Reports

Experimental Mucormycosis in the Healthy Rat

In a study of inflammation we observed germination and growth of *Rhizopus oryzae* spores which we had injected into Selye's "granuloma pouch" (*1*) in the healthy rat. Wright *et al.* (*2*) used this technique to study growth of another fungus, *Coccidioides immitis.*

Twenty-five-milliliter pneumoderms were produced on the backs of Wistar strain rats that weighed from 150 to 200 g each (*3*). A 0.5-milliliter saline suspension of 1,250,000 spores (estimated by hemocytometer count) of *Rhizopus oryzae* (Duke University, No. 2473) was injected into each pouch. Rats were sacrificed after 24 hours, 3 days, 6 days, 10 days, 4 weeks, and 6 weeks, respectively, and tissues were taken for histologic sections and cultures on Sabouraud's medium from the pouch, lungs, spleen, liver, and pancreas.

In all rats killed 24 hours after injection of spores, there was a gray-yellow exudate within the hyperemic, edematous, thickened wall of the pouch. In those killed after 6 days, yellow, cloudy fluid had accumulated and small gray-yellow nodules, 3 to 4 mm in diameter, appeared in a thickened wall. In those killed at the end of 4 weeks, several of the nodules appeared to have merged, with formation of raised gray-yellow areas in the wall of the pouch. The fluid in the pouch had not become grossly bloody. In one of the animals sacrificed at the end of 4 weeks, the pouch had collapsed and the small nodules had become walled off by fibrous tissue. No fluid was present. In the animals killed after 6 weeks, the pouches were collapsed, but the sites were marked by small, firm gray-white subcutaneous nodules.

Rhizopus oryzae grew in cultures from the pouch, but not from any organ, of each rat when sacrificed. Species of *Penicillium, Aspergillus,* or *Trichoderma* grew in cultures made from the lungs of most animals, but all cultures from other visceral organs remained sterile. It is felt that fungi in the lung were air-borne contaminants in the alveoli, as no fungi were demonstrated to be present in the histological sections.

In histological sections of the pouch at the end of 24 hours, there were hyperemia and edema. A granulocytic exudate was present, and hyphae of the fungus were present in the wall. After 6 days a granulomatous reaction was evident in the wall of the pouch. Nodules containing centrally located hyphae of the fungus and degenerating neutrophils were present. This central area was surrounded by multinucleated cells and macrophages or epithelioid cells. There was also a moderate degree of fibroblastic proliferation. At the end of 4 weeks fibrous tissue proliferation was more pronounced and surrounded or walled off the nodules; hyphae of the fungus were still present. There was very little difference between the 4- and 6-week-old pouches except for perhaps a slight increase in fibrous tissue.

Histological sections of the viscera were not remarkable except for the lungs, which in some of the animals contained very early lesions of so-called "enzootic bronchiectasis" (*4*). It has been stated that approximately 75 percent of rats, particularly of certain strains, have this lesion in their lungs by the time they are a year old (*5*). No fungi were seen in any of the organs sectioned.

Infection with *Rhizopus oryzae* was induced in a pneumoderm or Selye's pouch on the backs of normal healthy Wistar strain rats. The fungus excited a nonspecific inflammatory reaction which after 6 days became granulomatous in type, with the formation of gross nodules. Infection had continued for at least 6 weeks in the pouch but had not spread to the viscera; all histological sections and cultures of the latter were negative for *Rhizopus oryzae.* In several of the 4- and 6-week-old rats the inflammatory process had apparently subsided, at least to some degree, and the nodules had become walled off by fibrous tissue. This may indicate that the animals were beginning to overcome the infection. However, experiments are now in progress to see how long the animals will remain infected with the fungus.

The Selye's pouch offers an excellent tool for the study of mucormycosis in a physically defined space. The use of normal rather than metabolically altered animals may facilitate the understanding of the pathogenesis of this mycotic infection in human beings (*6*).

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References and Notes

1. H. Selye, *Proc. Soc. Exptl. Biol. Med.* 82, 328 (1953).
2. E. T. Wright, V. D. Newcomer, T. H. Sternberg, *J. Invest. Dermatol.* 26, 217 (1956).
3. The rats used in this study were secured from the J. H. Griffin Laboratory, Danville. Va.
4. J. B. Nelson, *Proc. Animal Care Panel* 7, 30 (1957).
5. E. J. Farris and J. Q. Griffith, Jr., *The Rat in Laboratory Investigation* (Lippincott, Philadelphia, 1949), p. 519.
6. This study is a preliminary report on mucormycosis in rats. It was supported by the Fluid Research Funds of the Bowman Gray School of Medicine of Wake Forest College.

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Evidence for a Blue-Sensitive Component in the Retina of the Gecko, Oedura monilis

In October 1955 there arrived from Australia nine living specimens of the gecko, *Oedura monilis.* A digitonin extract was prepared in accordance with the usual methods of visual pigment research (*1*). The alkaline extract was divided into two 0.5 ml portions. One of these was analyzed directly while to the second portion was added 0.05 ml of 0.1*M* NH_2OH. Both portions were then analyzed by the method of selective bleaching (*1*). The results of both analyses led to the same conclusions, so only the data obtained in the NH_2OH experiment will be summarized. The spectrum of the unbleached extract (Fig. 1, top, curve 1) is typical of a solution of visual pigment. After exposure for 124 minutes to a light at 606 mμ, the pigment was bleached to give curve 2 (Fig. 1, top). The selective change in density is given by the difference spectrum (Fig. 1, bottom, curve 1). This NH_2OH difference spectrum suggests that the retinal extract contained a photolabile component with an absorption maximum at about 518 mμ. Retinene is most probably the chromophore of this pigment. This is indicated by the fact that the oxime of retinene$_1$ (formed by adding NH_2OH to a solution of crystalline all *trans*-retinene$_1$ in digitonin) yielded a spectrum (indicated by *X*) which, when scaled properly coincided with the product of bleaching.

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