in a purple color similar to that caused by serotonin. The acid extract of the secretion of *B. asper* gave a light violet color with *p*-dimethylaminobenzaldehyde, and a faint purple color with the Steensma reagent, much less intense than that of *B. melanostictus*.

A second portion of the acid extract was injected intravenously into pithed cats. Rise of carotid blood pressure occurred with both *B. asper* and *B. melanostictus*. Equivalent doses showed that the extract of *B. melanostictus* was more active than that of *B. asper*. The pressor action was not blocked by ergotoxine or dibenamine. When the methanol extract was applied to the isolated rabbit ileum and uterus and the isolated guinea pig ileum and uterus, stimulation was observed with concentrations of 1:10,000 and 1:5000. These pharmacological results and color reactions are suggestive of the presence of indolethylamines, probably analogues of serotonin, in the parotoid secretions of the two species of Javanese toads. These bases also occur in the secretion of other toads (*11*). Of special interest is the absence of catecholamines, since no color was produced by Ewins' reaction (*12*) for epinephrine detection, even though concentrations of epinephrine of 1:500,000 gave a red color. These adrenergic substances have been found in certain species of toads (*1, 2, 13*).

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26 December 1961

Detection of Aspergillus flavus in Soil by Immunofluorescent Staining

Abstract. Strains of *Aspergillus flavus* grown in soil in the presence of buried slides could be detected on the slides by fluorescent antibody techniques. Staining with *A. flavus* antiserum labeled with fluorescein, followed by examination with fluorescence microscopy revealed characteristic fluorescence at sites of distribution of the homologous fungus. The specificity of the reaction and the absence of nonspecific absorption of antibody to soil materials suggest that the method may be useful in studying the ecology of the soil microflora.

Knowledge of ecological relationships among microorganisms in the soil is fundamental to the disciplines of microbiology, soil science, and plant pathology. Little specific information has accumulated as the techniques available to soil ecology sharply restrict the relationships that can be studied. The complexity of the soil environment, the small size and nondescript morphology of most soil microflora, and a lack of distinctive physiological features for much of the indigenous microflora, present inherent difficulties.

Soil ecology problems may be approached by both direct and indirect means. Among the indirect methods, elective culture techniques have been exploited most extensively in recent years in connection with interest in the ecology of soil fungi (*1*). Information obtained by elective culture and other indirect methods is derived at the expense of separating a given isolate from its soil environment. Direct methods also have intrinsic limitations but often afford a realistic view of microorganisms *in situ*. The Cholodny buried (contact) slide technique provides one of the best means for viewing some interrelationships in the soil environment, but the organisms viewed cannot be cultured and only rarely can be identified.

The usefulness of the buried slide would be greatly extended if microorganisms could be detected and recognized directly on the slide. Application of the immunofluorescent staining technique was considered as a possible means of detecting a particular microorganism on the buried slide. The promising results obtained thus far with this approach, substantial interest following an oral report (*2*), and the possible application of the technique to numerous ecological problems prompt this preliminary report.

Interest was centered on the soil fungus *Aspergillus flavus*. As this heterotrophic organism is capable of nitrate formation in pure culture, its ecology in natural environments is worthy of attention (*3*). The antigen was mycelium of *A. flavus* strain CS, cultured in a glucose, dicalcium glutamate, inorganic salts medium in shaken culture at *p*H 7.3. Mycelium was filtered, washed with sterile saline, minced in a blender, and suspended in saline at a concentration of 0.1 mg/ml (based on dry weight of mycelium) for intravenous injection into rabbits. Antiserum with agglutination titers of at least 1280 was fractionated with ethanol, and the crude gamma globulin was conjugated with fluorescein isothiocyanate (*4*).

A simple soil system was adopted to permit the development of the technique with a single known culture present on the slide. Centrifuge tubes, 25 to 30 cm in diameter, were half filled with air-dry loam soil and then sterilized. Sterile microscope slides were then inserted part way into the soil. The soil was moistened slightly with 3 to 5 ml of sterile glucose (0.1 percent) solution and inoculated with a few drops of a spore suspension of the test fungus. After 2 to 4 days of incubation, one surface was washed clean, and the other carefully flooded with distilled water to remove larger soil particles only. After drying briefly, the slide was gently heat fixed, placed in a moist chamber, and the unwashed face was flooded with labeled antiserum. After 30 minutes of contact with the antiserum, the slide was washed carefully with distilled water and dried at room temperature.

Preliminary examination of slides with bright-field microscopy and ordinary light often was helpful in locating fields of particular interest. Mycelium and spores usually were plentiful on the slides together with the soil minerals and the amorphous organic matter that is normally present on buried slides.

Slides were examined for fluorescence by means of a dark-field condenser with an HBO-200 mercury vapor light source. A primary filter (Corning 5840 or Corning 5860) and a colorless eyepiece barrier filter (Wratten 2B) were effective in suppressing the autofluorescence of soil particles and microorganisms, and they allowed the detection of the yellow green color emitted by the fluorescein. Fields were

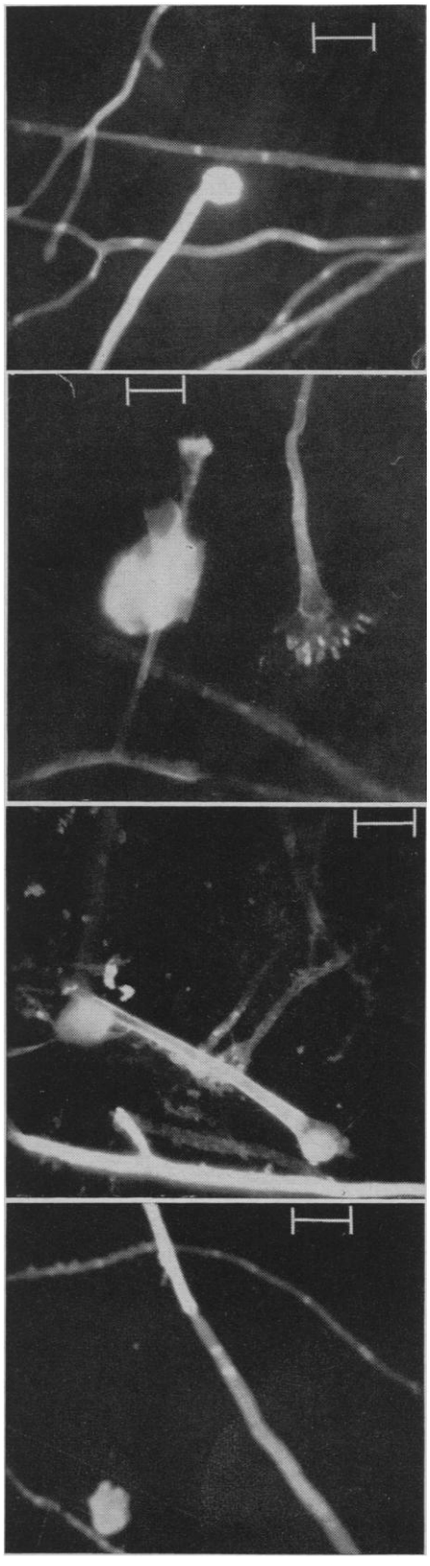

Fig. 1. Fields observed by fluorescence microscopy on slides that had been buried in soil inoculated with strains of *A. flavus*. All slides were stained with fluorescein labeled antiserum of *A. flavus* strain CS. Reference lines represent 50 μ. (Top and upper middle), *A. flavus*, strain CS structures; (lower middle), *A. flavus*, strain F9807; (bottom), *A. flavus*, strain F921.

observed with 10×, 20×, or 40× dry objectives.

Contact slides bearing the antigen but not stained with the antiserum gave no evidence of interfering fluorescence when examined with fluorescence microscopy. Fields were black to dark blue for the most part with nothing visible. Soil particles, when visible, fluoresced in bright blue or red most commonly, but occasionally minerals with yellow fluorescence were seen. Autofluorescence of *A. flavus* was barely apparent as faintly bluish white mycelium. Slides with or without the antigen gave little evidence of interference through nonspecific absorption of the fluorescein label to soil materials. Characteristic fluorescence was restricted to structures of the fungus antigen visible in the field. Figure 1*a* is a photomicrograph from a slide buried in soil, inoculated with the antigen strain of *A. flavus*, and treated with the immunofluorescent stain. The bright areas associated with the conidiophore and the enlarged apex of the conidiophore (vesicle) in the foreground, and the cross wall and hyphal segments in the background, reflect regions of most intense fluorescence. These light areas were brightly yellow green against the dark background when viewed through the microscope. The duller sections of hyphae visible in the photomicrograph reflect a lower intensity of fluorescence commonly associated with much of the hyphae. It is likely that the age of the mycelium has an effect on the intensity of the staining reaction. A conidiophore of *A. flavus* strain CS with some fluorescent conidiospores still in place is shown in Fig. 1*b*. The other conidiophore is partially obscured by a soil mineral which fluoresced as a bright blue.

Isolates of *A. flavus* other than the one used as the antigen reacted well when stained with labeled antiserum of *A. flavus* strain CS. Two such strains are shown in Figs. 1*c* and 1*d*. Immunofluorescence of strain F9807 in Fig. 1*c* was intense on the conidiophores and hyphae in the foreground; soil particles in the background appeared dark green or dark blue. A field with strain F921 is shown in Fig. 1*d* and again shows areas of good to intense fluorescence on the hyphae, with a blue soil mineral in the lower center.

The specificity of the staining reaction extended to numerous other isolates of *A. flavus* as well. Relatively little evidence of cross reactions with other fungi has been encountered. Work dealing with the specificity of the strain CS fluorescein labeled antibody will be reported in more detail (*5*).

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19 December 1961

Serotonin-like and Antiserotonin Properties of Psilocybin and Psilocin

Abstract. These psychotomimetic analogs of serotonin act like this hormone in some tests and against it in others.

The evidence which originally led Woolley and Shaw to suggest in 1954 that serotonin was concerned in normal mental processes and in schizophrenia was that a variety of antimetabolites of this hormone induced schizophrenia-like symptoms in normal persons (*1*). This evidence was interpreted at first to mean that cerebral deficiency of serotonin could be the basis of the mental disorder. Subsequently new evidence indicated that many of these hallucinogenic analogs of serotonin exerted a serotonin-like action on some kinds of tissue in addition to their antiserotonin effects (*2*). This and independent evidence obtained in other ways has led to the idea that hallucinations and other kinds of agitation may be related to cerebral excess of serotonin (*3*), and that depressions may be the result of cerebral deficiency of this hormone. In any event it is clear that many drugs which are relatives of serotonin and which affect the mind can be shown to have either serotonin-like or antiserotonin actions, depending on the nature of the test system. Consequently, it is of interest to determine whether newly discovered psychotomimetic agents also