(18) D. M. Maurice and M. V. Riley, in "Biochemistry of the Eye," C. N. Graymore, Ed., Academic, London and New York, 1970, pp. 6–16.
(19) J. H. Kim, K. Green, M. Martinez, and D. Paton, *Exp. Eye Res.*, **12**, 231 (1971).
(20) B. O. Hedbys and S. Mishima, *Exp. Eye Res.*, **5**, 221 (1966).
(21) E. R. Garrett and K. Schnelle, *J. Pharm. Sci.*, **60**, 833 (1971).
(22) G. L. Flynn, S. H. Yalkowsky, and T. J. Roseman, *J. Pharm. Sci.*, **63**, 479 (1974).
(23) I. Fatt in "Physiology of the Eye," Butterworth, Woburn, Mass., 1978, pp. 114–121.
(24) R. D. Schoenwald and J. A. Houseman, *Biopharm. Drug Dispos.*, (1982), in press.
(25) V. G. Levich, in "Physicochemical Hydrodynamics," Prentice Hall, Englewood Cliffs, N.J., 1962, pp. 40–46.
(26) N. Draper and H. Smith, in "Applied Regression Analysis," 2nd ed., Wiley, New York, N.Y., 1981, pp. 294–312.
(27) E. J. Lien and P. H. Wang, *J. Pharm. Sci.*, **69**, 648 (1980).
(28) E. J. Lien, A. A. Alhaider, and V. H.-L. Lee, Jr. *Parenter. Sci. Technol.*, **36**, 86 (1982).

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

HONG-SHIAN HUANG *, RONALD D. SCHOENWALD ˣ, and JOHN L. LACH

Received July 26, 1982, from the *Pharmaceutics Division, College of Pharmacy, University of Iowa, Iowa City, IA 52242.* Accepted for publication September 16, 1982. *Present Address: National Defense Medical Center, P.O. Box 8244-14, Taipei, Taiwan 107, ROC.

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 semiempirical 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],

[1] May & Baker LTD Research Laboratories.
[2] Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, Del.
[3] Warner-Lambert Co., 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.

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Table I—Permeability Coefficients and Hydration Levels for the Permeation of β-Blocking Agents Across Excised Corneal Preparations [a]

β-Blocking Agent	Intact Cornea		Stromal and Endothelial Layers		Epithelial and Stromal Layers		Stroma Only	
	P_{app} [b]	HL [c]	P_{app} [b]	HL [c]	P_{app} [b]	HL [c]	P_{app} [b]	HL [c]
Penbutolol	44.9(5.1)	80.3(1.4)	29.4(5.4)	90.4(0.5)	—	—	—	—
Bufuralol	57.0(6.8)	79.7(1.1)	40.2(1.7)	90.5(0.3)	48.1(0.3)	89.5(0.7)	39.5(1.7)	92.5(1.0)
Bevantolol	53.9(5.0)	79.4(0.7)	34.0(2.7)	90.1(0.4)	45.1(4.6)	90.2(0.5)	34.3(2.3)	93.0(0.4)
Propranolol	47.6(1.7)	79.5(0.4)	31.2(1.2)	89.4(0.7)	39.3(5.1)	90.3(0.3)	35.1(1.6)	91.7(0.3)
Levbunolol	16.4(1.4)	81.5(1.3)	25.3(1.7)	90.3(0.9)	—	—	—	—
Oxprenolol	25.1(1.2)	79.6(1.1)	31.0(1.1)	88.3(0.2)	26.1(1.6)	89.9(0.2)	36.7(4.0)	91.9(0.2)
Timolol	11.7(1.3)	77.4(2.9)	25.6(1.3)	87.8(0.6)	—	—	—	—
Metoprolol	22.0(1.6)	79.0(0.7)	28.2(2.5)	87.5(0.3)	23.0(0.9)	89.0(0.3)	33.7(1.0)	91.9(0.5)
Acebutolol	0.85(0.06)	76.3(0.90)	9.33(0.91)	86.1(2.5)	0.97(0.060)	89.9(0.5)	30.0(2.1)	92.6(0.3)
Sotalol	1.60(0.40)	77.5(1.5)	18.3(1.8)	90.9(0.8)	—	—	—	—
Nadolol	1.03(0.12)	77.1(0.7)	15.0(0.7)	91.3(1.4)	—	—	—	—
Atenolol	0.67(0.10)	77.0(1.7)	15.7(1.1)	91.7(0.4)	0.64(0.27)	87.6(0.5)	32.8(2.0)	92.0(0.2)

[a] Standard deviation in parentheses. [b] Apparent permeability coefficient (10^{-6} cm/sec); n = 4–8 for each determination. [c] Hydration level (percent of water in excised cornea following permeation experiment).

oxyprenolol hydrochloride[5], penbutolol sulfate[7], propranolol hydrochloride[8], sotalol hydrochloride[9], and timolol maleate[10]. The distribution coefficients used in this study, as well as the general procedure for determining the coefficients, were described in the previous paper (5).

Excised Cornea Procedure—Male New Zealand White rabbits[11], weighing 1.6–2.0 kg each, were sacrificed by injecting a bolus of air into the marginal ear vein. The experimental procedure for excising and mounting the corneas in the perfusion chamber were described previously (5). Four different corneal preparations were used in the permeability experiments: the intact cornea, stroma, epithelium–stroma, and endothelium–stroma.

The epithelium and/or endothelium was removed before mounting in the perfusion chamber. The entire epithelium was removed immediately after enucleation by scraping with the blunt end of a scalpel blade. The endothelium was removed after excising the cornea and attaching it to the corneal ring, but just prior to mounting in the perfusion chamber. It was removed by carefully and gently rubbing the endothelial surface with a cotton-tipped applicator (7, 8). The removal of endothelium could be detected with the aid of a dissecting microscope. Whenever a particular corneal layer was removed, the remaining layers were left undisturbed. Solutions used during the permeability experiments as well as sampling procedure, assay methodology, and permeability coefficient calculations were described in the previous paper (5).

Corneal Thickness—Following each permeability experiment, the corneal preparations were weighed and dried in an oven at 103° for 8–12 hr. The dried corneal mass was weighed so that the hydration level of the cornea during steady state could be determined. For a 2-kg rabbit the thickness of the cornea can be determined by:

$$q(\text{cm}) = \frac{0.42 + \text{H}}{100} \qquad \text{(Eq. 2)}$$

where H represents mg of water/mg of dry tissue (9).

The rabbit cornea can be divided into three distinct diffusional layers. The outer (epithelium), consisting of 6–10 cellular layers, is the most lipophilic. The inner layer, which is also lipophilic, consists of a single layer of endothelial cells. The middle layer (stroma) is a hydrophilic layer which accounts for 90% of the corneal thickness. The epithelium and endothelium control hydration and therefore normal thickness; however, when the cornea swells it is the stromal layer only which collects fluid and swells. Consequently, for a 2-kg rabbit the epithelial and endothelial thicknesses remain constant at 0.00385 and 0.0005 cm, respectively (10). From these values and from the experimentally determined hydration levels, Eq. 2 was used to correct for differences in stromal thickness for all corneal preparations. One particular result of stromal swelling is that its thickness but not its diameter increases, which is the reason for the linear form of Eq. 2.

Calculation of Corneal Layer Resistances—The total diffusional resistance, R_{app}, through the multilayered cornea is represented by (11):

$$R_{app} = \frac{1}{P_{app}} = \sum_{i=1}^{n} R_i = \sum \frac{h_i}{D_i A (\text{PC})_i} \qquad \text{(Eq. 3)}$$

where P_{app} is the experimentally measured permeability coefficient, i is the designation for each homogeneous barrier in a series of n barriers, h is the barrier thickness, A its surface area, D represents the effective diffusion coefficient, and PC represents the effective partition coefficient between the barrier and its adjacent phase.

The calculated permeability coefficients were converted to their reciprocals and expressed as resistances. Including the aqueous diffusional barrier, the apparent resistance of the excised cornea can be represented as a sum of barriers in a series:

$$R_{app} = R_T + R_{aq} \qquad \text{(Eq. 4)}$$

and

$$R_T = R_{epi} + R_{str} + R_{endo} \qquad \text{(Eq. 5)}$$

where R_{aq} is the sum of the aqueous diffusional resistances on each side of the cornea in the perfusion chamber and R_T is the sum of resistances of the significant layers of the cornea (epithelium, stroma, and endothelium).

The resistance of the aqueous diffusional layer in the perfusion chamber was 3.7×10^3 sec/cm using O_2–CO_2 gas (5:95) to induce stirring. R_{aq} was determined for atenolol by comparing the permeability coefficient of the drug at different stirring rates using a modified perfusion chamber equipped with a stainless steel stirrer (5); R_{aq} was then deducted from the apparent resistances to obtain the intrinsic resistance for intact cornea, stroma, epithelium–stroma, and stroma–endothelium for all compounds.

The thicknesses varied for each corneal preparation depending on whether the cornea was intact or the epithelium and/or endothelium was removed. To apply Eq. 5, resistances were corrected for experimentally induced differences in thicknesses. Since resistance is directly proportional to barrier thickness, the resistances could be corrected to the normal stromal thickness as existing in the intact cornea ($R_{str,int}$) by:

$$R_{str,int} = R_{str,swl}\left(\frac{h_{int}}{h_{swl}}\right) \qquad \text{(Eq. 6)}$$

Table II—Calculated Log Distribution Coefficients and Log Permeability Coefficients [a] for Epithelium, Stroma, Endothelium, and Intact Cornea

β-Blocking Agent	Log P_{epi}	Log P_{str}	Log P_{endo}	Log P_T [b]	Log DC [c]
Penbutolol	−2.23	−3.84	−3.94	−4.22	2.53
Bufuralol	−3.39	−3.80	−3.64	−4.14	2.31
Bevantolol	−3.05	−3.84	−3.90	−4.17	2.19
Propranolol	−3.11	−3.91	−3.97	−4.24	1.62
Levbunolol	−4.52	−3.89	−4.19	−4.76	0.72
Oxprenolol	−4.22	−3.87	−4.10	−4.56	0.69
Timolol	−4.74	−3.90	−4.27	−4.91	0.34
Metoprolol	−4.34	−3.92	−4.19	−4.62	0.28
Acebutolol	−6.00	−3.93	−4.95	−6.07	0.20
Nadolol	−5.95	−3.93	−4.58	−5.99	−0.82
Sotalol	−5.77	−3.95	−4.38	−5.79	−1.25
Atenolol	−6.22	−3.93	−4.53	−6.17	−1.52

[a] Permeability coefficients have the dimensions of cm/sec (n = 4–8 for each determination. [b] P_T represents the permeability coefficient for the excised intact cornea (n = 4–8); R_{aq} has been subtracted. [c] DC represents the distribution coefficient between octanol and Sorensen's buffer at pH 7.65.

[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] Morrison Rabbitry, West Branch, Iowa.

Table III—Comparison of the Excised Intact Corneal Resistance to the Total Resistance of the Three Corneal Composite Layers Obtained Separately From Various Corneal Preparations

β-Blocking Agent	$R_{epi} + R_{str} + R_{endo}$[a], 10^3 sec/cm	R_T[b] (Intact Cornea), 10^3 sec/cm	$(R_{epi} + R_{str} + R_{endo})/R_T$, %
Bufuralol	13.6	13.8	98.6
Bevantolol	16.4	14.9	110.0
Propranolol	19.1	17.3	110.0
Oxprenolol	37.2	36.1	103.0
Metoprolol	45.5	41.8	109.0
Acebutolol	1104.5	1177.3	93.8
Atenolol	1584.6	1491.3	106.0
			Average 104 ± 6%

[a] Determined from epithelium–stroma, stroma–endothelium, and stroma corneal preparations (n = 4–8). [b] Determined from intact corneas (n = 4–8).

where h is the stromal thickness and the subscripts int and swl represent intact cornea and swollen stroma, respectively. Equation 6 was also used for another purpose: R_{epi} could be calculated by subtracting $R_{str,swl}$ from $R_{epi/str}$. Although the stroma was swollen in both preparations, their thicknesses were not exactly equal; therefore, the $R_{str,swl}$ value was first adjusted to the same stromal thickness as occurred for $R_{epi/str}$. This was done using the hydration levels and Eqs. 2 and 6. By the same procedure R_{endo} was calculated from the resistance value experimentally determined for the stroma–endothelium corneal preparation.

RESULTS AND DISCUSSION

The permeability coefficients were obtained by linear regression of the quantity of drug penetrating the corneal preparation over time after steady state had been reached. Table I lists the averaged permeability coefficient of each β-blocking agent for intact cornea, stroma, epithelium–stroma, and stroma–endothelium; Table I also lists the hydration levels obtained for each corneal preparation. Excised intact cornea maintained its transparency and rarely exceeded a hydration level of 80% after 4 hr of permeation. If the drug concentration was above a certain level (which varied for each drug), swelling occurred and consequently the hydration level increased. This was a result of cationic drug interaction with the cornea. For each drug a concentration was used that did not induce intact corneal swelling. For stroma, epithelium–stroma, and stroma–endothelium, swelling could not be avoided since the removal of the epithelium and/or endothelium caused the swelling and not the drug. It was determined in preliminary experiments that swelling, and hence stromal thickness, gradually increased over time reaching 95% of maximum in 0.45, 1.0, and 0.30 hr for stroma, epithelium–stroma, and stroma–endothelium corneal preparations, respectively.

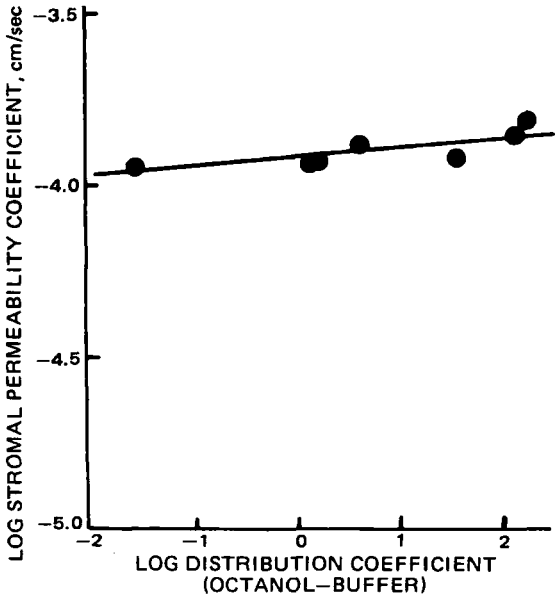

Figure 1—*Log–log plot of permeability coefficient through stroma (P_{str}) corrected to intact corneal thickness* versus *distribution coefficient (octanol–Sorenson's buffer, pH 7.65) for seven β-blocking agents. Linear regression: slope = 0.0292, intercept = −3.9098, and r = 0.8062.*

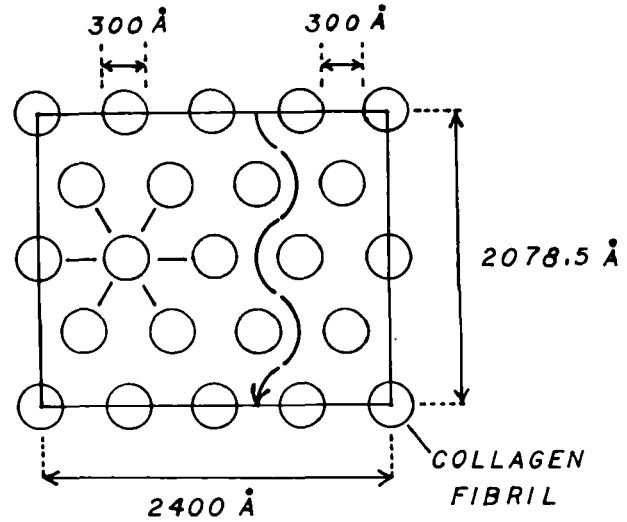

Figure 2—*Arrangement of collagen fibrils geometrically interspersed in the stroma as proposed to calculate the ratio of tortuosity to porosity (= 1.56) from stromal diffusion. The curved arrow between the fibrils represents the diffusional pathway of least resistance.*

Table II lists the calculated log values of R_{str}, R_{epi}, and R_{endo}. R_{str} was determined from $R_{str,swl}$ by correcting for thickness differences (Eq. 6) to the resistance expected in a normal cornea with a hydration level of 78.7%, the average obtained for all determinations of intact cornea (n = 43). Table III shows a comparison between the resistances of excised intact corneas, each determined from separate experiments, and that of the sum of three composite layers (Eq. 5) estimated from the various corneal preparations. Good agreement (106 ± 4%) exists for the seven β-blocking agents for which complete data were generated. These results indicate that the penetration of β-blocking agents through the excised rabbit cornea could be treated as three barriers in series.

Stromal Diffusion—Figure 1 represents a plot of log P_{str} *versus* log DC for the seven drugs for which complete permeability data were calculated. The plot shows a good linear relationship with a slope near zero (slope = 0.0292, intercept = 3.91, r = 0.778) indicating P_{str} is generally independent of DC. This is not surprising since the stroma contains 76–80% water. The remainder is composed mostly of collagen fibers and mucopolysaccharide, the latter of which is hydrophilic and responsible for the high water content of the stroma (10). Considering the large range of lipophilic/hydrophilic character covered by the seven drugs (over four log units), the results strongly suggest that drug is diffusing through the aqueous mucopolysaccharide medium of the stroma which is interspersed by a matrix of collagen fibers. The collagen fibrils, 300 Å in diameter, are arranged nearly parallel to one another with a fairly regular open spacing of ~300 Å between fibrils (10). The fibrils probably provide a high resistance to penetration and increase the diffusional path length as opposed to free diffusion through the aqueous stromal medium. Mathematically, resistance through the stroma can be defined by[12]:

$$R_{str} = \frac{h_{str}}{D_{str}(PC)} \qquad \text{(Eq. 7)}$$

where D_{str}, the effective diffusion coefficient in the stroma, can be expanded to (11):

$$D_{str} = \frac{D_{aq}\epsilon}{\tau} \qquad \text{(Eq. 8)}$$

In this equation, ϵ is the porosity (dimensionless) or volume fraction of the stroma, D_{aq} is the aqueous diffusion coefficient, and τ is the tortuosity (dimensionless) imposed by the geometrical arrangement of the stromal matrix. By combining Eqs. 7 and 8 and assuming PC as unity, stromal resistance can be defined by:

$$R_{str} = \frac{h_{str}\tau}{D_{aq}\epsilon} \qquad \text{(Eq. 9)}$$

[12] Equation 7 does not contain the A term in the denominator as shown in Eq. 3 because it is incorporated into the calculation of R_{str}; A = 1.087 cm² for 2-kg rabbits.

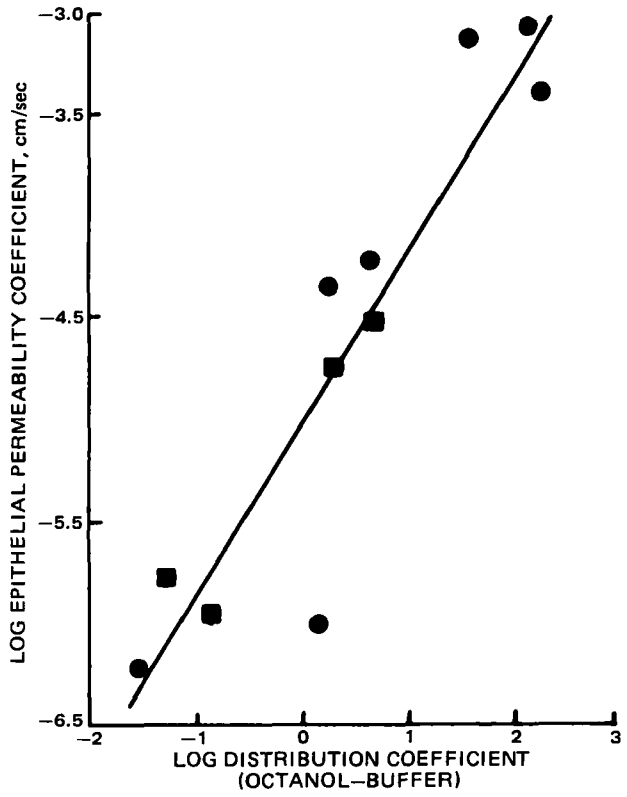

Figure 3—*Log–log plot of permeability coefficient through epithelium (P_{epi}) versus distribution coefficient (octanol–Sorensen's buffer, pH 7.65) for 11 β-blocking agents. Key: (●) determined from various corneal preparations, (■) calculated by difference using Eq. 5, regression results from Fig. 1, and permeability coefficient for intact cornea (penbutolol not shown). Linear regression: slope = 0.8505, intercept = −5.033 and* r = *0.9207.*

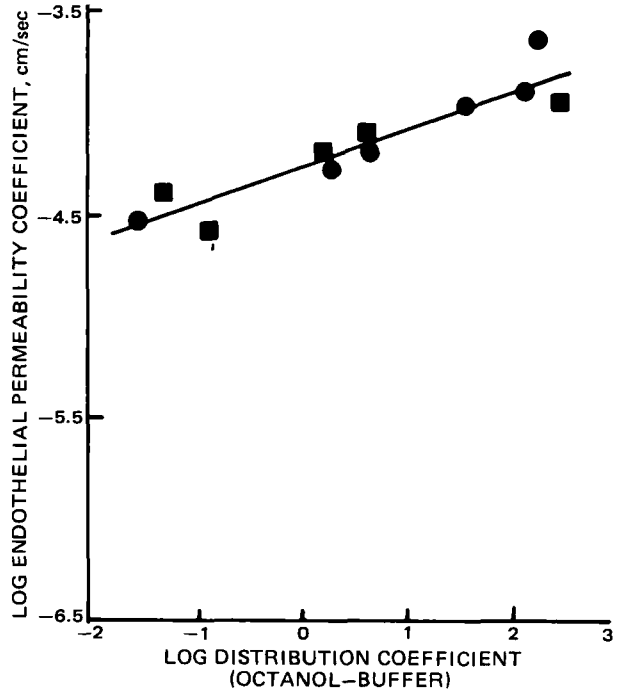

Figure 4—*Log–log plot of permeability coefficient through endothelium (P_{endo}) versus distribution coefficient (octanol–Sorensen's buffer, pH 7.65) for 11 β-blocking agents. Key: (●) determined from various corneal preparations (acebutolol not shown), (■) calculated by difference using Fig. 1 and permeability coefficient for intact cornea. Linear regression: slope = 0.1843, intercept = −4.2724, and* r = *0.9283.*

The collagen structure of the stroma can be depicted as in Fig. 2. Assuming that the average diffusional path of least resistance is midway between the geometrically arranged collagen fibrils, the porosity and tortuosity can be estimated as 0.773 and 1.21, respectively, resulting in a τ/ϵ ratio of 1.56.

In Eq. 9 both R_{str} and h_{str} are known from the experimental data. The averaged resistance for a normal stromal thickness of 0.03725 cm (78.7% hydration) is 7.83×10^3 sec/cm. To estimate D_{aq} for Eq. 9, the Sutherland–Einstein equation[13] and the well-established aqueous diffusion coefficient for benzoic acid (MW = 122), 1.1×10^5 cm²/sec at 25° (11), were used. After correcting for molecular weight, the β-blocking agents yielded an average diffusion coefficient of 7.5×10^{-6} cm²/sec in water. Temperature and viscosity differences between water and stromal medium were not included in the correction, although they should compensate for one another. These estimations yielded a τ/ϵ value of 1.58 for normal stroma. Therefore, good agreement exists between the τ/ϵ value for stroma predicted from geometrical considerations of collagen in Fig. 2, neglecting the mucopolysaccharide contribution to viscosity or structure.

Maurice (12) described a factor referred to as an obstruction of the stroma to diffusion or more specifically, as "how many times diffusion in tissue is slower than diffusion in saline at the same temperature." This factor is similar to the τ/ϵ ratio in Eqs. 7–9. Values for ^{134}Cs, ^{82}Br, and ^{24}Na ranged from 1.9 to 2.7, which agree fairly well with our value of 1.58 considering that the molecules studied and the methods used are quite different.

[13] The Sutherland-Einstein equation is:

$$D = \frac{RT}{6T\eta N}\left(\frac{4N}{3M\upsilon}\right)^{1/3}$$

where D is the diffusion coefficient, R is the gas constant, T is the temperature, N is Avogadro's number, M is the molecular weight, υ is the partial specific volume, and η is viscosity of the solvent. Assuming D is proportional to $(1/M)^{1/3}$ and that all other parameters remain unchanged, then the value D_{aq} for β-blocking agents with an average molecular weight of 288 is: $D_{aq} = (1.1 \times 10^{-5})(122/288)^{1/3} = 7.5 \times 10^{-6}$ cm²/sec.

Epithelial Diffusion—Figure 3 represents a linear plot of log P_{epi} *versus* log DC. Five drugs (penbutolol, levbunolol, timolol, nadolol, and sotalol) lack the permeability data for stroma and epithelium–stroma preparations so that individual resistances could not be directly calculated for all layers. However, because of the excellent fit between permeability and partitioning for seven of the drugs shown in Fig. 1, predictions for R_{str} could be obtained from the known DC value. Using the experimentally determined permeability data for intact cornea, R_T was calculated. Consequently R_{epi} could be determined from Eq. 5 by difference for the five drugs lacking the appropriate experimental data. The R_{epi} value for penbutolol was over one log unit from the least-squares fitted line (slope = 0.8505, intercept = −5.033, and r = 0.9207). Its deviation could be a consequence of the very small percentage contribution of the P_{epi} value estimated for penbutolol and, therefore, the large potential for error when taking its reciprocal. It is interesting to note that the slope in Fig. 3 is only slightly <1, suggesting that the lipophilic character of the epithelium is only slightly lower than octanol (a slope of 1 would indicate identical partitioning behavior).

Endothelial Diffusion—Figure 4 represents a plot of log P_{endo} *versus* log DC for all of the drugs except acebutolol, which had an outlying value. The R_{endo} value of acebutolol was small compared to R_{epi} and may have been subject to a relatively large error. The linear regression analysis

Table IV—Percent Contribution of the Resistance of Individual Corneal Layers to the Total Corneal Resistance

β-Blocking Agent	R_{epi}/R_T, %	R_{str}/R_T, %	R_{endo}/R_T, %	Log DC[a]
Penbutolol	1.0	46.0	53.0	2.53
Bufuralol	18.0	50.0	32.0	2.31
Bevantolol	7.0	44.0	49.0	2.19
Propranolol	7.0	45.0	48.0	1.62
Levbunolol	58.0	15.0	27.0	0.72
Oxprenolol	45.0	21.0	34.0	0.69
Timolol	68.0	9.0	23.0	0.34
Metoprolol	48.0	18.0	34.0	0.28
Acebutolol	91.0	1.0	8.0	0.20
Nadolol	95.0	1.0	4.0	−0.82
Sotalol	95.0	1.0	4.0	−1.25
Atenolol	97.5	0.5	2.0	−1.52

[a] The distribution coefficient is between octanol and Sorensen's buffer at pH 7.65, which is also the pH of the permeability experiments.

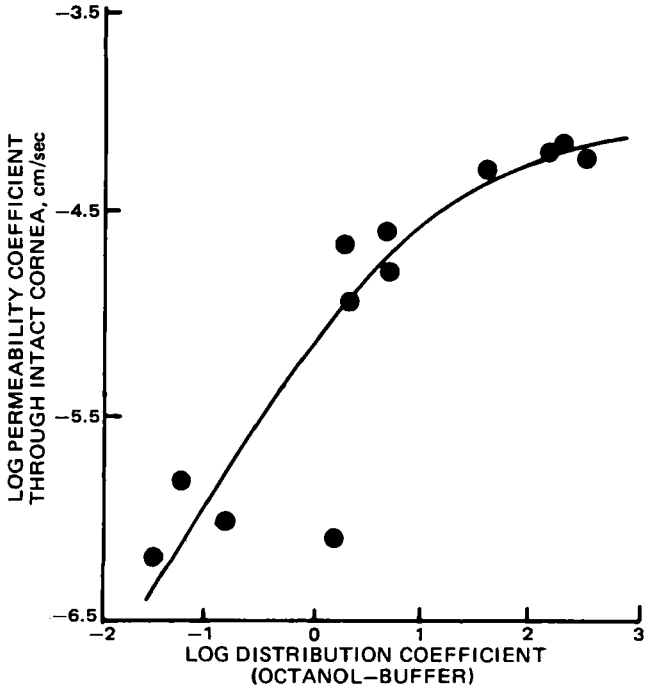

Figure 5—*Log–log plot of theoretical curve fitted to experimentally determined permeability coefficients through intact cornea (●) versus distribution coefficient (octanol–Sorensen's buffer, pH 7.65) for 12 β-blocking agents; computer-generated curve represents the sum of three composite corneal layers according to Eq. 20.*

produced a line with a slope and intercept intermediate between epithelial and stromal data (slope = 0.1843, intercept = −4.272, and r = 0.9282).

The intercepts from the linear regression in Figs. 2–4 represent the log P_T value for a compound with a log DC of zero. The permeability coefficients, in units of 10^{-6} cm/sec, are 9.27, 53.46, and 123.03, respectively, for epithelium, endothelium, and stroma for a compound whose DC equals 1. For a compound with this partitioning behavior, the epithelium is the rate-determining barrier. A β-blocking agent must be considerably more lipophilic before another layer becomes rate determining.

Relative Layer Contributions—Table IV shows the percent contribution of the resistances from each corneal layer to the total corneal resistance according to Eq. 5. The percent contribution of epithelial resistance increases as the drug lipophilicity decreases. Conversely, resistance decreases for stroma and endothelium as the lipophilicity of the drug decreases. Stroma is hydrophilic and expected to behave in this manner. However, the endothelium is considered lipophilic due to its cellular composition. Because the endothelium is only one cell thick and therefore does not present the tortuosity of the multilayered epithelium, it is possible that intercellular (pore) transport becomes significant for the more hydrophilic compounds.

For the most lipophilic compounds (penbutolol, bufuralol, bevantolol and propranolol), the stroma and endothelium offer the greater resistance. For the other more hydrophilic compounds, the epithelium is the most significant barrier to penetration.

Diffusional Model Relating DC to P_T—The total diffusional resistance, R_T, through the three-layer corneal membrane was generalized in Eq. 3, but can be expanded according to Eq. 5 to:

$$P_T = \frac{1}{R_T} = \frac{1}{\frac{h_1}{D_1(\mathrm{PC})_1} + \frac{h_2}{D_2(\mathrm{PC})_2} + \frac{h_3}{D_3(\mathrm{PC})_3}} \qquad \text{(Eq. 10)}$$

where all terms have been previously defined; for simplicity, the numerical subscripts 1, 2, and 3 are used for the epithelial, stromal, and endothelial layers, respectively. An attempt was made to determine if each corneal layer, with its own effective partition coefficient (PC), could be related to the distribution coefficient of an octanol–buffer (pH 7.65) system according to:

$$(\mathrm{PC})_i = \gamma_i(\mathrm{DC})^a \qquad \text{(Eq. 11)}$$

where γ is a proportionality constant and a represents a measure of the sensitivity of the biological partition coefficient (PC) of layer i to the *in vitro* distribution coefficient (DC).

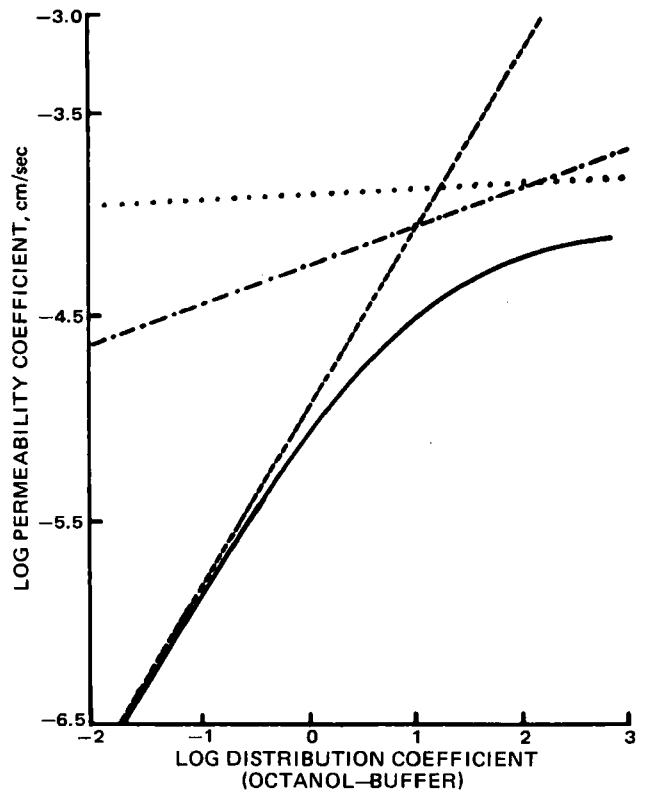

Figure 6—*Computer-generated log–log plots of permeability coefficient for intact cornea and three separate corneal layers* versus *distribution coefficient (octanol–Sorensen's buffer, pH 7.65). Key: (- - -) epithelium, (· · ·) stroma, (— · —) endothelium, (——) intact cornea generated from Eq. 20.*

The basis for use of Eq. 11 comes from the work of Collander (13), who studied the partition of organic compounds between higher alcohols and water. He found that there was a linear relationship among the log partition coefficients in two different solvent systems:

$$\log \mathrm{PC}_{S2} = a \cdot \log(\mathrm{PC}_{S1}) + b \qquad \text{(Eq. 12)}$$

where S1 and S2 represent two different solvent systems each containing water as the polar phase, but with different nonpolar phases. The octanol–water system has been used as a reference system for correlation to *in vivo* responses (14, 15). When other systems are used, conversion to the octanol–water system can be achieved through Eq. 12. Equation 11 can be converted to a form identical to Eq. 12, where S1 in Eq. 11 represents octanol–buffer (pH 7.65) and S2 represents a membrane–water system. Equation 11 can be substituted into Eq. 10 to give:

$$P_T = \frac{1}{\frac{h_1}{D_1\gamma_1(\mathrm{DC})^{a_1}} + \frac{h_2}{D_2\gamma_2(DC)^{a_2}} + \frac{h_3}{D_3\gamma_3(DC)^{a_3}}} \qquad \text{(Eq. 13)}$$

Combining fractions and rearranging gives:

$$P_T = \frac{(D_1\gamma_1/h_1)(\mathrm{DC})^{a_1}}{1 + (h_2D_1\gamma_1/h_1D_2\gamma_2)(\mathrm{DC})^{a_1-a_2} + (h_3D_1\gamma_1/h_1D_3\gamma_3)(\mathrm{DC})^{a_1-a_3}} \qquad \text{(Eq. 14)}$$

and combining constants leads to:

$$P_T = \frac{dx^e}{1 + fx^g + ix^j} \qquad \text{(Eq. 15)}$$

where x = DC, $d = D_1\gamma_1/h_1$, $e = a_1$, $f = h_2D_1\gamma_1/h_1D_2\gamma_2$, $g = a_1 - a_2$, $i = h_3D_1\gamma_1/h_1D_3\gamma_3$, and $j = a_1 - a_3$. For a single epithelial layer, $h_2 = h_3 = 0$ and Eq. 13 becomes:

$$P_{epi} = \frac{1}{R_{epi}} = \frac{D_1\gamma_1(\mathrm{DC})^{a_1}}{h_1} \qquad \text{(Eq. 16)}$$

Similarly:

$$P_{str} = \frac{1}{R_{str}} = \frac{D_2\gamma_2(\mathrm{DC})^{a_2}}{h_2} \qquad \text{(Eq. 17)}$$

Table V—Nonlinear Least-Squares Best Fit of the Relationship Between Permeability Coefficient and Distribution Coefficient (Octanol–Buffer) [a]

Corneal Layer	f_hP_h 10^{-6} cm/sec	$f_lD\gamma/h$, 10^{-6} cm/sec	a	Weight	RMS[b]	r[c]	DF[d]
Endothelium	15.19	21.380	0.3298	$(1/P)^{0.85}$	32.7	−0.275	9
	10.60	31.410	0.2709	$(1/P)^{0.50}$	138.2	−0.431	9
Epithelium	0.60[e]	9.346	0.7885	$(1/P)^{0.85}$	243.2	0.225	9
	0.60[e]	25.290	0.6152	$(1/P)^{0.50}$	2007.7	0.101	9

[a] Results apply to three barriers in series with parallel pathways assigned to epithelium and endothelium only; see Eq. 23 for an explanation of the symbols. [b] Residual mean square calculated by dividing residual sum of squares by degrees of freedom. [c] Correlation coefficient. [d] Degrees of freedom. [e] A fixed value for computer fitting represents the permeability coefficient from the most hydrophilic drug, atenolol.

and

$$P_{endo} = \frac{1}{R_{endo}} = \frac{D_3\gamma_3(DC)^{a3}}{h_3} \qquad \text{(Eq. 18)}$$

Equations 16–18 can be linearly rearranged to yield:

$$\log P_i = a_i \log (DC) + \log \frac{D_i\gamma_i}{n_i} \qquad \text{(Eq. 19)}$$

Equation 19 indicates that for a single corneal layer, the plot of log P_i *versus* log DC will show a linear relationship with a slope a_i and an intercept equal to log $D_i\gamma_i/h_i$. This requires that all drugs used in the plot have the same diffusion coefficient within layer i.

By substituting the intercepts and slopes from the log P_i *versus* log DC plots of the three corneal layers into Eq. 15, the following equation was obtained:

$$P_T = \frac{(9.27 \times 10^{-6})(DC)^{0.8505}}{1 + 0.0753(DC)^{0.8216} + 0.1734(DC)^{0.6662}} \qquad \text{(Eq. 20)}$$

Equation 20 is exactly the same form as Eq. 15. Figure 5 shows that the experimental data corresponding to the log P_T *versus* log DC plot for intact corneal permeability fits the curve represented by Eq. 20. The excellent fit further justifies our theoretical basis regarding corneal penetration through three composite layers acting as a sum of barriers in series.

Figure 6 is a combination of all the log P_i *versus* log DC curves for permeation through intact cornea, epithelium, stroma, and endothelium. It clearly shows that the permeabilities are rate determined by the epithelium for the four hydrophilic compounds (atenolol, sotalol, nadolol, and acebutolol). There are four intermediate lipophilic compounds (*i.e.*, metoprolol, timolol, oxyprenolol, and levbunolol) whose permeabilities are controlled by epithelium, endothelium, and stroma, in that order. The most lipophilic compounds fall on the plateau range, with their permeabilities controlled by endothelium and stroma; these compounds include propranolol, bufuralol, bevantolol, and penbutolol. It is interesting to note that linear processes (Fig. 6) can be added to produce curvilinear results. This occurs because of the small slope values (a in Eq. 19) of stroma and endothelium; therefore, a plateau is reached for the most lipophilic compounds.

The only outlier in Fig. 5 is acebutolol, for which the experimental permeability coefficient falls significantly below the theoretical curve represented by Eq. 20. By applying the Sutherland–Einstein equation to correct for the difference in molecular weight between acebutolol (336.4) and the remaining 11 β-blocking agents (289.6), the diffusion coefficient of acebutolol was found to be only 5% lower than the average. When the 5% correction is applied, the permeability coefficient is still significantly below the computer-generated line of best fit. Figures 3 and 4 indicate that log P_{epi} and log P_{endo} for acebutolol deviate from the observed trend, shown by their respective plots against log DC. This suggests that the decreased permeability coefficients of acebutolol through epithelium and endothelium, and not the stroma, account for the deviation of the experimental data from the curve. It was thought that acebutolol might cause a physiological or structural change in the epithelial or endothelial layers such that the permeation decreases. However, the experimental hydration level obtained following intact corneal permeability was within the normal range, suggesting that the corneal layers had not been altered in permeability. Still another possibility for the deviation for acebutolol may lie in its partitioning behavior. From a structural point of view, acebutolol may show exceptional hydrogen bonding ability compared to the other 11 β-blocking agents. The fact that the acebutolol structure contains acetyl, amide, and tertiary amine groups indicates that significantly more hydrogen bonding could occur compared with the other compounds, possibly leading to the deviation of acebutolol in Fig. 5.

Intercellular (or Aqueous Pore) Pathways in the Epithelial and Endothelial Layers—When two or more independent diffusional pathways exist in a given diffusional medium, the total permeability coefficient at steady state is (11):

$$P_T = f_1P_1 + f_2P_2 + \ldots + f_nP_n \qquad \text{(Eq. 21)}$$

where $f_1, f_2, \ldots f_n$ define the fractional areas of each route and $P_1, P_2, \ldots P_n$ are the individual permeability coefficients through each pathway. It has been suggested that there are two parallel pathways occurring for the diffusion of drugs across biological membranes (6, 16). One pathway is represented by hydrophilic channels that consist of pores or intercellular spaces, whereas the other pathway is represented by lipophilic transport across lipoid-like cell membranes. Hydrophilic molecules with low lipophilic partitioning behavior (low DC) are logically thought to diffuse through the hydrophilic channels, the path of least resistance. Applying these concepts to either the epithelium or endothelium, Eq. 21 becomes:

$$P_i = f_hP_h + f_lP_l \qquad \text{(Eq. 22)}$$

where subscripts h and l refer to hydrophilic and lipophilic transport, respectively. Since the hydrophilic channels are mostly filled with water, the diffusion coefficients for the β-blocking agents should approximate the diffusion coefficient in water for an average molecular weight of 288. The DC value for each drug can be assumed equal to 1. P_h becomes a

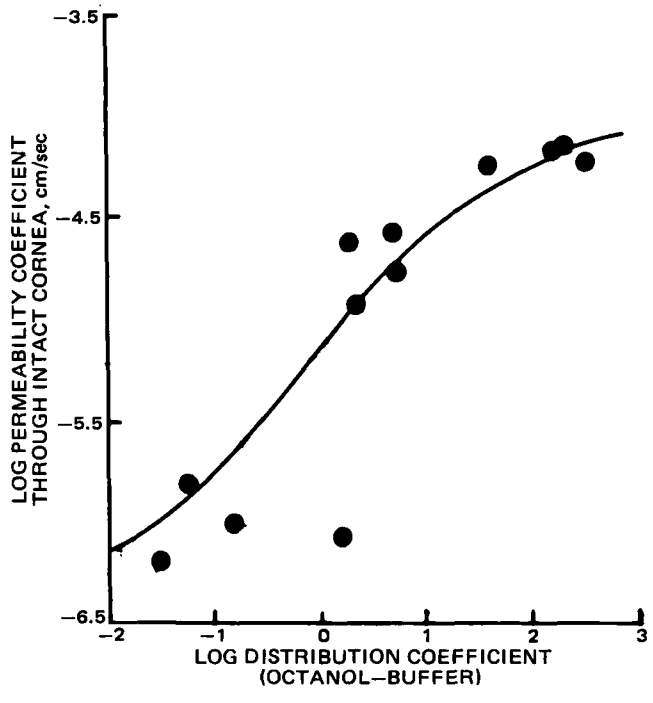

Figure 7—*Log–log plot of permeability coefficient* versus *distribution coefficient (octanol–Sorensen's buffer, pH 7.65). The theoretical curve represents the model with three barriers in series as well as intra- and intercellular parallel pathways for epithelium and endothelium. Key: (●) intact corneal permeability data, (—) computer-generated sigmoidal curve representing parallel pathways according to Eq. 24.*

[14] Nonlinear regression was performed using the BMDP3R program on an IBM370 computer.

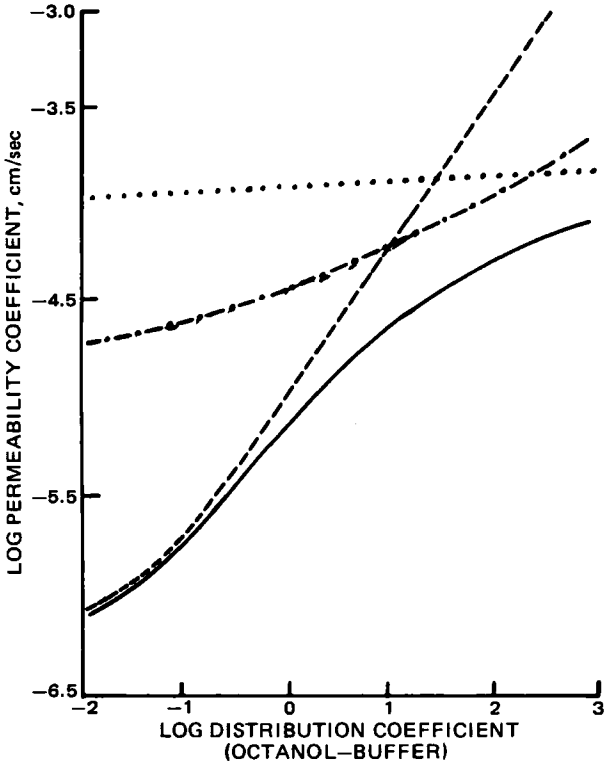

Figure 8—*Computer-generated log–log plots of permeability coefficient for intact cornea and three separate corneal layers* versus *distribution coefficient using (octanol–Sorensen's buffer, pH 7.65). The model includes intra- and intercellular pathways for epithelium and endothelium. Key: (- - -) epithelium, (· · ·) stroma, (-·-) endothelium, (—) intact cornea generated from Eq. 24.*

constant, but P_l is dependent on DC; thus, by combining Eqs. 3, 11, and 22 for either epithelial or endothelial transport:

$$P_i = f_h P_h + \frac{f_l D \gamma_i (\mathrm{DC})^{a_i}}{h_i} \qquad \text{(Eq. 23)}$$

Equation 23 can be fit by nonlinear regression[14] to either P_{epi} or P_{endo} *versus* DC to obtain estimates of $f_h P_h$ and $f_l D\gamma_i/h_i$.

Parallel Endothelial Pathways—Electron micrographs show that the boundries of adjoining endothelial cells are separated by ~200 Å (10). Based on an average endothelial thickness of 4.5 μm, as well as an experimentally determined P_{endo} of 2×10^{-5} cm/sec and a diffusion coefficient of 1.7×10^{-5} cm²/sec for ^{24}Na, Maurice (10, 17) estimated f_h in the endothelium to be 1/1720. Setting P_h equal to $D(\mathrm{PC})/h$ and assuming that PC = 1, h = 4.5 μm, and $D = 7.5 \times 10^{-6}$ cm²/sec[13], P_h becomes 1.7×10^{-2} cm/sec and $f_h P_h$ becomes 9.7×10^{-6} cm/sec. Therefore, 9.7×10^{-6} cm/sec was used as an initial estimate for the nonlinear fit to Eq. 23 for P_{endo} *versus* DC. Since f_l is nearly unity, the initial estimates for $D\gamma/h$ and a were obtained from the linear fit of log P_{endo} *versus* log DC (Fig. 4).

Weighting factors were used in the nonlinear fitting procedure since $f_h P_h$ is more likely determined by the hydrophilic compounds, which have much lower permeability and distribution coefficients than those of the lipophilic compounds. Table V shows the results of the computer fit for the endothelium. Using the weights of $(1/P_3)^{0.85}$ or $(1/P_3)^{0.5}$ the final estimates of $f_h P_h$ are reasonably close to the theoretically derived initial estimate. However, if the endothelial permeability coefficient for acebutolol, which is extremely low, is again taken as an outlier and discarded for the computer fitting, then $f_h P_h$ decreases to ~0. Consequently, a strict interpretation of the results is tenuous based on the variability of the data. The intercellular pathway for the endothelium cannot be confirmed from our nonlinear regression analysis unless a greater number of data points in the hydrophilic range are used.

Parallel Epithelial Pathways—Since the epithelium has 5–10 cellular layers, its intercellular pathway should have a large tortuosity and small area fraction. The epithelial permeability coefficient for the most hydrophilic drug, atenolol, is only 0.6×10^{-6} cm/sec. Assuming atenolol traversed the epithelium predominately through pores, the value of 0.6×10^{-6} cm/sec was assigned as an initial estimate for $f_h P_h$ for the nonlinear regression of epithelial permeation. The initial estimates for $D\gamma/h$ and a were obtained from the linear fit of log P_{epi} *versus* log DC (Fig. 3).

Despite the uncertainty defining the intercellular pathway, the computer fitting using Eq. 23 provides useful information for both epithelial and endothelial layers. As pointed out previously, the a-value indicates the sensitivity of the respective corneal layer to the change in the lipophilicity/hydrophilicity character of the penetrating drug. As shown in Table V, the slope values (a) are <0.35 for the endothelium. In contrast, the epithelium has a-values 2 times as high as that calculated for the endothelium. The results indicate that compared with endothelium, the epithelium is more sensitive to the lipophilicity of permeating compounds. These conclusions are consistent with the results of the linear regression fits of log P_i *versus* log DC.

The hydrophilic stroma contains very low cell counts and, therefore, the intercellular pathway model did not apply. The log P_{str} *versus* log DC data was fitted by linear regression. The parameter values from the line of best fit as well as those from the P_i *versus* DC nonlinear curve-fitting procedure were used to construct the following:

$$P_T = \frac{1}{\dfrac{10^6}{0.6 + 9.346 \cdot (\mathrm{DC})^{0.7885}} + \dfrac{10^6}{123.03 \cdot (\mathrm{DC})^{0.0289}} + \dfrac{10^6}{15.19 + 21.38 \cdot (\mathrm{DC})^{0.3298}}} \qquad \text{(Eq. 24)}$$

where the three terms in the denominator on the right-hand side represent the permeability coefficient through epithelium, stroma, and endothelium, respectively. According to Eq. 24, the computer-generated curve of log P_T *versus* log DC (Fig. 7) describes the experimental permeability coefficients through intact cornea. The lower limit of the curve represents the intercellular pathway while the plateau is controlled by the endothelium and stroma, overall resulting in a sigmoidal curve. Figure 8 shows the computer-fitted curves for stroma, endothelium, epithelium, and intact cornea. Unlike the linear log P_{str} *versus* log DC plot for stroma, the curves for epithelium and endothelium in Fig. 8 approach a minimum in the hydrophilic range.

Because of the variability in the log P_i values for the hydrophilic compounds, Figs. 7 and 8 do not describe the data any better than Figs. 5 and 6. Nevertheless, the sigmoidal curve obtained in Figs. 7 and 8 resemble the intestinal absorption profile proposed by Ho *et al.* (6) for similar data. These authors showed that a sigmoidal relationship existed between the rates of absorption and lipophilicity. Diffusion through the aqueous pore pathway in the intestine accounted for the lower limiting value represented by the most hydrophilic compounds, whereas at high lipophilicity, the absorption rate reached a plateau controlled by the aqueous boundary layer adjacent to the intestinal absorptive cell membrane. Corneal penetration rate, on the other hand, is limited for lipophilic β-blocking agents by the stroma, which is primarily an aqueous barrier on the cornea; however, the hydrophilic stromal barrier is a physiologically real and permanent barrier located within the cornea. Both Figs. 5 and 7 show that stroma and endothelium control the corneal permeation of lipophilic compounds in the absence of an aqueous diffusional (or boundary) layer.

REFERENCES

(1) E. J. Lien, in "Drug Design," Vol. V, E. J. Ariens, Ed., Academic, New York, N.Y., 1975, pp. 82–132.

(2) E. J. Lien and P. H. Wang, *J. Pharm. Sci.*, **69**, 648 (1980).

(3) E. J. Lien, A. A. Alhaider, and V. H. L. Lee, Jr. *Parenter. Sci. Technol.*, **36**, 86 (1982).

(4) N. Draper and H. Smith, in "Applied Regression Analysis," 2nd ed., Wiley, New York, N.Y., 1981, pp. 294–312.

(5) R. D. Schoenwald and H. S. Huang, *J. Pharm. Sci.*, **72**, 1266 (1983).

(6) N. F. H. Ho, J. Y. Park, W. Morozowich, and W. I. Higuchi, in "Design of Biopharmaceutical Properties through Prodrugs and Analogs," E. B. Roche, Ed., Am Pharm Assoc, Washington, D.C., 1977, pp. 136–227.

(7) J. H. Kim, K. Green, M. Martinez, and D. Paton, *Exp. Eye Res.*, **12**, 231 (1971).

(8) R. D. Schoenwald and J. A. Houseman, *Biopharm. Drug Dispos.*, **3**, 231 (1982).

(9) B. O. Hedbys and S. Mishima, *Exp. Eye Res.*, **5**, 221 (1966).

(10) I. Fatt, in "Physiology of the Eye," Butterworth, Woburn, Mass.,

1978, pp. 92–188.
(11) G. L. Flynn, S. H. Yalkowsky, and T. J. Roseman, *J. Pharm. Sci.*, 63, 479 (1974).
(12) D. M. Maurice, in "The Structure of the Eye," G. K. Smelser, Ed., Academic, New York and London, 1960, pp. 381–391.
(13) R. Collander, *Acta Chem. Scand.*, 5, 774 (1951).
(14) R. N. Smith, C. Hansch, and M. M. Ames, *J. Pharm. Sci.*, 64, 599 (1975).
(15) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).
(16) W. D. Stein, "Theoretical and Experimental Biology," Vol. 6, Academic, New York and London, 1967, pp. 73–74, 106–125.
(17) D. M. Maurice in "The Eye," Vol. 1, 2nd ed., H. Davson, Ed., Academic, New York, N.Y., 1969, pp. 6–8, 534–557.

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Corneal Penetration Behavior of β-Blocking Agents III: *In Vitro–In Vivo* Correlations

HONG-SHIAN HUANG *, RONALD D. SCHOENWALD ˣ, and JOHN L. LACH

Received July 26, 1982, from the *Pharmaceutics Division, College of Pharmacy, University of Iowa, Iowa City, IA 52242.* Accepted for publication September 4, 1982. *Present address: National Defense Medical Center, P.O. Box 8244-14, Taipei, Taiwan 107, ROC.

Abstract □ Aqueous humor levels were determined over time after the topical administration to rabbit eyes of 1% isotonic buffered (pH 7.3) solutions of three β-blocking agents, acebutolol hydrochloride, timolol maleate, and bufuralol hydrochloride (arranged in order of increasing lipophilicity). Corneal permeability coefficients, determined from a previous *in vitro* study, were inversely related to the observed time to peak for the three drugs, as expected. Two of the drugs, bufuralol and timolol, did not give the expected rank order for C_{max} and AUC, which could result from differences in distribution and/or elimination processes. Aqueous boundary layers were postulated for *in vivo* corneal permeability which suggested that bufuralol and timolol may have nearly identical effective permeability coefficients *in vivo*.

Keyphrases □ Permeability—acebutolol, timolol, bufuralol, excised rabbit corneas, pharmacokinetics, *in vitro–in vivo* correlations □ Acebutolol—corneal permeability in rabbits, pharmacokinetics, *in vitro–in vivo* correlations □ Timolol—corneal permeability in rabbits, pharmacokinetics, *in vitro–in vivo* correlations □ Bufuralol—corneal permeability in rabbits, pharmacokinetics, *in vitro–in vivo* correlations

In a previous report (1) the penetration behavior of 12 β-blocking agents measured across excised rabbit corneas was correlated with partitioning, which varied over a fourfold logarithmic range. Optimal penetration (log permeability coefficient) reached a maximum at a log distribution coefficient (octanol–buffer, pH 7.65) of ~2–3. Subsequent results (2) showed that a plateau was reached because the stroma, and to a lesser extent the endothelium, became the rate-controlling barrier for the most lipophilic compounds, while the epithelium acted as a rate-determining barrier for the hydrophilic compounds.

The purpose of this study was to determine if the corneal permeability coefficients of three compounds ranging widely in lipophilicity could be correlated with parameters obtained from the aqueous humor–time profile. The three drugs (in descending lipophilic order: bufuralol, timolol, and acebutolol) were administered as 1% isotonic, buffered (pH 7.3) solutions.

EXPERIMENTAL

Reagents and Materials—Isotonic, buffered (pH 7.3), 1% w/v solutions of acebutolol hydrochloride, timolol maleate, and bufuralol hydrochloride were prepared separately[1]. The reagents used for aqueous humor extraction and subsequent high-performance liquid chromatographic (HPL) assay were reagent- or UV spectrophotometry-grade chemicals. New Zealand White rabbits, 2 months of age and of either sex, weighing 1.6–2.0 kg were used for the experiments.

Topical Administration and Aqueous Humor Sampling—The rabbits were administered drug with their heads in an upright position while resting in a restraining box. The rabbits were returned to their cages when the sampling interval was >1 hr. A 50-μl volume was instilled onto the cornea of each eye while the lower lid was gently pulled away from the eye globe to form a pocket. The lower eyelid was held against the upper lid for 20 sec after instillation. Second and third instillations were given 2 and 4 min after the first application. The multiple-dose regimen was designed to give aqueous humor concentrations above the sensitivity of the assay. This especially applies to acebutolol hydrochloride, since its permeability was found to be the lowest.

At various postinstillation times, rabbits were sacrificed by a rapid injection of ~25 ml of air into the marginal ear vein. Each cornea was then quickly rinsed with 1 ml of normal saline solution to get rid of residual drug. The aqueous humor samples were withdrawn by puncture with a 26-gauge 0.95-cm needle attached to a 0.5-ml disposable syringe[2] through the corneal–scleral junction into the anterior chamber. The same syringe was used for the opposite eye of each rabbit in order to pool the aqueous humor of both eyes.

The sampling times for each drug are listed in Table I; each value represents an average of 4–12 rabbit eyes. The aqueous humor samples were left in the syringes and were assayed within a few hours. Although rabbit aqueous humor sample volumes varied from animal to animal (ranging from 0.25 to 0.35 ml), a constant volume of sample was used in the assay for each drug.

Extraction and Analyses—A mixer[3] was used to facilitate the mixing and extraction. In 10-ml, glass centrifuge tubes, aqueous humor samples of 0.25 ml were mixed with 0.1 ml of 0.5 *N* NaOH, extracted with 2.0 ml of methylene chloride, and centrifuged. After discarding the aqueous layer, the organic phase was extracted with 1.0 ml of 0.05 *N* sulfuric acid. The acidic aqueous phase was used for HPLC assay of acebutolol.

A 0.30-ml volume of aqueous humor sample was mixed with 0.1 ml of 1 *N* NaOH and extracted with 5 ml of heptane containing 4% isoamyl alcohol in a 10-ml glass centrifuge tube. No centrifugation was necessary

[1] 1% Acebutolol (as hydrochloride salt) contained the following vehicle ingredients: 0.184 g of $NaH_2PO_4 \cdot H_2O$, 0.758 g of Na_2HPO_4, and 0.288 g of NaCl/100 ml of solution. 1% Timolol (as maleate salt) contained the following vehicle ingredients: 0.947 g of Na_2HPO_4, 0.265 g of NaOH, and 0.332 g of NaCl/100 ml of solution. 1% Bufuralol (as hydrochloride salt) contained the following vehicle ingredients: 0.184 g of $NaH_2PO_4 \cdot H_2O$, 0.758 g of Na_2HPO_4, and 0.242 g of NaCl (1.0 g bufuralol hydrochloride)/100 ml of solution.

[2] Glaspack B-D, sterile disposable glass syringe; Becton, Dickinson, and Co., Rutherford, N.J.

[3] Vortex genie mixer, S8223; Scientific Products.

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