The authors investigated whether fluorescein sodium affects the in vitro endothelial function of rabbit corneas. As an index of this function, the transendothelial electrical potential difference (TEPD) was used.

The TEPD in a balanced salts and glucose (BSG) control solution increased for the first 30 min and then decayed slowly, reaching about 60% of its original value after 5 hr. When a BSG solution containing 5 μg/ml of fluorescein sodium was used, the TEPD time course was similar to the control solution. Since this fluorescein sodium concentration is about sevenfold higher than that seen in the anterior chamber of ocular patients, these results reassure users that no toxic effect of fluorescein is discernible at concentrations relevant to ophthalmic practice. With a fluorescein sodium concentration of 500 μg/ml, the TEPD decreased below control values after 4 hr of exposure, but such a concentration is approximately 5000-fold higher than that seen in the anterior chamber of patients. The adverse effect of fluorescein on TEPD is probably irrelevant for standard systemic clinical use.

Fluorescein sodium (or soluble fluorescein) is a fluorescent dye widely used in ophthalmic procedures. However, its influence on the various structures in the eye is not well known, and the possibility of a toxic effect has not been excluded. A compound similar to fluorescein sodium, Rose Bengal, is known to be phototoxic to corneal endothelial cells. Therefore, we investigated how fluorescein sodium affects the function of the corneal endothelial layer as evidenced by the transendothelial electrical potential difference (TEPD) generated by that layer.

The TEPD in a very sensitive index of endothelial transport. It is a manifestation of the activity of the endothelial fluid pump which maintains the cornea at the level of hydration required for transparency. In rabbit corneas at 37°C, the TEPD is approximately 0.8 mV (aqueous negative with respect to the stroma). A laboratory tool for many years, TEPD recently has been applied to evaluate endothelial function in the presence of corneal preservation solutions.

**Materials and Methods.** Animals: Three-kilogram male New Zealand white rabbits were killed with T-61 (Richard Orthotics, Germany) euthanasia solution (0.3 ml/kg) injected into the marginal ear vein. Their eyes were enucleated immediately using the technique of Dikstein and Maurice, and the epithelium was scraped off. These studies conformed to the ARVO Resolution on the Use of Animals in Research.

TEPD: The deepithelialized corneas were clamped between two cylindric hemichambers filled with Ringer's solution. An agar–saline-filled polyethylene tubing bridge attached to a calomel electrode was immersed into each hemichamber. The arrangement is shown schematically in Figure 1.

The TEPD between these two electrodes was measured with a Keithley 610C electrometer (Keithley Instruments, Inc., Cleveland, OH) and was plotted continuously on a chart recorder. The experimental chamber was enclosed in a Faraday cage to minimize electromagnetic interference. Small potential differences between electrodes and bridges were zeroed out with an adjustable series battery. A more extensive description of this technique has been given previously.

**Experimental Solutions:** The hemichambers were surrounded by thermal jackets, the temperature of which was maintained at 37°C. The solutions in both (termed stromal side and endothelial side) were aerated with a mixture of 5% CO2 and 95% air for oxygenation, stirring, and pH buffering.

For the control experiments, both hemichambers were filled with a solution containing balanced salts and glucose (BSG solution), a standard maintenance medium for the isolated endothelial preparation. This solution contained: NaCl, 110.4 mM; NaHCO3, 39.2 mM; KHCO3, 3.8 mM; KH2PO4, 1.0 mM; MgSO4·7H2O, 0.78 mM; CaCl2, 1.7 mM; and glucose, 6.9 mM. For the test experiments, fluorescein sodium (Sigma, St. Louis, MO) was dissolved directly into BSG.

**Results.** Our results are summarized in Figure 2. The TEPD in the control solution increased for the first 30 min and then decayed slowly, reaching about 60% of its original value after 5 hr. Such a time course is normal under in vitro conditions.

Two test concentrations of fluorescein sodium
were used; 5 μg/ml (or $13.3 \times 10^{-6}$ M/l) and 500 μg/ml (or $1.33 \times 10^{-3}$ M/l). At the lower fluorescein sodium concentration (test1, $13.3 \times 10^{-6}$ M/l), the TEPD was slightly (about 13%) higher than in the controls for approximately 2.5 hr. This difference was not statistically significant (by analysis of variance, $P < 0.11$). The TEPD was not different from the control values after this time. In contrast, at the higher fluorescein sodium concentration (test2, $1.33 \times 10^{-3}$ M/l), the TEPD was lower than in the controls. The difference was almost imperceptible at first but increased with time. After 5 hr, the TEPD with the test solution was only one half of the control value. For the last two points in our curves (times = 270 and 300 min), an analysis of variance found the difference between the values obtained with test2 solution and those with the control (BSG) solution to be significant ($P < 0.06$).

**Discussion.** Fluorescein conjugate concentrations in the human anterior chamber 90 min after oral ingestion have been reported to be between 50–100 ng/ml ($135–270 \times 10^{-9}$ M/l) in healthy subjects and as high as 740 ng/ml in postcataract patients. Thus even the lower fluorescein sodium concentration tested here was about sevenfold higher than the higher range of values seen in the anterior chamber clinically. Our results at the lower fluorescein concentration are therefore reassuring and suggest that no toxic effect of fluorescein is discernible at concentrations relevant to ophthalmic practice. This is all the more so since some of the anterior chamber fluorescence in patients may originate from systemic fluorescein sodium already detoxified to fluorescein glucuronide. On the other hand, fluorescein sodium at a millimolar concentration, or 5000 times larger than that seen in the anterior chamber, has an adverse effect on endothelial function (as evidenced by TEPD), especially after approximately 4 hr of exposure. However, such a high dose is so extreme that this adverse effect is probably irrelevant for standard systemic clinical use.
Key words: corneal endothelium, fluorescein sodium, transendothelial electrical potential difference

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