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Scanning Electron Microscopy of Hydrophilic Contact Lenses

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Hydrated hydrophilic lenses of three different manufacturers (Griffen, Kontur, and Bausch and Lomb) were prepared for scanning electron microscopy utilizing air drying, freeze drying, and critical point drying techniques. Air dried lenses demonstrated artifacts analogous to those commonly seen in soft biological tissues when prepared in this manner. The freeze and critical point dried specimens showed better preservation of surface detail. Surfaces did not appear to have a porous architecture and no pores were seen at magnifications to 36,000 times. Surfaces of two of the lenses demonstrated conspicuous polishing marks which appeared to predispose to the adherence of debris, following wearing and cleaning. *Pseudomonas aeruginosa* was noted on the posterior surface of one lens. Varying the tonicity of the hydrating solution appeared to have little effect on the surface morphology of the lenses.

HYDROPHILIC lenses have been reported to accept and release fluids and are presumed to have surfaces through which the fluid exchange can occur. The scanning electron microscope was utilized in this study to determine whether or not surface openings can be visualized in hydrophilic lenses and also to determine whether the surfaces of a variety of lenses from different manufacturers demonstrate distinctive morphologic differences. Different techniques have been utilized in the preparation of biologic specimens for scanning electron microscopy, endeavoring to minimize induced artifacts. It was anticipated that a nonbiologic polymer such as that composing the hydrophilic contact lens might be a useful vehicle to study the effects of varying preparation techniques on the surface morphology of biologic specimens. Consequently, the hydrated hydrophilic lenses were prepared by air drying, freeze drying, and critical point drying in hypotonic, isotonic, and hypertonic saline solutions. The lenses examined were produced by Kontur (A), Griffen (B), and Bausch and Lomb (C).

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Materials and Methods

Specimens.—Wedge-shaped segments of the three hydrated lenses were prepared for scanning electron microscopy as indicated below.

Preparation for Scanning Electron Microscopy.—All of the lenses were hydrated in 0.9 sodium chloride according to the manufacturer's recommendations. In addition, segments of lenses A and B were hydrated in a hypotonic solution (distilled water) and a hypertonic solution (saturated sodium chloride).

The segments destined for air drying were taken from their hydrating solutions of varying tonicity, and then without rinsing, were allowed to dry in air at room temperature for 24 hours.

The hydrated segments for freeze drying were transferred into 50% ethanol for ten minutes to initiate the dehydration. The specimens were then successively transferred into 80%, 95%, and 100% ethanol, residing for ten minutes in each solution. Following dehydration, the specimens were transferred to nonpolar amyl acetate and then frozen with liquid freon E1. The lens segments were placed in the freeze drier (Pearce Speed-Evac) for sublimation at −80 C at a vacuum of 1 mm Hg for 72 hours.

The segments for critical point drying were dehydrated in 50%, 80%, 95%, and 100% ethanol for ten minutes each and then transferred into amyl acetate. Following amyl acetate replacement, the segments were placed in the chamber of the "bomb" and liquid carbon dioxide at 15 C was passed through the chamber replacing the amyl acetate in the segments. The temperature was raised above the critical point (31 C for carbon dioxide), causing the

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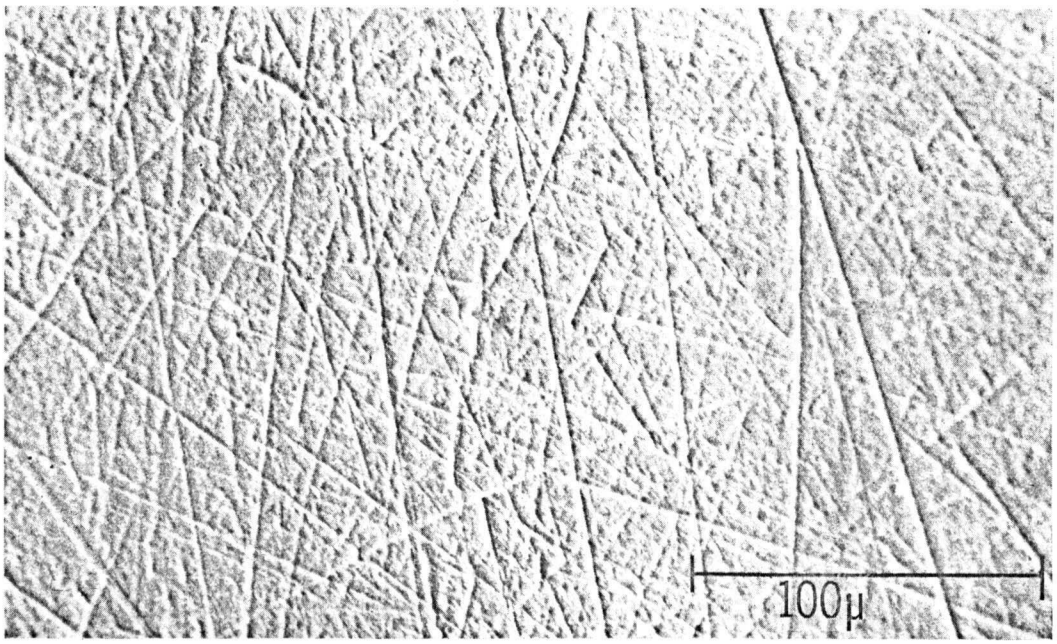
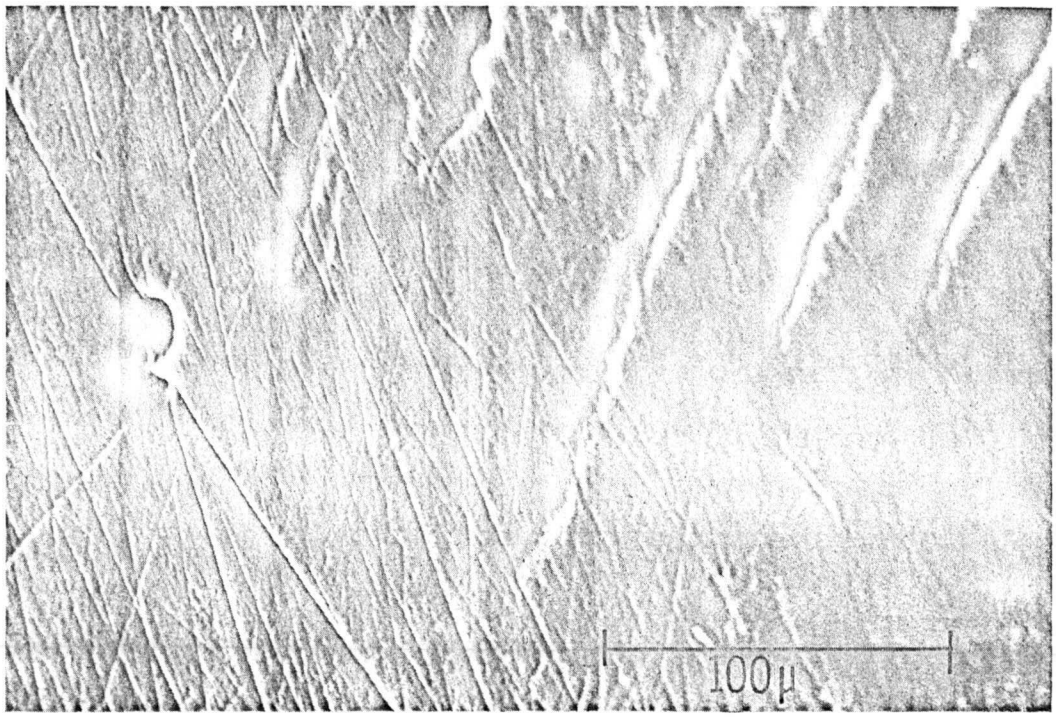


Fig 1.—Central surface of lens B demonstrating polishing marks (original magnification × 300).

Fig 2.—Central surface of lens B showing relatively wide and deep grooves in addition to usual polishing marks (original magnification ×300).



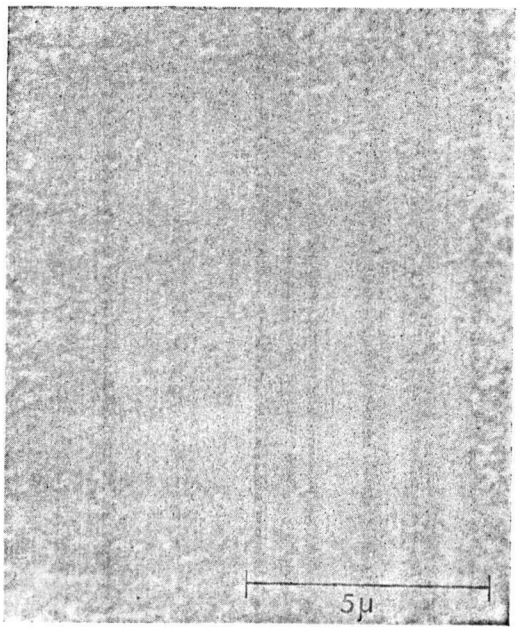


Fig 3.—Central, relatively smooth surface (lens C) (original magnification ×600).

liquid to become a gas which was gradually released from the dried segments.

Lenses A and B were cleaned according to the manufacturer's recommendations.

A.—Clean lens with Soaclens, then soak lens in micropore filtered 0.9% sodium chloride.

B.—Soak lens for five minutes in 3% hydrogen dioxide then rinse 30 seconds in 0.9% sodium chloride and sodium bicarbonate, then soak overnight in 0.9% sodium chloride.

Following drying, the specimens were coated with a conducting layer of gold and then placed on the stage of a modified scanning electron microscope (JEOL [JSM-1]). The stage was placed at a 45° angle with the scanning beam.

Photographs of the display cathode ray tube were made with type 42 roll film (Polaroid).

Results

The surface topography of lenses A and B is very similar and differs markedly from the lens C.

Contrary to expectations, the appearance of lenses prepared from hypertonic and hypotonic solutions did not materially differ from lenses hydrated in 0.9% sodium chloride except for an excess of salt crystals on the surfaces of the lens that had been in hypertonic solution. The lens segments prepared by freeze and critical point drying were morphologically very similar in appearance, and fine surface details could be appreciated. The fine surface details were considerably less distinct in the air dried segments. The figures in this paper were prepared from the freeze-dried specimens.

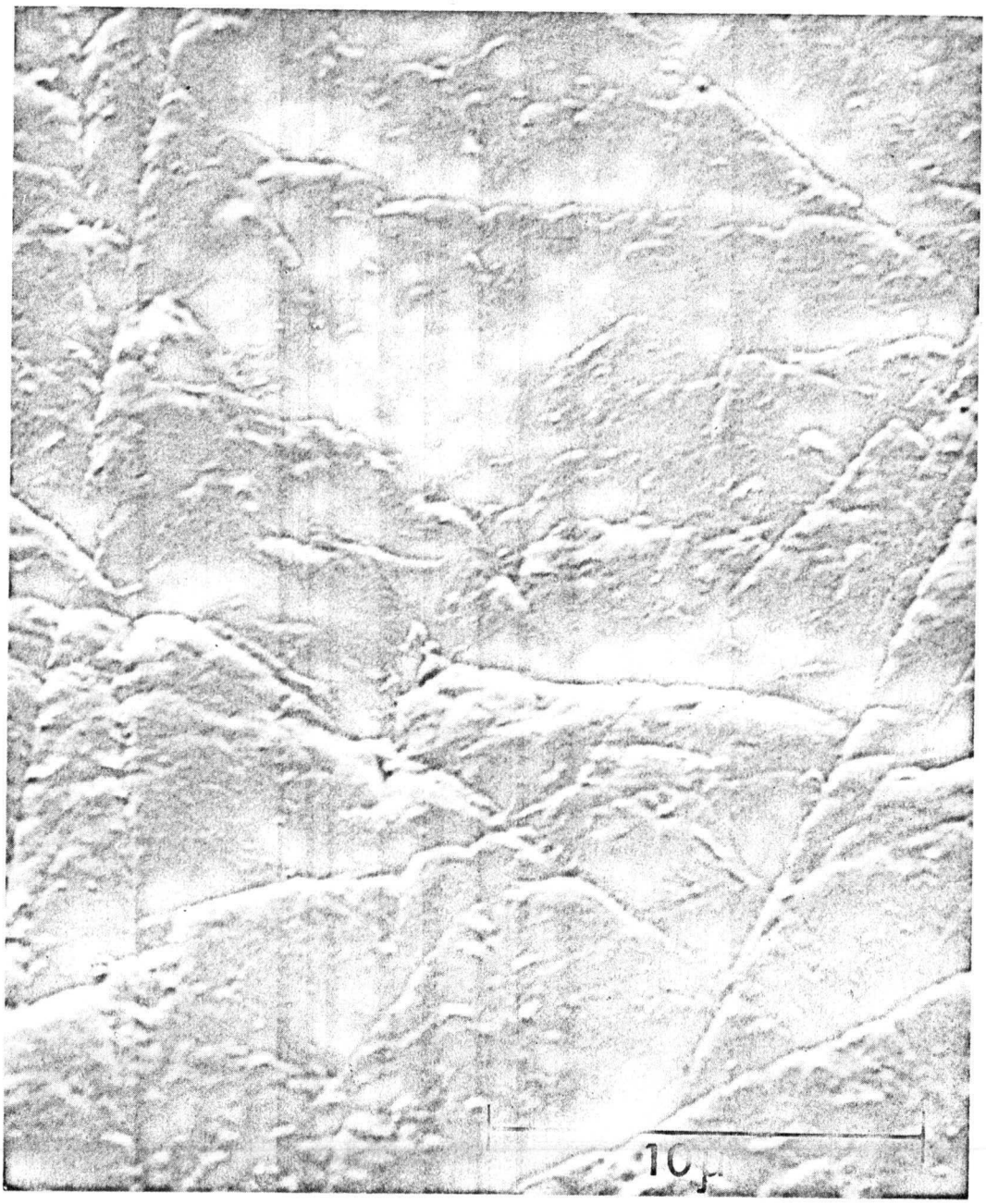
The front and back surfaces of both lenses A and B demonstrate the presence of randomly distributed polishing scratches (Fig 1, × 300). The back surface appears identical to the front. In addition to the polishing scratches, occasional large and deep grooves are found on the surface (Fig 2, × 300). No pores with this technique can be identified at this magnification nor at magnifications to 10,000 times. The central surface of the lens C (Fig 3, original magnification × 600) appears much smoother than that of lens B (Fig 4, × 3,000). Lens C was examined at magnifications to 36,000 times. The surfaces were smooth without evidence of a porous architecture. The periphery of the front surface of lens C is seen at lower magnification (Fig 5, original magnification × 600). The ridge at the edge (*single arrow*) and the peripheral grooves (*double arrows*) are presumed to be produced by edge treatment. More centrally (*C*) the surface appears smooth. The edge seen at high magnification (Fig 6, × 6,000) demonstrates the generally rough appearance of this portion of the lens. Figure 7 (× 1,000) compares the unworn back surface (*left*) of lens B against a lens B which has been worn (*right*) and then cleaned (see "Materials and Methods"). Debris presumed to be dried mucus adheres to the worn but cleaned lens. The depression seen at *left* is an artifact which will be described below. Figure 8 (original magnification × 3,000) is a higher power view of the debris remaining on the central portion of the posterior surface of lens B.

In Fig 9 (× 300) a comparison is made between the unworn lens A (*left*) and the worn lens A (*right*) after cleaning (see "Materials and Methods"). The worn lens A has considerable material remaining on its surface after having been cleaned. The ruptured blebs (*arrows*) seen at *right* are artifacts that will be described below.

At a higher magnification Fig 10 (original magnification × 3,000) the foreign material (*arrow*) on the worn lens A can be seen to

consist of rod-shaped structures 2μ to 4μ in length. This foreign material could only be found on the wearing surface of the lens. Culture of the soaking solution, micropore filtered 0.9% sodium chloride, from which this lens was obtained, revealed the presence of *Pseudomonas aeruginosa*. Scanning electron microscopy of the cultured colonies demonstrated collections of rod-shaped structures which appeared morphologically identical to those seen on lens A. Colonies of these structures are seen adjacent to a ruptured bleb (Fig 11, original magnification × 3,000). They appear to lie on the surface of

Fig 4.—Higher magnification of Fig 2 showing relatively rough appearance (lens B, original magnification ×3,000).



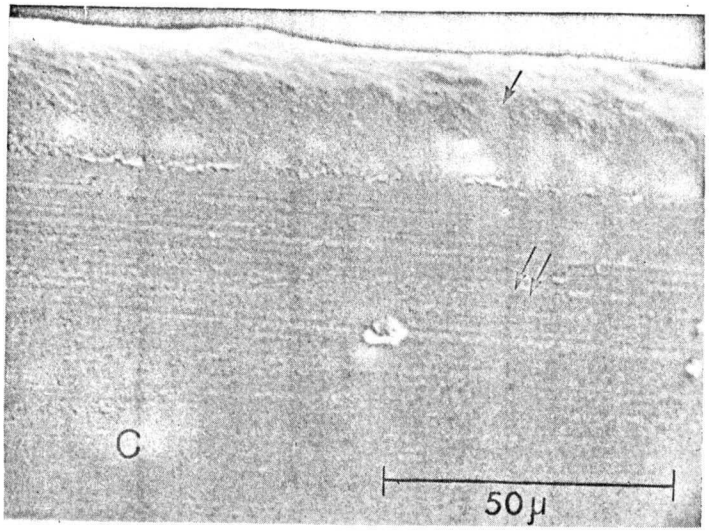


Fig 5.—Front surface (lens C). Ridge (**single arrow**) forms outer edge of lens. Circumferential peripheral grooves (**double arrows**) parallel ridge. Centrally (**C**) the surface becomes smooth (original magnification ×600).

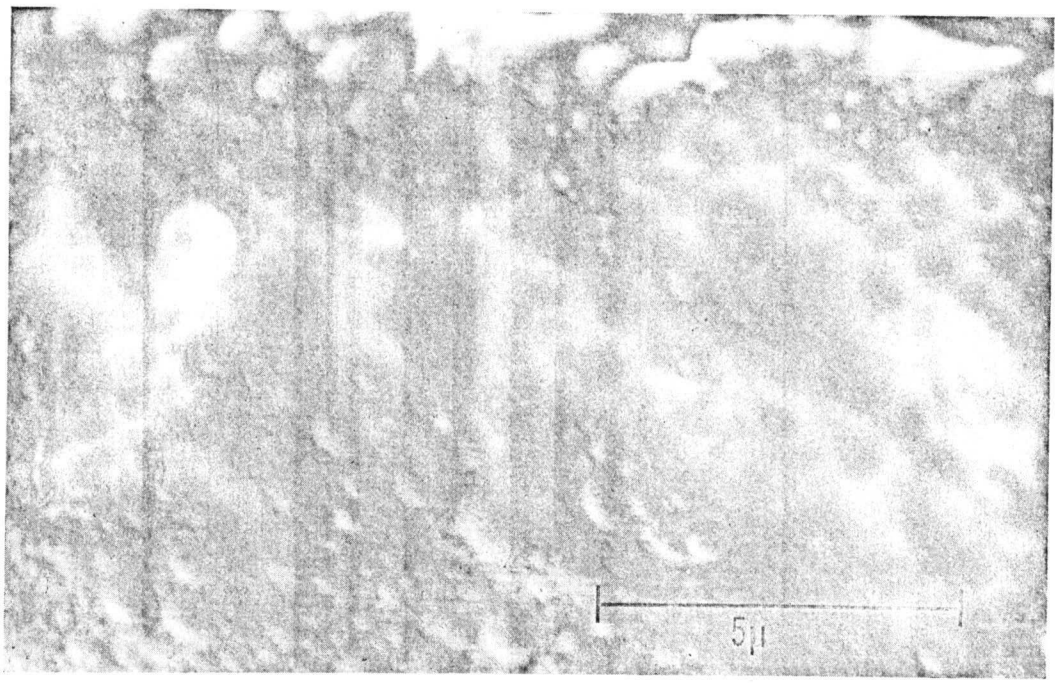


Fig 6.—Higher magnification of ridge (Fig 5) showing its generally rough appearance (lens C) (original magnification ×6,000).

the lens with no evidence of invasion of the lens.

Figure 12 (original magnification × 1,000) shows the surface of lens A which is unremarkable at *left* except for the presence of the polishing grooves. During examination of this area with the scanning electron beam, a bleb or blister appeared (*right*). The bleb was characterized by a lifting upwards of the surface as if it were covered by a thin skin. As the lifting process continued the skin ruptured along the course of the polishing grooves (*arrows*). The bleb formation could be produced on any part of the front and back surfaces of lenses A and B, but it could not be produced in lens C. When the orientation of lenses A and B was altered to expose the cut edge (Fig 13,

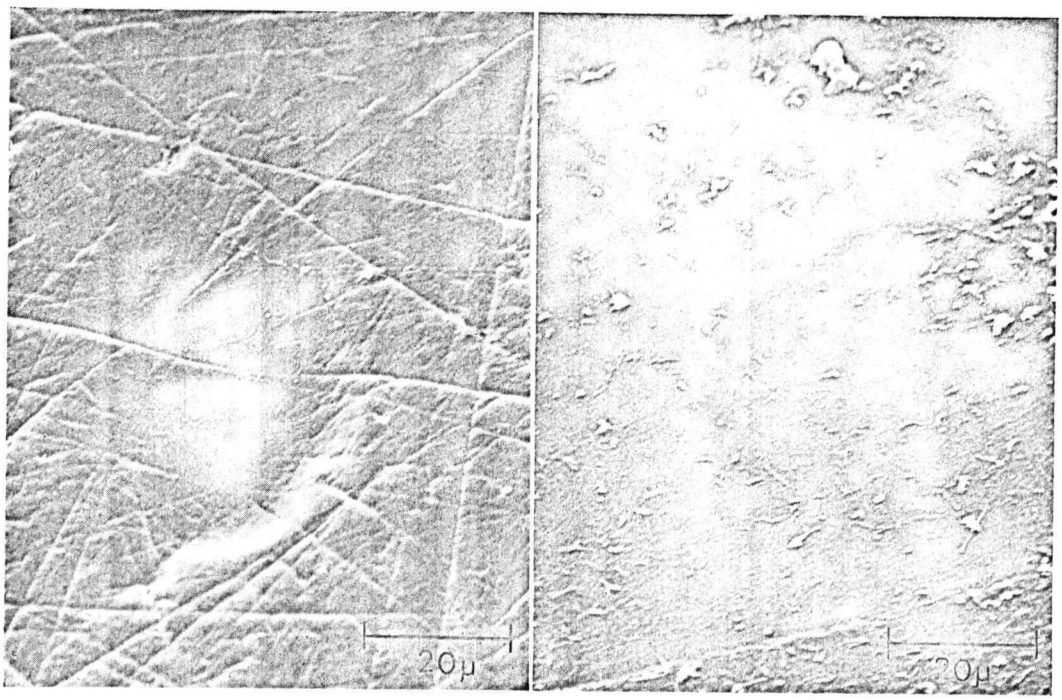


Fig 7.—**Left,** Unworn lens B compared with **(right)** lens worn and then cleaned by manufacturer's recommendations.

original magnification × 1,000) to the scanning beam, a different phenomenon occurred. Instead of forming blebs, deep cavitations (*arrow*) were produced in the substance of the lens.

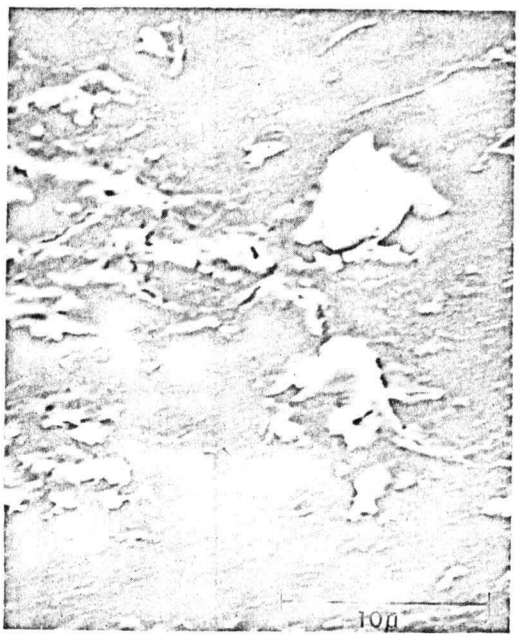


Fig 8.—Higher power view of worn, but cleaned lens B showing presence of wearing debris (original magnification ×3,000).

Comment

Boyde and Wood,[1] in a recent discussion, stated that air drying is a satisfactory technique for hard tissues, but they agree with most other investigators that soft biological specimens should not be studied by this technique because of the induced extreme distortion and loss of fine surface detail. Although the hydrophilic lens is not a biological specimen, it has some features in common with such specimens. The nonhydrated lens is brittle like some hard tissues; whereas the hydrated lens has certain similarities to soft tissues. The artifacts induced by air drying the hydrated hydrophilic lens are analagous to those seen in air dried soft biological specimens. Consequently, lenses prepared by air drying are subject to the same criticisms.

It is considered that freeze and critical point drying induce relatively little tissue distortion and that these techniques are approximately comparable in producing speci-

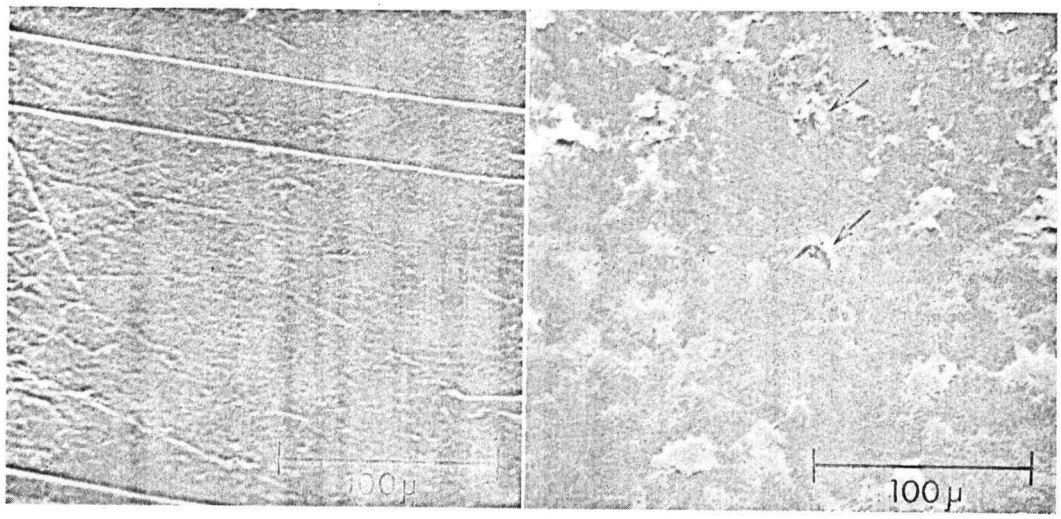
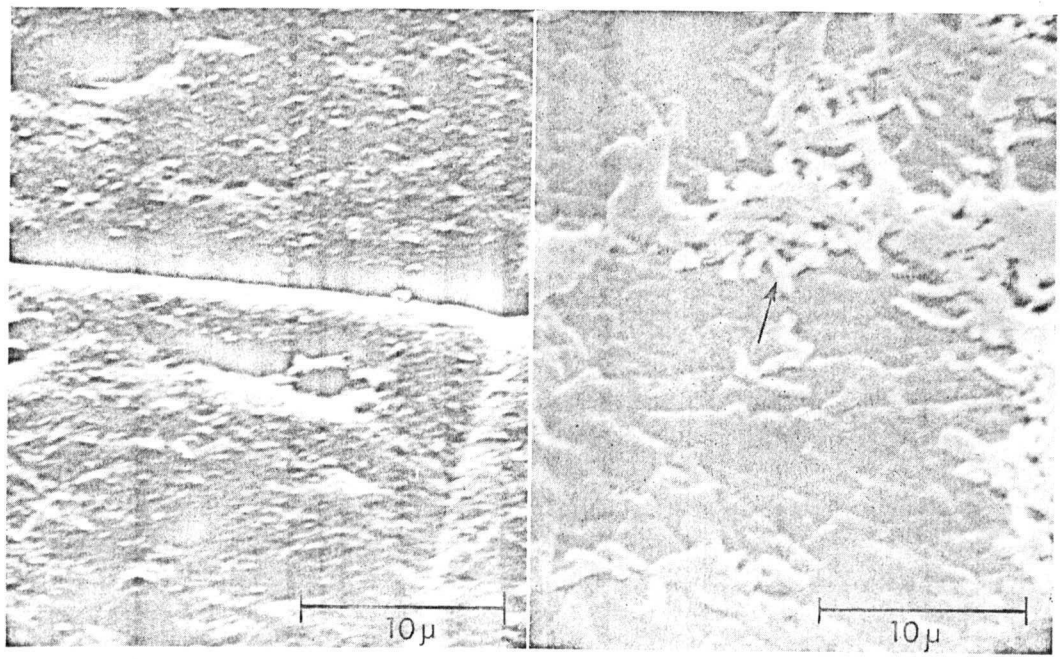


Fig 9.—**Left,** Unworn lens A compared with **(right)** lens worn and cleaned by manufacturer's recommendations. Note presence of ruptured blebs **(arrows)** (original magnification ×300).

Fig 10.—Higher power view of Fig 9 (lens A). **Left,** Unworn; **right,** worn. Rod-shaped structures 2μ to 4μ in length comprise debris **(arrow)** on worn but cleaned lens (original magnification ×3,000).



mens acceptable for scanning microscopy. Consistent with this is the fact that little observable difference in surface morphology was noted between the freeze and critical point dried hydrophilic lens segments.

The surfaces of these lenses do differ with different magnifications. Lens C is very smooth, whereas lenses A and B have relatively rough surfaces with grooves and scratches related to their manufacture.

The inner surface of lens B, which had been worn and then cleaned in the recommended manner, had some remaining debris presumed to be dried mucus probably related to the wearing; however, no bacteria were noted on this lens.

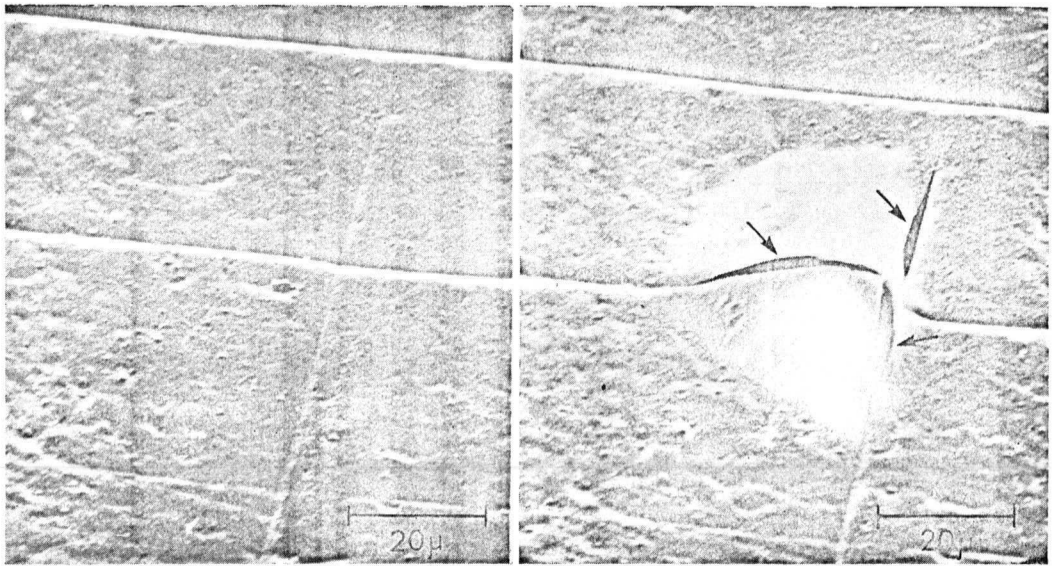
The inner surface of lens A which had previously been worn and then cleaned had

many large clumps of material present. These clumps consisted of debris and aggregates of rod-shaped structures 2μ to 4μ in length. It was only on the posterior surface of the lens that this material was found. The appearance of the rod-shaped structures suggested *Pseudomonas,* and this organism was cultured from the lens soaking solution. Scanning electron microscopy of the colonies grown in culture revealed rod-like structures identical to those noted on the lens. Another investigator[2] has reported the *Pseudomonas* contamination of these lenses and soaking solutions, and subsequently at the request of the California State Department of Public Health, lens A has been recalled. The lens A front surface is identical to the back surface with polishing scratches. Possibly the scratches and surface irregularities occurring in these lenses predispose to the adherence of mucus and other debris following wearing to permit a nidus to form where bacteria can aggregate. Hard contact lenses can be cleaned more effectively, and the debris can be more readily removed. Continual wearing appears to induce subtle changes in the surface architecture of the hydrophilic lenses which is manifested here by the surface scratches becoming less apparent.

Fig 11.—Ruptured bleb and rod-shaped structures on wearing surface (lens A) (original magnification ×3,000).

The work of Takahashi et al[3] on the permeability of hydrophilic lenses questions the functional porosity of hydrophilic lenses. They found that "the water permeability of the hydrophilic material is not greater than that of conventional methyl methacrylate" (hard hydrophilic contact lens). This finding is somewhat surprising, and further investigation of this important feature of the

Fig 12.—Lens A demonstrating formation of bleb along polishing grooves (**arrows**) (original magnification ×1,000).



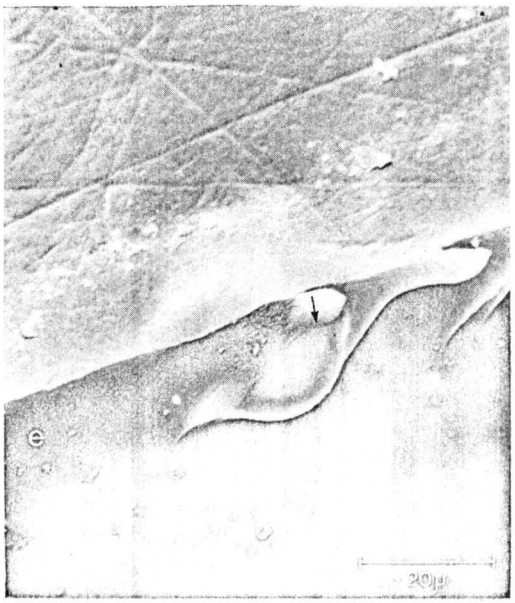


Fig 13.—Hole (arrow) formation occurring along cut edge (e) of lens B (original magnification ×1,000).

lenses will have to be performed before any definite conclusions about the permeability can be determined. In our study no pores or surface openings could be detected with the scanning electron microscope at magnifications to 36,000 times. It is possible that smaller pores are present but beyond the resolution of our instrument (~ 200 Angstroms) or, alternatively, that changes may be induced in the surface during the manufacturing process such as the heat produced during polishing which may obliterate the surface openings.

Induced Artifacts.—*Bleb and Hole Formation.*—In scanning over small areas of the outer and inner surfaces of lenses A and B, the beam of radiation induces a change in the surface. The surface skin raises upwards like a volcano producing a bleb which then ruptures or vents presumably to release gaseous material. This process has been recorded utilizing a videotape attachment (Ampex) to the scanning electron microscope operated at television-scan rate. It is well known that metals and some other substances form a surface "skin" when polished. This skin consists of an amorphous layer and is very thin. This phenomenon was described by Beilby[4] in 1903 and is known as the Beilby effect. It is possible that a similar skin forms on the surfaces of the lathed lenses. Scanning the cut edge of the specimen produces a hole rather than a bleb. The bleb formation apparently is related to the skin effect of the surface. Blebs could not be produced in lens C. Lenses A and B are presumed to differ from each other and from lens C in their composition. It can be postulated that the scanning beam produces a depolymerization of a substance which is present in lenses A and B but not in lens C, and that this results in the formation of a gas which expands and forces the surface skin upwards to produce a bleb which ruptures and permits the gas to escape. There is no skin on the cut surface so holes rather than blebs form there. The formation of blebs may be interpreted as further evidence against the presence of pores which penetrate the surface skin. It is also possible that the volume of gas produced at a rapid rate is too great to permit its escape through any tiny openings in the surface which may be present but not visible with this technique. It is not entirely clear whether these phenomena are produced by the electron radiation per se or by a nonspecific heating effect produced by the radiation.

Changes in Tonicity of the Hydrating Solution.—The changes in tonicity of the hydrating solutions appeared to have very little effect on the surface morphology examined by scanning electron microscopy. The only obvious change was the precipitation of salt crystals on the surface of the lens hydrated in hypertonic saline.

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Iris Wound Healing

Calvin Hanna, PhD, and F. Hampton Roy, MD, Little Rock, Ark

A longitudinal iridotomy was made in the rabbit between the major iris arteries. The wound edges remained in apposition, and soon after injury epithelial cells of the iris migrated and elongated to cover the wound edge. One day after injury epithelial, endothelial, and stromal cells began to undergo cell division. During the next several months, the wound gradually healed as new cells and collagen fibrils filled the defect. This is the first demonstration that iris tissue has the potential to heal and scar formation does not occur.

IT IS EASY to demonstrate that an iridectomy in man or rabbit does not heal. Fuchs[1] commented on this in 1896, and he noted the iris showed little or no tendency to scar formation in the absence of infection. In 33 cases of iridectomies in man, Henderson,[2] in 1907, found no evidence of healing. Likewise, Daniel,[3] in 1944, found an absence of healing following sector iridectomies in rabbits and suggested that the failure was due to a factor in the aqueous fluid. Further, when the human iris was kept in tissue culture fluid the cells grew very slowly.[4-7] Finally, Kobenhaun[8] observed a failure of the pigmented cells of irides to cover the defect after iridectomy accompanying cataract extraction in man.

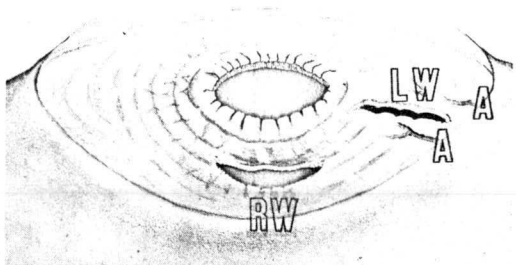
Teng et al,[9] in 1962, examined the irides of 18 eye bank eyes with previous iridectomies. They found that what little healing did occur involved the cells of the anterior surface of the iris.[9]

In each of these studies, the iris was injured in such a way that the wound gapped open. The iris has been taken as a prime example of a tissue that exhibits no potential for wound healing.[8,9] On the contrary, our studies show that the rabbit iris has all the potential for healing.

Materials and Methods

Forty-eight adult albino rabbits were anesthetized with intravenously administered 2% pentobarbital sodium followed by 400 units of intravenously administered heparin sodium. A small corneal incision near the limbus was made in both eyes and the iris herniated out through the wound. In 12 experiments an iridotomy was performed by cutting radially across the iris with scissors. The iris of the remaining animals was cut linearly between the two major arteries to spare the sphincter and to be paral-

Fig 1.—Iris, showing placement of iridotomy. Iridotomy in one group of rabbits was made across iris to give a radial wound (**RW**) which gaps open; bleeding temporarily is profuse. Linear wound (**LW**) was made between two main arteries (**A**) of iris, and edges remain together; bleeding was minimal.



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