Bicarbonate formation in cerebrospinal fluid: role in sodium transport and pH regulation

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MAREN, Thomas H. Bicarbonate formation in cerebrospinal fluid: role in sodium transport and pH regulation. Am. J. Physiol. 222(4): 885-899. 1972.—Two series of experiments were performed which clarified the role of HCO₃⁻ formation and movement into cerebrospinal fluid (CSF), and its relation to sodium transport and pH regulation. In the first, the rates of access of HCO₃⁻, Na⁺, and Cl⁻ from plasma to CSF were measured in the dogfish Squalus acanthias by means of their isotopes. The rate constant (kᵢ) values for the three ions were 1.9, 0.19, and 0.14 hr⁻¹, respectively. Assuming that these represent net influx, the composition of CSF as secreted has the same sodium concentration as plasma, with HCO₃⁻ much higher and Cl⁻ lower than plasma. The rate data and the effects of carbonic anhydrase inhibition suggest that HCO₃⁻ reaches the CSF by hydroxylation of gaseous CO₂ at the choroid plexus, and probably at glia. The relations among the rates of the three ions show that the movement of HCO₃⁻ has an important influence on the movement of sodium and of fluid. In the second series, the Pco₂ of the fish was elevated fourfold, and the effect upon CSF HCO₃⁻ was measured. The procedure caused a striking elevation of CSF HCO₃⁻ concentration, to fourfold normal, in about 3 hr. As a result, pH regulation was maintained in the CSF during a profound respiratory acidosis in the plasma.

The rate of HCO₃⁻ formation in CSF was reduced to half by carbonic anhydrase inhibition. The data of the two series agree in showing that HCO₃⁻ formation is a principal feature of CSF formation and that there is a clear homology to the formation of aqueous humor.

The following experiments are designed to compare the rates of transfer of Na⁺, Cl⁻, and HCO₃⁻ from plasma to cerebrospinal fluid (CSF) under identical conditions and to study the effect of carbonic anhydrase inhibition on these rates. The experiments are of two types: series I gives rates for the three ions based on isotope transfer, and series II shows the effect of hypercapnia on HCO₃⁻ transfer, using concentrations of "cold" ion.

Table 1 summarizes earlier work in this field. Although fragmentary, it shows that Na⁺ and Cl⁻ influx rate constants are about equal, and the constant for HCO₃⁻ is 30-100 times greater. Analyses of the data suggest that kᵢ for Na⁺ represents net flux inward at the secretory site for CSF (15) or aqueous humor (32). The few studies on HCO₃⁻ accession (11, 31) have not been analyzed from the point of view of net flux, although the rapid HCO₃⁻ appearance might be expected to lead to a HCO₃⁻-rich primary fluid. This has in fact been found for the aqueous humor (31, 32) and choroid plexus fluid (2).

Under conditions of ventriculocisternal perfusion in the cat, HCO₃⁻ accession to the artificial CSF was considerably more rapid than that of Cl⁻; both rates were reduced by carbonic anhydrase inhibition (38).

There is a large literature on the effect of plasma Pco₂ on CSF HCO₃⁻ (19). When Pco₂ is high, CSF HCO₃⁻ exceeds that in plasma (64); when plasma Pco₂ is low, CSF HCO₃⁻ is less than that of plasma (25). In all cases, HCO₃⁻ adjustment in CSF provides rapid pH control, superior to that in plasma. The same mechanism applies to brain interstitial fluid itself, following hypercapnia (67). These findings extend also to chronic hypo- and hypercapnia, in which CSF pH continues to be well regulated (10, 55).

Despite the wealth of data, events at the secretory site for CSF do not appear to have been interpreted in terms of the reaction CO₂ + OH⁻ → HCO₃⁻ nor has the role of carbonic anhydrase been evaluated.

The present experiments were carried out in the elasmobranch fish Squalus acanthias, commonly called the spiny dogfish. I have previously shown that the CSF in this species has characteristics common to other vertebrates: carbonic anhydrase in choroid plexus and brain, chloride excess in fluid (Table 3), and regulation of pH in the face of hypercapnia (34). The negative potential in CSF relative to blood (29) at all blood pH (53) implies an active process for anions in dogfish as has been shown for the cat (38). The rate of CSF production in S. acanthias is about 0.98 ml/hr (51); for a volume of 1.6 ml (39) this yields a rate constant for fluid turnover of 0.17 hr⁻¹, very close to mammalian values (15). As in higher animals, CSF K⁺ is closely regulated, independent of plasma concentration (13). S. acanthias has important anatomical and physiological virtues for the present experiment: CSF is easily drawn as a clear uncontaminated fluid near its site of production, and the volume is large enough so that consecutive sampling in the 50-µl range is feasible. The fish are sturdy and withstand handling well. They are an inexpensive, abundant, and disease-free species in which individual variation is low.

In this species, CSF is formed by the choroid plexus of the fourth ventricle and optic lobes, and probably brain tissue. It circulates freely from olfactory lobes caudal to optic lobes.

THE FOLLOWING EXPERIMENTS are designed to compare the rates of transfer of Na⁺, Cl⁻, and HCO₃⁻ from plasma to cerebrospinal fluid (CSF) under identical conditions and to study the effect of carbonic anhydrase inhibition on these rates. The experiments are of two types: series I gives rates for the three ions based on isotope transfer, and series II shows the effect of hypercapnia on HCO₃⁻ transfer, using concentrations of "cold" ion.

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equilibrium of uncatalyzed rates, mechanism I is used, since it is
to be about 20 times) at pH 7.4 (36).

Ref. This study

* Other sodium values: CSF; rat .019 (16), man (cisternal) .002,
  man (ventricle) .0096 (15). Anterior aquemex; rat .025, monkey .01,
  rabbit .009 (16). † Based on HCO$_3^-$ accumulation, from the
  base of plasma HCO$_3^-$ See Table 6 for rates based on plasma CO$_3$.
  ‡ From published curves which yield a $T_{2g}$ of about 2 min.

to cerebellum to fourth ventricle, and possibly to the spinal
cord. There is no subarachnoid space, and the exit route to
the veins has not been charted. The chemical analysis of the
fluid is given in Table 2.

Further basis for this work lies in earlier experiments on the
effect of carbonic anhydrase inhibition on CSF electrolytes and fluid formation in S. acanthias (34, 51). Since there is no
carbonic anhydrase in the kidney of this (or any) marine fish
(28, 34, 44), such experiments are simplified by lack of
renal response to inhibitors. The chief systemic effect
of acetazolamide or methazolamide is respiratory acidosis,
which as in all species, elevates CSF HC03- above that in
plasma. The characteristic Cl- excess in CSF is abolished
by carbonic anhydrase inhibition. The data indicate that plasma CO$_3$ is
about 2 mM. For the elevation of Pco$_2$, the
water was pumped into the fish as described just above.
The physical arrangement of bubbler, tubing, pump, and
fish was such that the measured Pco$_2$ of the gas (35 mm Hg)
was decreased to about 16 mm Hg in the fish in all experi-
ments performed. A small incision was made in the skull,
about 1 square inch of chondocranium removed, and the
optic lobes and cerebellar ventricles exposed for sequential
sampling. The cerebrospinal fluid was removed with a
27-gauge needle into a 0.25 ml glass syringe, which was
immediately sealed.

Blood was removed from the fish of both series by puncture
of the tail artery. The pH of whole blood was taken
anaerobically at once; no temperature correction was made,
since the temperature of the water (15-18 C) was usually
close to that of the air. The pK$_{H_2}$ was taken as 6.1 and as
0.045, the values measured for elasmobranch blood at
17 C by Albers and Pleschka (1). The blood was centrifuged
and plasma separated and stored in sealed syringes. Total
CO$_2$ was determined on plasma and CSF using the Kopp-
Natterlon microgasometer; chloride was determined on
plasma and CSF using Hg(NO$_3$)$_2$ titration. Chloride 36
and 4C were analyzed with a Mark 1 liquid scintillation
counter (Nuclear-Chicago); duplicate 0.1-ml samples were
counted, either for about 4 min or to 104 counts. Background
was 40 counts/min. Sodium 22 was counted in a well-type
counter (Nuclear-Chicago) ; duplicate 0.1-ml samples were
 counted.

Biological Laboratory, Salisbury Cove, Maine. The fish
were caught by line and trawl in Frenchman’s Bay and
kept for a day or two before the experiments in live-cars on
the dock.

In series I, the sodium and chloride experiments were done
with fish swimming freely in the live-car; the bicarbonate
experiments were done with fish swimming freely in a 25-gal
tank of seawater or immobilized in a box with perfusion of
gills with cooled oxygenated seawater (40). In the freely
swimming fish, a single terminal sample was taken from the
CSF at the time noted; data from the different fish were
combined to give the sequential information. Plasma was
sampled at the same time, and in some cases additional
plasma samples were obtained prior to CSF sampling. In
the immobilized animals the individual fish were sampled
sequentially.

In series II, the fish were restrained, lying on their ventral
surface, while 1-2 liters of seawater per minute were pumped
through their spiracles, thus perfusing the gills. It was found
that this procedure did not alter the Pco$_2$ of the plasma,
although the handling and immobilization did tend to lower
plasma HC03- about 2 mm. For the elevation of Pco$_2$, the
seawater was passed through a small bubble oxygenator
(Seals Corporation) to which 5% CO$_2$ in air was admitted.
This water was pumped into the fish as described just above.

At physiological pH and the normal situation involving
carbonic anhydrase, no distinction is made between mecha-
nisms 1 and 2 below:

$$
CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \quad (1)
$$

and

$$
HOH \rightarrow OH^- + H^+
$$

$$
CO_2 + OH^- \rightarrow HCO_3^- \quad (2)
$$

Thus, the terms hydration and hydroxylation of CO$_2$ are
used interchangeably. In both cases the overall reaction is
the formation of HCO$_3^-$ from gaseous CO$_2$. For the calcu-
lation of uncatalyzed rates, mechanism 1 is used, since it is
faster (about 20 times) at pH 7.4 (36).

METHODS

All experiments were done in the dogfish, S. acanthias, in
the summers of 1969 and 1970, at the Mount Desert Island
methazolamide is similar, except it penetrates the brain more rapidly. Both drugs have a long duration of action in this species \( T_1 = 1-2 \) days.

The rate constant \( k_{in} \) was calculated from the rate of change of concentration of isotope in CSF, divided by the midpoint plasma concentration for the period observed. This is an approximation based on the facts that plasma levels are reasonably constant during the periods used and that these periods were selected to give an approach to initial rates. The term \( k_{in} \) has the same mathematical meaning as in the expression used by Davson (15) and given in the Appendix. It is shown there that \( k_{in} \) is a composite term including clearance and volume. Attention is given to the clearance, which yields the critical value for amount of substance transferred from plasma to CSF per unit time.

RESULTS

Series 1: Rates of Ion Movement

In this series the rates of accession of labeled \( \text{Na}^+ \), \( \text{Cl}^- \), and \( \text{HCO}_3^- \) from plasma to CSF were measured. Conditions in the experiments were identical so that the rates of ion movement could be compared. A control series was run for each ion, and a series in which carbonic anhydrase was inhibited by the prior injection of acetazolamide (30 mg/kg) or methazolamide (30 or 50 mg/kg). It was established that the choroid plexus carbonic anhydrase of this species had the same susceptibility to these drugs in vitro as mammalian tissues, \( I_{50} \sim \sim 10^{-5} \) M. The relations among tissue enzyme concentration, dose of drug, and enzyme inhibition for this species have been given (34).

Sodium. Figure 1 shows the data. The decay of the isotope in plasma after the 1st hour in both control \( (A) \) and inhibited \( (B) \) fish yields a half-life of 11.5 hr. The critical 1- and 3-hr points appear firm, and the rate of change is small enough so that a reasonable approximation of constancy in plasma is achieved. Figure 14 shows that the \( k_{in} \) from 0 to 1 hr is 0.23 and from 1 to 3 hr is 0.19 min\(^{-1}\). In the latter period, plasma concentrations of \( ^{22}\text{Na} \) declined only slightly (72-64), and more confidence is put in this value. The 1- to 3-hr \( k_{in} \) for both \( ^{22}\text{Na} \) and \( ^{36}\text{Cl} \) will be used as the basis for calculations and comparisons with the inhibited situation.

Carbonic acid in plasma after the 1st hour in both control (A) and inhibited (B) fish yields a half-life of 11.5 hr. The critical 1- and 3-hr points appear firm, and the rate of change is small enough so that a reasonable approximation of constancy in plasma is achieved. Figure 1B yields 0.16 min\(^{-1}\) for the \( ^{22}\text{Na} \) during 1-3 hr following pretreatment with methazolamide.

The CSF/plasma ratio for isotope approaches, but does not reach, the equilibrium ratio for \( \text{Na}^+ \) in 6 hr. Neither plasma nor CSF sodium concentration changes during this period of carbonic anhydrase inhibition (Table 2), so the data of Fig. 1 are equivalent to specific activity.

Chloride. Figure 2 shows the data. From the 4- to 6-hr points, the plasma decay in control fish \( (A) \) yields a half-life of 11.5 hr, the same as sodium. If a fit is attempted from 2 to 6 hr, the half-life is 9.5 hr. When carbonic anhydrase is inhibited, these half-lives drop slightly to 9 and 7 hr, respectively. There is also a small decrease in cold chloride concentration in the plasma 6 hr after acetazolamide, which might in part be related to the coincident \( \text{HCO}_3^- \) increase (Table 2). In agreement with this, hypercapnia in \( S. \text{acanthias} \) induces a small fall in plasma \( \text{Cl}^- \), which appears secondary to the increase in plasma \( \text{HCO}_3^- \) (12). However, Fig. 2 shows that from 0.5 to 3 hr, plasma concentrations of \( ^{36}\text{Cl} \) are essentially identical in control and treated fish.

The normal or control \( k_{in} \) for \( ^{36}\text{Cl} \) was calculated from 0 to 1, 1 to 2, and 2 to 3 hr in Fig. 24, yielding values of 0.164, 0.150, and 0.135 hr\(^{-1}\), respectively. As noted above, the 1- to 3-hr data will be used for subsequent calculations, 0.14 hr\(^{-1}\). Following carbonic anhydrase inhibition at 0-1 and 1-3 hr, the \( k_{in} \) values were 0.167 and 0.142 hr\(^{-1}\).

### TABLE 2. Electrolyte composition of plasma and CSF of \( S. \text{acanthias} \): normal \( (A) \) and following carbonic anhydrase inhibition \( (B) \)

<table>
<thead>
<tr>
<th></th>
<th>( \text{Na}^+ ), mM</th>
<th>( \text{Cl}^- ), mM</th>
<th>( \text{HCO}_3^- ), mM</th>
<th>pH</th>
<th>( \text{Pco}_2 ), mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Plasma</td>
<td>255 254</td>
<td>239</td>
<td>233</td>
<td>7.7</td>
<td>11.6</td>
</tr>
<tr>
<td>CSF</td>
<td>271 268</td>
<td>264</td>
<td>236</td>
<td>8.3</td>
<td>16.6</td>
</tr>
</tbody>
</table>

Statistics are given in original publication (34). B refers to data 6 hr following 30 mg/kg iv acetazolamide. Pco\(_2\) values have been recalculated using \( \alpha \) factor of 0.049. The small discrepancy in internal agreement among \( \text{HCO}_3^- \), Pco\(_2\), and pH is due to different numbers of samples used for pH and total CO\(_2\) measurements. Further work (38) suggests that these animals had a slight respiratory acidosis due to handling. Freshly caught fish have a pH of 7.6-7.7 and \( \text{HCO}_3^- \) of about 6, yielding Pco\(_2\) of 4.
Clearly, there is no difference between control and drug-treated fish.

Figure 2 shows a small effect of inhibition on the $^{35}$Cl access to CSF at the 6 hr time. In the presence of nearly identical plasma concentrations, the CSF concentration was 13% lower in the inhibited than in the control fish. We should note that this difference is only significant at the 0.05-0.1 level for $P$; in the case of sodium there were insufficient data for any analysis of the 6-hr points. The isotopic chloride effect at 6 hr is similar to that observed for cold chloride (Table 2): the effect is smaller or absent at 2-3 hr (Fig. 2 and Fig. 4 of ref. 34). We shall return to this point in DISCUSSION.

**Bicarbonate.** The concentration of counts of total $^{14}$CO$_2$ in plasma and CSF following injection of NaH$^{14}$CO$_3$ is partitioned between $^{14}$CO$_2$ gas and H$^{14}$CO$_3^-$ ion. Table 3 and Fig. 3 show the data for the control (A) and for the inhibited (B) series. In Table 3 the concentration of label in plasma of each control fish at 3 min is set at 100, and all other isotope data are relative to this. The relative retention of isotope in the inhibited fish is evident by the higher counts in the plasma. In Fig. 3 a different convention is used: the concentrations of label in plasma of control and of inhibited fish at 3 min are each set at 100, in this way the data are normalized for the isotope retention, and decay rates in plasma and accession rates in CSF may be directly compared between control and treated fish.

The $^{14}$CO$_2$ partition in the plasma of control fish is based on equilibrium between $^{14}$CO$_2$ gas and H$^{14}$CO$_3^-$ at the measured pH (Table 3A), since there is carbonic anhydrase in the red cells (34) assuring near-instant equilibrium. During the first 6 min, rapid disappearance of label is due to

| Table 3. Accession of H$^{14}$CO$_3^-$ from plasma to CSF in S. acanthias box experiments |
|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| **3 min** | **6 min** | **12 min** | **Acid-Base Balance at 3 min** | **Notes** |
| Plasma | 100  | 94  | 6  | 60  | 36  | 4  | 41  | 38.5  | 2.5  | 7.29  | 5.2  | 7  | Isotopic CO$_2$ partition at equilibrium in plasma calculated from pH. In CSF, equilibrium not reached. |
| CSF | 11.5 | 5.5 | 6  | 26.7 | 22.7 | 4  | 30.4 | 35.9  | 2.5  | 7.55  | 9.2  | 7  | HCO$_3^-$/CO$_2$ ratio in plasma calculated from percent equilibrium using Roughton equation. See text. |
| Plasma | 255 | 242 | 10 | 136 | 128 | 8  | 94  | 87  | 7  | 7.12  | 6.4  | 12 |
| CSF | 20  | 10  | 10 | 44  | 36  | 8  | 64  | 5/  | /  | 7.40  | 10.4 | 12 |
mixing and distribution; based on decay from 12 to 60 min the plasma half-life is about 45 min (Fig. 3). It may be calculated from the metabolic rate of the fish and the size of the total CO2 pool that this decay represents the turnover of CO2 and its elimination across the gills.1

The partition of total \(^{14}\)CO2 in CSF is made on the basis that the gaseous phase in \((^{14}\)CO2) has the same concentration as that in plasma (Table 2). This is subtracted from the total \(^{14}\)CO2 in CSF to yield the \(^{14}\)HCO3\(^{-}\) concentration in the CSF. Table 3A gives the gradual accumulation of \(^{14}\)HCO3\(^{-}\) in CSF; equilibrium is not reached in 12 min. Equilibrium at this time would mean for the measured pII of 7.55 a \(^{14}\)HCO3\(^{-}\) concentration 28 times that of \(^{14}\)CO2, the same as that of the cold species. The data show \(^{14}\)HCO3\(^{-}/^{14}\)CO2 = 14. Between 6 and 12 min the rate of \(^{14}\)CO2 decay in the plasma is moderate enough so that a reasonable calculation of the rate constant for accumulation of \(^{14}\)HCO3\(^{-}\) in CSF can be made (Table 4). In the time considered, 2.2 units/min accumulate in the CSF. The average gaseous \(^{14}\)CO2 concentration is 3.2 units, where \(k_{in} = 0.69 \text{ min}^{-1}\). The same calculation, made for the 3- to 6-min time, yields a somewhat higher rate constant, although not statistically different. The mean of both periods for the eight control fish \((n = 16)\) yields a rate constant for entry of 0.915 \text{ min}^{-1}. This may be compared to the chemically determined values falling above it.

Since the fish of Table 3 were immobilized, it was necessary to find whether this distorted the normal rates. Accordingly, a group of five free-swimming fish were injected with the bicarbonate label; blood was taken at 3 min, and at 6 min blood and CSF were withdrawn for analysis. The data are shown in Table 5 and are quite similar to those of Table 3. Blood acid-base values as well as isotope concentrations were nearly identical. The lower rate constant \(0.65 \text{ min}^{-1}\) compared to 0.915 \text{ min}^{-1} (Table 4) is due to the fact that Table 5 data are for the period 0-6 min after isotope injection; in the first few minutes the rate is low due to delay in mixing and circulation time (note also low 0- to 3-min access in Table 3A). Thus, it appears that, in the short periods used, the acid-base physiology of the fish was not altered by immobilization and that the procedure of Table 3 represents normal function.

Tables 3B and 4 and Fig. 3B show the results of completely inhibiting carbonic anhydrase in all tissues of the fish by a large injection of acetazolamide 30 min before Na\(^{14}\)HCO3 was given. This produced the following series of events, due to inhibition of enzyme in red cell and choroid plexus.

1) The \(^{14}\)HCO3\(^{-}\) ion, immediately following its injection, can only form \(^{14}\)CO2 at the rate of the uncatalyzed reaction. Using the rate constant for dehydration at 16 C, and mathematical treatment equivalent to the Roughton equation (60), the half-time to equilibrium between HCO3\(^{-}\) and \(^{14}\)CO2 is about 4 min and to 90% equilibrium is 13 min. Table 3B gives the partition of total \(^{14}\)CO2 in the plasma according to the percent that has reached equilibrium between HCO3\(^{-}\) and \(^{14}\)CO2 at pH 7.12 at the time of sampling. This ranges from 40% at 3 min to 88% at 12 min. This disequilibrium accounts for the large initial retention of label in the blood as \(^{14}\)HCO3\(^{-}\), since the exit of total CO2

2 Specifically, the uncatalyzed reaction was run in the presence of 250 mM NaCl, 100 mM trimethylamine oxide, and 350 mM urea.

3 The hydration rate constant (HCO3\(^{-}\)) for 16 C was taken at 7 sec\(^{-1}\) (33). Using \(pK_a\) for \(^{14}\)HCO3 of 3.7 and the pH (from the determined value in the fish) of 7.12, the \(^{14}\)CO2 rate constant is 0.17 \text{ min}^{-1},

### Table 4. Formation rate constants of CSF \(^{14}\)HCO3\(^{-}\)

<table>
<thead>
<tr>
<th>Period, min</th>
<th>(^{14})HCO3(^{-}) in CSF</th>
<th>CSF Accum. (^{14})HCO3(^{-})/Min</th>
<th>Mean Plasma Conc., (^{14})CO2</th>
<th>(k_{in} \text{ min}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3-6</td>
<td>17.2</td>
<td>5.6</td>
<td>5.0</td>
<td>1.14 ± 0.25</td>
</tr>
<tr>
<td>6-12</td>
<td>13.2</td>
<td>2.2</td>
<td>3.2</td>
<td>0.69 ± 0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean 0.915</td>
</tr>
<tr>
<td>Inhibited</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6</td>
<td>26</td>
<td>9.7</td>
<td>9</td>
<td>0.96 ± 0.16</td>
</tr>
<tr>
<td>6-12</td>
<td>21</td>
<td>3.5</td>
<td>7.5</td>
<td>0.47 ± 0.10</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean 0.715</td>
</tr>
</tbody>
</table>

Data from Table 3. \(k_{in}\) values are means ± sr.

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1 Carbon dioxide production is 1.5 mmolles/hr for a 1-kg shark (58).

2 The accurately determined 25 C value of 2.1 min\(^{-1}\) (24) would yield about the same range for 16 C, assuming a \(Q_{10}\) of 2. Thus, the mean observed rate is about the same as that of the uncatalyzed reaction with some values falling above it.
from fish is a function of the fraction present as CO$_2$ gas. Table 3B shows that the ratio of plasma labeled HCO$_3^-$/CO$_2$ at 3 min is 24 and at 12 min is 11.4; at equilibrium for pH 7.12 the ratio should be 10.5. Cold total CO$_2$ is reasonably well equilibrated in peripheral blood in the steady state, even when the enzyme is inhibited (36). As the label approaches equilibrium, it does so at a lower HCO$_3^-$/CO$_2$ ratio than normal, since the Pco$_2$ in the inhibited fish is almost twice that of the normal. This enables unloading of total labeled CO$_2$ at a rate as fast as normal, even though the enzyme is inhibited. The same situation applies in the mammalian (8). It is for this reason that the decay rates in plasma, as shown in Fig. 3, are the same in the normal and inhibited fish.

2) In the CSF, gaseous $^{14}$CO$_2$ is taken to be at the same concentration as in plasma; CSF $^{14}$CO$_2$ is then entered in Table 3B as total minus gaseous $^{14}$CO$_2$, just as for controls. The rate constants of HCO$_3^-$ accumulation in CSF were then determined for the various periods based on the actual gaseous $^{14}$CO$_2$ concentrations in the plasma, taking disequilibrium into account (Table 3B). The 6- to 12-min period yields the most reliable data because the initial mixing period of plasma is past and the interval is longer than the first period; 21 units were formed, or 3.5 units min$^{-1}$. The mean gaseous $^{14}$CO$_2$ concentration was 7.5 units, yielding a rate constant of 0.47 min$^{-1}$ (Table 4), 32% less than the control rate constant.

The effect of inhibition is best seen in Fig. 3, in which the initial isotope concentration in plasma is set at 100 for both control (A) and inhibited (B) fish. The data show identical plasma decay curves of total $^{14}$CO$_2$ for A and B so that the accession to CSF should reflect the partition of HCO$_3^-$/CO$_2$ in plasma and the rate of hydration of CO$_2$ to HCO$_3^-$ at the secretory sites. Inhibition initially lowers gaseous $^{14}$CO$_2$ in plasma below control (3-6 min) because of disequilibrium. By the 6- to 12-min interval, however, the gaseous CO$_2$ concentration in plasma is the same in control and treated fish, but label has not reached equilibrium between plasma and CSF. This is the critical interval for measurement of inhibition. At this time there is a 35% reduction in rate following acetazolamide. In the next (12-32 min) period, control fish show label in CSF reaching the concentration in plasma (Fig. 3A); the inhibited fish show a small difference between CSF and plasma, which is not significant (Fig. 3B).

### Table 5. Accession of $^{14}$CO$_2$ from plasma to CSF in S. acanthias, free-swimming fish

<table>
<thead>
<tr>
<th>Period</th>
<th>Total $^{14}$CO$_2$</th>
<th>HCO$_3^-$</th>
<th>CO$_2$</th>
<th>Total $^{14}$CO$_2$</th>
<th>HCO$_3^-$</th>
<th>CO$_2$</th>
</tr>
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<tbody>
<tr>
<td>3 min</td>
<td>94</td>
<td>6</td>
<td>6</td>
<td>67</td>
<td>63</td>
<td>4</td>
</tr>
<tr>
<td>6 min</td>
<td>72</td>
<td>23</td>
<td>4</td>
<td>72</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>3-6 min</td>
<td>70</td>
<td>23</td>
<td>4</td>
<td>70</td>
<td>23</td>
<td>4</td>
</tr>
</tbody>
</table>

### Table 6. Rates of accession of ions to CSF and their concentration in newly formed fluid

<table>
<thead>
<tr>
<th>Ion</th>
<th>Plasma, mM</th>
<th>CSF, mM</th>
<th>$k_i$, hr$^{-1}$</th>
<th>$T_{1/2}$ to equil, hr</th>
<th>Accession Rate (col. 5), mM/hr</th>
<th>Concentration in Secreted Fluid, mM$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>255</td>
<td>271</td>
<td>.19</td>
<td>3.7</td>
<td>48</td>
<td>272</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>239</td>
<td>264</td>
<td>.14</td>
<td>5.0</td>
<td>33</td>
<td>185</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>7.7</td>
<td>8.3</td>
<td>1.9$^*$</td>
<td>37</td>
<td>14.5</td>
<td>82</td>
</tr>
</tbody>
</table>

* Accession rate (col. 5) mM/hr, X vol CSF (1.6 ml)/formation of CSF (0.28 ml/hr). † Calculated as if plasma HCO$_3^-$ were the base of the process, to facilitate comparison with other ions. Entries in columns 4-6 of this row refer to kinetics of HCO$_3^-$ in CSF, irrespective of origin. ‡ Calculated as HCO$_3^-$ formation from CO$_2$ (from Table 4).

Calculation of Transport Rates and Concentrations of Secreted Fluid

Table 6 shows the $k_i$ (col 3) and $T_{1/2}$ (col 4) of the three ions that have been studied. In this context HCO$_3^-$ is treated in the same way as Na$^+$ and Cl$^-$, i.e., the $k_i$ is based on plasma concentration of the ion, disregarding for the moment the mechanism of HCO$_3^-$ accumulation in CSF. In all cases, $k_i$ is based on initial rates of isotopic transfer, with reasonable agreement between two successive early periods (Figs. 1-3, and calculations given above).

The question arises whether $k_i$ is opposed at the site of fluid formation by a process in the reverse direction. For Na$^+$, the extensive analyses of mammalian data by Davson (15) indicate that it is not; $k_i$ for Na$^+$ represents net flux and is equivalent to the rate of fluid formation. The present data agree quite accurately with this, for $k_i$ for Na$^+$ (0.19 hr$^{-1}$) is equal to the formation rate constant for fluid (rate of formation, 0.28 ml/hr + volume of 1.6 ml = 0.175 hr$^{-1}$).
The latter data are from ref. 51, as amended in footnote 4 below. The calculation of the sodium concentration of newly secreted fluid then yields a value (270 mm) that indicates (since other cations are negligible) isotonicity with plasma, again in conformation with other work (2).

For Cl-, a similar calculation yields 185 mm (Table 6, col 6). There is no reason to invoke an opposing (CSF to blood) rate at the site of formation; the situation may be regarded as similar to that for Na+, where k-in is equivalent to net influx. If there were in fact a backrate for Cl-, Cl- concentration in newly formed fluid would be less than 185 mm and would therefore demand an even higher value for HICO3- (see below).

The calculation for HCO3- yields 82 mm in secreted fluid, at first an improbable value, but actually less than many measured or calculated concentrations in other fluids; for example, pancreatic juice, aqueous humor, or alkaline gland excretion in the skate (36). Put in another way, this states that the clearance of HCO3- is about 10 times that of Na+ or of fluid formation. Again, this is a maximum value, which implies no backflux. The fact that (Cl- + HCO3-) concentration in newly formed fluid so nearly equals that of Na+ argues for the reliability of the data; furthermore, the inherent nature of the process for HCO3- accumulation in CSF predicates against a large backflux, since a relatively nonpermeant ion is being formed. Since the data of Table 6 on composition of newly formed fluid depend in part on this argument, it is necessary to develop it now; it will also be evident that the conclusions are strongly supported by the experiments of series II below.

The transfer of 14C from plasma to CSF following injection of NaH14CO3 in plasma could follow several chemical pathways, but they are distinguishable. In the blood, the labeled bicarbonate is immediately partitioned as H14CO3- / 14CO2 at a ratio of about 30 (Table 6). It must be appreciated that the conversion is not automatic; it requires addition or loss of OH-, and for near-instantaneous rate, 

\[ \text{HCO}_3^- + \text{CO}_2 \rightarrow \text{H}_2\text{CO}_3 \]

This assumption is the basis for the bookkeeping of Table 6, col 6, in calculating the ionic composition of newly secreted fluid.

The calculated or theoretical composition of newly formed fluid in Table 6 (col 6) must be altered to the observed composition (col 2) by loss of HCO3- and addition of Cl-. This could be effected by addition of isotonic NaCl from brain (at pH 7) to the primary fluid, the pH of which is about 8.5. The neutralization and dissipation of HCO3- could also be mediated by acid metabolites from brain (i.e., lactic), although stoichiometry demands that this contribution be modest, since lactate does not accumulate in CSF.

Series II: Effect of Hypercapnia on Bicarbonate Formation in CSF—pH Control of CSF and Role of Carbonic Anhydrase

In these experiments, bicarbonate accumulation in CSF was studied by increasing the concentration of gaseous CO2 in plasma. This procedure is similar to a number of studies involving acute hypercapnia in mammals (59, for recent data in man and review of literature). The data of Figs. 4–6 have several new features: 1) the Pco2 is elevated fourfold, which is more than is generally possible in mammalian experiments; 2) sampling is done in a main ventricular cavity close to the site of fluid production; 3) most important, the effect of carbonic anhydrase inhibition is observed.

Figure 4 shows one of five experiments in which Pco2 was raised from 4 to 16 mm Hg by admitting 5% CO2 to aerated seawater which perfused the gills. Cerebrospinal fluid HCO3- showed a rapid rise, in which the sample taken 60 min after the onset of hypercapnia was 10 mm above the control. At that time there was no change in plasma HCO3-. CSF HCO3- continued to rise until it reached a plateau of 24 mm, about 4 times normal, 3 hr after the onset of hypercapnia. At that time plasma HCO3- was 7 mm, so there was a 17 mm gradient between plasma and CSF. The result of this adjustment was pH stabilization of the CSF, during a time when the induced hypercapnia caused a drop of about 0.5 pH unit in the plasma.

Figure 5 shows one of four experiments of the same type as that of Fig. 4, except that carbonic anhydrase was totally inhibited throughout the procedure. It will be observed that the injection of methazolamide itself caused an elevation of plasma Pco2; this is the typical response in this species (34). When 5% CO2 was admitted to the perfusate, the Pco2 continued to rise, and the final value was about the same as in Fig. 4. Examination of all nine experiments involving hypercapnia showed no difference between Pco2 in controls and following carbonic anhydrase inhibition. Figure 5 shows a relatively slow rise in CSF HCO3-; 60 min after the onset of hypercapnia, it was 5 mm above the control value. At 3 hr the CSF HCO3- was 18 mm and had not yet reached equilibrium. At this time there was a gradient of about 10 mm between plasma and CSF. It appears from a comparison of Figs. 4 and 5 that the effect of hypercapnia in the normal and in the carbonic anhydrase-inhibited fish was qualitatively the same; the difference was a quantitative one, with respect to the rate at which HICO3- entered the CSF.

Figure 6 bears out this idea. Here the data for all experiments are combined, showing the mean rates of HCO3- accumulation in the control and inhibited fish. It is clear that
carbonic anhydrase plays a role in this process, since the inhibited rate is one-half that of the control. The data suggest that CSF HCO₃⁻ would reach a plateau value in about 8 hr in the inhibited fish. This would agree with earlier data in which inhibition alone raised the Pco₂ in plasma, and elicited a slow rise in CSF HCO₃⁻, reaching a culmination in 6–20 hr (34).

DISCUSSION

These experiments may be considered in terms of the relations among the transfer rates to CSF of the several ions (series I) and in terms of the mechanism underlying the defense of CSF pH during change in acid-base balance (series II). Finally, I attempt a unifying view of the secretion of CSF and aqueous humor in vertebrates.

Series I: Transfer Rates

Normal fish. The rate of sodium entrance to the CSF in S. acanthias is essentially the same as that of the formation of new fluid. This principle has been discussed (15) and depends upon the fact that the Na⁺ concentration of newly formed fluid is the same as that of plasma. Table 6 shows that the half-life of Na⁺ transfer is 3.7 hr, the same as that obtained for fluid, using the figure of Oppelt et al. (51) for fluid formation of 5 μl/min, and CSF volume of 1.6 ml. Conversely, these two figures, together with the Na⁺ accession rate, yield a concentration of 271 mM in newly formed fluid, the same as that of plasma. The large (64 %) decrease effect of intraventricular ouabain on formation rate of CSF in this species (51) suggests that sodium transport is dependent upon ATPase, as it is at many other secretory sites.
Ouabain and acetazolamide both reduce Na+ transport to CSF and fluid production in the mammal (17), ouabain acting directly on Na+, acetazolamide by its control of HCO₃⁻ availability for pairing with Na+. Thus, the system depends both on ATPase and carbonic anhydrase. Ouabain and acetazolamide have about the same magnitude of effect and are not additive (17). The linkage between Na+ and HCO₃⁻ is supported by recent work using isolated choroid plexus of the frog, in which the sodium current was abolished when HCO₃⁻ was excluded from the medium (70).

The rate constant for Cl⁻ is 26% less than that for sodium. Taking into account the small difference in their plasma concentrations, Cl⁻ accession rate and concentration in the primary fluid is 31% less than that of sodium (Table 6). It has been shown that Cl⁻ concentration increases from choroid plexus to cistern (2), and the same sequence exists from primarily secreted aqueous humor to fluid in the anterior chamber (32). The means of adjustment between the primary and final fluid are suggested under series I results above.

The rate constants for sodium and chloride in dogfish are surprisingly close to those obtained for the mammal (Table 1).

The rate constant for HCO₃⁻ accumulation in the fish is 10-fold greater than that of sodium, assuming that in both cases \( k_{in} \) represents net inward flux. The HCO₃⁻ constant is about 1/12th that of the single study reported in the mammal (Table 1); this could reasonably be ascribed to differences in temperature and blood flow. It may be that the temperature dependence of the uncatalyzed (four- to sevenfold, 33, 35) and catalyzed (threefold, 40, 41) hydration of CO₂ between 16 and 37°C is greater than that of the ATPase system. As noted below, blood flow is a significant determinant of the HCO₃⁻ rate, but this would affect only that fraction of the total Na⁺ rate that is linked to HCO₃⁻.

**Inhibited rates.** The sodium rate constant \( k_{in} \) in *S. acanthias* is only slightly (16%) altered by carbonic anhydrase inhibition (Table 7). Since HCO₃⁻ formation (Fig. 3) and fluid flow (31), and Table 7 this study) are reduced by inhibition 37 and 28%, this appears at first an anomalous result. However, as \( k_{in} = \frac{a}{V} \) where \( a = \) clearance and \( V = \) volume of CSF (APPENDIX), it is essential to analyze these separately. This has not been done previously for either CSF or aqueous, despite the fact that in earlier studies in mammals the effect of acetazolamide on \( k_{in} \) for Na⁺ was always less than on fluid formation (APPENDIX).

Table 7 sets out the data in a way that shows the sodium transport in terms of the clearance (col 3, as ml/hr, \( a = k_{in}V \)) and the derived term which I shall call transfer (col 6, \( \mu \) mol/cm²/hr, \( a = \) plasma concentration). The data are given for controls and for the inhibited steady state, at which time we have determined that V is reduced from 1.6 to 1.2 ml (39). Rate constants (\( k_{in} \)) are those given in Figs. 1–7 and Tables 1–8, calculated as described in the introduction. It is assumed in the model that the rate constant determined in the first few hours of inhibition persists during the steady state. Fluid formation rates are from ref. 51. In terms of Na⁺ clearance (col 3) or transfer (col 6), inhibition causes a reduction of 33.5%, shared about equally with Cl⁻ and HCO₃⁻ (see below). It is also of interest that the sodium concentration of newly formed fluid appears to drop about 10%, which indicates a slightly hypotonic fluid (col 7).

Figure 2 and Table 7 show that carbonic anhydrase inhibition does not lower \( k_{in} \) for Cl⁻. However, since secretion rate and volume are lowered, the clearance of Cl⁻ and the related transfer term are lowered to the same degree, 25% (cols 2–6). The effect of inhibition on Cl⁻ concentration of freshly formed CSF is a slight elevation (col 7), but the final fluid has low Cl⁻ concentration in agreement with the hypotonicity revealed by the sodium data and the elevation of HCO₃⁻ concentration (34, and Table 2). The significant point here is that Cl⁻ transfer is reduced; Table 7 shows that this accounts for about half of the sodium decrement. The data emphasize that \( k_{in} \) is not the sole critical term, in circumstances where there are

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**Table 7. Model of ionic transport from plasma to CSF in *S. acanthias*—Normal values and steady state following carbonic anhydrase inhibition**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{in} ) hr⁻¹</td>
<td>1.9</td>
<td>1.6</td>
<td>30</td>
<td>255</td>
<td>76</td>
<td>272</td>
<td></td>
</tr>
<tr>
<td>( a/V ) ml/hr</td>
<td>1.2</td>
<td>1.2</td>
<td>90</td>
<td>228</td>
<td>52</td>
<td>45</td>
<td>245</td>
</tr>
<tr>
<td>Control</td>
<td>Inhibited</td>
<td>Control</td>
<td>Inhibited</td>
<td>1.2</td>
<td>1.2</td>
<td>14</td>
<td>16</td>
</tr>
</tbody>
</table>

---

* \( k_{in} \) values from Table 6 and Figs. 1–3. These data are applied to the steady-state situation by using the volume term (V, col 2) of control and inhibited steady states (39). See text.
changes in V (Appendix). The clearance (col 3) or transfer term (col 6) denotes the effect of inhibition (or any change) on ion movement; it is significant that when V is maintained constant by ventriculocisternal perfusion (in the cat) k_{in} for Cl\(^-\) is reduced.

The net result of inhibition is therefore to decrease Cl\(^-\) transfer, although the calculated concentration of newly formed fluid rises slightly (Table 7). Earlier studies in dogfish (34) and man (16) show that acetazolamide lowers Cl\(^-\) concentration in the measured fluid at the steady state (Table 2). Current experiments show that hypercapnia increases Cl\(^-\) concentration in CSF and that this increase is abolished by acetazolamide (42). The "chloride effect" is either due to an actual Cl\(^-\) receptor function of carbonic anhydrase, or secondary to gradients imposed by the movement of HCO\(_3\)\(^-\) and Na\(^+\) (Table 7). This matter has been discussed previously (37, 38).

Consider the reduction by acetazolamide of the accession of \(^{14}\)C\(_2\)O_3 from plasma to CSF, in terms of k_{in} and a (Table 7). The rate constant k_{in} (in terms of HCO\(_3\)\(^-\)) is reduced from 1.9 hr\(^{-1}\) in controls to 1.9 hr\(^{-1}\) in inhibited fish. Note that this inhibited k_{in} is determined about 45 min after injection of drug, when V is essentially unchanged from controls.\(^5\) The assumption here is that k_{in} determined before the new inhibited steady state is reached, remains constant throughout inhibition. This value, 1.2 hr\(^{-1}\), taken with the inhibited steady-state volume, yields a of 1.4 ml/hr, about half of normal. Alternatively, if a is calculated from the inhibited rate constant and the initial volume (1.6 ml), its value is 1.9 ml/hr. Use of this alternative model would not greatly change the interpretation of these experiments.

The inhibited rate constant, based on CO\(_2\) (Table 4), is about the same as the chemical hydration rate constant for CO\(_2\) at this temperature. We have compared the hydration rate constant, previously determined in dilute buffer, KCl, NaCl, and NaHCO\(_3\) solutions (36, 45), with that determined in a solution approximating shark plasma, and there was no difference, despite the high NaCl and urea concentrations.\(^6\)

The rapid accumulation of HCO\(_3\)\(^-\) in CSF of dogfish and cat (11, 38) indicates that our earlier suggestion that CSF formation involves H\(^+\) secretion was incorrect (36). I was misled by the apparent acidity of CSF in certain species, which is not a general characteristic (rat, ref. 54; choroid plexus fluid of cat, ref. 1) and, in any case, not the true pH of primarily secreted fluid (Table 6), which would have a high pH and HCO\(_3\)\(^-\) concentration. Further comparative data may be found in the excellent recent review by Cserr (13).

The data summarized in Table 7 show that under normal circumstances at 15–18 C carbonic anhydrase has only a moderate role in CSF HCO\(_3\)\(^-\) production and Na\(^+\) transport in dogfish, even though it is clear that the general mechanism of HCO\(_3\)\(^-\) accumulation is through hydration of CO\(_2\). The fact that the uncatalyzed reaction has a major role here is not really unusual; in the mammalian pancreas, for example, the rate of HCO\(_3\)\(^-\) formation is accounted for half by the catalyzed and half by the uncatalyzed reaction (36). It may be speculated that if the fish were in colder water during migration (for instance at 6 C), the uncatalyzed rate would be half that of the present experiments (35), but the enzymic contribution would keep the normal total rate the same.

In man, CSF HCO\(_3\)\(^-\) concentration is reduced by acetazolamide (5, 41). In the steady state, starting 24 hr after a large dose, CSF HCO\(_3\)\(^-\) is about 16 mm.\(^6\) This is not secondary to the renal loss of HCO\(_3\)\(^-\) and metabolic acidosis of plasma, since HCl does not lower CSF HCO\(_3\)\(^-\), when plasma HCO\(_3\)\(^-\) is as low as 15 mm (15). The effect of acetazolamide on CSF HCO\(_3\)\(^-\) appears to be a direct effect on the secretory site. Fluid production, sodium k_{in}, and pressure in CSF are lowered in all mammals (36); the volume effect is not known.

### Series II: Effect of Hypercapnia—Limiting Effect of Blood Flow and pH Control of CSF

The data of Fig. 4 and 6A are fundamentally like those obtained in numerous mammalian experiments (10, 30, 55, 59, 64). In all species, hypercapnia causes an elevation of CSF HCO\(_3\)\(^-\) greater than that of plasma HCO\(_3\)\(^-\). There are similar data for brain, which in hypercapnia show an elevated HCO\(_3\)\(^-\) concentration, usually measured in comparison to muscle (6, 46, 49, 54, 67). These and other studies show (3, 25, 48, 61, 62) that the pH of CSF is regulated in all acid-base abnormalities, whether they be low or high pH, or of respiratory or metabolic origin (19). There has been some question as to the efficacy of CSF pH regulation in chronic respiratory acidosis in man (10, 55); however, an examination of the data shows clearly that CSF does indeed "buffer" to protect pH in this situation to within 0.1 unit of normal.

The nature of pH regulation of CSF has not been clear. In the last few years, attention has been given to the CSF blood potential and its relation to H\(^+\) secretion in the direction of fluid formation (61, 62). Our experiments, showing HCO\(_3\)\(^-\) accumulation in both cat (38) and dogfish (Fig. 3) CSF by a carbonic anhydrase mechanism, point to a different mechanism. Also relevant is the fact that the CSF potential is negative in the dogfish (29, 53), but positive in mammals (27, 62), while the underlying chemical mechanisms, including the response to hypercapnia, are the same in the two species.

It is of interest that spinal fluid collected below a ligation of the cervical cord shows the usual HCO\(_3\)\(^-\) elevation in response to hypercapnia that we have seen to be typical of CSF and brain interstitial fluid. It was concluded that hydration of CO\(_2\) is not confined to choroid plexus, but occurs throughout the brain (63). The obvious loci for the reaction are glia, which contain a high concentration of

\(^{5}\)Since inhibition decreases fluid formation about 0.1 ml/hr, the mean volume of CSF during HCO\(_3\)\(^-\) measurement is essentially unchanged, and during Na\(^+\) and Cl\(^-\) measurements was about 1.4 ml.
carbonic anhydrase (23). The following calculation shows that blood flow through the choroid plexus is not sufficient to supply CO₂ at the observed rates of HCO₃⁻ formation in the CSF.

The maximum rate of delivery of a substance from blood to CSF and brain (assuming it is distributed in total brain fluid) is set by the blood flow and total fluid volume: in the cat 30 ml/min and 18 ml, respectively. The quotient of these gives the maximum rate constant, 1.6 min⁻¹. This assumes perfect mixing and sampling. The highest value recorded is 0.3 min⁻¹ for ethanol (15); the rough estimate for total CO₂ transport in this species, taken from Table 1, is 0.35 min⁻¹ (11). Choroid plexus blood flow is about 0.2 % of that of the brain or .065 ml/min (66). The maximum rate constant for delivery into the total fluid volume of the cat brain (18 ml) from the plexus is then 0.0036 min⁻¹; into CSF (4 ml), 0.016 min⁻¹. Thus, sodium transport in the mammal (Table 1) can be mediated at the choroid plexus, but not that of CO₂, which is 40-80 times faster.

In the dogfish, relations are similar. Cardiac output is about 60 ml/min (58); we can only guess as to brain blood flow, but in view of the small brain size (0.25 % of body wt) we might say that 1/10 of cardiac output goes to the brain. If the brain fluid volume is taken as 4 ml, this would yield a maximal rate constant of 1.5 min⁻¹ or 90 hr⁻¹—somewhat higher than observed data for CO₂ accumulation (55 hr⁻¹) in the present experiments (Table 6). Clearly, the rapid synthesis of brain and CSF HCO₃⁻ must occur throughout the central nervous system.

Since blood flow is critical to HCO₃⁻ accumulation, and there appears a relation between this process and Na⁺ and fluid transport, it is clear why respiratory alkalosis produces a profound reduction in CSF formation (50). In this circumstance both the concentration of CO₂ and the flow of blood are reduced; in the presence of carbonic anhydrase the first of these should not be limiting, but actual delivery of substrate appears to be critical.²

The hypercapnia experiments show not only the chemistry underlying accession of HCO₃⁻ to CSF, but also the role of carbonic anhydrase. Figures 5 and 6B show that methazolamide cuts the rate of CSF accession of HCO₃⁻ to half during hypercapnia. This can only mean that we are dealing with a carbonic anhydrase-mediated reaction, and there appears a relation between this process and Na⁺ transport, it is clear why respiratory alkalosis postulate for CSF has essentially no limit and appears to be controlled by the physiological requirement for pH normalcy.

I shall compare the rates of CSF accumulation of H⁺CO₃⁻ (Table 6) with those of cold HCO₃⁻ in hypercapnia (Table 8). This will throw light on both the mechanism and stoichiometry. The data of Table 6, per se, do not permit a discrimination between transport of ionic HCO₃⁻ and that of CO₂ followed by hydroxylation to yield HCO₃⁻ in CSF; but on other grounds, including the magnitude of the rate and the effects of carbonic anhydrase inhibition, the latter appears the correct mechanism. In Fig. 6, CSF HCO₃⁻ is increased when plasma Pco₂ (but to far less extent plasma HCO₃⁻) is elevated, thus permitting an unambiguous evaluation of the rate relative to Pco₂.

The first two rows of Table 8 show the rates taken from the isotope experiments, in the presence of or adjusted for normal Pco₂. The "effective gradient" (col 1, 2) is taken here to be the Pco₂ of plasma, on the basis that the CO₂ is used at the secretory sites at about the rate at which it is generated and delivered. This has been discussed above in relation to blood flow. A further point supporting equality between normal plasma Pco₂ and effective gradient is that the calculated uncatalyzed rate using plasma CO₂ as substrate (0.27 mm) and the first-order rate constant for hydration of CO₂ at 16°C (0.85 min⁻¹) yields a value (13.7 mm hr⁻¹) reasonably close to the observed rate in vivo following inhibition of enzyme (9.2 mm hr⁻¹, row 2, Table 8).

Turning now to the data from the cold experiments (rows 3-5, Table 8), consider first the inhibited fish at

<table>
<thead>
<tr>
<th>Pco₂ (mm Hg)</th>
<th>CSF HCO₃⁻, mm/hr</th>
<th>Situation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Fish</td>
<td>6 6</td>
<td>14.5</td>
</tr>
<tr>
<td>First day of carbonic anhydrase inhibition (34)</td>
<td>11 7</td>
<td>11.3</td>
</tr>
<tr>
<td>Hypercapnia (Fig. 6)</td>
<td>16 8</td>
<td>20.5</td>
</tr>
<tr>
<td>Hypercapnia + inhibition (Fig. 6)</td>
<td>16 8</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Column 1 gives the observed Pco₂. Column 2 shows the hypothetical gradient, explained in the text. Columns 3 and 4 give the observed turnover of HCO₃⁻ in the normal fish (row 1) and the approximate rate of the uncatalyzed turnover at normal Pco₂ (row 2). The remaining data show the accumulation of HCO₃⁻ during the unsteady state caused by elevation of Pco₂. The difference (In – Out) shown for rows 4 and 5 corresponds to the accumulation rate of CSF HCO₃⁻ in Fig. 6.
by a one-way process. There are no previous studies on HCO$_3^-$ from this aspect, so it is expected that the elevation of plasma CO$_2$ does not now represent the effective gradient. In the model of Table 8, a Pco$_2$ gradient of 8 mm Hg (0.36 mm) accounts for the accession rate in the hypercapnic inhibited situation, as the following calculation shows: from Fig. 3 or Table 7 the inhibited rate constant is 63% of the control; related to gaseous CO$_2$ this yields an inhibited rate constant of 55 hr$^{-1}$ (Table 6). From the rate of 12.5 mm hr$^{-1}$ (Table 8, row 5), the calculated substrate concentration is then 0.36 mm. The reason the full plasma Pco$_2$ (i.e., 16 mm Hg) is not used as the gradient probably has to do with the fact that high CO$_2$ gradients cannot be built up due to rapid diffusion of gas and that there is a limitation on HCO$_3^-$ transport after it is formed. Plainly, the system is geared to small Pco$_2$ gradients or changes. Note that the increase in HCO$_3^-$ input is not matched by increase in output until the proper CSF HCO$_3^-$ concentration is achieved for normal pH.

Finally, row 3 shows in model form what happens when carbonic anhydrase is inhibited in the absence of exogenous CO$_2$, i.e., simple injection of acetazolamide or methazolamide (34). The induced respiratory acidosis secondary to inhibition of the blood enzyme raises CSF HCO$_3^-$, even though the enzyme at the CSF secretory sites is also inhibited. Although the actual elevation of formation rate is modest compared to the experiment of Fig. 6, it was adequate to bring the CSF HCO$_3^-$ to about 20 mm in 18 hr and maintain CSF pH normal in the face of respiratory acidosis in the blood.

**Evidence Supporting Idea that HCO$_3^-$ Influx by Formation from CO$_2$ Represents Net Flux**

The idea that Na$^+$ influx at the choroid plexus and possibly other secretory sites (47) represents net flux from plasma to CSF is well accepted (16). Little attention has been given to Cl$^-$, but charge balance demands that most, if not all, Cl$^-$ follows a similar pattern to Na$^+$. There are no previous studies on HCO$_3^-$ from this aspect, so it is worthwhile summarizing the evidence that suggests that HCO$_3^-$ is also accumulated in CSF largely, if not entirely, by a one-way process.

1) HCO$_3^-$ accession to CSF is rapid because it is not based on ionic movement. However, once formed in CSF it would be analogous to Na$^+$ and Cl$^-$, which exit largely by bulk flow, rather than backflux of the ion.

2) Calculation of the transfer rates and concentrations of Na$^+$ and Cl$^-$ in newly formed CSF leaves an anion deficit of about 30%, which corresponds to the rates and concentrations for HCO$_3^-$ (Tables 6 and 7).

3) Evidence for net HCO$_3^-$ accumulation in CSF arises from both isotope data and cold experiments during hypercapnia. These yield the same values for rates of plasma CO$_2$ to CSF HCO$_3^-$ conversion, if the effective hypercapnic gradient is set at about 8 mm Hg (Table 6).

4) The effect of carbonic anhydrase inhibition is to decrease k$_{in}$ and clearance of HCO$_3^-$ This speaks for formation of HCO$_3^-$ at the sites of the enzyme, choroid plexus, and glia.

5) There is a clear analogy to net formation of HCO$_3^-$ in the aqueous humor (32) and other systems (36). This is discussed in the following section.

6) Earlier work by Pappenheimer's group (Fig. 1 of ref. 19) suggested that HCO$_3^-$ ion moved from CSF to blood. The data showed that high or low HCO$_3^-$ in artificial CSF moved toward its normal value of 22 mm. These experiments are equally compatible with the present idea that the level of CSF HCO$_3^-$ is regulated by its rate of formation from CO$_2$.

In summary, net HCO$_3^-$ formation appears to be a principal aspect of CSF physiology. The numbers derived (Tables 7 and 8) arc maximal ones and should be regarded for the present as models until full knowledge of backfluxes and exchanges with brain are known.

**General View of CSF and Aqueous Humor Formation**

The first view that a primary step in the secretion of aqueous humor was the conversion of OH$^-$ to HCO$_3^-$ in the ciliary epithelium was due to Jonas Friedenwald (21). This led directly to the use of acetazolamide in the treatment of glaucoma (3), for meanwhile, carbonic anhydrase had been discovered in the ciliary process (69). The rabbit showed a high concentration of HCO$_3^-$ in the aqueous humor and even higher calculated value in the primarily secreted fluid, about 90 mm (32). The rate of HCO$_3^-$ accumulation in aqueous was reduced by acetazolamide (31). Data of Kinsey and Reddy (31) summarized in Table 1 (summarized in Table 7) suggest that the turnover of HCO$_3^-$ is so rapid that it can account for essentially all of Na$^+$ and thus of fluid formation. The situation became confused later by the emphasis on differing ratios of the concentration of HCO$_3^-$ and Cl$^-$ between plasma and aqueous of various species (4). It was thought possible that the HCO$_3^-$ accumulation mechanism was not a general one or a specific manifestation of secretory activity (14). In the primate, for example, there is a Cl$^-$ excess in the anterior aqueous and a slight HCO$_3^-$ deficit, and this was thought to represent a different mechanism from that of Friedenwald (21). However, the finding that fish possess the HCO$_3^-$ accumulatory mechanism (18, 34) and that all mammals respond to acetazolamide with a lowering of fluid formation in the eye led to the idea that the Friedenwald scheme applies to all vertebrates (36). The fundamental rate analysis (32) has only been done for the rabbit, but there is no reason to believe that it would turn out differently in other species. The varying ratios of HCO$_3^-$ between anterior aqueous and plasma in mammals, from 0.67 to 1.35 (14), may be
regarded as representing secondary changes due to acid metabolites from lens and retina, as well as interaction with the vitreous.

In this connection, it must be mentioned that the leading monograph in the field of eye physiology (14) rejects this line of argument, due chiefly to the apparent failure of acetazolamide to reduce 22Na turnover (as \( k_{in} \)) in the eye (but see APPENDIX) and insufficient attention to the decisive experiment showing the effect of carbonic anhydrase inhibition on the rate of HCO\(_3^-\) formation in the aqueous (31).

In the case of CSF, HCO\(_3^-\) accumulation, by what is precisely the Friedenwald eye mechanism, does not appear to have been considered before. This has been largely because the CSF appeared in most species as a slightly acidic fluid with a Cl\(^-\) excess and because of emphasis on fluid-to-plasma ion ratios, rather than rates (15). There has also been concern with the potential between CSF and plasma as a driving force for H\(^+\) and HCO\(_3^-\) (61, 62). As with the eye, these appear now to be secondary phenomena and unrelated to the primary process. Elevation of Pco\(_2\) in the cat, for example, decreases the CSF potential from +2.3 to -4 mV (52) at a time when CSF HCO\(_3^-\) is increasing. The crucial experiment is analogous to that of Kinsey and Reddy (31) for the aqueous: the demonstration that the rapid accession of labeled HCO\(_3^-\) to the CSF is slowed by acetazolamide (38 and present series I; Fig. 3). Present series II shows that the accession of cold HCO\(_3^-\) to CSF under the stimulus of hypercapnia is also reduced by acetazolamide. The decrease in CSF formation (36) is thus visualized in relation to the CO\(_2\)-carbonic anhydrase system, particularly since HCO\(_3^-\) formation is enough to match considerable sodium (Table 6).

The decrease in Cl\(^-\) transport seen in both aqueous and CSF after acetazolamide (22, 38) might be viewed as secondary to the decrease in HCO\(_3^-\) and Na\(^+\) transport, to interference with a carbonic anhydrase-Cl\(^-\) reaction, or to a pH-HCO\(_3^-\) effect on the sodium-transport mechanism. This first could occur if the normal rapid HCO\(_3^-\) transfer is an exchange reaction. This may, for the present, be regarded as tentative, since the control of respiration in the face of altered CO\(_2\) equilibria in CSF (7, 20) has not been directly studied under conditions of carbonic anhydrase inhibition. However, the principal finding of the past 20 years, beginning with the work of Leusen and extending to many others (7), may be shortly and accurately summarized as follows: resting ventilation is a simple function of CSF and interstitial brain [H\(^+\)] (20). The present data suggest the delicate chemical and secretory mechanisms whereby [H\(^+\)] in these fluids is regulated. We should perhaps not be surprised by the finding that this vital control system, like so many others in the animal kingdom, is fashioned in simple and elegant terms from the commonest molecules in nature: carbon dioxide and water.

**APPENDIX**

A persistent problem in cerebrospinal and ocular fluid dynamics has been the disparity between Na\(^+\) turnover rate and fluid turnover rate following carbonic anhydrase inhibition. In the normal, the Na\(^+\) rate and fluid production rate are the same for both CSF and aqueous humor (14, 15). However, when acetazolamide is given and a new steady state established, the following is found when Na\(^+\) is measured by the rate of appearance of isotope in the fluid, and formation of fluid independently by a variety of means, including inulin dilution, tonography, and exit of labeled albumin: in the rabbit aqueous, less than 10% change in the \( k_{in} \) for Na\(^+\); about 50% decrease in fluid formation (4, 16, 26; reviewed in refs. 14 and 36). In CSF of rat, dog, and rabbit, there is a 23-35% decrease in Na\(^+\) \( k_{in} \) compared to a 30-65% decrease in fluid production (16, 17). Notably, however, when the aqueous humor was artificially perfused at a rate 4 times normal flow, both formation rate and 22Na turnover from plasma to fluid were decreased to the same degree (40%) by acetazolamide (29).

The discrepancy between effects of acetazolamide on 22Na and fluid turnover is also an issue in the present paper, since the change in fluid formation (51) is accompanied by a lesser change in 22Na \( k_{in} \) (Table 7). It appeared important to inquire into the problem, with attention to the meaning of the 22Na \( k_{in} \).

The derivation of \( k_{in} \) is given by Davson (15) for the general case of a transfer constant, and applied by him to the isotopic rates. When the plasma concentration of the substance (\( C_{in} \)) is maintained constant, and the concentration within the compartment (\( G_{in} \) is meas-
ured at various times \( t \), the rate constants \( k_{in} \) and \( k_{out} \) are evaluated as follows:

\[
\frac{dC}{dt} = k_{in}C_{out} - k_{out}C_{in}
\]

(1)

For the case as for sodium where the equilibrium values of \( C_{out} \) and \( C_{in} \) are approximately unity, \( k \sim k_{out} \). Integrating:

\[
C_{in} = C_{out}(1 - e^{-k_{out}t})
\]

(2)

which is usually rearranged to give

\[
\ln \left( \frac{1 - C_{in}}{C_{out}} \right) = -k_{out}t
\]

(3)

Equation 3 is plotted to yield \( k_{out} \); this is equivalent to the treatment in the present paper which yields \( k_{in} \) by the plot of Fig. 1 as described in the text.

The foregoing treatment is based on changes in concentration within the compartment, and tacitly implies no change in volume. However, acetazolamide lowers the rate of fluid formation and could reasonably be expected to lower the volume of the CSF or aqueous. In any case, it would be desirable to have a formulation based on the transfer of amounts of sodium (or any substance). Dr. A. S. V. Burgen kindly suggested this approach, with the following derivation. Let \( Q_{in} \) = amount of material transferred into the fluid compartment from the concentration in plasma, \( C_{out} \), which is again kept constant. The concentration within the compartment is designated \( C_{in} \) as above. Then

\[
\frac{dQ_{in}}{dt} = a_{in}C_{out} - a_{out}C_{in}
\]

(4)

where \( a \) has the units of volume per time, and the same meaning as renal clearance.

As above, in the steady state \( C_{in} = C_{out} \), so that \( a = a_{in} = a_{out} \).

\[
\frac{dQ_{in}}{dt} = (C_{out} - C_{in})a
\]

(5)

also, in the steady state

\[
\frac{dQ_{in}}{dt} = V\frac{dC_{in}}{dt}
\]

(6)

where \( V \) = volume of fluid compartment.

Equating 5 and 6 yields

\[
\frac{dC_{in}}{dt} = \frac{a}{V}(C_{out} - C_{in})
\]

(7)

Integrating for the boundary conditions \( t = 0 \) and \( C_{in} = 0 \)

\[
C_{in} = C_{out}(1 - e^{-\frac{t}{k_{out}}})
\]

(8)

This is the same as equation 2, with \( k \sim k_{out} \). Since the rate constant \( k \) as defined in equation 1 is equal to clearance divided by volume, the substitution could have been made directly in equation 2. Dr. Burgen’s derivation above, however, makes it clear that amounts of substance passing across have been considered.

Equation 8 shows that if \( a \) and \( V \) change to the same degree, the change in \( C_{in} \) with time (the modality measured) will be unchanged. This \( C_{in} \) unaffected is the finding for aqueous humor \( ^{31}Na \) in several species following acetazolamide, shown particularly well in ref. 16. In these circumstances, fluid formation is lowered by half (4), and we might expect that sodium turnover would be affected the same way (14). Changes in \( V \) were not measured, but an approach to a new inhibited steady state had been achieved by giving a large dose of drug 30-90 min before isotope, depending on protocol or species. This interval is long enough for considerable turnover of aqueous at the rate, i.e., since (in rabbit) this normal production rate is 4 ml/min and the volume is 300 ml. We can only deduce that the new aqueous production at rate \( a/2 \) was into a volume of \( V/2 \). On the other hand, the change of \( C_{in} \) with time for \( ^{31}Na \) in rabbit CSF (and hence \( k_{in} \) or \( a/V \)) is lowered some 35% by acetazolamide (16), so the presumption is that in CSF \( a \) is reduced to a greater extent than \( V \).

In the present experiments \( a \) and \( V \) change their relations differently with inhibition, depending on the ion. For Na, \( a/V \) decreased slightly; for Cl- \( a/V \) was constant; for HCO3- \( a/V \) decreased considerably (Table 7). To evaluate these factors properly, such matters as the timing of dose in relation to the measurements, the fate of the drug, and the flow and volume characteristics of the chamber are all of critical importance.

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