HISTOLOGY OF EXPERIMENTAL MURINE CRYPTOCOCCOSIS *

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This report is an outgrowth of a study in which strains of *Cryptococcus neoformans* were isolated from a number of pigeon nests in the Greater Cincinnati (Ohio) area.[1] The investigation followed that of Emmons[2] who reported the isolation of Cryptococcus from pigeon nests in Washington, D.C. In our own survey,[1] material was collected from 107 pigeon nests in order to document the concept that this organism had a wide geographic distribution. In 41 of the nests Cryptococcus was demonstrated by animal inoculation and culture. In the present paper we will describe the histologic changes produced in experimental animals after inoculation with material containing Cryptococci.

MATERIAL AND METHODS

The bird droppings procured from the pigeon nests were suspended in a mixture of antibiotic agents and saline solution (2,000 units each of penicillin and streptomycin per ml.) and introduced intraperitoneally into mice. The organs of each animal which died spontaneously or was sacrificed after 5 weeks were used for cultures on Sabouraud's glucose agar at 25° C. As indicated above, strains of *Cryptococcus neoformans* were isolated from 41 nests.

Male mice were inoculated with 3-day-old cultures of these Cryptococcus strains intraperitoneally (0.5 ml.) and intracerebrally (0.05 ml.). The suspension was uniformly heavy, but no actual cell counts were made. The animals began to die 6 days after inoculation, and surviving animals were sacrificed after approximately 5 weeks. Cultures were made from the liver and spleen of each animal. Specimens of liver, spleen, brain, lung, heart, and pancreas were fixed in 10 per cent formalin solution and sectioned in paraffin. In addition to this material, sections of these organs from the 367 mice which had been inoculated directly with pigeon dropping suspension were also studied. The sections were stained with hematoxylin and eosin, mucicarmine,[3] and by Grocott's[4] and Gridley's methods.[5]

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Observations

A great variation was observed in morbidity and mortality of experimental animals, and no clear-cut pattern of response was established. More animals died following intracerebral than after intraperitoneal inoculation, and the organisms were more often demonstrable in the brain and lung in the former group. At necropsy some animals exhibited hepatosplenomegaly, but this feature was not indicative of

Table I

Description of Source of Tissue Used in This Study

Material injected	Route	Number of animals		
		Injected	Dead (spontaneously)	Sacrificed
Suspension from pigeon nests	Intraperitoneal	367	162	205
Culture suspension	Intracerebral	46	29	17
Culture suspension	Intraperitoneal	46	11	35

infection with the Cryptococcus, since the cultures from some livers and spleens were negative. Furthermore, the yeast cells were not always demonstrated in sections of these organs even when inflammatory lesions were present, or when Cryptococcus appeared in the cultures. For convenience in this histologic analysis, the lesions as encountered in each organ will be enumerated. Since our purpose is to describe the lesions, there appears to be no need to indicate which were produced by direct inoculation of nest material or which by inoculation of culture suspension. In the direct suspension group, the lung, liver, spleen, and retroperitoneal tissue were more often the seat of lesions whereas in the animals inoculated with culture suspension there was also a marked involvement of brain, leptomeninges and fewer lesions in the pancreas.

The colonies of Cryptococci in culture showed variation in color and texture. This was the case even in instances in which they were isolated from the same pigeon nest. In tissue sections, however, the yeast cells were structurally indistinguishable from each other. Their average diameter without the capsules was 6.5 μ and with the capsule 17 μ. Most yeast cells were round or only slightly ovoid and had a variable width of capsule surrounding each organism. Budding was occasionally seen. In general, the appearance of the yeast cells was identical with that seen in human torulosis.[6]

Examination of Tissue Sections

Lungs. These consistently revealed the most severe inflammatory responses to the yeast cells. One type of lesion was characterized by diffuse inflammation and another by localized granuloma formation consisting for the most part of histiocytic elements, accompanied by occasional epithelioid cells and rare giant cells of the Langhans type. The presence of organisms in the alveoli or in the interstitial tissue without any noticeable reaction was also observed. The large number of yeast cells noted in some instances was striking, and the tissue alterations were thought to be the result of simple mechanical pressure rather than destructive inflammation. The thick capsules of the Cryptococci caused marked distention of alveoli with rupture of the interalveolar septa and pressure atrophy of adjacent tissues. In certain instances this culminated in the production of discrete areas of necrosis.

Diffuse inflammatory reaction was manifested by hyperemia and the accumulation of neutrophils in the lumens of the affected capillaries. Congestion was occasionally quite pronounced and was associated with alveolar edema. The edema fluid contained varying numbers of leukocytes. The exudate ranged from a scanty infiltration with widely distributed leukocytes to cellular aggregates gathered in perivascular and peribronchial tissues. Occasionally there were purulent bronchitis, peribronchitis, bronchopneumonia, and multiple minute abscesses. In addition to neutrophils there was almost always an intermixture of mononuclear cells (Fig. 1). Microscopic foci of atelectasis were induced by the widening of interalveolar septa containing yeast cells and inflammatory elements. The desquamation of alveolar lining cells mimicked a cellular inflammatory exudate, especially if with these elements there were also yeast cells.

The other common form of response was characterized by a diffuse or discrete granulomatous reaction in the pulmonary parenchyma. Yeast cells were readily recognized in the granulomas which were initially characterized by focal histiocytic proliferation. The yeast cells either remained free or were engulfed by macrophages. Present also were multinucleated foreign body giant cells (Fig. 2). The granulomatous lesions appeared around the blood vessels and bronchi. Necrosis in the center of the lesions was not observed (Fig. 3). The granulomas were characteristically discrete but exhibited a peripheral zone containing lymphocytes and scattered histiocytes. Neither fibrous encapsulation nor major scarring were encountered during the period of

investigation. Rarely, the lungs were so heavily studded with granulomas, and the component histiocytes so closely resembled epithelioid cells that the pattern of sarcoidosis was simulated. A disproportionately large number of yeast cells appeared in those lungs which showed minimal or no inflammatory response. On the other hand, the number of Cryptococci in the granulomas was always small, and in a few cases no organisms were demonstrable microscopically at all.

Liver. The incidence of hepatic lesions was exceeded only by that of the lung and the brain. As in the lung, there was a range of alteration from the presence of organisms without related inflammation to the development of granulomas. The encapsulated organisms singly or in clusters often caused pressure atrophy of adjacent tissue or distortion of liver architecture. The large, almost empty cavities thus produced, unaccompanied by an inflammatory reaction, caused a "Swiss cheese"-like appearance. In the presence of smaller clusters of Cryptococci, contiguous liver cells were flattened and stretched about the mass of yeast cells, simulating thin capsule formation.

In addition to the features described, the liver exhibited congestion and an acute or chronic inflammatory exudate, often with perivascular location. The cellular reaction, when restricted to the vicinity of a vessel, maintained a peculiar circumscribed appearance which resembled a tubercle and, indeed, was occasionally composed of epithelioid and giant cells. Not all of the lesions contained fungi, and when yeast forms were present, the exudate was often quite sparse. Granulomas were observed more often following inoculation of culture suspension intraperitoneally than following inoculation of pigeon dung suspension. The phagocytic activity of the macrophages was essentially identical with that observed in the lung.

Spleen. Clear cut splenic lesions were observed only in animals inoculated with culture suspension. The introduction of the pigeon nest preparation resulted in the appearance of a few solitary Cryptococci in the organ without obvious reaction apart from hyperemia. With the culture suspension the response varied from hyperemia to granuloma formation. In a few cases there were circumscribed foci of necrosis in which no yeast cells were recognized. Occasionally, the spleen was so heavily studded with yeast cells that the architecture was obscured, and though the pattern was that of an overwhelming infection, there was almost no inflammatory reaction. In those instances in which inflammatory cell infiltration and granuloma formation occurred, epithelioid cells and giant cells were evident. They appeared, in the main, in the splenic hilar area and extended to adjacent peritoneal tissue (Fig. 4).

Pancreas. This organ revealed less alteration than the liver or spleen. The lesion consisted of either a diffuse inflammatory cellular process or a perivascular microgranuloma formation. In addition, groups of Cryptococci were collected in tissue spaces unaccompanied by inflammatory response. The neutrophil or mononuclear infiltration was often localized to the surface of the pancreas or appeared in the peripancreatic tissues. In a few cases, small foci of necrosis were observed within the parenchyma.

Heart. The heart exhibited no inflammatory reaction. Scattered solitary fungi or small clusters of yeast cells appeared in the interstitial tissue or were embedded directly in muscle fibers. The presence of the large organisms caused pressure atrophy and was manifested in tissue sections by the appearance of small, sharply outlined holes (Fig. 5).

Meninges and Brain. With the exception of the lung, the meninges and the brain were the most frequent sites of involvement. The fungi were usually easily identified with the mucicarmine stain and were found either clustered focally or distributed fairly evenly in the leptomeninges (Fig. 6). The inflammatory response in the meninges was characterized in some cases by hyperemia or edema and in others by a cellular infiltration with polymorphonuclear leukocytes, lymphocytes, or monocytes. An exudate consisting entirely of lymphocytes was more often encountered (Fig. 7). The fungi were found lying free or in macrophages. The tissue response in some cases was granulomatous, and in others was of "meningothelial" nature. The "meningothelial" reaction was characterized by diffusely distributed lymphocytes producing a very cellular appearance in the subarachnoid region. In such cases the quantity of demonstrable fungi was small. In those with granulomatous lesions, neutrophils and lymphocytes were scanty.

In each instance the brain contained numerous fungi, but the cellular response to these was variable. The presence of free fungi in the lumens of blood vessels was reasonable indication that the brain lesions were of embolic nature (Fig. 8). Conglomerate masses of fungi were found in both the gray and white brain substance. Capsule development was often so pronounced that large holes were created with resemblance to "Swiss cheese" (Fig. 9). In these cysts fungi were quite obvious even without special stains. The multiplication of yeast cells created pressure effects on brain tissue which were rarely associated with cellular exudation. However, this was not always the case, and in many sections discrete round cell collections or nodular microglial reactions were observed. In some instances direct extension from the meninges was associated with lesions in the cortex along the perforating vessels. This form of propagation was particularly well demonstrated if the

plane of section happened to be in the axis of the vessel. One could then observe a perivascular cellular exudate consisting predominantly of either neutrophils or lymphocytes accompanied by smaller numbers of histiocytes. Small leukocytic conglomerations resembling microabscesses were also observed. The yeast cells were easily recognized in the perivascular spaces, where they appeared singly or in aggregates. The latter often led to cyst formation. In sections stained with hematoxylin and eosin, the paradoxical pattern of empty holes crossed by denuded vessels was noted. Mucicarmine stains, however, always demonstrated the presence of fungi in these cavities.

Discussion

The cellular response to *Cryptococcus neoformans* is nonspecific, variable and pleomorphic. Much of the variation probably reflects the histologic nature of the organ concerned. One never can be sure, however, whether the reaction in a given period will be of exudative or granulomatous character. Although the yeast cells can be demonstrated readily in the majority of cases, isolated lesions may be recognized when the organisms are not detected.

The pathologic lesions of experimental cryptococcosis have been analyzed by Levine, Zimmerman and Scorza,[7] who have provided a detailed description of the histologic features, especially in the skin and brain. The organisms used in their studies were isolated from human cases of cryptococcosis. While they established the existence of relatively prompt inflammatory reaction in certain organs and a delayed reaction in the brain, they also noted a relative immunity of striated muscle in spite of heavy inflammation in adjacent tissues. This to some degree parallels our own observations in the myocardium. Littman and Zimmerman[6] in an admirable monograph discussed the problem of cryptococcosis in detail from both the laboratory and clinical aspects. However, they did not discuss the histologic lesions in experimentally infected animals very extensively.

Summary

The histologic lesions in experimental animals inoculated with *Cryptococcus neoformans* have been described. The strains of Cryptococcus used in this experiment were isolated from pigeon nests in the Cincinnati area. Brain and lungs were the most frequent sites of lesions. The histologic pattern was found to vary from organ to organ even in the same animal. The heart muscle failed to respond with inflammation to the invasion by yeast cells.

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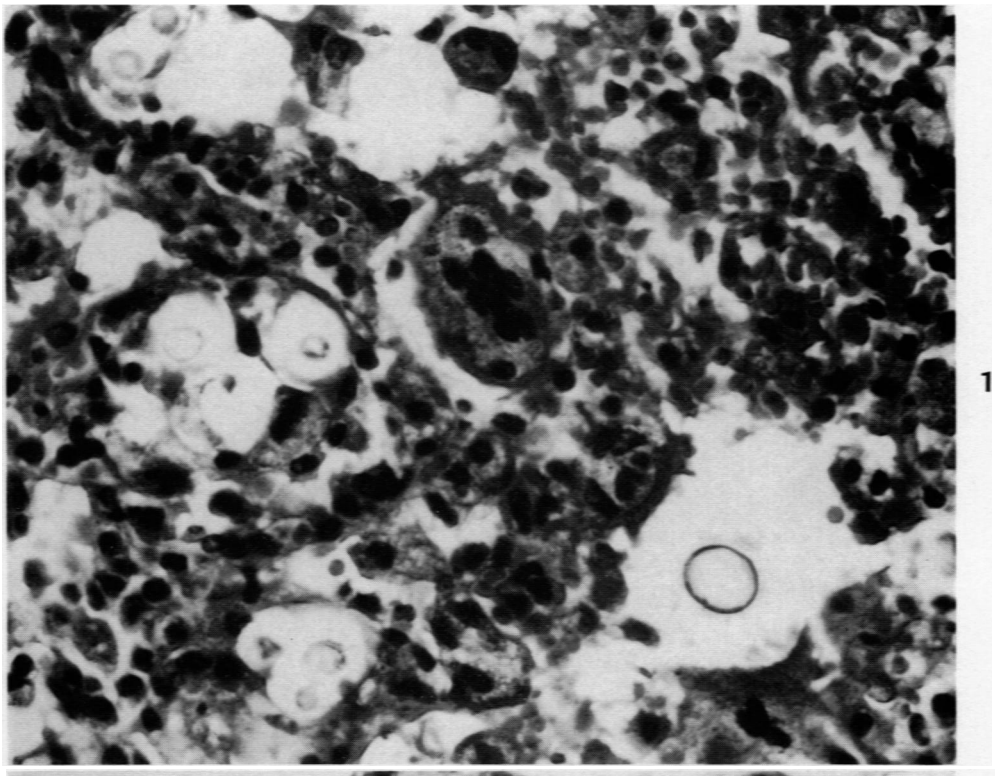
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Pneumonitis following inoculation with Cryptococcus (strain 38). Some yeast cells lie within the cytoplasm of macrophages. Mouse, spontaneous death on seventh day. Hematoxylin and eosin stain. × 500.

FIG. 2. Inflammatory giant cell response in lung of mouse inoculated intraperitoneally with Cryptococcus (strain 38). Hematoxylin and eosin stain. × 500.



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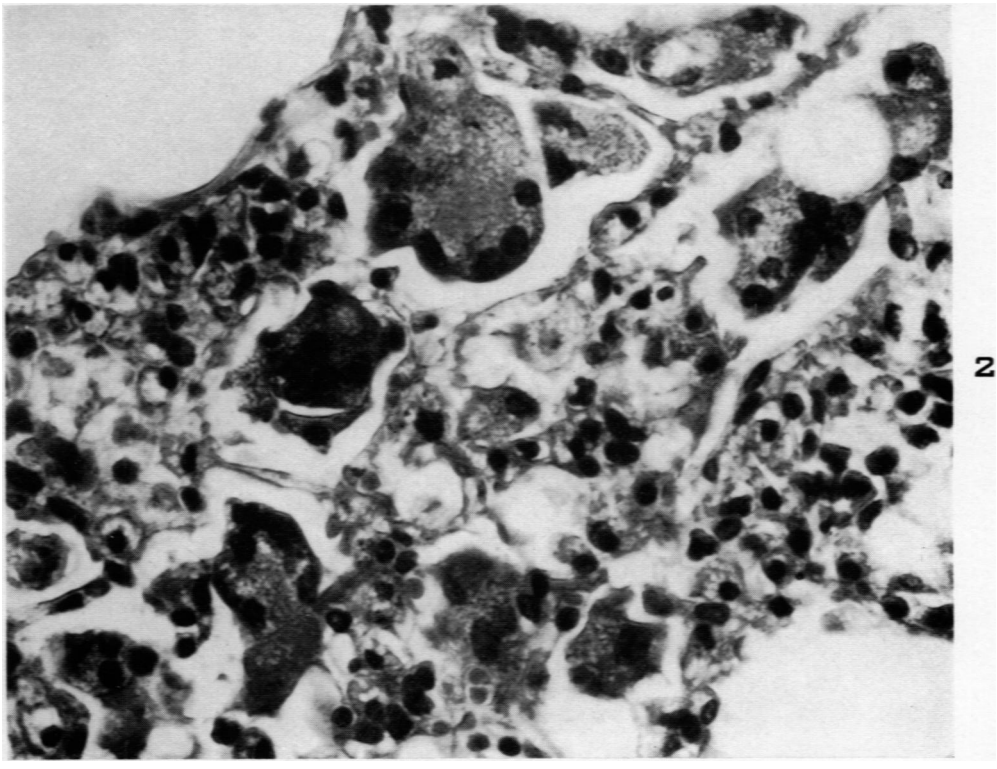

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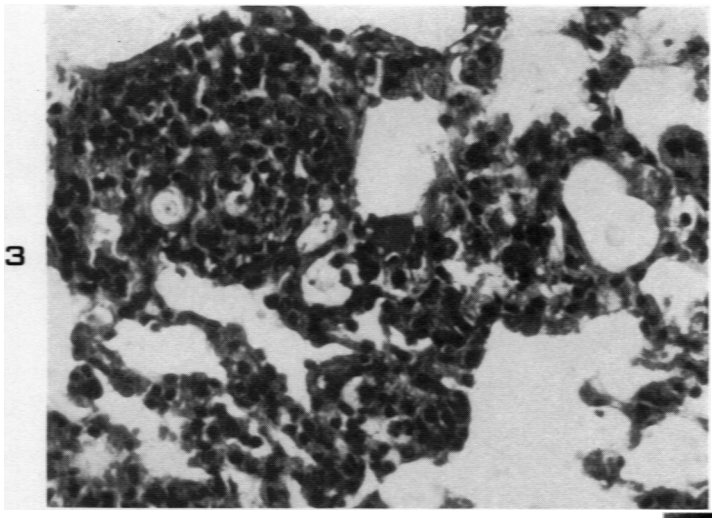
FIG. 3. Micrograniulomatous lesion in mouse lung, obviously caused by Cryptococcus (strain 69). Inoculation with a suspension from a pigeon nest. Hematoxylin and eosin stain. × 250.

FIG. 4. The splenic hilus consistently shows inflammation after intraperitoneal inoculation. (strain 52.) Hematoxylin and eosin stain. × 250.

FIG. 5. Heart of a mouse inoculated intraperitoneally, death on 23rd day (strain 9). The yeast cells are lodged in heart muscle and interstitial tissue without manifest cellular reaction. Hematoxylin and eosin stain. × 500.

FIG. 6. Diffuse distribution of yeast cells in meninges 30 days after intraperitoneal inoculation with Cryptococcus (strain 48). Mucicarmine stain. × 500.



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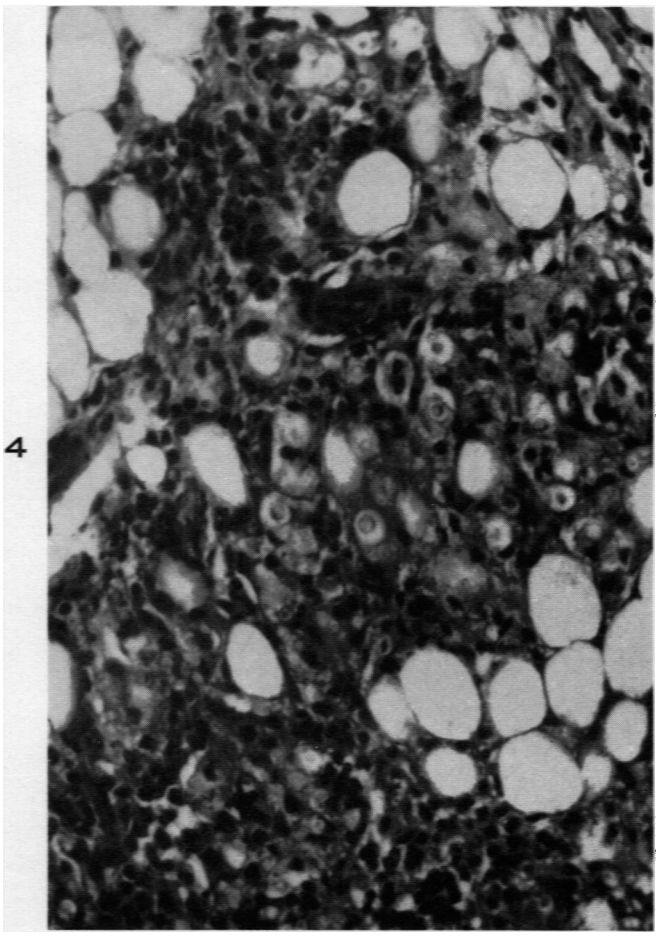

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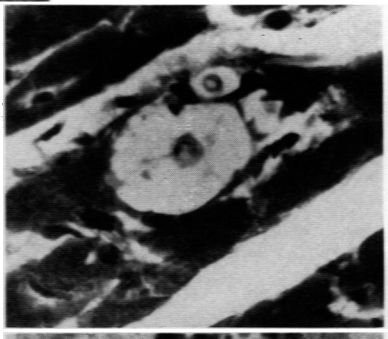

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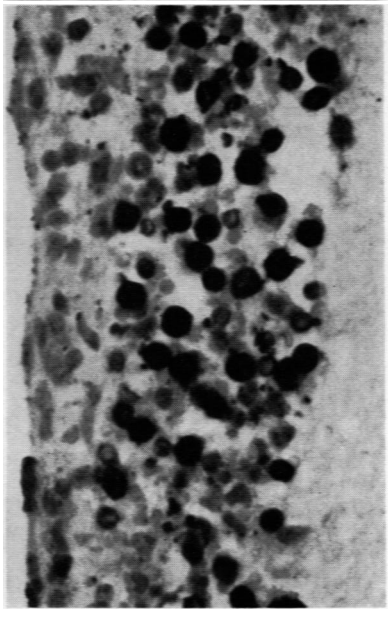

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FIG. 7. Cryptococcal meningitis with exudate dipping into a sulcus, 9 days after intracerebral inoculation (strain 51). Hematoxylin and eosin stain. × 250.

FIG. 8. Cryptococcus yeast cell "in transit" in a pulmonary vessel, 30 days after intracerebral inoculation (strain 54). Hematoxylin and eosin stain. × 1,000.

FIG. 9. Aggregation of Cryptococci in subcortical cerebral area without reaction in the immediate vicinity. "Swiss cheese" appearance. In contrast, note perivascular encephalitis in the same field (without organisms). Mouse, 80 days after intracerebral inoculation (strain 23). Hematoxylin and eosin stain. × 250.