Occurs more frequently in males. While more cases are detected in individuals in the second to fourth decade, no age is exempt. Poor oral hygiene appears to be an important contributing factor in its incidence.

CLINICAL DISEASE

The infection may be cervicofacial, thoracic, abdominal, cutaneous or generalized. The clinical symptoms depend upon the location of the lesions.

CERVICOFACIAL: Injury or trauma to the oral tissues is an important predisposing factor. The first symptom is a painless tumefaction which later becomes firm and "woody" with little inflammatory tissue reaction. As the disease progresses the tissue tends to break down and form multiple draining sinus tracts. Characteristic of actinomycosis is the healing of sinus tracts with scar formation in one area with appearance of new sinuses in other areas.

THORACIC TYPE: This form of the disease often begins as a mild infection resembling early tuberculosis. However, the lesions are more frequently located in the bases of the lungs. As the infection progresses, the pleurae become involved, and multiple draining sinuses may develop.

ABDOMINAL: The ileocecal region or the adnexae of the female are frequent primary sites of infection. Early symptoms may simulate appendicitis or salpingitis. The disease may remain localized or it may progress and spread to other abdominal organs. The infection may not be suspected until draining sinuses develop or until it is discovered during an operation.

CUTANEOUS: This rarely remains a superficial infection. Invasion of the subcutaneous tissues and underlying structures, such as bone, occurs frequently.

GENERALIZED: Any of the more localized types of actinomycosis may eventually involve other organs or tissues either by direct extension or by hematogenous spread.

X-RAY DIAGNOSIS

The pulmonary lesions are generally bilateral and are more frequently located in the lower lobes although the upper lobes may be involved (Fig. 4). There may be areas of infiltration or massive consolidation of the lung (Fig. 5). When the pleurae are involved in the process there is thickening and fluid may accumulate in the cavities. Both destructive and proliferative changes are produced in bone. The lesion may be a localized osteomyelitis with slight periosteal reaction, or it may be massive with cyst formation. The mandible is the bone most frequently involved (Fig. 6).

LABORATORY DIAGNOSIS

MATERIAL FOR CULTURE: Sputum or exudate should be spread in a sterile Petri dish and examined under bright light for the presence of the characteristic "sulfur granules" (Fig. 7). These appear as small yellow to light tan flecks or "granules" dispersed throughout the material. Occasionally they are not present or they are few in number so that repeated search must be made for them. Curettement of the wall or irrigation of the sinus using slight pressure dislodges the "granules" and washes them out in the exudate. The gauze which covers the draining sinuses should be inspected carefully since colonies of organisms may become entangled in the meshes. These can be removed and examined under the microscope.

MICROSCOPIC EXAMINATION: "Granules" are transferred to a slide and crushed beneath a cover slip. They appear as round or lobulated masses of intertwining mycelium, the filaments of which measure approximately 1 micron in width. The ends of the hyphae which radiate peripherally from the central mass of filaments may or may not appear clubbed (Figs. 8 and 9). Filaments of the fungus are gram positive when stained by Gram's method. In the absence of characteristic "sulfur granules" the presence of gram positive branching filaments should lead to a suspicion of actinomycosis. *A. Israeli* can be grown with relative ease if the material is free from secondary bacterial contamination and a suitable culture medium is employed. Best results will be obtained when material is aspirated from