centrations of about 20 μg/ml, while 50-100 μg/ml is required for MS 222.

Dr. T. F. Muther of this department had independent evidence that neither MS 222 nor IBA have central action, but that "anesthesia" is due to blockade of peripheral neurotransmission or neuromuscular junction or both. He stimulated the posterior part of the spinal column of goldfish electrically. There was a correlation between the decrease in tail reflex contractions and depth of anesthesia as measured by rate of opercular movements.

It is of passing interest that MS 222 has been regarded as general central anesthetic for cold-blooded animals for 50 years. There appears to be a serious information and communication gap between warm-blooded investigators and the poikilotherms.

I thank Mr. Robert Welliver and Mr. Robert Woodworth for excellent assistance.

1969 #23

TRANSFER RATES OF CO_2 AND CL^- FROM PLASMA TO CEREBROSPINAL FLUID (CSF) IN Squalus acanthias: EFFECT OF CARBONIC ANHYDRASE INHIBITION

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We wished to find the rate at which the major anions are transferred from plasma to CSF. $NaH^{14}CO_3$ and $Na^{36}Cl$ were injected into the caudal artery, and the accumulation of label in the cerebellar ventricle of the dogfish was measured. Cold concentrations of these anions were also monitored, along with appropriate measurements of anions in the plasma. Carbonic anhydrase was inhibited by injecting acetazolamide (30 mg/kg) intravascularly 15-30 minutes before injection of the label. Enzyme in choroid plexus, as well as in gill and red cells, will be completely inhibited by this procedure (Physiol. Rev. 47:595, 1967).

CO_2. Two different protocols were used. (1) Fish in the box, with continuous perfusion of the gills (Comp. Biochem. Physiol. 26:853, 1968) and small opening in the skull, through which CSF could be sampled at intervals. (2) Fish in 25 gallon tank of fresh sea water, freely swimming. CSF was sampled 6 minutes after injection of label. The usual dose of carbon label was 40 μcuries.

1. Table 1 gives data from the box experiment. The concentration of labeled CO_2 (all forms) in the three minute control (untreated) plasma sample is set as 100, and all other counts are relative to this. Up to 12 minutes CO_2 disappearance rate from plasma is like that in the free swimming fish (see below) and in accord with the rate of metabolism and volumes of fluid recorded by Robin and Murdaugh (Sharks, Skates and Rays, ed. by Gilbert et al, Baltimore: Johns Hopkins Press, 1967). In the acetazolamide-treated animals, this rate is unchanged, but there is an initial retention of the label, leading to higher total CO_2 counts; also there is a higher proportion of gaseous CO_2. This leads to a much higher concentration of labeled gaseous CO_2 than controls (last column). This is the expected effect of carbonic anhydrase inhibition in this species (Comp. Biochem. Physiol. 5:201, 1962).

The uptake of total CO_2 into the CSF was roughly linear for the first 12 minutes of the experiment, when it reached the level in the plasma. To analyze the role of carbonic anhydrase in CO_2 transport the rates of transfer from plasma to CSF in the control and acetazolamide treated animals were compared. In each case, these rates were related to the concentration of gaseous

Table 1

UPTAKE OF $^{14}CO_2$ FROM PLASMA TO CSF IN S. acanthias FOLLOWING INJECTION OF $NaH^{14}CO_3$ AT 0 TIME

Box Experiments

	Counts relative to control plasma at 3 min = 100			Acid-base at 3-6 min			
	Concentration units			pH	CO_2 mM	p CO_2 mm Hg	Counts gaseous CO_2
	3 min	6 min	12 min				
Controls							
Plasma	100	60	41	7.27	5.2	7	6.8
CSF	12	27	38	-	9.2	-	-
Acetazolamide at -30 min							
Plasma	255	136	94	7.12	6.4	12	24.3
CSF	20	44	89	-	10.4	-	-

n = 8 for each group.

CO_2 in the plasma, on the assumption that this species moves very much more rapidly than CHO_3^- and that hydration of this species to HCO_3^- at the choroid plexus is responsible for the accumulation of total CO_2 in CSF.

Table 1 shows that for the control fish in the first six minutes, 27 concentration units of total CO_2 appeared in the CSF, or 4.5 units per minute. In the same period, the rate in the inhibited fish was 7.3 units per minute. These rates are divided by the appropriate concentration of labeled gaseous CO_2 at the three minute (mid-point) time to yield a rate constant for uptake: For the control the value is 0.67 min^{-1} and for the inhibited (uncatalyzed) it is 0.30 min^{-1}.

2. Table 2 gives data for the free-swimming fish. It will be noted that the acid-base equilibria as well as the uptake rates of CO_2 into CSF agree very well with those of Table 1, indicating that metabolism of the fish, for at least 6 and probably 12 minutes in the box, is reasonably normal. In the free-swimming fish, we also studied the disappearance of the carbon label for 60 minutes with the following results: the label became rapidly diluted about 20 fold during the first 20 minutes, indicating its transfer from plasma to total body water. Thereafter it decays with a half life of about 30 minutes, which is close to the maximal rate for diffusion of a substance across the gill (Bull. MDIBL 7:51, 1967).

In the free-swimming fish, the rate constant for uptake of CO_2 from plasma to CSF in the controls was 0.92 min^{-1}, and in the inhibited 0.32 min^{-1}.

These rate constants indicate the participation of carbonic anhydrase in the reaction. It is also of interest to compare them with the first order rate constant for the hydration of CO_2 at 14.2 C, which is 0.68 min^{-1} (J. Pharm. Expt. Ther. 139:129, 1963). This represents the rate in an indeal chemical system, without enzyme. Our inhibited rate in the fish is about half of this, which seems to be reasonably close, considering the very large differences in chemical and physiological systems. Our mean control rate is somewhat more than this, with a considerable range, so that in some fish the rates were about three times greater than the theoretical maxi-

Table 2

UPTAKE OF $^{14}CO_2$ FROM PLASMA TO CSF IN S. acanthias FOLLOWING INJECTION OF $NaH^{14}CO_3$ AT 0 TIME

Free Swimming Fish

	Counts relative to control plasma at 3 min = 100		Acid-base at 3-6 min			
	Concentration units		pH	CO_2 mM	p CO_2 mm Hg	Counts gaseous CO_2
	3 min	6 min				
Controls						
Plasma	100	64	7.30	5.3	6	6.7
CSF	-	37	-	7.5	-	-
Acetazolamide at -30 min						
Plasma	297	168	7.15	6.9	13	27
CSF	-	52	-	11.3	-	-

n = 5 for each group.

mum for the uncatalyzed reaction; thus the enzyme appears necessary to sustain physiological rates.

The thirteen pairs of control and inhibited fish (of Tables 1 and 2) were analyzed for significance. The variation of means for control fish was ±19%; for inhibited was ±11%. P was between 0.05 and 0.01. The conclusion that acetazolamide has an effect is different from our preliminary and very tentative observations of last year (Bull. MDIBL 8:12, 1968).

Chloride. The entrance of Cl^- into CSF was far too slow to allow study in the box or tank as described above; experiments were done in the live car. 20 μcuries of $^{36}Cl^-$ were injected, and the plasma concentration in controls 12 minutes later was taken as 100. At six hours this value was 41. The initial isotope concentration in the inhibited fish was slightly higher: the decay rate was the same. The higher levels are possibly due to inhibition of ^-Cl transport by gill (discussed in Physiol. Rev. 47:595, 1967).

These decay curves included at least two slopes, which probably reflects slow distribution of the anion more than excretion. From Burger's (Physiol. Zoology 35:205, 1962 and Comp. Biochem. Physiol. 19:649, 1966) data we may calculate that the half-time of chloride in this species is about 60 hours, about 1/4 of this being excreted each by the kidney and gill, and 1/2 by the rectal gland.

Access of Cl^- from plasma to CSF had an approximate half-time of 3 hours in both control and treated fish. The first order rate constant is thus 0.004 min^{-1}, or several hundred fold slower than that for the HCO_3^- - CO_2 system. Table 3 shows an analysis of the system at six hours, which yields the suggestion, but not proof, that Cl^- transport may also be dependent upon carbonic anhydrase. These data confirm our earlier studies which showed that the concentration of Cl^- in CSF is reduced by acetazolamide (Comp. Biochem. Physiol. 5:201, 1962), and show small changes in the rate of uptake of the labeled ion. It may also be mentioned that the effect of

Table 3

UPTAKE OF $^{36}Cl^-$ FROM PLASMA TO CSF. PLASMA COUNTS AT 12 MINUTES IN CONTROLS SET AT 100.

	Concentration units						Cl^- conc. (mM)	
	12 min	30 min	1 hr	2 hr	3 hr	6 hr*	0 time	3-6 hrs†
Controls								
Plasma (n = 6)	100	81	68	58	52	41	239	246
CSF (n)	4	10	-	14	20	39	-	263
	(2)	(1)		(2)	(1)	(6)		
Acetazolamide at -30 min								
Plasma (n = 6)	120	102	85	68	60	48	240	244
CSF (n)	6	12	-	23	34	31	-	253
	(2)	(1)		(1)	(1)	(6)	(n = 9)	

*The difference in plasma/CSF counts between control and treated fish at 6 hours is significant at the level of P between 0.05 and 0.1.

†The difference in CSF Cl^- concentration between control and treated fish is significant at $P < 0.05$.

acetazolamide in decreasing CSF flow in this species is relatively small (Oppelt, et al, Comp. Biochem. Physiol. 17:857, 1966).

We conclude that the carbonic anhydrase system is involved in the rapid accumulation of HCO_3^- ion in the CSF; the physiological significance of this is not certain, but a reasonable possibility is its role in the control of respiration (cf. Pappenheimer et al, Am. J. Physiol. 208:436, 1965). A role of this enzyme in Cl^- accumulation is also suggested. It is the reduction of ionic movement by acetazolamide that is responsible for the decrease in CSF flow.

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1969 #24

EFFECT OF A DILUTE ENVIRONMENT ON CEREBRO-SPINAL FLUID AND BRAIN ELECTROLYTES IN THE ELASMOBRANCH, Squalus acanthias

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Elasmobranchs are capable of surviving over a broad range of osmotic environments. Short term experiments have shown that the lemon shark and the marine skate are capable of survival in dilute sea water. No studies are available concerning the impact of a dilute environment on the electrolyte metabolism of the cerebro-spinal fluid or of the brain. Such data should be important since maintenance of a normal electrolyte environment in the brain would be critical for survival of the animal. Accordingly, the effect of a dilute environment on steady state concentrations of sodium, potassium and chloride in the cerebro-spinal fluid and brain and of water content of the brain in marine elasmobranchs was studied.