TISSUE REACTIONS IN HUMAN BLASTOMYCOSIS *

AN ANALYSIS OF TISSUE FROM TWENTY-THREE CASES

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In North Carolina many human cases of blastomycosis have been encountered. Reports on them have dealt primarily with the general and immunologic features of the disease,[1] or with particular features such as laboratory diagnostic,[2] gynecologic,[3] cardiac,[4] cutaneous[5] and mycologic [6] considerations. From animal experimentation in connection with these human cases it has become apparent that the mouse is preeminently suited to the experimental production of blastomycosis.[7] The comparatively abundant histologic material available has permitted a restudy of the reaction of the tissues of man to this large fungous organism. Since it has been stated that blastomycosis and tuberculosis are practically identical histopathologically,[8,9] this aspect of the reaction has been approached with especial interest.

In a comprehensive analysis of blastomycosis, Stober,[10] who based his conclusions on a large group of especially well studied cases, stated: "The changes most characteristic of blastomycosis are the cutaneous ulcerations, the deep and superficial abscesses, and the often tubercle-like nodules in the viscera." The emphasis in this quotation is on ulceration and abscess formation, although it is indicated that tubercle-like nodules occur. Others have adopted about the same view.

In more recent years the emphasis in papers on blastomycosis appears to have shifted somewhat. One might be led to believe that the tissue response in blastomycosis is identical with that in tuberculosis. Medlar [8] suggested this view in a report of two cases of pulmonary blastomycosis. D'Aunoy and Beven,[9] in a report of 26 cases, 16 of which were cutaneous, went so far as to state that "only the presence of the specific organisms either active or dead, allowed making an histological differentiation from tuberculosis in any of the cases." I supported this opinion in a case report,[3] but have now altered my view.

The observations to be reported reëmphasize the importance of abscess formation in blastomycosis, while they do not deny the occurrence, in some cases, of lesions like those commonly seen in tuberculosis. In most instances in which a differentiation was to be made by biopsy,

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the histologic appearance was thought to be highly characteristic. The presence of microscopic abscesses, certainly not common in tuberculosis, stimulated search for blastomycetes. On the other hand, it is not desirable to make the definite histopathologic diagnosis of blastomycosis without demonstration of blastomycetes.

All available clinical and pathologic data were analyzed in 23 cases of human blastomycosis from which tissue had been obtained. These occurred in the 10-year period ending in July, 1940. Twenty had been studied clinically at Duke Hospital. In 3, tissue came from other hospitals in North Carolina. Several patients were seen at Duke Hospital during the same decade from whom no biopsy had been obtained. For valuable data upon 11 of the 23 cases the reader is referred to other papers.[1,3,4]

For the present study, data were recorded in relation to the time when the tissues were obtained for examination, and the following facts

TABLE I

Human Blastomycosis, 23 Cases

Ages: 5 to 70 years

Race: Colored, 16; White, 7

Sex: Male, 20; Female, 3

Tissue available:
- Autopsies, 4
- Bone resections, 4
- Deep tissue resections, 2
- Skin biopsies or resections, 13

Involvement:
- Generalized, 7 cases
- Thoracic, 3 cases
- Skin, 13 cases (single lesions, 9 cases; multiple lesions, 4 cases)

Duration before histologic examination: 6 weeks to 13 years

Organism cultured: 16 cases

Microscopic sections:
- Viable organisms: always present
- Dead organisms: often present
- Polymorphonuclear abscesses: always present
- Giant cells: always present
- Caseation: present in generalized cases and many "deep" cases; not noted in skin cases

and observations in each case were tabulated: Race, age, sex, first manifestations of the disease, clinical course, proved or inferred involvement of the body, duration of the disease up to the time of histologic examination and outcome. The gross and histologic appearances of the lesions were studied in the light of additional data: whether iodides had been administered just before tissue was obtained, the results of skin tests and complement-fixation tests and the degree of

toxicity of the patient. In histologic study a quantitative estimate was made of each of the following features: viable blastomycetes, dead blastomycetes, polymorphonuclear abscesses, giant cells and caseation.

Basis of Diagnosis

In 16 of the 23 cases, *Blastomyces dermatitidis* was recovered from lesions, and identified by cultural methods in the Department of Bacteriology. The mycologic and other criteria for identification in these cases are given by Conant and Martin.[6] The organism grew as a budding yeast at body temperature, and as a mold, showing mycelial threads and lateral conidia, at room temperature.

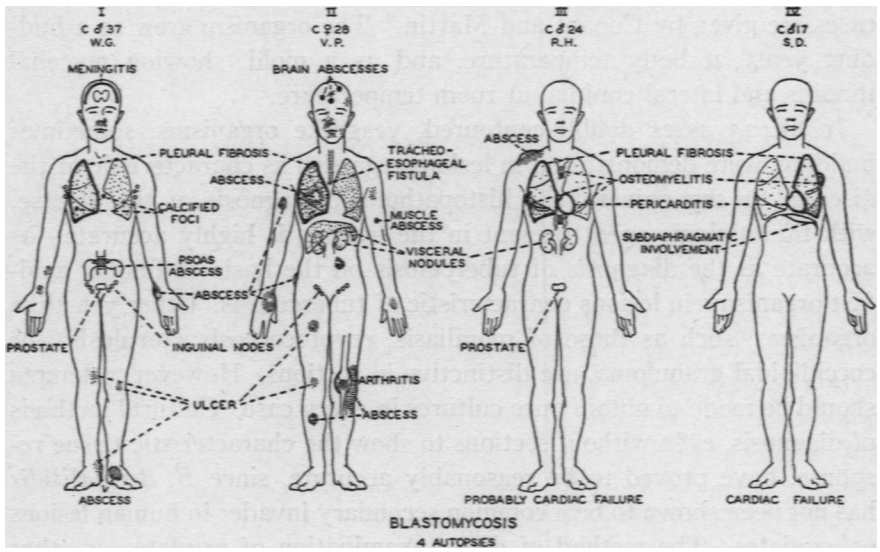
In all 23 cases double-contoured, yeastlike organisms, sometimes budding, were demonstrated in lesions regarded as characteristic of the disease. In my opinion, the histopathologic diagnosis of the disease, with the etiologic agent present in the lesions, is highly accurate—as accurate as the diagnosis of tuberculosis on the basis of finding acid-fast organisms in lesions characteristic of tuberculosis. Other yeastlike organisms, such as those of moniliasis, cryptococcosis (torulosis), or coccidioidal granuloma, are distinctive in sections. However, attempts should be made to obtain pure cultures in every case. Cultural methods of diagnosis, even without sections to show the characteristic tissue response, have proved to be reasonably accurate, since *B. dermatitidis* has not been shown to be a common secondary invader in human lesions or exudates. The method of direct examination of exudates or other material in 10 per cent sodium hydroxide should, of course, precede all other methods. In obscure pulmonary conditions in which blastomycosis is suspected, a skin test and complement-fixation reaction should be done.[11]

Involvement and Spread

The extent of involvement of the body was determined satisfactorily in the four cases upon which autopsies were done, as indicated in Text-Figure 1. In all four the lungs were involved. In cases 1, 2 and 3 there must have been hematogenous dissemination since lesions occurred throughout the body. In case 4 the lesions were confined to the thoracic region, and all could have extended directly from the oldest pulmonary lesion in the left lower lobe. This is the distinction between systemic and thoracic cases.

From the clinical histories it was thought that the portal of entry in the cases examined by autopsy was the respiratory tract. Therefore, thorough search was made for a primary pulmonary lesion such as occurs in tuberculosis, or for an old healed blastomycotic lesion which might have altered the susceptibility of the individual to reinfection.

An "oldest" lesion was identified in cases 2, 3 and 4. In case 2 this was a ragged abscess cavity in the right middle lobe (Fig. 8); in case 3, blastomycotic scarring of the upper portion of the right lower lobe; and in case 4, blastomycotic scarring at the base of the left lung. In case 1 calcified foci were found in the right lower lobe and in the draining hilar node, which were as likely to be tuberculous as blastomycotic,



Text-Figure 1. The location of lesions in four cases of blastomycosis studied at autopsy.

if not more so. No organisms were demonstrable in the calcified lesions. Dense pleural fibrosis occurred in each case on the side which appeared to be primarily affected.

Thus in the four cases coming to autopsy there was no close analogy to adult or reinfection tuberculosis. The process began in one portion of the lungs, developed there for a time, and then spread to the rest of the lungs and usually to the general circulation. No stage of massive mediastinal blastomycotic lymphadenitis occurred, analogous to childhood or first-infection tuberculosis.

Lymphatic spread was noted as from lesions on the extremities to axillary or inguinal nodes, and from lungs to peribronchial nodes. Hence the lymphatic system was not immune to blastomycosis. The thoracic duct appeared to be normal, grossly and microscopically, in three cases. This does not prove that transmission of organisms cannot occur by this duct, but suggests that lymphangitis is not common in blastomycosis, in contrast to sporotrichosis.

Direct extension was a frequent and important method of spread:

from bone to skin surfaces, from spine to iliopsoas abscesses, from lung to pleura, and probably from pleura to pericardium. In case 2 a tracheo-esophageal fistula had formed in connection with a blastomycotic lymph node which lay between these channels.

The cases from which specimens of bone or deep tissues had been obtained were also systemic or thoracic. There was no evidence for primary bone involvement as from penetrating wounds.

In the cutaneous group, those examples with multiple ulcers widely separated on the body and those with roentgenographic evidence of "fibrosis of the lungs" suggested the possibility that some cutaneous lesions might have originated hematogenously, perhaps from a subclinical pulmonary focus. The only alternative explanation for widely separated skin lesions is auto-inoculation.

Most of the cases with cutaneous involvement were thought to be primary, probably from direct inoculation, as in wound infection, although precise information on this point was unobtainable. Cases are on record of cutaneous blastomycosis following traumatic injuries,[12] and cutaneous blastomycosis can be produced in monkeys by intracutaneous injections.[13] In most of our cases spread from single cutaneous lesions appeared to be by peripheral extension, either direct or by the cutaneous lymphatics. The cutaneous ulcerations found in the patients of autopsies no. 1 and 2 seemed to be of hematogenous origin.

Toxicity

A close correlation between toxicity and systemic or deep involvement was noted. Most of the patients with cutaneous lesions were ambulatory and "non-toxic." All of those who came to autopsy were judged to be 4 plus (++++) in respect to toxicity, on the basis of temperature, blood-cell studies and weakness. In three, death appeared to be due directly to blastomycotic involvement; in one, to heart failure secondary to constrictive blastomycotic pericarditis (case 4). An element of heart failure may have been present in case 3 also, and on the same basis.[4]

Study of the tissues suggested that the toxicity of the patient was related to two features: (1) large numbers of organisms and (2) caseation. In material obtained by autopsy and from deep lesions, organisms were often present in enormous masses, almost in pure culture, as in the brain abscess (Figs. 2 and 9) of autopsy no. 2. Organisms were often dead in the central portions of such masses, reminiscent of the findings in experimental blastomycosis in mice. In the cutaneous cases, on the other hand, organisms occurred singly or in pairs (Fig. 1) and the lesion was composed of cellular (polymorphonuclear) exudate, or granulation tissue and hyperplastic epithelium.

Duration and Outcome

The patients who came to autopsy had the disease for periods of between 3 and 24 months, so far as could be judged from their histories and physical evidence. Of the patients who had bone lesions, two died and two were living, 2 and 4 years after onset, without evidence of blastomycosis. One, a child of 5 years, who had osteomyelitis of a rib and miliary pulmonary involvement, revealed by x-ray examination, became free from evidence of the disease without iodides or other forms of therapy except drainage of the focus of osteomyelitis. Another patient was cured by a combination of therapeutic procedures.[14]

Of the two patients with resections of deep tissues, one is living and apparently free of blastomycosis 7 years after the onset of symptoms[3] (Martin and Smith,[1] case no. 10). The other patient with thoracic involvement and a supraclavicular subcutaneous abscess, cannot be traced.

Of the 13 patients with cutaneous involvement, two died 4 and 5 years after the onset, apparently of blastomycosis. Two showed no blastomycosis 2 and 5 years after onset. Most of the cutaneous cases have remained such without generalization, and have improved or have been cured by various forms of therapy. One has had a duration of 13 years, with involvement of the skin of the entire thorax and neck, but without impairment of general health.

Fundamental Gross Characteristics

The pyogenic character of the disease was usually impressive. Three of the patients coming to autopsy presented massive, fluctuating, subcutaneous abscesses (Text-Fig. 1) and all of them had osteomyelitis with sinuses extending to the skin or to joint surfaces. Brain, psoas and intramuscular (Figs. 3 and 4) abscesses were noted. The pus was usually pink. In a patient with a deep lesion, subjected to laparotomy, 25 cc. of creamy pus came from the tubes and pelvic peritoneal recesses.[3] In the cutaneous cases pus could be obtained from miliary abscesses at the periphery of the lesions.

Grossly the appearance of caseation was sometimes noted. In autopsy no. 2, the cut surface of the lung showed "large confluent areas of caseation from which pus can be squeezed." In autopsy no. 1, the fresh lungs at the time of autopsy showed "scattered, small yellow abscesses." After fixation, the same foci were spoken of as tubercles. In autopsy no. 3 the term "tubercles" was used for the pulmonary nodules, but it was noted that central softening was more apparent than in tuberculosis.

Fundamental Microscopic Characteristics

In every case the polymorphonuclear neutrophil was a prominent cell type (Figs. 1 to 5) and usually it occurred in abscesses. In two cases with deep lesions, true abscesses were not present and the polymorphonuclear reaction was spoken of as diffuse. Sometimes the polymorphonuclear neutrophils occurred in the interstices between blastomycetes, as in the massive lesions of the generalized type; and sometimes the opposite relationship existed, *i.e.*, one or two blastomycetes occurred in a mass of pus, as in the sparser involvement of the cutaneous cases. The former type of involvement is well illustrated by Mallory.[15] Eosinophils were occasionally prominent. Giant cells (Fig. 5) were noted in every case, but were sometimes very rare. Usually blastomycetes, either living or dead, occurred in giant cells, but in a few instances no organisms were noted. Large mononuclear cells were not prominent.

Caseation was not found in the lesions of the solely cutaneous cases, nor in the skin lesions of the cases coming to autopsy. In fact, the skin lesions of the latter group closely resembled those of the "pure" skin cases. Abundant caseation was noted in autopsies nos. 1, 3 (Fig. 6) and 4, and in all the cases with osseous and deep lesions except two. In specimens obtained at autopsy it could sometimes be determined, from the presence of shadows of dead blastomycetes, that caseous material was composed largely of dead fungi. This was true of lesions of the heart, brain, prostate and bone. In some of the caseous pulmonary lesions, on the other hand, no shadows were noted in the caseous material. It was not evident whether the necrosis was due to proteolytic enzymes of the contained polymorphonuclear neutrophils, or to the poor nutrition of those blastomycetes which lay centrally, or to other factors.

Fibrosis was frequently prominent (Fig. 7). In many lesions fibrosis about abscesses had developed a heavy collagenous component.

Viable organisms were always present, free or within giant cells. Dead organisms, free or within giant cells, were present in material obtained by autopsy, and in most of the cutaneous lesions. The differentiation between viable and dead organisms was made on the basis of staining reaction. Blastomycetes appearing as shadows were obviously dead. Forms in which the central portion of the organism no longer absorbed any hematoxylin were also considered dead. The validity of this differentiation was supported by the appearance, in sections, of the heat-killed blastomycetes injected experimentally into mice.

From this analysis, it is clear that the microscopic characteristics of blastomycosis are not identical with those of tuberculosis. In the tabu-

lation, similarity to tuberculosis was designated 1 plus (+) in seven cases and 2 plus (++) in one case. But even in these cases, other areas deviated from the characteristics of tuberculosis.

Iodides

An attempt was made to correlate the histologic picture and the number of living or dead organisms with the administration of iodides. No clear-cut conclusions could be adduced. In four of the cases massive doses had been given shortly before histologic examination. Two of these were cases examined at autopsy. Comparison with the other two similarly examined, in which iodides had not been given, showed no essential differences with respect to the organisms, the degree of caseation, or the fibrosis. In a surviving systemic case [3] in which laparotomy had been performed, desensitization and iodide therapy were carried out according to the method of Martin and Smith.[11] In the pelvic material there were blastomycotic tubercles with more fibrosis about them than in any other case (Fig. 7). In the same region, however, there were other areas with pus and organisms, and with all stages of inflammatory reaction and repair. (The reader is referred to the illustrations in the paper by Hamblen, Baker and Martin.[3]) In a cutaneous case, healing of lesions with dense fibrosis, devoid of blastomycetes, occurred. Iodides and desensitization had been used, but x-ray treatment had also been given. Thus the evidence as to the effect of iodides on the histologic picture of blastomycosis is not clear; possibly fibrosis is stimulated. Clinically, the administration of potassium iodides by mouth is said to promote marked improvement in the majority of cutaneous cases, but in the systemic cases the results are very discouraging.[11]

It has not been possible to evaluate histologically the effect of x-ray treatment in this series, since there were not enough biopsies after such treatment. Clinically the cutaneous lesions appear to undergo regression and fibrosis with x-ray treatment,[16] but usually too many factors have been present in the therapy to evaluate any one of them properly.

Immunologic and Allergic Responses

The pathologic analysis was made with the following observations of Martin and Smith [1] in mind:

"Antibodies can be found in the sera of patients who are heavily infected; they persist until death unless the infectious process is overcome or greatly reduced.

"Some patients develop a condition of hypersensitiveness to the infecting fungus, and this allergic state diminishes in the terminal stages of the disease."

A correlation of histopathologic details with such clinical observations could be made only in so far as these details were associated with

either severe systemic infection or with mild, localized cutaneous infection. When antibodies were high or when the patient was in the terminal anergic state, great numbers of organisms were present and caseous necrosis was often noted.

Discussion

In affirming the characteristic nature of the tissue response in blastomycosis, I am impelled to comment on the opposite point of view as expressed by Medlar.[8] He emphasized the identity of the gross and microscopic pathology in fungous and tuberculous infections in connection with a report of two cases of pulmonary blastomycosis. This statement seems to me to be far too sweeping even if the problem is approached as Medlar approached it, by comparison of the blastomycotic reaction with the histogenesis of tuberculosis, with special regard to the polymorphonuclear neutrophil, caseation and the presence of reticulum. In generalizing about blastomycosis, moreover, he used case reports of pulmonary blastomycosis alone upon which to base his opinion. It has been shown above that these are the very cases which may resemble tuberculosis most strongly.

Abscess, a circumscribed collection of pus, with the connotation of a liquid state in the unfixed condition at body temperature, may occur in tuberculosis, it is true. But if 23 cases of tuberculosis were accepted *seriatim*, the observer would certainly be far less impressed by the presence of abscesses grossly and microscopically than in the 23 cases of blastomycosis here reported.

The reverse of this would be true with respect to caseation. The term caseation is applied to the gross characteristics of the necrotic material commonly seen in tuberculous infection. If this term is applied to the necrotic material commonly seen in blastomycotic infection, in some instances the microscopic character of caseation in the two diseases will correspond closely. But in other instances, as has been pointed out, blastomycotic caseation may consist largely of masses of dead blastomycetes and not of reacting cells of the host. In tuberculosis this situation is encountered only in those very rare instances in which masses of stained tubercle bacilli can be perceived in a histologic section with the naked eye.

Miller,[17] using Bielschowsky's silver method (which is excellent to demonstrate blastomycetes and reticulum), showed that the growth and transformation of collagenous tissue differed in no way in the tubercle of blastomycosis from that in the tubercle of tuberculosis. While this is a point of resemblance between the reactions of the two diseases, it should be remembered that the production of reticulum

and collagen is a general pathologic process related to a stage in repair, and not peculiarly associated with these diseases, nor with all stages of these diseases.

Summary and Conclusions

1. Among 23 cases of human blastomycosis (13 cutaneous and 10 generalized or thoracic) complete autopsy was available in 4, and histologic material in the remaining.

2. The formation of abscesses was an impressive gross feature of the generalized cases, and polymorphonuclear foci were noted microscopically in all 23 cases. Giant cells were always present. Caseation was present in the generalized and thoracic cases, but was not noted in the cutaneous cases.

3. Human blastomycosis was interpreted as being primarily pyogenic, with prominence of polymorphonuclear neutrophils. Some lesions of some cases, especially in the systemic group, closely resembled the lesions of tuberculosis.

4. The terminal stage of systemic blastomycosis in man corresponded closely to the experimental disease in the mouse. Masses of blastomycetes occurred with necrosis, producing a toxic effect upon the patient. In the cutaneous cases, in contrast, organisms were usually moderate in numbers, caseous necrosis was usually absent and the lesion was composed of miliary abscesses, granulation tissue and hyperplastic epidermis. There was little toxic effect upon the patients.

5. These observations on the nature of toxicity in blastomycosis suggest that therapeutically a fungicide is not desirable in the severe systemic cases, since too much necrotic blastomycotic material is already present.

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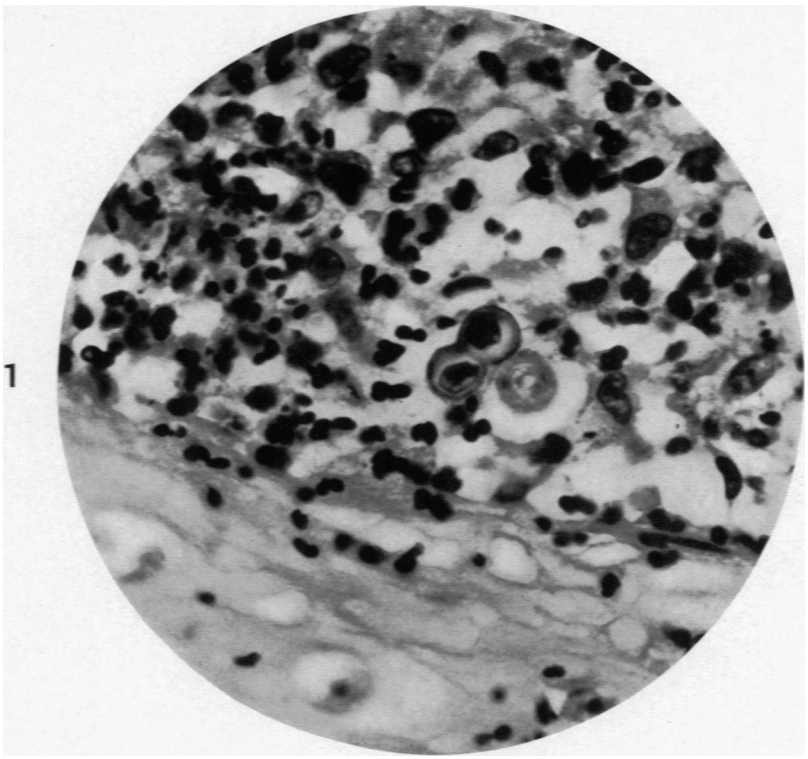
DESCRIPTION OF PLATES

PLATE 78

FIG. 1. Microscopic abscess in cutaneous blastomycosis. Blastomycetes, one budding, are free in the center. There is a predominantly polymorphonuclear response, but other types of cells are present. The epidermis appears below. From skin of nose of white man, 63 years old, with multiple cutaneous lesions. Biopsy was taken 3 years after onset of disease. The patient was living in 1938, 2 years later, without evidence of blastomycosis. × 705.

FIG. 2. Massive growth of blastomycetes in an abscess of the human brain, autopsy no. 2. (Photograph of the gross specimen is shown in Fig. 9.) Viable blastomycetes, from the edge of the abscess, are above; and necrosis, toward the center of the abscess, is below and to the left. × 705.



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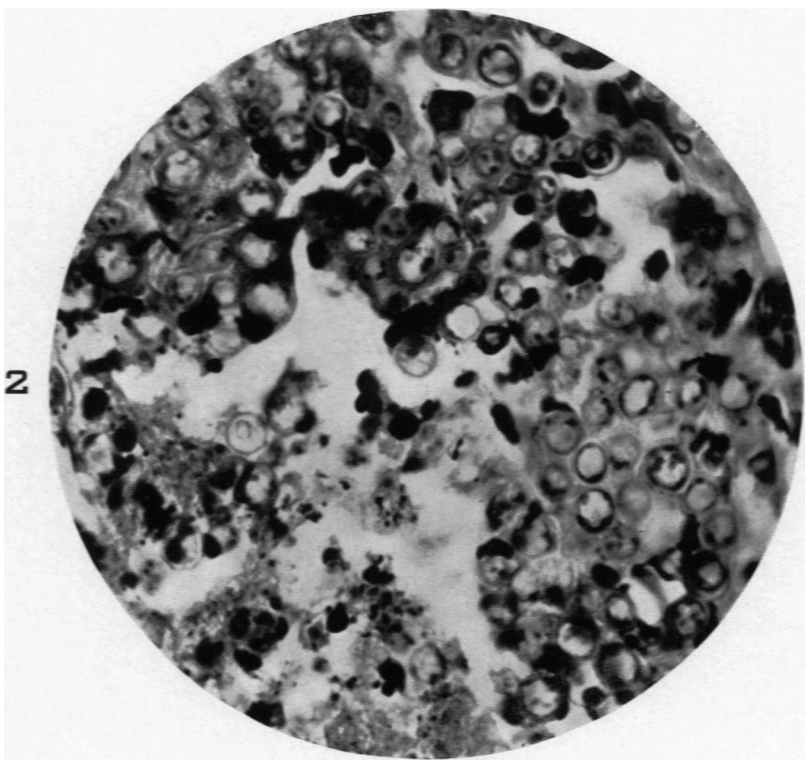

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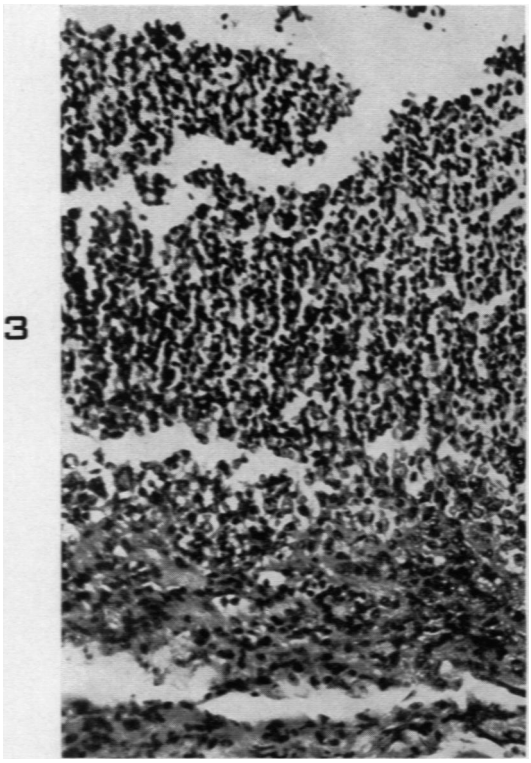
PLATE 79

FIG. 3. Wall of an abscess, and exudate. in muscle of upper arm, autopsy no. 2. × 174.

FIG. 4. Higher power magnification of area in Figure 3 to show free blastomycetes with cellular response rich in polymorphonuclear cells. Mononuclear cells are also numerous. × 760.

FIG. 5. Abscess and giant cells containing blastomycetes in an osteomyelitic case. This was a surgical specimen. Osteomyelitis of elbow and rib in a white male, 30 years old. The patient was apparently cured by a variety of therapeutic methods and was well 2 years after onset. (See report of Martin and Jones.[14]) × 174.



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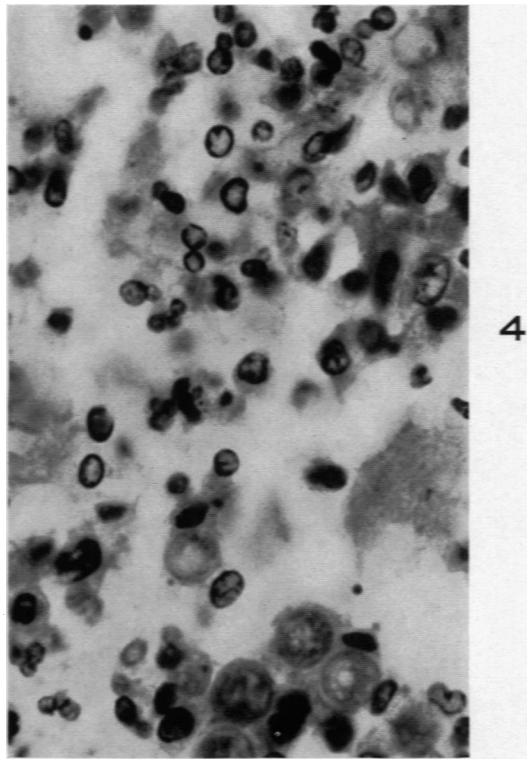

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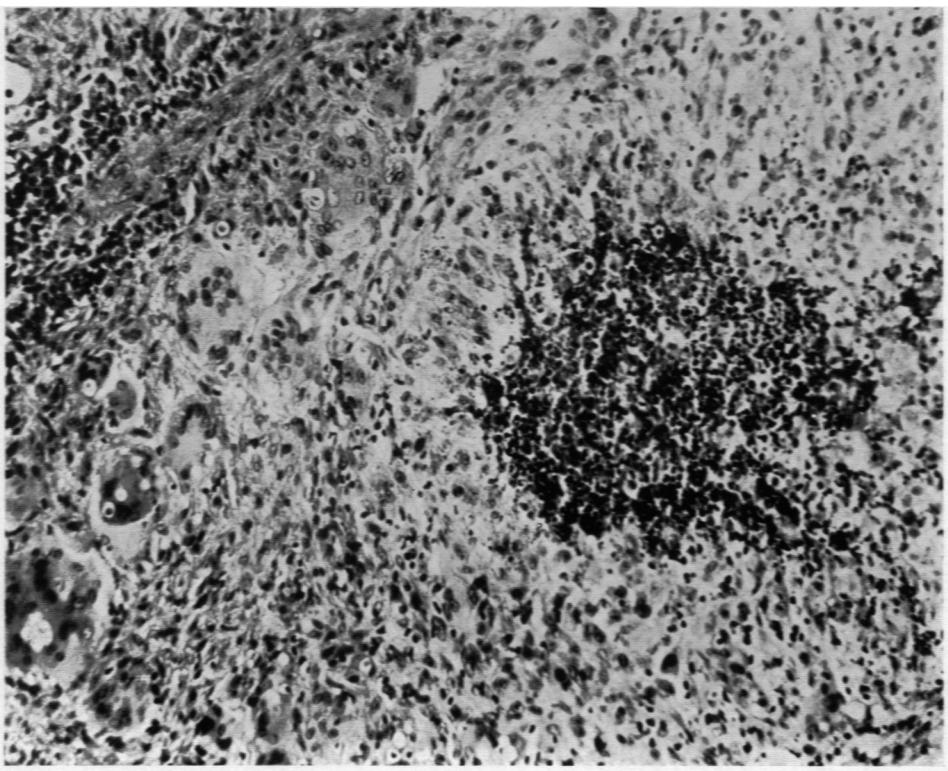

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PLATE 80

FIG. 6. Caseous pulmonary lesion in autopsy no. 3, upon a case of generalized blastomycosis. Shadows of necrotic blastomycetes were seen in several levels of this lesion and in the adjacent giant cells. Acid-fast bacilli were not found in sections appropriately stained. × 174.

FIG. 7. Hard tubercles in peritoneal reaction about blastomycosis of fallopian tubes. Surgical specimen from white woman, 27 years of age, with generalized blastomycosis, now (1941) apparently free of the disease, 7 years after onset.[3] Blastomycetes were present in adjacent tubercles. Acid-fast bacilli were not found. × 174.

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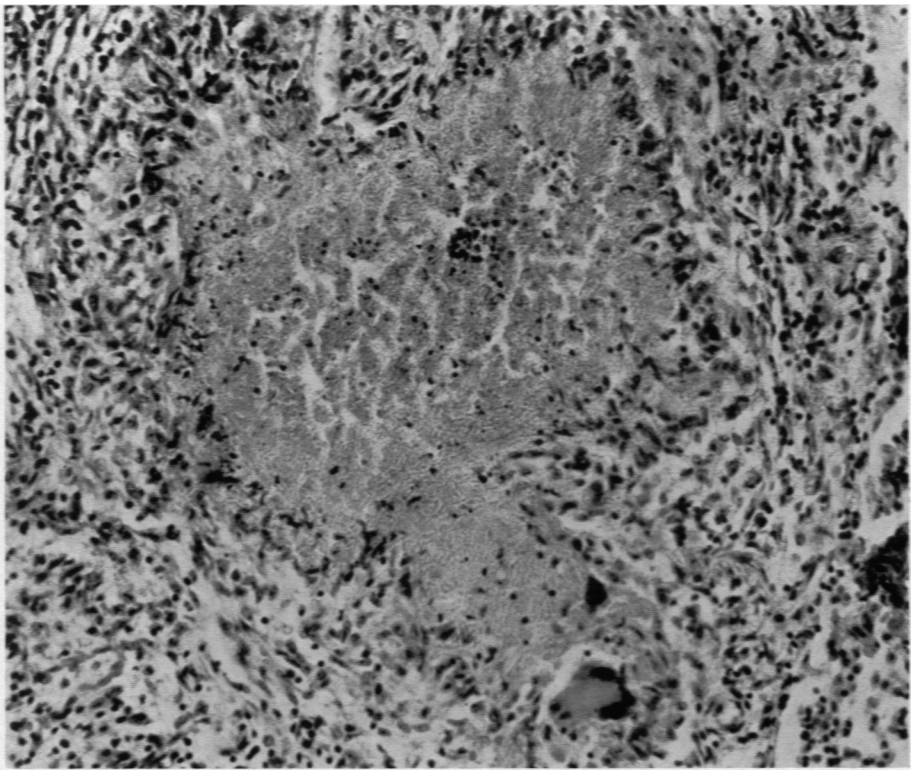

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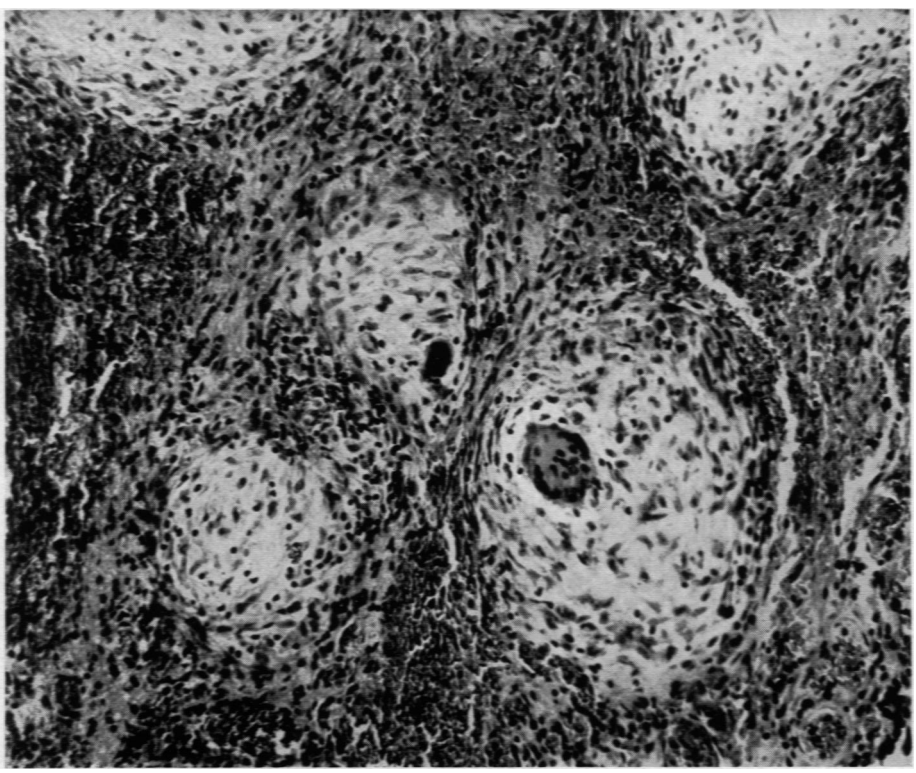
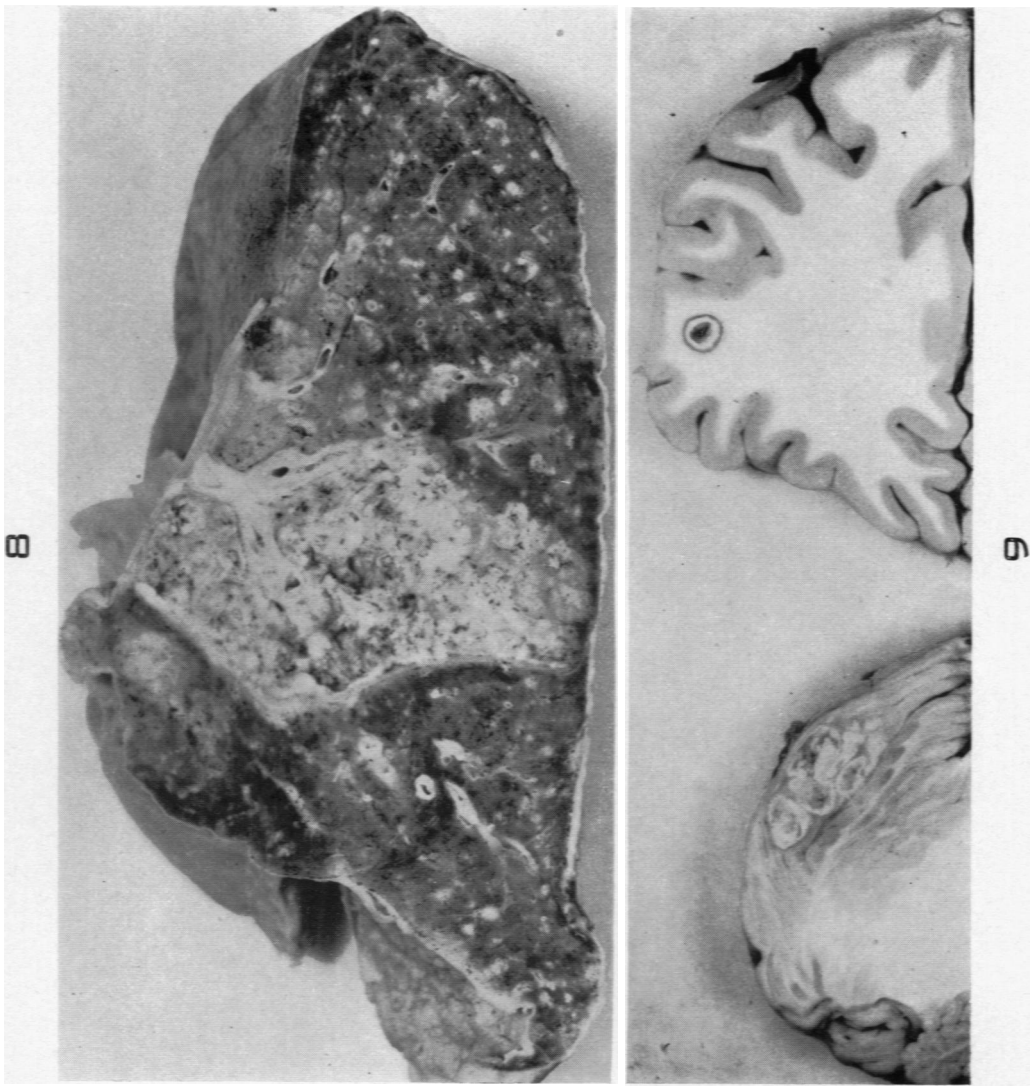


PLATE 81

FIG. 8. Blastomycotic pulmonary abscess (autopsy no. 2).

FIG. 9. Blastomycotic brain abscesses (autopsy no. 2).



Baker Experimental Blastomycosis in Mice