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PILOT STUDIES ON THE RATE OF HCO_3^- FORMATION IN CSF IN S. acanthias: ROLE OF CARBONIC ANHYDRASE

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An unsolved problem in cerebrospinal fluid (CSF) physiology is the means whereby CO_2 and H^+ equilibria are maintained in the CSF, and as corollary, the role of carbonic anhydrase in the choroid plexus. As one model for such a chemical system, the accumulation of $H^{14}CO_3^-$ in the aqueous humor of the rabbit was studied by Kinsey and Reddy (see analysis of their experiment in Phys. Rev. 47:595, 1967). It was clear that excess HCO_3^- and low $[H^+]$ in this fluid was brought about by hydration of CO_2, catalyzed by carbonic anhydrase in the ciliary process.

Although the choroid plexus of all vertebrates contains carbonic anhydrase, CSF does not have excess HCO_3^-, and the effect of inhibiting carbonic anhydrase is generally to raise CSF HCO_3^- over that in plasma, an effect particularly striking in Squalus acanthias (Comp. Biochem. & Physiol. 5:201, 1962).

S. acanthias was placed in a box, and the spiracles perfused with sea water, cooled and circulated as previously described (Comp. Biochem. & Physiol. 26:853, 1968). The brain was exposed, so that CSF could be sampled at intervals through a small needle, and the dorsal aorta was cannulated.

In three control fish, $NaH^{14}CO_3$ was injected at 0 time, and CSF and plasma were sampled at 5, 12, 20 and 60 minutes. In a given fish, only 2 CSF samples were taken. Radioactive carbon was measured by liquid scintillation, and CO_2 and pH of the fluids were measured. Three other fish were treated the same way, except that they received 30 mg/kg i.v. of acetazolamide at 30 minutes before injection of labeled $NaHCO_3$. In this interval they swam freely in the live-car.

Plasma and CSF HCO_3^- was constant throughout the experiment (about 8 mM) in both control and acetazolamide treated fish. pH was relatively constant (about 7.2); the acidosis doubtless reflecting some respiratory underfunction due to the abnormal environment. The specific activity of the plasma declined about 6-fold from 5 to 60 minutes after injection of the label in both control and treated groups, reflecting distribution and gill excretion. Table 1 gives data from a control fish and 2 treated, for the initial period, when CSF label was increasing. In each case the carbon counts at five minutes in plasma are normalized to 100, and other counts are relative to this.

In treating these data, the assumption is made that gaseous CO_2 passes rapidly and freely from plasma to CSF. We ask the question whether total CO_2 in CSF (largely HCO_3^-) is formed from CO_2, either catalyzed or uncatalyzed, or arrives there by another process, such as slow diffusion of HCO_3^-. There are several approaches to this: First we can compare the rates of HCO_3^- accumulation in CSF with known hydration rates of CO_2; second we study the effect of carbonic anhydrase inhibition on the rates.

Table 1 gives the type of data used for these analyses, although not enough experiments have been done for more than a tentative conclusion. The counts have the dimension of concentration units, so are suitable for insertion in rate equations. From the pH of 7.2 and the total counts in plasma at 5 minutes set at 100 the counts (or relative concentration units) of CO_2 gas

Table 1

RELATIVE COUNTS OF TOTAL $^{14}CO_2$ IN THE DOGFISH

	Plasma		CSF	
	5 min	12 min	5 min	12 min
Control	100	37	20	40
Treated*	100	45	6	11
	100	56	18	29

*30 mg/kg acetazolamide i.v. 30 minutes before injection of $NaH^{14}CO_3$.

are obtained; at this time the value is 8. This number can be entered as the substrate (S) in the rate equation for the uncatalyzed reaction

$$V_{unc.} = (S)\ k_1$$

where k_1 is the hydration rate constant at 14° C, 0.7 min^{-1}. Thus the uncatalyzed rate would provide the accumulation of 5.6 units per minute. We find from the table that the control rate (from 5 to 12 minutes) was 3 units per minute; treated fish were less but the same order of magnitude. Thus there appeared no catalytic component; this would, if present, make the HCO_3^- accumulation instantaneous, as in the eye (Kinsey and Reddy, vide supra). The fact that the physiological rates are comparable to the noncatalytic suggests that we are seeing an inevitable chemical process—akin to CO_2 being generated into a buffer—and not one of importance in physiological regulation. The non-participation of carbonic anhydrase in this process is borne out by the fact that the rates in the inhibited fish show no great (although there is some) difference from the controls. The two treated fish show variability and clearly more experiments are needed. Tentatively, the data suggest that carbonic anhydrase has a different role in CSF chemistry—perhaps associated with Cl^- or H^+ secretion, as in the stomach and salt gland (Phys. Rev. 47:595, 1967).

In conclusion, it appears that the rates of $H^{14}CO_3^-$ appearance (and those of other isotopes) in CSF can readily be studied in S. acanthias, over a brief period of time. Data thus far point away from a primary process involving plasma $CO_2 \rightarrow$ CSF HCO_3^-, since carbonic anhydrase is not involved in the reaction.

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CONDITIONS OF INHIBITION OF MORPHOGENESIS AND MACROMOLECULAR SYNTHESES IN *Fundulus heteroclitus* EMBRYOS

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The determination of conditions under which embryogenesis is altered or inhibited has long been useful in the search to understand the normal process. Preliminary studies on certain in-