The rates of ion movement from plasma to aqueous humor in the dogfish, *Squalus acanthias*

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Aqueous humor dynamics were studied in the dogfish, *Squalus acanthias*, using isotopically labeled inulin, Na⁺, Cl⁻, and HCO₃⁻. Fluid production was 100 ul per hour, with a turnover rate constant of 0.4 hr⁻¹, about half that of mammals. The ciliary process contains carbonic anhydrase and Na-K-ATPase. Diffusion of Na⁺ and Cl⁻ from plasma to aqueous was four to five times greater than flow, and from aqueous to vitreous, about 15 times greater. Sodium and chloride secretion is masked by the diffusion process; neither ouabain nor acetazolamide yield measurable effects on accumulation of these isotopes. HCO₃⁻ accumulation in aqueous was very rapid and was reduced by inhibition of carbonic anhydrase. Analyses of the data suggest that newly formed aqueous has similar Na⁺ concentration to plasma, but is high in HCO₃⁻ and low in Cl⁻. This anion pattern resembles mammalian aqueous humor, and cerebrospinal fluid and endolymph of other vertebrates. These and other data suggest that constant features of formation of these fluids in all phyla are sodium transport and formation of HCO₃⁻ from CO₂.

Keywords: aqueous humor, *Squalus acanthias*, elasmobranch, transport rates, carbonic anhydrase, Na⁺, Cl⁻, HCO₃⁻.

The roles of the several ions in aqueous humor production must be assessed by studying their comparative rates of movement, together with their steady-state concentrations. Only one such study has been done, the truly remarkable work by Kinsey and Reddy fifteen years ago. With meticulous attention both to details of sampling the two chambers of rabbit eye and to the mathematics of transport, they were able to conclude the following: sodium enters the posterior chamber largely (75 per cent) by secretion, i.e., one-way net transfer from plasma with newly secreted fluid, and this fluid has about the same sodium concentration (145 mM) as plasma and measured posterior chamber fluid. Chloride enters largely by diffusion. Its calculated concentration in newly secreted fluid is very low (53 mM), but increases as chamber fluid...
Rates of ion movement in dogfish eye

Exchanges with components of plasma and ocular tissues. HCO₃⁻ is a special case; gaseous CO₂ is converted to the ion in the ciliary epithelium with a half-time < 1 minute. The concentration of HCO₃⁻ in newly formed fluid could be gauged only by the difference between sodium and chloride, and was taken to be about 90 mM. An important additional finding was that the rate of HCO₃⁻ formation in aqueous was greatly slowed by inhibition of carbonic anhydrase.

These and related findings by others have recently been discussed, particularly with respect to the chemistry of HCO₃⁻ formation and the mechanism by which carbonic anhydrase inhibitors reduce the secretion of aqueous humor. Certain points, however, still demanded investigation, and the dogfish, Squalus acanthias, appeared very suitable. These points were:

1. The question of whether there are important species differences in the chemistry of aqueous humor formation among primates, dog, and rabbit. Investigation of a "stem vertebrate" should throw light on this.

2. Direct measurement of rates of HCO₃⁻ formation, which had not previously been done, and comparison of these rates with those of Na⁺ and Cl⁻ in one species.

3. Certain experiments with acetazolamide, ouabain, and CO₂ gas on the processes of ion accumulation.

The ionic composition of the aqueous humor in this elasmobranch has been studied, and the presence of the important enzymes, carbonic anhydrase and Na-K-ATPase, has been shown. From comparisons made between responses in chemical composition to acetazolamide in the dogfish and rabbit, it seemed that the basic secretory mechanisms might be similar, and might represent a generalized vertebrate pattern. To validate this critical issue, the following study of rates was undertaken.

Methods and materials

The biological methods, chemical analyses, drug use, isotope counting, and calculations have all been described. It is emphasized that the fish were swimming in the live cars throughout the experiment. For isotope and drug injections they were held by the tail and immersed in the sea; the injection was made into tail vein or artery. Fish were removed from the sea momentarily for sampling of aqueous humor. Each eye was used only once, but the second eye of the fish was often used for a later sample. For experiments in which eyes were kept out of water and for those involving elevated PCO₂, fish were brought to the laboratory and perfused through their spiracles with cold, oxygenated seawater. An important procedural point is that before H¹⁴CO⁻ was injected into fish treated with acetazolamide, the isotope solution was mixed with fresh fish blood to ensure equilibration between H⁻¹⁴CO⁻ and HCO₃⁻.

The rate of aqueous humor formation was measured with the fish immobilized in a trough-like wooden holder, while the gills were perfused with seawater through the spiracles at 1.5 L. per minute. The method is essentially that of Oppelt, using a dilution of ¹⁴C-inulin. The lateral conjunctiva, just posterior to the limbus, was grasped with a fine tweezer, and two 22-gauge needles, for inflow and outflow, were introduced into the anterior chamber. These were connected by polyethylene tubing to a Harvard infusion/withdrawal pump. To facilitate mixing, 5 mm. shafts of steel were introduced to the eye through the needle, and were rotated inside the chamber by an external magnet. It was found that this did not affect results, and was later discontinued. Perfusion rates were initially varied from 3 to 68 μl per minute, but in most experiments the rate was 14 μl per minute. At this rate, we observed that a fluorescein marker in the perfusion fluid filled the pupil and anterior chamber in about eight minutes. The perfusion fluid contained the solutes of plasma or aqueous which are essentially the same. To this "shark Ringers" was added heparin and radioactive inulin. The experiment could be run for about four hours.

Methazolamide (50 mg. per kilogram intravenous) was used to inhibit completely carboxic anhydrase in the isotope experiments reported here. This treatment is comparable to earlier experiments cited in which acetazolamide was used, since the two drugs have similar inhibitory potency, Kᵢ against carbonic anhydrase in this and most other vertebrate tissues being about 2 × 10⁻⁹ M. At the dose given, the degree of inhibition is about 99.99 per cent. Intravenous ouabain (40 to 60 μg per kilogram) was used to inhibit Na-K-ATPase, but this dose killed half the fish in six hours. If the drug is largely distributed in extracellular fluid and the Kᵢ for the enzyme is about 0.5 μM, the degree of inhibition in vivo is roughly 50 per cent.
Results

Flow, volumes, and concentration relations. Fig. 1 shows the rate of production of aqueous humor. The mean of 14 control periods ± S.E. was $1.72 \pm 0.34 \mu l$ per minute, or $100 \mu l$ per hour. Steady rates were achieved over a four-hour period. By careful withdrawal of aqueous into micro-syringes, the volume was estimated at 0.25 ml. The fluid turnover rate is thus $0.0067 \text{ min}^{-1}$ or 0.4 hr.$^{-1}$. It will be recognized that this is about half of the mammalian value.

The vitreous volume was estimated by weight as 3 ml.

Concentrations (mM) of ions in plasma and aqueous, respectively, are essentially those reported previously\textsuperscript{4}: Na\textsuperscript{+}, 255, 279; Cl\textsuperscript{-}, 239, 253; and HCO\textsubscript{3}\textsuperscript{-}, 7.7, 8.5. A few estimations of vitreous showed all three ions to be approximately the same as the plasma-aqueous range; close analyses were not necessary for the present study.

Entry of ions from plasma to aqueous: (1) sodium, (2) chloride, and (3) bicarbonate. The relative "units" of isotopes used in the graphs are those of concentration. All were measured on 0.1 ml of fluid, but this volume cancels out in the calculations of rate constants. Zero time is that of injection of isotope, and the times noted in text and figures follow from this. The concentration in plasma at 10 minutes is set at 100 for $^{22}\text{Na}^+$ and $^{35}\text{Cl}^-$; at 2.5 minutes it is set at 100 for $^{14}\text{HCO}_3\text{-}$.

(1) Sodium. Fig. 2 shows the accession of $^{22}\text{Na}^+$ from plasma to aqueous humor. In the first 10 minutes, there is rapid distribution of isotope, with a fast phase of decline in plasma (not shown). Thereafter, the concentration of isotope declines, but gradually, so that at hourly intervals it is constant enough to permit calculations of rate constants for ion accession to cerebrospinal fluid\textsuperscript{7} and endolymph.\textsuperscript{9} In the present case, the first sample of aqueous (15 minutes) showed counts $\%$ that of the maximum achieved. Clearly, a rapid process was at work and the data were not amenable to calculation of a rate constant, which depends on measurements during the initial periods, i.e., well before equilibrium is approached. However, secretion of sodium is estimated by the fluid formation rate as follows. We assume that newly formed fluid is approximately isotonic with
plasma, and since sodium is essentially the only cation, this nascent fluid will have the same sodium concentration as plasma, thus the same turnover rate. The concentration of isotopic sodium (relative to plasma) entering by secretion in a given time should be the same as the fluid turnover at that time. Thus in 15 minutes the volume secreted is 25 μl; dividing by the volume of aqueous (250 μl) yields 0.1. However, Fig. 2 shows that at 15 minutes the isotopic sodium concentration is 0.21 of plasma. Thus it appears that at least as much sodium enters by diffusion as by secretion.

Put in another way: since Na⁺ is the only cation involved (K⁺ and others are less than 5 per cent of Na⁺), we may be confident in saying that its secretory rate is the same as that of aqueous itself; the first-order rate constant (k = 0.4 hr⁻¹) and halftime to equilibrium (1.7 hours) for Na⁺ are then equivalent to those for fluid turnover.

(2) Chloride. The situation for 32Cl (Fig. 3) is essentially the same as analyzed above for sodium. Here we have very reliable data at 30 minutes, showing 23 units in aqueous (n = 5), with supporting data for 20 units at 15 minutes. Analysis for the secretory component is similar to that for sodium, since the plasma isotope decay is the same, and we assume provisionally that the chloride concentration in secreted fluid is no greater than in plasma. Thus, in 15 minutes the isotope concentration for the secretion process should be, at most, 10, as for sodium. Clearly the measured isotope concentration units are greater, indicating rapid diffusion of the anion. The shape of the curve is such that initial rates were too rapid to measure accurately. The diffusion component can be stated, as for sodium, to be at least as great as, and probably greater than, the secretory.

For chloride, we cannot assign a secretory rate constant equivalent to flow, as we did for sodium, since there is a second major anion, HCO₃⁻, accumulating in aqueous. A fundamental problem in this field is assigning the relative roles of Cl⁻ and HCO₃⁻ in secreted fluid. In the following section we shall arrive at a tentative HCO₃⁻ rate and concentration, from which chloride values may be calculated (Discussion).
(3) Bicarbonate. Fig. 4 shows the accession of total $^{14}$CO$_2$ to aqueous. Since this is 97 per cent HCO$_3^-$ (pH of dogfish plasma is about 7.7), the data are essentially those for the ion. Under normal conditions, the process is well toward equilibrium at the first (3.5 minutes) sample, and a rate constant cannot be calculated. However, when carbonic anhydrase is inhibited, the 3.5 and 7 minute samples ($n = 29$) do permit accurate calculation, by the simple relation

$$k_n = \frac{\text{units accumulated/time}}{\text{mean units in plasma}} = 0.03 \text{ min}^{-1} \text{ or } 1.8 \text{ hr}^{-1}.$$

This experiment also suggests that the mechanism of HCO$_3^-$ accession, i.e., formation of the ion from CO$_2$ in plasma, is different from ion transport (i.e., sodium or chloride) since it is so much faster.

From $k_n$ in the inhibited or uncatalyzed state we may make the critical calculation of the concentration of HCO$_3^-$ in newly formed fluid; $k_n$ (1.8 hr.$^{-1}$) $\times$ plasma concentration (7.7 mM) yields the rate of accumulation, 14 mM hr.$^{-1}$. The concentration in newly formed fluid is then given by the expression:

$$\text{Rate of accumulation (14 mM hr}^{-1}) \times \frac{\text{volume (250 } \mu\text{l})}{\text{flow (100 } \mu\text{l/hr.})} = 35 \text{ mM.}$$

The early part of Fig. 4 shows that the normal HCO$_3^-$ rate is at least twice the uncatalyzed and, since inhibition does not change volume or flow in this species, the concentration of HCO$_3^-$ in uninhibited newly formed fluid may be 70 mM or more (Table I). This calculation is based on the assumption that there is no appreciable back flux of formed HCO$_3^-$ in the inhibited situation. This matter will be treated in the Discussion. It is interesting that Kinsey and Reddy's data on HCO$_3^-$ accumulation in the posterior chamber of rabbit yield data similar to ours: in the normal HCO$_3^-$ accumulation is too fast to yield a rate constant, but in the acetazolamide-treated animal a rough constant of 0.16 min.$^{-1}$ may be calculated, which yields a HCO$_3^-$ concentration of about 60 mM in newly formed fluid. However, the exact concentration of HCO$_3^-$ in nascent fluid is not crucial; we do suggest that it is
Fig. 4. Accession of total $^{14}$CO$_2$ to aqueous humor from plasma in S. acanthias. Isotope injected at 0 time. All counts relative to plasma 100 at 2.5 minutes. Plasma curve is the same for normal fish and following carbonic anhydrase inhibition. Lowest curve (CAI) shows aqueous following injection of 50 mg. per kilogram methazolamide 30 minutes before the isotope. Standard errors of the mean are shown.

Table I. Rate constants and accession of ions to aqueous humor of S. acanthias

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Aqueous</th>
<th>$k_{vol}$ hr.$^{-1}$</th>
<th>Accession rate mM hr.$^{-1}$ (col 1 x col 3)</th>
<th>Concentration of newly formed fluid mM$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>255</td>
<td>279</td>
<td>0.4†</td>
<td>100</td>
<td>250</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>239</td>
<td>253</td>
<td>0.31‡</td>
<td>72</td>
<td>180</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>7.7</td>
<td>8.5</td>
<td>$-$3.6§</td>
<td>28</td>
<td>70</td>
</tr>
</tbody>
</table>

*Col 4 x volume aqueous (0.25 mL).
†From formation rate of fluid. See text.
‡By difference of accession rates Na$^+$-HCO$_3^-$.
§From two times the uncatalyzed $k_{vol}$, as a means of approximating a minimum catalytic constant. See text.

strikingly higher than in plasma, as it is for fish and cat cerebrospinal fluid$^5$, $11$ and aqueous humor of rabbit.$^1$

Fig. 4 shows fairly rapid equilibration of HCO$_3^-$ between plasma and aqueous; clearly HCO$_3^-$ is formed so rapidly in aqueous that its diffusion out into the vitreous (or elsewhere) has relatively little influence on the approach to equilibrium, compared to the situation for Na$^+$ (Fig. 2) and Cl$^-$ (Fig. 3).

Exit pathways from aqueous: role of vitreous. Fig. 1 shows that the vitreous at one hour has a considerable concentration of $^{24}$Na, 11.5 units. We calculate a rate constant for the passage of $^{24}$Na$^+$ from aqueous to vitreous, using the data of the first hour, when the concentration in the aqueous is about 23 units. Then

$$11.5 \, \text{hr.}^{-1} \times \text{vol. vitreous (3 ml.)} \div 23 \times \text{vol. aqueous (0.25 ml.)} = 6 \, \text{hr.}^{-1}.$$  

This rate constant is 15 times greater than that of secretion of fluid (and sodium) from plasma to aqueous, and is therefore largely (93 per cent) exchange diffusion of the ion. This aqueous to vitreous exchange is much greater than plasma to aqueous exchange, an expected finding since there are cell barriers between plasma and aqueous, but aqueous and vitreous are virtually continuous structures.$^1$ These relations fit the late (20 hour) data of Fig. 2, showing that isotopic equilibrium is not
reached between aqueous and plasma; $^{22}$Na$^+$ is drained too rapidly from aqueous to vitreous.

Fig. 3 shows similar relations for chloride, except that diffusion from aqueous to vitreous seems to be somewhat slower than that for sodium. Still the process is rapid enough to deplete isotope from aqueous, so that equilibrium with plasma is not achieved in 20 hours.

Fig. 4 shows that (unlike Na$^+$ and Cl$^-$) H$_{14}$CO$_3$ in aqueous reaches equilibrium with plasma in 40 minutes (normal) or 90 minutes (carbonic anhydrase inhibited). Clearly this is because HCO$_3$ formation in aqueous (1.8 hr.$^{-1}$ inhibited; twice or more this value in normal fish) is about as fast as ionic diffusion from aqueous to vitreous, as judged by the data given just above for Na$^+$ and Cl$^-$. The large diffusional exchange of sodium between aqueous and vitreous (6 hr.$^{-1}$) would mask the component due to fluid flow even if all the aqueous drained into the vitreous (0.4 hr.$^{-1}$). Since there is no identifiable canal of Schlemm nor aqueous veins in this species (also personal communication from Dr. Lee Jampol), it is likely that vitreous is an outflow pathway, but details remain to be discovered.

The question also arises as to whether Na$^+$ and Cl$^-$ in aqueous are exchanging, across the cornea, with ions in the sea. This seems unlikely, since there is a considerable gradient for both ions across the cornea; seawater is 450 mM in NaCl. However, this point was studied directly by repeating the experiments of Figs. 2 and 3 with fish out of the sea, arranged as described under Methods, and with their eyes out of water. For both Na$^+$ and Cl$^-$, eight experiments were done, half at one hour and half at three hours. The Cl$^-$ data were indistinguishable from those of Fig. 3. The Na$^+$ data yielded points for aqueous about 25 per cent below those of Fig. 2, indicating either a modest component of corneal diffusion or a reduction of Na$^+$ inflow due to the modification of the procedure.

**Effect of elevation of $P_{CO_2}$.** In a variety of secretory systems, CO$_2$ has been shown to alter rates of fluid and ion output$^{10}$. in cases where HCO$_3$ is the measured product, elevation of $P_{CO_2}$ markedly increases its formation, as we have shown for cerebrospinal fluid and endolymph.$^{1,9}$ This was tested for aqueous humor, with the results shown in Fig. 5. It is apparent that the aqueous does not behave like these other fluids, at first a surprising finding, since we believe the secretory mechanisms are the same. Fig. 5 shows, however, that the $P_{CO_2}$ of aqueous humor itself does not rise in the face of systemic respiratory acidosis. This shows that the cornea is sufficiently permeable so that a high concentration of CO$_2$ cannot be maintained in the aqueous, unlike the situation in the relatively closed cerebrospinal fluid and endolymph.$^8$ Either the $P_{CO_2}$ gradient from plasma to aqueous is so steep that it is relatively low at the secretory site itself, or the diffusion of CO$_2$ from aqueous into the sea serves to keep the HCO$_3$ low.

Elevation of plasma CO$_2$ can also be induced by acetazolamide or methazolamide, due to inhibition of red cell carbonic anhydrase. The effect is very prominent in fish because they do not hyperventilate.$^3$ Table II shows that acetazolamide increases the HCO$_3$ concentration of cerebrospinal fluid and endolymph, just
Table II. Effect of acetazolamide* on CO₂ equilibria of body fluids in *S. acanthias*

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Control pH</th>
<th>Control Pco₂ mm Hg</th>
<th>Control HCO₃⁻ mM</th>
<th>2 hours pH</th>
<th>2 hours Pco₂ mm Hg</th>
<th>2 hours HCO₃⁻ mM</th>
<th>2 hours pH</th>
<th>2 hours Pco₂ mm Hg</th>
<th>20 hours HCO₃⁻ mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>7.61</td>
<td>7.4</td>
<td>7.14</td>
<td>7.7</td>
<td>7.5</td>
<td>16</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>7.68</td>
<td>5</td>
<td>7.80</td>
<td>7.63</td>
<td>7.14</td>
<td>15</td>
<td>13</td>
<td>11</td>
<td>8.4</td>
</tr>
<tr>
<td>Endolymph</td>
<td>7.80</td>
<td>5</td>
<td>7.30</td>
<td>7.11</td>
<td>7.31</td>
<td>6</td>
<td>4.7</td>
<td>7.60</td>
<td>6</td>
</tr>
<tr>
<td>Aqueous</td>
<td>7.63</td>
<td>5</td>
<td>8.4</td>
<td>7.30</td>
<td>(15)</td>
<td>6</td>
<td>4.7</td>
<td>7.60</td>
<td>(16)</td>
</tr>
</tbody>
</table>

*Thirty milligrams per kilogram intravenously. Full inhibition is maintained through this time; half life is 1.5 days. From refs. 4 and 5, with additional pH data for aqueous. Figures in parentheses are Pco₂ taken from plasma.

like CO₂ gas. Again, aqueous humor HCO₃⁻ is not affected in this way since its Pco₂ does not rise. Table II shows the early decrease in aqueous humor HCO₃⁻ concentration following acetazolamide, due to decrease in its formation (Fig. 4). Although acetazolamide also yields a decrease in HCO₃⁻ formation in cerebrospinal fluid and endolymph, the effect of hypercapnia leads ultimately to an increase in its concentration in these fluids (Table II).

**Effects of methazolamide and ouabain on accession of ions.**

*Methazolamide.* The effects upon HCO₃⁻ have already been discussed (see above and Fig. 4), and fit with the finding of carbonic anhydrase in the ciliary process. Methazolamide was also used in the experiment on Na⁺ and Cl⁻ accession. The drug was given intravenously (50 mg per kilogram) 30 minutes before isotope. Accumulation in aqueous was studied from 0.25 to 3 hours, in 28 experiments for Na⁺ and 17 for Cl⁻. There was no drug effect; data were identical to controls as shown in Figs. 2 and 3. This result fits our analysis above, that a large component of both Na⁺ and Cl⁻ enter by diffusion. The portion of Na⁺ entering with the moiety of HCO₃⁻ affected by acetazolamide is small (see Discussion) and would be masked by the diffusion process.

The effect of acetazolamide on aqueous humor flow was studied in five fish. The value was 1.79 ± 0.27 µl per minute, which is indistinguishable from control data (Fig 1).

**Ouabain.** This was given at 40 to 60 µg per kilogram 30 minutes before isotope. Six experiments each were done for Na⁺ and Cl⁻, half at 0.5 hour, and half at 2 hours. Six experiments were done for HCO₃⁻ at 3 to 6 minutes. The drug gave no effects; all data fell in the range of the controls shown in Figs. 2 through 4.

**Discussion**

Two main points will be considered: (1) the chemistry underlying aqueous humor formation, and (2) the dynamics of flow. Both will be viewed in their comparative aspects, to inquire whether a general vertebrate pattern is discernible in our data.

**Chemistry.** The chemistry has been analyzed only in terms of the HCO₃⁻ accumulating system, which involves carbonic anhydrase; however, there is presumptive evidence that the Na-K-ATPase system is also at work. The primary finding is that methazolamide reduces HCO₃⁻ accession to aqueous; presumably the reaction is plasma CO₂ + OH⁻ (at secretory side of cell) → aqueous HCO₃⁻ + carbonic anhydrase.

This is the same process that occurs in the rabbit. The calculated concentration of HCO₃⁻ in new fluid is also of fundamental importance, and rests on whether the isotopic rate truly measures net accumulation of fluid or merely reflects diffusional exchange of isotope with unlabeled ion. The situation for HCO₃⁻ is complicated by its equilibrium relation with CO₂ and requires discussion.
Fig. 6. Early stages of accumulation of isotopic HCO₃⁻ in aqueous humor. Starred °C and pathways indicate movement of H¹⁴CO⁻ following its injection into plasma. CA = carbonic anhydrase. Step 4 (uncatalyzed) or step 2 (catalyzed, in cell) can deplete cold HCO₃⁻ by back diffusion of CO₂ from aqueous (step 5, arrow toward cell and plasma). But label of neither species is depleted from aqueous since it is still moving toward equilibrium, i.e., building up in aqueous. Thus, measured isotopic rate may exceed true net rates of formation (step 1) and transfer (step 3) of HCO₃⁻ to aqueous. Inhibition of carbonic anhydrase slows formation (step 1) and, ultimately, accumulation of HCO₃⁻ in aqueous.

We portray the situation in Fig. 6. The general secretory mechanism involves the production of a high concentration of OH⁻ at the cell border, and we show here only how isotopic HCO₃⁻ may be accumulated and the possibility for exchange with unlabeled CO₂. The central point is that HCO₃⁻ accumulates in high concentrations on the secretory side and in the nascent fluid. As a first approximation, we take this to be a unidirectional process, with the gradient furnished by the OH⁻ (step 1). As HCO₃⁻ is formed however, it comes into equilibrium with CO₂; presumably, this can occur either in the cell (step 2) or in the aqueous (step 4). At step 2 carbonic anhydrase is involved; at step 4 it is not. Thus the rapidly diffusible species, CO₂ can deplete HCO₃⁻ at steps 4 and 5. In the isotope experiment, the labeled pair H¹⁴CO⁻-¹⁴CO₂ mixes with HCO₃⁻-CO₂ in the cell and in the aqueous; CO₂ then back diffuses (step 5) depleting unlabeled HCO₃⁻. Thus the rate of H¹⁴CO⁻ accumulation may overstate the net HCO₃⁻ gain. However, when carbonic anhydrase is inhibited or when the reaction is confined to the aqueous the process HCO₃⁻ → CO₂ (steps 2 and 4) is slow and limits back diffusion and depletion. The uncatalyzed halftime at pH 7.4 and 16° is about 10 minutes. Thus, if we hold that the OH⁻ gradient (step 1) is unchanged by inhibition and that HCO₃⁻ formed at the cell border moves one way down its concentration gradient into aqueous (step 3), the rate from the inhibited experiment may truly measure net formation. Dehydration steps 2 and 4 are now both slow relative to the formation rate of HCO₃⁻. Thus the isotopic rate may measure the net rate. These relations are shown in Table III, in model form. This model conforms to the present data and
Table III. Model for rate constants of HCO$_3^-$ accumulation in posterior aqueous.

<table>
<thead>
<tr>
<th></th>
<th>Formulas</th>
<th>Back movement</th>
<th>Net HCO$_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH$^-$/CO$_2$</td>
<td>HCO$_3^-$/CO$_2$</td>
<td></td>
</tr>
<tr>
<td>Control:</td>
<td></td>
<td></td>
<td>10 (may be too fast to measure, cf. Fig. 4)</td>
</tr>
<tr>
<td>H$^+$CO$_3^-$</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Inhibited = uncatalyzed:</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>H$^+$CO$_3^-$</td>
<td>1</td>
<td>0</td>
<td>1 (observed, Fig. 4)</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Isotopic rates from early measurements, when there is large gradient of label from plasma to aqueous.
Entries of zero are not intended to be absolute, only small in relation to other numbers.

Depletion by the CO$_2$ back-diffusion route is not inevitable for the normal, uninhibited situation, since it depends on the balance of these several factors and volume relations in the system. In the case of HCO$_3^-$ accession to the cerebrospinal fluid in dogfish, the label did appear to measure net formation, in the normal state. This suggests that dehydration at step 2 was not dominant. In other situations, particularly in the mammalian aqueous and cerebrospinal fluid, isotopic accession appeared complicated by back diffusion. The difference, at least in part, is explainable on the basis of temperature. At 16°, uncatalyzed rates are 1/2 those at 37°; specifically, the halftime for HCO$_3^-$ dehydration at 37° is 100 seconds, which is about the time that isotopic equilibrium is achieved and the mammalian experiment finished. But at the lower temperature of the fish the accumulation of isotopic HCO$_3^-$ is observed at 1 to 6 minutes (Fig. 4 and Reference 7) when only a small fraction of HCO$_3^-$ in the fluid is dehydrated.

In both our work in fish and that of Kinsey and Reddy in rabbit, the uncatalyzed accumulation of H$^+$CO$_3^-$ in aqueous could be measured, and seemed consistent with a net rate (see the model of Table III). From their data with acetazolamide we calculate (see Results: HCO$_3^-$ entry) the concentration of HCO$_3^-$ in nascent fluid as about 60 mM; our value is 35 mM. In both cases this value is some three to five times that of HCO$_3^-$ concentration in plasma.

In the control uninhibited fish (as in the control rabbit of Kinsey and Reddy) the net isotopic rate is too rapid to measure. Could we have taken serial samples before 2 minutes, we might have measured a net isotopic rate twice or more what we found in the inhibited state (Fig. 4). We take our experimentally determined concentration of HCO$_3^-$ in newly formed fluid during inhibition (35 mM) as equivalent to net accumulation of cold ion (Table III) and approximate the normal value at about twice this concentration based on effects of inhibition in a number of physiologic systems. The absolute value is of no importance other than the fact that it is much higher than plasma, and that carbonic anhydrase inhibition reduces it. We see no other way to explain the findings of Fig. 4, than formation of HCO$_3^-$ in aqueous as shown in Fig. 6.
If HCO$_3^-$ in nascent fluid is 70 mM, it is the counter ion for about 30 per cent of Na$^+$; in the rabbit the comparable value is 60 per cent. It is also of interest that newly formed cerebrospinal fluid in the dogfish has an HCO$_3^-$ concentration of 82 mM.$^7$

Table I attempts to approximate the concentrations of ions in newly formed aqueous. Presumably, Na$^+$ is actively transported via the Na-K-ATPase system in concentration isotonic with plasma. HCO$_3^-$, as described, is formed in high concentration. Cl$^-$ is in deficit. This agrees with data from the rabbit.$^1$

In *S. acanthias*, when carbonic anhydrase is inhibited, flow does not measurably decrease; this is different from all mammals. However, the difference almost certainly lies in the fact that the ratio of Na$^+$/HCO$_3^-$ in mammalian body fluids is about 6, while in the dogfish it is 35. Thus when HCO$_3^-$ formation is reduced in this species of fish, the influence on sodium is not great enough to affect flow.

Failure of acetazolamide or ouabain to reduce isotopic Na$^+$ and Cl$^-$ transport to aqueous in this species can readily be explained by the large component of exchange diffusion for these ions. It is almost certainly for the same reason that acetazolamide has failed to reduce $^{22}$Na$^+$ transfer from plasma to aqueous in mammals, which had been a vexing question.$^{14}$ Ouabain did decrease Na$^+$ transport in the rabbit under special conditions of blocking aqueous outflow.$^{15}$ When the diffusional component is reduced by perfusion, both drugs reduce isotopic Na$^+$ and Cl$^-$ transport into cat aqueous.$^{16}$

**Dynamics of flow of aqueous fluid and ions.** Fluid formation in *S. acanthias* is about half that of the rabbit; the volume is roughly the same, so the rate-constant for flow is also half. It seems reasonable to assume that the fluid is formed from plasma in the ciliary body, since the transport enzymes are found there. The outflow route is not known, but in view of the absence of any structure analogous to the canal of Schlemm$^6$ and the very large diffusion pathway into the vitreous, it is likely that this is a primary drainage route.

The large component of diffusion both from plasma to aqueous, and aqueous to vitreous is roughly comparable to that for the rabbit.$^1$ Fig. 6 shows a model of secretory and diffusional rates for the three ions. This diagram serves to summarize the various findings and their interrelations: the importance of HCO$_3^-$ in the secretory process due to its very rapid formation and appearance in aqueous; the large diffusional component for sodium and chloride between plasma and aqueous which can mask drug effects; the very large diffusion between aqueous and vitreous, greatly delaying isotopic equilibrium of $^{22}$Na$^+$ and $^{36}$Cl$^-$ between aqueous and plasma; the possibility that the vitreous is a sink, not only for diffusion but for formed fluid.

In general, then, the present data, when compared to those for mammals, do suggest a constant vertebrate pattern with respect to both the chemistry and physiology of aqueous humor formation. The central common points are: newly formed fluid at ciliary process, rapid formation of HCO$_3^-$ from CO$_2$, and transport of sodium. Outflow is probably different among species as are quantitative relations between secretion and diffusion of the ions, but we regard these as secondary matters.

Equally interesting with the fact of a primary vertebrate pattern for aqueous secretion is that formation of cerebrospinal fluid$^7$ and endolymph$^6$ has the same general character.

**REFERENCES**

3. Maren, T. H.: HCO$_3^-$ formation in aqueous humor: mechanism and relation to the treat-