The Relations Between Ionic and Non-Ionic Diffusion of Sulfonamides Across the Rabbit Cornea

Lidia M. Jankowska, Amir Bar-Ilan, and Thomas H. Maren

We studied the transcorneal permeability of five carbonic anhydrase inhibitors, with particular attention to the passage of these acidic compounds in their ionized and nonionized forms. Their pKs varied from 5.9 to 8.0, and CHCl₃/pH 7.2 buffer partition coefficients from 10⁻⁴ to 25. Solutions of appropriate pH of each compound were applied to the cornea in steady state, and the rates of passage to the anterior chamber for each compound in each form were measured. The rate constants for the ionized forms were surprisingly high, only four- to sevenfold less than those for the uncharged species. Thus these compounds penetrate the cornea in both forms, and the data show that by increasing the pH of solutions, and thereby solubility, the overall rates of accumulation in the eye are increased. Invest Ophthalmol Vis Sci 27:29-37, 1986

This study is a part of our general program to develop new carbonic anhydrase inhibitors which could be applied topically to the eye for treatment of glaucoma. We have shown that when certain sulfonamides of relatively low pKₐ are applied to the cornea in the form of their soluble sodium salts, drug reaches the anterior and posterior chambers and the ciliary body, and intraocular pressure is lowered. However, we did not determine quantitatively whether the permeability was due to the ionized or the nonionized form; this is the substance of the present report.

The generally accepted concept is that it is mainly the nonionized form of any drug which penetrates biological membranes including the cornea. This is confirmed by many studies, and is known as the non-ionic diffusion or the pH-partition hypothesis. Despite the data suggesting penetration of the ionic form of drug through the cornea, the classical view, that epithelium is relatively impermeable to electrolytes, still seems to dominate ocular pharmacology. In our study on the ability of topical application of sulfonamide carbonic anhydrase inhibitors to lower intraocular pressure, we obtained results which showed a deviation from classical theory: Penetration of some lipophobic compounds was surprisingly high if compared to that predicted by theory and suggested that the ionic form of these weak organic acids penetrates the cornea to a significant extent. This may have practical importance, since the sodium salts of sulfonamides are much more water soluble than their free acid forms, and total penetration of sulfonamide into the eye can be increased to the point where concentration of a drug is high enough to inhibit carbonic anhydrase in ciliary processes and lower aqueous flow and pressure. We believe that this is one route to the discovery of new drugs of this class for the treatment of glaucoma.

The generated transcorneal rate constants for penetration represent the overall passage of sulfonamide through the cornea and they are not intended to address the more complex questions implicit in problems of all the different layers of the cornea and the problem of sequestration of certain drugs in the stroma. We will consider this aspect of sulfonamide pharmacology in subsequent publications. The main thrust of the present paper is the contribution of the nonionized and ionized forms of the molecule to the total transcorneal passage of the sulfonamide and how this knowledge may be useful in the drug design.

Materials and Methods

Drugs

Investigated sulfonamides are very potent inhibitors of carbonic anhydrase with \( K_i \) ranging from \( 10^{-8} \) M to \( 10^{-9} \) M. They are weak organic acids of low molecular weight, and their properties are given in Table...
Table I. pK, Molecular weight, water solubility and organic solvent/aqueous buffer partition coefficient for seven sulfonamides

<table>
<thead>
<tr>
<th>Drug</th>
<th>pK</th>
<th>MW</th>
<th>Water solubility g/l</th>
<th>Ether buffer pK = 7.2</th>
<th>Ether CHCl3/buffer pH = 7.2</th>
<th>pH = 9* CHCl3/buffer</th>
<th>pH = 5</th>
<th>pH = 7.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benztolamide</td>
<td>3.4</td>
<td>320</td>
<td>0.4</td>
<td>10^{-3}</td>
<td>0.013†</td>
<td>0.014</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Bromacetazolamide</td>
<td>5.9</td>
<td>301</td>
<td>0.3</td>
<td>0.03</td>
<td>4 × 10^{-3}</td>
<td>3 × 10^{-4}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-imino-4-methyl-1,3,4-thiadiazoline-2-</td>
<td>7.7</td>
<td>195</td>
<td>27</td>
<td>0.12</td>
<td>0.013†</td>
<td>0.014</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>sulfonamide</td>
<td>7.4</td>
<td>236</td>
<td>1.2</td>
<td>0.6</td>
<td>0.13</td>
<td>0.06</td>
<td>2 × 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>Methazolamide</td>
<td>6.6</td>
<td>290</td>
<td>2.3</td>
<td>6</td>
<td>0.5</td>
<td>0.3</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Trifluormethazolamide</td>
<td>7.0</td>
<td>339</td>
<td>0.08</td>
<td>56</td>
<td>14</td>
<td>4</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Ethoxzolamide</td>
<td>8.0</td>
<td>258</td>
<td>0.01</td>
<td>140</td>
<td>30</td>
<td>25</td>
<td>0.4 (at pH 10)</td>
<td></td>
</tr>
</tbody>
</table>

* When these numbers are corrected for the solubility of chloroform or ether in buffer, they suggest complete insolubility (at the limit of the method) of the anion in the lipid solvent.
† For benzolamide this value was obtained at pH = 1.

1. Note that their pK values vary from 5.9, as in the case of bromacetazolamide, to 8 for ethoxzolamide. Water solubility also changes to a great degree. The most hydrophobic, ethoxzolamide, is 230 times less water-soluble than trifluormethazolamide, which is the most hydrophilic drug in the free acid form. The extremely high water solubility value (27 g/l) for 5-imino-4-methyl-1,3,4-thiadiazoline-2-sulfonamide is irrelevant in this context, since this compound is a zwitterion and is always in an ionized state. Lipid solubility differs greatly. Chloroform/buffer partition coefficients show that at pH 7.2, the difference is about 80,000 between the least lipophilic, bromacetazolamide, and the most lipophilic, ethoxzolamide. Such enormous differences make these compounds very interesting for comparison of their penetrability properties. More relevant information about the physicochemical properties of these compounds can be found in Maren et al.1

Two ranges of pH were investigated for each sulfonamide: one, below the pKα in order for the compound to be mainly in the undissociated, free acid form, and one above pKα in order to obtain a solution with the anion as the prevailing form.

Saturated solutions of the sulfonamides were used in order to have a constant concentration of the acidic form of the sulfonamide with only the concentration of the ionic form changing with pH. In one set of experiments with methazolamide, two concentrations were used at the high pH range: one was saturated solution and the second had the same concentration as used at the acidic range for this drug, namely 5 mM. In the case of 5-imino-4-methyl-1,3,4-thiadiazoline-2-sulfonamide, the saturated solutions were not used because of the extremely high water solubility of this compound.

The saturated solutions were prepared by suspending slightly more than the calculated amount for solubility at a given pH in the proper volume of phosphate buffer of desired pH or 0.9% NaCl and vigorously mixing for a few minutes. The pH of the suspension was determined and in the cases of saline solutions the required volume of 1 N NaOH was added to increase pH to the desired value. The suspension was shaken again for a few minutes, then centrifuged, and the pH of the supernatant determined again. These supernatants were used immediately for the experiments. Concentrations of sulfonamides in these supernatants were determined by the method of Maren, Ash, and Bailey.18

Experimental Procedure In Vivo

White male and female albino rabbits 2-3 kg body weight, anesthetized with sodium pentobarbital (30-40 mg/kg i.p.) were used for this study. The animals were placed on their sides and their eye lids were kept widely open with hemostats attached gently to the fur surrounding the eye. In this way, a small pocket was created out of the lids, to load 0.5 ml of a solution of a studied compound in the pocket for a given time, usually 10 or 25 min. At the end of this time, remaining fluid was withdrawn from the lids pocket with a syringe. These investigations were done in accordance to the ARVO Resolution on the Use of Animals in Research.

The pH and concentration of a sulfonamide in the applied solutions were determined just before application, and immediately after the experiment in the fluid recovered from the pocket. Assays of the drug
were also performed as soon as possible in the aqueous humor samples (200–250 µl) aspirated from the anterior chamber. Trifluromethazolamide, however, was handled differently in the analysis as described earlier, since it alone is subject to hydrolysis. The other compounds are not altered. Aqueous humor was prepared for assay as follows: after removing the studied solution from the pocket, the cornea was washed thoroughly with a large volume of warm water to rinse away traces of the original solution which might contaminate intraocular samples. To be sure that the surface of the cornea was free from the stock solution, 0.5 ml of physiological saline was instilled in the pocket on the rinsed cornea for a moment; then this fluid was also withdrawn for drug concentration assays. In all experiments, concentration of sulfonamide in this sample was either undetectable (below 1 µM) or much below the concentration of a drug in the aqueous sample. Sampling of aqueous humor was done in the following manner: the tip of a half-inch 27-gauge needle attached to the tuberculin syringe was pushed through the cornea into the anterior chamber and the whole aqueous humor was withdrawn. The concentration of each compound in the anterior aqueous was determined by the method of Maren, Ash, and Bailey.

This procedure allows achievement of near constant external conditions (regarding pH and concentration of drug) because of the large amount of fluid instilled on the eye. This also makes it possible to generate first order rate constants for entry of a drug into anterior chamber.

It must be noted that the experimental conditions in this model do not alter penetration of the sulfonamide through the cornea. Prolonged time of exposure seems to have no significant effect on the permeability of the cornea, since the 60 min of exposure of the eye to bromacetazolamide resulted in essentially the same rate constants as obtained from 10 min of exposure in this study. The same applies to trichloromethazolamide.

As far as increased concentration of external solution is concerned, it does not affect permeability of the cornea in this study either, since experiments with methazolamide at two different concentrations at high pH range give the same rate constant for accession.

The absence of direct pH effect on the cornea was already studied by Friedenwald et al (no damage) and Maurice (no change in permeability).

Experimental Procedure In Vitro

Three drugs were used for in vitro study: bromacetazolamide, methazolamide, and trichlormethazolamide.

Drugs in their free acid form were dissolved in buffered glutathione bicarbonate Ringer solutions. These were adjusted to pH above and below pKₐ as in the in vivo experiments. The method of O’Brien and Edelhauser was employed for the penetration of sulfonamides through the cornea. Excised rabbit corneas with epithelium intact or removed were mounted in a Lucite-block-perfusion system with a corneal holder that was modified according to the method of Dikstein and Maurice. This method prevents trauma to the corneal epithelium and distortion of the corneal curvature during clamping. Corneas were exposed from epithelial and endothelial sides to the constantly mixed solutions in reservoirs (6 ml from each side). The solution of drug was placed in the epithelial chamber. Its passage through the cornea was measured by sampling fluid from the epithelial and the endothelial chamber every 30 min for 4 hr. Solutions with modified pH were applied to both sides of the cornea. Temperature was maintained at 37°C. There was no pressure difference across the cornea. More details on this technique are given in ref. 21.

Calculations

The following symbols and equations were used:

\[ C_{\text{out}}: \] concentration of sulfonamide in the solution outside of the eye

\[ * \] On the rest of the Figures bromacetazolamide is denoted as "B."
Table 2. Concentration of sulfonamide outside of the eye and in the anterior chamber aqueous after topical application of saturated solutions for 10 minutes at two pHs

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>pH</th>
<th>( C_{\text{out}} ) (mM) Mean ± S.E.M.</th>
<th>( C_{\text{in}} ) (µM) Mean ± S.E.M.</th>
<th>( C_{\text{in}} \times 1000 )</th>
<th>( C_{\text{out}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzolamide</td>
<td>15</td>
<td>7.4</td>
<td>145.4 ± 5.3</td>
<td>29.1 ± 6.74</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>Bromacetazolamide</td>
<td>7</td>
<td>5.5</td>
<td>1.4 ± 0.12</td>
<td>0.3 ± 0.01</td>
<td>0.2</td>
<td>7</td>
</tr>
<tr>
<td>Ethoxzolamide</td>
<td>6</td>
<td>8.4</td>
<td>0.2 ± 0.02</td>
<td>4.0 ± 0.42</td>
<td>0.04</td>
<td>6</td>
</tr>
<tr>
<td>Methazolamide</td>
<td>10</td>
<td>5.9</td>
<td>4.4 ± 0.14</td>
<td>4.8 ± 0.6</td>
<td>1.1</td>
<td>9</td>
</tr>
<tr>
<td>Trichlormethazolamide</td>
<td>6</td>
<td>7.9</td>
<td>40.6 ± 1.31</td>
<td>22.2 ± 4.5</td>
<td>0.6</td>
<td>6</td>
</tr>
<tr>
<td>Trifluormethazolamide*</td>
<td>10</td>
<td>6.3</td>
<td>0.134 ± 0.002</td>
<td>3.4 ± 1.07</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7.6</td>
<td>1.6 ± 0.002</td>
<td>17 ± 3.3</td>
<td>11</td>
<td>6</td>
</tr>
</tbody>
</table>

* Time of exposure for Trifluormethazolamide was 25 minutes. † Concentration made to match that of lower pH.

\[
\text{pH}_{\text{out}}: \quad \text{pH of solution outside of the eye} \\
\text{C}_{\text{in}}: \quad \text{concentration of a drug in the anterior chamber} \\
\text{V}: \quad \text{accession rate of a drug to the anterior chamber, as} \\
\text{V} = \frac{\text{C}_{\text{in}}, \mu\text{M}}{\text{hr of exposure}} = \mu\text{M/hr} \\
\text{k}_{\text{in}}: \quad \text{first order rate constant} \\
\text{k}_{\text{a}}: \quad \text{nonionic rate constant} \\
\text{k}_{\text{b}}: \quad \text{ionic rate constant}
\]

The following symbols were used for two pH values:

- a and \( a_1 \): concentrations of nonionic form
- b and \( b_1 \): concentrations of ionic form
- V and \( V_1 \): accession rates of sum of both forms of drugs

Concentration of acidic form at each pH was calculated from the Henderson-Hasselbalch equation:

\[
\text{pH} = \text{pK} + \log \frac{b}{a},
\]

where

\[
a + b = C_{\text{out}}.
\]

Then

\[
a = \frac{C_{\text{out}}}{1 + \text{antilog} \ (\text{pH} - \text{pK})}.
\]

To calculate rate constants for nonionic and ionic forms, the basic assumption is that:

\[
\text{V} = k_a \cdot a + k_b \cdot b \quad \text{for the first pH}
\]

and

\[
V_1 = k_a \cdot a_1 + k_b \cdot b_1 \quad \text{for the other pH}.
\]

Solving these two equations gives formulas for \( k_a \) and \( k_b \).

\[
k_a = \frac{Vb_1 - V_1b}{ab_1 - a_1b}
\]

\[
k_b = \frac{V_1a - Va_1}{ab_1 - a_1b}
\]

**Results**

Table 1 presents physicochemical properties of the compounds, which were already noted in the Material and Methods section.

**In Vivo**

Figure 1 demonstrates corneal permeability of the compounds in the living rabbit plotted against lipid/buffer at pH 7.2 partition coefficient. As one can see, the pattern does not follow exactly the lipophilicity of these drugs. This applies specifically to the three compounds of relatively low lipophilicity: benzolamide, bromacetazolamide, and methazolamide. All of these deviate upward from the line, methazolamide threefold, bromacetazolamide sevenfold and benzolamide more than one thousandfold. As we shall show, the reason for this is that nonionized or lipid insoluble form has appreciable corneal permeability of its own.

Table 2 demonstrates the concentrations of sulfonamide in the external solutions (\( C_{\text{out}} \)), their pHs, and concentrations in anterior chamber (\( C_{\text{in}} \)). The factor \( C_{\text{in}}/C_{\text{out}} \) compares the penetrating properties of the compounds at different pH. Where the acidic form dominates, penetration is two- to fivefold greater than for the ionic form. For benzolamide only the alkaline range of pH was analyzed, because due to low pK of this drug it was impossible to investigate the acidic form.
Figure 2 shows the rate of entry of sulfonamide to the anterior chamber plotted against pH of applied solution, when saturated solutions of drugs are used at each pH. The solubility of the nonionized form determines the saturation of the solution by nonpolar species, and this remains constant at all pH's. However, as the pH is raised, the concentration of the anions increases, and this allows the permeability of this form to be distinguished. Increase in the rate appears because of a notable contribution of the anion; for trichlormethazolamide, increase is fivefold; for bromacetazolamide, increase is about fifteenfold.

Table 3 shows the dependence of the first order total rate constant upon ionization. Note that $k_{in}$ varies only two- to fourfold, despite large changes in ionization at the two studied pH conditions. The smallest difference between $k_{ins}$ obtained for 5-imino-4-methyl-$\Delta$-1,3,4-thiadiazole-2-sulfonamide reflects the fact that this is essentially the difference between rate constants of the two ionic forms.

Figure 3 is the graphical presentation of this relationship. Benzolamide is not present on this figure because there is no experimental value for $k_{in}$ below pK of this compound. Ampholytic sulfonamide is also omitted.

Table 4 shows $k_{in}$ at physiological pH and at the pK_a of each compound. The separate $k_{in}$ for acid and for anion were calculated, and data show that the ratio of rate constants of the two forms is somewhat different for each of the drugs, varying from 3.9-7.3.

Figure 4 plots rate constants of the nonionic form of these drugs against organic solvent/buffer partition coefficients at a pH where they are totally in the acid form. This reveals that lipid solubility is related to the rate constant for the diffusional process; the rate constant of penetration of the nonionic form increases as lipophilicity increases, and here there is no departure from linearity as in the plot of Figure 1. This confirms the idea that the high values to the left of Figure 1 are due to ionic diffusion; these are absent (Fig. 4) when only nonionic forms are studied.

**In Vitro**

Table 5 shows the experiments performed in vitro. The results confirm our data obtained in vivo. The three sulfonamides chosen for these experiments differ greatly in their lipid solubility. The rate constants of penetration through the cornea / Jonkowsko et al. 33

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**Table 3. First order rate constants at pH above and below pK of sulfonamides**

<table>
<thead>
<tr>
<th>Sulfonamide</th>
<th>$pK_a$</th>
<th>$k_{in} \times 10^{3}/hr \pm S.E.M.$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzolamide</td>
<td>7.4</td>
<td>1.2 ± 0.28</td>
<td>15</td>
</tr>
<tr>
<td>Bromacetazolamide</td>
<td>5.55</td>
<td>0.82 ± 0.05</td>
<td>6</td>
</tr>
<tr>
<td>5-imino-4-methyl-$\Delta$-1,3,4-Thiadiazole-2-sulfonamide</td>
<td>7.3</td>
<td>0.23 ± 0.08</td>
<td>7</td>
</tr>
<tr>
<td>Methazolamide</td>
<td>5.86</td>
<td>6.8 ± 1.09</td>
<td>8</td>
</tr>
<tr>
<td>Trifluormethazolamide</td>
<td>7.9</td>
<td>3.7 ± 0.8</td>
<td>7</td>
</tr>
<tr>
<td>Trichlormethazolamide</td>
<td>7.5</td>
<td>10 ± 1.4</td>
<td>7</td>
</tr>
<tr>
<td>Ethoxzolamide</td>
<td>6.15</td>
<td>230 ± 39</td>
<td>8</td>
</tr>
</tbody>
</table>

* Saturated solutions are used in each experiment.

† This sulfonamide is an ampholyte and these $k_{in}$ represent rate constants for two ionic forms.

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**Figures:**

Fig. 2. Accession rates of sulfonamides to anterior chamber of the rabbit eye vs pH. Concentration of the nonionized form is maintained constant.

Fig. 3. First order rate constant dependency of total drug on pH of solution applied to the living eye.
Table 4. Rate constants of sulfonamides at the pKₐ, and calculated rate constants of nonionic and ionic forms

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percentage of HA at pH 7.5</th>
<th>( k_{in} \times 10^3/hr ) at pH 7.5</th>
<th>( k_a \times 10^3/hr ) at pKₐa</th>
<th>Factor of ( k_a ) to ionic form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromacetazolamide</td>
<td>2.4</td>
<td>0.23</td>
<td>0.65</td>
<td>5.5</td>
</tr>
<tr>
<td>Methazolamide</td>
<td>44.2</td>
<td>3.75</td>
<td>5.15</td>
<td>2.1</td>
</tr>
<tr>
<td>Trifluormethazolamide</td>
<td>11.2</td>
<td>14</td>
<td>22.5</td>
<td>8</td>
</tr>
<tr>
<td>Trichlormethazolamide</td>
<td>24</td>
<td>75.6</td>
<td>125</td>
<td>30</td>
</tr>
<tr>
<td>Ethoxzolamide</td>
<td>80</td>
<td>230</td>
<td>165</td>
<td>40</td>
</tr>
</tbody>
</table>

Derivation for \( k_a \) and \( k_b \), see Calculations Section.

Undissociated form correlates positively with lipophilicity. Of particular significance here are the experiments with epithelium removed. Considering the free acid form, we find that as lipid solubility increases for these three compounds, participation of the corneal resistance represented by the epithelium drops from 98% to 0%. For the anionic forms of the three compounds, the epithelial barrier changes only threefold.

Figure 5 presents the relationship between in vitro permeability and lipid solubility of these three drugs at their pKₐ, thus normalizing the participation of each form among the 3 compounds. Again we see that the barrier for the penetrating compound is the corneal epithelium. After the epithelium is removed, total rate constants for all three sulfonamides are essentially the same.

**Permeability Constant**

The \( k_{in} \) values are converted to permeability constants \( (P) \) by the relation

\[
P = k_{in} \cdot \frac{\text{volume on the endothelial side}}{\text{corneal area}}
\]

The volume \( (V) \) of the solution on the endothelial side in the in vitro experiments is 6 ml (see Methods); in vivo anterior aqueous volume = 0.25 ml; the corneal areas \( (A) \) in vitro and in vivo are 1.3 cm², and 2.1 cm² respectively.

Thus:

\[
\frac{k_{in}^{vivo}}{k_{in}^{vitr}} = \frac{V^{vitr}}{V^{vivo}} \times \frac{A^{vitr}}{A^{vivo}} = 40
\]

Comparing Table 5 with Table 3 shows, that the observed ratios of \( k_{in} \) of the three drugs as free acids are much less: Bromacetazolamide = 1.6, Methazolamide = 1.2, and Trichloromethazolamide = 8.4. The reason for this difference is not clear, nor is it an essential part of our study. It may be caused by a parallel leakage pathway due to edge effects in clamping the cornea. If so, this would be a greater factor for hydrophilic drugs, since for these ones the permeability of the epithelium is low.

**Discussion**

Our data (Fig. 1) show that the penetration of sulfonamide carbonic anhydrase inhibitors through the rabbit cornea deviates from the pH-partition hypothesis. This can be explained by the penetration of the anionic form of these drugs at rates only an order of
Table 5. Experimental and theoretical corneal permeability in vitro at acidic and alkaline pH for three sulfonamides: Effect of epithelium removed

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH 5.4</th>
<th>7.6</th>
<th>8.6</th>
<th>6</th>
<th>7.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromacetazolamide</td>
<td>0.52</td>
<td>0.29</td>
<td>1.5</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Methazolamide</td>
<td>1.76</td>
<td>1.3</td>
<td>6.9</td>
<td>31</td>
<td>143.0</td>
</tr>
<tr>
<td>Trichlormethazolamide</td>
<td>26.3</td>
<td>23</td>
<td>17</td>
<td>106.1</td>
<td></td>
</tr>
<tr>
<td>Factor of Epith Removed</td>
<td>38</td>
<td>22</td>
<td>4.5</td>
<td>14.5</td>
<td>7</td>
</tr>
</tbody>
</table>

First order rate constants show that although the nonionic form penetrates the cornea more rapidly than the ionic, the ratio is only 4–7:1. On the basis of this we predict that the first order rate constant for acidic form of benzolamide is probably between 4.7 and 8.7. This would also fit perfectly into the Figure 4 placing this compound between bromacetazolamide and methazolamide. There are a few papers on the influence of pH on penetration of pilocarpine into the eye that are worthy of mention in this context. Anderson and Cowle reported their data essentially inconclusive as to the relative penetration of ionic and non-ionic forms, but their range of pH (4–6.5) was improper for pilocarpine, a base of pKa = 7.12. Ramer and Gasset found an approximately twofold difference between penetration of nonpolar and polar forms of pilocarpine.
Also the use of quaternary amines as carbachol and echothiophate support the concept that the ionized forms of drugs are penetrating through the cornea to some degree. Recent data on bioavailability of topically applied pyrilamine, histamine and cinmetidine show penetration of dissociated form into the eye.15

The cornea contains several layers which have different properties. For pharmacokinetic studies, it generally is useful to consider three major compartments which act as differential solubility barriers: first, a lipoidal epithelium; next, stroma, due to its chemical composition considered as a hydrophilic barrier; and third, again a lipoidal part of the cornea, endothelium. The drug almost certainly has the same degree of ionization regardless of external pH after it crossed the corneal epithelium, since all these structures are probably buffered to body pH. This also makes comparisons of a drug at two pH's at a fixed time valid.

Table 5 shows that when the epithelium is removed, the passage of poorly (bromacetazolamide) or modestly (methazolamide) lipid soluble drugs is increased, both in ionic and nonionic forms. The same is true for the ionized form of the lipophilic trichlormethazolamide. However, for the nonionized trichlormethazolamide, the epithelium is no barrier. As lipid solubility increases, the role of the epithelial barrier decreases, as shown by the "factors" in the last column of Table 5. It will be noted that the differences in the effect of epithelium among the drugs are far greater for the nonionized forms—38-fold—than for the ionized forms—3-fold.

We conclude that the transcorneal penetration of weak acids is the summation of the processes of nonionic and ionic diffusion. While the intrinsic rate constant of the former is the greater by some fivefold for a given compound, the much greater solubility of the anion dictates that at certain regions of pK and pH, the ionic rate may dominate. For example, at pH 7.5, 11% of trifluoromethazolamide is nonionic (Table 4). The solubility of the free acid is 8 mM (Table 1), and the total solubility at pH 7.5 is 63 mM (Table 2), so the contribution of the ionic form in external solution is 7 times greater than that of the undissociated molecule. However, the decrease in rate constant from the acidic form (0.037/hr) to that at pH 7.5 (0.014/hr) is only 2.5-fold, so under these conditions ionic penetration dominates. Specifically, at limits of solubility at pH 7.5, and from $k_{in}$ of Table 4, we calculate

\[
\text{acid (nonionic) rate } 8 \text{ mM } \times 0.037/\text{hr} = 0.3 \text{ mM/hr} \\
\text{ionic rate } 55 \text{ mM } \times 0.008/\text{hr} = 0.44 \text{ mM/hr}
\]

The sum of 0.74 mM/hr is within experimental error of the observed rate at pH 7.5 of 63 mM $\times 0.014/\text{hr} = 0.88 \text{ mM/hr}.$

Such relationships appear rather surprising, but have been foreshadowed by the findings of ionic diffusion of weak acids in the intestine (ref 25–29). Extention to other tissues will be of clear pharmacological interest. To recapitulate: (1) Lipid solubility is a major factor which governs transcorneal penetration of sulfonamides. (2) Total rate constant for penetration is the summation of the rate constants of the dissociated and undissociated form. (3) The nonionized molecule penetrates the cornea only four- to sevenfold faster than its ionic form.

Key words: pH—partition theory, cornea, topical application, penetration of the ion, aqueous humor, carbonic anhydrase inhibitors

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