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

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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 50-100 times greater. Analyses of the data suggest that k in 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).

The following 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.
the summers of 1969 and 1970, at the Mount Desert Island

faster (about 20 times) at pH 7.4 (36).

Thus, the terms hydration and hydroxylation of CO₂ are

involved in the actual formation of CSF. In hypercapnia, the

rate, and that in hypercapnia, are reduced by carbonic

anhydrase, no distinction is made between mecha-

nisms I and 2 below:

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^- \quad (1)$$

and

$$\text{HOH} \rightarrow \text{OH}^- + \text{H}^+ \quad (2)$$

Thus, the terms hydration and hydroxylation of CO₂ are

used interchangeably. In both cases the overall reaction is

the formation of HCO₃⁻ from gaseous CO₂. 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

Biological Laboratory, Salisbury Cove, Maine. The fish

were caught by handline or 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-cars; 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

taken 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 Pco₂ of the plasma,

although the handling and immobilization did tend to lower

plasma HCO₃⁻ about 2 mm. For the elevation of Pco₂, the

seawater was passed through a small bubble oxygcnator

(Seals Corporation) to which 5 % CO₂ in air was admitted.

This water was pumped into the fish as described just above.

The physical arrangement of bubbler, tubing, pump, and

fish was such that the measured Pco₂ 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 pKₐ was taken as 6.1 and α 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

CO₂ was determined on plasma and CSF using the Kopp-

Natelson microgasometer; chloride was determined on

plasma and CSF using Hg(NO₃)₂ titration. Chloride 36 and

¹⁴C 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 10⁶ counts. Background

was 40 counts/min. Sodium 22 was counted in a well-type

gamma counter for 10 min. In all ²²Na and ³⁶Cl experiments,

the counts in 0.1 ml plasma at 18 min were set at 100. There

was no significant difference in actual counts for these points

between control and inhibited fish. In the case of ¹⁴CO₂

counts, the plasma of the control fish at 3 min after injec-
tion of the isotope was set at 100; at this time the level in

the inhibited fish is much higher, as shown in the data.

All drugs and solutions were injected into the tail vein

of the fish. Solutions of ions were given at zero time, and

drug usually 30 min earlier. Acetazolamide or methazol-

amide was used in doses large enough to inhibit > 99.99 %
carbonic anhydrase (34, 36). The pharmacology of ace-
tazolamide in S. acanthias has been reported (34); that of

| TABLE 1. Rate constants of transfer from plasma to CSF and aqueous humor (min⁻¹) |
|---|---|---|---|---|---|---|
|     | Dogfish CSF | Dog CSF Cisternal | Rabbit CSF Cisternal | Cat CSF Cisternal | Dog Ant. Aqueous | Rabbit Ant. Aqueous Secreted Component |
| Na⁺ | 0.0032 | 0.0073 | 0.041 | 0.025 | 0.060 | 0.060 |
| Cl⁻ | 0.0033 | 0.0077 | 0.19 | 0.060 | >3 | 0.6 |

* Other sodium values: CSF; rat .019 (16), man (cisternal) .002, man (ventricle) .0036 (15). Anterior aquenix; rat .025, monkey .01, rabbit .009 (16). † Based on HCO₃⁻ accumulation, from the base of plasma HCO₃⁻. See Table 6 for rates based on plasma CO₂.

‡ From published curves which yield a T½ of about 2 min.
BICARBONATE FORMATION IN CSF

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 I: Rates of Ion Movement

In this series the rates of accession of labeled $Na^+$, $Cl^-$, and $HCO_3^-$ 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, $I_{Mr} = \sim 10^{-8} \text{ m}$. 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 $^{22}Na$ declined only slightly (72-64), and more confidence is put in this value. The 1- to 3-hr $k_{in}$ for both $^{22}Na$ and $^{36}Cl$ will be used as the basis for calculations and comparisons with the inhibited situation. Figure 1B yields 0.16 min$^{-1}$ for the $^{22}Na$ during 1-3 hr following pretreatment with methazolamide.

The CSF/plasma ratio for isotope approaches, but does not reach, the equilibrium ratio for $Na^+$ 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 $HCO_3^-$ increase (Table 2). In agreement with this, hypercapnia in $S.$ acanthias induces a small fall in plasma $Cl^-$, which appears secondary to the increase in plasma $HCO_3^-$ (12). However, Fig. 2 shows that from 0.5 to 3 hr, plasma concentrations of $^{36}Cl$ are essentially identical in control and treated fish.

The normal or control $k_{in}$ for $^{36}Cl$ 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 $S.$ acanthias: normal (A) and following carbonic anhydrase inhibition (B)

<table>
<thead>
<tr>
<th></th>
<th>$Na^+$, mm</th>
<th>$Cl^-$, mm</th>
<th>$HCO_3^-$, mm</th>
<th>pH</th>
<th>$Pco_2$, 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</td>
<td>254</td>
<td>239</td>
<td>233</td>
<td>7.7</td>
</tr>
<tr>
<td>CSF</td>
<td>271</td>
<td>268</td>
<td>264</td>
<td>236</td>
<td>8.3</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 $\Delta$ factor of 0.045. The small discrepancy in internal agreement among $HCO_3^-$, $Pco_2$, and pH is due to different numbers of samples used for pH and total CO2 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 $HCO_3^-$ of about 5, 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 $^{36}$Cl accession 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

<table>
<thead>
<tr>
<th></th>
<th>$^{14}$CO$_2$ Counts Relative to Control Plasma at 3 min</th>
<th>Acid-Base Balance at 3 min</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 min</td>
<td>6 min</td>
<td>12 min</td>
</tr>
<tr>
<td></td>
<td>HCO$_3^-$</td>
<td>CO$_2$</td>
<td>HCO$_3^-$</td>
</tr>
<tr>
<td>A) Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>100</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>CSF</td>
<td>11.5</td>
<td>5.5</td>
<td>6</td>
</tr>
<tr>
<td>B) Acetazolamide at -30 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>255</td>
<td>242</td>
<td>10</td>
</tr>
<tr>
<td>CSF</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Percent equilibrium in plasma</td>
<td>40</td>
<td>63</td>
<td>88</td>
</tr>
</tbody>
</table>

Isotopic CO$_2$ partition at equilibrium in plasma, calculated from pH.

In CSF, equilibrium not reached.

HCO$_3^-$/CO$_2$ ratio in plasma calculated from percent equilibrium using Roughton equation. See text.
Carbon dioxide production is 1.5 mmoles/hr for a 1-kg shark. 
The partition of total \( \Delta \mathrm{HCO}_3^- \) in CSF is made on the basis of \( \Delta \mathrm{HCO}_3^- \) in plasma and the assumption that Table 3A represents normal function.

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 4 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 min\(^{-1}\)) compared to 0.915 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 \( \Delta \mathrm{HCO}_3^- \) was given. This produced the following series of events, due to inhibition of enzyme in red cell and choroid plexus.

1) The \( \Delta \mathrm{HCO}_3^- \) ion, immediately following its injection, can only form \( \Delta \mathrm{CO}_2 \) 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 \( \Delta \mathrm{HCO}_3^- \) and \( \Delta \mathrm{CO}_2 \) is about 4 min and to 90% equilibrium is 13 min. The accurately determined 25 C value of 2.1 min\(^{-1}\) (24) would yield about the same range for 16 C, assuming a \( Q_{16} \) of 2. Thus, the mean observed rate is about the same as that of the uncatalyzed reaction with some values falling above it.

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from fish is a function of the fraction present as CO₂ gas.

Table 3B shows that the ratio of plasma labeled HCO₃⁻/CO₂ 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 CO₂ 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 HCO₃⁻/CO₂ ratio than normal, since the Pco₂ in the inhibited fish is almost twice that of the normal. This enables unloading of total labeled CO₂ at a rate as fast as normal, even though the enzyme is inhibited. The same situation applies in the mammal (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 H¹⁴CO₂ is taken to be at the same concentration as in plasma; CSF H¹⁴CO₂⁻ is then entered in Table 3B as total minus gaseous H¹⁴CO₂, just as for controls. The rate constants of H¹⁴CO₂⁻ accumulation in CSF were then determined for the various periods based on the actual gaseous H¹⁴CO₂ 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 H¹⁴CO₂ 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 H¹⁴CO₂ for A and B so that the accession to CSF should reflect the partition of HCO₃⁻/CO₂ in plasma and the rate of hydration of CO₂ to HCO₃⁻ at the secretory sites. Inhibition initially lowers gaseous H¹⁴CO₂ in plasma below control (3-6 min) because of disequilibrium. By the 6- to 12-min interval, however, the gaseous CO₂ concentration in plasma is about 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).

Calculation of Transport Rates and Concentrations of Secreted Fluid

Table 6 shows the kᵢₙ (col. 3) and Tₛₙ (col. 4) of the three ions that have been studied. In this context HCO₃⁻ is treated in the same way as Na⁺ and Cl⁻, i.e., the kᵢₙ is based on plasma concentration of the ion, disregarding for the moment the mechanism of HCO₃⁻ accumulation in CSF. In all cases, kᵢₙ 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 kᵢₙ 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; kᵢₙ for Na⁺ represents net flux and is equivalent to the rate of fluid formation. The present data agree quite accurately with this, for kᵢₙ 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).

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**Table 5. Accession of H¹⁴CO₂⁻ from plasma to CSF in S. acanthias, free-swimming fish**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Total</th>
<th>HCO₃⁻</th>
<th>CO₂</th>
<th>HCO₃⁻</th>
<th>CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>100</td>
<td>94</td>
<td>6</td>
<td>63</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>94</td>
<td>63</td>
<td>4</td>
<td>6</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>kᵢₙ, hr⁻¹</th>
<th>Tₛₙ to equil, hr</th>
<th>Accession Rate (col. 5) mm/hr, × vol CSF (1.6 ml)/formation of CSF (0.28 ml/hr)</th>
<th>Concentration of Secreted Fluid, mM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>255</td>
<td>271</td>
<td>3.7</td>
<td>48</td>
<td>272.0</td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td>239</td>
<td>264</td>
<td>3.0</td>
<td>33</td>
<td>183.0</td>
<td></td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>7.7</td>
<td>8.3</td>
<td>1.9</td>
<td>37</td>
<td>14.5</td>
<td>82.0</td>
</tr>
</tbody>
</table>

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* Equilibria of isotope from total CO₂ counts at 6 min calculated as 63%, and at 3 min as 40%, as in Table 3. † From the 6-min uptake of H₁⁴CO₃⁻ to CSF, divided by the midpoint (3 min) plasma ¹⁴C0₂. Because of the rapid changes in these early periods these kᵢₙ values are only approximate.
BICARBONATE FORMATION IN CSF

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 kH+ 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 HCO3– (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 injections of Na14CO3 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, carbonic anhydrase. How does the equilibrium mixture of HCO3– and CO2 (labeled or unlabeled) reach the CSF? (labeled or unlabeled) reach the CSF at the observed rates? There are only two possibilities, as ionic HCO3– or as CO2. The first is unlikely, since the observed rate is far greater than that of Cl– (Table 6) or of any known movement of ions into CSF (15). The second is reasonable, but then the data demand an additional step, since the concentration of label in CSF rapidly becomes (for the given pH and Pco2) that of a new equilibrium mixture of HCO3– and CO2. Clearly, new HCO3– must be formed, and the question is, How and where is this done? CSF has almost no buffer properties; fast conversion of CO2 to HCO3– in the fluid itself may be ruled out. Since the choroid plexus and the glia of brain are secretory tissues containing carbonic anhydrase, and inhibition of the enzyme lowers the rate of bicarbonate formation in CSF, it is clear that the enzyme is responsible for this process. The transfer of 14C from plasma to CSF involves 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, carbonic anhydrase. How does the equilibrium mixture of HCO3– and CO2 (labeled or unlabeled) reach the CSF at the observed rates? There are only two possibilities, as ionic HCO3– or as CO2. The first is unlikely, since the observed rate is far greater than that of Cl– (Table 6) or of any known movement of ions into CSF (15). The second is reasonable, but then the data demand an additional step, since the concentration of label in CSF rapidly becomes (for the given pH and Pco2) that of a new equilibrium mixture of HCO3– and CO2. Clearly, new HCO3– must be formed, and the question is, How and where is this done? CSF has almost no buffer properties; fast conversion of CO2 to HCO3– in the fluid itself may be ruled out. Since the choroid plexus and the glia of brain are secretory tissues containing carbonic anhydrase, and inhibition of the enzyme lowers the rate of appearance of H14CO3– in CSF, its formation in these tissues from plasma 14CO2 is almost certainly the mechanism underlying the data of Tables 3–6 and Fig. 3. Under these conditions, the newly impermeant ion (HCO3–) in the CSF would not be expected to show any large element of backflux. 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 HCO3– 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 Pₐ₀₂ 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.
BICARBONATE FORMATION IN CSF

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⁻ available 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 kₘ 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 kₘ for 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 kₘ = 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 kₘ 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 = kₘV) and the derived term which I shall call transfer (col 6, μoles/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 (kₘ) 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 35.5 %, 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 kₘ 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 kₘ 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>
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<th>1</th>
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<tr>
<td></td>
<td>kₘ*</td>
<td>V (ml/hr)</td>
<td>(1 X 10⁻⁶ V)</td>
<td>flow (ml/min)</td>
<td>Plasma (μoles/hr)</td>
<td>transfer (μoles/hr)</td>
<td>New CSF</td>
</tr>
<tr>
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<td>ml/hr</td>
<td>ml/hr</td>
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<td>.28</td>
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<td>.19</td>
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<td>ml/hr</td>
<td>ml/hr</td>
<td>μoles/hr</td>
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<td>.22</td>
<td>.28</td>
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<td>.17</td>
<td>.20</td>
<td>239</td>
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<td>200</td>
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<tr>
<td>HCO₃⁻</td>
<td>hr⁻¹</td>
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<td>ml/hr</td>
<td>ml/hr</td>
<td>ml/hr</td>
<td>μoles/hr</td>
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<td>.28</td>
<td>7.7</td>
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<td>1.4</td>
<td>.20</td>
<td>7.7</td>
<td>11</td>
<td>55</td>
</tr>
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* kₘ values from Table 6 and Figs. 1–5. These data are applied to the steady-state situation by using the volume term (V, col 2) of controls and inhibited steady states (50). 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 (38).

The net result of inhibition is therefore to decrease $Cl^-$ transfer, although the calculated concentration of newly formed constant by ventriculocisternal perfusion (in the cat) $k_{in}$ for $Cl^-$ is reduced (38).

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The rapid accumulation of $HC0_3^-$ 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 $HC0_3^-$ concentration. Further comparative data may be found in the excellent recent review (19).

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The data summarized in Table 7 show that under normal circumstances at 15–18°C carboxic anhydrase has only a moderate role in CSF $HC0_3^-$ production and $Na^+$ transport in dogfish, even though it is clear that the general mechanism of $HC0_3^-$ accumulation is through hydration of $CO_2$. The fact that the un catalyzed reaction has a major role here is not really unusual; in the mammalian pancreas, for example, the rate of $HC0_3^-$ 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 of the present experiments (35), but the enzymic contribution would keep the normal total rate the same.

In man, CSF $HC0_3^-$ concentration is reduced by acetazolamide (5, 41). In the steady state, starting 24 hr after a large dose, CSF $HC0_3^-$ is about 16 pH. This is not secondary to the renal loss of $HC0_3^-$ and metabolic acidosis of plasma, since HCl does not lower CSF $HC0_3^-$, when plasma $HC0_3^-$ is as low as 15 pH (19). The effect of acetazolamide on CSF $HC0_3^-$ 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 $HC0_3^-$ greater than that of plasma $HC0_3^-$. There are similar data for brain, which in hypercapnia show an elevated $HC0_3^-$ 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 $HC0_3^-$ 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 $HC0_3^-$ 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...
BICARBONATE FORMATION IN CSF

Carboxylic 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 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 some 40-80 times faster.

In the dogfish, relations are similar. Cardiac output is about 60 ml/min (58); we 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.

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, HCOS⁻. Inhibition does not alter the fundamental process; Figs. 4 and 5 appear qualitatively similar. Only the rate of CSF HCO₃⁻ formation is altered so that the steady-state concentration would be reached in 6-8 hr, rather than 3-4 hr as in the uninhibited. The new steady state is reached when the CSF HCO₃⁻ concentration becomes 4 times normal (24 mm), then matching the experimental elevation of Pco₂ and maintaining normal pH. This experiment shows the process at hand with particular clarity because it is possible to make a large change in Pco₂. The profound difference between buffering in plasma and in CSF is well illustrated, since at the relatively low Pco₃ range of these experiments the chemical buffering in plasma is limited (12), while the active formation mechanism which we 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 discrimination between transport of ionic HCO₃⁻ and that of other species. Table 8 shows the effect of carbonic anhydrase inhibition; it is clear that 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” (row 1, 2) is taken here to be the Pco₂ of plasma, on the basis that the CO₂ is used at the secretory site 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. Evidence by bulk flow, rather than backflux of the ion.

worthwhile summarizing the evidence that suggests that it would be analogous to Na+ and Cl-, which exit largely based on ionic movement. However: once formed in CSF if not all, Cl- follows a similar pattern to Na+. There are possibly other secretory sites (47) represents net flux from plasma to CSF is well accepted (16). Little attention has been given to Cl- (i.e., of 16 mm Hg) is not used as the gradient probably has to do with the fact that high CO2 gradients cannot be built up due to rapid diffusion of gas and that there is a limitation on HCO3- transport after it is formed. Plainly, the system is geared to small Pco2 gradients or changes. Note that the increase in HCO3- input is not matched by increase in output until the proper CSF HCO3- concentration is achieved for normal pH.

Row 4 of Table 0 gives the increase in HCO3- formation induced by elevated CO2 when carbonic anhydrase is normally active (taken from data of Fig. 6). The theoretical rate of HCO3- formation based on enzyme concentration would yield a value hundreds of times greater than observed (36). The actual limitation, as discussed above, is probably blood flow; by analogy to other systems studied there may be also a limitation on the transport of HCO3- from cell to CSF following its formation within the cell (36).

Finally, row 3 shows in model form what happens when carbonic anhydrase is inhibited in the absence of exogenous CO2, i.e., simple injection of acetazolamide or methazolamide (34). The induced respiratory acidosis secondary to inhibition of the blood enzyme raises CSF HCO3-, 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 HCO3- to about 20 mm in 18 hr and maintain CSF pH normal in the face of respiratory acidosis in the blood.

Evidences Supporting Idea that HCO3- Influx by Formation from CO2 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 HCO3- from this aspect, so it is worthwhile summarizing the evidence that suggests that HCO3- is also accumulated in CSF largely, if not entirely, by a one-way process.

1) HCO3- 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 HCO3- (Tables 6 and 7).

3) Evidence for net HCO3- accumulation in CSF arises from both isotope data and cold experiments during hypercapnia. These yield the same values for rates of plasma CO2 to CSF HCO3- conversion, if the effective hypercapnic gradient is set at about 8 mm Hg (Table 0).

4) The effect of carbonic anhydrase inhibition is to decrease kHCO3- and clearance of HCO3-. This speaks for formation of HCO3-, at the sites of the enzyme, choroid plexus, and glia.

5) There is a clear analogy to net formation of HCO3- 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 HCO3- ion moved from CSF to blood. The data showed that high or low HCO3- 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 HCO3- is regulated by its rate of formation from CO2.

In summary, net HCO3- formation appears to be a principal aspect of CSF physiology. The numbers derived (Tables 7 and 8) are 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 HCO3- 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 HCO3- in the aqueous humor and even higher calculated value in the primarily secreted fluid, about 90 mM (32). The rate of HCO3- accumulation in aqueous was reduced by acetazolamide (31). Data of Kinsey and Reddy (31) (summarized in Table 1) suggest that the turnover of HCO3- 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 HCO3- and Cl- between plasma and aqueous of various species (4); it was thought possible that the HCO3- 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 HCO3- deficit, and this was thought to represent a different mechanism from that of Friedenwald (21). However, the finding that fish possess the HCO3- 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 HCO3- 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 \(^{22}\)Na 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\(^+\) or 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 +2.5 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 establishes a Cl\(^-\) gradient from plasma to primarily secreted fluid; this would be diminished during enzyme inhibition (Table 7). The second is possible since Cl\(^-\) is an inhibitor of carbonic anhydrase, and thus there is a site on the protein for the anion (36, 38). The third could occur if an ATPase possibly involved in Cl\(^-\) transport were sensitive to HCO\(_3^-\) or pH. This appears the case for oxytomic cells of stomach and gall, tissues also rich in carbonic anhydrase (68).

Thus, there appears a general homology between the chemistry of secretion of CSF and aqueous humor, regardless of species. The scheme may be visualized in Fig. 7. Secretory cells engage in the protolysis of water; for this fundamental process there is some cellular property or locus that imposes direction. In this case OH\(^-\) is at the fluid side, where it reacts with CO\(_2\) to form HCO\(_3^-\), either with or without carbonic anhydrase. The enzyme, like the cell itself, is a OH\(^-\) separator or activator. Thus, HCO\(_3^-\) gradients are developed; the counterion for transport is sodium, with the energy derived from ATPase. The proton from water is buffered by hemoglobin in the blood. Sodium and HCO\(_3^-\) are intimately linked so that inhibition of either Na- K-ATPase or carbonic anhydrase produces similar effects (17) on Na\(^+\) transport and fluid formation in CSF. This process is quite general and the fundamental steps of protolysis of water, hydroxyl attack upon CO\(_2\), and proton buffering are common to all animal, and possibly plant, cells containing carbonic anhydrase, as well as some that carry out the process at the nonenzymic rate (37).

Relation of Present Findings to Chemical Control of Respiration by CSF

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 \(^{22}\)Na turnover from plasma to fluid were decreased to the same degree (40%) by acetazolamide (22).

The discrepancy between effects of acetazolamide on \(^{22}\)Na 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 \(^{22}\)Na \(k_{in}\) (Table 7). It appeared important to inquire into the problem, with attention to the meaning of the \(^{22}\)Na \(k_{in}\).

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 (\(C_{pl}\)) is maintained constant, and the concentration within the compartment (\(C_{in}\)) is meas-
ures 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, $k = k_{out} - k_{in}$. Integrating:

$$C_{in} = C_{out}(1 - e^{-kt})$$

(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 $C_{in} = 0$

$$C_{in} = C_{out}(1 - e^{-kt})$$

(8)

This is the same as equation 2, with $k = a/V$. 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 $^{22}Na$ in several species following acetazolamide, shown particularly well in ref. 18. In these circumstances, fluid formation is lowered by half ($t$), 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 new rate, 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 $^{22}Na$ 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 $HCO_3^-$, $a/V$ 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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