Further basis for the small or absent effect of methazolamide on CSF dynamics in S. acanthias (compared with the mammal) may be again the very large systemic respiratory acidosis induced in this species. This would tend to increase flow, since CSF HCO_3^- turnover is rapid, and responds to high pCO_2 even when carbonic anhydrase is inhibited (Maren and Kent, Bull. MDIBL, this issue). Thus we postulate that the systemic effect of high plasma pCO_2 drives the key reaction in choroid plexus (to CSF HCO_3^-, which directly or indirectly affects flow) past the enzymic inhibition in the tissue. High substrate thus replaces enzyme to yield adequate rates. There is an interesting precedent for this; Rawls (Bull. MDIBL 4, part 4, 58, 1962) could not alter rectal gland secretion in the intact dogfish by methazolamide, and advanced the same explanation. Palmer was then able to show that in vitro the rectal gland did respond (Bull. MDIBL 5, part 2, 32, 1966). An important experiment in the present context will be the effect of hypercapnia upon CSF flow, Cl^- and Na^+ accession.

In summary, the principal finding is documentation of comparative rates and rate constants for transfer of Na^+, Cl^- and HCO_3^- from plasma to CSF. The rate of Na^+ movement is matched by that of $Cl^- + HCO_3^-$. The large component of HCO_3^- transfer, arising from hydration of CO_2, makes it possible to visualize how inhibition of carbonic anhydrase can reduce CSF Na^+ accession and flow in the mammal. I thank Mr. Barry Dvorchik and Mr. Robert Woodworth for their careful work.

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1970 #28

THE EFFECT OF HYPERCAPNIA ON CEREBROSPINAL FLUID (CSF) HCO_3^- FORMATION IN S. acanthias

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From studies of the transfer of plasma $^{14}CO_2$ to CSF $H^{14}CO_3^-$ in both cat (Maren and Broder, J. Pharm. Exptl. Therap. 172:197-202, 1970) and dogfish (Maren et al, Bull. MDIBL 9:33, 1970) it was deduced that this process may play an important role in CSF formation and pH regulation. We have tested this directly, by the effect of raising plasma pCO_2 upon CSF HCO_3^-, and how this is altered by carbonic anhydrase inhibition.

Fish were taken from the live car at 0 time and arranged in the laboratory for perfusion of gills and blood sampling as described by Peirce and Kent (Bull. MDIBL 8:49, 1968). The brain was exposed through a 1-2 cm opening in the cranium, and CSF samples (50-100 μl) withdrawn through a 27 gauge needle. Fish could be maintained for many hours in this control situation with the development of some metabolic acidosis but no respiratory acidosis and no change in CSF HCO_3^- concentration.

At 30 to 60 minutes after the start of the procedure 5% CO_2 in oxygen was admitted to the seawater perfusate. Figure 1 shows one of five experiments of this type. Plasma showed a typical respiratory acidosis with a slow and moderate elevation of HCO_3^- concentration. CSF, on the other hand, showed a rapid and marked elevation of HCO_3^-, so that its pH was maintained at near-normal levels. At the end of the experiment, CSF HCO_3^- concentration had risen 4-fold, exactly matching the rise in pCO_2.

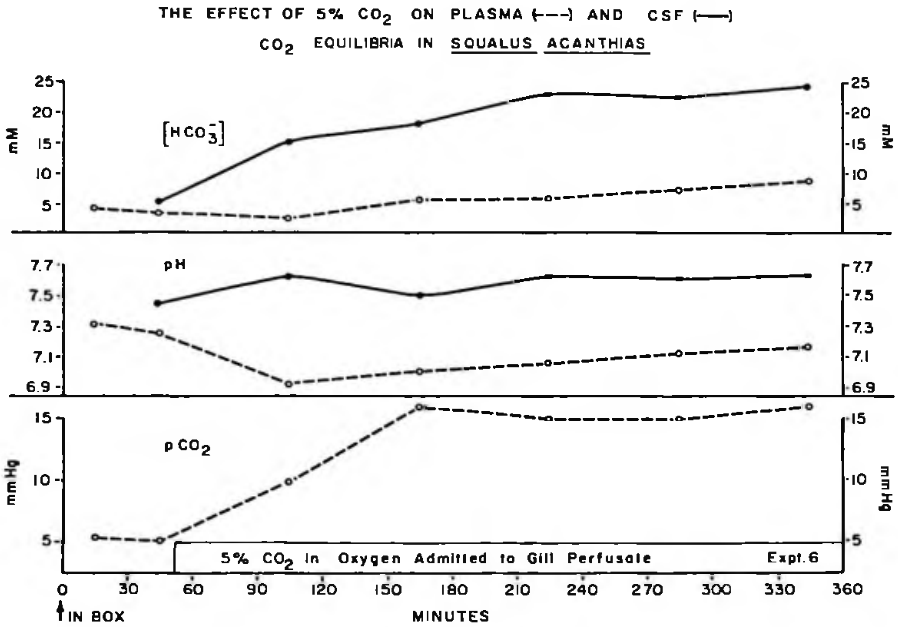

Figure 1. Showing the rise in CSF HCO_3^- following hypercapnis in dogfish. In 2 of the five experiments, including this, the rise in plasma pCO_2 was delayed.

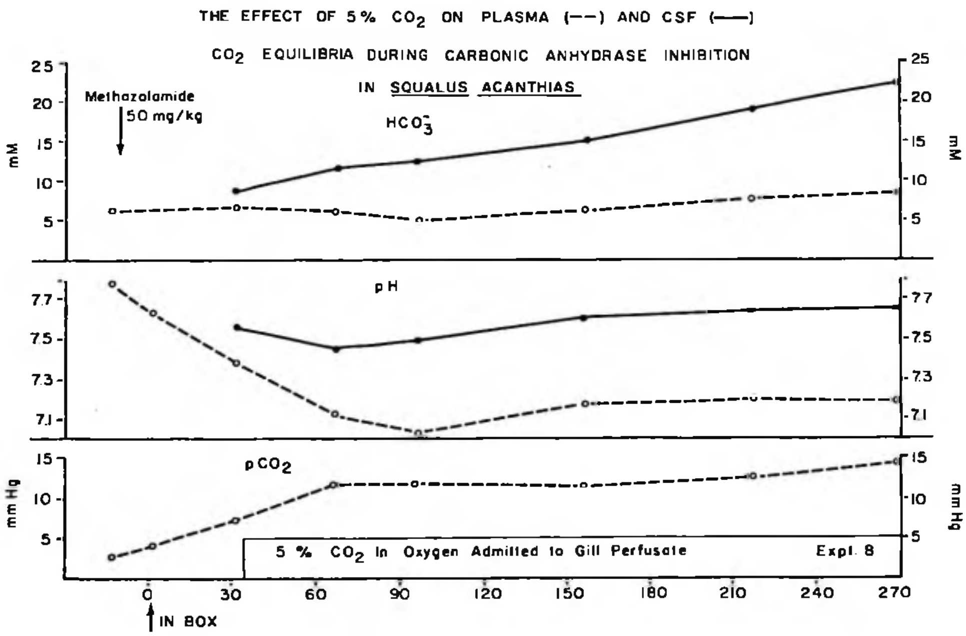

Figure 2. As Figure 1, but also showing electrolyte values from the fish while swimming freely (first point), followed by i.v. methazolamide. Fifteen minutes later the fish was put in the experimental box. Note the rise in pCO_2 due to inhibition of carbonic anhydrase, before CO_2 was given; but during CO_2 administration the pCO_2 was the same as that in Figure 1. The plasma half-life of methazolamide in this species is 1-2 days. This dose is 5X that needed for total physiological inhibition.

Figure 2 shows one of four experiments similar to that of Figure 1, except that carbonic anhydrase was totally inhibited throughout the procedure. Plasma pCO_2 was the same in the two situations, since it was dependent on the level in the ambient water. Results in Figure 2 were qualitatively like those in the uninhibited fish (Figure 1) but quantitatively different in that CSF HCO_3^- rose more slowly.

Figure 3 shows the mean data for plasma and CSF HCO_3^- in all nine experiments. Inhibition of carbonic anhydrase cuts the rate of HCO_3^- formation in CSF to half the normal value. This agrees with the effect of inhibition on the rate constant for transfer of plasma $^{14}CO_2$ to CSF $H^{14}CO_3^-$ (Bull. MDIBL 9:33, 1969).

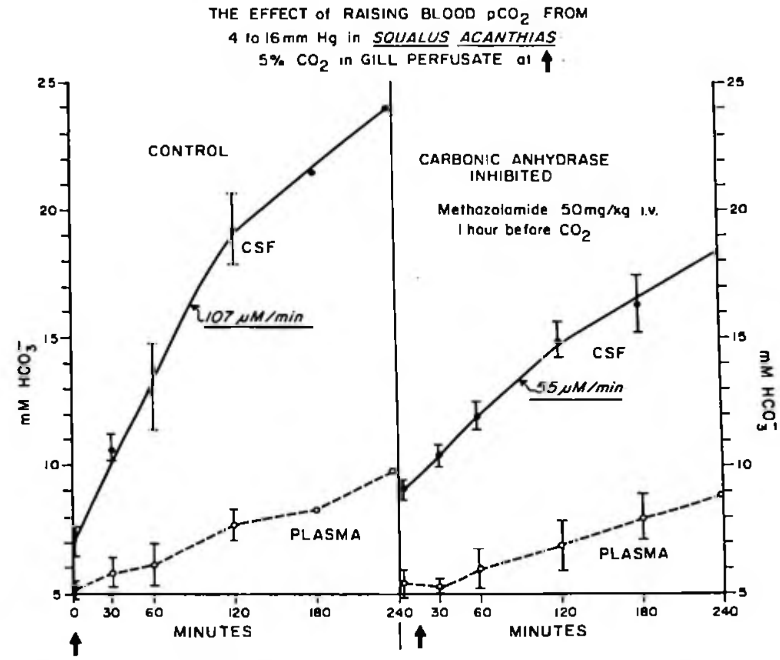

Figure 3. Showing mean data ± S.E. from all experiments, for plasma and CSF HCO_3^-. n = 3-6 except where no S.E. is shown, n = 2.

The data strongly support the idea that the choroid plexus and other secretory cells in brain containing carbonic anhydrase have the function of polarizing water to H^+ and OH^- ions. CO_2 reacts with the latter to form HCO_3^-, which diffuses or is secreted into CSF. This provides a rapid means of regulating CSF pH in the face of alterations in blood pCO_2, and may be the chemical means by which a close feedback control of respiration is achieved. We thank Mr. Robert Woodworth and Miss Marjorie Peirce for their excellent assistance.

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1970 #29

IN VIVO IONIC EXCHANGE THROUGH THE SKIN OF THE FROG <u>Rana clamitans</u>

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