Fig. 1.—A, Cryptococcus neoformans in adrenal gland. The large yeast cells are surrounded by a clear halo, which represents the capsule. No mycelium is formed. A. F. I. P. accession no. 170021. Bauer stain; × 435.

B, Histoplasma capsulatum in lymph node. The small yeast cells are typically crowded together in large phagocytic cells, and there is no mycelium. A. F. I. P. accession no. 190797. Hematoxylin and eosin stain; × 435.

C, mucormycosis of lung. The hyphae are very large and nonseptate, and show marked irregularity of contour, with lateral branching. Yeast cells are not present. A. F. I. P. accession no. 218946. Bauer stain; × 525.

D, Candida in kidney. Budding yeast cells and septate mycelium are seen. The hyphae are relatively small, show end to end growth with little lateral branching. A. F. I. P. accession no. 264305. Bauer stain; × 525.

E, same as D, showing organisms predominantly in yeast phase. Bauer stain; × 355.

F, pulmonary aspergillosis. The hyphae are larger than those of Candida but smaller than those of Mucor. Like that of Candida, the mycelium is septate but shows more lateral branching. The rounded forms are not spores but are hyphae cut transversely. A. F. I. P. accession no. 232320. Bauer stain; × 525.

G, “sulfur granule” of actinomycosis. The filamentous hyphae are much smaller than those of Candida and Aspergillus, and spore forms are never seen. A. F. I. P. accession no. 167249; × 525.
admitted to Gallinger Municipal Hospital on Aug. 14, 1939, with a history of obstipation, hemorrhagic spots on the palms, and chills and fever of two days’ duration. For three weeks preceding admission she had noted numbness, stiffness and pain in the right leg, cramping abdominal pain, nausea and vomiting. On admission her temperature was 100.2 F., pulse rate 130, respirations 24 and blood pressure 130 systolic and 84 diastolic. Petechiae were present in the palms and nail beds. There was a loud presystolic murmur. The abdomen was tender and rigid. Her course was characterized by persistent fever, with a temperature of 101 F., and abdominal pain and vomiting were the major complaints. Occult blood was present in vomitus and stools. A Candida was isolated from the blood and identified as C. guilliermondi by Dr. C. W. Emmons. Nine days after admission the abdominal pain became more intense and the patient went into shock. She died 12 hours later.

At autopsy a huge, fungating vegetation, measuring 2 by 5 cm., involved about two thirds of the circumference of the mitral valve. There was evidence of underlying rheumatic disease, with fusion of the leaflets and fibrosis of the chordae tendineae, but the heart weighed only 250 Gm. Embolization of the mesenteric vessels had occurred, with infarction of the ileum. Five small perforations and generalized peritonitis were noted. Infarcts of the spleen and the kidneys were also present. Sections of the vegetation revealed that it was engrafted on a thickened, sclerotic mitral leaflet (fig. 2). Its entire bulk was composed of fungus elements and an acellular hyaline substance in which no inflammatory cells were seen. A thin layer of fibrin and leukocytes capped its surface. On low power examination the vegetation had a striking foliate pattern, due to the undulating manner of growth of the fungus at the periphery. High power examination of hematoxylin and eosin sections showed faintly staining mycelium and an abundance of small spores. With the Bauer stain, hyphae were seen to better advantage, although small rounded spores predominated (fig. 2 B). The mycelium was typical of Candida with respect to size, septation and end to end growth with little lateral branching.

CASE 2 (“Aspergillus endocarditis”).—A 25 year old white soldier, on April 16, 1946, fell from a train, sustaining multiple injuries, the most significant of which was partial amputation of the left leg. The leg was surgically amputated below the knee the same day, and use of penicillin was started. The next day roentgenograms revealed a fractured scapula. There was a 4 inch (10 cm.) retraction of the skin at the site of amputation. The stump remained edematous, foul smelling and semilecrotic. On April 25, amputation above the knee was performed. Cultures revealed gram-positive and gram-negative rods and gram-positive spore-forming rods. Low grade fever persisted in spite of transfusions and penicillin. A month later pain developed in spite of transfusions and penicillin. A month later pain developed in the stump and in the lumbar region. A temperature of 99 to 103 F. persisted. On May 26 right popliteal embolism occurred. At this time a to and fro murmur could be heard over the entire precordium. The unusual blood pressure of 130 systolic and 150 diastolic was recorded. Papaverine hydrochloride was given, and paraverterbral sympathetic blocks and embolectomy were performed; administration of penicillin was continued. On June 1 his temperature rose to 104 F., and signs of vascular insufficiency of the right leg appeared. Systolic and diastolic murmurs were heard, and there was a systolic precordial thrill. Blood cultures produced no growth. The spleen became palpable on June 4. Venous distention and a gallop rhythm appeared the next day. On June 6 the patient went into shock, became irrational and died. Autopsy again revealed the enormous spherical vegetations which seem to be characteristic of mycotic endocarditis (fig. 3 A). These vegetations, which were of firm consistency and measured up to 2.5 cm. in diameter, involved predominantly the right cusp of the aortic valve. They also extended onto the adjacent mem-
Fig 2 (case 1).—A, Candida guilliermondii endocarditis of mitral valve. Note the foliate pattern of growth. A. F. I. P. accession no. 292649. Hematoxylin and eosin stain; X 10.

B, same as A. The majority of the organisms are in the yeast phase. Eosin-azure stain; X 525.
Fig. 3 (case 2).—A, Aspergillus endocarditis. These vegetations on the aortic valve are of large size and spherical contour. The arrow indicates the site of an erosion which penetrated through the membranous portion of the interventricular septum. Walter Reed Negative 7292. A. F. I. P. accession no. 190959.

B, vegetation on aortic valve. Hematoxylin and eosin stain; X 7.

C, large vacuolated septate hyphae, tightly packed, without inflammatory cells, in a section from an aortic vegetation. Bauer stain; X 355.

D, high power view of hyphae. They are larger than those of Candida, stain less regularly and show more lateral branching. Bauer stain; X 1500.
branous portion of the interventricular septum. Ulceration had penetrated through this portion of the septum, creating a passage 4 by 1 mm. A vegetation had also formed on the septal cusp of the tricuspid valve. The heart weighed 385 Gm. There was evidence of renal embolization. A recent hemorrhagic infarct was present in the right kidney, and several small, older lesions were seen in the left kidney.

Microscopically the aortic valve cusps showed intense fibroblastic proliferation with the presence of typical Anitschkow myocytes. A zone of advanced edematous degeneration of the valvular connective tissue was present. In the area where the vegetation was attached, there were acute collagenous necrosis and intense mobilization of segmented granulocytes. As in case 1, the vegetation was composed of a massive fungous growth with a foliate pattern (fig. 3B). In this case, however, most of the growth was composed of septate mycelium, larger in size than that in case 1, while spore forms were much less numerous (fig. 3C and D). Bauer's stain was helpful in demonstrating the typical lateral branching of Aspergillus.

In the myocardium, foci of interstitial myocarditis were seen. In the kidney, ischemic infarcts were present. About these there were small arteries containing emboli. An exudate of segmented granulocytes was noted in the lumen, while histiocytic proliferation, with formation of giant cells of the Langhans type, occurred in the vessel walls. Some of these histiocytic cells contained fragments of hyphae.

CASE 3 ("Aspergillus endocarditis")—A 32 year old white woman was hospitalized for two weeks in July 1947 because of fever, nausea, and easy fatigability. No diagnosis was established, and subsequent to her discharge pain developed in the epigastric region and the left upper quadrant of the abdomen. In August she was hospitalized again, for three weeks, because of fever of undetermined origin. Numerous blood cultures were negative. Penicillin and sulfadiazine were given, without response. In November she had an episode of pain in the flank with fever.

On Jan. 17, 1948 she noted numbness of the right leg. Ten days later this became more severe and she was again admitted to the hospital. There were signs of embolism of the right leg. Harsh apical aortic murmurs were heard. The liver was enlarged and the spleen tender. In spite of 42 negative blood cultures during this stay in the hospital, the patient was treated intensively for bacterial endocarditis, receiving 84,000,000 units of penicillin, without response. Streptomycin was also given, without benefit. On June 23 signs and symptoms of left femoral embolism appeared. Her condition continued to deteriorate. On August 14 she awoke coughing and dyspeptic, and died suddenly.

The heart revealed the residua of old rheumatic disease but, as in case 1, there was no evidence of functional impairment, as the weight was only 275 Gm. In addition to the old process, the aortic valve showed perforations of the right anterior cusp and friable, grayish vegetations of the right anterior and posterior cusps. The latter were large and lobulated and measured 2 by 2.5 cm (fig. 4A). Extensive embolization had occurred. A firm, gray to red, lamellated thrombus filled the terminal 2 cm. of the aorta and both common iliac arteries. Extensive infarction of the right kidney, both adrenal glands and the spleen were found.

Hematoxylin and eosin as well as gram-stained sections of the vegetations showed only minute spores, which originally were interpreted as Histoplasma capsulatum (fig. 4B). With the Bauer stain these spores appeared much larger and showed great variation in size and shape. Although spore forms predominated, foci in which branching septate hyphae were developing could also be found, indicating Aspergillus infection (fig. 4C). Sections of the infarcted adrenals, how-
Fig. 4 (case 3).—A, Aspergillus endocarditis of aortic valve. A. F. I. P. accession no. 204556. Bauer stain; × 6.

B, small yeast cells with no visible mycelium. Gram stain; × 435.

C, Aspergillus showing many more organisms, including definite mycelial elements. The spore forms are much larger than those of Candida; compare with D and E in figure 1. Bauer stain; × 435.
ever, revealed only spore forms. These showed a striking tendency to be clumped together within the cytoplasm of large mononuclear cells, further simulating H. capsulatum.

PROBLEMS IN DIFFERENTIAL DIAGNOSIS

Typical Candida and Aspergillus organisms are easily recognized by the criteria previously given. Difficulty arises when mycelial filaments either are not visible, owing to staining difficulties, or are not recognized. Thus in another case studied the failure to recognize mycelium led to a diagnosis of cryptococcosis, while in case 3 the mycelium was not visible in the original slides submitted.

A second source of difficulty involves morphological variation in the mycelial structure. Variations in size, type of branching and degree of septation, especially when the organisms are not abundant, may make accurate diagnosis impossible.

A third problem is centered about the presence of spores in addition to hyphae. In some reported cases of aspergillosis a number of photomicrographs which are captioned as showing spores actually show only hyphae cut in cross section. In most instances spores are not seen in deep-seated mucormycosis or aspergillosis, although they are usually recognized in surface lesions. Candida infections, on the other hand, usually include large numbers of spores—even in deep tissue. In case 3 the large number of Aspergillus spores and the absence of hyphae in the adrenal are considered unusual.

Another confusing feature in some cases of aspergillosis is the presence of “actinomycetoid bodies” resembling the sulfur granules of actinomycosis and mycetoma. High power examination, however, reveals the filaments to be much larger than those of Actinomyces and Nocardia. According to Henrici, actinomycetoid forms are a manifestation of hypersensitivity appearing in the later stages of the disease.

The obvious conclusion is that all too frequently an unequivocal diagnosis cannot be made on the basis of morphological evidence in tissue sections. Weed and Dahlin properly emphasized the need for bacteriologic and mycologic examination of tissue specimens. In only one of the three cases presented here was a cultural diagnosis established. In the other two the specific mycologic diagnosis is only probable, and cultural studies would have been necessary to establish a definitive diagnosis.

One tried and useful procedure for routine handling of autopsy and surgical specimens is that suggested by Littman. A duplicate portion of a given lesion is placed in a sealed sterile container in the refrigerator at 0 zero C. If pathological examination reveals the lesion to be of

infectious origin, appropriate cultural studies may then be performed on
the refrigerated sample. This method circumvents unnecessary culturing
of all specimens, an impossible task in most laboratories.

Another suggestion for the clinical pathologist and bacteriologist is
in regard to the finding of "nonpathogens" or "contaminants" in
cultures. It seems not unlikely, for example, that among the very large
number of "negative blood cultures" in case 3 some may have yielded
aspergilli. Such a finding, especially if repeated, in a case of endo-
carditis (or any other infectious process) in which the condition is not
responding to adequate antibiotic therapy should create at least a
suspicion of the correct diagnosis.

ETIOLOGIC FACTORS

Antibiotic and Chemotherapeutic Drugs.—It is well established that
none of the antibiotics are effective against these fungi. Actually the
reverse appears to be the case. Foley and Winter, working in Dr.
Sidney Farber’s laboratory at the Children’s Medical Center in Boston,
have demonstrated that penicillin enhances the growth and pathogenicity
of Candida stellatoidea and Candida albicans.

Dr. Farber and his associates at the Children’s Hospital in Boston
have had a remarkable experience since the introduction of antibiotic
therapy. They have observed seven cases in which disseminated
moniliasis occurred in children who were being treated with various
chemotherapeutic agents for other diseases. The majority received both
penicillin and aureomycin. In five instances the original disease was
leukemia, and in each of these cases the primary site of moniliasis was
the esophagus or the intestine. Most of these children showed widespread
fungus infection at autopsy. The fact that no similar cases were observed
prior to 1946 definitely implicates the newer antibiotic and chemo-
therapeutic agents in the genesis of these mycotic infections. These most
interesting cases will be reported in detail by Dr. Farber and his
associates.

Another case in point is that of Geiger and co-workers. Their
patient had twice been successfully treated with penicillin for bacterial
endocarditis. During the second illness, while penicillin was being given,
"folliculitis" of the scalp developed. Though the bacterial infection
was brought under control, a fatal C. albicans endocarditis ensued.

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10. Farber, S.: Personal communication to the author.

Case 2 is another which supports the thesis that penicillin increases the virulence of certain mycotic infections. The soldier received penicillin from the day of his injury to that of his death, and yet he was usually febrile and became progressively worse. In case 3 the condition of the patient steadily deteriorated in spite of prolonged administration of penicillin and streptomycin. It is not inconceivable that in these two cases the antibiotics may have permitted localized mycotic lesions to become disseminated. In this connection the case of generalized aspergillosis reported by Cawley'2 is of interest. The child, who died at 8 years of age, had experienced repeated episodes of pulmonary aspergillosis due to Aspergillus fumigatus, presumably since the age of 2 weeks. Although at the age of 4 years he had pleural effusion and osteomyelitis of a rib, it was only after treatment with sulfonamide drugs and penicillin that dissemination occurred. Autopsy revealed abscesses at numerous sites (brain, dura, heart, lungs, mediastinum, lymph nodes, spleen, liver, right kidney and right ankle) from which A. fumigatus was isolated by cultural technics.

The recently reported case of Grekin, Cawley and Zheutlin'3 may also be related to antibiotic therapy. A 22 year old man had influenza symptoms for which he was given penicillin. He became more severely ill and expectorated thick, purulent sputum. Another complaint was a sore mouth, and the oral mucosa was reddened and covered with a patchy exudate. An eruption of the erythema multiforme type was also present. Sulfadiazine, aureomycin and streptomycin were given in that order, but his condition became steadily worse. The disease process disseminated, and at autopsy fungi, both spores and hyphae, were found in various tissues (brain, myocardium, lung, thyroid, duodenum and kidney). Cultures of pulmonary tissue revealed a pure growth of A. fumigatus.

Just how the antibiotic drugs stimulate the growth of fungi is not completely understood, although there is evidence that stimulation occurs. Besides this growth-enhancing effect there is the possibility that a growth-restraining substance normally produced by the natural bacterial flora of the intestine is lacking. It is known that at least some of the sulfonamides, streptomycin, aureomycin and chloromycetin® seriously alter the intestinal flora.

Bone Marrow Depressants.—By interfering with the cellular defense mechanisms, marrow-depressing drugs may also be etiological factors in some of these infections. It is interesting to note the frequency of

oropharyngeal moniliasis in patients receiving anti-folic-acid drugs. Furthermore, in five of the seven cases in which Farber found disseminated moniliasis the patients were children who were being treated for acute leukemia with these drugs. A similar case was mentioned by Dr. E. C. Rice in the paper which he read before the Washington Society of Pathologists April 1, 1950.

Lesions of Mucous Membranes.—It seems not unreasonable to assume that pathologic conditions of mucous membranes from various causes may be associated with a sharp diminution of resistance to microbial invasion. Evidence is accumulating which suggests that fungi which ordinarily have but little invasive power may infect lesions of mucous membranes and even penetrate the mucosa to gain access to underlying lymphatics and blood vessels. A variety of mucous membrane lesions may thus set the stage for moniliasis. Hewitt and Williams recently reported that in six patients receiving chloromycetin a dark brown discoloration of the tongue developed; culture in three instances revealed C. albicans.

Orally given streptomycin, aureomycin and chloromycetin (chloramphenicol) result in sterilization of the bowel, the stools becoming soft and odorless. A complication of this alteration of intestinal flora is the development ofavitaminoses consequent to the removal of vitamin-synthesizing bacteria. Mucous membrane lesions on a vitamin deficiency basis (especially ariboflavinosis) may therefore appear suddenly and may account for the rectal burning and diarrhea which some patients experience. Genital lesions apparently due to estrogen deficiency may also appear, and these, too, are readily infected by Candida.

In the leukemic patient, mucous membrane lesions of two additional types have been observed in the alimentary tract: hemorrhagic ulceration incident to administration of folic acid antagonists, and leukemic infiltration. Leukemic infiltrations compromise the blood supply of the mucosa, resulting in necrosis. If the intestine is sterilized with respect to its bacterial flora, mycotic infection of these necrotic foci may become clinically important. Such a sequence of events is suggested by the fact that in each of Farber's leukemic patients with disseminated moniliasis the portal of entry was the alimentary tract.

Diabetes.—This disease is usually thought of as a predisposing factor in moniliasis of the skin and genitalia. It also appears to be a constant

feature of mucormycosis of the central nervous system.\textsuperscript{17} In disseminated moniliasis and aspergillosis, however, diabetes has not seemed important.

**Age.**—Figure 5 shows an even distribution of cases of disseminated moniliasis, and the over-all effect of age in this collected series is regarded as unimportant. It must also be recalled that seven of the 13 cases charted in the first two decades are those from the Boston Children’s Hospital. Of necessity, these cases were drawn from a select population, and the relative importance of age as compared with other factors is considered minimal.

**Debility.**—Another predisposing factor often mentioned is debility; yet study of the reported cases fails to show that disseminated moniliasis is usually a complication of debilitating diseases.

**Drug Addiction.**—Persons who inject heroin hydrochloride intravenously are especially susceptible to endocarditis. Wikler and co-workers\textsuperscript{18} in 1942 reported that in a two year period mycotic endocarditis had been recognized in five drug addicts. Most interesting is the fact that in each instance the organism was reported to be a “non-pathogen.” Candida parakrussei was isolated four times, while in the fifth case (case 1 of this report) the organism was C. guilliermondii.

**Metastasis.**—In a number of the reported cases primary oropharyngeal moniliasis metastasized to a distant organ. Metastasis had occurred in most of the cases in which the lesions were meningeal and cerebral, and in each instance, when the species was identified, the organism was

\[\text{Fig. 5. The age distribution of the patients in 35 cases of disseminated moniliasis is charted according to decades. The criterion of dissemination in these collected cases is the presence of Candida in an organ to which access could be gained only by way of the blood stream. Cases of generalized cutaneous, oropharyngeal, bronchopulmonary, gastrointestinal and genital moniliasis were, therefore, excluded.}\]

C. albicans. In other cases, however, it appears that isolated infections developed without any apparent superficial focus.

Previously Reported Cases

It is not within the scope of this paper to review the previously reported cases of disseminated mycosis due to Candida and Aspergillus. With respect to endocarditis, four reported cases of Candida endocarditis are acceptable, and two other unpublished cases were referred to in the report of Wikler and associates. Several possible, but not acceptable, cases have been reported. No published cases of Aspergillus endocarditis were found. Ocular moniliasis leading to blindness and enucleation occurred in Miale's case, the patient dying four years later of meningitis.

Expectations for the Future

The potential danger of prolonged antibiotic therapy for a patient who harbors a focus of fungus infection has already been discussed. This danger increases as new antibiotics and oral preparations are introduced and their cost lowered by mass production. By the same token, such toxic drugs as nitrogen mustard and anti-folic-acid compounds that have gained widespread use may be expected to result in an increased incidence of unusual infections, both bacterial and mycotic. The clinical pathologist can no longer disregard certain organisms which in the past he has been able to dismiss as contaminants. The anatomic pathologists must familiarize themselves with the varied forms of different fungi in tissue sections and the equally varied tissue responses to these organisms.

Summary

This presentation has been based on a study of three cases of disseminated mycotic disease which illustrate the difficulties of differentiating Candida, Aspergillus, Mucor, Cryptococcus and Histoplasma...
as they appear in tissue sections. In one case the causative organism was a Candida; in the other two, Aspergillus.

Variations in staining qualities and morphological features often confuse the histopathological diagnosis of fungus disease, and accurate identification depends on cultural studies.

Factors of importance in the genesis of these infections include the administration of antibiotic drugs and marrow depressants, drug addiction and metastasis of superficial lesions.

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