Table 2B shows that carbonic anhydrase inhibition decreases $Na^+$ and $Cl^-$ entry to CSF during hypercapnia. The small cation rise ($Na^+$ = 3 mM) is now accompanied by a small anion rise ($Cl^-$ + $HCO_3^-$ = 7.7 mM).

These data support the idea (Am. J. Physiol., in press 1972) that a significant amount of $Na^+$ transport into CSF is linked to $HCO_3^-$ formation. The precise nature of the $Cl^-$ effect is not yet known; either it is secondary to the transfer of $Na^+$ and $HCO_3^-$, or it represents a function of carbonic anhydrase (or closely related protein also inhibited by sulfonamides) in $Cl^-$ transport as such.

In this model utilizing hypercapnia, carbonic anhydrase inhibition decreases the transport of $Na^+$, $Cl^-$, and $HCO_3^-$ into the CSF. It is interesting to observe that in micro-puncture studies of the proximal tubule, inhibition also decreases the reabsorption of all three ions (Kunau, J. Clin. Invest., in press 1972).

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1971 #28

## MEASUREMENT OF CEREBROSPINAL FLUID VOLUME IN *S. acanthias* DURING CARBONIC ANHYDRASE INHIBITION

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In studying the relations between ion movements and fluid production of CSF (Maren, Bull. Mt. Desert Island Biological Lab., 9:33, 1969, and 10:44, 1970) knowledge of the volume of CSF of *S. acanthias* became important. Oppelt *et al.* (Comp. Biochem. Physiol., 17:857, 1966) showed that intravascular or intraventricular administration of a carbonic anhydrase inhibitor reduced CSF production rate by 28%. The effect of inhibition on CSF volume was not determined. We have now measured the volume of CSF before and after carbonic anhydrase inhibition. The method was the dilution of Blue Dextran 2000, a carbohydrate polymer of molecular weight about $2 \times 10^6$, which appears to have no affinity for tissue.

Fourteen male fish of 1-2 kg were removed from live cars, and a portion of the chondrocranium removed so that a thin layer of cartilage remained above the brain. The cerebellum was located visibly, 0.25 ml of CSF withdrawn and 0.25 ml of Blue Dextran (3000 $\mu$g/ml in shark Ringer) injected into the cerebellar ventricle. Half of the fish had been given 50 mg/kg i.v. of methazolamide, a powerful carbonic anhydrase inhibitor with a half life of 1.5 days, 7-8 hours before the dye was injected. The fish were returned to the live cars where they swam freely for 15 minutes. They were then killed, and all CSF was withdrawn. Following centrifugation, aliquots of the clear blue solution were measured into 3-4 volumes of shark Ringer solution and absorbance measured at 650 nm against a standard curve of Blue Dextran.

From dye dilution the volume (V) of the CSF in controls was (mean $\pm$ S.E.) 1.67 $\pm$ 0.16 ml. From fish pretreated with methazolamide V = 1.22 $\pm$ 0.04 ml. In each series, withdrawal of all CSF from the cavities yielded a volume about 0.3 ml less than measured with dye. Both the dye and

direct measurements show that carbonic anhydrase inhibition reduces the CSF volume by about 27%.

For V = 1.67 ml and production rate of 0.28 ml/hr (Oppelt, ibid.) the normal rate constant for CSF turnover is 0.17 $hr^{-1}$, whence the half life is 4.1 hours. By 8 hours 75% of the fluid has turned over.

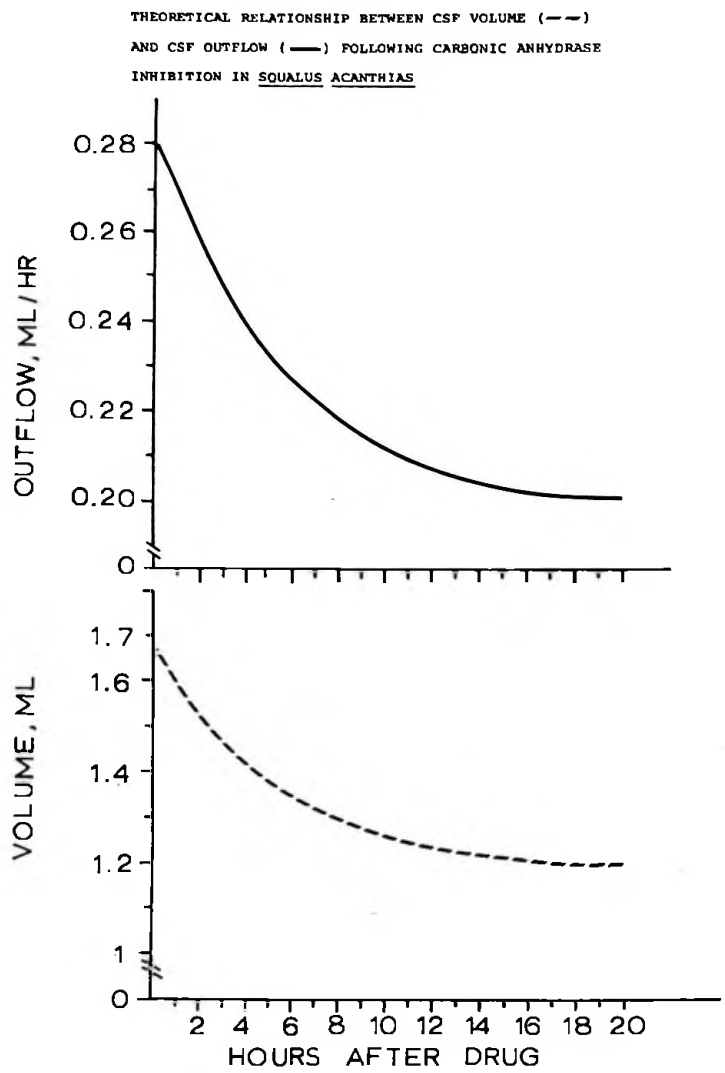


Figure 1

The figure shows the approach to the new inhibited steady state for both V and outflow of CSF. The model is constructed for hourly changes; of course the changes are occurring continuously. From Oppelt's (ibid.) data the formation rate of CSF during carbonic anhydrase inhibition is 0.20 ml/hr, or 0.08 ml/hr less than normal. At the end of the first hour V is then 1.67 - 0.08 = 1.59 ml, assuming that outflow during this time was at the normal rate, i.e. proportional to the volume at 0 time. The outflow for each successive hour was calculated by proportion to V at the start of that hour. Thus for the second hour $\frac{\text{outflow}}{1.59 \text{ ml}} = \frac{\text{normal outflow} = .28 \text{ ml/hr}}{\text{normal volume} = 1.67 \text{ ml}}$ whence outflow is 0.27 ml/hr.

Successive volumes, in turn, were calculated from the decrements generated by the difference

between outflow and the new production rate of 0.2 ml/hr: thus for V at time t $^{\nu}V_t = V_{t=t-1} + (0.2 - outflow_{t-1})$ where t–1 are the measurements for the previous hour and all units are ml.

The Figure shows that by theory the new inhibited steady state is achieved at about 16 hours. In this model V at 8 hours is 1.30 ml. We measured 1.22 ± .04 ml. Out data and this model thus furnish reliable evidence for flow and volume changes in CSF during carbonic anhydrase inhibition.

1971 #29

## HYPERCAPNIA DOES NOT ELEVATE $pCO_2$ IN THE AQUEOUS HUMOR OF *S. acanthias*

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Ten years ago (Comp. Biochem. Physiol., 5:201, 1962) one of us (T.H.M.) obtained the following type data 6 hours following carbonic anhydrase inhibition in the spiny dogfish, *Squalus acanthias* (Table 1).

TABLE 1. $CO_2$ Equilibria in CSF and Aqueous Humor During Carbonic Anhydrase Inhibition* (Maren, Comp. Biochem. Physiol., 5:201, 1962)

| | PLASMA | | | CSF | | | AQUEOUS | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | $pCO_2$ mm Hg | $HCO_3^-$ mM | pH | $pCO_2$ mm Hg | $HCO_3^-$ mM | pH | $pCO_2$ mm Hg | $HCO_3^-$ mM |
| Control | 7.56 | 6 | 7.6 | 7.66 | 5 | 8.3 | 7.63 | 6 | 8.3 |
| Inhibited | 7.47 | 11 | 11.5 | 7.58 | 12 | 16.3 | - | - | 9.6** |

* $pCO_2$ has been recalculated using $\alpha = 0.045$.

** Total $CO_2$

The three fluids have essentially the same composition in the normal untreated fish. Following 30 mg/kg acetazolamide, there were striking changes: (1) Respiratory acidosis in plasma due to inhibition of red cell carbonic anhydrase. (2) Large elevation of CSF $HCO_3^-$ *exceeding* that in plasma. This is now known to be due to formation of new $HCO_3^-$ from gaseous $CO_2$ at the site of CSF formation (Maren, Welliver and Istin, this journal, paper #27, and references cited). (3) But no change in aqueous humor total $CO_2$, so that its concentration is *less* than that of plasma.

The divergence between aqueous humor and CSF appears at odds with our current work (Am.