visualize the fluorescent epithelial cells with less fluorescent nuclei. The corneal epithelial cellular autofluorescence was most likely due to nicotinamide adenine dinucleotide phosphate. At 200 µm, the collagen pattern at the wound is irregularly arranged, which is in sharp contrast to the orthogonal packing of adjacent lamella found in normal stroma (Figure 1H). At imaging depths of 400 µm and beyond, regions lacking in SHG collagen were observed. In addition, the collagen fibers outside of the wound tended to align parallel to the wound edges. At the imaging depths of 1000 and 1200 µm, intense fluorescent lining (possibly from detached uveal tissue) along the wound edge was found (Figure 1I).

For comparison, the histological image is shown in Figure 2. Both the surface epithelial cells and the V-shaped corneal wound were visible. At greater depths, we also found granulation tissue (with cells). The existence of the corneal wound and granulation tissue may explain the lack of SHG collagen fibers within the wound.

Comment. Previously, it was shown that multiphoton microscopy can be used to image autofluorescent epithelial cells and SHG collagen fibers within the stroma of normal porcine cornea. In this study, we extended this approach to the investigation of the structural alterations of a full-thickness linear corneal scar. The structural alteration of the cornea along the linear scar can be identified using the multiphoton technique without histological procedures. With additional refinement of scanning technology (increase in imaging speed) and a better characterization of possible tissue photodamage, multiphoton microscopy may be developed into a clinical diagnostic tool for in vivo monitoring and lead to a better understanding of corneal wound healing processes.

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Histopathologic Findings in Naturally Preserved Mummified Human Eyes

This study was undertaken to assess the suitability of 2 pre-Columbian, naturally preserved, mummified human globes for contemporary histopathologic analysis, to successfully rehydrate and process the unembalmed specimens, and to describe the gross and microscopic findings. The ability to analyze ancient human remains may expand our understanding of the prevalence of ophthalmic diseases and possible correlations involving diet, lifestyle, and heredity. Histopathologic analysis was performed on the 2 naturally preserved eyes from the Atacama Desert of northern Chile. One eye was from a 2-year-old boy and the other from a 23-year-old woman. Modifications to traditional tissue dehydration techniques were used. Mummified ocular tissues were successfully rehydrated. Processed tissues survived paraffin embedding and microtome sectioning. Tissue sections absorbed various conventional tissue stains. Light microscopy revealed uveal tissue, retinal pigmented epithelia, and intact sclera. Structures representing the inner lining of the embryonic optic cup were not recovered. Naturally preserved mummified tissues are amenable to contemporary histopathologic analysis. The
findings in this pilot study suggest that further study of mummified eyes may expand current understanding of cultural practices and disease processes afflicting ancient peoples. We demonstrated a nondestructive method for the successful reconstitution and histopathologic examination of spontaneously mummified human ocular remains.

Mummified eyes survive the centuries better than any other human organs. When paleopathologists examine a mummy head, the eyes are found 93% of the time.1 Given the consistent presence of ocular structures in mummified remains, it is a paradox that they have not been more extensively studied. The study of mummified tissues has offered new insights into contemporary disease processes that plagued ancient civilizations. As an example, Salo et al2 demonstrated the presence of Mycobacterium tuberculosis through polymerase chain reaction analysis of lung tissue from a 1000-year-old Peruvian mummy, showing that M tuberculosis existed in the Americas 5 centuries before European contact. Patterns of disease prevalence may also be defined in ancient populations, conferring an understanding of humankind’s relationship with environmental pathogens through the ages. Once again using polymerase chain reaction analysis, Aufderheide et al3 found a 40% prevalence of Chagas disease in mummies from the Atacama Desert, including Acha man, one of the oldest known mummies, dating back nearly 9000 years.

Although the classic archetype is the shrouded Egyptian mummy, studying Egyptian mummy eyes is fraught with problems because the embalmers often made no attempt to preserve the eyes. They removed the collapsed globes with their dull clouded corneas and replaced them with shells, linens, or painted onions—artificial eyes for the afterlife.4 Postmortem Egyptian mummy eyes were preserved with natron, a carbonate salt, as a desiccant. Resins that accumulated in the tissues from the embalming process also make the rehydration less successful.1 Primitive techniques developed for artificial physical preservation confounded modern-day attempts at chemical reconstitution, often yielding an amorphous mass of uninterpretable matter.

With this understanding, we developed a pilot study to examine mummified human ocular tissue from the Atacama Desert. Known as one of the driest places on earth, this desert is a stark plain spanning 960 km from southern Peru into northern Chile. In addition to the arid climate, the absence of groundwater and the chemical composition of the sandy desert soil naturally mummify tissues.5 The dead inhabitants were not embalmed but simply placed in shallow pits just beneath the surface of the desert.1 We describe a successful method for rehydration of ancient mummified ocular tissues and describe our histopathologic findings.

Report of Cases. Dissected orbital contents from 2 mummies discovered in the Atacama Desert were acquired from the University of Minnesota, Duluth. Conventional carbon dating techniques were used. The first specimen was from a 2-year-old boy dating back to 1000 AD, and the second specimen came from a 23-year-old woman whose remains dated back to 1250 AD. We received approval from the University of California, Davis, Human Anatomical Specimen Authorization Committee, to conduct the study. Examination of the desiccated tissue disclosed fragile tan-brown fragments of lightweight tissue without recognizable physical anatomical landmarks. Using an empirically modified formula based on the previously described Sandison technique, the mummified specimens were successfully rehydrated using a solution composed of 3 parts 96% ethanol, 5 parts 1% aqueous formalin, and 2 parts 5% aqueous sodium carbonate. The specimens were immersed for 24 hours. Visual inspection of the rehydrated specimens confirmed that the tissue represented orbital contents. Gross findings included extraocular muscle, orbital bone, and hair-bearing periorbital skin (Figure 1). Deep inside the orbit, the flattened globe was collapsed on itself and adhered to the roof of the orbit. Once rehydrated, mummified tissue must be chemically preserved or it will quickly disintegrate. Rehydrated tissues were preserved in 4% buffered formalin. After 24 hours of formalin fixation, the specimens were manually sectioned and chemically preserved before representative portions were embedded into paraffin blocks. Microtome sections averaging 5 µm thick were floated in a water bath, mounted on glass slides, dried, and subsequently stained with either hematoxylin-eosin or periodic acid–Schiff. Cover glasses were
applied, and the stained slides were then examined by light microscopy.

Naturally preserved mummified ocular tissues were successfully rehydrated. Gross and microscopic anatomical features were identified in both specimens. Cellular structures remained intact following fixation. Sections of paraffin-embedded, chemically preserved tissue were satisfactorily stained. Examination of microscopic slides confirmed the presence of connective tissue structures, uvea, and pigment epithelia within a collapsed globe (Figure 2). Optic nerve tissue was also identifiable, although the axonal elements had long disappeared. Periodic acid–Schiff staining vividly highlighted basement membranes, including those situated within the pars plicata and the Bruch membrane. We identified a fragment of crystalline lens tissue in the globe taken from the 23-year-old woman. Much more advanced tissue deterioration was observed in the eye of the 2-year-old boy. A large tissue defect was identified in this globe. An intact amber crystalline lens was visible inside. Surprisingly, this lens was remarkably well preserved, and individual lens fibers could be visualized under microscopy. Furthermore, the child’s lens resembled contemporary lens ticular tissue after fixation and staining (Figure 3). Corneal and retinal structures were absent in both specimens. No cell nuclei or surface epithelial layers were found in any of the specimens.

Comment. To our knowledge, this is the first time naturally preserved, mummified ocular tissues from the Atacama Desert region have been successfully rehydrated and examined. These were spontaneously desiccated tissues that had been spared the use of artificial desiccants, and we found the tissue that remained after centuries of existing in the desiccated state survived rehydration, fixation, and chemical processing. Not surprisingly, we were left with the more durable of the ocular tissues to examine centuries later. Uveal tissue, which functions to absorb energy and protect the more delicate neurosensory retina, and the sclera, a vital mechanical barrier to the insults of an unforgiving environment, were well preserved. Collagenous structures, including the Bruch membrane and the perineural support structures of the optic nerve, also survived. Finally, the crystalline lens, which by design passively permits the unencumbered passage of light energy, was also well preserved.

Curiously, the lens of the 2-year-old specimen was nearly perfectly preserved, whereas only a small particle of lens was found in the 23-year-old female specimen. This variability of tissue preservation in mummified specimens from the Atacama Desert has been observed by others. It has been speculated that this is a manifestation of nonuniform soil nitrate concentrations. Many different factors likely contribute. For example, soil temperature variation is directly affected by original depth of burial.
Conspicuously absent were retinal tissues and blood vessels. They likely did not survive 1 week after burial in the conditions offered by the Atacama Desert. This is not surprising given that these delicate tissues do not escape autolytic disintegration in vivo following ischemic events.

Mummification describes the preservation of soft tissues by various mechanisms that resist the usual enzymatic and microbial degradation processes of postmortem decay. Soft tissues may be preserved by desiccation, chemical processing, or freezing temperatures that retard the enzymatic cascade of tissue degeneration.

In this pilot study, the sample size was small and the calculated age at death was relatively young. Unlike the specimens in our study, evidence of atherosclerosis has been identified in the coronary arteries of bodies mummified through freezing. Similarly, ocular tissues mummified via freezing would be best suited for the study of ancient retinal and retinal-vascular diseases, such as diabetes mellitus, hypertension, and age-related macular degeneration. This type of investigation could lead to new understandings of contemporary eye diseases and help to define the prevalence and existence of these disease processes in the ancient world.

An expansion of the present pilot study may reveal evidence of prior trauma, ancient cataract procedures, and, perhaps, ophthalmic infections. Intraocular and orbital tumors might also be identified. Molecular medicine techniques have been successfully applied to identify infectious pathogens, including *M tuberculosis* and *Trypanosoma cruzi*. In future studies, polymerase chain reaction analysis may also be applied to ophthalmic tissues if the stigmata of infectious diseases are identified. Human herpesvirus and *Acanthamoeba* could potentially be identified. Enzyme-linked immunosorbent assay techniques may also be applied to identify ancient ophthalmic parasitic infestations, such as toxocariasis, toxoplasmosis, cystercerosis, or onchocerciasis.

A more complete ancient epidemiologic database may help us gain perspective on the pathobiological features of human ocular disease processes. The ability to identify cancers of the eye, and perhaps their prevalence, may indicate the contributions of the environment on this disease process. Recognizing the prevalence and patterns of systemic diseases in the ancient world might confer new understandings of the relationship between diet, lifestyle, and these chronic diseases. We have taken only the first step and have shown that these tissues can be successfully reconstituted and made available for 21st-century investigative laboratory techniques. Further work must be done to unlock this ancient and fascinating database.

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**5.** Aufderheide AC. Progress in soft tissue paleopathology *JAMA*. 2000;284(20):2571-2573.


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**The Fusarium Keratitis Outbreak: Not Done Yet?**

In February 2006, several clusters of patients with Fusarium keratitis were reported in Singapore and in Hong Kong. Because initial findings suggested an association between the use of Bausch & Lomb’s ReNu with MoistureLoc solution (Rochester, New York) and the development of this infection, the manufacturer voluntarily suspended all sales of the product in these locations. One month later, the Centers for Disease Control and Prevention (CDC) received a report of 3 cases of *Fusarium* keratitis in New Jersey and, subsequently, several clusters of contact lens–related Fusarium keratitis were reported in the literature, including cases in unlikely temperate-climate locations such as San Francisco, California. After preliminary findings suggested a link between the infections and the ReNu with MoistureLoc product, on May 15, 2006, Bausch & Lomb withdrew this product from the world market.

The CDC has subsequently published a summary of the multistate outbreak of *Fusarium* keratitis associated with contact lens use in the United States, and they concluded that the Bausch & Lomb ReNu product was indeed associated with the outbreak. In this study, only 2 of the 164 patients with confirmed *Fusarium* keratitis used a non–Bausch & Lomb solution.

After the withdrawal of the ReNu with MoistureLoc product, there was a dramatic decrease in the number of cases of contact lens–related *Fusarium* keratitis that were reported to the CDC, and it appeared that the *Fusarium* keratitis outbreak was finished. In this study, we report 4 cases of *Fusarium* keratitis that presented to us between July 1, 2006, and November 1, 2006, after the withdrawal of Bausch & Lomb’s ReNu with MoistureLoc from the world market. In all cases, cultures from the cornea yielded *Fusarium* species, and none of the patients were using Bausch & Lomb’s ReNu with MoistureLoc product.

Institutional review board approval was obtained to perform this retrospective study, and the patients’ clinical data are shown in Table 1. All patients were female and ranged in age from 12 to 42 years. The patients were initially treated for 2 to 21 days for a bacterial or herpetic keratitis in 3 of the 4 cases, and for a corneal abrasion in 1 case. All pa-