The Effects of Carbonic Anhydrase Inhibitors on Aqueous Humor Chemistry and Dynamics

Amir Dar-Ilian, Ness I. Pessah, and Thomas H. Maren

The effects of topical application of the carbonic anhydrase inhibitor trifluormethazolamide (TFM) on intraocular pressure (IOP), ascorbate and CO₂ concentrations in aqueous humor, and aqueous humor flow were studied in rabbits. These effects were compared with those produced by systemic treatment with methazolamide. The decrease in IOP observed after TFM was accompanied by changes in the composition of the aqueous humor. Posterior aqueous ascorbate concentration showed a marked increase (up to 1.7-fold), whereas the anterior aqueous ascorbate did not change significantly. Similar changes were found in rabbits after systemic treatment with methazolamide. A small but statistically significant decrease in the CO₂ content of both posterior and anterior aqueous was observed after topical TFM application. Methazolamide yielded a more profound lowering in the CO₂ content of the aqueous humor, a reflection of the significant decrease in plasma CO₂ content. For topical TFM or systemic methazolamide doses yielding complete inhibition of carbonic anhydrase in the eye, a 55–59% reduction of aqueous flow was calculated from the ascorbate data using the Kinsey and Palm equation. However, a 31–42% reduction in aqueous flow was obtained from the same data using an equation based only on posterior chamber data. The reasons for using only posterior aqueous ascorbate data for calculating the changes in aqueous humor flow are discussed. Invest Ophthalmol Vis Sci 25:1198–1205, 1984

Although carbonic anhydrase inhibitors given parenterally are widely used for the treatment of glaucoma, topical applications of these inhibitors have failed previously to cause a reduction of intraocular pressure (IOP). It was shown only recently that the reduction of IOP by topical use of carbonic anhydrase inhibitors is feasible. Trifluormethazolamide (TFM)—one of the 11 compounds tested—was reported to reduce IOP in the rabbit and aqueous flow in the cat.

The aims of this study were: (1) to explore further the effect of TFM on aqueous humor dynamics by measuring changes in the concentration of ascorbate and total CO₂ in the anterior and posterior aqueous humors; and (2) to compare the effects of the topical treatment with TFM to those produced by systemic treatment with methazolamide.

Materials and Methods

Adult male New Zealand albino rabbits, weighing 2.3–3.3 kg, average weight 2.8 kg, were used in all experiments. The experimental procedures employed here conform to the ARVO Resolution on the Use of Animals in Research. Animals were kept in the laboratory, in individual cages with access to food and water, for at least 1 day before the experiment. During this period, their intraocular pressure (IOP) was recorded intermittently, using a Digilab model 30R pneumotonometer. The pneumotonometer readings were matched with a three-point standard pressure reading, performed at least twice a day, using the Digilab Calibration Verifier. One drop of 0.5% propracaine (Ophthetic-Allergan Pharmaceuticals, Inc.), diluted 1:2 with saline, was instilled in each eye immediately prior to each set of IOP measurements. All animals that showed a consistent difference in IOP between eyes, or any sign of eye irritation, were not used in these experiments.

For topical drug application, the animals were anesthetized with an intraperitoneal (i.p.) injection of 70 mg/kg sodium thiamylal (Bio-Tal, Bio-Ceutic Laboratories, Inc.). The eyelids were protracted by attaching a hemostat to the fur, 1.5–2.0 cm from the lid margin. By gentle application of minimal leverage to the hemostat, the eyelids were raised slightly, thus creating an enlarged cul-de-sac. This method of eyelids protraction prevented any mechanical irritation to the conjunctiva.

A 3% (100 mM) trifluormethazolamide (TFM) solution in saline was prepared immediately prior to
the application by neutralization of the free acid to pH 7.8 with NaOH. A volume of 500 μl of this solution was instilled into the enlarged cul-de-sac, making sure that the cornea was covered completely. Exposure time was 5 or 25 min. The eye was washed with large volumes of warm water. Control eye was washed in the same way. Animals regained their righting reflexes 30–40 min after induction of anesthesia. Control experiments with sham-treated animals (saline application) did not reveal any detrimental effect of this method of drug application on IOP.

IOP of both eyes was recorded at 1-hr intervals. Three to four hours after the drug was washed from their eyes, animals were anesthetized with i.p. injection of 40 mg/kg pentobarbital. The needle of a 50 or 100 μl Hamilton syringe (26 G) was inserted into the posterior chamber, through the sclera. The needle tip could be seen behind the iris and its position ascertained. After withdrawal of the posterior aqueous sample, an anterior aqueous humor sample was obtained with a syringe and a 27-gauge needle placed in the anterior chamber through the cornea.

In addition to the 3% TFM treatment, a group of eight rabbits was treated with a 5% solution of TFM (pH 8). Exposure time was 10 min. IOP measurements were carried out as in the other groups. Aqueous humor samples for ascorbate determination were obtained from five of these rabbits.

Systemic Treatment

The rabbits were injected subcutaneously (s.c.) with 50 mg/kg of methazolamide (50 mg/ml suspended in 0.9% NaCl, pH adjusted to 7.3–7.4). A second injection was administered 10 hr after the first one. Posterior and anterior aqueous samples were obtained as described before, 3–4 hr after the last methazolamide treatment. Blood was obtained by heart puncture. IOP was measured prior to the aqueous and blood sampling. This reading ("IOP treated") was matched with an IOP reading obtained from the rabbit at the same time of the day, 1 day before the beginning of the methazolamide treatment ("IOP control").

Ascorbic Acid Determination

The method used here was adapted from Pirie and modification recommended by D. E. Gäasterland (personal communication). It is based on the decrease of absorbance of a standard dye solution that follows addition of ascorbic acid. The standard solution contained 2 ml of 0.1 M, pH 6.8, phosphate buffer solution, 0.8 ml of dye solution containing 0.9 mM of 2,6-dichlorophenol-indophenol and 2.5 mM NaHCO₃. Final volume was adjusted to 5 ml with water. This was mixed well and its absorbance read immediately at 600 nm using a Gilford model 252 spectrophotometer coupled to a Beckman DU model 2400 spectrophotometer. An aqueous humor sample (25–50 μl) was added to the standard solution, mixed well and read as mentioned above. A standard curve was constructed using L-ascorbic acid (Sigma). The curve was linear over the range of values measured here (0–4.5 mM). The average variation between duplicates of standard solution, anterior and posterior aqueous samples did not exceed 5%.

Aqueous samples were stored on ice until assayed, within 10 min of sampling. No significant change in ascorbic acid concentration was found in a series of anterior aqueous samples, stored on ice and assayed at 5, 10, 15, and 20 min of storage.

Total CO₂ was measured in 30 μl aqueous humor samples using a Natelson microgasometer model 600 with a motorized shaker attachment, at room temperature (24 ± 1°C). CO₂ in plasma was measured immediately after separation of the blood.

Assay of drug levels in anterior aqueous, plasma, and red blood cells (RBC) was carried out according to Maren et al.

Statistical Analysis

The results are expressed by mean ± standard error (SE) unless stated otherwise. Student's t-test was used to compute the significance of the differences between groups. Correlation and regression coefficients were calculated using the least squares method.

Results

Topical TFM Treatment

Intraocular pressure: The time course of changes in IOP after topical application of 3% TFM for 25 min is presented in Figure 1. The use of sodium thiamylal (an ultra-short-acting thiobarbiturate) resulted in a temporary decrease in IOP. By 3 hr, the IOP of the control eye was not significantly different from that measured prior to drug application. By 4 hr, the IOP was somewhat higher than that measured prior to drug treatment. A similar rise in IOP also was observed in untreated rabbits and is part of the diurnal variations in IOP (Bar-Ilan, unpublished data).

The average difference in IOP between the treated and control eyes, which was +0.4 ± 0.3 mmHg (n = 16) prior to the 25-min application of 3% TFM, changed to −2.3 ± 0.3 mmHg (n = 16) 3–4 hr later. This difference is significant at P < 0.01.

Application of 5% TFM for 10 min also resulted in a decrease in IOP. The average difference in IOP between the treated and the control eyes, which was −0.3 ± 0.3 mmHg (n = 8) prior to drug application,
increased to $-1.2 \pm 0.4$ mmHg 3-4 hr later. This difference is significant at $P < 0.05$.

Exposure for 5 min to TFM resulted only in a small decrease in IOP of the treated eye, as compared with the control eye. The average difference in IOP, between the two eyes, which was $-0.2 \pm 0.3$ mmHg (mean $\pm$ SE, $n = 16$) before drug application, became $-0.9 \pm 0.4$ mmHg ($n = 16$) 3-4 hr later. This difference is not significant at $P < 0.05$. Since in other experimental groups (Tables 1, 2) this dose resulted in a small but statistically significant ($P < 0.05$) effect, its hypotensive effect is equivocal.

**Ascorbate and CO$_2$ content in aqueous humor:**
Concentrations of ascorbate in anterior and posterior aqueous humor samples, obtained from the different experimental groups 3-4 hr after drug exposure terminated, are presented in Table 1. Treatment with TFM resulted in an increase in ascorbate concentration in posterior aqueous humor but never a significant change in anterior aqueous. The 25-min treatment increased posterior ascorbate 1.7-fold; 10-min and 5-min treatments, 1.37- and 1.38-fold, respectively.

Becker$^9$ showed that changes in the concentration of ascorbate in aqueous humor following pharmacologic intervention yielded an estimate of the relative change in aqueous humor flow. The calculation was based on the simplified formula of Kinsey and Palm$^5$ and Friedenwald and Becker.$^6$

$$K_F = \frac{C_{AC} - C_{PL}}{C_{PC} - C_{AC}}$$

$K_F =$ the coefficient of transfer by flow into and out of the anterior chamber; $K_D =$ the coefficient of transfer of diffusion between blood and anterior chamber; $C_{AC} =$ steady state concentration in the anterior chamber; $C_{PC} =$ steady state concentration in the posterior chamber; and $C_{PL} =$ steady state concentration in the plasma.

The relative change in aqueous flow $\Delta F$ is calculated by:

$$\Delta F = \left( \frac{K_F}{K_D} \right)_{\text{control}} - \left( \frac{K_F}{K_D} \right)_{\text{treated}} \times \frac{100}{\left( \frac{K_F}{K_D} \right)_{\text{control}}}$$

The $\Delta F$ values obtained here are: control: $-5.2 \pm 8.4\%$ ($n = 12$); 3% TFM, 25 min: $-55.0 \pm 8.3\%$ ($n = 9$); 5% TFM, 10 min: $-39.0 \pm 4.9\%$ ($n = 5$); and 3% TFM, 5 min: $-37.5 \pm 8.1\%$ ($n = 10$).

A linear relationship was found between the decrease in IOP and the calculated $\Delta F$ (Fig. 2). This relationship can be described by the regression line:

$$\Delta IOP = 0.053 \Delta F + 0.735; \ (r = 0.966).$$

$\Delta IOP$ in aqueous humor, as measured in the different experimental groups, again 3-4 hr after the end of treatment, is presented in Table 2. The reduction in IOP caused by the 3% TFM application for 25 min was accompanied by a significant but small decrease in CO$_2$ content in both anterior (4 mM) and posterior

### Table 1. Ascorbate concentration in aqueous humor and changes in intraocular pressure in rabbits treated topically with TFM$^*$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control eye (C)</th>
<th>Treated eye (T)</th>
<th>$\Delta IOP (T-C)$ mmHg</th>
<th>n**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$1.13 \pm 0.09$</td>
<td>$1.18 \pm 0.08$</td>
<td>$+0.3 \pm 0.2$</td>
<td>12</td>
</tr>
<tr>
<td>3% TFM (5 min)</td>
<td>$1.04 \pm 0.18$</td>
<td>$1.12 \pm 0.12$</td>
<td>$-1.1 \pm 0.4$</td>
<td>10</td>
</tr>
<tr>
<td>3% TFM (25 min)</td>
<td>$1.40 \pm 0.22$</td>
<td>$1.63 \pm 0.19$</td>
<td>$-2.2 \pm 0.5$</td>
<td>7</td>
</tr>
<tr>
<td>5% TFM (10 min)</td>
<td>$1.35 \pm 0.11$</td>
<td>$1.48 \pm 0.21$</td>
<td>$-1.0 \pm 1.1$</td>
<td>5</td>
</tr>
</tbody>
</table>

$^*$ Values are mean $\pm$ SE.

$\dagger$ A.C. = anterior chamber.

$\ddagger$ P.C. = posterior chamber.

$\dagger\dagger$ Significantly different from control at $P < 0.01$. $P < 0.05$, and $P < 0.10$.

$**$ n = number of animals.
Table 2. CO₂ Concentration in aqueous humor and changes in intraocular pressure in rabbits treated with TFM*

| CO₂ (mM) | Control eye (C) | Treated eye (T) | ΔIOP (T-C) | n
|---------|----------------|----------------|-------------|-----
|         | A.C.†          | P.C.‡          | A.C.        | P.C. | mmHg     |   
| Control | 34.7 ± 0.8     | 38.8 ± 1.0     | 34.9 ± 0.8  | 39.1 ± 1.4     | +0.1 ± 0.2 | 10 
| 3% TFM (5 min) | 35.5 ± 1.2     | 39.0 ± 1.4     | 36.4 ± 1.8  | 37.8 ± 1.6     | −0.9 ± 0.5ξ | 7   
| 3% TFM (25 min) | 35.1 ± 0.7     | 39.0 ± 0.9     | 32.1 ± 0.7† | 36.3 ± 1.0†    | −2.0 ± 0.59 | 9

* Values are mean ± SE.
† A.C. = anterior chamber.
‡ P.C. = posterior chamber.
ξ Significantly different from control at: P < 0.05; †P < 0.01.
‖ n = number of animals.

(2.5 mM) aqueous. The CO₂ decrease for the 5-min treatment was statistically insignificant despite the significant decrease in pressure observed in this group of rabbits.

Systemic Methazolamide

Twenty-four hours after the onset of treatment, methazolamide yielded a mean plasma level of 66.1 ± 8.0 μM (n = 10). The concentration of methazolamide in the red blood cells was 98.0 ± 5.5 μM (n = 16). The anterior aqueous concentration was 12.2 ± 1.2 μM (n = 22). These drug levels ensure a complete inhibition of the carbonic anhydrase in the ciliary processes.7

An average decrease of −4.4 ± 0.3 mmHg (n = 14) was observed in IOP, 3–4 hr after the last administration of methazolamide (Table 3). Ascorbate concentration in anterior aqueous of these rabbits was no different from that of the control group. A significant (P < 0.01) increase (1.63-fold) was observed in their posterior aqueous ascorbate concentration (Table 3). A calculated relative change in aqueous flow (ΔF) of −58.3 ± 6.0 (n = 8) was obtained from the ascorbate data using the Kinsey-Palm formulation as described above.

The CO₂ content in plasma and aqueous of rabbits treated with methazolamide was significantly (P < 0.01) lower than the corresponding levels in the control group. The 33% decrease in plasma CO₂ content was accompanied by a decrease of 38% in anterior and 24% in posterior aqueous CO₂ content (Table 3).

Discussion

Methodology

The method of drug application employed here has been used previously in the study of topically applied carbonic anhydrase inhibitors since it allows a stricter control over the amount of drug applied than would be possible with the common "clinical" method.1 Using the same procedure here makes it possible to correlate the pharmacokinetics and pharmacodynamics of these drugs.

The IOP values obtained here for the control conditions are in excellent agreement with those reported by other investigators using a pneumotonometer calibrated for the rabbit eye,8–10 although the pneumotonometer used here was not calibrated for the rabbit eye.

General anesthesia with barbiturates has been shown previously to affect IOP and aqueous humor dynamics in the rabbit.11,12 Preliminary experiments with the ultra-short-acting barbiturates used here showed that the initial reduction in IOP diminished within the first 2–3 hr following the induction of anesthesia, and no measurable effects on IOP could be demonstrated afterwards. In order to minimize
any possible effect of the anesthetic, the data was analyzed and presented not as absolute values, but in terms of relative changes (ΔIOP, ΔF; see Materials and Methods), calculated separately for each animal, the contralateral eye serving as a control. We assumed that any possible change in aqueous humor dynamics induced by the anesthetic would affect both eyes equally.

Ascorbate

The average ascorbate concentration in anterior aqueous obtained here for control eyes is 1.20 ± 0.08 mM (n = 31). This is in good agreement with values reported by other investigators, 1.16 ± 0.09, 1.28 ± 0.05, 0.96, 1.32 ± 0.11, 1.08 ± 0.04.

Table 1 shows that the topical administration of TFM did not significantly change the ascorbate concentration in the anterior aqueous humor. It is important to notice that this is also the case for systemic inhibition as shown clearly by Table 3 for methazolamide. These data are in agreement with the early studies of Becker who showed that 6 hr after acetazolamide the concentration of ascorbate in the anterior aqueous changed only from 1.30 to 1.47 mM. Inspection of the individual data show that this change is not significant. These findings have an important bearing on the theoretic treatment of the increase in the posterior aqueous ascorbate concentration, which appears to be dose dependent (Table 1). The largest increase was for the 3% solution applied for 25 min, 1.7-fold in the concentration.

This may be compared with the results after systemic administration of methazolamide (Table 3), which yields almost exactly the same change, an increase in concentration of 1.65-fold. Becker’s data are remarkably close to this. Following systemic acetazolamide, the equivalent change was 1.5-fold.

CO₂ Content

The average CO₂ concentration in control eyes, obtained here was 35.0 ± 0.4 mM (n = 37). This is slightly higher than the data previously reported, which ranges between 35.0 ± 0.6 (n = 37) for posterior aqueous. These values are within the range of total CO₂[HCO₃⁻] values reported by other investigators. Treatment with topical TFM resulted in a small but statistically significant decrease in the CO₂ concentration of both anterior and posterior chambers (Table 2). Again, this may be considered somewhat dose or time related; note that longer exposure to TFM resulted in a larger decrease. It should be emphasized that the maximum decreases seen here, approximately 3 mM, are only about 8% of the total concentration of CO₂ present, so that for the present purposes the CO₂ concentrations are relatively unchanged. This is in agreement with earlier studies in dogs in which, 1 hr after systemic acetazolamide the aqueous humor concentrations of CO₂ are relatively unchanged, even though there is a marked decrease in flow and pressure at that time.

We suppose that HCO₃⁻ is formed by the catalytic hydroxylation of CO₂ in a milieu where there is a relative OH⁻ gradient at the apical cell surface, and CO₂ is freely diffusible. In a way still not completely understood, HCO₃⁻ is the pilot ion for the movement of fluid, i.e., its synthesis and movement controls flow. This being so, we should expect that the decrease in HCO₃⁻ formation be accompanied by a decrease in flow so that the concentration should remain relatively unchanged. That is the crucial finding of the present experiments and supports the idea that HCO₃⁻ and fluid move together. The data in Table 3 are presented because there have been rela-

### Table 3. The effect of methazolamide on IOP, ascorbic acid and CO₂ concentration in aqueous humor and plasma of rabbits*

<table>
<thead>
<tr>
<th></th>
<th>A.C.†</th>
<th>P.C.‡</th>
<th>Plasma</th>
<th>A.C.</th>
<th>P.C.</th>
<th>Plasma</th>
<th>ΔIOP (T-C) (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbate (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (C)</td>
<td>1.20 ± 0.08</td>
<td>2.02 ± 0.15</td>
<td>—</td>
<td>1.18 ± 0.14</td>
<td>3.30 ± 0.34</td>
<td>—</td>
<td>−4.6 ± 0.6</td>
</tr>
<tr>
<td>Treated (T)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbate (mM)</td>
<td>35.0 ± 0.4</td>
<td>39.0 ± 0.6</td>
<td>23.3 ± 1.1</td>
<td>21.8 ± 1.4²</td>
<td>29.5 ± 1.8²</td>
<td>15.7 ± 0.7²</td>
<td>−4.2 ± 0.3</td>
</tr>
<tr>
<td>CO₂ (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (C)</td>
<td>39.0 ± 0.6</td>
<td>—</td>
<td></td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated (T)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂ (mM)</td>
<td>23.3 ± 1.1</td>
<td>—</td>
<td></td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are mean ± SE; number of animals in parenthesis.
† A.C. = anterior chamber.
‡ P.C. = posterior chamber.
² Significantly different from control at P < 0.01.
Table 4. The effect of carbonic anhydrase inhibitors on the CO₂ content of aqueous humor in rabbits

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Treatment</th>
<th>% Δ[HCO₃⁻]</th>
<th>% ΔCO₂</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A.C.*</td>
<td>P.C.†</td>
<td></td>
</tr>
<tr>
<td><strong>Systemic application</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intact rabbits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>(1) 100 mg/kg i.v. + 25 mg/kg i.v. every 25 min for 6 hr</td>
<td>−24.7 ± 1.5</td>
<td>−23.9 ± 1.9</td>
<td>Becker¹⁴</td>
</tr>
<tr>
<td></td>
<td>(2) 100 mg/kg i.v. + 50 mg/kg i.v. every hour sampling after 2.5 hr</td>
<td>−26.9</td>
<td>− —</td>
<td>Langham and Lee¹⁶</td>
</tr>
<tr>
<td>Dichlorophenamide</td>
<td>30 mg/kg i.v. + 15 mg/kg i.p. every 2 hr for 4–6 hr</td>
<td>−33.1 ± 2.4</td>
<td>−32.7 ± 3.8</td>
<td>Constant and Falch¹⁵</td>
</tr>
<tr>
<td>Methazolamide</td>
<td>50 mg/kg s.c. twice daily sampling 3–4 hr after last treatment</td>
<td>−36.5 ± 2.7</td>
<td>−27.3 ± 3.4</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Nephrectomized rabbits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>(1) 100 mg/kg i.v. sampling 6 hr after treatment</td>
<td>−19.4 ± 1.2</td>
<td>−19.3 ± 1.5</td>
<td>Becker¹⁴</td>
</tr>
<tr>
<td></td>
<td>(2) 5 mg/kg i.v. sampling after 4 hr</td>
<td>−42.0</td>
<td>− —</td>
<td>Friedman et al²³</td>
</tr>
<tr>
<td><strong>Topical application</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trifluormethazolamide</td>
<td>(1) 3% solution, 500 μl on cornea for 5 min</td>
<td>−6.4 ± 1.7</td>
<td>−5.5 ± 1.2</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>(2) 3% solution, 500 μl on cornea for 25 min</td>
<td>−8.3 ± 1.2</td>
<td>−6.9 ± 1.3</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>(3) 2.5% suspension, 50 μl drop, 1–5 drops every 5 min</td>
<td>−13.0</td>
<td>− —</td>
<td>Stein et al.²</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Number of eyes in parenthesis.

%Δ[HCO₃⁻] or ΔCO₂ were calculated using:

\[
\text{[HCO₃⁻]}_{\text{Treated}} - \text{[HCO₃⁻]}_{\text{Control}} / \text{[HCO₃⁻]}_{\text{Control}} \times 100
\]


tively little data on experiments with carbonic anhydrase inhibition past a few hours yielding a steady state for ion concentrations in the aqueous fluids. The data show, as expected, a striking decrease (8 mM) in plasma HCO₃⁻ 24 hr after continuous exposure to systemic inhibition. The posterior chamber has decreased 10 mM and the anterior chamber about 13 mM. However, because of the very large changes in plasma, CO₂ concentration, pH and pCO₂ (not shown here),¹⁷⁻¹⁹ we regard that the interpretation of the CO₂ chemistry in the anterior and posterior chambers cannot reveal anything of interest about the mechanisms of the ocular effect.

In Table 4, we have compiled previous experiments on CO₂ and from this it is readily seen that the change in the anterior aqueous is usually close to that in the posterior aqueous, irrespective of whether the drug is given systematically or topically. We conclude from this that the two chambers are, with respect to CO₂ content, basically in close contact and may (almost) be considered as a single vessel.

Aqueous Flow Related to the Ascorbate Data

The data on ascorbate first were calculated as described above using the equation of Kinsey and Palm, which takes into account the concentrations of ascorbate in the anterior and posterior aqueous humors. However, an examination of our own data (Table 1) as well as those of others using different drugs has revealed that the concentration in the anterior aqueous does not change following drug intervention.¹¹⁻¹²,¹⁴,²⁴ The reason for this is not entirely clear. The selection of posterior chamber concentration of ascorbate as a means of measuring flow, an idea which originated with Becker,⁶,¹⁴ seems to us to be entirely sound on theoretic grounds. It is based on the fact that the secretion of ascorbate is independent of flow of aqueous so that as flow is reduced, the concentration of ascorbate rises in direct proportion. It is theoretically the same type of physiologic experiment as done for the cerebrospinal fluid formation by Pappenheimer²⁰ and adapted by Oppelt²¹ to the eye, in which a constant inflow of a marker such as inulin is diluted by new formation of cerebrospinal or aqueous fluid and the concentration of the foreign substance is a measure of flow. The only difference here is that, fortunately, ascorbate is a native substance to the eye. On analysis of the Kinsey-Palm formula, we can see no cogent reason for retaining the expression for concentration in the anterior chamber. The reasons for use of this term as included in the early papers by Kinsey and Palm⁵ and by Becker⁶ but presently do not appear relevant and, in fact, were not derived originally for ascorbate.

On the contrary, the inclusion of the term for the anterior aqueous introduces a systematic arithmetical distortion into the dilution system just described.
This is appreciated readily if we compare the expression X-A/Y-A with the simple expression X/Y. In this comparison, A is the anterior concentration before or after treatment, X is the concentration in the posterior chamber after treatment, while Y is the concentration in the posterior chamber before treatment. Inspection of these two expressions reveals that the former will always be greater than the latter, that is to say, X-A/Y-A is greater than X/Y. This means that the relative changes of X to Y are falsely enlarged by the inclusion of the expression for A. Table 5 compares the calculation of the change in flow used by the Kinsey-Palm formula with the simple expression based on dilution of the posterior chamber alone, that is the comparison of the concentration terms X to Y. As an example, if the concentration in the anterior chamber (A) is 1 (recalling it does not change by treatment), and the concentration of Y is 2, which increases to 3 (=X) after treatment, X-A/Y-A yields a 50% decrease in flow while X/Y yields only a 33% decrease.

Table 5 shows how this works out practically. If we take the numbers for the largest dose of the carbonic anhydrase inhibitors which yield complete inhibition of the enzyme, it is evident that the Kinsey-Palm formula yields values of 54.5-58.7% reduction in flow. The expression based only on posterior chamber data, however, yields a value of 31.2-41.6% reduction. This is a considerable difference.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% ΔF</th>
</tr>
</thead>
<tbody>
<tr>
<td>3% TFM, 5 min (applied to cornea)</td>
<td>-37.5</td>
</tr>
<tr>
<td>3% TFM, 25 min (applied to cornea)</td>
<td>-55.0</td>
</tr>
<tr>
<td>5% TFM, 10 min (applied to cornea)</td>
<td>-39.0</td>
</tr>
<tr>
<td>Methazolamide (50 mg/kg, s.c. twice daily)</td>
<td>-58.7</td>
</tr>
<tr>
<td>Acetazolamide (100 mg/kg, i.v.) (Becker, 1955, intact rabbits)</td>
<td>-54.5</td>
</tr>
<tr>
<td>Ouabain (0.5 μg into the vitreous) (Becker, 1963)</td>
<td>-75.1</td>
</tr>
<tr>
<td>Halothane anesthesia (Krupin et al, 1980)</td>
<td>-48.9</td>
</tr>
<tr>
<td>Phenobarbital anesthesia (Becker et al 1970)</td>
<td>-30.8</td>
</tr>
</tbody>
</table>

Y = posterior aqueous concentration of ascorbate, before treatment.
X = posterior aqueous concentration of ascorbate, after treatment.
A = anterior aqueous concentration of ascorbate (virtually unchanged by treatment).

et al22 concluded that the maximum effect was some 30-40% lowering of the outflow. Using the IOP recovery rate after rapid infusion of 20% NaCl as an index of aqueous humor formation,23 it was found that treatment with 3% TFM for 25 min resulted in a decrease of 41% in aqueous humor formation (Bar-llan, unpublished data). Our present belief is that the right-hand column of Table 5 represents reliable flow data and that the left-hand column of this table represents a systematic, arithmetical, overcalculation as indicated above. This is true for any drug, as the data for ouabain, halothane, and phenobarbital show.

It is our hope that the future use of topical carbonic anhydrase inhibitors for local inhibition in the ciliary processes will lead to a more accurate quantification of the contribution of this enzyme to the secretion and dynamics of aqueous humor.

Key words: aqueous humor flow, aqueous humor ascorbate, intraocular pressure, trifluoromethazolamide, topical carbonic anhydrase inhibitor, methazolamide

References