SYSTEMIC NORTH AMERICAN BLASTOMYCOSIS
REPORT OF A CASE WITH CULTURAL STUDIES OF THE ETIOLOGIC AGENT AND OBSERVATIONS ON THE EFFECT OF STREPTOMYCIN AND PENICILLIN IN VITRO *

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North American blastomycosis is a fungus disease caused by *Blastomyces dermatitidis*. It is manifested in two clinical forms, cutaneous and systemic. The former, beginning as a small pimple or pustule on the hands, face, wrists, ankles, or other exposed site, or in an area injured by abrasion, proceeds to a chronic or subacute ulcerating lesion of the skin. Skin lesions of cutaneous blastomycosis are characteristically single, spread slowly and peripherally, and may cause extensive involvement with no evidence of visceral infection. The cutaneous form of the disease is seldom fatal and usually responds to treatment with iodides and x-ray therapy. Systemic blastomycosis, on the other hand, is a fatal disease ordinarily characterized by pulmonary involvement and widely disseminated lesions in the subcutaneous tissues, bones, joints, central nervous system, and internal organs.[1] In contrast with the cutaneous type, skin lesions in this form of the disease are multiple instead of single and are widely distributed over the skin on unexposed parts of the body such as the abdomen, back, and thighs. Both cutaneous and systemic manifestations of blastomycosis are caused by the same fungus.

The characteristic reaction of the tissues to the organism in either systemic or cutaneous blastomycosis is abscess formation with chronic granulomatous inflammation, giant cells, necrosis, and fibrosis. Numerous abscesses of almost microscopic size are found in skin lesions, while old lesions show signs of chronic inflammation and scarring. There is extensive epithelial hyperplasia about the margins of the ulcerating skin lesions. Although the tissue reaction in the systemic form of the disease is usually pyogenic, the presence of caseation necrosis and tubercle formation may cause it to be confused with tuberculosis.[2] Nevertheless, the tissue reactions in blastomycosis and tuberculosis are not identical,[3] the most obvious difference being the suppurative reaction in blastomycosis. Comprehensive reviews of the subject are available in the excellent papers of Martin and Smith,[4] of Baker,[5] and

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of Friedman and Signorelli.[5] Cases of North American blastomycosis have been reported almost exclusively from the United States. Proved and presumptive cases have been found in 28 states, with Illinois, Louisiana, Kentucky, Tennessee, Virginia, and North Carolina furnishing the larger numbers.

The purposes of the following report are (1) to present the clinical and pathologic features of a case of fatal systemic North American blastomycosis involving the meninges as well as other organs of the body; (2) to present and discuss the method of primary isolation and identification of *B. dermatitidis;* and (3) to describe tests of the resistance of the fungus *in vitro* to streptomycin and penicillin.

Report of Case

A 20-year-old, colored, male laborer entered Charity Hospital on May 16, 1946, because of tender cutaneous nodules which had grown progressively larger and more painful over the preceding 3 weeks. He had been well prior to the present illness and had had no symptoms referable to the gastrointestinal, genito-urinary, or nervous systems. For a period of 3 months he had had slight afternoon fever, night sweats, and blood-streaked sputum. He had been employed as a laborer in an oil field in central Mississippi but reported no prolonged contact with irritating dusts or chemicals. The past history was otherwise noncontributory.

Physical Examination. The patient was lying flat in bed without dyspnea, and was oriented and alert; his respiratory rate was 23; pulse, 94; and temperature, 99.4° F. He was well developed but poorly nourished and appeared severely ill. On the left forehead there was a large encrusted lesion, measuring 2 by 3 cm., which was sharply demarcated from the surrounding normal skin. Pus could be expressed from beneath the eschar. There were no lesions of the mucous membranes. Multiple subcutaneous, fluctuant masses, painful to palpation and varying in diameter from 2 to 5 cm., were present over the arms, forearms, thighs, and legs. There was no palpable enlargement of the superficial lymph nodes. The lungs were clear to percussion and auscultation. The heart was not enlarged and no abnormal sounds were heard. There were no palpably enlarged viscera in the abdomen and no abdominal tenderness or rigidity. There was no edema of the extremities. No signs of meningismus were elicited.

Laboratory Findings (on admission). *Blood:* Hemoglobin, 7 gm. per 100 cc.; red blood cells, 2.75 million per cmm.; white blood cells, 15,350 per cmm. Differential blood cell count: 63 per cent polymorphonuclear leukocytes, 13 per cent immature polymorphonuclear leukocytes, 4 per cent monocytes, 20 per cent lymphocytes. Sedimentation rate (Wintrobe) was 67 mm. in 20 minutes, 78 mm. in 1 hour. The Mantoux test was negative with 1:10,000 dilution of old tuberculin. The Kline and Kolmer tests were negative. *Urine (voided specimen)*: Yellow, acid; specific gravity, 1.012; albumin, negative; sugar, negative; 2 to 3 red blood cells and 5 to 6 white blood cells per high power field.

Clinical Course. A roentgenogram of the chest taken on admission revealed multiple infiltrations of pin-head size throughout both lung fields (Fig. 1). Since the findings were compatible with miliary tuberculosis, the patient was admitted directly to the Tuberculosis Unit. Sputum examinations (24-hour concentrations) on three occasions revealed no acid-fast bacilli. The vital capacity was recorded as 1,600 cc. The patient was febrile with sharp, irregular fluctuations in temperature up to 102° F. He showed severe muscular weakness throughout the period of observation and frequently required codeine for relief of chest pain.

Toward the end of the first hospital week in the Tuberculosis Unit, the patient became irrational and it was feared that he had developed tuberculous meningitis. A spinal puncture yielded clear cerebrospinal fluid under an initial pressure of 210 mm. of water. The fluid contained 10 cells per cmm., and 31 mg. per cent of protein. The Kline and Kolmer tests were negative; the colloidal gold test gave no reaction.

Cultures of sputum collected on May 24 showed growth of fungi after 6 days, but the organisms were not definitely identified. Pus was then aspirated from the subcutaneous abscesses and examined microscopically in a moist mount. Numerous doubly contoured bodies, measuring approximately 6 to 16 μ in diameter, were seen. Several of the organisms possessed single buds extending from the periphery of the cell. A diagnosis of blastomycosis was entertained and on the 18th hospital day the patient was transferred to the Isolation Unit for treatment.

Upon admission to the Isolation Unit, the patient was anemic, emaciated, edematous, dyspneic, and irrational. Crepitant râles and dullness were present in the base of the left lung and in the left axilla. The heart rate was rapid but regular and a systolic murmur was heard at the apex. The liver was tender and extended 3 finger-breadths below the costal margin. No other masses were felt in the abdomen. The deep reflexes were active and equal. Nuchal rigidity was present, as was Kernig's sign. The subcutaneous abscesses were still present and were quite painful. Microscopic examination of pus from the ulcer on the forehead revealed many oval, doubly contoured, highly refractile bodies which developed small peripheral buds when left at room temperature for 24 hours on a sealed slide mount.

The patient was given a high-vitamin, soft diet, and sedation as needed. He required increasing amounts of narcotics to control the pain in the chest. A transfusion of 500 cc. of whole blood and repeated infusions of normal saline and glucose solution were administered. However, he failed rapidly. His respiratory rate increased to 52 per minute and he became more irrational, experiencing visual and auditory hallucinations during the last 2 days of life. He gradually lapsed into coma, refused all food, and expired on the 22nd hospital day, approximately 4 months after the onset of symptoms.

Clinical Diagnosis: Systemic blastomycosis.

Autopsy Report

Autopsy was performed 2½ hours after death. The body was that of a well developed, poorly nourished, colored, adult male weighing 120 lbs. and measuring 177 cm. in length. Marked pedal edema was present. Granulating ulcers of the skin with slightly raised, irregular edges and necrotic, hemorrhagic bases were present as follows: lesion, 3 cm. in diameter, on the left side of the forehead; two lesions, 3 and 1.5 cm. in diameter, respectively, on the right side of the chin; lesion, 1 cm. in diameter, under the chin; lesion on the right hip, 3 by 1 cm. in diameter. Small, firm, cervical lymph nodes were palpable on the left side of the neck. Round to oval subcutaneous abscesses varying from 3 to 8 cm. in diameter were noted in the following sites: the left side of the neck below the angle of the jaw, the midportion of the inner aspect of the right forearm, below the elbow on the medial aspect of the left forearm, and the calf of the left leg. The pus in these cavities was creamy and varied from pale yellow to greenish gray or bloody. A draining sinus, 1 cm. in diameter, was present in the thoracic wall

in the 4th intercostal space about 1 cm. to the right of the sternum. A necrotic portion of the 4th rib was exposed in the depths of this sinus but there was no communication with the pleural cavity.

The peritoneal surfaces were smooth and shiny. The cavity contained about 500 cc. of clear yellow fluid. The liver extended 4 cm. below the xiphoid and 3 cm. below the costal margins. The abdominal viscera were grossly normal. The mesenteric lymph nodes were not enlarged. The pleural cavities contained no free fluid. The surfaces of the lungs were smooth and shiny anteriorly, but posteriorly the pleural space on the right was obliterated by firm, fibrous adhesions which also involved the diaphragmatic pleura. An abscess, 2 cm. in diameter, was present in the intercostal muscles of the second interspace just to the right of the sternum. The abscess did not communicate with the skin or pleural cavities but had almost ruptured through the parietal pleura. It was filled with thick, yellow-green pus. The pericardial cavity appeared normal. The heart weighed 200 gm. and appeared to be normal. The lungs together weighed 2,490 gm. Innumerable small, shiny nodules, measuring 2 to 3 mm. in diameter, were visible and palpable beneath the pleura of all surfaces of both lungs. Similar discrete nodules were found throughout the substances of both lungs (Fig. 2). On section, the nodules were firm and yellow without obvious central liquefaction. All lobes were rubbery, subcrepitant, and congested. The hilar and mediastinal lymph nodes were moderately enlarged, firm, and pink. The bronchi were filled with red, frothy fluid. The spleen weighed 220 gm. On section, the parenchyma was reddish purple and studded throughout with firm, yellow nodules resembling those in the lungs. The liver weighed 1,660 gm. Scattered small, yellow nodules were present beneath the capsule but were not found elsewhere in the parenchyma of the liver. The right and left kidneys weighed 176 and 180 gm., respectively. The cortical surfaces were smooth, reddish brown, and were elevated by numerous firm, yellowish nodules similar to those already described. On section, the cortices were similarly studded with firm, yellow nodules measuring up to 8 mm. in diameter. The prostate gland was slightly enlarged and soft. On section an abscess, 0.8 cm. in diameter, was present in the right lateral lobe. Its cavity was filled with thick, yellow pus. The gastrointestinal tract and the remaining viscera were of normal appearance.

The brain weighed 1,330 gm. It was soft and edematous, the gyri were flattened, and the sulci narrowed. The dura mater was not thickened or otherwise abnormal except in a region 3 cm. wide running along the superior sagittal sinus for a distance of 5 cm. in the occipital region. In this region, the under surface of the dura was tenaciously

adherent to a semi-necrotic mass of friable, yellow tissue, 2 to 12 mm. thick, which spread laterally about 3 cm. on either side of the sinus and extended down between the occipital lobes along the falx cerebri to its inferior margin. The lining of the superior sagittal sinus in this region was roughened and granular but the lumen was not occluded. An attempt was made to lift the dura and the adherent necrotic material from the brain surface, but no clear line of demarcation was present. Necrosis extended into the brain to a depth of several mm. over the medial and superior surfaces of the parietal and occipital lobes (Fig. 4). The leptomeninges of the base of the brain, cerebellum, pons, and medulla were covered by a tenacious, thick, yellow-green exudate, while the meninges covering the convexities of the cerebral hemispheres appeared normal. The vessels of the leptomeninges were moderately congested.

Microscopic Examination

The heart was histologically normal except for slight edema of the pericardium.

The lungs showed numerous uniformly distributed focal areas of necrosis and suppuration, each surrounded by a granulomatous zone. These granulomata varied greatly in size, the larger ones corresponding to the nodules seen grossly. The granulomata occupied about one-half of the area of the sections examined; some were poorly delimited and others were surrounded by a zone of fibrous tissue. They consisted of necrotic débris, polymorphonuclear neutrophils, epithelioid cells, and round or ovoid yeast-like bodies measuring 8 to 12 μ in diameter. The yeast-like bodies had a highly refractile, doubly contoured cell wall surrounding a granular, central, cytoplasmic mass. Many large multinucleated giant cells were present chiefly at the periphery of the granulomata and some of them contained yeast-like bodies in their cytoplasm (Fig. 3). The alveolar architecture was destroyed within the granulomata but the intervening pulmonary tissue showed remarkably little change except for edema fluid in occasional alveoli.

The spleen contained focal granulomatous and necrotic areas similar to those in the lungs. Some of these lesions contained few, and others many, yeast-like organisms, either free or within giant cells. The intervening splenic pulp was normal except for moderate fibrosis. The malpighian bodies were well preserved and were seldom involved.

The liver showed widely scattered, small, granulomatous lesions containing yeast-like bodies, but was otherwise normal. The gallbladder, pancreas, gastrointestinal tract, and adrenals were normal.

The kidneys contained sharply delimited regions of necrosis, granu-

lomatous inflammation, and suppuration. Many yeast-like organisms were present in these lesions, both free and within the cytoplasm of giant cells. There were degenerative changes of the epithelium of the convoluted tubules and occasional hyaline and granular casts in tubular lumina. The urinary bladder and testes were normal.

The prostate contained multiple abscesses. Many yeast-like organisms were present in the abscess cavities, in the neighboring pus-filled glands, and in the cytoplasm of large multinucleated giant cells.

The mediastinal lymph nodes showed diffuse infiltration with polymorphonuclear neutrophils and marked interstitial fibrosis. Many yeast-like bodies and giant cells were present.

The skin from the lesions on the forehead showed extensive fibrosis of the dermis and subcutaneous tissues. There were many irregular regions of suppuration and granulomatous inflammation surrounded by dense fibrous tissue. Within these regions yeast-like bodies were present, both free and within giant cells.

In the right occipital lobe of the brain and near the midline, the superficial layers of the cortex were destroyed and replaced by granulation tissue and inflammatory exudate. The exudate contained many polymorphonuclear neutrophils together with lymphocytes, plasma cells, and macrophages. Scattered throughout were numerous round, doubly contoured yeast-like bodies, varying in diameter from 6.4 to 12.3 μ. Some of these showed budding (Fig. 11). The cortical tissue beneath the region of superficial destruction showed marked edema and degenerative changes of nerve cells. Gliosis was not apparent. The spaces of the overlying leptomeninges were filled with similar exudate containing many yeast-like organisms. In neighboring regions the meninges contained inflammatory exudate but there was no destruction of the underlying cortex. Sections from several other regions showed no inflammatory changes in the meninges or in the brain.

The cerebellum showed extensive superficial destruction and inflammatory changes of the meninges similar to those described. The cerebral peduncles, pons, and basal ganglia were normal. Extensive inflammatory changes were present in the leptomeninges covering the medulla, and numerous yeast-like bodies were found.

A mass of granulomatous tissue exceeding 1 cm. in thickness was attached to the internal surface of the dura mater in the region of the falx cerebri. Much of this mass was completely necrotic, but in many regions innumerable yeast-like bodies were present, mingled with giant cells, polymorphonuclear neutrophils, macrophages, plasma cells, and lymphocytes. Such collections were in many instances completely surrounded by collagenous fibrous tissue. New blood vessels and fibro-

blasts were present except in regions of necrosis and suppuration. The superior sagittal sinus was almost completely surrounded by tissue of this character and in one region the wall was destroyed. Granulation tissue containing many organisms had replaced the wall of the sinus in this region and had encroached on the lumen.

Anatomic Diagnosis. Systemic blastomycosis with involvement of the skin, subcutaneous tissues, lungs, liver, spleen, kidneys, prostate, bone, meninges, superior sagittal sinus, and brain.

Summary of Pathologic Findings. This case of systemic blastomycosis was characterized by widespread abscesses and granulomatous foci in which the polymorphonuclear neutrophil was the predominating cell. In all of these lesions there were numerous round, oval, yeast-like bodies, either free or within giant cells, measuring 4.6 to 14.0 μ in diameter, which resembled *B. dermatitidis*. Lesions were found in the lungs, liver, spleen, and kidneys. In addition, there were cutaneous lesions of the face which lacked the typical gross appearance of blastomycotic ulcers but nevertheless contained organisms. Subcutaneous abscesses and lesions of the ribs, brain, and meninges were present also. Grossly and microscopically, the lesions in the lungs bore some resemblance to miliary tubercles, but were more like miliary abscesses in that the granulomatous zones were heavily infiltrated with polymorphonuclear neutrophils and their centers were suppurative rather than caseous. The histologic characteristics of the lesions in this case were similar to those described by Baker.[3]

Primary Isolation and Identification of the Fungus

The method of primary isolation of the fungus was similar to those recommended by Benham[6] and Martin and Smith.[7] Direct examination of exudates was made by means of unstained moist mounts, in saline solution and in 10 to 20 per cent NaOH, and by Wright's staining. Numerous spherical, doubly contoured, thick-walled, yeast-like cells were seen. Exudate was streaked by the gridiron technic on agar plates containing 5 per cent sheep's blood with heart infusion base and Sabouraud's dextrose agar (pH 5.6). Incubation was at 37° C. and room temperature, respectively. Growth on Sabouraud's agar, at room temperature, was not apparent until 7 days after inoculation, when many small colonies of a white, filamentous mold appeared along the lines of streaking.

Growth at 37° C. Growth appeared on blood agar on the fourth day of incubation in the form of numerous well isolated, tiny, filamentous, fungus colonies growing along the lines of streaking. Transfer of one colony from this plate to a blood agar slant resulted in the development

4 days later of a mealy, adherent growth resembling the growth of *Mycobacterium tuberculosis* on glycerin media. A giant colony of this type is shown in Figure 5. Microscopic examination of the growth revealed numerous large, round, thick-walled, doubly contoured, single or budding cells, 5.7 to 16.4 μ in diameter, and thin, segmented mycelial strands (Fig. 7). Upon second and third serial transfers to blood agar slant and Sabouraud's agar, the culture underwent characteristic gross and microscopic changes with complete suppression of mycelium formation and development of only large budding cells. The progression of changes in the microscopic appearance of the culture is illustrated in Figures 7, 8, and 9. The third serial transfer of the organism produced a mealy, wrinkled, cerebriform and friable growth of fungus which possessed no cottony, aerial mycelium. It was noted that frequent transfers favored the mealy type of growth.

Growth Characteristics at Room Temperature. A single transfer of the organism growing at 37° C. to either Sabouraud's or blood agar at room temperature caused the prompt reversion of structure, with development of an abundant wooly, white, aerial mycelium (Fig. 6). Further study of the organism growing in a Henrici-type slide culture [8] at room temperature revealed the development of abundant aerial mycelium and numerous oval to round conidia, 3 to 4 μ in diameter, which were attached to hyphae near the segmentations. Other round to pear-shaped conidia of the same size developed on lateral sterigmata of varying lengths (Fig. 10). Submerged mycelium developed raquette hyphae and thick, swollen structures resembling intercalary chlamydospores, 7.5 to 18 μ in diameter.

Growth Characteristics on Carrot Slant. At 37° C., the organism produced well isolated, warty, wrinkled, cerebriform colonies similar to those produced on blood agar, but considerably more raised. Ascospore formation was not observed at any time. The new strain of fungus appeared to be identical in all its characteristics with a stock laboratory strain of *Blastomyces dermatitidis*.*

Cultural Variation at 37° C. After several subcultures on blood agar and prolonged incubation at 37° C., minute prickly elevations consisting of closely packed filaments of true mycelium appeared on the surface of the growth. These were considered to be abortive coremia representing an attempt of the fungus to revert to the myceliated state. Both the stock and newly isolated strains of *B. dermatitidis* exhibited this characteristic, and also showed the other two cultural variations described for the fungus by Henrici.[8] These forms were: (1) mealy colony, unicellular form at 37° C. (Figs. 5, 7, 8, and 9); (2) prickly,

* Furnished by Dr. Norman F. Conant, Duke University, Durham, N.C.

abortive coremia at 37° C.; and (3) wooly, mycelial form at room temperature (Figs. 6 and 10). In summary, it may be said that the morphologic appearance of *B. dermatitidis* on agar medium is variable, depending on temperature of incubation, age of the culture, and number and rapidity of transfers from the original isolation. Recent studies by Levine and Ordal [9] on factors influencing the structure of *B. dermatitidis* on peptone glucose medium showed that the wooly, mycelial form was predominant in cultures growing from "room temperature" through 33° C., and the mealy, yeast-like form from 35° C. through 37° C.

Biochemical Reactions. Both the newly isolated strain of *B. dermatitidis* and the stock laboratory strain, which had been maintained in the budding cell stage at 37° C., were inoculated into beef infusion broth, pH 6.8, containing 1 per cent of one of the following sugars: maltose, lactose, sucrose, and dextrose. At 37° C., both strains developed abundant, colorless, submerged mycelium in the liquid media rather than the fragmented, mealy type of growth of the organism on solid media at 37° C. Cultures were maintained for 6 weeks, during which time growth of the fungus filled the media, but failed to produce any discernible gas or change in reaction. This does not preclude, however, the utilization of any of the carbohydrates by either strain of Blastomyces.

In Vitro Resistance of Blastomyces Dermatitidis to Streptomycin and Penicillin

Little information is available concerning the effect of the antibiotics, streptomycin and penicillin, on *B. dermatitidis*. Since the accepted method of treatment of North American blastomycosis with iodides and x-ray therapy is not particularly effective, the *in vitro* action of streptomycin and penicillin against the organism was thought worthy of examination.

Series of tubes of Sabouraud's dextrose broth and nutrient broth containing quantities of streptomycin hydrochloride (Merck) and sodium penicillin (Wyeth) varying from 0.1 to 200 units per cc. were inoculated with standard laboratory strains of *Staphylococcus aureus* and *Escherichia coli* as control organisms, and with two strains of *Blastomyces dermatitidis,* the newly isolated and the laboratory stock culture. The fungus inoculum consisted of 6-day-old budding cells, maintained in the unicellular state on blood agar at 37° C. Incubation was at 37° C. and observations were made at suitable intervals. The two strains of the fungus were found resistant to concentrations of streptomycin and penicillin up to 200 units per cc., as illustrated in

Table I. In order to verify these results, the "window" method of Cooke,[10] originally devised for assay of penicillin and for the determination of penicillin sensitivity of bacteria, was used to determine the penicillin and streptomycin sensitivity of *B. dermatitidis*. Blood agar was used as the culture medium. Both strains of Blastomyces again were found to be insensitive to concentrations up to 200 units per cc.

TABLE I

In Vitro Resistance of Blastomyces dermatitidis to Streptomycin and Penicillin

Concentration	Growth in Sabouraud's dextrose broth			
Units per cc.	*Blastomyces dermatitidis**	*Blastomyces dermatitidis* (Conant)	*Staphylococcus aureus*†	*Escherichia coli*†
Streptomycin hydrochloride				
0	+	+	+	+
0.1	+	+	+	+
0.5	+	+	+	+
1.0	+	+	+	+
5	+	+	+	−
10	+	+	±	−
20	+	+	−	−
50	+	+	−	−
100	+	+	−	−
200	+	+	−	−
Sodium penicillin				
0	+	+	+	
0.1	+	+	+	
0.5	+	+	−	
1.0	+	+	−	
5	+	+	−	
10	+	+	−	
20	+	+	−	
50	+	+	−	
100	+	+	−	
200	+	+	−	

* Isolated from case of fatal North American blastomycosis.
† Same results in nutrient broth.

of either antibiotic. The fact that penicillin did not affect the growth of two strains of *B. dermatitidis* when cultured at 37° C. on liquid or solid medium is in keeping with the clinical failure of penicillin therapy in a case of North American blastomycosis reported by Herrell, Nichols, and Heilman.[11] The results obtained by us are in agreement with the recent observations of Meyer and Ordal [12] who reported that streptomycin and penicillin were not fungistatic to one strain of *B. dermatitidis*.

DISCUSSION

Demonstration of Fungal Elements in Exudates

Pus and exudates are cleared by placing them in a drop of 10 to 20 per cent sodium hydroxide (or potassium hydroxide), mounting a

coverslip on the preparation and gently warming the slide. Since numerous artifacts are easily confused with round or oblong fungal cells, a vaseline-rimmed mount of the fresh untreated specimen may be kept at room temperature for 24 to 48 hours and examined for either budding cells or development of hyphal germ tubes and hyphal elements from the suspected fungal spores.

Routine Gram staining of smears for demonstration of fungal elements is unsatisfactory because of the distorting effect of heat fixation. Gram stains of alcohol-fixed smears or direct Wright stains generally yield better results. Direct examination of unstained exudates in our case of blastomycosis disclosed the presence of numerous nonbudding, oval, thick-walled, refractile bodies, which, when maintained in a moist, untreated mount at room temperature for 24 hours, formed single, small buds compatible in structural characteristics with cells of *B. dermatitidis*. The wall of the oval cell was thick, highly refractile, and appeared as a bright band bordered by two thin, dark lines, which imparted a doubly contoured appearance to the unstained cell. The cell wall of the single bud, on the other hand, was considerably thinner than that of the parent cell.

Although the finding of budding, thick-walled, doubly contoured cells is suggestive of Blastomyces, it should be stressed that the doubly contoured appearance is by no means confined to this genus. Budding cells of certain species of Torula and Candida possess double contours which may be even more prominent than those of Blastomyces. Immature spherules of *Coccidioides immitis*, not possessing endospores, appear doubly contoured and resemble nonbudding forms of *Blastomyces dermatitidis* and *Paracoccidioides* (*Blastomyces*) *brasiliensis*. Furthermore, Cryptococcus cells possess rather thick cell walls and present double contours, as well as the spherical or oblong cells of Geotrichum. The discovery of budding, thick-walled, doubly contoured cells in exudates, although highly suggestive of *Blastomyces dermatitidis*, should, therefore, not be considered as ultimate proof of diagnosis of North American blastomycosis. As aptly phrased by Martin and Smith: [1]

"The diagnosis can be proved unequivocably only by the isolation and identification of the infecting organism, *Blastomyces dermatitidis*. However, from the history and clinical appearance of the infection, direct examination of material from the lesions, skin tests, and complement fixation reactions, tentative diagnoses can be made which should stimulate efforts toward isolation of the fungus."

Baker [3] believed that the tissue reaction produced by *B. dermatitidis* is sufficiently distinctive to permit histopathologic diagnosis with a high degree of accuracy if Blastomyces-like organisms are present in the lesion, but attempts should be made to obtain pure cultures in every case.

TABLE II

Microscopic Features of Oval, Spherical, or Oblong Fungal Cells More or Less Resembling Yeasts and Capable of Producing Granulomatous Lesions in Tissues

Disease and organism	In exudates[2,8,18]	In stained tissue section[2,8,18]	Measured size in culture and tissue section						Literature		
			Cell type or shape	Age	Temperature of incubation	Medium	Width x length: Smallest size	Width x length: Largest size	Source	Size	Reference no.
				days			*μ*	*μ*		*μ*	
North American Blastomycosis									Culture#	7–18	13
									Exudate	3–24	8
									Exudate	5–20	14
*Blastomyces dermatitidis**	Spherical, thick-walled, yeast-like organisms appearing doubly contoured and forming single buds.	Similar to appearance in exudates. Double contour more prominent due to shrinking of cytoplasm. Cytoplasm stains with variable intensity.	Oval	5	37°C.	Blood agar	4.6 x 5.7	12.8 x 16.4	Exudate	7–18	13
									Exudate	8–15	2
						Tissue section	4.6 x 6.6	12.3 x 14.0	Tissue	3–24	8
									Tissue	5–20	14
									Tissue	8–20	6
South American Blastomycosis									Culture#	3–25	13
									Exudate	10–60	2
									Exudate	10–60	8
Paracoccidioides (Blastomyces) brasiliensis†	Spherical, thick-walled, yeast-like organisms forming multiple buds.	Some forms are indistinguishable from *B. dermatitidis;* others show minute, dark staining projections about periphery of parent cell which represent multiple buds. Minute buds found free and within cytoplasm of giant cells. Large, multiple peripheral buds from parent cell also seen.	Parent cell	10	37°C.	SAB¶	8.2 x 8.2	18.9 x 18.9	Exudate	1–30	14
									Tissue	10–60	17
									Tissue	1–30	14
			Single bud	10	37°C.	SAB	4.6 x 4.6	9.1 x 9.1	Exudate	10–30	2
			Tuberculate buds of parent cell	10	37°C.	SAB	1.6 x 1.6	4.6 x 4.6	Exudate	1–10	2
Geotrichosis											
Geotrichum sp.†	Spherical, rectangular, or oblong, thick walled cells. May be confused with *B. dermatitidis.*	Not reported.	Oval	6	Room	SAB	5.7 x 7.4	8.2 x 10.7	Culture#	4–12	2
			Oval	40	Room	SAB	4.9 x 8.2	10.7 x 10.7	Exudate	4–10	2
			Oblong	6	Room	SAB	5.7 x 8.2	6.6 x 16.4	Exudate	4–8	2
			Oblong	40	Room	SAB	4.9 x 8.2	4.6 x 20.5			

Cryptococcosis									Culture #	4–5	6
									Exudate	5–20	2
Cryptococcus neoformans†	Oval to spherical, forming single buds, thick-walled, yeast-like organisms; wide refractile capsule demonstrable in India ink preparation.	Oval to spherical organisms surrounded by irregular, nonstaining, mucinous capsule; single buds; best seen in Gram-stained sections.	Oval	5	Room	SAB	2.5 x 2.5	6.6 x 8.2	Exudate	5–10	14
									Exudate	2–15	8
			Oval	35	Room	SAB	2.5 x 2.5	6.6 x 8.2	Tissue	5–15	6
									Tissue	5–10	14
Moniliasis											
Candida albicans†	Small, oval, thin-walled, yeast-like organisms, forming single buds.	May be confused with lymphocytes in preparations stained with hematoxylin and eosin, but not in Gram-stained sections; cell stains darkly with Gram stain but capsule stains poorly. Both oval cells and mycelial elements are present. Histopathologic diagnosis difficult without culture.	Oval	5	Room	SAB	3.3 x 3.3	8.2 x 8.2	Culture #	3–6	18
									Exudate	2.5–4	2
			Oval	40	Room	SAB	2.5 x 2.5	9.0 x 9.0	Tissue	4	2
Coccidioidomycosis									Exudate	2–80	14
									Exudate	20–80	2
Coccidioides immitis†	Nonbudding, thick-walled spherules filled with numerous endospores which are released in tissues and which increase in size with maturity. Immature spherules not containing endospores are doubly contoured and resemble nonbudding forms of *B. dermatitidis* and *P. brasiliensis*.	Many forms indistinguishable from nonbudding forms of *B. dermatitidis* and *P. brasiliensis*; heavy doubly contoured walls; material within cell stains irregularly, darker just adjacent to capsule than in center of cell. Absence of budding and presence of endospores are diagnostic.	Spherule‡			Tissue section	12.8 x 16.4	46.0 x 46.0	Exudate	5–50	8
									Tissue	2–80	14
									Tissue	2–60	15
									Tissue	20–80	8
									Tissue	6–50	6
			Endospore‡			Tissue section	2.5 x 2.5	5.7 x 5.7	Exudate	2–5	2
									Tissue	1–4	8

TABLE II (Continued)

Disease and organism	In exudates[2,6,12]	In stained tissue section[2,6,12]	Measured size in culture and tissue section						Literature		
			Cell type or shape	Age	Temperature of incubation	Medium	Width x length: Smallest size	Width x length: Largest size	Source	Size	Reference no.
				days			μ	μ		μ	
Chromoblastomycosis *Hormodendrum compactum*† *Hormodendrum pedrosoi*† *Phialophora verrucosa*†	All three forms are single or clustered, round, thick-walled, dark brown bodies; multiply by splitting and not by budding.	All three forms identical; brown, round, clustered, septate cells.	Oval conidia	14	Room	SAB	1.6 x 1.6	2.0 x 2.0	Culture#	1.5–3.0	16
			Oval conidia§	14	Room	SAB	1.6 x 1.6	1.6 x 2.5	Culture# Culture#	1.5-2.5§ 1.5-6.0△	16 2
			Oval conidia	14	Room	SAB	1.6 x 1.6	2.5 x 4.6	Culture#	1.5-4.0	16
Rhinosporidiosis *Rhinosporidium seeberi*	Round to oval spores possessing thin cell walls and containing several granules of uniform size. Spores mature to large sporangia.	Numerous large sporangia which are empty or filled with many oval, thin-walled spores; the latter may also be found free in tissues.	Sporangia‖			Tissue section	21 x 21	177 x 206	Tissue	50-300	17
			Spores‖			Tissue section	3 x 3.8	12 x 12	Tissue	8–9	17

* Isolated from a case of fatal systemic blastomycosis.
† Furnished by Dr. N. F. Conant, Duke University, Durham, N.C.
‡ One tissue section.
§ Phialophora type of conidia.
‖ Nasal mucosa, one case.
¶ Sabouraud's agar.
# Age of culture not reported.
△ Hormodendrum type of conidia.

In support of these statements, we might point to the wide variety of fungi, as listed in Table II, which appear in exudates as oval, spherical, or oblong, refractile, fungal cells more or less resembling yeasts and capable of causing granulomatous lesions in tissues. In Table II there are also presented careful measurements of fungal cells and spores made at different ages of culture by one of us (M.L.L.) and compared with sizes reported in the literature. In this study, measurements were made of only one representative culture. Strain variation was not investigated. In one study of strain variation of Microsporum by Conant,[19] spore dimensions were found to be too variable for species differentiation. The wide variation in dimensions between the smallest and largest spore in a given culture of fungus and between strains of the same species is presumably dependent upon many factors of growth and development, none of which were studied here. Differences in spore size among the several genera of fungi presented in Table II are sufficient, however, to permit rough identification of the fungi in human exudates and stained tissues. Cultures should be attempted in every case in which a fungus disease is suspected.

Technic of Primary Isolation

Media suitable for *B. dermatitidis*, as well as for other fungi, are Sabouraud's dextrose or maltose agar incubated at room temperature, and blood agar incubated at 37° C. Specimens are streaked by the gridiron technic on both media in such a manner as to yield isolated fungal colonies. Growth of pathogenic fungi on primary isolation will appear in most cases within 7 to 10 days on Sabouraud's dextrose agar at room temperature, but may sometimes require as long as 16 days. Many air-borne nonpathogenic fungi, which are laboratory contaminants, on the other hand, produce abundant, rapidly spreading aerial mycelium in as short a time as 48 to 72 hours and may overgrow the entire plate. Care must be taken to minimize contamination by air-borne fungal spores during the pouring, storage, and inoculation of the media.* The use of a small, closed room, rendered relatively dust-free and kept shut, will increase the proportion of successful primary isolations. Once a pure culture of a fungus is obtained, it is easier to maintain its purity on Sabouraud's agar slant than on an agar plate because of the reduced exposure to air-borne fungal spores. As soon as visible, filamentous, mold-like colonies develop in the agar along

* A new agar medium for primary isolation of fungi, obviating many of the difficulties due to air-borne contamination and inhibiting bacteria, recently has been developed by the senior author and is now undergoing clinical trial.

An account of this medium has been published since this manuscript was submitted. Littman, M. L., A culture medium for the primary isolation of fungi. *Science*, 1947, 106, 109–111.

the lines of streaking, one entire small colony is dug out with a platinum loop and transferred to a Sabouraud's agar slant. Although the young colony will usually not have developed spores, the vegetative mycelium is nevertheless quite capable of further growth and subsequent sporulation in the transplant.

All fungi incubating at room temperature are kept in a darkened cabinet since direct sunlight may inhibit growth. Streaking of a blood agar plate with fresh exudate and incubation at 37° C., as recommended by Martin and Smith,[7] and Conant *et al.*,[2] will increase the likelihood of successful primary isolation. When the fungus is obtained in the pure state, free of bacteria and other fungi, attempts at identification may be continued by the slide culture technic, giant colony study, identification of characteristic sporulating elements in special media, and studies of animal pathogenicity and tissue reactions. If suitable facilities are not available locally, a pure culture may be sent to a specialized laboratory for identification.

Primary isolation and subsequent identification of pathogenic fungi from lesions in patients usually is not a difficult task for an experienced mycologist. It does present real difficulties to the bacteriologist or pathologist who is unfamiliar with the special methods and media required. Demonstration and primary isolation of certain fungi from mycotic lesions are simple if certain basic procedures are followed in the treatment of the exudate:

Direct Examination. (1) Mount in 10 per cent NaOH; (2) Gram stain or Wright stain; (3) incubation of unstained saline mount to detect sprouting or budding; (4) rough identification of the fungus with the help of Table II (spherical or oval fungal cells only).

Culture. (1) Streaking on Sabouraud's and blood agar plates, and incubation at room temperature and 37° C., respectively; (2) early transfer of young mold colony to Sabouraud's agar slant; (3) slide culture; (4) giant colony study; (5) sporulating media; (6) animal pathogenicity; (7) animal tissue reactions.

Summary and Conclusions

A case of fatal North American blastomycosis presented, on autopsy, involvement of the skin, subcutaneous tissues, lungs, liver, spleen, kidneys, prostate, bone, meninges, superior sagittal sinus, and brain. Histopathologic examination showed widespread abscesses and granulomatous foci in which the polymorphonuclear neutrophil was the predominating inflammatory cell.

Cultures isolated from exudates and from organs obtained at autopsy were identified as *Blastomyces dermatitidis*. Budding cell forms of the

fungus were found to vary in size in culture from 4.6 by 5.7 μ (width and length) to 12.8 by 16.4 μ, and in various organs of the body from 4.6 by 6.6 μ to 12.3 by 14.0 μ.

Two strains of *B. dermatitidis* were found to be resistant to streptomycin and penicillin in concentrations up to 200 units per cc. For this reason it is not likely that either of these antibiotics will prove effective in the clinical treatment of North American blastomycosis.

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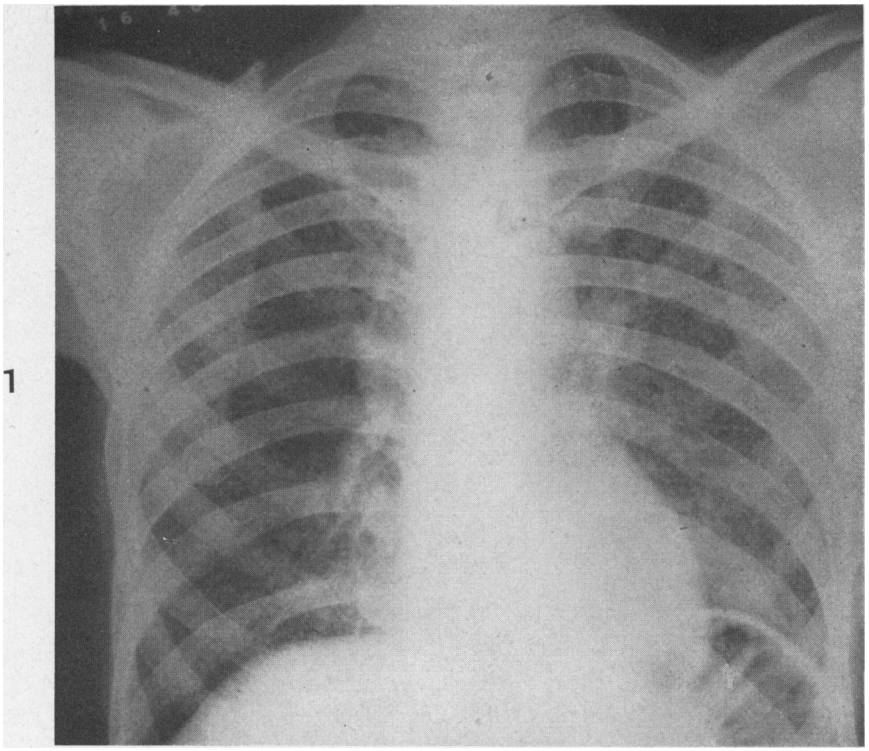
DESCRIPTION OF PLATES

PLATE 66

FIG. 1. Roentgenogram of the chest showing diffuse bilateral, miliary, pulmonary lesions in North American blastomycosis. Heart and mediastinal structures are within normal limits. The picture is compatible with miliary tuberculosis.

FIG. 2. Lung. The surface made by cutting shows firm, yellow nodules with no apparent central liquefaction.



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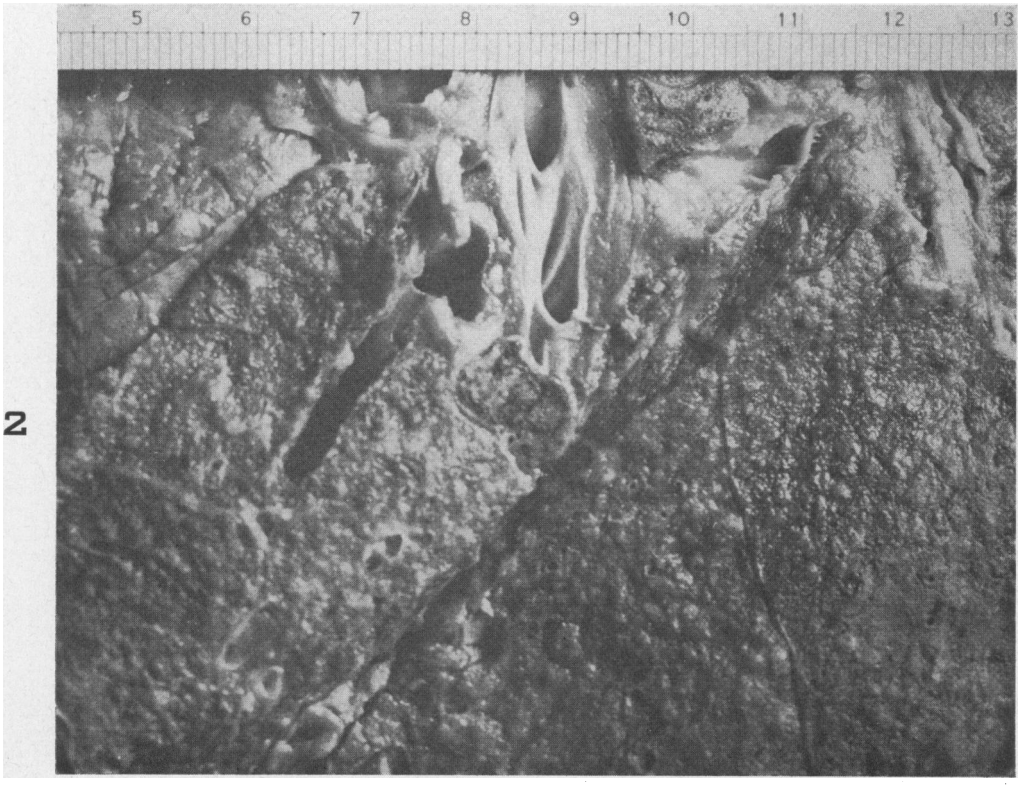


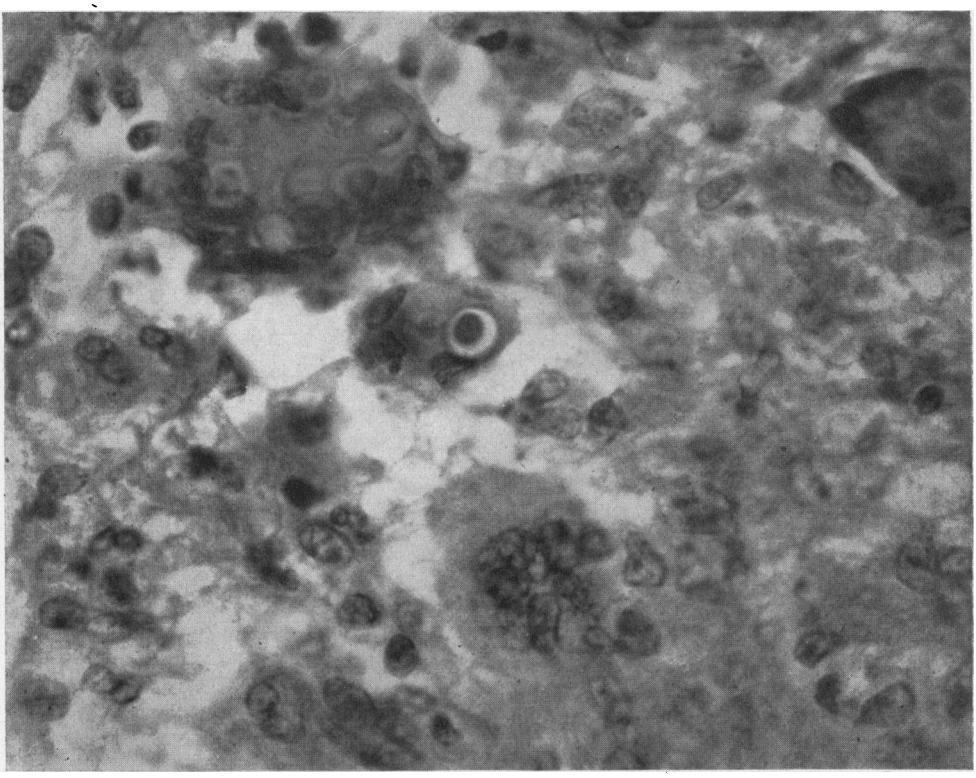
2

PLATE 67

FIG. 3. Lung. Photomicrograph of the periphery of a focal granuloma showing epithelioid cells and giant cells containing thick-walled, doubly contoured yeast-like bodies of *B. dermatitidis*. × 1000.

FIG. 4. Brain. Superficial necrosis of the cortex of the parietal-occipital lobes near the midline. Of note are the thickening of the dura mater and the presence of a granulomatous exudate attached to the inner surface of the falx cerebri.

3



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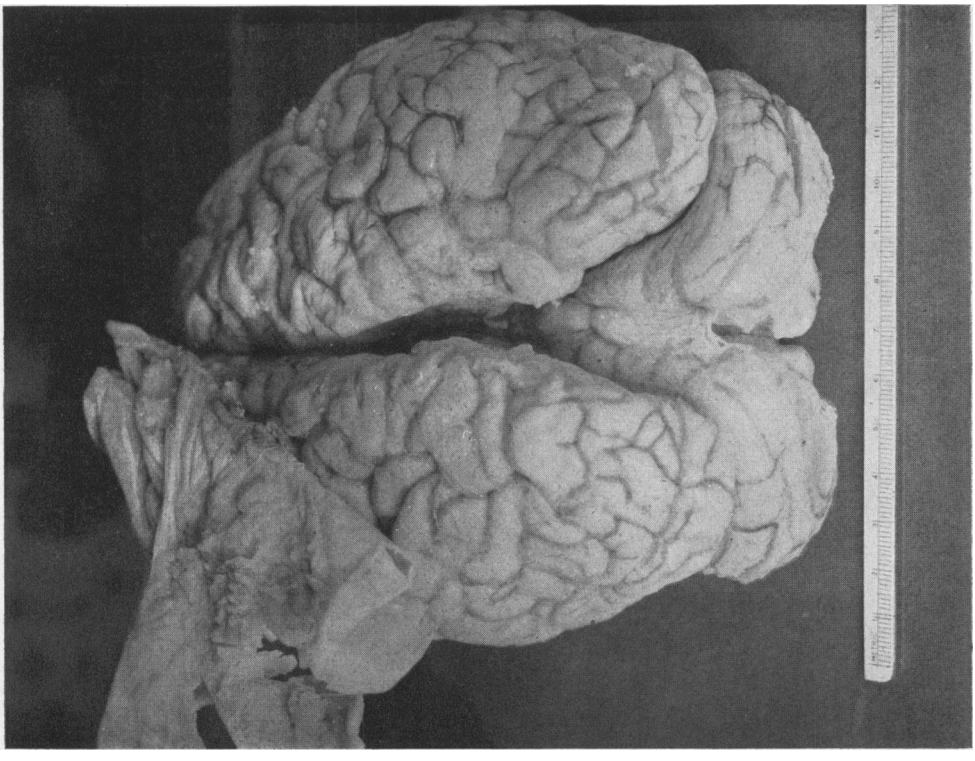
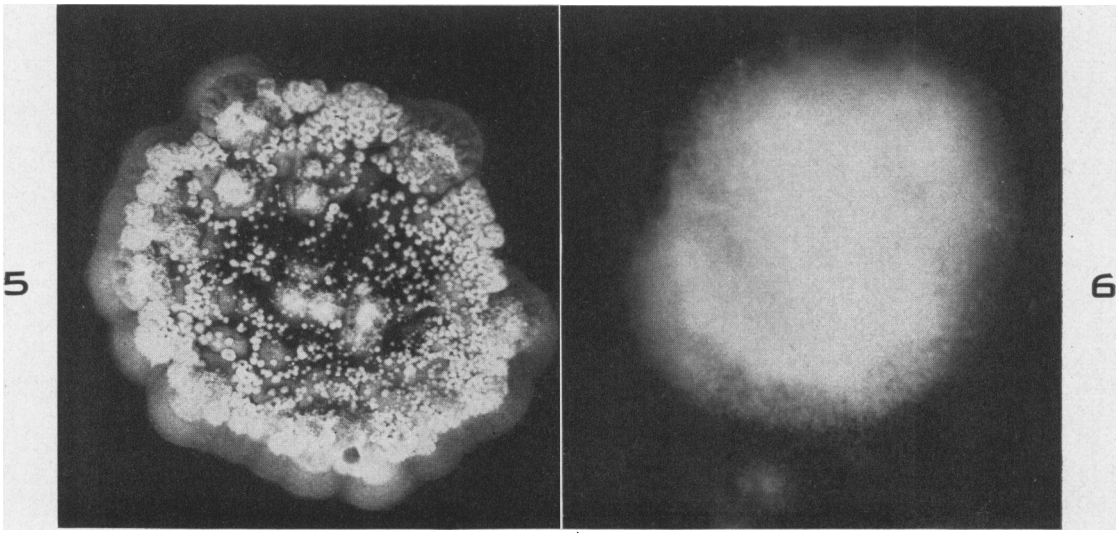


PLATE 68

FIG. 5. Giant colony of *B. dermatitidis,* incubated at 37° C. on Sabouraud's dextrose agar, showing typical mealy, wrinkled, cerebriform growth. The light gray periphery of the colony represents that portion of the growth which has penetrated into the agar. First transfer of newly isolated mold from growth at room temperature. (Mealy colony, original size.)

FIG. 6. Giant colony of *B. dermatitidis* incubated at room temperature on Sabouraud's dextrose agar, showing wooly mycelial appearance. (Wooly mycelial colony, original size.)

FIG. 7. Microscopic appearance of giant colony grown at 37° C., as seen in Figure 5. There are numerous round, doubly contoured, single or budding cells as well as numerous fragmented mycelial strands. First transfer from wooly growth at room temperature. (Mealy state.) × 1000.



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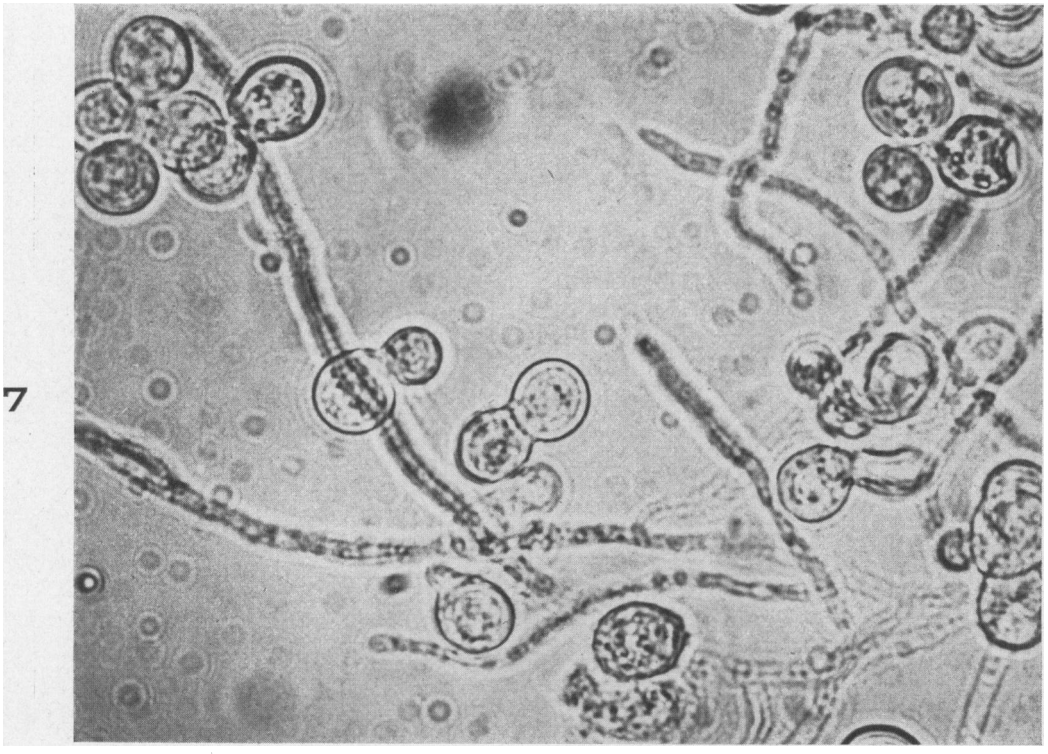

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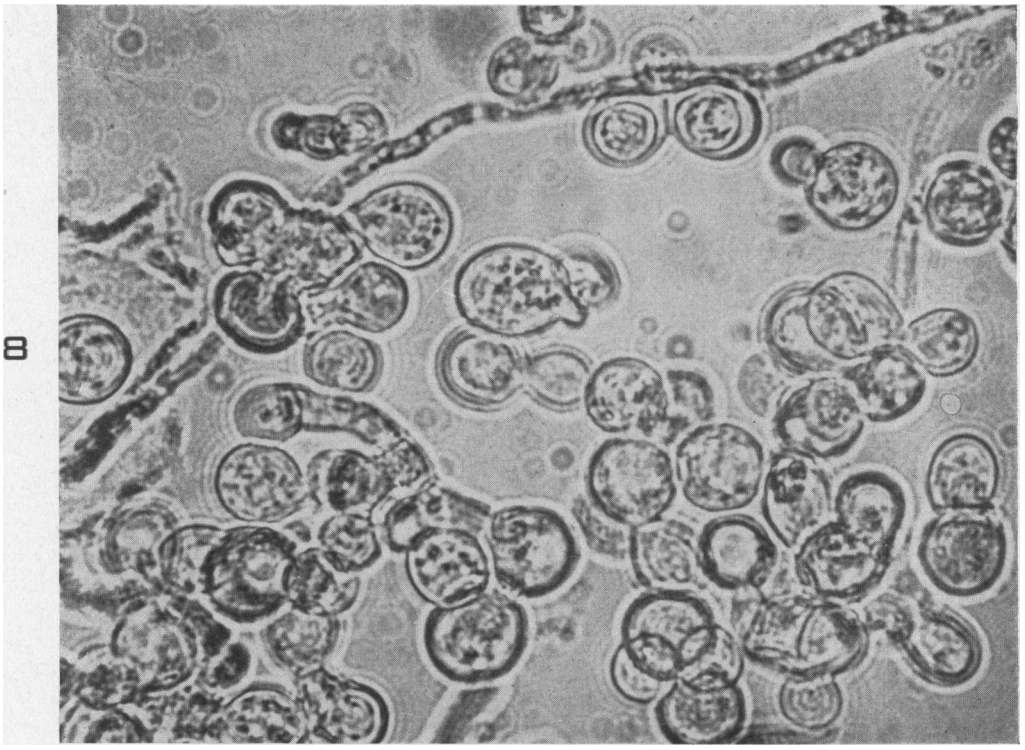
Littman, Wicker, and Warren North American Blastomycosis

PLATE 69

FIG. 8. *B. dermatitidis,* second transfer, growth at 37° C. on Sabouraud's dextrose agar. There are numerous budding cells and relatively few mycelial strands. Age, 5 days. (Mealy state.) × 1000.

FIG. 9. *B. dermatitidis,* third transfer, growing at 37° C. on Sabouraud's dextrose agar. There are numerous budding cells and very few mycelial strands. Age, 5 days. (Mealy state.) × 1000.



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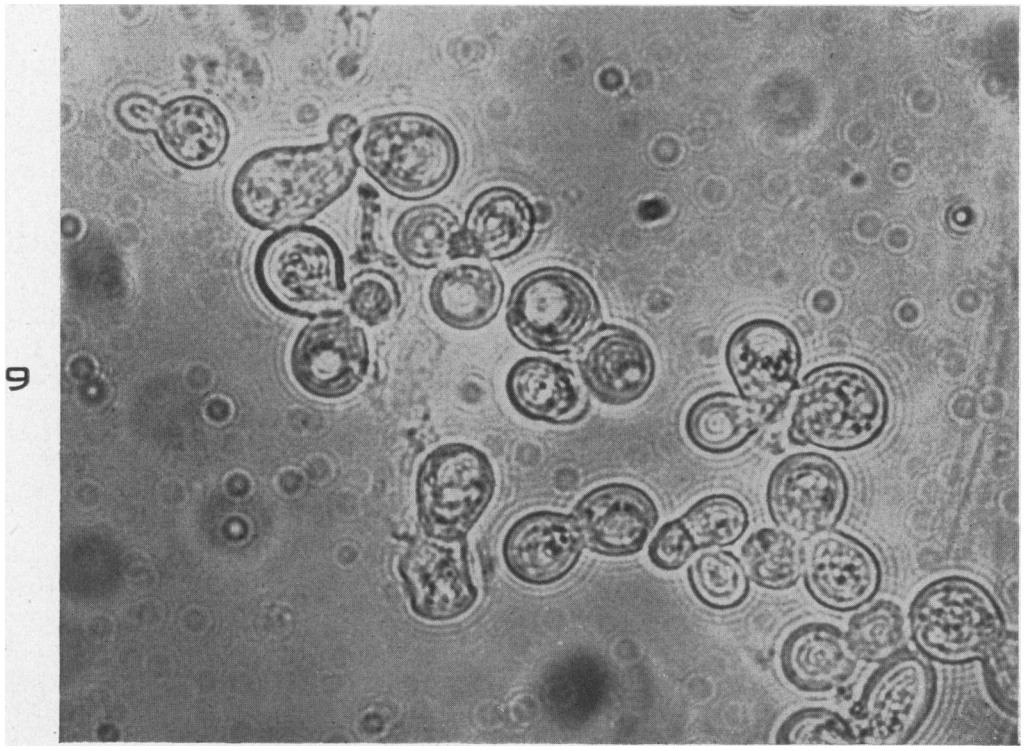

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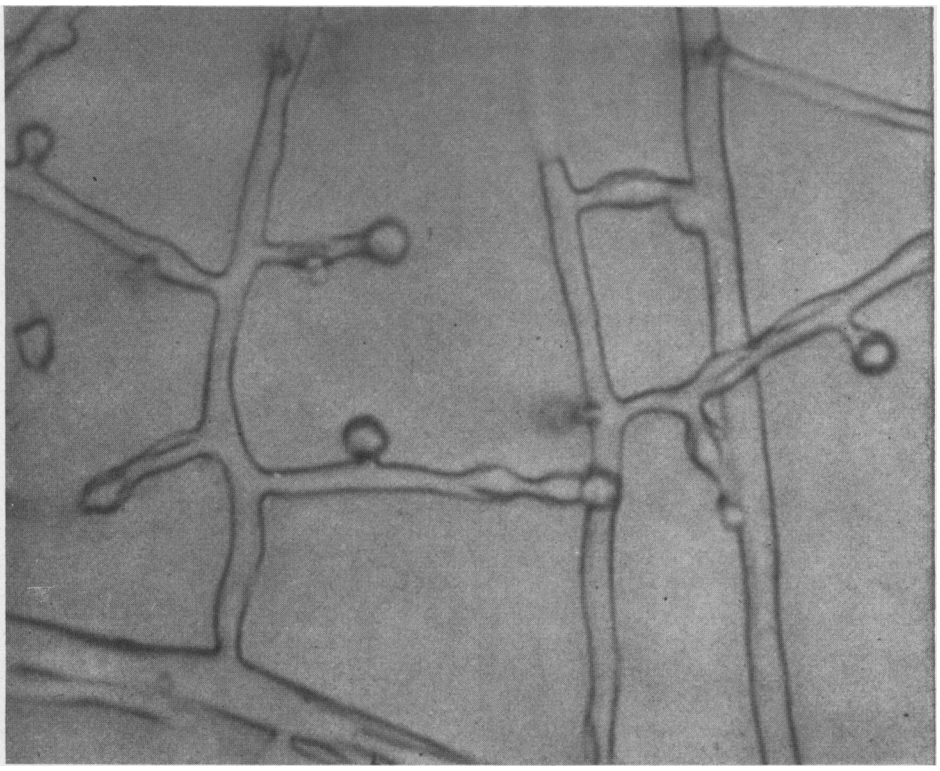
PLATE 70

FIG. 10. Aerial mycelium of *B. dermatitidis* growing in a Henrici-type slide culture at room temperature. Oval, sessile conidia and other oval to pear-shaped conidia are attached to lateral sterigmata. Age, 14 days. (Wooly mycelial state.) × 1200.

FIG. 11. Brain. Photomicrograph of involved portion of the right occipital lobe, showing numerous thick-walled budding cells, varying in diameter from 6.4 to 12.3 μ. Cytoplasmic staining of the fungal cells varies in intensity. × 1000.

10



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