

# EXPERIMENTAL INFECTION IN THE RABBIT WITH *TRICHOSPORON CAPITATUM*

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## ABSTRACT

GILBERT, WALTER R., JR. (Duke University Medical Center, Durham, N.C.) AND BERNARD F. FETTER. Experimental infection in the rabbit with *Trichosporon capitatum*. *J. Bacteriol.* **84**:961-966. 1962.—Fifteen New Zealand white rabbits were inoculated intravenously with a saline suspension of spores and hyphal fragments of *Trichosporon capitatum*. Nine of the animals succumbed spontaneously. The others were sacrificed at varying intervals. Autopsies revealed mycotic abscesses in the kidneys, which ruptured through into the tubules. The fungus also invaded the brain and heart, where it evoked a mixed reaction. The organism was recovered from the animals at the time of autopsy.

This report concerns the experimental production of a heretofore undescribed mycosis in the rabbit. The fungus was isolated from the sputum of a patient with pulmonary tuberculosis and mistaken for *Geotrichum candidum* during preliminary morphological examination. Because of its peculiar experimental pathogenicity, the organism was thoroughly studied morphologically as well as physiologically and identified as *Trichosporon capitatum* (Lodder and Kreger-Van Rij, 1952). To our knowledge, there has been no previous report of animal inoculation with this fungus.

## MATERIALS AND METHODS

The fungus was grown in approximately 100 ml of Sabouraud Liquid Medium (Difco) in 2-liter Erlenmeyer flasks for a period of 10 to 14 days at 37 C. This method of culture was chosen so that a large surface would be available for growth. At the end of the growth period, the flasks were shaken vigorously to fragment the chains of arthrospores and to disperse the blastospores. The material was filtered through eight layers of sterile cotton gauze. The resulting filtrate

was centrifuged at  $1,300 \times g$  for 15 min. The sediment was resuspended to prepare a 4% suspension of cells in sterile saline for injection.

Fifteen stock New Zealand white rabbits, weighing between 2 and 3 kg were used. During the study, they were fed equal amounts of Purina Rabbit Chow and oats. Each animal received a single injection of 1 ml of the 4% suspension of fungus into the marginal ear vein. The rabbits which did not die spontaneously were killed by intravenous injection of Nembutal at varying intervals following inoculation (Table 1). Complete autopsies were performed on 12 animals and cultures were made from the kidney, liver, and lung.

## RESULTS

Of the 15 animals, 9 died spontaneously: 3 on the 4th day, 4 on the 5th day, 1 on the 7th day, and 1 on the 18th day. The three animals which died on the 4th day were unavailable for autopsy. The results of autopsy and culture from the remaining 12 animals are reported in Table 1.

Striking changes were produced in all kidneys examined. Characteristically, mycotic abscesses formed predominantly in the cortex and sometimes in the medulla (Fig. 1). These frequently extended into the tubules (Fig. 2 and 3). In the kidney of the animal killed on the 16th day, we saw pyelonephritic scarring (Fig. 4). Hyphae were microscopically demonstrated in the kidneys of 11 animals. The presence of the organism in the kidneys of the remaining animal was determined by a positive culture as well as by the presence of an inflammatory reaction.

Hyphae were seen in the hearts of five animals. In some instances, the organisms were present without surrounding cellular infiltrate. In other instances, they were associated with either acute reaction (Fig. 5) and necrosis, or reaction of the large mononuclear cell. Occasionally, no organisms could be seen in areas of mononuclear reaction (Fig. 6).

TABLE 1. *Histological and cultural results*

Animal no., duration of disease	Heart	Lung	Brain	Kidney	Cultures		
					Lung	Liver	Kid- ney
27 Killed at 2 days	Fungi in areas of acute reaction and areas of no reaction. Gran- ulomata without fungi.	Negative	Fungi in areas of acute reaction and areas of no reaction.	Fungi in acute reaction in tubules.	+	+	+
28 Killed at 4 days	Fungi in areas of acute reaction and areas of granulomata.	Negative	Fungi in thrombosed meningeal vessels and in brain with little or no reaction.	Fungi in abscesses. Fungi in pelvis.	+	+	+
36 Died at 5 days	Negative	Several foci of large mononuclear cells. No fungi.	Fungi in areas of acute reaction and areas of no reaction.	Fungi in abscesses.	+	+	+
37 Died at 5 days	Fungi in areas of acute reaction. Occasional granulomata.	Fungi in thrombosed vessel.	Fungi in areas of acute reaction and areas of no reaction.	Fungi in abscesses.	-	+	+
38 Died at 5 days	Fungi in areas of acute reaction. Rare granuloma.	No fungi. One throm- bosed vessel.	Fungi in areas of acute reaction and areas of no reaction.	Fungi in abscesses.	+	+	+
39 Died at 5 days	Fungi in single focus of acute re- action.	One focus acute peri- vascular reaction.	Fungi in areas of acute reaction and in areas of no reaction.	Fungi in abscesses.	+	+	+
35 Died at 7 days	Occasional focus lymphocytes.	Fungi in thrombosed vessel.	Fungi in areas of acute reaction.	Fungi in abscesses.	+	+	+
30 Killed at 8 days	Negative	No fungi. Rare granu- loma.	Occasional glial scars.	Rare fungus in acute re- action in tubules.	+	+	+
41 Killed at 12 days	Negative	Negative	Fungi in areas of acute reaction and of chronic reaction.	Fungi in acute reaction in tubules.	+	+	+
33 Killed at 14 days	Negative	Negative	Fungi in areas of mixed cellular reaction.	Fungi in acute reaction in tubules.	+	-	+
34 Killed at 16 days	Negative	Negative	No fungi in rare small area of mixed reaction.	No fungi seen in acute reaction in tubules.	+	-	+
40 Died at 18 days	Large focus of mononuclear in- filtrate. No fungi.	Negative	Occasional glial scars.	Fungi in abscesses.	-	+	-

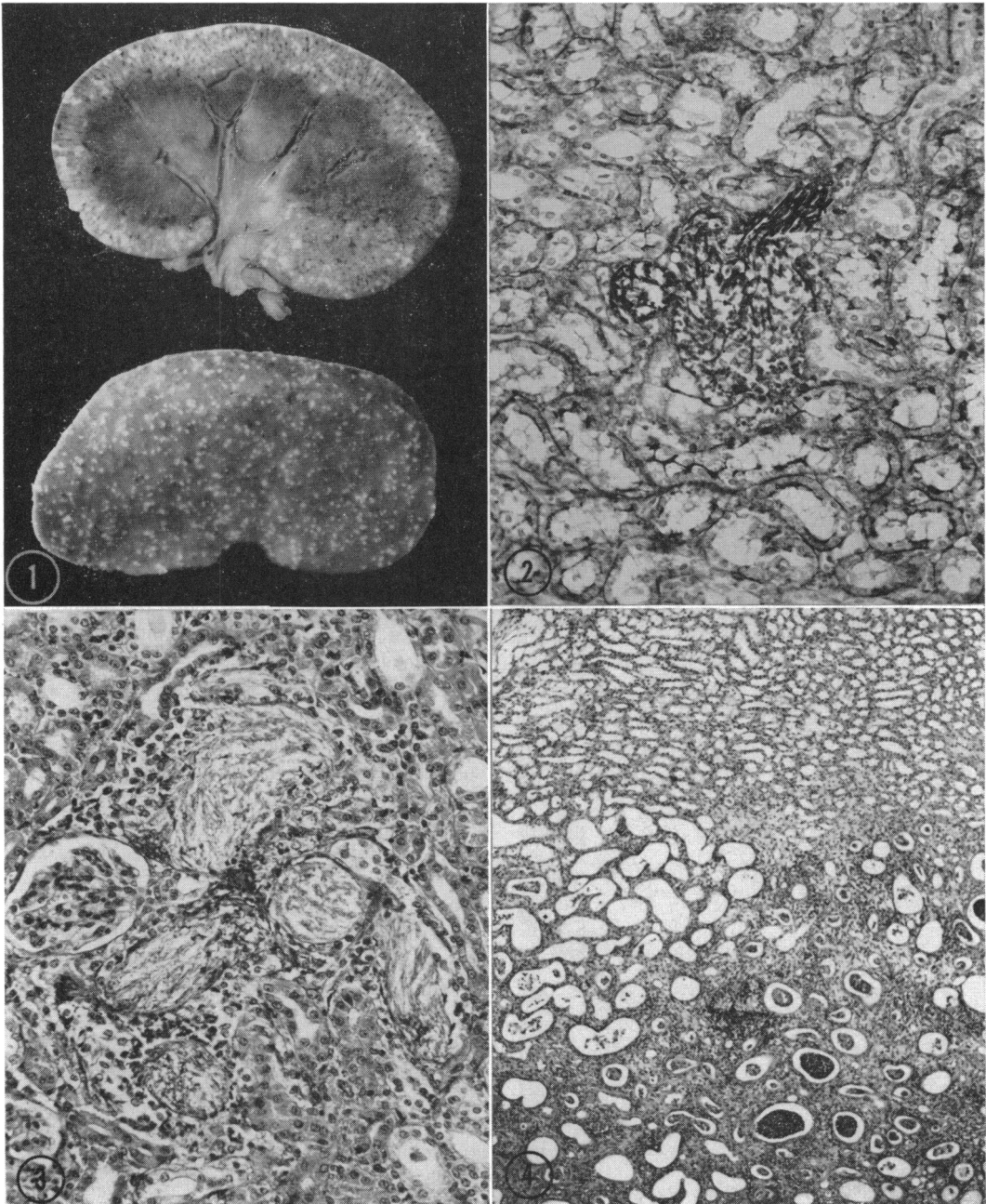


FIG. 1. Characteristic gross kidney lesion. The small white spots represent abscesses.

FIG. 2. Animal no. 27 killed on 2nd day. Numerous hyphae are in the kidney tubules with an associated neutrophilic exudate. Methenamine silver stain. 228 X.

FIG. 3. Animal no. 28 killed on the 4th day. In the kidney tubules, there are occasional viable hyphae. Much of the material in the lumina suggests necrotic hyphae. Hematoxylin and eosin stain. 228 X.

FIG. 4. Animal no. 24 killed on 16th day. In this photo, the margin of a pyelonephritic scar and adjacent normal medullary tissue can be seen. Hematoxylin and eosin stain. 67 X.

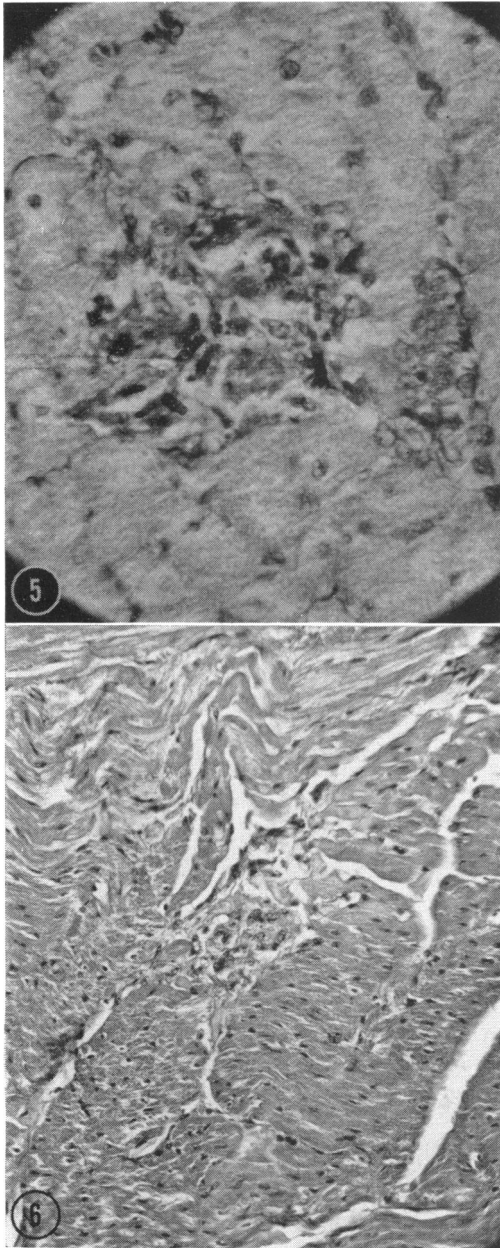


FIG. 5. Animal no. 27 killed on 2nd day. Numerous hyphae are seen in a small zone of neutrophilic infiltration in the heart. Methenamine silver stain. 593  $\times$ .

FIG. 6. Animal no. 28 killed on 4th day. In the center is a small group of large mononuclear cells in the heart. No fungi are seen. Hematoxylin and eosin stain. 228  $\times$ .

In nine of the animals, fungi were demonstrable in the brain. As in the heart, both mononuclear (Fig. 7) and polymorphonuclear responses (Fig. 8 and 9) were evoked. Organisms were also seen without surrounding phagocytes (Fig. 10).

Fungi were seen in the lungs of only two animals, although cultures were positive in nine. Reaction to the organism was slight and consisted either of a small focus of mononuclear cells or a thrombus associated with hyphae which did not proliferate through the wall of the occluded vessel.

#### DISCUSSION

From the results, it is possible to draw certain conclusions concerning the pathogenesis of this disease in the rabbit. After intravenous inoculation, the organism is carried to the lungs. In this site, it causes little disease. Some of the organisms are mechanically stopped in the capillary network, and thrombi form about them. Rarely, the organism finds its way to the lung parenchyma and provokes the mononuclear cells. Still other organisms pass through the pulmonary capillaries and become disseminated by the systemic circulation.

Although the organism is disseminated, only three organs are found in which growth is of any significance: the kidney, brain, and heart. The fact that cultures of the liver and lung are frequently positive in the absence of tissue reaction suggests that a persistent mycemia accompanies infection of the kidney, brain, and heart.

When the organism reaches the kidney, an abscess is produced. This abscess, usually cortical, enlarges and frequently ruptures into the tubular system. The organism then may pass from the kidney in the urine, since it was seen in the renal pelvis of one animal. The renal abscess formation with tubular invasion resembles that seen in experimental candidiasis (Evans and Winner, 1954; Muster, 1945). We did not see the glomerular hyaline necrosis reported by Evans and Winner (1954) in this latter disease.

The disease in the heart and brain is interesting in that the organism does not evoke the same reaction in all instances. In some cases, there are neutrophils reacting to the organism; in some cases, large mononuclear cells; and in still other cases, there is no reaction. We are at a loss to explain the absence of reaction. In Fig. 10,

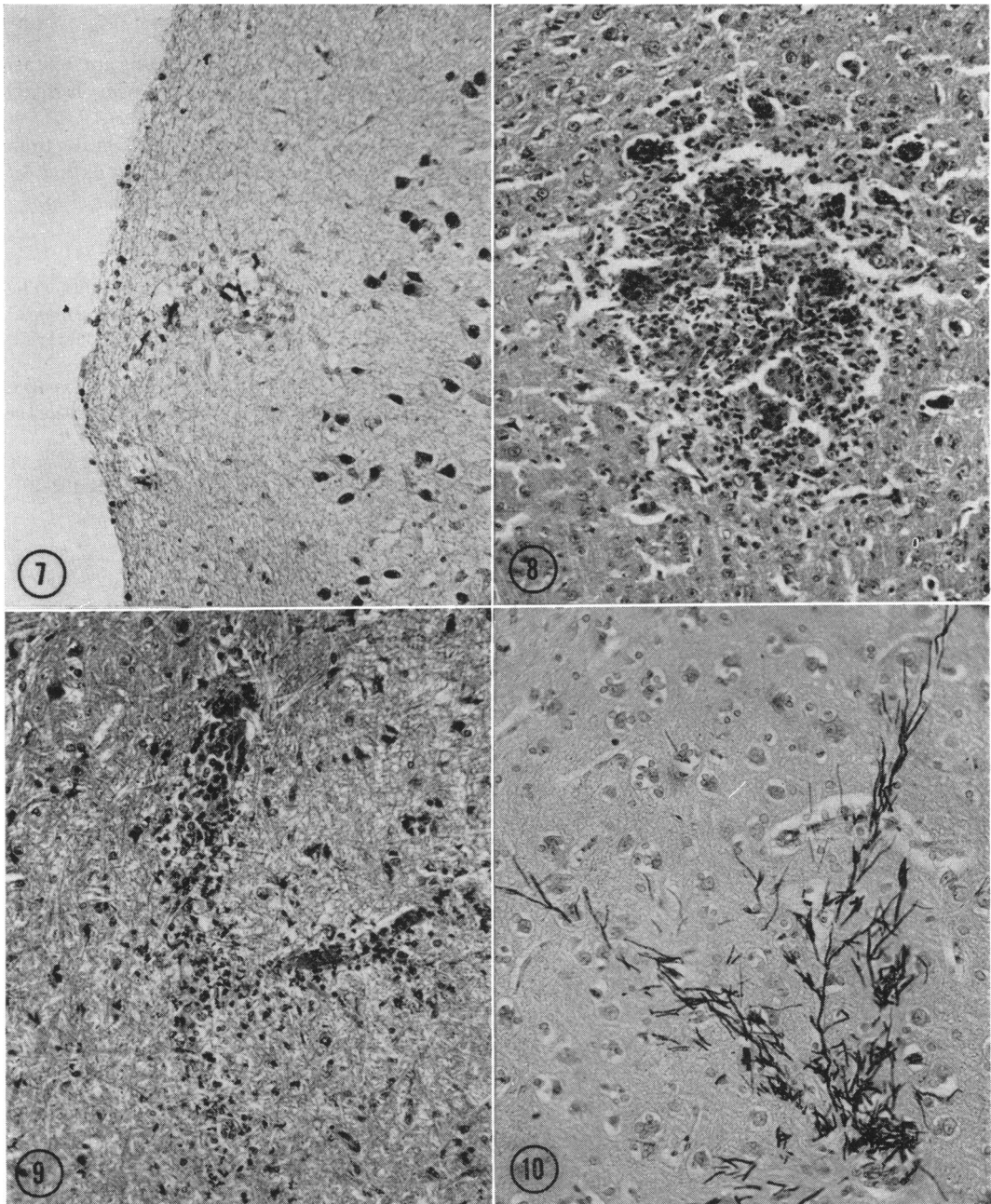


FIG. 7. Animal no. 27 killed on 2nd day. A few hyphae are seen centrally surrounded by mononuclear cells in the brain. Methenamine silver stain. 228 X.

FIG. 8. Animal no. 34 killed on 16th day. There is a zone of mixed neutrophilic and mononuclear reaction with giant cell formation. No fungi were seen in this lesion. Hematoxylin and eosin stain. 171 X.

FIG. 9. Animal no. 28 killed on 4th day. There is vascular thrombosis with surrounding neutrophils and mononuclear cells. In the brain tissues adjacent to the vessels, there are numerous organisms. Hematoxylin and eosin stain. 228 X.

FIG. 10. Animal no. 27 killed on 2nd day. There is a luxuriant growth of organisms in the brain in the absence of cellular reaction. Methenamine silver stain. 228 X.

numerous hyphae are seen in the brain of an animal killed on the 2nd day. Since the tissues were fixed immediately after death, we cannot explain the presence of the organism as post-mortem growth. The complete absence of reaction suggests the picture seen in some cases of human meningitis caused by *Cryptococcus neoformans*.

The organism here described is very pathogenic for the rabbit, since 9 of 15 animals died in a period of 18 days. Seven of these animals died on the 4th or 5th day. The number of animals dying spontaneously is probably lower than it would have been had some of the animals not been killed. It should be emphasized that the rabbits used were normal. No attempt was made to alter the immune response of the rabbits in any way nor was any attempt made to alter the pathogenicity of the fungus.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

- EVANS, W. E. D., AND H. I. WINNER. 1954. The histogenesis of the lesions in experimental moniliasis in rabbits. *J. Pathol. Bacteriol.* **67**:531-535.
- LODDER, J., AND N. J. W. KREGER-VAN RIJ. 1952. *The yeasts*, p. 634-636. Interscience Publishers, Inc., New York.
- MUSTER, I. 1945. Contribution à la diagnose de *Candida albicans*. *Dermatologica* **91**:102-107.