Abstract—To elucidate the pharmacokinetics of local anesthetics with respect to corneal permeability in the rabbit, we examined the relationship between the corneal permeability velocities of three agents, cocaine·HCl, procaine·HCl and tetracaine·HCl and corneal hydration. The corneal permeability velocity constants (k) of these three ester-type local anesthetics were approximately 0.5–6.0×10⁻⁶ cm/sec and the membrane permeability constants of these agents were approximately 0.5–4.0×10⁻⁷ cm²/sec, whereas the rabbit corneal hydration values were 3.2–4.2. Tetracaine·HCl with the strongest topical anesthetic action showed the greatest corneal hydration and the smallest corneal permeability velocity constant among these local anesthetics. Rabbit corneal permeability decreased with increasing molecular length of the agents. Permeability of these local anesthetics in the rabbit cornea appears to result from passive transport. As corneal hydration values and the corneal permeability constant increased with greater topical anesthetic activity, it appears that the degree of inhibition of Na⁺-K⁺ ATPase activity is associated with the order of topical anesthetic activity in a similar manner as general anesthetics.

Murmann et al. developed an experimental method for evaluation of topical anesthetic activity in which they utilized the blink reflex that occurs in response to touching of the rabbit cornea with a foreign substance (1). Chance and Lobstein found higher sensitivity of the guinea pig cornea compared to the rabbit cornea, and the apparent variability of this response was proportional to the dose of the local anesthetics. Based on their results, they proposed a procedure for obtaining the ED₅₀ values (2). Much research has been done on the mechanism of local anesthetic activity (3–5), but there are few reports on the pharmacokinetics of topical anesthetics in rabbit eyeball. In order to examine the filtration of local anesthetics into the eyeball when they are applied topically in the conjunctiva, it is necessary to determine intraocular permeation through the cornea.

In order to clarify the basic transport of the three ester-type local anesthetics into the eyeball, the interrelation between permeability and the properties of the drugs were examined pharmacokinetically using isolated rabbit cornea (6).

Materials and Methods

Albino rabbits weighing 3–4 kg were killed by air embolism. The eyeballs were isolated rapidly, and the sclera was excised along the external margin 2–3 mm from the corneal outline. After removing the lens, the ciliary body and iris from the anterior chamber of the eyeball, the sclerocorneal specimen was placed in the center of a corneal permeability experimental chamber (Fig. 1) (7).
Samples of cocaine·HCl, procaine·HCl and tetracaine·HCl were prepared at concentrations of 0.25, 0.5 and 1%, dissolved in an artificial tear solution (8) and the solutions were adjusted to an osmotic pressure of 290 mOsm/L and pH 7.15. A sample solution (3 ml) and artificial aqueous humor solution (9) were placed into the tear side and aqueous humor side of the chamber, respectively. The corneal permeability of the agents was facilitated by stirring during incubation at 35°C for 10, 30 and 60 min. At 10-min intervals, 0.01 N-HCl solution was added to the artificial aqueous humor solution to maintain the pH level at 7.15. After incubation, the volumes of solutions on both sides of the chamber were measured. In addition, the amount of anesthetic on the aqueous humor side and the residual quantity on the tear side were also measured with an UV spectrophotometer (cocaine·HCl: wave length 233 nm, procaine·HCl: 278 nm and tetracaine·HCl: 311 nm).

The corneal permeability velocity constant \((k)\) is expressed by the formula:

\[ k = \frac{V_2}{2A t} \ln \frac{C_0 - 2C_2}{C_0} \]

The corneal permeability constant \((p)\) is expressed by the formula:

\[ p = \frac{1}{t(1/V_1 + 1/V_2)} \ln \frac{1-(C_2/C_1)}{1+(V_2/V_1)(C_2/C_1)} \times A \]

where, \(t\): incubation time (second), \(C_0\): initial concentration of sample solution, \(C_1\): sample concentration in the tear side after \(t\) seconds, \(C_2\): sample concentration in the aqueous humor side after \(t\) seconds, \(V_1\): the sample volume in the tear side, \(V_2\): the sample volume in aqueous humor side, \(A\): the permeable area of cornea, \(Jx\): the thickness of cornea.

For the determination of corneal hydration, the wet weight (mg) of only the corneal permeable area (7 mm in diameter) was determined immediately after excision from the sclerocorneal specimen and removal of surface water on the cornea with a filter paper after incubation.

After drying the corneal portion using a thermostat vacuum desiccator with silica gel for 12 hr at 100°C, the dry corneal weight (mg) was measured for calculating corneal hydration by the equation:

\[ \text{corneal hydration} = \frac{\text{corneal wet weight (mg)} - \text{corneal dry weight (mg)}}{\text{corneal dry weight (mg)}} \]

Blank experiments were performed using the artificial tear solution without local anesthetics by a similar procedure (7, 10, 11).

**Results**

The corneal hydration values at the 3 incubation periods ranged from 3.4 to 3.6 at 10 min, from 3.9 to 4.1 at 30 min and from 3.9 to 4.2 at 60 min (Table 1). These values were approximate to the normal corneal hydration (ranged from 3.34 to 3.54) (12).

In general, the order of corneal hydration in these samples were: tetracaine·HCl > cocaine·HCl > procaine·HCl (Table 1). Moreover, as demonstrated by the blank experiments, no cornea turbidity was found within 60 min of incubation. Based on these findings, the amount of sample solution of the agents diffusing into the tear side, corneal permeability velocity constants \((k)\), and permeability constants of membrane \((p)\) were determined.

Regardless of the concentrations of these samples, the \(k\) values were approximately constant. The respective \(k\) values of local anesthetics after a 10-min incubation were approximately: 3.5 x 10^{-6} cm/sec for cocaine·HCl, 3.0 x 10^{-6} cm/sec for procaine·HCl, and 0.3 x 10^{-6} cm/sec for tetracaine·HCl, and at 60 min, 6.1 x 10^{-6} cm/sec for cocaine·HCl, 4.2 x 10^{-6} cm/sec for procaine·HCl and 1.5 x 10^{-6} cm/sec for tetracaine·HCl. Comparison of the \(k\) values at incubation times of 30 min with those at 10 min showed increases of approximately 30% for cocaine·HCl and 20% for procaine·HCl at all concentrations (Fig. 2).

The \(p\) values after a 10-min incubation...
were approximately \(1.2 \times 10^{-7}\) cm\(^2\)/sec for cocaine•HCl, \(1.0 \times 10^{-7}\) cm\(^2\)/sec for procaine•HCl, and \(0.5 \times 10^{-7}\) cm\(^2\)/sec for tetracaine•HCl, and at 60 min, \(1.6 \times 10^{-7}\) cm\(^2\)/sec for cocaine•HCl, \(1.8 \times 10^{-7}\) cm\(^2\)/sec for procaine•HCl and \(3.5 \times 10^{-7}\) cm\(^2\)/sec for tetracaine•HCl. Compared to these values, those at incubation times of 30 min were increased approximately 1.2-fold for cocaine•HCl, 1.4-fold for procaine•HCl and 1.6-fold for tetracaine•HCl (Fig. 3).

### Table 1. Effect of local anesthetics concentration and incubation time on hydration of rabbit cornea in vitro

| Local anesthetics concentration (%) | Incubation time (min) |
|-----------------------------------|------------------------|
|                                   | 10         | 30          | 60          |
| 0                                 | 3.2±0.3    | 3.2±0.1     | 3.5±0.2     |
| Cocaine•HCl                       | 0.25       | 3.4±0.1     | 3.9±0.1     | 4.0±0.3     |
|                                   | 0.50       | 3.5±0.2     | 4.1±0.3     | 4.2±0.1     |
|                                   | 1.00       | 3.5±0.3     | 3.9±0.2     | 4.0±0.2     |
| Procaine•HCl                      | 0.25       | 3.4±0.1     | 3.9±0.3     | 3.9±0.2     |
|                                   | 0.50       | 3.5±0.1     | 3.9±0.2     | 3.9±0.1     |
|                                   | 1.00       | 3.6±0.3     | 3.9±0.2     | 3.9±0.4     |
| Tetracaine•HCl                    | 0.25       | 3.4±0.2     | 4.0±0.2     | 4.1±0.3     |
|                                   | 0.50       | 3.5±0.1     | 4.1±0.2     | 4.1±0.2     |
|                                   | 1.00       | 3.6±0.3     | 4.1±0.2     | 4.1±0.1     |

Values represent the mean±standard error for 5 experiments.

**Fig. 2.** Permeability velocity constant \((k)\) of ester-type local anesthetics in rabbit cornea. Permeation time: 10 min (○), 30 min (×) and 60 min (△)

Discussion

An essential factor for maintenance of corneal transparency is that hydration of the corneal stroma layer be kept constantly by the function of corneal epithelial and endothelial layer. Many reports have shown that the external environment for lacrimation and aqueous humor secretion is involved in the increase in corneal hydration (13).

The increase in corneal hydration, i.e., the...
corneal swelling phenomenon, is largely influenced by the interaction between the mucopolysaccharides and ions in the corneal stroma layer, as well as the inflow of water into matrical constituents of the corneal stroma layer and swelling of the matrix substances. The inflow of water to the corneal stroma layer is controlled by the Na⁺-K⁺ ATPase in the endothelial and epithelial layers which is known to have a large barrier effect (14) and depends largely on the biological function of both layers, especially the endothelial layer. Furthermore, participation of this barrier effect on drug permeability has been confirmed. Accordingly, the order of corneal hydration values permits the estimation of the degree of inhibition of Na⁺-K⁺ ATPase activity, i.e., inhibition of the barrier effect in the corneal epithelial and endothelial layers.

Moreover, toxicity produced by intravenous administration in a rabbit and the degree of corneal swelling indicated by corneal hydration measurement showed almost the same tendency, that is, the order of tetracaine·HCl>cocaine·HCl>procaine·HCl (Table 1); therefore, this order seems to be equal to the degree of inhibition of Na⁺-K⁺ ATPase activity by the three local anesthetics in a similar manner as general anesthetics (15). On the other hand, tetracaine·HCl with the highest specific activity among the three agents tended to induce more remarkable swelling of the cornea than the two others. Corneal hydration was almost same as the normal values, and turbid corneas were not found within 60 min of incubation.

With respect to the corneal permeability of the three agents, the corneal permeability velocity constant (k) and the corneal permeability constant (p) tended to increase with the incubation time, both the k and p values tended to be more dependent on the incubation time than on the concentration (Figs. 2 and 3).

This may be due to swelling of the corneal stroma layer with water and the consequent decrease in the barrier effect of both layers of the cornea. The increase in permeability of these local anesthetics as a result of corneal swelling appears to be caused by expansion of the spaces in the corneal tissue (16).

The increased corneal permeability with increasing corneal hydration values suggests passive transport that depends on the intramembranous diffusion due to the local anesthetic concentration. The corneal permeability constant (p) is mostly defined by the porosity of the membrane and the type of solute. Generally, permeability of the solute is examined using a membrane found in a homogeneous structure.
The present investigation of permeability in the rabbit cornea showed that the three anesthetics yielded almost equal p values with an incubation time of either 10 or 30 min, but these values were comparable to 2/7–1/10 the values obtained for bupranolol used in glaucoma therapy under the same conditions (7) (Fig. 3).

On the other hand, when the local anesthetics are applied to the mucous membrane, it is evident that the absorption velocity of drugs is necessarily slow, thus the interrelation between corneal permeability and permeability velocity (k) was examined. The finding showed that the corneal permeability velocity constants were in the order of tetracaine-HCl<procaine·HCl<cocaine·HCl, irrespective of the incubation times; generally, these values were comparable to 1/4–1/10 the value for bupranolol (7).

Topical anesthetic action is in the order of tetracaine·HCl>cocaine·HCl>procaine·HCl. It seems that tetracaine·HCl with the lowest corneal permeability velocity constant and the strongest topical anesthetic action has the greatest affinity for the sensory nerve of rabbit cornea among the three agents employed.

However, taking into account rabbit corneal permeability and topical anesthetic action, it seems that procaine·HCl has extremely weaker affinity for the sensory nerve of rabbit cornea than tetracaine·HCl and cocaine·HCl.

A correlation was found between rabbit corneal permeability, topical anesthetic action and fat solubility in these local anesthetics. Moreover, a correlation was found between rabbit corneal permeability and molecular length of these agents (tetracaine·HCl 17.8 Å>procaine·HCl 12.9 Å>cocaine·HCl 11.5 Å).

Tetracaine·HCl with the strongest topical anesthetic activity showed the highest corneal hydration and corneal permeability constant, possibly due to the higher degree of inhibition of Na⁺-K⁺ ATPase.

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