between outflow and the new production rate of 0.2 ml/hr: thus for V at time t $V_t = V_t = _{t-1} + (0.2 - outflow_{t-1})$ 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 $1.22 \pm .04$ 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$_2$	HCO$_3^-$	pH	pCO$_2$	HCO$_3^-$	pH	pCO$_2$	HCO$_3^-$
		mm Hg	mM		mm Hg	mM		mm Hg	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 $\propto = 0.045$.

** 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.

J. Physiol., in press, 1972) which shows that the chemistry of HCO_3^- formation (and probably Na^+ and Cl^- transport) is the same in the two fluids. The possibility remained that the aqueous humor (and by inference the ciliary process) does not exhibit a raised pCO_2 in response to hypercapnia; this had not been measured in the experiments of Table 1.

Hypercapnia was induced by 5% CO_2 added to sea water perfusing the gills, in the same experiments as described in paper #27 above. Total CO_2 and pH were measured in plasma, CSF and aqueous humor at the same time. Table 2 shows clearly that during very large elevation of pCO_2

TABLE 2. Failure of Aqueous pCO_2 and HCO_3^- to Rise
During Hypercapnia in S. acanthias.

	PLASMA	CSF	AQUEOUS
pH	7.15	7.52	7.52
HCO_3^- mM	8.2	16.5	4.4
pCO_2 mm Hg	16	14	4

Hypercapnia was induced by 5% CO_2 in gill perfusate for 3 hours. Expt. 18, representative of n = 5.

pH and total CO_2 measured. HCO_3^- and pCO_2 from nomogram in following paper, #   .

in plasma and CSF, there is no change in the aqueous. The high pCO_2 increases HCO_3^- in CSF; there is no change in aqueous HCO_3^-. By analogy we may reason that in Table 1 the pH and pCO_2 of the aqueous were the same in inhibited as in control fish.

Since there is carbonic anhydrase in the ciliary process of *Squalus acanthias* and since acetazolamide acutely (2 hrs) lowers aqueous HCO_3^- concentration (Comp. Biochem. Physiol., 5:201, 1962), the system plasma $CO_2 \rightarrow$ aqueous HCO_3^- is at work here, as in all vertebrates. The present experiments show only that pCO_2 cannot be elevated in the ocular fluid in this species.

It is unlikely that CO_2 fails to diffuse into the eye at the elevated pCO_2 of arterial blood. More likely the gas diffuses readily out through the cornea. In the experiments of Table 1 the fish were freely swimming; in that of Table 2 the gills were perfused with cold sea water but the eyes were exposed to the air.

Whether the mammalian eye fails to respond to hypercapnia in this way remains to be clarified. Newman (Trans. Assoc. Am. Physicians, 71:85, 1958) demonstrated the rapid movement of CO_2 inward or outward across the human cornea. However, Langham (Brit. J. Ophthalmol., 41:65, 1957) showed that the aqueous maintained a high pCO_2 of 64 mm Hg after acetazolamide was given to rabbits with ligation of renal arteries. Here the effect of enzyme inhibition locally and hypercapnia were not dissociated. Fatt and Bieber (Expt. Eye Res., 7:103, 1968) showed the kinetics of CO_2 diffusion from the cornea, and that the rate of CO_2 production is not much greater than the rate at which the gas can diffuse into the open air. It may follow then that the pCO_2 of

the eye can be maintained at normal (=normal body pCO_2) by metabolic CO_2 production, but when body pCO_2 is elevated, diffusion from the cornea is adequate to bring aqueous pCO_2 to the normal value.

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A NOMOGRAM FOR CO_2 EQUILIBRIA IN COLD WATER ELASMOBRANCHS

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Nomograms for CO_2 equilibria in mammalian physiology texts or monographs cannot be used for most fish work, because they do not extend to low pCO_2 and HCO_3^- values, and are based on CO_2 solubility at 37° in plasma of about 300 mOsm.