The ocular surface of 74 subjects was assessed with the biomicroscope after sequential instillations of fluorescein (SSI) and again after a single instillation of Ophthetic® that was followed by sequential instillations of fluorescein (SSII). Twenty-six eyes (17.6%) stained after SSI and 89 eyes (60%) stained after SSII. The use of Ophthetic seems to enhance the nonuniform penetration of fluorescein into the epithelium in specific regions of the cornea of some subjects. The clinical significance of this finding is unknown.

Eye practitioners continually strive for improved techniques for screening their potential contact lens patients. The use of fluorescein to assess the ocular surface is a well-known technique. In the healthy eye, fluorescein does not stain corneal epithelial cells. Fluorescein staining of the cornea occurs only if fluorescein penetrates at sites where there is an interrupted continuity of the epithelial surface and then into the intercellular spaces between the cells.

Fluorescein has been observed in the corneal stroma when it has remained on the ocular surface over a period of time. Fluorescein staining of the corneal epithelium of apparently healthy human eyes has been described after a single instillation of fluorescein and after sequential instillation of fluorescein. The sequential instillation of fluorescein is a very sensitive method of revealing corneal surface lesions or areas that may not stain after a single instillation of fluorescein. Significant staining after the sequential instillation of fluorescein has also been associated with contact lens intolerance.

In the interest of enhancing the corneal staining phenomenon that may be observed after the sequential instillation of fluorescein (SSI), and of developing a more discriminating method for the selection of contact lens candidates, we reviewed the use and effects of certain agents that have been considered for increasing corneal permeability in rabbits and humans. Proxymetacaine 0.5% (propriacaine hydrochloride) and benzalkonium chloride have been associated with an increase in corneal permeability to fluorescein.

Materials and Methods

Seventy-four volunteer subjects (148 eyes) between the ages of 19 and 50, with an unremarkable ocular history, apparently healthy eyes and normal corneas on slit-lamp examination, were recruited after informed consent was obtained. These patients did not have any recent history of contact lens wear or use of topical ocular preparations.

Fluorescein was applied to the superior bulbar conjunctiva six times at 3 min intervals using Ayerst Fluor-i-strips A.T.® (fluorescein (9 mg) blended with chlorobutanol, polysorbate and boric acid impregnated in a paper strip; manufactured by Ayerst Laboratories, New York, NY) applicators wetted and saturated with buffered nonpreserved saline solution.

A single drop of Ophthetic® (proparacaine hydrochloride 0.5%; benzalkonium chloride 0.01% in an unbuffered medium with a nominal pH of 5.5; manufactured by Allergan Pharmaceuticals, Irvine, CA) topical anesthetic was then instilled into the inferior fornix and was then followed 2 min later by another five applications of fluorescein to the superior bulbar conjunctiva in the manner described above. For purposes of evaluation, the cornea was divided into numbered areas which were used to record and locate the areas that stained with fluorescein (Fig. 1). Evaluation of fluorescein staining of the cornea was made using a Zeiss 30SL slit lamp with a cobalt blue filter at maximal illumination. A wratten yellow filter No. 12 was attached to the front of the observation microscope to enhance the observed fluorescence. Observations were recorded approximately 60 seconds after the first and last instillations of fluorescein, respectively. Careful attention was paid to the patients'
blink rate; they were instructed to blink every 10 seconds on a regular basis, and this was supervised by the observers. One observer (JEJ) did all the recording.

Results

Twenty-six eyes (17.6%) presented staining and 122 eyes (82.4%) did not stain after the initial sequential staining procedure (SSI). After the instillation of anesthetic and subsequent instillation of fluorescein (SSII), 89 eyes (60%) stained and 59 eyes (40%) did not stain. Ten of these eyes had equal staining after SSI and SSII. No eyes showed less staining after SSII than after SSI. Sixty-three of the 122 nonstaining eyes in SSI (51.6%) presented with significant corneal staining after SSII (see flow diagram, Fig. 2). The incidence of staining in the various regions of the cornea prior to the use of anesthetic can be seen in Figure 3. The incidence of corneal staining in the various regions of the cornea subsequent to the topical instillation of Ophthetic and sequential instillation of fluorescein (SSII) can be seen in Figure 4.

Examples of various types of staining can be seen in the Figures 5–9.

Discussion

The possible etiologies of corneal staining observed after SSI have been discussed by Korb and Herman. To explore the etiology of the enhanced corneal staining observed in this experiment it is important to consider the agents used in this study. The ingredients mixed with fluorescein in Fluor-i-strips have been shown not to cause or enhance corneal staining in rabbits. One of the ingredients, chlorobutanol, does not produce toxic effects when used topically in rabbit eyes unless use has been sustained over a long period of time.

The ingredients of Ophthetic must also be considered. Although a single dose of 0.01% benzalkonium chloride has been reported to affect the rabbit cornea after the in vivo instillation of 0.02% benzalkonium chloride applied three times (1 min exposure), no definite structural changes ascribed to benzalkonium chloride could be detected. In human subjects it does not produce a significant permeability change in the epithelium to fluorescein. Proparacaine hydrochloride at 0.5% does not demonstrate any significant plasma membrane effects on the surface epithelial cells in rabbit corneas.

The effect on the epithelium of Ophthaine® (proparacaine hydrochloride 0.5%, benzalkonium chloride 0.01%, chlorobutanol 0.2%; Squibb Mark, Inc., New Brunswick, NJ), a product with similar composition to that of Ophthetic, instilled in human sub-

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<td>3 (13.6%)</td>
<td>4 (18.2%)</td>
<td>9 (34.6%)</td>
<td>14 (53.9%)</td>
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Fig. 3. The incidence of staining in the various regions of the cornea prior to the instillation of Ophthetic (SSI).

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<td>14 (18.0%)</td>
<td>21 (26.1%)</td>
<td>18 (23.1%)</td>
<td>78 (100.0%)</td>
<td>10 (12.8%)</td>
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Fig. 4. The incidence of staining in the various regions of the cornea subsequent to the instillation of Ophthetic and sequential instillation of fluorescein (SSII).
jects caused no immediate differences in sloughing rate of epithelial cells. The sloughing rate increased only gradually over a period of hours.  

Since so many subjects demonstrated this staining phenomenon we considered whether the staining was physiological. Kikkawa found that in rabbits staining with fluorescein without anesthetic was quite common and was associated with the normal physiological process of desquamation. However, Korb and Herman have associated significant corneal staining after the sequential instillation of fluorescein with contact lens intolerance, which suggests a pathological etiology. These authors have indicated that they found it impossible to determine the transition point between corneal staining which might be considered physiological and that which is pathological.

In pursuing a physiological theory of corneal staining, it has been shown that under normal conditions some surface epithelial cells are shed, leaving behind "crater-like" holes in the surface cell layer. Al-
Corneal Staining After Topical Anesthetic (SSII) / Josephson and Coffey

No. 7

1099

Although there has been no investigation of the staining characteristics of these holes, there are reports of more mitotic activity in the peripheral regions of the cornea than in the center. Perhaps the "craters" referred to present more frequently in the corneal periphery where the mitotic activity is highest. It is possible that these craters may even facilitate the spread of fluorescein between the cells in the immediate area as part of a process of fluorescein penetration, as originally suggested by Norn. The ideas presented in the proposed physiological theory of corneal staining may relate to our observations and those of Norn, that corneal staining with healthy eyes occurs most frequently in the corneal periphery. However, it is difficult to explain the dynamics of the nonstaining cornea if this is truly a normal physiological phenomenon.

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References