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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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.

29 January 1958

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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The results suggest that this gecko contains in its retina a photosensitive pigment comparable to that found in other geckos (*1*). This unusual group of visual pigments is characterized by absorption spectra located, not in the general region of 500 mμ, the typical position for the visual pigments of terrestrial animals, but in the general region of 520 mμ. The pigment from *Oedura* is very probably a visual pigment. This is indicated by its photosensitivity, by the presence of retinene, and by the fact that the difference spectrum agrees well with the construction based on Dartnall's nomogram (*2*) for the visual pigments. The possible biological significance of this group of visual pigments has been discussed in a previous report (*1*).

The spectrum obtained (Fig. 1, top, curve 2) after the initial bleaching with light at 606 mμ is very informative because it possesses a distinct upward inflection in the region of 460 mμ. This is an important point because it suggests the presence, in the solution from which the 518_1 pigment had been removed, of a blue-absorbing component. The next two exposures show that this inflection was abolished by light of wavelength shorter than 606 mμ. The first of these exposures was to light at 560 mμ. This caused a very small change, but the important information conveyed by this bleaching was that the 518_1 pigment had, in fact, all been removed by the previous long exposure to light at 606 mμ. The final exposure was to white light. This led to a disappearance of the inflection (Fig. 1, top, curve 4). The difference spectrum which resulted from these two final bleachings (Fig. 1, bottom, curve 3) revealed the occurrence of a small but definite density loss, maximal at about 457 mμ, and a density gain at about 360 mμ.

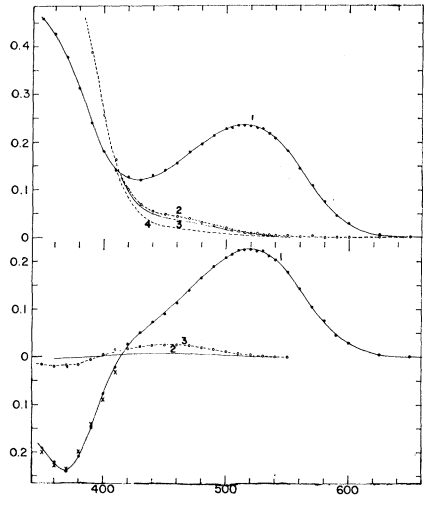

Fig. 1. (Top) Curve 1, absorption curve of unbleached extract; curve 2, result of exposure to light at 606 mμ; curve 3, result of exposure for 125 minutes to light at 560 mμ; curve 4, result of exposure to tungsten light (40 watts) for 10 minutes. (Bottom) Corresponding difference spectra. Curve 1 is the 1–2 difference spectrum. The points indicated as *X* show the data obtained with a retinene$_1$ oxime in 2-percent digitonin. Curve 2 is the 2–3 difference spectrum; curve 3, the 2–4 difference spectrum.

The evidence supports the idea that a blue-sensitive pigment was in fact a component of the original retinal extract of this gecko. The results of both experiments point to the fact that, following removal of the 518_1 pigment, there occurred in response to appropriate illumination a selective change as described. The important feature of this finding is that it was obtainable in an extract containing NH_2OH, a substance which is known to be useful in preventing isomerizing or other side reactions of the products of bleaching. Moreover, the presence of a clear inflection in the spectrum following the removal of the 518_1 pigment is evidence which cannot be easily ignored. The only point in question in this case is whether the blue-absorbing pigment was a component of the original unbleached extract or was a product formed as a result of bleaching the 518_1 pigment. It could, for example, have been a regenerated pigment. Hydroxylamine, by combining with retinene, effectively prevents regeneration in extracts of the retina. In any case no evidence was obtained of regeneration during the 45-minute period which was required to determine the absorption curve. The argument that a secondarily formed photosensitive pigment was present is not easily disposed of; to refute it would require experiments in which the main pigment is left untouched. Whether such experiments can ever be satisfactorily carried out is questionable; lack of animals has thus far prevented attempts in this direction.

A comparison of the difference spectra of this presumed blue-sensitive pigment (a pigment with peak absorption at 457 mμ was assumed) with the curve constructed from Dartnall's nomogram (*2*) shows that the data fit well, considering the small magnitude of the difference spectra. This agreement accords with the idea that the *Oedura* retina contains a visual pigment with absorption in the blue region of the spectrum. The photolabile substance riboflavin has been detected in the vertebrate retina (*3*). It is clear, however, that the blue-sensitive pigment of *Oedura* is not riboflavin. A solution of riboflavin in 2-percent digitonin yielded, after illumination, a difference spectrum with two peaks, one at 373 mμ and the second at about 453 mμ. The 453 mμ was significantly narrower than the 457 mμ spectrum of *Oedura*.

The literature on visual pigments includes a number of references (*4*) which suggest the occurrence of blue-sensitive pigments in the retinae of various vertebrates. Some of these claims are based on inadequate or even questionable experimental procedures, so it is not surprising that the claims have provoked criticism. This report, which is the first account of a blue-sensitive component in the retinae of lizards, is unique for two reasons: (i) Isomerizing actions, which could confuse the interpretation, were reduced to a minimum, and (ii) the pigment in question was demonstrated to be present in the extract before the bleaching employed to remove it.

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5. This study was aided by a grant from the Division of Research Grants and Fellowships, National Institutes of Health, U.S. Public Health Service, and by a grant from the University of California Board of Research.

10 December 1957

A Mechanism for Pyridine-Nucleotide-Dependent Dehydrogenases

Most dehydrogenases, which utilize DPN (*1, 2*) or TPN as hydrogen acceptors, can be classified as either alcohol or aldehyde dehydrogenases. A large body of evidence, accumulated in recent years, indicate that fundamental differences exist between these two classes of enzymes. The enzymes which can be classified as "alcohol" dehydrogenases oxidize primary alcohols to aldehydes, secondary alcohols to ketones, primary amines to ketones and ammonia, and hemiacetals to lactones (*3*). Examples of each subgroup are alcohol dehydrogenase, lactic dehydrogenase, glutamic dehydrogenase, and glucose-6-phosphate dehydrogenase, respectively. The mechanism proposed in this paper is meant to be applicable only to this group of enzymes and is not to be applied to aldehyde dehydrogenases.

In recent years great effort has been expended in elucidating detailed properties of these dehydrogenases. Among the many significant findings, some require special enumeration. First, the enzymes act by direct hydrogen transfer in a stereospecific fashion both with respect to the substrate and with respect to the pyridine ring (*4*). The reversible oxidation-reduction site in the coenzyme molecule is the *para* position of the pyridine ring (*5*). Second, it has been shown that