In two patients with fungal keratitis, direct examination of corneal biopsy specimens showed positive fungal elements, but cultures of biopsy specimens failed to disclose fungal growth. We compared the value of direct examination and culture of biopsy specimens in the diagnosis of keratomycosis in rabbits with experimental fungal keratitis caused by *Fusarium solani*, *Aspergillus fumigatus*, and *Candida albicans*. Cultures disclosed seven specimens (70%) positive for *Candida* and eight (80%) for *Fusarium* and *Aspergillus* keratitis, whereas direct examination showed positive fungal elements of *Fusarium*, *Aspergillus*, and *Candida* in all specimens.

The incidence of keratomycosis has increased dramatically in recent decades. Its diagnosis remains a difficult problem, although the history and clinical appearance may suggest fungal keratitis. The definitive diagnosis of keratomycosis requires confirmation of the existence of fungi in the corneal lesion by direct examination or cultures. In this study, we describe two patients with keratomycosis whose cultures were negative for fungi but in whom the diagnosis was made by direct examination of the biopsy specimens. To determine whether direct examination or culture of biopsy specimens is superior in the diagnosis of fungal keratitis in experimental keratomycosis, we inoculated 30 rabbits with *Fusarium solani*, *Aspergillus fumigatus*, and *Candida albicans* and performed both direct examination and culture.

**Case Reports**

**Case 1**
A 53-year-old man was examined on Feb. 7, 1985, because he had been struck in his right eye with vegetable matter. The patient was treated with antibiotic eyedrops for a corneal ulcer. The lesion progressed without improvement and he was referred to another ophthalmologist. The ulcer was treated with antibiotic and corticosteroid eyedrops. Despite this treatment, the lesion worsened. The patient was referred to our institution for examination and treatment on Feb. 27, 1985.

Visual acuity in the right eye was 20/200. Slit-lamp examination showed a 4-mm white corneal lesion in the central portion of the right eye. The conjunctiva was severely injected. The border of the ulcer was irregular (Fig. 1). Its depth was approximately one half of the corneal thickness. There were many folds in Descemet's membrane, as well as a severe reaction with hypopyon in the anterior chamber. The left eye was normal. We suspected fungal...
infection from the history and clinical appearance. We took four sterile corneal biopsy specimens from the same portion of the lesion with a razor blade and corneal forceps. Two specimens were examined with ink-potassium hydroxide preparation by light microscopy. Two specimens were inoculated on Sabouraud glucose agar and brain-heart infusion broth respectively and cultured at 25 C. Fungal elements were found in the corneal stroma by direct microscopic examination of the biopsy specimens (Fig. 2), although two cultures failed to disclose the fungal growth. Natamycin and miconazole eyedrops were started immediately. Two months later, the lesion had healed completely except for a slight nebula at the site of the fungal process.

Case 2
A 41-year-old man was struck in his left eye while cutting grass on March 19, 1985. The eye became irritated after a few days and he was referred to an ophthalmologist who prescribed antibiotic eyedrops for a corneal ulcer. However, the lesion failed to improve. Another ophthalmologist prescribed antibiotic and corticosteroid eyedrops for the ulcer. The corneal lesion did not respond to this treatment. The patient was referred to our institution for examination and treatment on April 9, 1985.

Visual acuity in the left eye was 20/100. Slit-lamp examination disclosed a severely injected conjunctiva and central corneal ulcer. The lesion was approximately 4 mm in diameter and about one third the depth of the cornea. The border of the lesion was irregular and the surface was somewhat elevated. There were many folds in Descemet's membrane, as well as a severe reaction with hypopyon in the anterior chamber. The right eye was normal.

We suspected fungal keratitis from his history and clinical appearance. Four sterile corneal biopsies were taken from the same portion of the lesion with a razor blade and corneal forceps and were processed in the same manner as those in Case 1. Septate hyphae were seen in the corneal stroma by direct microscopic examination, but no fungal growth was obtained in culture. Three weeks later, when the cultured corneal specimens were examined with ink-potassium hydroxide and light microscopy, we found fungal elements in the specimens. In this case, failure of the culture was attributed to dead fungi in the corneal stroma. The ulcer healed completely except for a slight nebula at the site of fungal process after an eight-week regimen of natamycin and miconazole eyedrops.

### Material and Methods
We used 30 New Zealand white rabbits weighing approximately 2 kg each in this study.

Isolates of *F. solani* and *A. fumigatus* were grown on Sabouraud glucose agar for seven days at 25 C. An isolate of *C. albicans* was grown on the same medium for three days at 25 C. Microconidia of *F. solani*, spores of *A. fumigatus*, and yeast cells of *C. albicans* were suspended in saline, counted with a hemacytometer, and adjusted to a concentration of $2 \times 10^6$ /ml. We followed a previously described inoculation procedure.5–10 The animals were anesthetized with intramuscular ketamine and xylazine. Proparacaine hydrochloride was applied topically and an eyelid speculum was inserted. We inserted a 27-gauge needle into the central corneal stroma. A second blunt-tipped 27-gauge needle, inserted through the same needle track, was used to create an intralamellar pocket. We were careful not to pene-
trate the deep cornea or enter the anterior chamber. Each type of inoculum (10 μl containing 2 × 10⁴ organisms) was injected with a microsyringe into intralamellar pockets in the right eyes of ten rabbits.

On the tenth day after fungus inoculation, we obtained biopsy specimens. The rabbits were anesthetized with ketamine and xylazine, proparacaine hydrochloride was applied topically, and an eyelid speculum was inserted. The epithelium and necrotic debris over the lesion were removed. Three corneal biopsy specimens were taken from the right eye with a razor blade and corneal forceps. At the margin of the lesion, the stroma was incised to about one half the corneal thickness, and small pieces of tissue were removed. Three specimens were taken from the same portion of the corneal lesion.

In each rabbit, corneal biopsy specimens for direct examination were chosen from the three specimens at random. The other two specimens were examined by culture. The biopsy specimen was soaked with the ink-potassium hydroxide preparation and examined by light microscopy as described previously.⁸,⁹ The remaining two biopsy specimens were inoculated on Sabouraud glucose agar and brain-heart infusion broth, respectively, and cultured at 25 C. The media contained no inhibitors. The specimens were observed by light microscopy at low magnification for growing fungi beginning the day after inoculation. The cultures were terminated three weeks after inoculation.

After the biopsies, several eyes were enucleated, fixed in formalin, and mounted in paraffin. Sections were stained with periodic acid-Schiff and hematoxylin and eosin for histopathologic studies.

### Results

All 30 inoculated right eyes of the 30 rabbits developed corneal lesions. In the ten eyes with *F. solani* keratitis, large corneal ulcers, hypopyons, neovascularization from the corneoscleral limbus, and injection of the iris were seen on the tenth day after inoculation (Fig. 3). In the ten eyes with *A. fumigatus* keratitis, the clinical features were similar to those with *F. solani* keratitis, although the infection was somewhat more severe in the *Aspergillus*-inoculated eyes. In the ten eyes with *C. albicans* keratitis, the major clinical features were moderate corneal ulcers, small hypopyons, and iris injection on the tenth day after inoculation.

In eyes with *Fusarium* keratitis, all biopsy specimens were positive for fungal elements (Fig. 4) by direct examination, whereas only eight of the cultures were positive for fungal growth. In eyes with *Aspergillus* keratitis, direct examination disclosed fungal elements in all biopsy specimens, although only eight of the cultures showed positive fungal growth. In the *Candida* group, all specimens were positive by direct examination, but only seven cultures grew fungi. The results of the cultures were identical in both Sabouraud glucose agar and brain-heart infusion broth.
Pseudohyphae and yeast cells are seen in the deep stroma and the anterior chamber surrounded by inflammatory cells in an eye with *C. albicans* keratitis ten days after inoculation (periodic acid-Schiff, x400).

Histopathologic studies in these experiments showed findings essentially identical to those described earlier. In *F. solani* and *A. fumigatus* keratitis, we found fungi in the deep stroma or the anterior chamber, or both, as well as a few fungi in the superficial cornea. In *C. albicans* keratitis, yeast cells and pseudohyphae were located in the deeper corneal stroma as a mass or in the anterior chamber, or both, and were surrounded by inflammatory cells. Few fungal elements were found in the superficial cornea (Fig. 5).

**Discussion**

Early and accurate diagnosis is of prime importance in successfully treating keratomycosis. Cultures ordinarily require a minimum of 48 to 72 hours for diagnosis. Moreover, as shown in our cases, cultures sometimes give false-negative results. In a previously reported case, direct examination of the biopsy specimen showed fungal elements in the corneal stroma, but the cultures failed to grow fungi. In this study, after we failed to find fungus in the cultures, we reexamined the cultured specimens with ink-potassium hydroxide preparation by light microscopy and found fungal elements in the specimens. In these cases, failure of the cultures can be attributed to dead fungi in the stroma.

We also experienced false-positive results in cultures. Because fungi exist almost everywhere, contamination can occur easily when the biopsy specimens are inoculated on the medium. To inhibit this contamination, some kinds of inhibitors are generally used with the medium. However, since ocular pathogens are also apt to be destroyed by these inhibitors, we do not use inhibitors in the culture medium for fungal keratitis. Dish plate medium is commonly used for fungal cultures, but this type of container is easily contaminated by fungi from the air or the breath of the technician. We used oblique medium in a test tube because this type of container is more difficult to contaminate. We gently put the corneal specimen on the medium in the test tube in a sterile manner. Beginning on the day after inoculation, we observed the specimen at low magnification by light microscopy. The specimen was declared positive only when the fungal growth was found to extend directly from the specimen.

We used ink-potassium hydroxide preparation for direct examination of the corneal stroma. This method is employed routinely by mycologists and dermatologists for the diagnosis of mycoses, although it is not frequently used in ophthalmology. The potassium hydroxide solution weakens the corneal lamellar structure, allowing the specimens to be flattened, and the ink, which has fine particles, attaches to the cell walls of the fungi. The fungal elements, which are delineated as blue material in the stroma, are easily identifiable.

The results of this study demonstrate that direct examination of biopsy specimens is superior to culture in the diagnosis of fungal keratitis, although the results of culture are important to identify the species of fungus and to measure the sensitivity of the fungus to antifungal drugs.

**References**