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Corneal Penetration Behavior of β-Blocking Agents I: Physicochemical Factors

RONALD D. SCHOENWALD [x] and HONG-SHIAN HUANG [*]

Abstract □ Rabbit corneas were excised and mounted in a chamber to determine the permeability characteristics of a group of β-blocking agents which varied in octanol–water partitioning over a fourfold logarithmic range. From the permeability rate at steady state, permeability coefficients (pH 7.65) were determined. For each drug the distribution coefficient and pK_a were measured, permitting the partition coefficients to be estimated. Various correlations were determined for the log permeability coefficient as a sum of log functions of the partition (or distribution) coefficient, molecular weight, and/or degree of ionization. The best fit, as judged by a high correlation coefficient (r = 0.9756) and lack of systematic deviation, was represented by: log P_T = 0.623 log DC − 0.108(log DC)^2 − 5.0268.

Keyphrases □ β-Blocking agents—permeability characteristics, excised rabbit corneas, physicochemical factors □ Permeability—β-blocking agents, excised rabbit corneas, physicochemical factors □ Ophthalmic drugs—β-blocking agents, corneal permeability, rabbits, physicochemical factors

Whenever an ophthalmic drug is applied topically to the eye, only a small amount (<10%) actually penetrates the cornea and reaches the internal eye tissues (1–3). Precorneal factors, such as rapid drainage by the nasolacrimal apparatus and noncorneal absorption, account for the poor absorption (4). As a result, optimal absorption depends on achieving a rapid penetration rate across the cornea to minimize the competing, but nonabsorptive rate factors. Rapid penetration either permits a lower dose to be administered or, in the case of an inactive drug, leads to the development of a clinically effective drug.

The penetration potential of a drug with regard to its chemical structure can be assessed by the use of the partition coefficient of the drug. This has been shown for the cornea by Schoenwald and Ward (5) and by Mosher and Mikkelson (6). Schoenwald and Ward (5) determined the permeability rates across excised rabbit corneas for 11 steroids. Permeability coefficients for each steroid were calculated, and their logarithms were plotted against their respective log octanol–water partition coefficients. A

parabolic relationship fit the data, with optimal permeability observed at a log partition coefficient of 2.9. Likewise, Mosher and Mikkelson (6) determined the *in vitro* corneal transport of *n*-alkyl-*p*-aminobenzoate ester homologues. For this series a parabolic equation also fit the data; optimal permeability was observed at a log partition coefficient of 2.5–2.6 (*n*-propyl homologue).

Although relative potency is a significant factor, a rapid penetration rate can contribute significantly to effectiveness. For example, prednisolone acetate (1% ophthalmic suspension) has been ranked as the most effective topical anti-inflammatory agent when the epithelium of the inflamed cornea is intact (7), whereas prednisolone (equally potent orally) is not effective topically. The prodrug di-
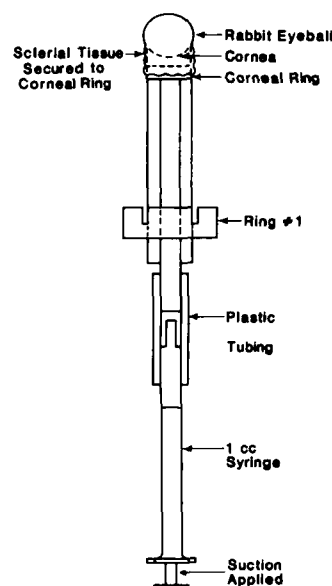

Figure 1—*Corneal holder for excised corneal preparation used in the permeability experiment.*

Table I—Chemical Structures, pK_a, and Partition Coefficients of β-Blocking Agents

Structure	Compound	pK_a	PC^a
Very lipophilic:			
OCH$_2$–CHOH–CH$_2$–NH–C(CH$_3$)$_3$	Penbut-olol	9.26	14,200.0
CHOH–CH$_2$–NH–C(CH$_3$)$_3$ (benzofuran, CH$_2$CH$_3$)	Bufuralol	8.97	4,460.0
CHOH–CH$_2$NH–CH$_2$CH$_2$– (OCH$_3$, OCH$_3$); CH$_2$–O–(phenyl)–CH$_3$	Bevant-olol	8.38	1010.0
OCH$_2$–CHOH–CH$_2$–NH–CH(CH$_3$)$_2$ (naphthalene)	Propra-nolol	9.23	1640.0
Lipophilic:			
OCH$_2$–CHOH–CH$_2$–NH–C(CH$_3$)$_3$ (tetralone)	Levbu-nolol	9.32	249.0
O–CH$_2$–CHOH–CH$_2$–NH–CH(CH$_3$)$_2$; OCH$_2$–CH=CH$_2$	Oxpren-olol	9.32	235.0
CH$_2$–CHOH–CH$_2$–NH–C(CH$_3$)$_3$; O ; N$_{\text{S}}$N–morpholine	Timolol	9.21	82.0
OCH$_2$–CHOH–CH$_2$–NH–CH(CH$_3$)$_2$; CH$_2$CH$_2$OCH$_3$	Metop-rolol	9.24	76.0
Hydrophilic:			
O–CH$_2$–CHOH–CH$_2$–NH–CH(CH$_3$)$_2$; COCH$_3$; NHOC–CH$_2$CH$_2$CH$_3$	Acebut-olol	9.20	59.0

Table I—*Continued*

Structure	Compound	pK_a	PC^a
Hydrophilic:			
CHOH–CH$_2$–NH–CH(CH$_3$)$_2$; NHSO$_2$CH$_3$	Sotalol	pK_{a1} = 8.15 pK_{a2} = 9.65	0.24
OCH$_2$–CHOH–CH$_2$–NH–C(CH$_3$)$_3$; OH–H, H–OH (tetralin)	Nadolol	9.39	8.5
OCH$_2$–CHOH–CH$_2$–NH–CH(CH$_3$)$_2$; CH$_2$CONH$_2$	Atenol-ol	9.32	1.46

^{a} Octanol–aqueous partition coefficient; the distribution coefficient was determined at pH 7.4 and 35° and converted through Eq. 2 to PC.

pivefrin is another example of a drug with improved corneal penetration when compared with the parent drug, epinephrine (8). A more rapid penetration rate for the prodrug has led to use of a reduced dosage and the observation of less ocular side effects.

The β-blocking agent timolol was introduced commercially to treat glaucoma following topical instillation of eye drops. Propranolol (9), atenolol (10), metoprolol (11), and practolol (10) also lower intraocular pressure, whereas nadolol and sotalol appear not to (12), even though nadolol is approximately equal in potency to propranolol. The purpose of this study was to compare the permeability of a series of β-blocking agents with a fourfold range in partitioning behavior across excised rabbit corneas to determine if optimal permeability can be identified.

EXPERIMENTAL

Drugs—β-Blocking agents used in the experiments included acebutolol hydrochloride[1], atenolol[2], beventolol hydrochloride[3], bufuralol hydrochloride[4], levbunolol hydrochloride[3], metoprolol tartrate[5], nadolol[6], oxyprenolol hydrochloride[5], penbutolol sulfate[7], propranolol hydrochloride[8], sotalol hydrochloride[9], and timolol maleate[10]. Structures of each drug are shown in Table I.

Potentiometric Titration Method for the Determination of pK_a—A pH meter[11] connected to a titrator[12] and equipped with a combination electrode[13] was used. The titrator was equipped with a 1.0-ml, syringe-type buret and was used for all titrations in the study. The buret was attached to a delivery tip capable of accurately metering 0.005

[1] May & Baker LTD Research Laboratories.
[2] Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, Del.
[3] Warner-Lambert Company, Pharmaceutical Research Division, Ann Arbor, Mich.
[4] Roche Products LTD, Research Department.
[5] CIBA Pharmaceutical Co., Division of CIBA-GEIGY Corp., Summit, N.J.
[6] E. R. Squibb & Sons, Inc., Princeton, N.J.
[7] Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.
[8] Ayerst Laboratories, Inc., New York, N.Y.
[9] Mead Johnson & Company, Evansville, Ind.
[10] Merck Sharp & Dohme Research Lab, Division of Merck & Co., Inc., Rahway, N.J.
[11] Model 620, Fisher Accumet pH meter.
[12] Metrohm Multi-Dosimat E415, Herisau, Switzerland.
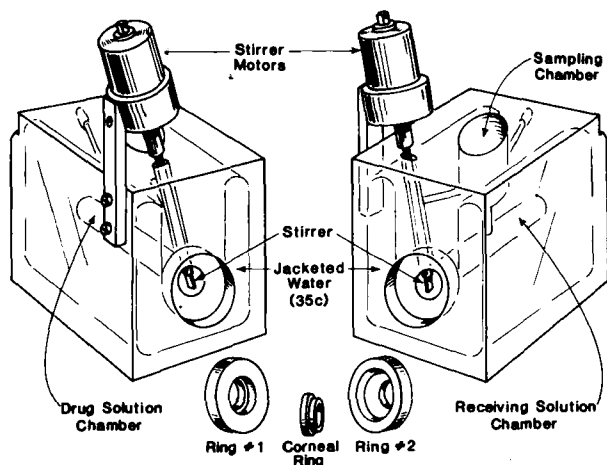[13] Metrohm AG 9100, Herisau, Switzerland.

Figure 2—*Modified perfusion chamber with the installation of two stirrers; the corneal rings and chambers are also pictured.*

ml of titrant into the titration cell. The cell had a capacity of 50 ml and was surrounded by a jacket through which 35° water was circulating. A 0.29-mg/ml solution (1 mM) of a salt of each β-blocking agent was prepared for titration. In the event that the drug species was a free base, an equivalent amount of hydrochloric acid was added. For some highly lipophilic drugs, such as penbutolol and bufuralol, a concentration as low as 100 μg/ml was used to prevent precipitation during drug titration.

An aliquot (25–40 ml) of drug solution was accurately transferred to the titration cell, maintained at 35°. Nitrogen continuously flowed over the sample solution to prevent carbon dioxide absorption from the surrounding air. At each titration interval, a volume of 0.005–0.02 ml of titrant was added while stirring. For the majority of β-blocking agents, which have pK_a values of 8–9.4 at 35°, the titration usually started at pH ~6 and ended at pH 10 or 11. A modified Gran plot was used to determine the K_a for each compound (13) with the exception of sotalol, which contained two K_a values determined by the Speakman method (14).

Determination of Distribution Coefficients (15)—Sorensen's phosphate buffer (pH 7.38) was prepared from monobasic potassium phosphate and dibasic sodium phosphate. The buffer and octanol were mutually saturated at 35° before use. The distribution coefficient at 35° was determined by dissolving drug in the aqueous-buffer phase and shaking intermittently with octanol at 35° for 5 hr to reach a distribution equilibrium. The volume ratio of octanol and buffer depended on the lipophilicity of the drug. The volumes of each phase were chosen so that drug concentration in the aqueous phase, before and after extraction, could be measured by high-performance liquid chromatography (HPLC). Centrifugation was used to separate the two phases.

The distribution coefficient (DC) was calculated by:

$$DC = \frac{(C_b - C_a)V_w}{C_b V_o} \qquad (Eq. 1)$$

where C_b and C_a represent the concentrations in the aqueous-buffer phase before and after distribution, respectively; V_w represents the volume of the aqueous phase; and V_o, the volume of the octanol phase. The partition coefficient (PC) was calculated from the distribution coefficient by:

$$PC = DC \left(1 + \frac{1}{antilog\ (pH - pK_a)}\right) \qquad (Eq. 2)$$

The pH was measured from the buffered phase at 35° after distribution was complete. All distribution coefficients reported here were measured at pH 7.4, but through the use of Eq. 2 were converted to pH 7.65, the pH of the excised corneal experiments.

Excised Cornea Procedure—Male New Zealand White rabbits[14], weighing 1.6–2.0 kg each, were sacrificed by injecting a bolus of air into the marginal ear vein. The intact eye, along with the lids and conjunctival sac, was then enucleated. The exposed cornea of the enucleated eye was carefully placed on a corneal holder, which maintained the cornea curvature and held the eye in place (5, 16, 17). Various tissues of the eye were dissected leaving the cornea, a small ring of scleral tissue, and the palpebral conjunctiva, which was tied to the corneal ring (Fig. 1).

The conjunctival and scleral tissue served as a gasket and permitted

Table II—Experimental Conditions for the HPLC Assay of β-Blocking Agents

Drug	Column[a]	Wavelength, nm	Methanol, %[b]	Flow Rate, ml/min
Acebutolol	A	254	20, 38	2
Atenolol	A	254	20, 7.5	2
Bevantolol	B	254	28, 30	2
Bufuralol	B	254	28, 30	2
Levbunolol	B	254	28, 47	2
Metoprolol	A	280	35, 35	2
Nadolol	A	254	20, 31	2
Oxprenolol	B	280	22, 15	2.5
Penbutolol	B	254	25, 62.2	2
Propranolol	B	254	28, 23	2
Sotalol	A	254	42[c], 30	2.5
Timolol	A	254	42[c], 30	2.5

[a] (A) μ-Bondapak C18; (B) μ-Bondapak CN. Waters Associates, Milford, Mass. [b] The two numbers represent the percentage of methanol in the mobile phase for partitioning and corneal permeability determinations, respectively; the aqueous phase contained 1.5% acetic acid and was adjusted to pH 4 by sodium hydroxide. [c] The aqueous phase consisted of 58% 0.005 M heptanesulfonic acid and 1% acetic acid; the flow rate was 2.0 ml/min for these conditions.

the cornea to be suspended within the corneal ring, which was then positioned between rings 1 and 2 and placed in the center of the perfusion chamber. The chamber was made from acrylic plastic[15] and was jacketed to maintain the cornea and the perfusion solution at 35° (5, 16, 17).

Bicarbonated Ringer's solution was modified (17) to preserve tissue integrity of an excised cornea over 6 hr and used throughout the perfusion studies. It was prepared in two parts: Part I was composed of sodium chloride (12.4 g/liter), potassium chloride (0.716 g/liter), monobasic sodium phosphate monohydrate (0.206 g/liter), and sodium bicarbonate (4.908 g/liter); part II was composed of calcium chloride dihydrate (0.230 g/liter), magnesium chloride hexahydrate (0.318 g/liter), glucose (1.80 g/liter), and oxidized glutathione[16] (0.184 g/liter). Both parts were stored in the refrigerator and were used in ~3 weeks to prevent mold growth. Equal volumes of parts I and II were mixed prior to use.

Within 20–40 min of death, the cornea was mounted and clamped between two cylindrical compartments of the perfusion chamber. A measured volume (7.0 ml) of bicarbonated Ringer's solution was added first to the endothelial side as the receiving solution to prevent the cornea from buckling. An equal volume of solution containing a β-blocking agent was then added to the epithelial side as the drug solution. The perfusion chamber system was designed in such a way that the height of the receiving solution was slightly higher than that of the drug solution to ensure that the cornea would not buckle during the course of the experiment. A mixture of O_2-CO_2 (95:5) was bubbled through the fluids in both chambers for 10 min to achieve a pH of 7.65 before being added to the perfusion chamber. Circulation of fluid inside each half chamber was induced immediately by bubbling the same gas mixture through at a rate of three to five bubbles/sec to maintain the solution at a constant pH of 7.65.

Samples ranged from 0.1 to 0.5 ml depending on the assay sensitivity of each drug. Samples were withdrawn from the receiving chamber (*i.e.*, endothelial side) over a 4-hr period. An equal volume of solution was immediately added to the receiving solution to maintain a constant volume. The first sample was withdrawn within 2 min after starting the permeation and served as a control to detect leakage and rapid penetration. Subsequent samples were taken approximately every 40 min through the 4-hr period.

The sampling method for the corneal permeability experiments of levbunolol and nadolol varied from other drugs in that equal volumes of solutions (0.1 ml) were removed from both sides of cornea. In this way, equal volumes on both sides were maintained throughout the experiment.

After each permeability experiment, the cornea was trimmed of excess scleral tissue and conjunctiva, weighed, and dried in an oven overnight at 103°. After each cornea was dried, it was reweighed so that the hydration level of the wet cornea could be determined. The normal cornea has a hydration level of 76–80% (18). If manipulation of the cornea or if the drug itself led to damage of the epithelium and/or endothelium, then the hydration level would rise (83–92%) and the data were discarded.

Determination of Aqueous Diffusional Layer Resistance in the Perfusion Chamber—Fluid circulation in the chamber was provided
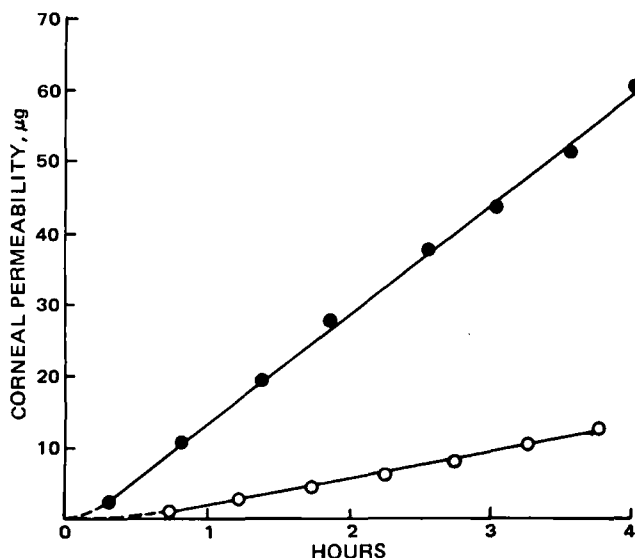
Figure 3—*Permeability rate of propranolol (●) and atenolol (○) across single excised rabbit corneas including linear regression lines; the initial concentrations for propranolol and atenolol were 85 and 2000 μg/ml, respectively.*

Figure 4—*Nonlinear regression results of the diffusional resistance of atenolol to stirring rate through excised rabbit stromal preparations. Each point is the average of 3–6 determinations; standard deviations were ≤10% of the mean.*

by maintaining a rate of three to five gas bubbles/sec. This maintains good mixing within each half chamber, as can be shown by adding a drop of colored solution into either half of the chamber and observing the homogeneous color that occurs in <1 min. However, to accurately determine the resistance of the cornea, it was necessary to detect and measure the magnitude of aqueous diffusional layer resistance, R_{aq}; consequently, the stirring was modified for these experiments.

The perfusion chamber was modified by installing two stirrers, one on each side of the cornea, with the center of each stirrer affixed 1 cm from the center of the corneal ring. Figure 2 depicts the modified perfusion chamber and rings used in mounting the cornea. In preliminary experiments it was observed that different rates of stirring induced varying degrees of swelling due to mechanical injury of the epithelium and endothelium.

An important purpose of the epithelium and endothelium is to control the thickness of the cornea by maintaining hydration levels at ~ 78%. By completely removing the epithelium and endothelium, the remaining stromal layer reached a constant and maximal thickness within the first 30 min of stirring such that subsequent changes in the stirring rate had no effect. The epithelium was removed by scraping with the blunt end of a scalpel blade. The endothelium was gently rubbed off with a cotton-tipped applicator (19). The removal of endothelium could be detected with the aid of a dissecting microscope. The epithelium was removed immediately following enucleation; the endothelium was removed just prior to mounting in the perfusion chamber. Atenolol (500 μg/ml) was chosen as the diffusing substance for these experiments. Since the aqueous diffusional barrier is independent of drug, these results were interpreted for the other β-blocking agents as well.

Once drug was placed adjacent to the cornea, the stirring speed was increased in steps every 30 min over a 4-hr period. The apparent permeability coefficients were determined for each 30-min increment. A sample was removed for atenolol analysis at the beginning and end of each 30-min period; both samples were used to calculate the permeability coefficient for each stirring speed. To minimize biological variability, each cornea was used to generate five or six permeability coefficients over a period of 4 hr.

Drug Assay—An HPLC method was used for analysis of each drug. The HPLC system included an injector[17], solvent delivery system, UV-absorption detector[17], column[18], and recorder[19]. The injector was equipped with different-sized loops, ranging from 50 μl to 200 ml, which enabled the injection of an accurate volume of sample solution. A solution of known concentration was used as an external standard. Each sample solution was divided so that two injections could be made and the results averaged.
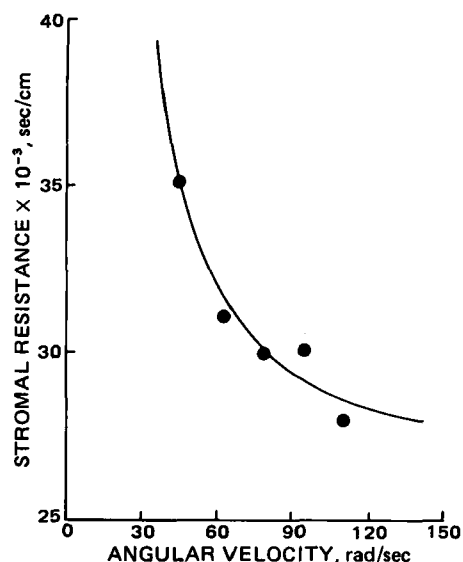
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[17] Model 7125 injector; Rheodyne, Cotati, CA 94928.
[18] M-6000 A solvent delivery system, Model 440 absorbance detector, μ-Bondapak C18, and μ-Bondapak CN Columns; Waters Associates, Milford, MA 01757.
[19] Model 5211-1; OmniScribe; Houston Instruments, Austin, Tex.

The mobile phases consisted of varying ratios of methanol and deaerated, deionized water containing 1.5% acetic acid, adjusted to pH 4 using sodium hydroxide. One exception was the mobile phase for sotalol and timolol, which utilized 0.005 M heptanesulfonic acid (to increase retention time) and 1% acetic acid. Table II lists the types of columns used, wavelengths at which UV measurements were made, methanol content of the mobile phase, and mobile phase flow rate. Methanol percentages in the mobile phases varied depending on whether partitioning or permeability experiments had been conducted. Drug solutions withdrawn from the endothelial side following corneal permeability contain polar extracts from the cornea which were eluted from the column within 1–3 min. In all experiments the retention times for the drugs were between 4 and 12 min. Linearity existed over the concentrations employed for each β-blocking agent (correlation coefficients >0.99).

Calculation of Permeability Coefficients—The apparent permeability coefficient (P_{app}, cm/sec) was determined by (19):

$$P_{app} = \frac{\Delta Q}{\Delta t (3600) A C_0}$$

(Eq. 3)

where the term $\Delta Q/\Delta t$ is the permeability rate (*i.e.*, steady-state flux, μg/hr) of drug across each excised cornea, C_0 is the initial drug concentration (μg/ml), A is the corneal surface area (cm²), and 3600 is the conversion of hours to seconds. Corrections of C_0 were made to account for the sample volume removed over time and subsequently replaced with blank solution.

The corneal thickness increases with hydration, and the permeability coefficient is inversely proportional to barrier thickness. Therefore, it was important to determine corneal thicknesses. For a 2-kg rabbit, the corneal thickness (h) can be determined by:

$$h \text{ (cm)} = \frac{0.42 + H}{100}$$

(Eq. 4)

where H is the mg of water/mg of dry tissue (20). When stromal thicknesses were swollen due to epithelial and endothelial removal, the stroma resistances, $R_{str,swl}$, that were used to assess the aqueous diffusional barrier were corrected to the normal stromal thickness as existing in intact cornea (*i.e.*, $R_{str,int}$) by:

$$R_{str,int} = R_{str,swl} \left(\frac{h_{int}}{h_{swl}}\right)$$

(Eq. 5)

where subscripts int and swl represent intact cornea and swollen stroma, respectively.

RESULTS AND DISCUSSION

The pK_a and partition coefficients of each β-blocking agent are listed in Table I. Although the aromatic substituents varied substantially for the series, these were too far removed from the amino group to exert much

of an effect on the pK_a values. Therefore, most pK_a values were within a narrow range (8.97–9.65). The pK_a of bevantolol was slightly lower (8.38) because of the electron-withdrawing effect of the ethoxybenzyl substituent. Sotalol has two pK_a values, 8.15 and 9.72, which are close to one another and compare reasonably well to the values of 8.30 and 9.80 published by Garrett and Schnelle (21) using the potentiometric titration method at 25°. The anilino group in sotalol acts as a weak acid as a result of the electron-withdrawing effect of the neighboring sulfonyl group. Ionization of the anilino group accounts for spectral shifts (21) and correlates with the pK_a of 8.15. The second pK_a, 9.72, was then assigned to the protonated amine group in the alkyl side chain of sotalol.

The distribution coefficients obtained from the extraction method using octanol and Sorenson's buffer varied over a fourfold log range. The range in partitioning behavior of the series is a consequence of the differences in aromatic substitution. The partitioning results permitted the compounds to be grouped as very lipophilic, lipophilic, or hydrophilic, classifications which were predictable from structural considerations. In addition to the amino group, the hydrophilic compounds also contained relatively polar substituents on the aromatic ring. Therefore, hydrogen bonding interactions with water are greater for atenolol and acebutolol, which contain amido groups, for nadolol, which contains a dihydroxy function and for sotalol, which contains a sulfonamido moiety. The very lipophilic compounds, on the other hand, contain hydrophobic substituents. The cyclopentyl group on the benzene ring gives penbutolol a high distribution and partition coefficient, whereas the furanyl group imparts lipophilicity to bufuralol. The ethoxybenzyl substituent in bevantolol not only lowers its pK_a, but also increases its lipophilicity. The high partition coefficient of propranolol is a result of the high lipophilic contribution of naphthalene. Based on the partitioning results of the very lipophilic and hydrophilic compounds, the remaining compounds (levbunolol, oxyprenolol, timolol, and metoprolol) appear to contain substituents of an intermediate nature as far as polarity.

Corneal Permeability—The permeability coefficients of each β-blocking agent were obtained by linear regression of the steady-state flux. Figure 3 shows a plot of Q versus t for propranolol and atenolol across excised rabbit corneas. The data points closely fit the least-square regression line once steady state has been reached. The lag time, defined by the linear intercept on the time axis, is related to the time required to reach steady-state permeation; more specifically, it is inversely related to the permeability coefficient. Consequently, the more rapidly penetrating compounds will have a shorter lag time and a greater steady-state flux. The slope of the straight line ($\Delta Q / \Delta t$), was substituted into Eq. 3 to obtain the apparent permeability coefficient. The apparent permeability coefficient also contains any aqueous diffusional layers that may exist on each side of the cornea.

Mathematical Model Relating Stirring Rate to Aqueous Diffusional Layer Resistance—The total diffusional resistance, R_{app}, through a multilayered barrier is represented by (22):

$$R_{app} = \frac{1}{P_{app}} = \sum_{i=1}^{n} R_i = \sum \frac{h_i}{D_i A(PC)_i} \qquad \text{(Eq. 6)}$$

where i represents each homogeneous barrier in series, n represents the total number of barriers, h represents barrier thickness, A represents surface area, D represents the diffusion coefficient, and PC represents the partition coefficient (22). With regard to significant diffusional layers, the rabbit cornea possesses two main tissue types: the lipophilic epithelium and endothelium, and the hydrophilic stroma. Assuming the existence of an aqueous diffusional barrier, the apparent resistance of the cornea can be considered as layers in series (23, 24) or:

$$R_{app} = \frac{1}{P_{app}} = R_{epi} + R_{str} + R_{endo} + R_{aq} \qquad \text{(Eq. 7)}$$

or

$$R_{app} = R_T + R_{aq} \qquad \text{(Eq. 8)}$$

where R_{aq} represents the sum of aqueous diffusional resistances on each side of the cornea and R_T represents the sum of the resistances of the corneal layers (epithelium, stroma, and endothelium).

According to the Nernst theory (25), there is a thin layer of static liquid of thickness h_{aq} immediately adjacent to a solid body. Outside of the static liquid layer is the well-stirred bulk solution. Experimental determinations have shown that the aqueous diffusional layer thickness, h_{aq}, can be expressed as:

$$h_{aq} = V^{-n} \qquad \text{(Eq. 9)}$$

where V is the velocity of the moving liquid. The exponent n depends

on the experimental conditions ranging from $n = 0.33$ to ≥ 1. The thickness measurements representing the diffusional layer are apparent and not real. For example, experimental measurements have shown that the liquid retains its mobility down to a distance from the solid surface smaller than h_{aq}. Despite the fact that the Nernst theory may not exactly represent the diffusional behavior at the interface of liquid and solid, it can be used empirically to calculate R_{aq} (25).

In determining the resistance of the β-blocking agents across excised rabbit corneas within the stirred perfusion chamber, the following equation, which combines Eqs. 6–9, was considered:

$$R_{app} = R_{str} + \frac{h_{aq1}}{DA(PC)} + \frac{h_{aq2}}{DA(PC)} \qquad \text{(Eq. 10)}$$

where h_{aq1} and h_{aq2} are the aqueous diffusional layer thicknesses on each side of the mounted cornea at a given stirring rate, and R_{str} represents the membrane resistance for the stroma. R_{app} is measured experimentally at a specific stirring rate, i.e., $1/P_{app}$.

By assigning $h_{aq} = h_{aq1} + h_{aq2}$ and substituting V^{-n} for h_{aq}, then Eq. 10 can be expressed as:

$$R_{app} = R_{str} + \frac{V^{-n}}{DA(PC)} \qquad \text{(Eq. 11)}$$

In the modified perfusion chamber, the stirrer is at the center of a circle 1 cm in diameter which contacts tangentially with the membrane and the perfusion chamber wall. Assuming that the liquid velocity tangential to the membrane, V, is proportional to the angular velocity of stirring, v, then Eq. 9 becomes:

$$R_{app} = R_{str} + \frac{(mv)^{-n}}{DA(PC)} \qquad \text{(Eq. 12)}$$

where m is a proportionality factor between the liquid velocity (cm/sec) and the angular velocity (rad/sec)[20]. To perform the nonlinear regression analysis the diffusion coefficient (D) was approximated by 1×10^{-5} cm^2/sec, an appropriate estimate for compounds of 200–300 molecular weight (22); PC was assigned a value of 1 for the aqueous system, and A was assigned a value 1.087 cm^2, which represented the surface area of the cornea used throughout the study. The remaining unknown parameter values (R_T, m, and n) were determined from the nonlinear regression analysis[21]. Figure 4 shows the results for atenolol permeation through excised stromal preparations with stirring rates varying from 425 to 1050 rpm. The computer-generated parameter values substituted into Eq. 10 become:

$$R_{app} = 26.8 \times 10^3 + 100,000 \, (0.1006v)^{-1.67} \qquad \text{(Eq. 13)}$$

where 26.8×10^3 sec/cm represents the intrinsic stromal resistance, R_{str}. The apparent stromal resistance to atenolol permeation was 30.5×10^3 sec/cm. This latter value represents the experimental conditions for the perfusion chamber when stirred with the bubbling action of O_2–CO_2. The difference between the two resistances ($R_{app} - R_{str}$) is 3.7×10^3 sec/cm and represents the aqueous diffusional layer resistance, R_{aq}. This value was used in determining the intrinsic membrane resistances for the other β-blocking agents.

Permeability versus Partitioning Correlations—Figure 5 shows a plot of log P_T [22] against log PC; Table III contains the calculated parameter values. The data, although somewhat scattered, shows a plateau region for the very lipophilic compounds (propranolol, bufuralol, bevantolol, and penbutolol). The rate-determining factor responsible for the plateau region is not a result of the aqueous diffusion layer, since its contribution was subtracted from the experimentally determined permeability coefficients. The permeability rate is probably controlled by the hydrophilic stroma for these very lipophilic compounds. The relatively poor permeability shown for the hydrophilic compounds nadolol and sotalol possibly explains their poor potential for lowering intraocular pressure.

Multiple regression analyses[23] (26) were performed on the data to find the best set of parameters to describe the change in log P_T with a change in either log PC or log DC. Although the ranges in molecular weight and pK_a were relatively narrow for the β-blocking agents selected for study,
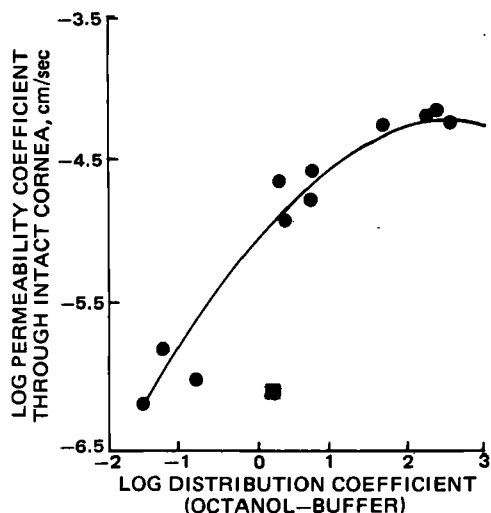
Figure 5—*Log–log plot of permeability coefficient (pH 7.65) and distribution coefficient (pH 7.65). The regression curve is represented by:* log P_T = 0.623 log DC − 0.108 (log DC)^2 − 5.0268, where r = 0.9756 and n = 11; acebutolol (■) is included in the figure, but not in the regression curve.

a log MW term and a log DI[24] term were included in the analysis. Molecular weight (MW) is inversely related to diffusion and has been shown to improve correlations of this type (27, 28). Because of the plateau region (Fig. 5), a (log PC)^2 or (log DC)^2 term was also included. All possible subsets were analyzed, beginning with the intercept plus one parameter, then the intercept plus two parameters, etc., up to the single set representing the intercept plus the maximum of four parameters. The correlation coefficient (r) and systematic deviation were used as the criteria to judge the best fit. The best fit for log P_T was represented as a function of all the parameters:

$$\log P_T = 1.01 \log PC - 0.115(\log PC)^2 - 5.64 \log MW$$
$$- 10.4 \log DI + 7.27 \qquad \text{(Eq. 14)}$$
$$r = 0.9272 \qquad p = 0.0041 \qquad n = 12$$

Both molecular weight and degree of ionization showed the expected inverse relationship to permeability. However, with either the molecular weight or degree of ionization term omitted from the regression analysis, the correlation coefficient was reduced only minimally to 0.8989 or 0.8678, respectively. With both molecular weight and degree of ionization removed, the correlation coefficient was 0.8560. With only the log PC term and the intercept, the regression analysis yielded a correlation coefficient of 0.8523. This latter linear regression line, however, shows systematic deviation at the plateau region and was not considered an acceptable fit to the data.

When DC was substituted for PC the multiple regression analyses produced an equally good fit:

$$\log P_T = 0.681 \log DC - 0.123(\log DC)^2 - 5.04 \log MW$$
$$- 2.64 \log DI + 7.22 \qquad \text{(Eq. 15)}$$
$$r = 0.9282 \qquad p = 0.0040 \qquad n = 12$$

When the degree of ionization was removed from consideration in Eq. 15, the correlation coefficient was reduced slightly to 0.9223. The lack of improvement from considering the degree of ionization is understandable, since the distribution and permeability coefficients represent the data at the same pH. By excluding the molecular weight term and (log DC)^2, the correlation coefficient was 0.8908 illustrating the small, but necessary, contribution of the squared term when systematic deviation is considered.

The hydrophilic acebutolol deviated the most from any regression line. By considering acebutolol as an outlier and excluding it from the regression analysis, the correlation coefficients increased. For example, the best regression lines yielded:

$$\log P_T = 0.972 \log PC - 0.112(\log PC)^2 - 2.71 \log MW$$
$$- 9.26 \log DI + 0.219 \qquad \text{(Eq. 16)}$$

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[24] DI represents degree of ionization and was calculated from: DI = 1/[1 + antilog (pH − pK_a)].

Table III—Permeability Coefficients and Physical Constants of β-Blocking Agents[a]

Drug	log P_T, cm/sec	log DC	log PC	log MW	log DI
Penbutolol	−4.22	2.53	4.15	2.46	−0.0106
Bufuralol	−4.14	2.31	3.65	2.44	−0.0200
Bevantolol	−4.17	2.19	3.00	2.50	−0.0740
Propranolol	−4.24	1.62	3.21	2.41	−0.0114
Levbunolol	−4.76	0.72	2.40	2.51	−0.0092
Oxprenolol	−4.56	0.69	2.37	2.42	−0.0092
Timolol	−4.91	0.34	1.91	2.49	−0.0119
Metoprolol	−4.62	0.28	1.88	2.50	−0.0110
Acebutolol	−6.07	0.20	1.77	2.52	−0.0119
Nadolol	−5.99	−0.82	0.93	2.49	−0.0079
Sotalol	−5.79	−1.25	−0.62	2.43	−0.0040
Atenolol	−6.17	−1.52	0.16	2.42	−0.0092

[a] P_T represents the permeability coefficient across the intact excised rabbit cornea; DC represents distribution coefficient; PC represents partition coefficient; MW represents molecular weight; DI represents degree of ionization.

$$r = 0.9696 \qquad p = 0.0008 \qquad n = 11$$

$$\log P_T = 0.623 \log DC - 0.108(\log DC)^2 - 5.0268 \qquad \text{(Eq. 17)}$$

$$r = 0.9756 \qquad p < 0.00009 \qquad n = 11$$

Equation 17 predicts an optimum log DC value of 2.88, determined by setting d log P_T/d log DC equal to zero and solving for log DC. However, there is no experimental evidence that a parabola would best describe the data. Compounds of greater lipophilicity than penbutolol could not be obtained to test this phenomenon.

Although correlations of this type are helpful in predicting useful molecular modifications, extrapolation to *in vivo* ophthalmic bioavailability must take into consideration solubility, the short residence time of instilled drops in the eye, and rapid metabolism or poor distribution to the target tissue. For example, a drug may have ideal partitioning behavior, but if it is not soluble, its concentration in tears will be too low to achieve an adequate penetration rate since the penetration rate is equal to the permeability coefficient multiplied by tear concentration. If a suspension is formulated because of the poor drug solubility, expulsion of the particles by the eye may take place before solubilization occurs, resulting in lower bioavailability.

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Corneal Penetration Behavior of β-Blocking Agents II: Assessment of Barrier Contributions

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Abstract □ Rabbit corneas were excised and mounted in a chamber to determine the permeability characteristics of a group of β-blocking agents. By measuring the permeability rate of each drug across intact cornea, stroma alone, epithelium–stroma, and stroma–endothelium, it was possible to determine the resistance to penetration for each corneal layer. The reciprocal of the sum of resistances for the epithelium, stroma, and endothelium equaled the experimentally determined permeability coefficient for the intact cornea (104 ± 6.0%). Thus, the penetration of β-blocking agents through the excised rabbit cornea could be treated as three barriers in series. For hydrophilic compounds, the epithelium was the rate-determining barrier. The endothelium offered less resistance, whereas the stroma offered only very minimal resistance. The lipophilic compounds penetrated the excised cornea more rapidly. However, the stroma became rate-determining for the most lipophilic compounds (penbutolol, bufuralol, bevantolol, and propranolol). Although the octanol–buffer (pH 7.65) distribution coefficient of these compounds varied over a fourfold logarithmic range, the permeability coefficient was considered nearly constant [3.4×10^{-5} (±0.34) cm/sec] for stroma. Also, the ratios of tortuosity to porosity for the stromal layer were 1.58 ± 0.15. These results suggest that drug diffuses through an aqueous media of gel-like mucopolysaccharide interspersed by a matrix of collagen fibrils. From further analyses intra- and intercellular pathways for epithelium and endothelium were added to the model resulting in a sigmoidal representation of permeability coefficient *versus* distribution coefficient. However, the intercellular (pore) pathway could not be adequately quantified because of the variation in the data for very hydrophilic compounds.

Keyphrases □ β-Blocking agents—permeability characteristics, excised rabbit corneas, barrier contributions □ Permeability—β-blocking agents, excised rabbit corneas, barrier contributions □ Ophthalmic drugs—β-blocking agents, corneal permeability, rabbits, barrier contributions

To optimize the penetration rate of drugs across biological membranes, quantitative multiple regression analyses are conducted to relate permeability to various physicochemical factors (1–3). These factors are often related through a sum of log terms, including partition coefficient, molecular weight, and degree of ionization. With the use of a digital computer and the appropriate algorithms, the regression analysis can be performed by a stepwise addition or deletion of each term or by comparing all possible subsets of the terms (4). In this way the significance of each term can be ascertained. Once all relevant physiochemical properties have been defined, an optimal chemical structure can be proposed. This semi-empirical approach, however, does not characterize the biological limitations imposed by the membrane, such as the significance of parallel aqueous pore pathways or limiting diffusional layers.

The permeability coefficients (P_T) of 12 β-blocking agents through excised rabbit corneas mounted in a perfusion chamber at pH 7.65 were determined in the previous paper (5). Through multiple regression analyses (excluding one outlier), log P_T could be related to partitioning factors by:

$$\log P_T = 0.6228 \log DC - 0.1081(\log DC)^2 - 5.03$$
$$r = 0.9756 \quad p < 0.00009 \quad n = 11 \qquad \text{(Eq. 1)}$$

where DC represents the octanol–buffer (pH 7.65) distribution coefficient. Neither a log molecular weight term nor a log degree of ionization term significantly improved the correlation. The parabolic equation represented in Eq. 1 predicted optimal penetrability at a log DC value of 2.88, the apex of the parabola. However, the experimental data (log P_T *versus* log DC) was curvilinear, leveling off to a plateau such that the asymptotic transport model of Ho *et al.* (6) could be applied. It is the purpose of this study to determine the limiting biological factors governing the steady-state flux of β-blocking agents across the multilayered excised rabbit cornea.

EXPERIMENTAL

Drugs—β-Blocking agents used in the experiments were acebutolol hydrochloride[1], atenolol[2], bevantolol hydrochloride[3], bufuralol hydrochloride[4], levbunolol hydrochloride[3], metoprolol tartrate[5], nadolol[6],