EXPERIMENTAL CRYPTOCOCCOSIS (TORULOSIS)*

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Cryptococcosis (torulosis), caused by the pathogenic yeast-like fungus *Cryptococcus neoformans,* is a disease which presents numerous unsolved problems relative to portal of entry, immunology, histopathology, selectivity of localization, natural history, diagnosis, and therapy. Studies of human material[1-3] have indicated the respiratory tract as the portal of entry, and have shown poor immunologic responses to the infection. The histopathology is characterized by a granulomatous inflammation or by a minimal inflammatory reaction, with preferential localization in the central nervous system. The disease tends to progress, although instances of healing are recorded. The study of human material has obvious limitations for solution of the stated problems, and the experimental investigations recorded here were designed to shed additional light on some of them, particularly the histopathologic features of early lesions and the natural history of infections produced by different routes of inoculation.

METHODS

Three pathogenic strains of *C. neoformans* were supplied by Dr. Rhoda Benham. Strain 1499.20 and strain 1499.57 were isolated from human cases of cryptococcosis in 1951 and 1952, respectively, and strain 1499.62 (Sanfelice's strain) was received from the Centralbureau voor Schimmelcultures in 1953. Cultures were grown in Mycophil broth‡ at room temperature for 3 to 5 days, including 16 hours of mechanical agitation. The broth cultures were centrifuged and the cells were suspended in saline solution. Samples of the saline suspension plated immediately prior to inoculation on Sabouraud's agar for incubation at room temperature and on blood agar for incubation at 37° C. demonstrated no fungal or bacterial contaminants. When dilutions of the inoculum were required, sterile saline solution was employed as diluent. The yeast cells were counted with the aid of a Levy chamber.

Subcutaneous inoculations were made at the midline of the lower

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abdomen, 1 day after shaving. Intraperitoneal inoculations were made into the lower abdomen, without prior shaving. Intracerebral inoculations were made with a 25 gauge hypodermic needle. The skull was entered just behind and medial to the ear, with the needle directed anteriorly, ventrally, and toward the midline. No anesthetic was required. The volume of each inoculation was 0.1 cc.

Necropsies were done on all animals that died during the experiment or that were sacrificed by ether inhalation. Cultures were prepared on Sabouraud's agar and blood agar, from brain, lung, liver, and any subcutaneous lesions. Tissues were fixed in 10 per cent formalin and embedded in paraffin. Routine sections of brain, lung, heart, liver, spleen, kidney, and skin were stained with hematoxylin and eosin and with the periodic acid-Schiff (PAS) stain.

Results

Subcutaneous Inoculation

Twenty-nine female A albino mice (average age, 3½ months) were inoculated subcutaneously with 13 million yeast cells in a saline suspension from a 3-day broth culture of *C. neoformans*, strain 1499.20. They were sacrificed at intervals of 1 day to 5 months.

On the first day after inoculation, palpable nodules were present at the site of injection in all animals. Microscopically, there were collections of closely packed polymorphonuclear neutrophils in the subcutaneous adipose tissue. Yeast cells were visible in sections stained with hematoxylin and eosin, but were rendered more prominent by the PAS stain. They were round or oval with occasional buds. The cell wall was deeply stained by the Schiff reagent and frequently showed a deep indentation or fold. Rarely was the presence of soluble capsular material indicated by an unstained space or halo. The mass of inflammatory cells was traversed by persisting strands of connective tissue and cutaneous maximus muscle. A few polymorphonuclear leukocytes were present in the surrounding adipose tissue and the overlying dermis, but not in the subjacent muscle of the abdominal wall.

On the second day, the inflammatory subcutaneous mass was larger, well demarcated, and composed of innumerable polymorphonuclear leukocytes and yeast cells with complete dissolution of tissue, that is, a true abscess (Fig. 1). The adipose tissue around the abscess was edematous, and contained numerous spindle-shaped fibroblasts. There were diffuse infiltrations of lymphocytes and polymorphonuclear leukocytes, and small collections of these cells surrounded congested

capillaries. In some animals, other smaller subcutaneous abscesses were present. Each of the abscesses contained many yeast cells, and yeasts were not seen outside of the abscesses. Probably, movement of the needle during injection of the suspension was responsible for the multiple abscesses.

On the third day, the subcutaneous abscesses reached maximal size (0.5 to 0.7 cm. in diameter). There was a tendency for the yeast cells to be clustered in the center of the abscess. The overlying dermis and epidermis were thin and necrotic (Fig. 2).

By the fifth day, one third of the animals showed ulcerated lesions and the necrotic skin was separated or desquamated. Expelled with it was part of the contents of the abscess, namely, necrotic polymorphonuclear leukocytes and large numbers of yeast cells. The base of the ulcer was usually well demarcated by a wall of inflammatory cells and young fibrous tissue. The epidermis at the margins of the lesion showed downward proliferation. By the seventh day, ulceration was detected in one half of the animals (Fig. 3).

On the 13th and 20th days, proliferation of capillaries and fibroblasts and infiltration by lymphocytes and plasma cells were much more conspicuous around the abscesses. An occasional multinucleated giant cell of Langhans' type was observed. The yeast cells were concentrated in the center of the abscesses which now contained many macrophages with vacuolated cytoplasm (Fig. 4). Some of the macrophages contained yeast cells. The striated muscle of the abdominal wall showed little or no alteration despite the intense inflammation in the subcutis immediately above.

After the 13th day, more and more of the palpable nodules disappeared. Sections of skin after 20 days showed nothing beyond fibrosis with a few inflammatory cells; no yeast cells were demonstrated (Fig. 5).

Only two of the 29 mice showed signs of systemic illness during the experiment. At the end of 1 month they were sacrificed. Disseminated cryptococcosis was confirmed by cultures and histologic sections. Extensive involvement of the brain was demonstrated in both animals, and involvement of heart, liver, and kidney in one. Sections of the abdominal wall of one of these mice showed a healing cryptococcal granuloma involving peritoneum and abdominal muscles. It is likely that dissemination in this case was due to an inadvertent peritoneal and intramuscular inoculation.

Absence of disseminated infection in the other 27 mice was indicated by histologic examinations of brain, heart, lung, liver, spleen, and kidney. In addition, cultures of brain, lung, and liver of 15 mice

were sterile. The necropsies were performed and the cultures made on the last eight mice after an interval of 5 months.

Pure cultures of *C. neoformans* were recovered from the subcutaneous abscesses of two animals during this experiment. Occasionally bacteria were cultured from the ulcerated lesions. (Including all the experiments in which subcutaneous abscesses were produced, pure cultures of *C. neoformans* were recovered on 15 occasions.)

In contrast with the results just described, subcutaneous inoculation of heat-killed cryptococcal cells elicited a transitory polymorphonuclear infiltrate on the first day. The infiltrate and the killed yeast cells could not be detected in sections by the third day.

Intracerebral Inoculation

Seventeen female mice of the same strain and average age were inoculated intracerebrally with the same dosage of cryptococci in saline suspension as was used for the subcutaneous inoculations. Within 5 days, all of these animals died or were sacrificed when moribund. In order to increase the survival periods, groups of five mice were inoculated with four serial 10-fold dilutions of the yeast cell suspension.

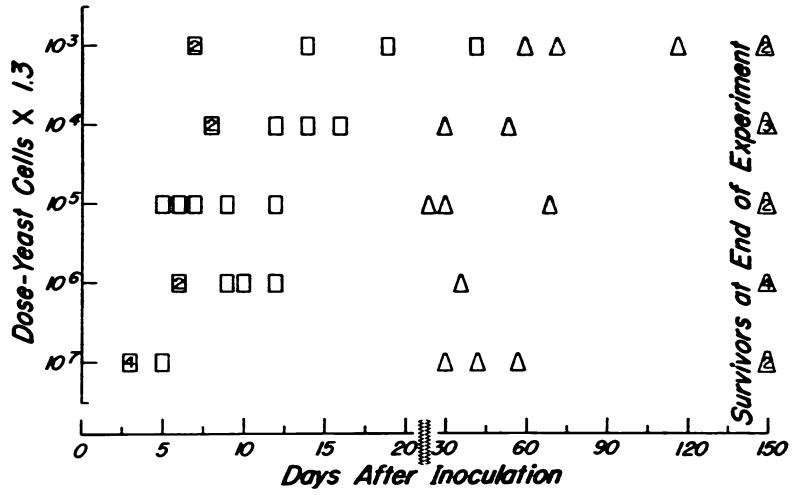
On the first day after inoculation, sections revealed small, irregular, cystic spaces in the cerebral parenchyma. The spaces contained many yeast cells and fresh blood cells, and were traversed by narrow strands of persisting cerebral tissue. The yeast cells were small, round or oval, and frequently possessed buds or were arranged in short chains or small clusters (Fig. 7). Broad, clear haloes suggested the presence of abundant, soluble, capsular material. The yeast cells were observed also in the ventricles and meninges. There was no tissue reaction in the brain, and only a few polymorphonuclear leukocytes were in the meninges.

On the second day there was still no reaction in the brain, but polymorphonuclear leukocytes were in the infected areas of the meninges. Extension of the infection from the meninges into the underlying perivascular spaces was observed.

On the third day (Figs. 6 and 7) the masses of yeast cells in the brain were larger and still unattended by any inflammatory response except for a few small foci in relation to the choroid plexus or choroid fissure. The meninges were uniformly infected, and contained few polymorphonuclear leukocytes as compared with the large number of yeast cells. Although the inflammatory response was greater in the meninges than in the brain, it never approached the intensity observed in the subcutaneous tissues.

From the 6th to the 16th day, observations were made on animals which had received much smaller inoculative doses (1,300 to 1,300,000 yeast cells). Several of the animals had predominately meningeal involvement, with a more conspicuous inflammatory reaction. Mononuclear cells, phagocytized yeast cells, and early meningeal fibrosis were increasingly prominent. Small or large masses of yeasts with relatively few inflammatory cells were observed in the substance of the brain or filling dilated ventricles. There was no evidence of encapsulation of these foci, and alteration of surrounding tissue was minimal; but there was increasing phagocytosis of the yeast cells (Fig. 8).

All the doses of *C. neoformans* proved lethal when injected in the brain, and the survival period was inversely proportional to the dose (Text-fig. 1).



Text-figure 1. Mortality from experimental cryptococcosis. Groups of five mice received intracerebral (squares) or intraperitoneal (triangles) inoculation of *C. neoformans* in serial 10-fold dilutions.

Dissemination of infection occurred in all of these animals. Metastases were observed (in order of frequency) in liver, heart, lung, kidney, and spleen. Yeast cells were observed in the capillaries of these organs as early as the first day after inoculation. The liver was involved with greatest severity as well as greatest frequency. By the second day, the small foci of yeast cells in the hepatic sinusoids were infiltrated by polymorphonuclear leukocytes. On the third day these areas resembled miliary abscesses (Fig. 9). In most cases observed thereafter, the liver was studded with such lesions, and there was gradual replacement of polymorphonuclear leukocytes by macrophages

containing yeast cells. In several animals, the severe inflammation in the liver was in striking contrast to the minimal reaction in the brain (Figs. 6, 7, and 9). The degree of inflammatory response in the liver varied among the individual animals. It was generally much greater, however, than in the other organs.

Intraperitoneal Inoculation

Seventeen female mice of similar strain and average age were inoculated intraperitoneally with the same saline suspension used for the subcutaneous and intracerebral inoculations. In order to compare the lethality of intraperitoneal and intracerebral routes of inoculation, groups of five mice were inoculated with four serial decimal dilutions of this saline suspension.

On the first day after inoculation, no changes were detected in sections of liver, spleen, kidney, lung, heart, and brain. On the second and third days, there was a small amount of purulent exudate on the surfaces of the spleen and liver. Yeast cells were present in this exudate and in small numbers in the parenchyma of these organs.

On the 7th and 13th days, the liver showed miliary abscesses. Disseminated cerebral lesions showed much less inflammation than the hepatic lesions. A comparable difference in inflammatory response was noted previously when the brain was the primary site of inoculation.

After 2 weeks, the same progressive changes, as described previously, were observed in the lesions.

C. neoformans proved far less lethal by the intraperitoneal route than by the intracerebral route (confirming Emmons' data)[4] and the mortality rate was not proportional to dosage (Text-fig. 1). Between 2 weeks and 5 months after inoculation, 23 animals died or were sacrificed. Cryptococcal infection in 12 animals was confirmed by cultures or histologic slides. The brain was involved in seven, the liver in six, and the heart, lungs, spleen, and kidneys in one animal with widely disseminated lesions, and the perirenal adipose tissue in one animal. The involvement of the liver was slight, and sometimes detected only by culture. Involvement of the brain was more frequent and usually took the form of large lesions that had caused motor signs during life. As in man, the cerebral lesions were the significant ones.

Two animals (sacrificed after 2 and 5 months) showed involvement of the muscles and subcutis of the abdominal wall, without other lesions. Histologically, both cases were characterized by a combined purulent and granulomatous reaction (Fig. 10). The granulomas contained multinucleated giant cells with yeast cells in their cyto-

plasm. The localization of these lesions was probably due to inadvertent intramuscular inoculation. The granulomatous response may be related to the site and chronicity of the infection.*

No evidence of infection was detected in cultures or sections of 11 animals. Nine of these were examined 5 months after inoculation.

Observations with Other Strains of C. neoformans

In order to determine whether the histopathology and course of the infection varied with the strain of *C. neoformans*, the preceding experiments were repeated with two other strains. Groups of eight female A albino mice, of an average age of 3 months, were inoculated with a saline suspension from a 5-day broth culture by the subcutaneous, intraperitoneal, or intracerebral routes. The doses were 8.2 million cells for strain 1499.62 and 7.3 million cells for strain 1499.57. The animals died or were sacrificed at various intervals; the survivors were sacrificed at 4 months.

After subcutaneous inoculation of strain 1499.62 the lesions appeared more slowly, grew larger, and lasted longer than comparable lesions produced by strain 1499.20. Strain 1499.57 produced even larger and longer-lasting lesions. Both strains produced an early histopathologic response characterized by formation of an abscess, as was noted for strain 1499.20, and all lesions ulcerated. Strain 1499.57 was different from the others in that the proliferation of yeast cells outstripped the inflammatory response by the sixth day. Older lesions showed phagocytosis of yeast cells, but minimal infiltration of polymorphonuclear and mononuclear inflammatory cells, and variable fibrosis and encapsulation (Fig. 11). The yeast cells were larger and had broad haloes, indicating copious quantities of capsular material. Despite this histopathologic difference, the lesions tended to heal and only one animal in each group showed dissemination to the brain.

No differences were noted following intracerebral inoculation with the two strains. All animals died within 5 days. The cerebral lesions showed the characteristic absence of inflammatory response in marked contrast to the subcutaneous lesions of equal age. There was dissemination of infection to all organs examined.

Following intraperitoneal inoculation, all of the animals died or were sacrificed within 2 months except for one in each group which had no histologic or cultural evidence of disease at 4 months. All

* The absence of such a distinct granulomatous response in the series of animals inoculated subcutaneously may be related to the rapid healing of these lesions.

other animals had disseminated disease. Again the brain was the site of predilection and the cerebral lesions were more numerous, larger, and showed much less inflammatory response than those in other organs. Figures 13 and 14 illustrate severe involvement of the brain approaching the "soap-bubble" appearance sometimes observed in human cases.

Subcutaneous Inoculations in Rats and Rabbits

The development of abscesses following subcutaneous inoculation in mice was a finding of sufficient interest to warrant confirmation in other species. Ten female albino rats of Wistar strain, averaging 130 gm. in weight, and four female New Zealand rabbits, averaging 1,760 gm. in weight, were inoculated subcutaneously with saline suspension of 25.6 million cells from a 4 day broth of *C. neoformans*, strain 1499.20. Local lesions developed in all animals. None showed evidence of systemic illness during life, and none showed histologic or cultural evidence of disseminated disease after death.

Five rats were sacrificed and studied histologically at intervals from the 4th to the 27th day. The development of well encapsulated abscesses followed by ulceration closely paralleled the results in mice. Multinucleated giant cells containing yeasts were more numerous than in lesions of mice by the 17th day. The lesions regressed and were undetectable on the 79th day when the remaining five rats were sacrificed.

The four rabbits were sacrificed between 4 and 27 days after inoculation. Ulceration was observed in only one rabbit, but in other respects the histopathologic lesions were the same as those of rats.

Discussion

The most important result of this experiment was the difference in degree and type of inflammatory response to *C. neoformans* in various organs. The prompt outpouring of polymorphonuclear leukocytes in the subcutaneous tissues, followed by lymphocytes, plasma cells, macrophages, fibrosis, and encapsulation, was in striking contrast to the delayed, weak, and irregular reaction in the brain. The visceral organs were intermediate in reactivity.

The healing of subcutaneous infections with only occasional dissemination, the universal and prompt lethality of cerebral infections, and the intermediate reactivity of intraperitoneal infections probably were related directly to the characteristics of the inflammatory response. Preferential localization in the brain following intraperitoneal

inoculation can be interpreted as a lack of resistance to circulating yeast cells, based in turn on the minimal inflammatory response. The small size and many buds of the yeast cells in lesions of the brain indicate active growth in a favorable medium, while the larger size and fewer buds in subcutaneous abscesses probably are due to an unfavorable environment for growth.[5] The similarity of the cerebral lesions in human and experimental cryptococcosis suggest that the same explanation may account for the predilection for the human central nervous system. In animals as in humans the lesions are not only more numerous in the brain than elsewhere, but are larger and more significant in producing symptoms and a fatal outcome.

Variability in the outcome of experimental inoculations is to be expected, based on differences in the dose, virulence, and strain of *C. neoformans,* and on differences in species, strain, age, and individual resistance of the experimental animals. Some authors have found disseminated disease following subcutaneous inoculation in the mouse,[2,6] rat,[2] and guinea pig, [1,2,6,7] while in other instances complete localization and healing of subcutaneous lesions have been recorded for the mouse,[8] guinea pig,[2] dog,[9] and monkey.[9,10] Under the conditions of our experiments, localization and healing of subcutaneous infections have been the rule in the mouse, rat, and rabbit. Instances of healing of human infections localized to skin, lung, or bone have been recorded.[2,3]

Dissemination of infection following subcutaneous inoculation occurred in two of our mice even though the skin lesions showed partial and complete healing, and similar observations are cited by Freeman.[1] This may explain the occurrence of disseminated cryptococcosis in humans despite the absence of a detectable portal of entry. In view of the isolation of cryptococci from normal human skin[11] and from soil,[12] the skin as well as lung must be considered a possible portal of entry.

The formation of true abscesses has not been considered common in human or experimental cryptococcosis, but it has been recorded in a few instances.[13-17] The explanation is probably that early lesions have not been studied histologically. Baker and Haugen[18] found numerous polymorphonuclear neutrophils with a few abscesses in five of 26 human cases. They suggested that hypersensitivity with increased tissue destruction might be the cause of the pyogenic responses. They also stated that "*C. neoformans* is a bland and inert agent when it first invades the body, and it produces no perceptible necrosis or inflammation in adjacent tissues. . . . There is no indication of a

primary neutrophilic response." Clearly, these suggestions are not applicable to the pyogenic response observed immediately following all subcutaneous inoculations under the particular conditions of our experiment.

Attempts to Alter the Course of Experimental Cryptococcosis

C. neoformans usually is considered to have little immunogenic activity, although useful antisera have been prepared in rabbits by some workers.[19,20] Hoff[21] attempted to produce active immunity in mice by injecting heat-killed cryptococcal cells, but he found only slight prolongation of life following intraperitoneal challenge. In view of the usually self-limited character of subcutaneous infections under the experimental conditions described here, we attempted to detect evidence of active immunity during the healing phase of subcutaneous lesions.

Sixteen female C57-black mice, of an average age of 4 months, were inoculated subcutaneously with 15.4 million cells of *C. neoformans,* strain 1499.20. Sixteen control mice received sterile saline injections. On the 14th day, when the subcutaneous lesions were regressing, all animals were challenged by the injection into the brain of 190,000 cells of the same strain. By the 13th day after challenge, all mice of the control series and 14 of the experimental series were dead. The survival of two mice of the experimental series to the 20th day does not, in our opinion, constitute a significant difference. Under the conditions of this experiment, we could not demonstrate active immunity associated with healing subcutaneous lesions.

It proved easier to decrease, rather than increase, the resistance of mice to experimental cryptococcosis. Cortisone is known to decrease inflammatory response.[22] Cortisone and radiation are reported to act synergistically in increasing susceptibility to fungi and other infectious agents.[23] Although the possibility of a relation between human cryptococcosis and cortisone therapy has been raised,[24,25] there is as yet little evidence for this thesis.[17] Increased dissemination of experimental cryptococcosis due to cortisone has been reported following intraperitoneal inoculation of rats,[26] while only minimal enhancement occurs following intraperitoneal infection of mice.[27] In view of the usually localized nature of subcutaneous infection in our experiments, it was of interest to determine whether cortisone would cause dissemination.

Five groups of six female A albino mice (average age, 2½ months) were inoculated subcutaneously with 9.6 million cells of *C. neoformans,* strain 1499.20. One group was untreated, and served as a

control. The second group was subjected to daily manual trauma of the subcutaneous lesions. The third group received 500 r. of whole body x-radiation 20 hours prior to inoculation. The fourth group received 2.5 mg. of cortisone acetate in 0.1 cc. of saline solution intraperitoneally, 1½ hours before inoculation. The dose was repeated after 1 week. The fifth group received both cortisone and x-radiation in dosages as detailed above. All surviving animals were sacrificed after 3 weeks and evidence of disseminated disease sought by cultures and histologic sections.

The control mice developed subcutaneous lesions which ulcerated and regressed as usual. When they were sacrificed, all appeared healthy and the lesions had almost completely healed. There was no evidence of dissemination except for a positive culture from the liver of one animal. The lesions which had been traumatized showed ulceration, healed more slowly, and a few yeast cells still were present in the healing abscesses. Granulomatous inflammation with multinucleated giant cells was more conspicuous than usual around the abscesses. There was, however, no dissemination of infection.

The appearance of the lesions was the same in the irradiated group as in the controls, but healing was a little slower. Two of the animals that died during the experiment showed impaired inflammatory response in the subcutis and widely disseminated lesions. Cultures of the lungs of one of the four remaining mice taken at the termination of the experiment revealed cryptococci.

Cortisone had a much more dramatic effect on the course of subcutaneous cryptococcal lesions. The appearance of palpable lesions was delayed 3 to 5 days after inoculation. The early lesions remained much smaller than those of the controls, but they grew progressively, and after 13 days were much larger than in the control mice. Only one lesion ulcerated despite the large size. Dissemination occurred in all animals, and three of them died before the end of the experiment. Sections of the subcutaneous lesions showed poor or absent inflammatory response, and absence of encapsulation. As noted in other experiments, the brain was the site of predilection and usually had the largest metastatic foci. The delayed appearance, small size of early lesions, and absence of ulceration could be related to the paucity or absence of polymorphonuclear leukocytes. The large size of late subcutaneous lesions was due to the rapid, unbridled proliferation of yeast cells.

The results with combined cortisone and x-radiation paralleled those with cortisone alone, except that all animals died by the 12th day. Disseminated disease was present in all animals examined, but

it was less prominent and widespread, with little involvement of brain, probably due to the early demise of the animals. Again, lack of inflammation was notable in the subcutaneous lesions (Fig. 12).

SUMMARY

Experimental cryptococcosis was produced by subcutaneous, intracerebral, and intraperitoneal inoculations using three strains of *Cryptococcus neoformans*. The subcutaneous lesions were characterized by early leukocytic response, abscess formation, encapsulation, healing, and only occasional instances of dissemination. Lesions of the brain were characterized by proliferation of the yeast cells to form large lesions with minimal inflammation and with universal dissemination. More inflammation was noted in the meninges, but here, too, the reaction was weak and irregular.

Intraperitoneal inoculations were attended by an intermediate degree of inflammatory response (usually most marked in the liver) and were also intermediate with respect to dissemination and lethality. The predilection for the brain in disseminated infection was thought to be directly related to the minimal inflammatory response in cerebral tissue.

The healing subcutaneous cryptococcal abscesses were not associated with active immunity against subsequent intracerebral challenge.

The inflammatory response to subcutaneous inoculation of *C. neoformans* was abolished by cortisone, and, to a lesser extent, by x-radiation. This was accompanied by an increased dissemination of the infection.

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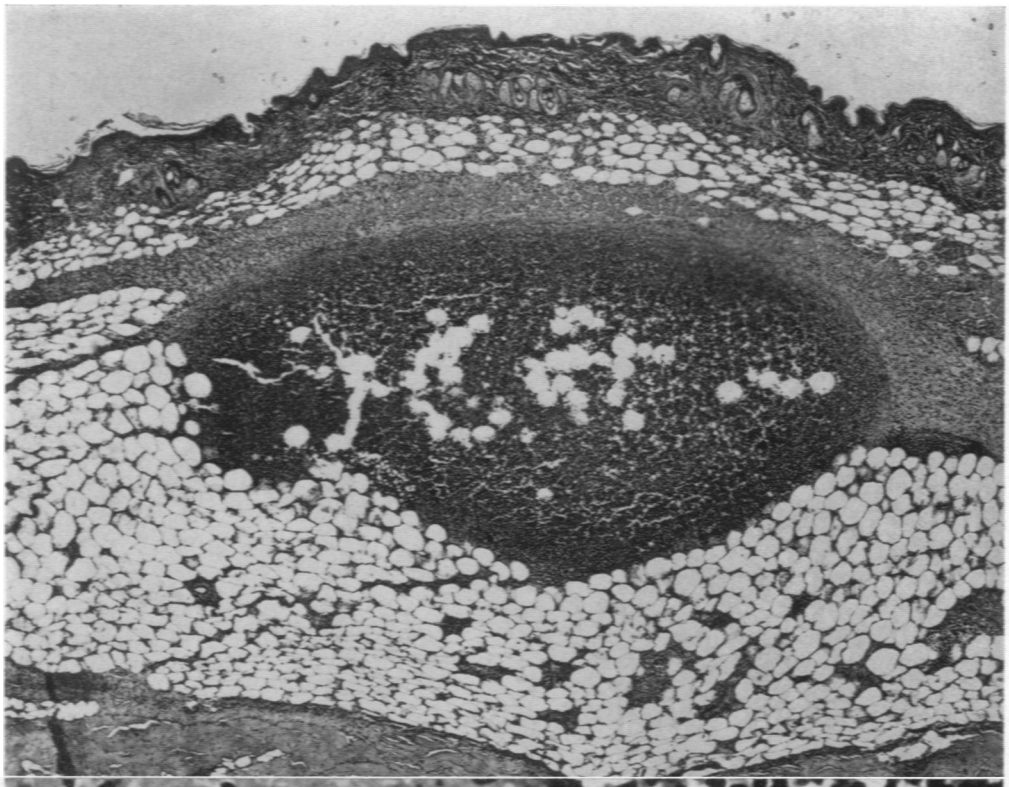
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Abscess in subcutis 2 days after inoculation of *Cryptococcus neoformans*, strain 1499.20. Hematoxylin and eosin stain. × 45.

FIG. 2. Cryptococcal cells in center of abscess, 3 days after inoculation. The faintly stained cells are polymorphonuclear leukocytes. Periodic acid-Schiff (PAS) stain. × 440.



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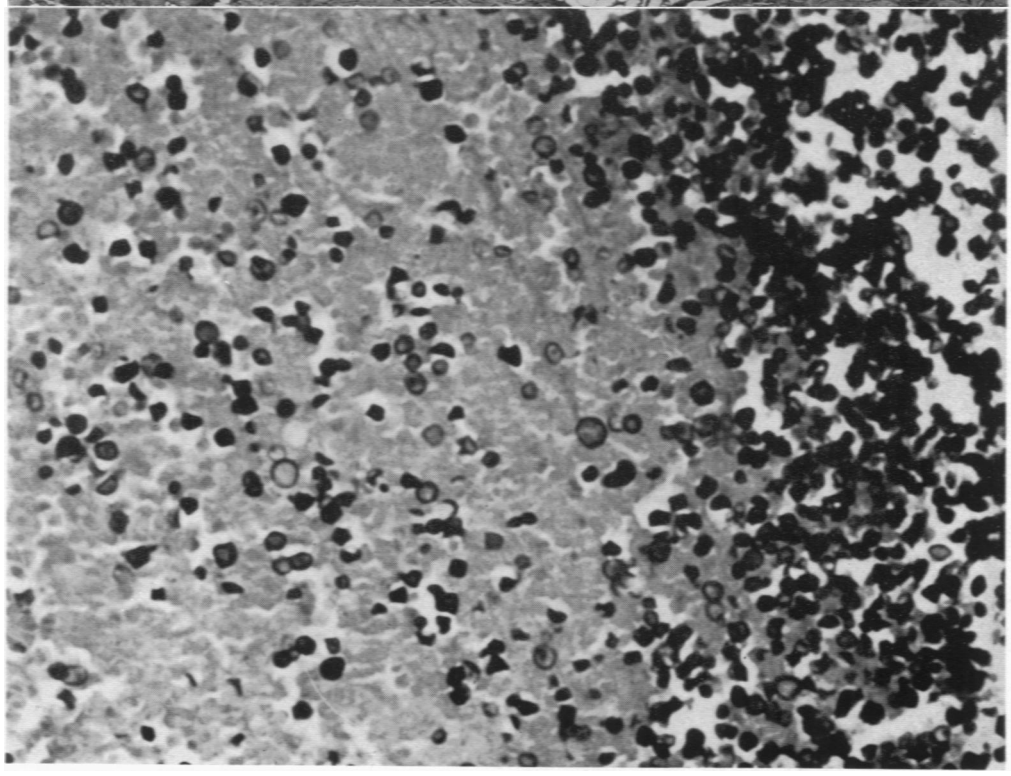

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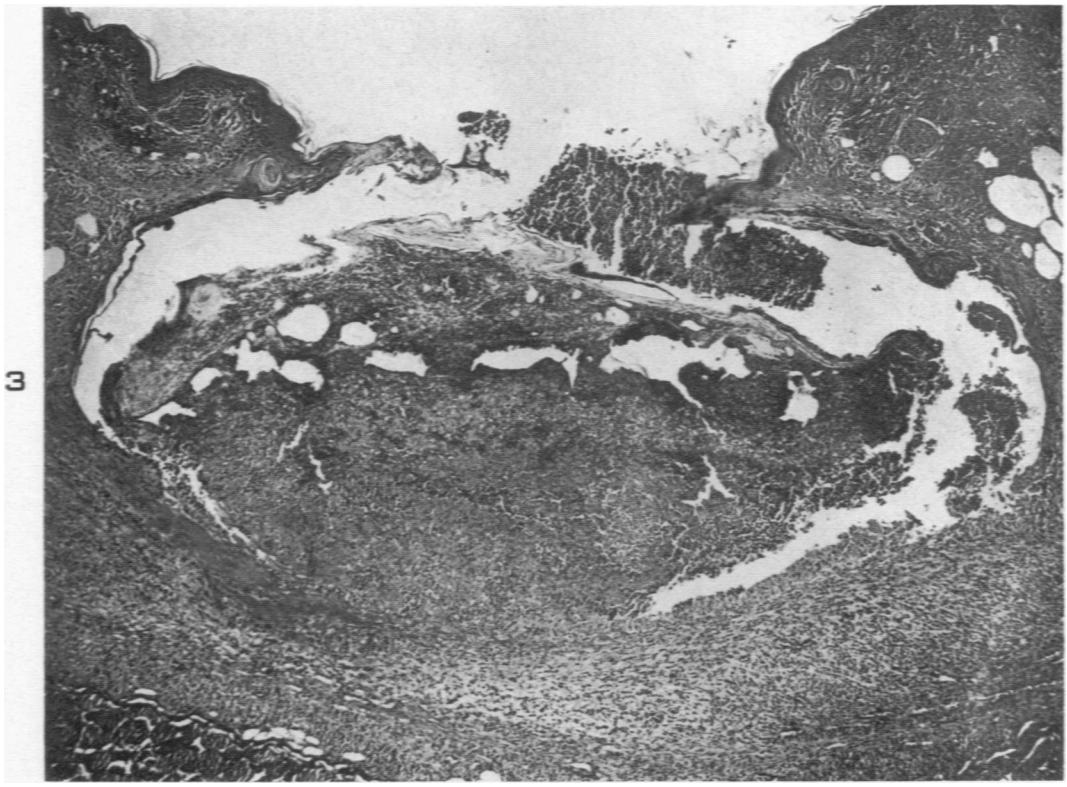
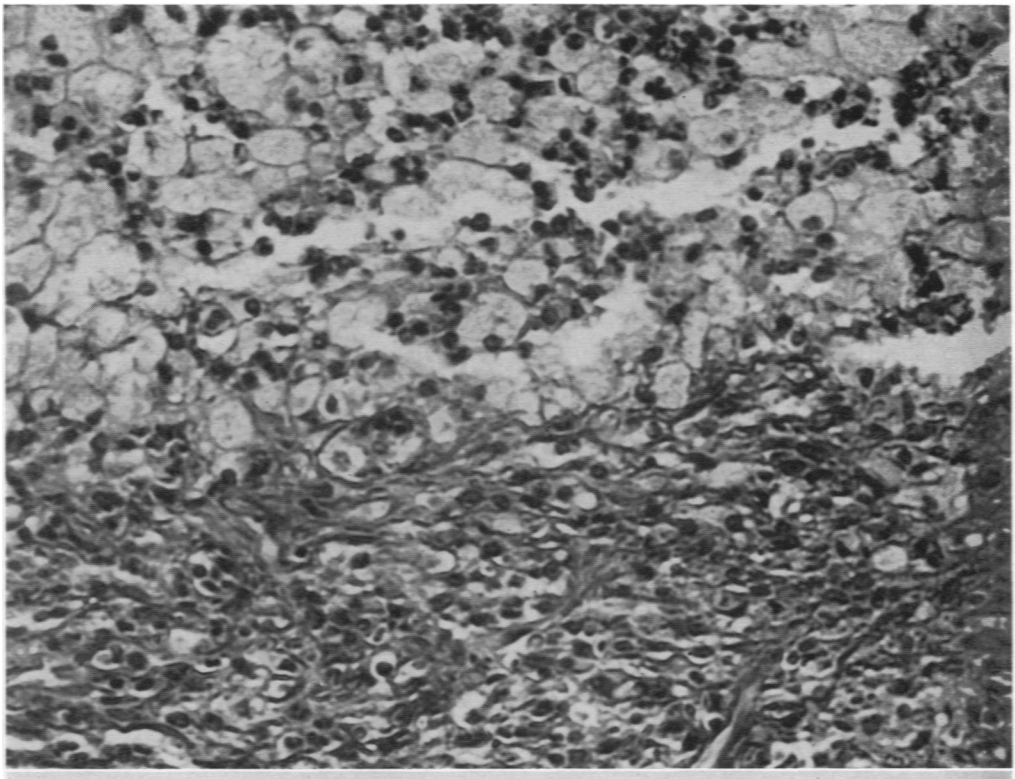


FIG. 3. Ulceration with separation of necrotic skin and contents of abscess, 7 days after inoculation. There is downward proliferation of the epidermis and walling off of the base of the abscess. Hematoxylin and eosin stain. × 61.

FIG. 4. Foamy macrophages and polymorphonuclear leukocytes in the center of the lesion (above) with surrounding wall of proliferated connective tissue infiltrated by lymphocytes and plasma cells (below), 20 days after inoculation. Hematoxylin and eosin stain. × 440.

FIG. 5. Fibrosis of dermis and subcutis, 41 days after inoculation. Hematoxylin and eosin stain. × 120.



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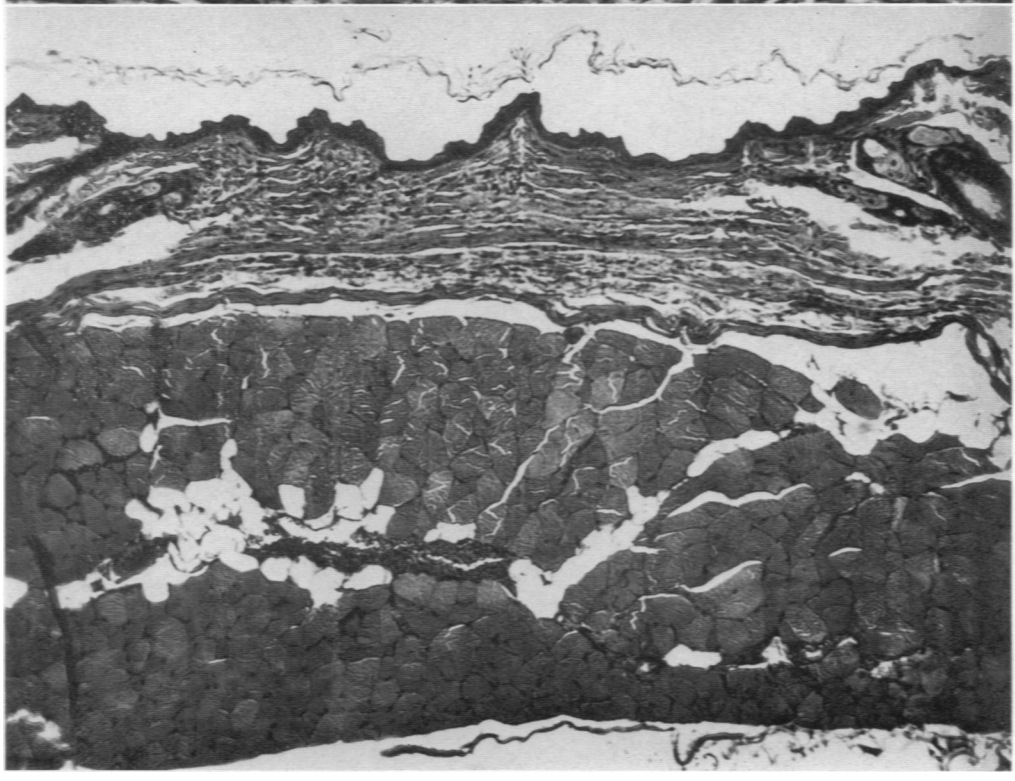

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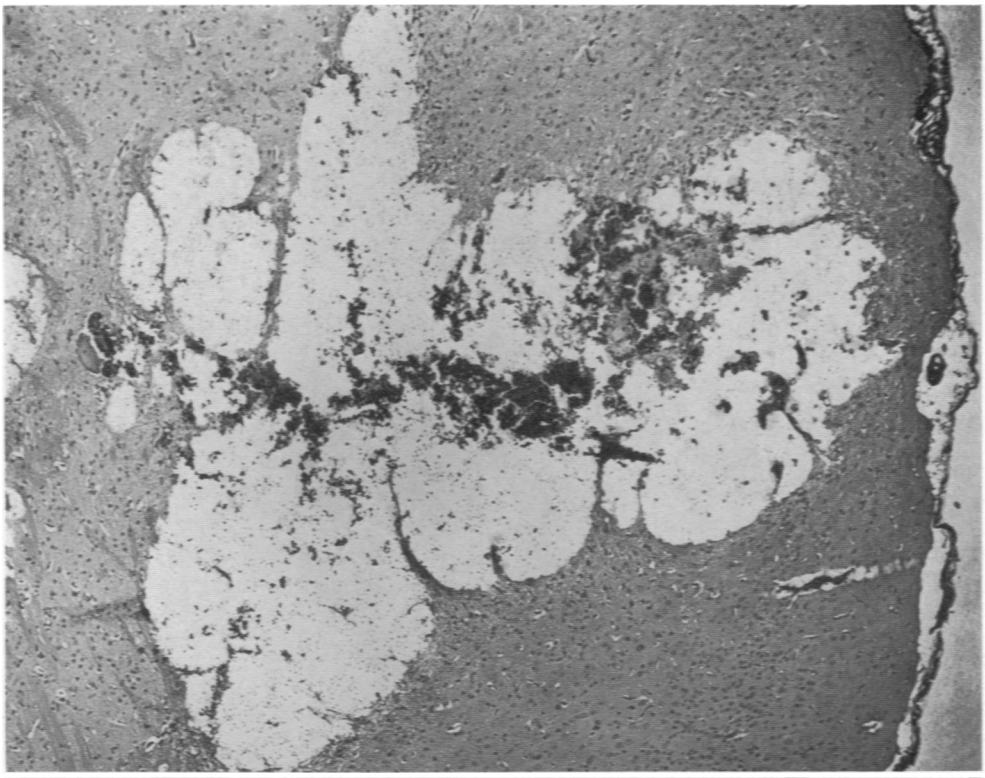
FIG. 6. Lesions of brain and meninges, 3 days after intracerebral inoculation. The clear areas contain many yeast cells (small faint dots), and fresh blood (dark material in center), but there is no inflammatory response. Hematoxylin and eosin stain. × 65.

FIG. 7. Edge of lesion of brain depicted in Figure 6. The darkly stained cryptococcal cells show innumerable buds and the clear areas probably represent soluble capsular polysaccharide. There is no inflammatory response. PAS stain. × 440.



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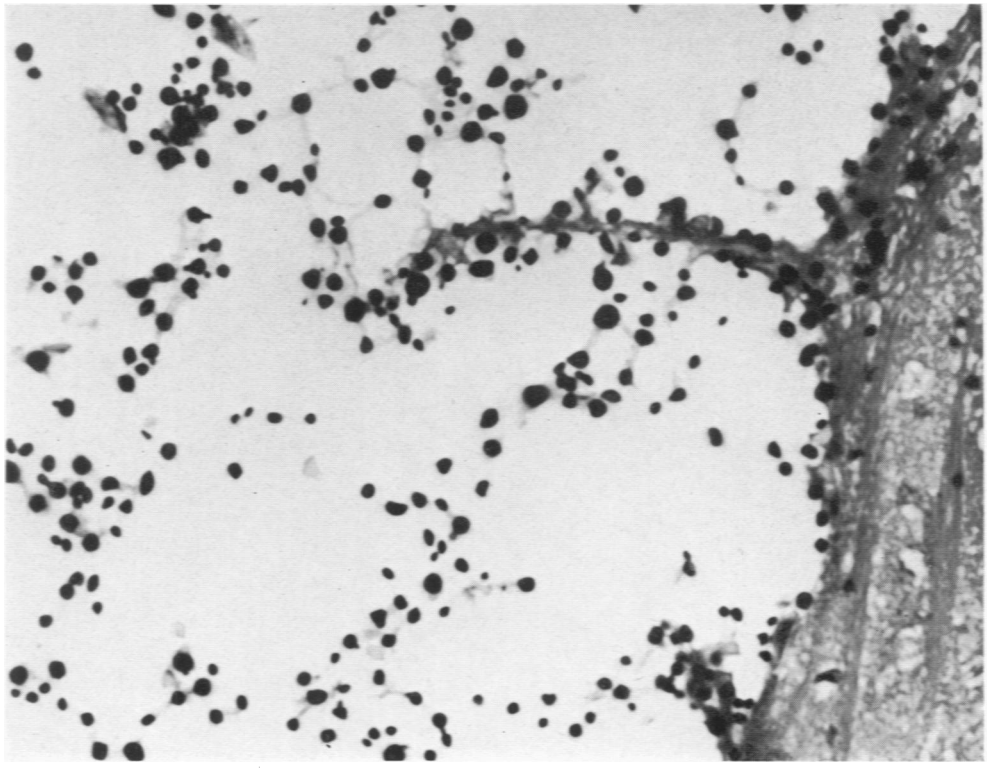

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FIG. 8. Edge of lesion of brain. 16 days after intracerebral inoculation. Many of the faintly stained cryptococci are within the cytoplasm of darkly stained phagocytes. There is no encapsulation of the lesion. Hematoxylin and eosin stain. × 440.

FIG. 9. Miliary abscess in liver, 3 days after intracerebral inoculation (same animal as that from which Figures 6 and 7 were taken). Yeast cells are few and faintly stained (arrow). Hematoxylin and eosin stain. × 440.

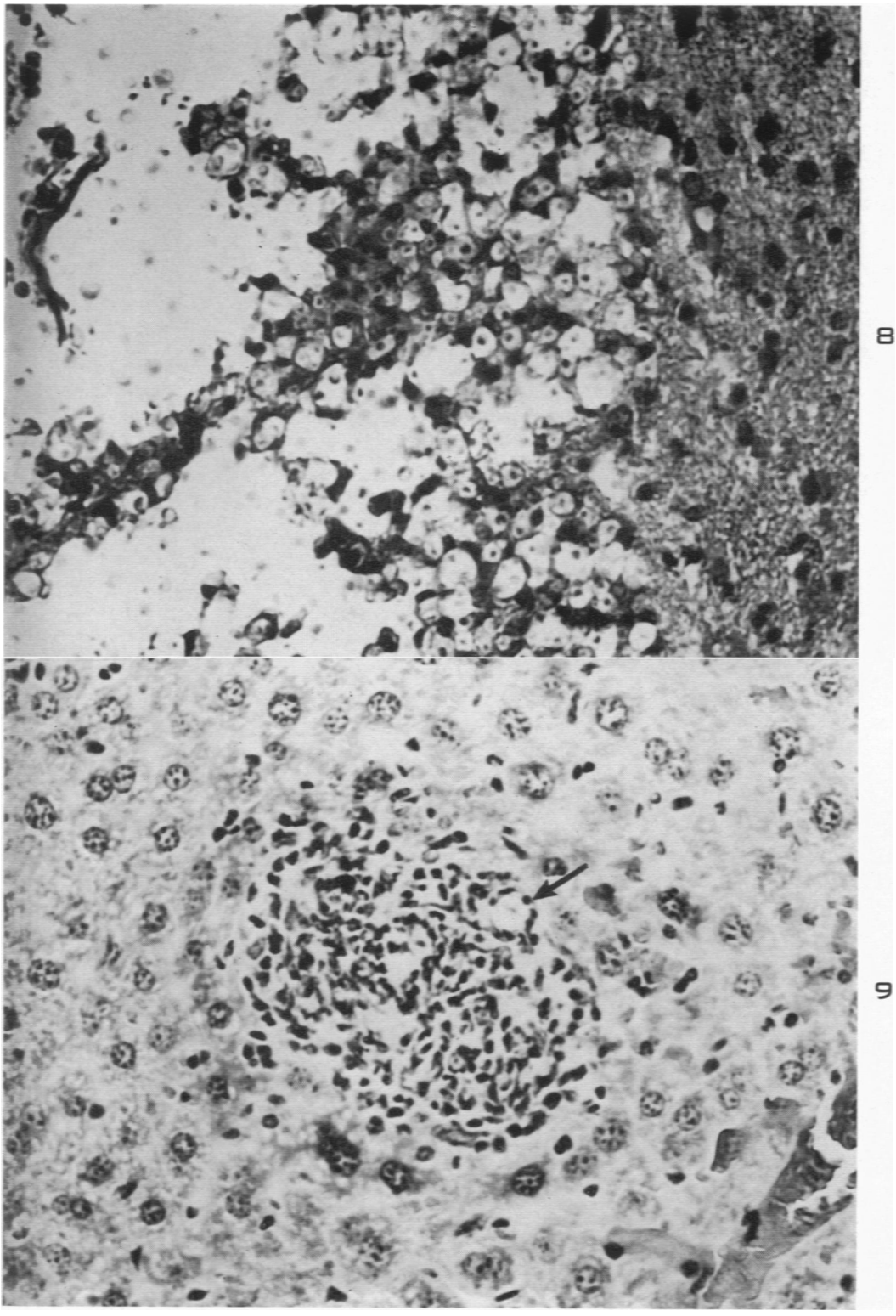

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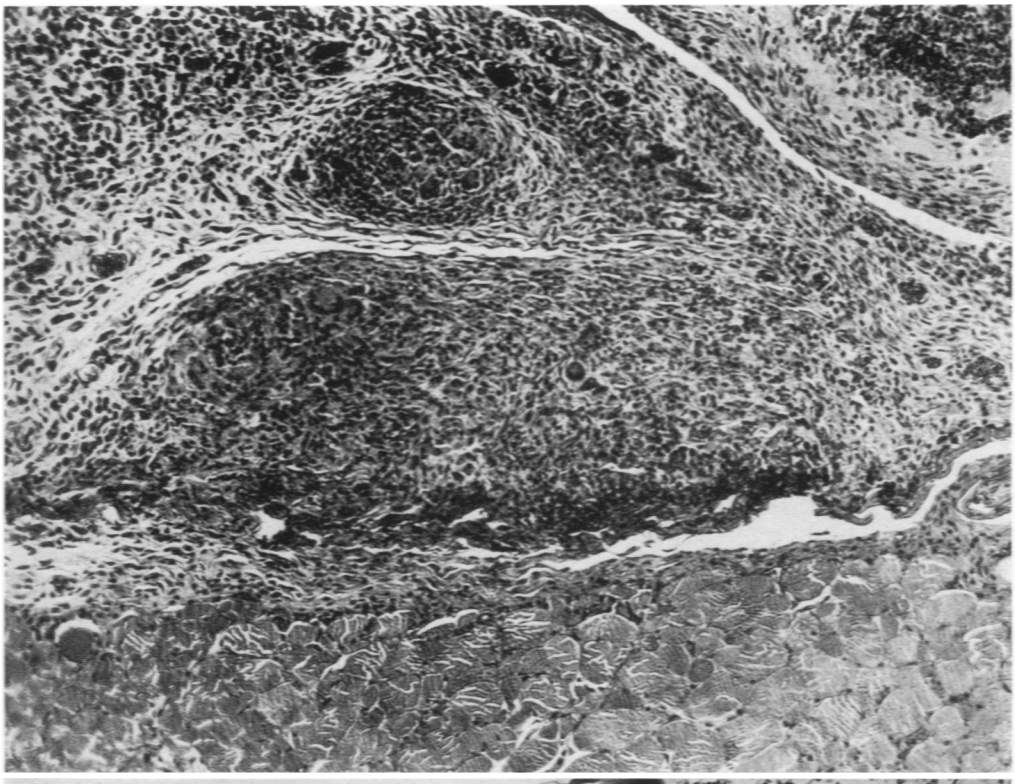
FIG. 10. Granulomatous reaction with numerous giant cells in abdominal wall, probably due to inadvertent intramuscular inoculation. Hematoxylin and eosin stain. × 120.

FIG. 11. Subcutaneous lesion 15 days after inoculation with *C. neoformans*, strain 1499.57. The lesion is large and poorly encapsulated, and contains innumerable darkly stained yeast cells with but few inflammatory cells. PAS stain. × 65.



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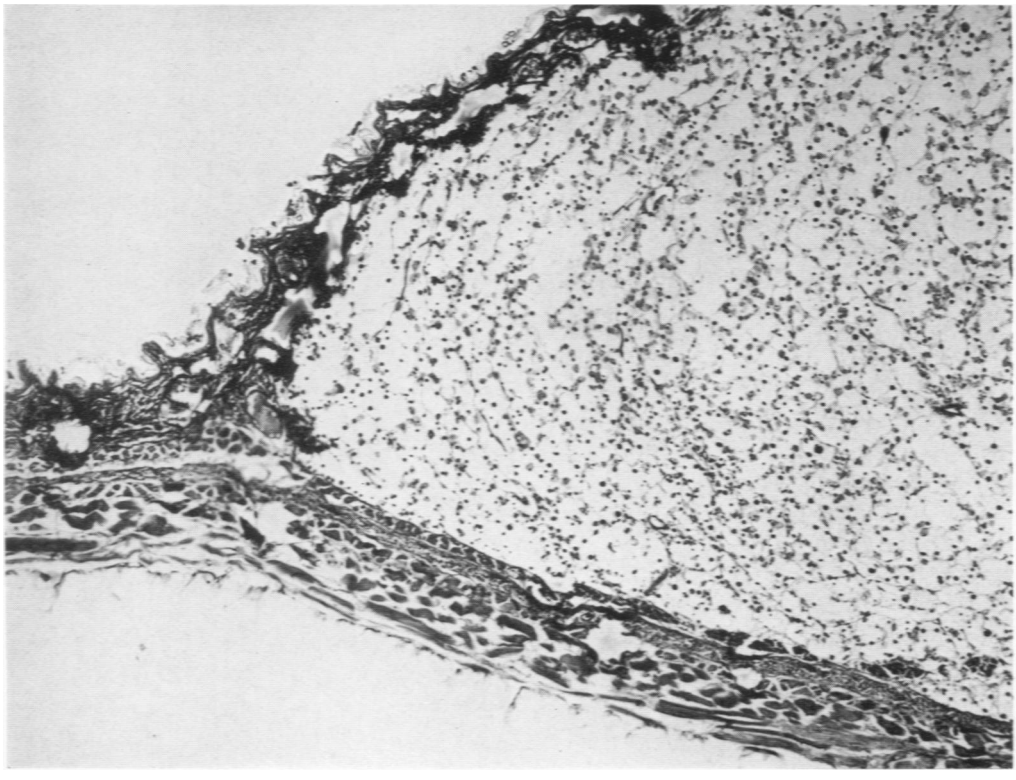

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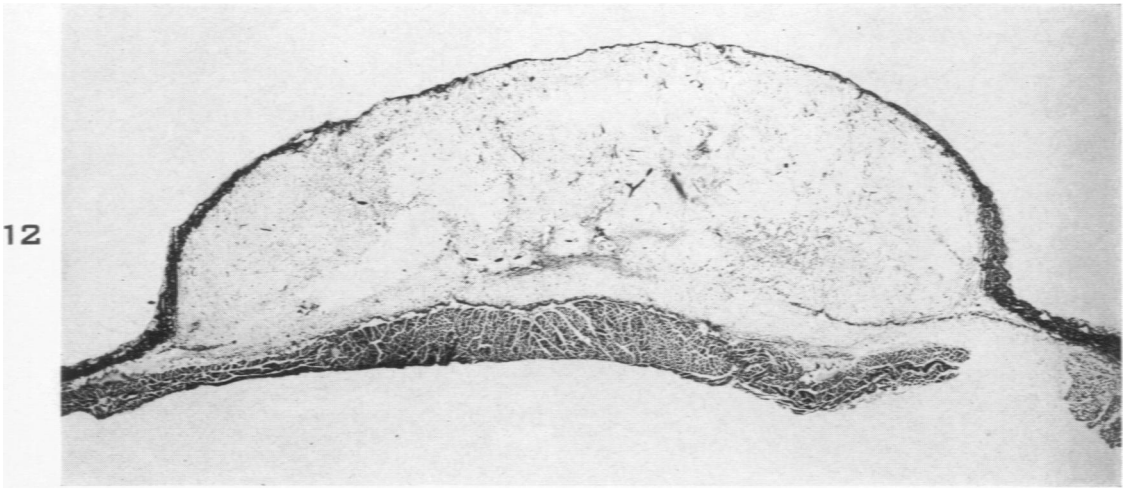
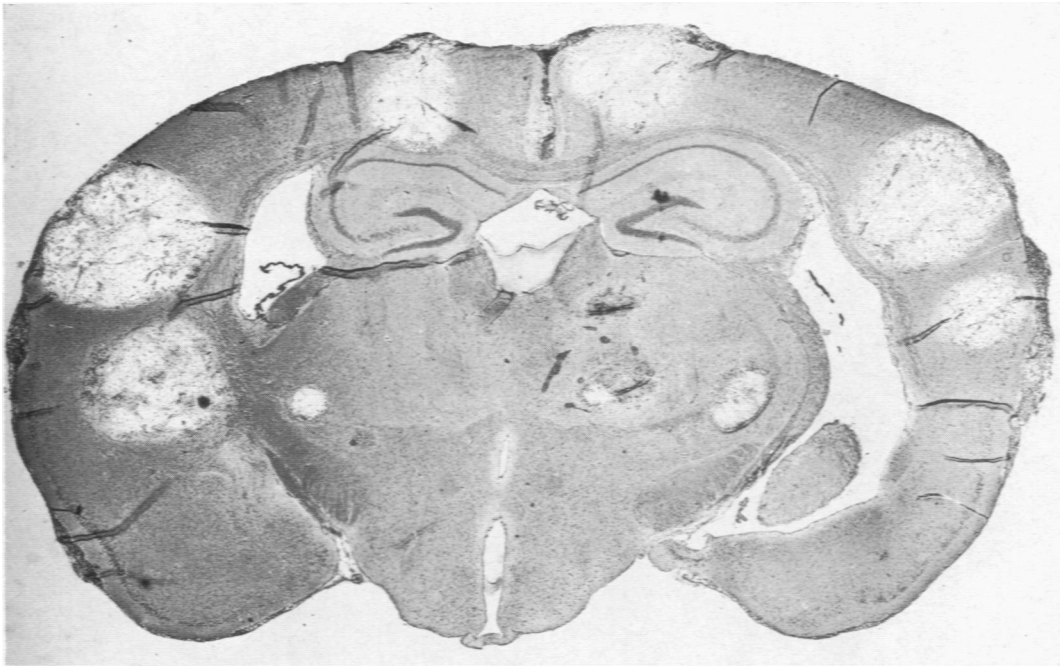


FIG. 12. Subcutaneous lesion, 12 days after inoculation of *C. neoformans*, strain 1499.20, in a mouse treated with cortisone and x-radiation. There is no inflammation or encapsulation, and no ulceration despite the large size of the lesion. Hematoxylin and eosin stain. × 15.

FIGS. 13 and 14. Severe involvement of brain, 29 days after intraperitoneal inoculation with *C. neoformans*, strain 1499.62. The lesions are pale in the section stained by hematoxylin and eosin (Fig. 13) because of the large, clear, capsular haloes and the minimal inflammatory response. They are dark in the section colored by the PAS stain (Fig. 14) because of the innumerable darkly stained yeast cells. × 18.



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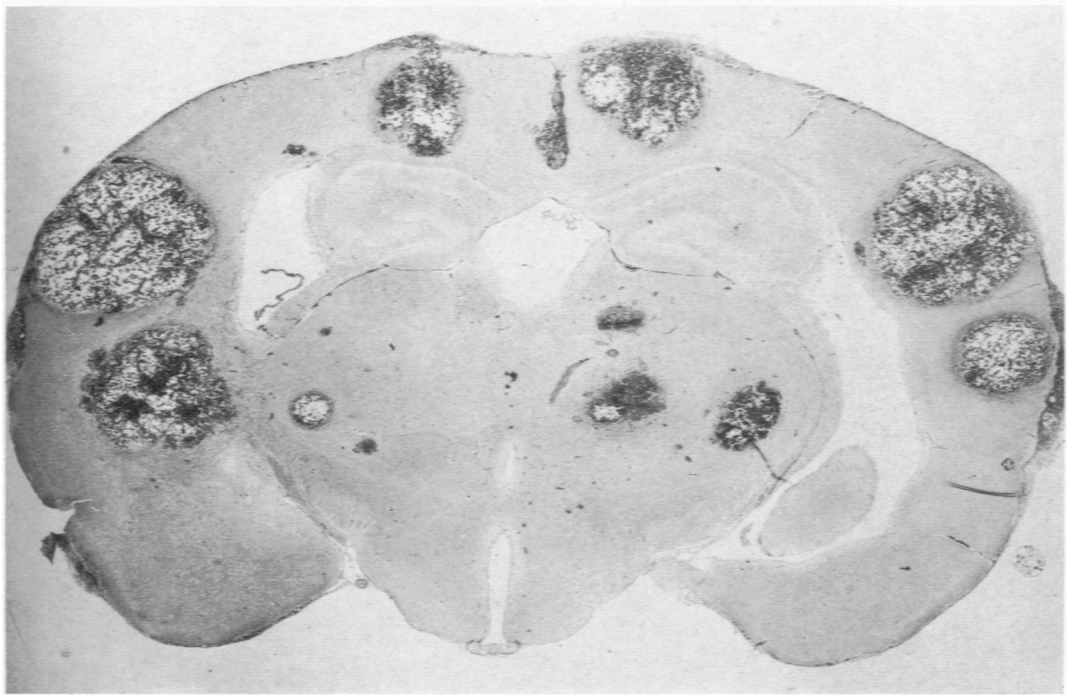

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