1969). Instead, water absorption increased to approximately sea water adapted levels after 3 days in sea water (Table 1). This increase in water absorption was not accompanied by an increase in Na-K-ATPase level of gut mucosa which was not significantly different in eels from fresh water (0.69 ± 0.07 μMoles P_i/mg protein · hr, n = 5 at 15° C) and after 3 days in sea water (0.72 ± 0.12, n = 5) but was significantly higher in eels adapted to sea water for 2 weeks (1.44 ± 0.07, n = 5, $p < 0.001$). Water absorption by intestines from sea water adapted eels was inhibited 51% by 10^{-4}M 2,4-dinitrophenol (DNP) (Table 1). When fresh water adapted eels were injected with cortisol (400 μgm/100 gm · day of cortisol hemisuccinate) for 3 days, gut water absorption was not significantly different ($p < 0.001$) from that in sea water adapted eels (Table 2).

Table 2

EFFECT OF HYDROCORTISONE INJECTION ON WATER ABSORPTION BY INTESTINAL SACS FROM FRESHWATER EELS

Days of hydrocortisone injection	0	1-1/2	3	10
Water absorption (μl/gm.hr.)	85 ± 14 (15)	250 ± 58 (10)	357 ± 85 (7)	363 ± 62 (8)

Although most of the increase in water absorption following sea water adaptation was ouabain sensitive, a large component of water absorption was not ouabain or diamox sensitive. An increase equal to the increase in water absorption during sea water adaptation can be produced in 3 days in fresh water adapted eels by hydrocortisone injection. Although Na-K-ATPase may be important in water absorption, gut water absorption can increase markedly without increase in Na-K-ATPase and in sea water adapted eels only 50% of water absorption was inhibited by 2,4-dinitrophenol which would eliminate ATP as a substrate for Na-K-ATPase. Water absorption by eel intestine appears to be linked to more than one salt absorptive mechanism but further studies which directly measure salt transport are required in order to define these processes more accurately.

This work was supported by National Institutes of Health Grant AM 09975.

1970 #27

RATES OF SODIUM, CHLORIDE, AND BICARBONATE ACCESSION TO CEREBROSPINAL FLUID (CSF) IN Squalus acanthias

Thomas H. Maren, Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Fla.

This study is part of a general program concerned with the relations among ion movements fluid production, and physiological role of CSF. In the accompanying paper (Report #28) the movement of CO_2 between plasma and CSF has been studied and its physiological role suggested. In this paper the rates of radioactive sodium and chloride loss from plasma and entry into CSF we studied, followed by an analysis of the effect of carbonic anhydrase inhibition on these processe The data are the first to show the quantitative relations for CSF entry among the three major

ions, Na^+, Cl^-, and HCO_3^-, in the same species.

^{22}Na or ^{36}Cl (10 μcuries and 20 μcuries respectively) was injected intravenously into dogfish. Plasma was sampled 18 minutes later, and this concentration of isotope was set as 100. Fish swam in the live car, and plasma and CSF were sampled at the times noted in the Figures. After 18 minutes, only one sample of each fluid was taken from each fish. The numbers of fish at each point are shown in the Figures, along with the relative concentrations of isotopes and their standard errors. A total of 113 fish were used, including some from the past year's study of chloride metabolism (Bull. MDIBL 9:33, 1969). In about half of the fish a large dose of methazolamide (30 mg/kg i.v.) was injected 30 minutes before the isotope, totally inhibiting carbonic anhydrase for the duration of the experiment.

Sodium (Figure 1). The decay in plasma yielded a half-life of about 11.5 hours. Carbonic

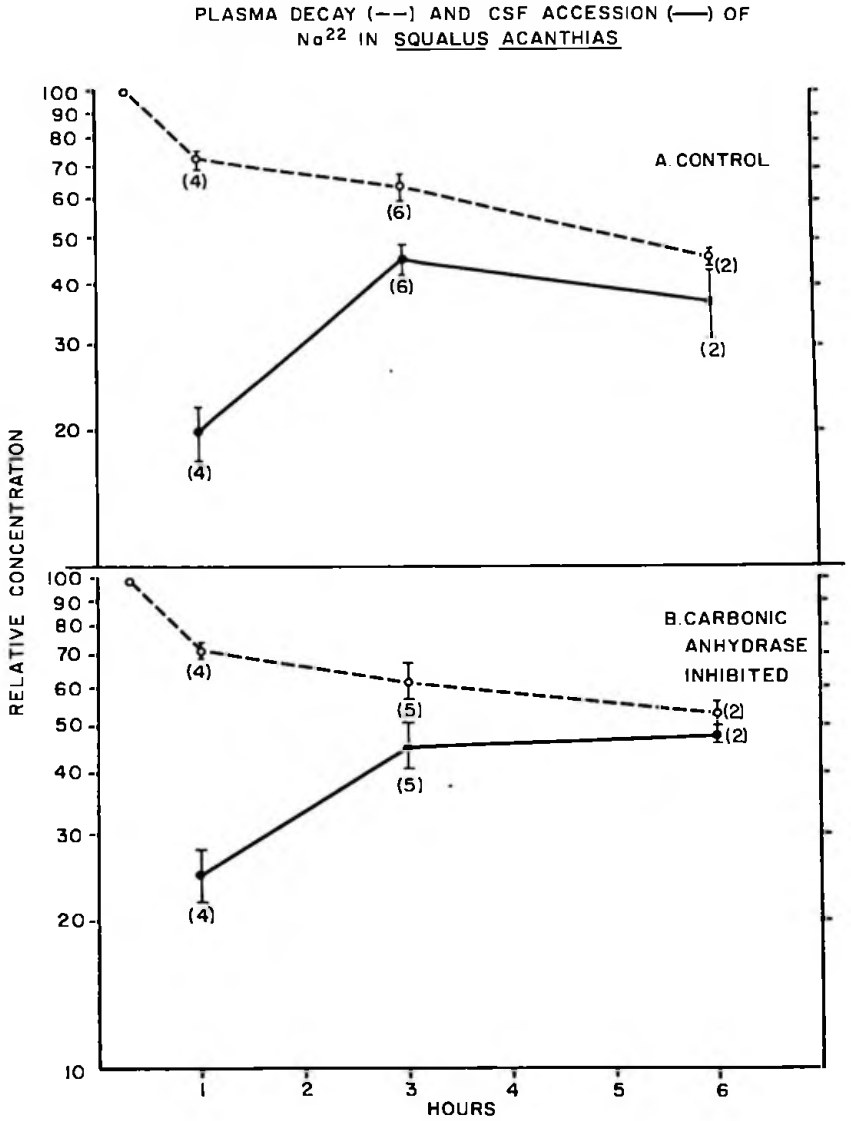

Figure 1.

anhydrase inhibition did not alter this time. Note that the precise value of the plasma half-life is not certain; more points are needed at 6 hours and beyond. But it is certain that the 1-3 hour figures for plasma ^{22}Na are identical for control and inhibited fish.

The rates of accession of sodium from plasma to CSF were calculated as apparent rate constants (k_{in}) for the periods 0-1 and 1-3 hours after injection of isotope, as follows, with data taken from Figure 1.

$$k_{in} = \frac{\text{rate of change of concentration in CSF}}{\text{mid point plasma concentration}}$$

Mean k_{in} values for these two periods for control (A) and inhibited (B) fish were between 0.15 and 0.28 $hour^{-1}$, and were not different between (A) and (B). The overall mean was 0.21 $hour^{-1}$. This yields a mean half time (t 1/2) of 3.3 hours for sodium to reach concentration in CSF equal to plasma, calculated from $t\ 1/2 = \frac{\ln 2}{k_{in}}$. There are inadequate data for rate analysis at 6 hours.

Chloride (Figure 2). The plasma decay in control fish (A) yielded a half-life of 11.5 hours when the 4, 5 and 6 hour points were used. It will be noted that these are precisely linear. If a fit is attempted from 2-6 hours, plasma half-life = 9.5 hours. When carbonic anhydrase is inhibited, these half-lives drop slightly, to 9 and 7 hours respectively. The significance of this faster chloride decay is not certain, either statistically or physiologically; but it might reflect HCO_3^- retention (Maren, Comp. Biochem. & Physiol. 5:201, 1962). Figure 4 of this earlier study also shows a small drop (4-7 mM) in plasma [Cl^-] 2-6 hours after carbonic anhydrase inhibition.

Figure 2 shows that plasma ^{36}Cl concentrations from 1/2 to 2 hours are essentially identical between control and inhibited fish, simplifying study of accession of the isotope to CSF. Using the equation above for k_{in}, the values for ^{36}Cl were calculated from 0-1 and 1-3 hours for both control (A) and inhibited (B) fish. The four values fell between 0.150 - 0.167 hr^{-1}, with mean of 0.158 and no significant difference between (A) and (B). The mean t 1/2 = 4.4 hours.

Figure 2 shows a small effect of inhibition at the 6 hour time, when the ^{36}Cl concentration (B) is 13% lower than control (A). This agrees well with our finding of a fall of 28 mM (from 264 mM) in CSF Cl^- 6 hours after acetazolamide. As in the present experiments, no effect was observed at 2 hours (Comp. Biochem. Physiol. 5:201, 1962).

Relation of Findings to CSF Chemistry and Production. The 34% more rapid rate of ^{22}Na entrance than ^{36}Cl can be explained on the basis of the very rapid entry of HCO_3^-, which reaches equilibrium in CSF from plasma with a t 1/2 of 10 minutes (calculated as above from Tables 1 and 2 in Maren et al, Bull. MDIBL 9:33, 1969). This is 20 times faster than the ^{22}Na rate, but the Na^+ concentration in plasma or CSF is about 43 times that of HCO_3^-. Clearly the sodium entry is shared between Cl^- and HCO_3^-. Although a full quantitative balance is not possible in the absence of rates for K^+ and possibly of other ions, the last column of Table 1 shows the rates of accession of the major ions as they enter the CSF. The only assumption here is that the entering fluid has the composition of plasma. There is remarkably good agreement between Na^+ and (Cl^- + HCO_3^-).

The t 1/2 for Cl^- closely approximates that for formation of CSF itself. Using 4 μl per minute for a 4-5 kg dogfish (Oppelt, Patlak, Zubrod, and Rall, Comp. Biochem. Physiol. 12:171, 1964), we can calculate the turnover if we know the CSF volume accurately. Oppelt's value of 4-5 ml (yielding half-time renewal rate of 10 hours) appeared too high, and we, with Dr. Joseph Fenstermacher, re-investigated this by carefully withdrawing all the fluid from all cavities in

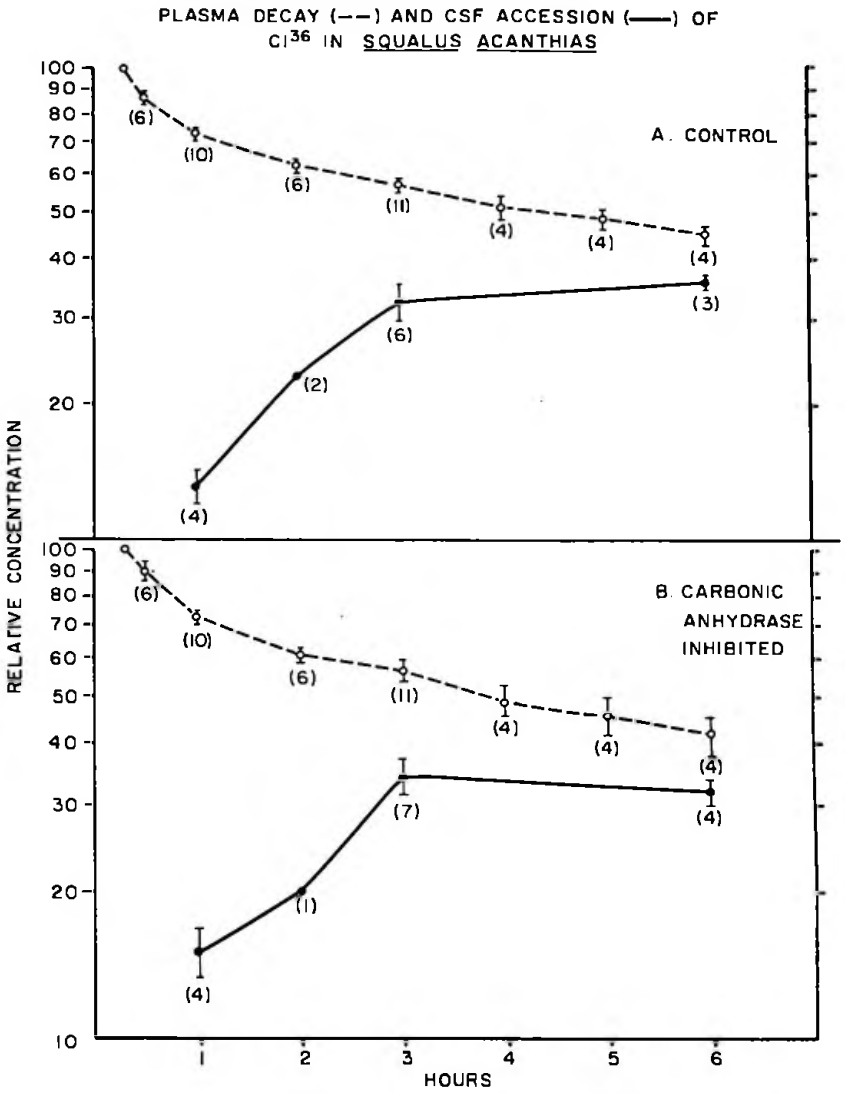

Figure 2.

the brain. We obtained 1-2 ml on each of 6 fish tested. Dr. Fenstermacher obtained a mean value of 1.1 ml on 5 fish of 1.1 - 1.6 kg, and 1.8 ml on 3 fish of 3.7 - 6 kg. Assuming that this slightly underestimates the value, we used 2 ml, yielding $\frac{240 \text{ l/hr}}{2000 \ \mu\text{l}} = .12 \text{ hr}^{-1}$ for the formation rate constant. The half time for fluid renewal is then 4.2 hours which may be compared to the 5 1/2 for chloride accession of 4.4 hours. These relations are summarized in Table 1.

Effect of Carbonic Anhydrase Inhibitors. Figures 1 and 2 and the calculated rate constants for 0-3 hours show no discernible effect of methazolamide upon the accession of sodium or chloride ion to CSF. The dynamics of the system are such that if there were a notable effect on the production of CSF, this would be reflected in the transfer of these ions. At the one hour time, for example, the volume normally moved into (and out of) the CSF is small (240 μl) compared to the total volume (2 ml). Thus any change in ion entry would be reflected in the concentration

Table 1

RATES OF ACCESSION OF IONS AND FLUID TO CSF

	Plasma	CSF	t 1/2 Plasma to CSF	k_{in}	Plasma conc. x k_{in}
	mM		Hours	Hr^{-1}	mM/hr
Na^+	255	271	2.2	.210	54
K^+	4	4	unknown	-	-
Cl^-	239	264	4.4	.158	38
HCO_3^-	5-7	6-9	0.17	4.1	24
Fluid	-	-	4.2	.12	-

Text gives calculations.

of isotope in CSF. Similarly, any change in rate of fluid formation (assuming that composition of formed fluid is reasonably fixed at the levels of plasma and CSF) would be reflected in the isotope data. That is precisely what is found in mammalian experiments, in which carbonic anhydrase inhibition reduces both flow and ^{22}Na entry about 50% (Davson, Physiology of the Cerebrospinal Fluid, p. 134, Little Brown, 1967).

The only evidence for drug effect in the present data is the small (13%) reduction in ^{36}Cl concentration at the six hour time, as compared to controls. Although this is not statistically significant, its importance is magnified by the fact that we had obtained the same effect (11% fall, $n = 17$, $P < 0.01$) for cold chloride, as described above. There is insufficient data for sodium to analyze this small a change. If the primary drug effect were in the rate of the secreted fluid (with constant composition), we should expect an early difference in CSF concentration of isotope between control and treated fish, disappearing later as old CSF was replaced with new. Just the opposite is found, suggesting that during inhibition new fluid has a slightly lower chloride concentration, a fact which becomes manifest in sampled CSF when most of the fluid has turned over. It should also be recognized that a primary change in fluid formation rate of 10% could not be detected. The reason for the decrease in chloride concentration at six hours following methazolamide or acetazolamide is most probably the concomitant rise in CSF HCO_3^- which follows carbonic anhydrase inhibition due to the very substantial (3-fold) elevation of plasma pCO_2 in this species (Comp. Biochem. Physiol. 5:201, 1962). There may also be some small effect on flow itself contributing to the chloride effect, which could not be detected in the present experiments. Oppelt et al had noted, in three fish of this species, a reduction in flow (measured by venticulo-cisternal perfusion) of 14-41% following i.v. acetazolamide (Comp. Biochem. Physiol. 12:171, 1964).

We may inquire why there is little effect of methazolamide on CSF flow or sodium or chloride compared to the situation in the mammal. There is carbonic anhydrase in choroid plexus of these fish (Comp. Biochem. Physiol. 5:201, 1962); whether there is large dependency on enzyme for normal flow is doubtful since Oppelt, Adamson, Zubrod, and Rall (Comp. Biochem. Physiol. 17:857, 1966) found that intraventricular acetazolamide only reduced flow 28% (range 40-43%, $n = 3$) compared with about 50% for the rabbit by the same technique (Pollay and Davson, Brain 86:137, 1963). These experiments are cited because they avoid systemic effects.

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.

Support was from Grant GM 16932-02 from the National Institutes of Health.

1970 #28

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

Thomas H. Maren and Barbara B. Kent, Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Fla., and Emory University, Atlanta, Ga.

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.