JOURNAL OF CLINICAL MICROBIOLOGY, July 1986, p. 21–25
0095-1137/86/070021-05$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 24, No. 1

Morphology and Ultrastructure of Fungi in Extended-Wear Soft Contact Lenses

R. B. SIMMONS,[1] J. R. BUFFINGTON,[1] M. WARD,[2] L. A. WILSON,[2] AND D. G. AHEARN[1]*

Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, Georgia 30303,[1] and the Department of Ophthalmology, School of Medicine, Emory University, Atlanta, Georgia 30322[2]

Received 30 January 1986/Accepted 8 April 1986

Filamentous fungi of the genera *Acremonium*, *Aspergillus*, *Alternaria*, *Cladosporium*, *Curvularia*, and *Fusarium* penetrated the matrix of soft contact lenses both during normal usage and in laboratory studies. Growth of the fungi within the lens matrix increased with increasing water content of the lens. Hyphae within the lens were coiled. Some species penetrated completely through the lens within 96 h. More frequent cleaning and disinfection of extended-wear soft contact lenses is recommended.

The growth of fungi in extended-wear soft contact lenses has been documented for lenses both in use (1, 2, 4, 6) and in laboratory studies (3, 7). Yamaguchi et al. (7) challenged 45- and 73.5%-water-content lenses on Sabouraud dextrose agar with *Fusarium solani* and *Aspergillus flavus*. These fungi penetrated both lenses, but appeared to grow more vigorously into the lenses with higher water content. Wilson and Ahearn (6) reported on fungal growth in 11 extended-wear soft contact lenses that, in most instances, appeared to have been contaminated while they were on the eye. In two cases, the same fungi isolated from the contaminated lenses were obtained also from corneal ulcers immediately underlying the fungal contamination on the soft lens. In this report, we examine the morphology and ultrastructure of the fungi in selected lenses from the study of Wilson and Ahearn (6) and in new lenses challenged in the laboratory.

MATERIALS AND METHODS

Cultures. The fungi *Curvularia lunata* (two isolates), *Cladosporium cladosporioides*, *Fusarium verticillioides*, *Acremonium* sp., and *Aspergillus* sp. were isolated from extended-wear contact lenses (6). Additional isolates, *Aspergillus fumigatus* and *Alternaria alternata*, were obtained from our stock culture collection of isolates which originated from extended-wear lenses. All cultures were maintained on Mycological Agar slants (Difco Laboratories) at room temperature (22 to 25°C). Conidiation was induced on 250 ml of potato-dextrose agar (Difco) in 1-liter Erlenmeyer flasks with incubation at 22 to 24°C for 5 to 14 days. Conidia were washed from the agar with 0.01 M phosphate-buffered saline (pH 7.0) and were concentrated by filtration through glass wool followed by centrifugation to give 10^6 to 10^8 conidiospores per ml. The conidium suspensions were kept for up to 30 days at 2 to 3°C. New, sterile, soft contact lenses, one per flask, were placed in 20 ml of balanced salts (NaCl, 0.49 g; KCl, 0.075 g; $CaCl_2$, 0.048 g; $MgCl_2$, 0.03 g; $C_2H_3NaO_2$, 0.39 g; $C_6H_6Na_2O_7$, 0.17 g; and H_2O, 100 ml). The flasks were inoculated with 10^2 to 10^4 conidia and incubated on a rotary shaker. In preliminary testing, all fungi were screened for their capacity to penetrate hydroxyethylmethacrylate (HEMA)-type hydrogel lenses of 38, 43, 55, 71, and 79% water content.

Microscopy. Contact lenses removed from the eyes of patients and new lenses inoculated in the laboratory were examined by light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). For TEM studies, lenses were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) plus 0.5%

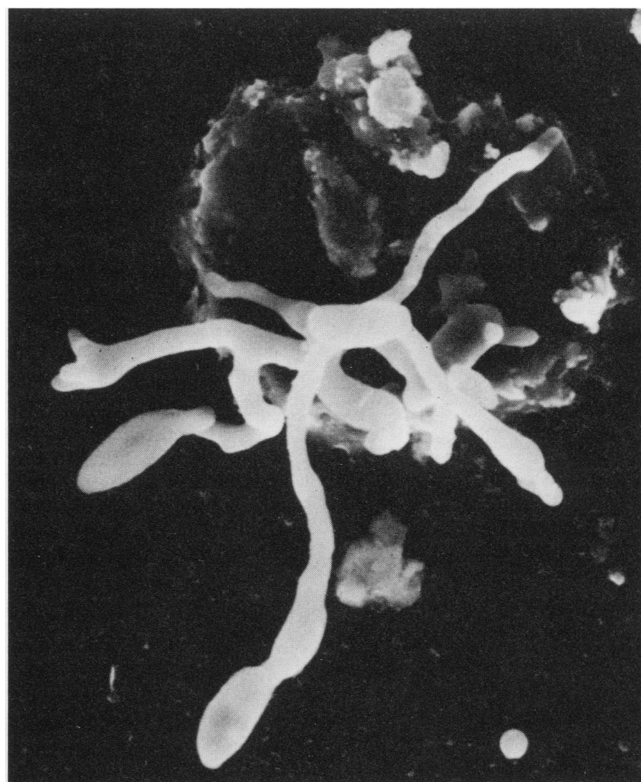

FIG. 1. SEM of germinating conidia of *Fusarium verticillioides* attached to a salt deposit on the surface of a 45%-water-content lens. ×5,200.

* Corresponding author.

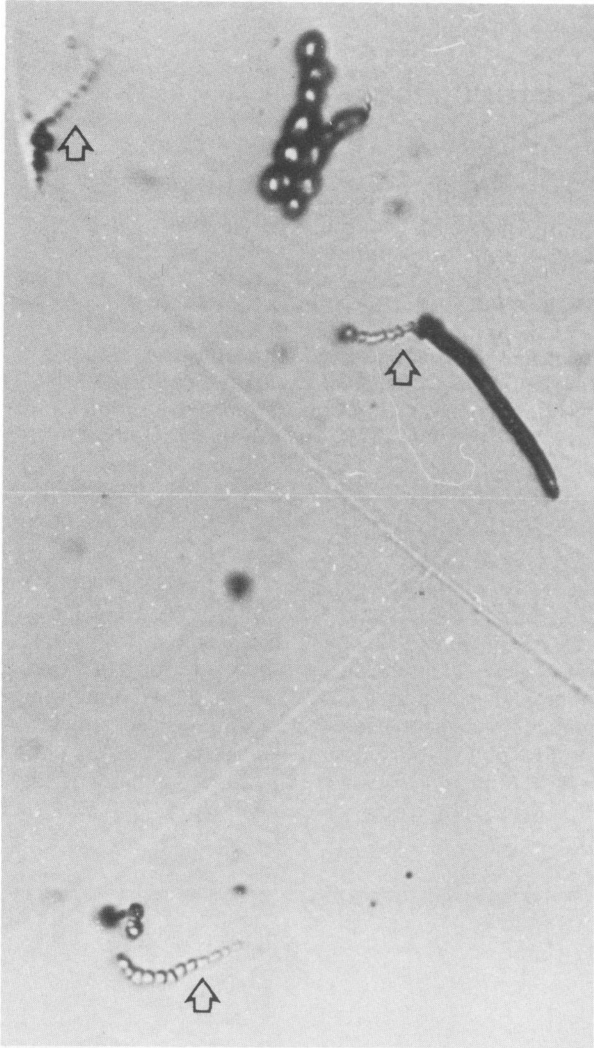

FIG. 2. Coiled and tapered penetration pegs from conidia and hyphal fragments in the matrix of a 55%-water-content lens. ×1,000.

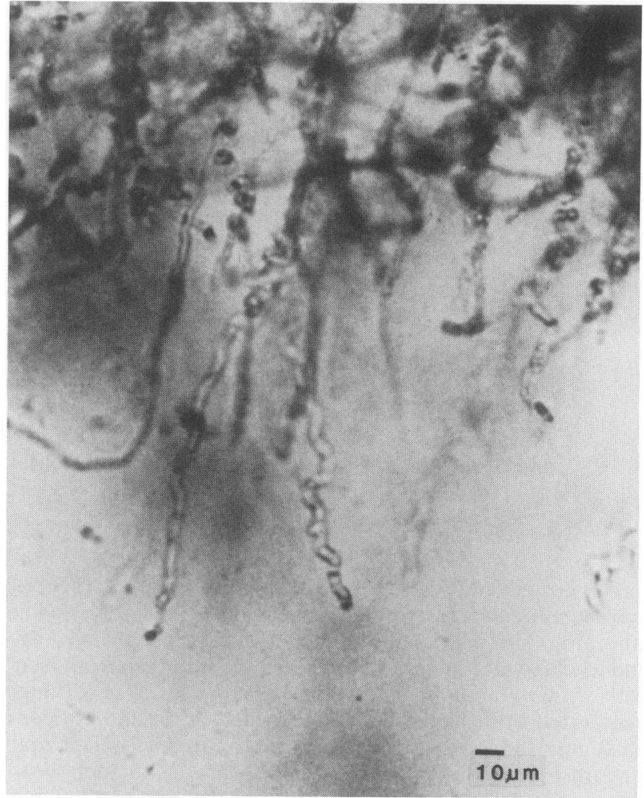

FIG. 3. Coiled hyphae of *Curvularia lunata* penetrating the matrix from the anterior surface of a 79%-water-content lens taken directly from the patient.

sucrose for 1 h at room temperature and then were rinsed three times (10 min each) in 0.1 M cacodylate buffer plus 6% sucrose. Postfixation was in 1.0% OsO_4 in 0.1 M sodium cacodylate buffer plus 4% sucrose for 1 h at room temperature, with three 10-min postfixation periods in 0.1 M sodium cacodylate buffer plus 4% sucrose. The lenses were dehydrated in a graded ethanol series, and slices 2 mm thick were embedded in either L. R. White or Araldite 6005 resin. Samples embedded in L. R. White resin were polymerized in gel caps at 60°C for 24 h. Araldite 6005-embedded samples were polymerized at 48°C for 24 h in flat embedding molds. Sections were cut with glass knives and mounted on Formvar-coated parallel-bar grids (100 mesh). The preparations were stained with 1.0% aqueous uranyl acetate for 3 min, followed by lead citrate for 1 min (5). Specimens were examined with a JEOL 100 CXII-SEG TEM operating at 80 kV.

Samples for study by SEM consisted of one-fourth of a contaminated lens. Specimens were processed through ethanol dehydration as for TEM and then placed in a Balzer CPD-020 critical-point drying unit. Critical-point drying was carried out with CO_2 as the transitional fluid-drying gas. Specimens were loaded into the unit under 100% ethanol, which was then replaced by successive changes of liquid CO_2 (at approximately 6°C and 50 atm [1 atm = 101.29 kPa]). The CO_2 was removed (temperature and pressure were raised to the critical points, i.e., 40°C and 80 atm, for gradual release of the gas). Specimens were sputter coated with gold and examined in a JEOL 35-CF SEM. For light microscopy, sections were cut directly from unfixed lenses, or 5-µm sections from L. R. White-embedded material were cut with glass knives.

RESULTS

All fungal isolates attached to and penetrated into all lens types. The isolates of *Acremonium*, *Aspergillus*, and *Fusarium* species exhibited increased growth with increasing water content of the lens. In comparison, the isolates of *Curvularia*, *Alternaria*, and *Cladosporium* species grew more vigorously on all lenses, but best growth was demonstrated on lenses of 55% water content and higher. The general morphology of the various fungi in all lenses was similar.

Acremonium sp., *Fusarium verticillioides*, and *Aspergillus fumigatus* usually required 120 to 168 h of incubation in the balanced salts solution with lenses having 55% or higher water content to exhibit readily detectable penetration. When germinating on lenses, the conidia of these fungi seemed to be more often associated with deposits, in contrast to the dematiaceous fungi (Fig. 1). When conidia of *Cladosporium cladosporioides* germinated on a new 55%-

FIG. 4. SEM of hyphae of *Curvularia lunata* emerging from posterior surface of a 55%-water-content lens challenged in the laboratory. Coiled hyphae are evident under the surface of the lens. ×3,640.

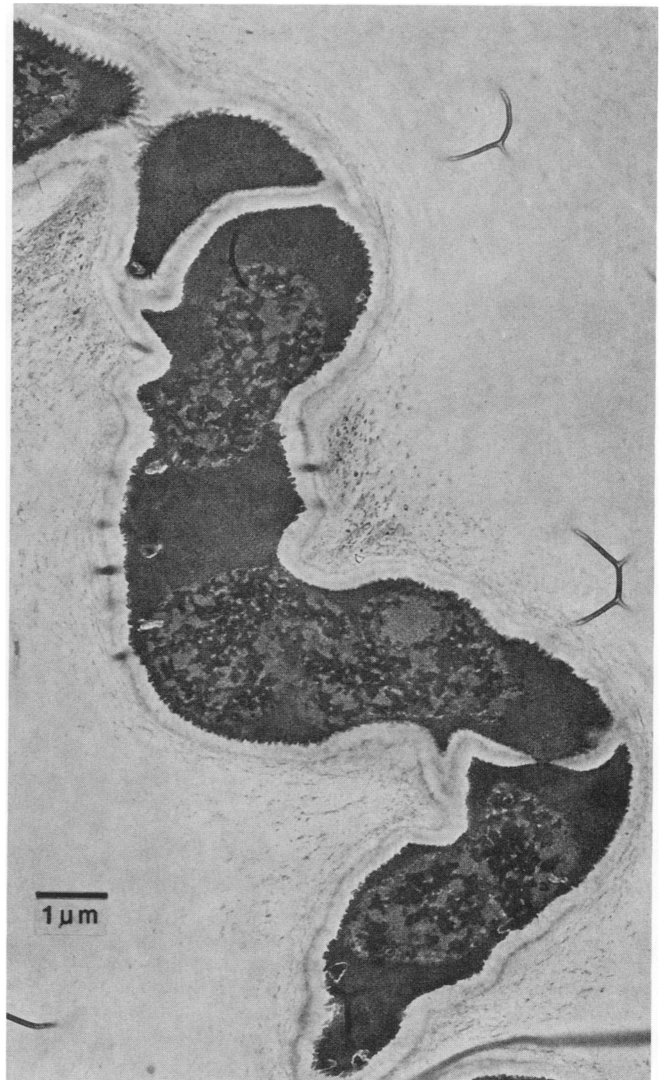

FIG. 5. TEM of transverse section of coiled hypha of *Curvularia lunata* in a 55%-water-content lens challenged in the laboratory. Scattered electron-dense spots peripheral to the hyphae were typical of all fungi.

water-content lens, they either produced flattened appressoriumlike cells on the lens surface or penetrated the lens with a finely coiled and tapered hyphal element within the first 12 h (Fig. 2). These penetration pegs enlarged and branched, but typically remained coiled. The growth of *Curvularia lunata* in a 79%-water-content lens (lidofilcon B) taken directly from a patient is shown in Fig. 3. The hyphae were tightly coiled, ramifying into the lens from the anterior surface. In balanced salts solution, conidia of *Curvularia lunata* germinated and penetrated into and through a new 55%-water-content lens within 96 h. The coiled hyphae were evident under SEM (Fig. 4) and TEM (Fig. 5). Spattered patterns of electron-dense material bordering hyphal elements occurred in the lens. *Cladosporium cladosporioides* in a 55%-water-content lens from a patient showed similar structures and, in some regions, possible hydrolysis of the lens material (Fig. 6). The thallus grew on the surface of the lens (Fig. 7), as well as ramifying through the lens matrix. Surface growth of *Fusarium* sp. was produced even when the lens was on the eye (Fig. 8).

DISCUSSION

Numerous observations of soft contact lenses have reported fungal growth including hyphal penetration of the lens matrix (1–4, 6, 7). Several in vitro studies suggest that the penetration of the lens matrix may result in physical and metabolic degradation of the lens (3, 7). Although these studies did not elaborate on the adherence, penetration, and morphology of the fungal hyphae, their illustrations are compatible with our observations.

Flattened branches or areas on hyphae resembling appressoria appeared to cement hyphal elements or germ tubes to the soft-lens surface. Some hyphae adhered primarily to ridges or crevices or occasionally to small salt deposits on the lenses, but adherence to smooth surfaces also was observed. Tightly coiled hyphae, at first finely tapered, resembling the styletlike infection pegs of plant pathogens, appeared common to our fungi, particularly the melanin-pigmented species. As in the study of Yamaguchi et al. (7), electron-dense material surrounded hyphae within the lens matrix, suggesting metabolic alteration of the lens.

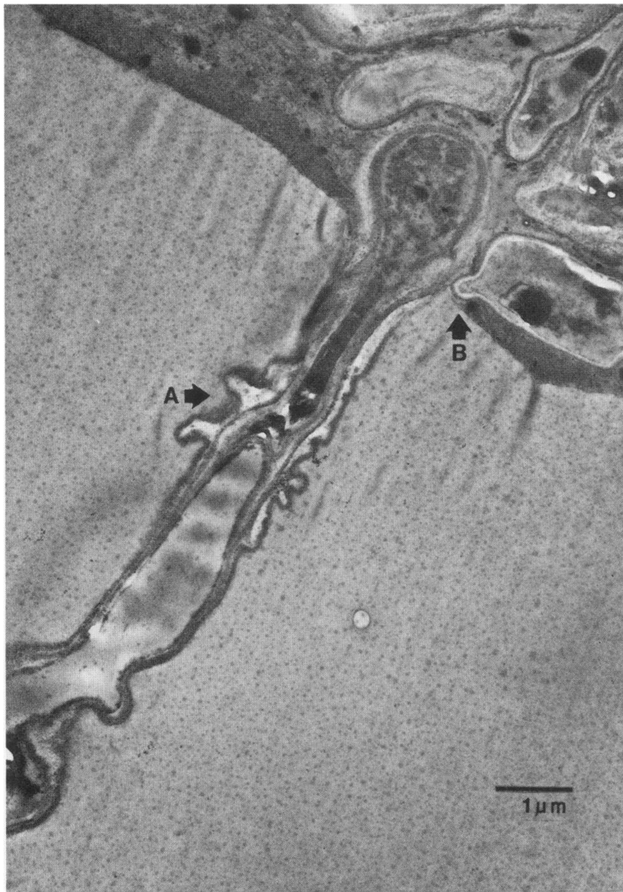

FIG. 6. TEM of transverse section of hyphal element of *Cladosporium cladosporioides* penetrating from the anterior surface into the matrix of an extended-wear soft contact lens. Areas of apparent lens hydrolysis border the hypha (A). Cell on surface of the lens appears to be producing an early-stage penetration peg (B).

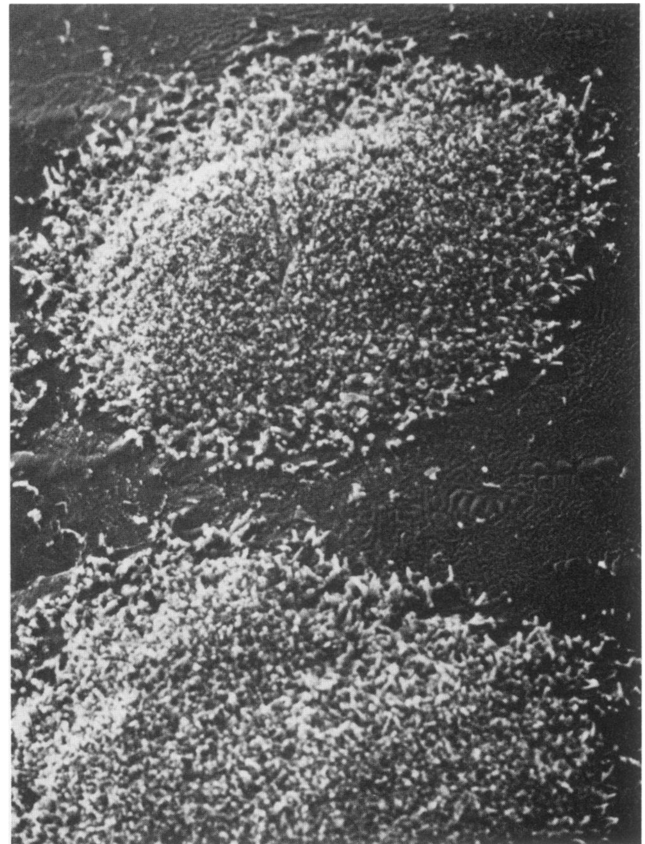

FIG. 7. SEM of growth of *Cladosporium cladosporioides* on the anterior surface of a 55%-water-content lens taken directly from the lens case of a patient. ×468.

The matrix of a soft contact lens, particularly a lens of 55% or higher water content, thus provided a suitable habitat for the growth of various saprophytic fungi that are adventitious agents of corneal ulcers (6). Washed conidia apparently contained enough endogenous nitrogen (or nitrogen-containing contaminants in new lenses were in sufficient quantity) to support growth from one surface through the opposite surface of a lens. Perhaps amide groups in certain lenses are available to various fungi. When the lens is on the eye, nitrogen and carbon necessary for fungal growth probably would be provided by the host. In in vitro tests, the penetration process for 55%-water-content lenses required only about 4 days for certain fungi and 7 days or more for other fungi. We presume that fungi could penetrate an extended-wear lens on the eye in about the same time. We inoculated our lenses in vitro with between 10^2 and 10^4 conidia per 20 ml. This number is not unrealistic for conidium populations encountered in the environment, and a single germinating conidium could colonize a lens. Two of

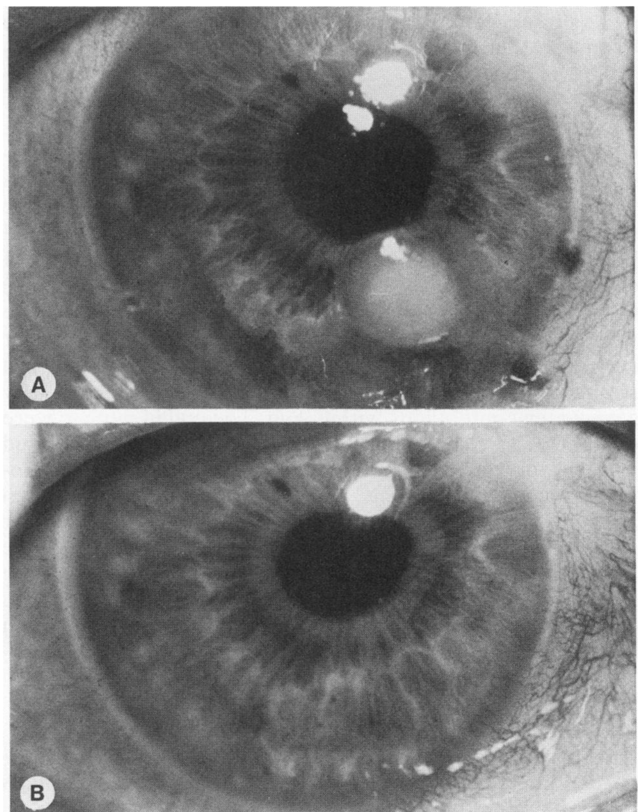

FIG. 8. Irritated eye of patient wearing an extended-wear soft contact lens (79% water content) with colony of *Fusarium* sp. on the surface and growing into soft-lens matrix (A). Same eye 2 days after removal of the contact lens (B).

the fungi that penetrated lenses most rapidly, *Curvularia lunata* and *Cladosporium cladosporioides*, which had been isolated from corneal ulcers and extended-wear lenses (6), were common airborne contaminants in Georgia during the summer and fall of 1985. The latter species in particular was isolated commonly in high numbers from shower curtains and bathroom tiles and, on two occasions, from contact lens cases (unpublished data).

The general recommendation from the literature is that lenses with fungal deposits be replaced. We agree with this recommendation. Nevertheless, we have found that contact lens wearers and some practitioners are not aware of the fungal nature of some deposits, and we believe that more frequent and more regular cleaning and disinfection of extended-wear contact lenses is advisable. Activities in moldy habitats (e.g., raking leaves, cleaning dusty areas, and showering in rooms with moldy plastic curtains or tiles) should be avoided while wearing current extended-wear lenses, or the lenses should be cleaned and disinfected after such activities.

LITERATURE CITED

1. **Berger, R. O., and B. W. Streeten.** 1981. Fungal growth in aphakic soft contact lenses. Am. J. Ophthalmol. **91**:630–633.
2. **Dreyer, V., O. A. Jensen, and J. J. Prause.** 1979. Morphological, histochemical and x-ray microanalytical examination of deposits on soft contact lenses in extended wearing. Acta Ophthalmol. **57**:847–859.
3. **Filippi, J. A., R. M. Pfister, and R. M. Hill.** 1973. Penetration of hydrophilic contact lenses by *Aspergillus fumigatus*. Am. J. Ophthalmol. **50**:553–557.
4. **Hara, J., Y. Tanaka, and S. Harino.** 1984. Histochemical examination of deposits on soft contact lenses in extended wear. Folia Ophthalmol. Jpn. **35**:1956–1960.
5. **Venable, J. H., and R. Coggeshall.** 1965. A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. **25**:407.
6. **Wilson, L. A., and D. G. Ahearn.** 1986. Association of fungi with extended-wear soft contact lenses. Am. J. Ophthalmol. **101**:434–436.
7. **Yamaguchi, T., A. Hubbard, A. Fukushima, T. Kimura, and H. E. Kaufman.** 1984. Fungus growth on soft contact lenses with different water contents. Contact Lens Assoc. Ophthalmol. J. **10**:166–171.