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THE RATES OF ACCESSION OF SODIUM, CHLORIDE, AND HCO_3^- TO AQUEOUS HUMOR IN *Squalus acanthias*

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An unresolved problem in aqueous humor dynamics in mammals has been the role of the various ions in the genesis of fluid production. Attention has been focused on which anion is in excess in the posterior chamber (HCO_3^- in rabbit, Cl^- in primate, both in dog), leading to speculations (unfounded, we believe) that the fundamental process may be different in the different species. In an attempt to resolve this problem we have studied the transfer of the major ions from plasma to aqueous humor in a typical "primitive" vertebrate, *S. acanthias*. Comparison is afforded with similar data obtained by Kinsey in the rabbit (in Membranes and Ion Transport, Vol. 3, ed. E.E. Bittar, Wiley, 1971).

A general account of the electrolyte physiology of the eye and techniques of sampling in *S. acanthias* are available from Maren (Comp. Biochem. Physiol., Vol. 5, 193, 1962) who also reported the presence of sulfonamide-sensitive carbonic anhydrase in the ciliary body. Jampol and Forrest (Expt. Eye Res., Vol. 13, 315, 1972) provided important anatomical details of the eye of *S. acanthias* and found Na^+-K^+ATPase in the ciliary body.

Results of our preliminary experiments (Expt. Eye Res., Vol. 16, 403-411, 1973) are superseded by this report.

Fish were used within two days of capture. Experiments were done with fish swimming in live-car from which they were removed only momentarily for sampling of aqueous humor. Only one sample was ever taken from a single eye but in some experiments each eye was used for a different point of time. In some experiments fish were brough to the laboratory and arranged in a box for perfusion with oxygenated sea water through the spiracles so that the eyes were not immersed in water.

The general plan was to inject $^{22}Na^+$, $^{36}Cl^-$, or $H^{14}CO_3^-$ at zero time and to follow the accumulation in the aqueous coincidentally with measurement of plasma concentration. In addition the vitreous humor was sampled at a few critical times by dissection of the eye or by withdrawal of the entire fluid contents (about 3 ml) from the posterior portion. This sampling occurred after the anterior (aqueous) fluid volume (about 0.2 ml) had been withdrawn with the needle tip in view through the iris opening. We report the kinetics of uptake of these ions into the aqueous in normal fish and in fish with carbonic anhydrase inhibited by methazolamide and Na^+-K^+ATPase inhibited by ouabain.

Sodium

Figure 1 shows the accession of $^{22}Na^+$ to aqueous humor. The ratio of cold sodium aqueous to pla is 1.08. This ratio was not achieved by isotope during the 20 hours of the experiment (isotopic ratio at 20 hours = 0.75). However initial access was surprisingly rapid, the isotopic ratio at 15-30 minutes being about 0.25. The data are consistent with loss of sodium from the small aqueous pool into the larger (by 10-15 fold) vitreous reservoir. The various volumes and rate constants are such that the equilibrium state among plasma, aqueous and vitreous (sodium concentrations of which are roughly equal) is reached slowly. Kinsey (in Membranes and Ion Transport, ed. by Bittar, Vol. 3, p. 185,

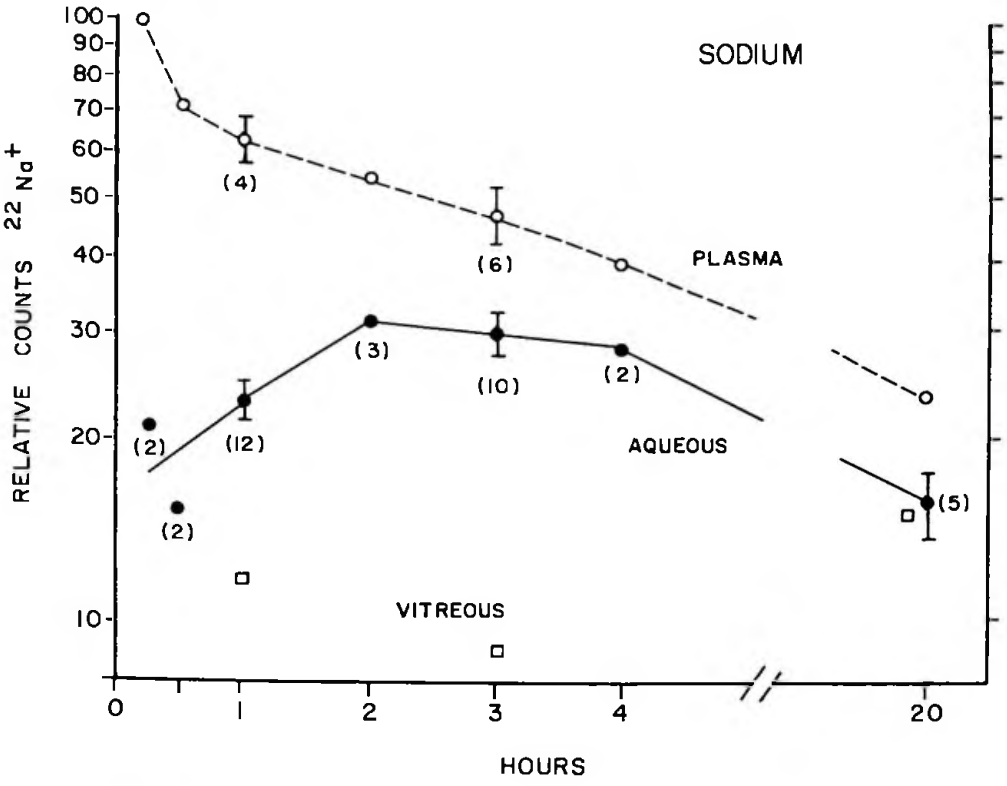

Figure 1. Accession of ^{22}Na from plasma to aqueous and vitreous humor of *S. acanthias*. Isotope injected at time 0. All counts relative to plasma = 100 at 10 minutes.

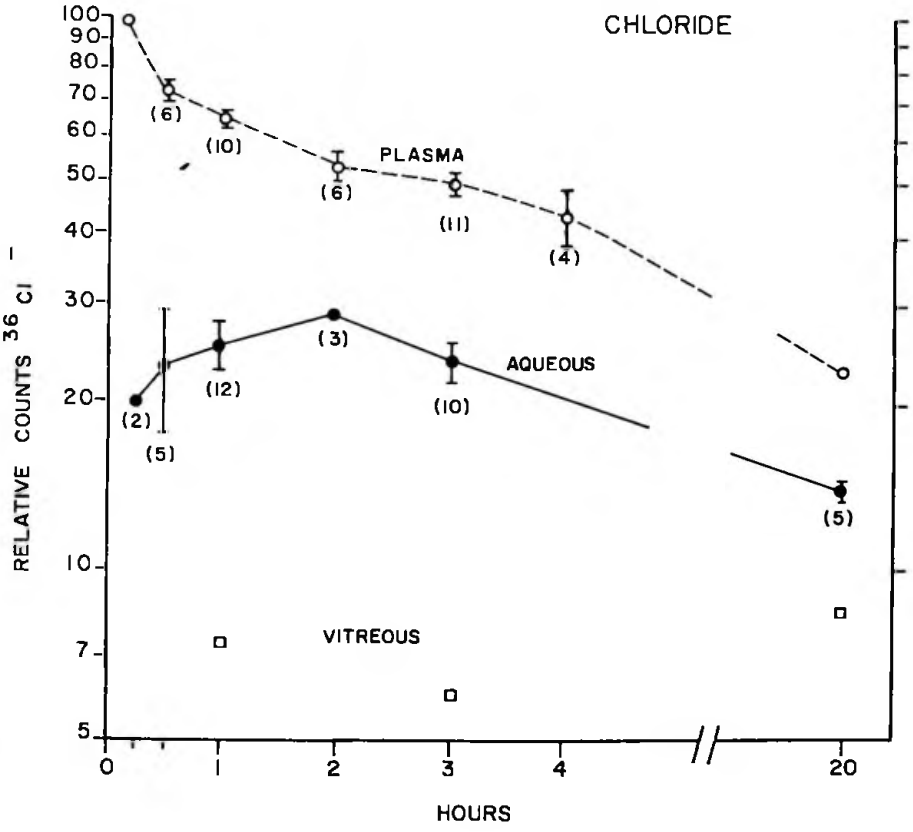

Figure 2. Accession of ^{36}Cl from plasma to aqueous and vitreous humor of *S. acanthias*. See Figure 1 legend.

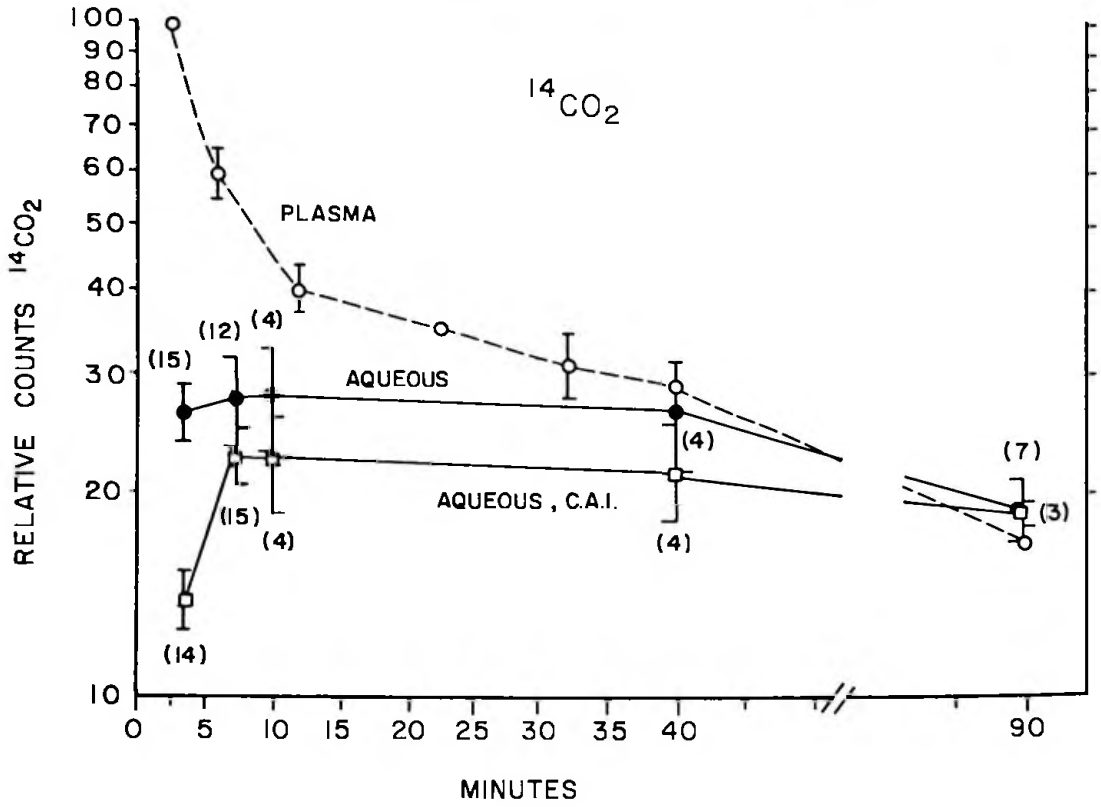

Figure 3. Accession of total $^{14}CO_2$ to aqueous humor from plasma in *S. acanthias*. Isotope injected at 0 time. Plasma curve is the same for normal fish and following carbonic anhydrase inhibition (C.A.I.) which was achieved by injecting 50 mg/kg methazolamide 30 minutes before the isotope. Standard errors of the mean are shown, except where replicates = 3.

1971) has described a very similar situation in the rabbit.

Our chief goal was the measurement of the initial rates of isotope entry in relation to the rate of fluid production. We can calculate, on the basis of the 15-30 minute sodium data, a rate constant for entry of

$$\frac{\text{40 units/hr. accumulating in aqueous}}{\text{plasma concentration of about 80 units}}$$

or 0.5 hr^{-1}. This value is minimal since the shape of the curve suggests that the rate may have been faster before 15 minutes. Thereafter it levels off rapidly.

Chloride

Figure 2 shows that the kinetics of $^{36}Cl^-$ are similar to those of sodium. The shape of the curve of the chloride concentration in the aqueous suggests that diffusion of chloride to the vitreous keeps the isotopic concentration in aqueous even lower than that of sodium. The 20-hour ratio of aqueous: plasma for $^{36}Cl^-$ is 0.61. The initial rate constant for isotope entry of chloride is somewhat faster than of sodium, about 0.65 hr^{-1}.

Bicarbonate

Figure 3 shows that HCO_3^- accession is extremely rapid, reaching an aqueous to plasma ratio

of about 0.3 within three minutes. A rate constant, calculated as described above for sodium, would yield a value of about 6 hr^{-1} or 10 - 12 times that for Na^+ or Cl^-. It is reasonable to assume (see section below on effect of methazolamide) that this value does not represent transport of HCO_3^- but formation of HCO_3^- at the ciliary body from gaseous CO_2 in plasma. Figure 3 shows that the equilibrium ratio of HCO_3^- between aqueous humor and plasma of about 1.1 is achieved within 40 minutes. In this case the formation of HCO_3^- at the ciliary body and its accumulation in aqueous is much more rapid than its diffusion into the vitreous so that isotopic equilibrium is reached readily between plasma and aqueous.

Sodium and Chloride Accession with the fish eyes out of water

It was important to find whether any Na^+ or Cl^- in aqueous (in the type of experiment shown in Figure 1 and 2) was exchanging across the cornea with sea water. Fish were kept in the laboratory as described above so that their eyes were not exposed to sea water. In aqueous humor taken one to three hours after isotope injection the concentrations of $^{22}Na^+$ or $^{36}Cl^-$ were no different from those experiments shown in Figure 1 and 2 in which fish were swimming in the sea. It seemed clear that the cornea is impermeable to these ions.

Effect of Methazolamide and Ouabain

Complete inhibition of carbonic anhydrase was achieved by intravenous injection of 50mg/kg methazolamide 30 minutes before giving the isotope. Sampling was done in precisely the same manner as in Figure 1 - 3. These was no effect on the accession of $^{22}Na^+$ in a total of 28 experiments over 0.5 to three hours. Similarly there was no effect on $^{36}Cl^-$ in 17 experiments over the same time. Figure 3 however shows that carbonic anhydrase inhibition did slow the access of HCO_3^- to the aqueous in the first 3.5 minutes from control (26 counts; n = 15) to treated (14 counts; n = 14). At later times there was no difference between control and treated animals. This effect of carbonic anhydrase inhibition is consistent with the hypothesis that HCO_3^- is not transported as such from plasma to to aqueous but that plasma CO_2 is hydrated to HCO_3^- at the secretory site (the ciliary body) and then diffuses into the aqueous.

The temporal effects of inhibition are also consistent with the kinetics of the uncatalyzed hydration of CO_2. The first order rate constant in about 1 min^{-1} at 16° which means that for essentially a complete reaction, say 99 percent, the time required would be five minutes. In agreement with this, the inhibited reaction in the fish requires some seven minutes but the normal catalytic reaction is complete in three minutes or less (Figure 3).

Ouabain was given intravenously at 40-60 µg/kg, a dose which was lethal within six hrs. in half the fish. Despite this however data were collected for $^{22}Na^+$ and $^{36}Cl^-$ at 0.5 and two hours for three fish each. Results showed an accumulation of isotope close to the control. It was concluded that either the process under study was not susceptible to Na^+-K^+ ATPase inhibition or possibly that the level of inhibition reached was not great enough to yield physiological effects.

Synthesis of Findings in Terms of Aqueous Humor Production

These data are interpreted in relation to: A) formation of aqueous humor, and B) mechanisms underlying the ion transport.

A) Aqueous humor is formed in *S. acanthias* at the rate of 0.06 ml/hr (Wistrand and Talalay, this Bulletin). The volume of fluid is approximately 0.25 ml so that the rate constant for turnover

is 0.24 hr^{-1}. The present data (summarized in Table 1) show that the measured k_{in} for Na^+ and Cl^- are twice or more this value indicating that at least half of the entrance of isotope was due to diffusion, i.e., $^{22}Na^+$ exchanging for $^{23}Na^+$, with no net transfer of ion. It is significant that Kinsey (vide supra) made qualitatively similar findings in the rabbit. In this circumstance the calculation of the concentrations of sodium and chloride in aqueous, based on isotope entry, yields impossibly high values (Table 1). The last column of Table 1 gives possible ionic concentrations in secreted fluid, setting sodium as equivalent to that in plasma water (i.e., the net sodium rate is equivalent to fluid production rate) and assuming the (maximal) limiting case that HCO_3^- concentration may be calculated from the isotopic K_{in}. Cl^- is set as $Na^+ - HCO_3^-$.

HCO_3^- accumulates in aqueous much faster than Na^+ or Cl^- or fluid itself. The data suggest that the process is *formation* of HCO_3^- from plasma CO_2; if trans-cellular ionic movement were involved, we might expect HCO_3^- appearance to be as slow as the movement of Na^+ or Cl^-. The presumption rather is that the rapid formation HCO_3^- may be a pilot ion in the movement of sodium and fluid. The value of 180mM (Table 1) for newly secreted fluid is very high but not impossible;

TABLE 1

NORMAL ENTRY RATES OF IONS
FROM PLASMA TO AQUEOUS IN *S. acanthias*

	Plasma	Aqueous	K_{in}*	Conc. in Secreted Fluid	
				From Isotope Entry+	Assuming isotonic Na, and HCO_3^- from Isotope Entry
	mM	mM	hr^{-1}	mM	mM
Na	255	279	0.50	540	280
Cl^-	239	253	0.65	650	100
HCO_3^-	7.7	8.5	6	180	180

+ $\frac{\text{Plasma conc.} \cdot k_{in} \cdot \text{vol aqueous (0.24ml)}}{\text{aqueous formation rate (0.06 ml/hr)}}$

*From initial isotopic rates

if Na^+ in newly secreted aqueous were isotonic to plasma water, i.e., 280 mM, two-thirds of it could be conceivably be matched by HCO_3^- with subsequent $Cl^- - HCO_3^-$ exchange to yield the measured values of the aqueous. It is of interest that we have shown that the measured concentrations of cold HCO_3^- in another secretion of the elasmobranch — the alkaline gland of the skate — is 200 - 300 mM (Comp. Biochem. Physiol. Vol. 10, p. 1, 1963).

B) Since more than half of measured sodium and chloride entry is by diffusion or isotope exchange (Table 1) it is expected that neither ouabain nor acetazolamide produces any effect on these rates.

Methazolamide clearly reduced the initial entrance of HCO_3^- (Figure 3) and the residual rate can be accounted for by the uncatalyzed hydration (or hydroxylation) of CO_2. Thus it is likely

that all of HCO_3^- accumulation in aqueous is due to formation of the ion rather than HCO_3^- transport as such. We showed many years ago (Comp. Biochem. Physiol. Vol. 5, p. 201, 1962) that carbonic anhydrase inhibition reduced the concentration of cold HCO_3^- in the aqueous humor of *S. acanthias.*

A general formulation of the process of aqueous humor formation in the elasmobranch follows: HCO_3^- formation from CO_2 is the most rapid process; Na^+ in part travels with it as a necessary counter-ion. Sodium entry may also be mediated by Na^+-K^+ ATPase, but clear evidence on this point will be difficult to obtain since a large portion of isotopic Na^+ (and Cl^-) entry is by exchange diffusion. Flow of aqueous must be at least in part dependent upon HCO_3^- formation and Na^+ transfer.

The significant fact emerges that these properties and quantitative relations in a major subclass of fish are also found in the rabbit and presumably in man. Slowing of aqueous humor flow by inhibition of the carbonic anhydrase catalyzed formation of HCO_3^- and the associated Na^+ transfer is the mechanism by which acetazolamide, methazolamide, and other sulfonamides reduce intra-ocular pressure in glaucoma. We conclude that the chemistry of aqueous humor formation is remarkably stable throughout the vertebrate phyla. (This work was supportedy by NIH grant GM 16934.)

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MINIMAL DDE-DISRUPTION OF OSMOREGULATION IN MALLARD TYPE DUCKS *Anas platyrhynchos*

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Studies reporting that organochlorine contaminants inhibit ATPases (Cutkomp *et. al.*, Chem.-Biol. Interactions, 3,439, 1971) and disrupt osmoregulatory events in marine fish (Kinter *et al.*, Environ. Health Perspectives, 1, 169, 1972) have led us to consider organochlorine inhibition of avian nasal gland Na-K-ATPase as a contributing factor in the recent large kills of young seabirds that have been reported on both sides of the Atlantic. Recently Friend and coworkers (Bull. Environ. Contam. Toxicol., 9, 49, 1973) studied the effect of DDE feeding (10-1000 ppm) on nasal gland secretion in the mallard and found decreased nasal gland function in response to an intravenous salt load in pesticide treated animals maintained on fresh water. These authors however did not observe this effect in mallards maintained on a one percent sodium chloride solution.

As our preliminary experiments with ducks showed a large variability in nasal gland function in response to intravenously administered salt, we elected to examine the effects of DDE feeding on nasal gland Na-K-ATPase and plasma electrolytes. To this end white Pekin ducks (hatched and raised at the laboratory) and mallards of a local strain (both *Anas platyrhynchos*) about three months of age were divided into paired flocks of about five birds each and fed *ad lib* duck breeder pellets (kindly donated by Agway, Inc.). The experimental flock had 100 ppm p,p'-DDE (Aldrich