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THE ELECTROLYTE PHYSIOLOGY OF THE ENDOLYMPH IN *Squalus Acanthias*

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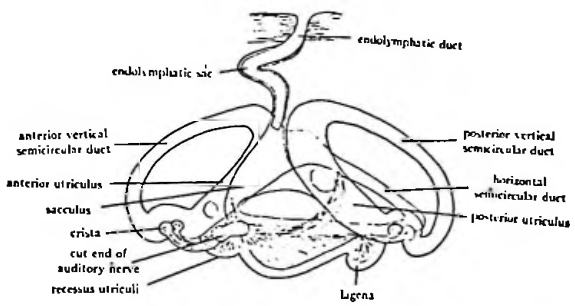
Relatively little is known about the electrolyte physiology of the endolymph, particularly with respect to the regulation of its ionic composition. Elasmobranch species offer large advantages for such a study because of the relative accessibility of the ear and the considerable volume (0.2-0.3 ml) of the endolymph. The anatomy of the internal ear (after Retzius, 1881) in *S. acanthias* may be found in L.H. Hyman, "Comparative Vertebrate Anatomy," University of Chicago Press, 1942, p. 461 (Fig. 1).

Figure 1



—The right internal ear of the dogfish, *Squalus acanthias* (after Retzius, 1881), viewed from the inner side.

Murray and Potts (Comp. Biochem. Physiol. 2:65, 1961) summarized previous work on the labyrinthine fluids of various species and showed clearly that in elasmobranchs (they used chiefly *Raja clavata*) the potassium concentration was very high which seems to be a general characteristic of vertebrates. Little has been done in either fish or mammal on the *rates* of accession of ions to endolymph, and this is the first work in which the four major ions have been studied together so that overall transport characteristics could begin to be defined.

We have measured the following in endolymph of the dogfish, *S. acanthias*: Normal ion composition, with a note on the histology of tissues of the inner ear; Effect of pCO_2 on ion composition; Carbonic anhydrase activity of inner ear tissues; Effect of acetazolamide on ion concentration; Effect of ethacrynic acid; Rates of transfer of Na^+, Cl^+, Rb^+ (for K^+) and CO_2/HCO_3^- from plasma to endolymph; Effect of acetazolamide on rates of ion transfer.

Fish were caught within two days of the experiment in which interval they were kept in live cars under the dock. When fish were brought to the laboratory they were immediately perfused with one to two liters of cold oxygenated sea water through the spiracles. For elevation of pCO_2, the sea water was passed through a bubbling device to which five percent CO_2 was admitted. Exposure of the inner ear was made by dissection from the dorsal surface of the head and could be accomplished

within about 15 minutes during which time the animal was perfused, gently restrained, and given sodium pentobarbital. Following isotope injection sampling was done at intervals from 20 minutes to four and a half days, each point of time representing a terminal sample in a single fish. Except for some of the very short (under one hour) experiments and those involving admission of CO_2, which were done during perfusion in the laboratory, the fish swam freely in the live cars in the intervals between injection of drug or isotope and sampling. Analytical methods, basis for calculation, and other aspects of the procedures have been described (Am. J. Physiol. 222: 885, 1972).

Normal Ion Composition and Histology. Table 1 shows the concentration of ions in the endolymph compared with those of perilymph and plasma. The endolymph was taken from the sacculus and perilymph from the dorsal space above the sacculus, perilymph thus being in direct contact with the chondocranium. The endolymph differs strikingly from plasma with regard to concentration of all the ions measured: Na^+ is less, while K^+, Cl^- and HCO_3^- are more. Note that the data show good stoichiometry in that (Na) + (K) ≅ (Cl) + (HCO_3). The urea concentration was 280 mM compared to plasma of 350 mM. The four ions + urea = 936 milliosmoles per liter.

TABLE 1

ELECTROLYTE CONCENTRATION IN ENDOLYMPH AND PERILYMPH OF *S. ACANTHIAS* (mM)

	ENDOLYMPH				PERILYMPH				PLASMA			
	Na^+	K^+	Cl^-	CO_2	Na^+	K^+	Cl^-	CO_2	Na^+	K^+	Cl^-	CO_2
Conc.	223	107	314	11.2	253	3.3	264	10.5	256	4.1	230	7.6
S.E. mean	4	3	4	0.8	5	0.3	5	0.5	2	0.1	3	0.6
n	14	13	11	7	9	9	4	5	15	15	13	10

Dr. W.L. Doyle studied the structure of these tissues with the following findings:

Preliminary electron microscopic survey of the lateral wall of the sacculus of *Squalus* reveals a bilayered epithelium supported on an interdigitating fibrous basement membrane. The basal cells have abundant smooth endoplasmic reticulum and clear vacuoles with relatively few mitochondria. The superficial layer exhibits cells in two stages. In some areas patches of superficial cells are filled apically with large clear vacuoles and in many of these cells the apical cell membranes were ruptured. In most areas the superficial cells were characterized by complex interdigitation with basal cells, elaborate development of Golgi areas and abundant distended smooth endoplasmic reticulum. Granular endoplasmic reticulum was sparse and mitochondria only moderately abundant. The nuclei were irregular in contour and scatter cells of this type displayed large intranuclear single crystalloids. The general morphology of the epithelium is consistent with moderately active secretion of fluid. Further study is required but there is reasonable correspondence in cell structure to that of the stria vascularis of higher forms. As yet we have found no intraepithelial capillaries in the wall of the sacculus but some are present in the semicircular canals where the epithelium is non-secretory.

Effect of Hypercapnia. Table 2 shows that five percent CO_2 added to the perfusing sea water, which raises the pCO_2 of the fish from 4 to about 18 mm Hg, has a slight or negligible effect upon

the ionic composition of the endolymph or on total CO_2 concentration. Data shown (single experiment for three hours) are representative of six in which hypercapnia was induced for periods of 75-270 minutes, and endolymph CO_2 ranged from 10-14 mM with no indication of an increase with time. This is in striking contrast to the effect of pCO_2 upon total CO_2 concentration in CSF, also shown in Table 2, and more fully documented in our earlier study (Am. J. Physiol. 222: 885, 1972). Table 2 also shows that pCO_2 elevation did not substantially affect the total CO_2 concentration of perilymph, cranial fluid or aqueous humor.

TABLE 2

EFFECT OF 3 HOURS HYPERCAPNIA† ON ENDOLYMPH CHEMISTRY OF *S. acanthias* (mM)

ENDOLYMPH				PLASMA				CO_2 OF OTHER FLUIDS*			
Na^+	K^+	Cl^-	CO_2	Na^+	K^+	Cl^-	CO_2	Aqueous	CSF	Peri-lymph	Cranial Fluid
213	96	313	10	264	4.9	230	9	11	22	7	6

† 5% CO_2 added to water perfusing the spiracles and gills.

* Normal values of these fluids 6-10 mM.

Carbonic Anhydrase Activity of Inner Ear Tissues. Six samples of sacculus wall showed concentrations of carbonic anhydrase ranging from 11-105 units/gram. Low values in this range might be artifactual if samples contain relatively high amounts of non-secretory tissue. The method is a modification of our earlier one (J. Pharmacol. Exp. Ther. 130: 26, 1960) using now a relatively high concentration of barbital buffer and bromthymol blue as indicator. In this system human blood has a unitage of 6,000/ml and that from *S. acanthias*, 550. The carbonic anhydrase activity of sacculus wall was completely inhibited by 10^{-4} M methazolamide in the final solution.

The canals contained carbonic anhydrase, in the same range as described for the sacculus, and also inhibited by methazolamide. The endolymphatic duct contained enzyme only in the doubtful range of activity (10-20 units/gram on this scale).

Effect of Acetazolamide on Ion Concentration. Carbonic anhydrase was inhibited *in vivo* by the intravenous injection of 50-100 mg/kg acetazolamide. The effect upon the endolymph was to raise the total CO_2 gradually, with a peak concentration of 22 mM (from normal of 11 mM) at 24 hours. This was similar to the rise in CSF CO_2 concentration (Comp. Biochem. Physiol. 5: 201, 1962) except, that in parallel experiments now carried out, the rate of rise was much faster in the CSF. With the rise of total CO_2 (essentially HCO_3^-), there was a corresponding decline in Cl^- concentration of endolymph with no clear change in sodium or potassium.

This effect is interpreted as due to the elevation of pCO_2 in the fish, following carbonic anhydrase inhibition. This elevation drives the formation of HCO_3^- in certain secretory tissues (via the uncatalyzed hydration of CO_2) even during inhibition of the enzyme within the secretory cells. The reason that high CO_2 alone (for one to four hours) is effective in CSF and not in endolymph (Table 2) is because of the differing rates of HCO_3^- formation at the two sites (see below): indeed after one hour of carbonic anhydrase inhibition, endolymph CO_2 had not increased but that of CSF was

elevated about 5 mM.

Ethacrynic Acid. This drug was given because Cohn, Gordes, and Brusilow (Science 171:910, 1971) reported a striking decline in the $[K^+]$ of dog endolymph within 10 minutes after its intravenous injection. We gave 30 mg/kg i.v. to two dogfish; in the first the endolymph was sampled at two and four hours, in the second at 21 hours. There was no change in endolymph $[K^+]$ from normal, but plasma $[K^+]$ was raised about 1 mM. We do not know if this is a significant change.

Rates of Ion Transfer from Plasma to Endolymph. These were determined with the use of the isotopes ^{22}Na, ^{36}Cl, $H^{14}CO_3$, and ^{86}Rb. The latter is used as a marker for K. From the plasma and endolymph concentrations at successive times, a first order rate constant for entry, k_{in}, was calculated in the same way as described for CSF (Am. J. Physiol. 222:885, 1971).

Sodium k_{in} was determined in five fish, at times ranging from one to 23 hours. Data from one to two hours yielded about 2 X higher k_{in} than those from four to 23 hours; whether this is an artifact of the method, spontaneous variation or real effect is not known. The k_{in} given is the mean for all periods. Table 3 shows this rate constant to be small, 0.0062 hr^{-1}. Multiplying this by the plasma concentration yields the accession rate, 1.6 mM hr^{-1}. This means that the sodium pool turns over in about 140 hours.

TABLE 3

RATES OF ENTRY OF IONS FROM PLASMA TO ENDOLYMPH

	Plasma mM	Plasma t 1/2 hrs	k_{in} hr^{-1}	Accession Rate mM hr^{-1} Col.1 x Col.3	k_{out} [b] hr^{-1}	(n)
Na^+	256	12	0.0062 ± .0013	1.6	0.0071	5
Cl^-	230	12	0.0084 ± .0016	1.9	0.0060	5
Rb^+ (K+)	4.1	75	0.22 ± .03	0.9	0.0084	13
HCO_3^-	7.3	0.6	0.17 ± .02	1.2	0.11	6
CO_2	0.3	0.6	4.1[a] ± 0.5	1.2[a]	--	

[a] Calculated as plasma CO_2 → endolymph HCO_3^-

[b] $k_{out} = \frac{k_{in} \times \text{plasma conc. (Accession rate)}}{\text{endolymph conc.}}$

Chloride k_{in} was also determined in five fish from one to 23 hours. As with sodium the shorter times tended to give higher k_{in} values; again data given are the mean for all periods. The quantitative rate data are comparable to those for sodium (Table 3). These rates for sodium and chloride are about five percent those of accession to CSF in the same species (Am. J. Physiol. 222:885, 1972).

Rubidium k_{in} was determined in 13 fish from four to 114 hours. Early data were no different from late data. The k_{in} was very much greater (about 30X) than that for Na^+ or Cl^-, but the accession rate is of the same order of magnitude (Table 3). The turnover time for Rb (or K, on the assumption that Rb is an ideal marker) is about 110 hours. Important evidence equating K with Rb is the near identity of the normal cold K $\frac{\text{endolymph}}{\text{plasma}}$ ratio of 26 (Table 1) with the mean ^{86}Rb

$\frac{\text{endolymph}}{\text{plasma}}$ ratio of 31 in three fish 90 - 114 hours after isotope injection. In the cat ^{42}K reaches $\frac{\text{endolymph}}{\text{plasma}}$ equilibrium in about 48 hours (Choo and Tabowitz, Annals of Otol., Rhinol., and Laryngol. 74:140, 1965).

HCO_3^- entry was studied in six dogfish, 40-70 minutes after injection of the isotope. Table 3 shows the rate constants calculated both as if HCO_3^- were transferred from plasma to endolymph and as if HCO_3^- were formed from plasma CO_2 gas. The latter is the preferred mechanism as indicated by the effect of carbonic anhydrase inhibition (see next section): the critical value of accession rate is the same whichever the mechanism. This rate is eight percent that of accession of HCO_3^- to CSF in the same species (Am. J. Physiol. 222:885, 1972).

Table 3 shows that the accession rates of all four ions are of the same order of magnitude. $Na^+ + K^+ = 2.5$ mM hr^{-1} which may be compared with $Cl^- + HCO_3^- = 3.1$ mM hr^{-1}. Considering the various sources of error and the fact that the numbers of experiments are not great, this appears to be reasonable agreement.

Table 3 also shows k_{out} values for the four ions. In view of the dominance of Cl^- concentration in the endolymph, its half time for exit, 115 hours, may be a rough measure of fluid movement.

Effect of Acetazolamide on Rates of Ion Transfer. Acetazolamide (80 mg/kg i.v.) was given intravenously 24 hours before the injection of isotopic sodium or chloride or rubidium (Table 4). Sodium uptake into endolymph was measured 1/2, 1-1/2, and 24 hours after injection of isotope, yielding a mean of 0.0087 min^{-1} which is higher but not significantly different from controls (Table 3). Similarly chloride was tested in three fish, one at two and two at 22 hours after isotope. The mean k_{in} was 0.0070 min^{-1} (Table 4), close to that of controls (Table 3).

TABLE 4

ENTRY OF IONS FROM PLASMA TO ENDOLYMPH DURING CARBONIC ANHYDRASE INHIBITION[a]

	Plasma mM	k_{in} hr^{-1}	Accession Rate mM hr^{-1}	k_{out}	(n)
Na^+	256	0.0087 ± .0019	2.2	0.01	3
Cl^-	230	0.0070 ± .0015	1.6	0.005	3
Rb^+ (K^+)	4.1	0.21	0.9	0.0084	2
HCO_3^-	14.0	0.063 ± .024	0.9	0.043	3
CO_2	0.6	1.5[c] ± 0.6	0.9[c]	--	
HCO_3^- [b]	8.0	0.075 ± .004	0.6	0.050	3
CO_2	0.6	1.0[c] ± .05	0.6[c]	--	

[a] Acetazolamide 80 mg/kg i.v. given 24 hours before isotope except as noted in footnote *b*. Plasma half-lives of isotopes are unaffected.

[b] Acetazolamide 50 mg/kg i.v. given 1/2 hour before isotope.

[c] Calculated as plasma $CO_2 \rightarrow$ endolymph HCO_3^-.

Rubidium was tested in two fish at 1-1/2 and at 48 hours after injection of label. The mean k_{in} was 0.21 hr^{-1} (Table 4), similar to controls (Table 3).

HCO_3^- label was tested in two ways: 24 hours after acetazolamide (as for the other ions) and 30-40 minutes after giving the inhibitor. Six fish were used, divided equally between the two protocols. HCO_3^- label was analyzed in endolymph and plasma one hour after injection. Table 4 shows the rate constants calculated for both direct transfer of HCO_3^- and for $CO_2 \rightarrow HCO_3^-$, in the same manner as for the control data of Table 3. Following both prolonged and acute carbonic anhydrase inhibition, the k_{in} and accession rates are lowered. There are not enough data to tell if there is a real difference between acute and chronic inhibition.

The decrease in accession rate of HCO_3^- following acetazolamide (about 0.5 mM hr^{-1}), while significant, is not great enough in magnitude to show a measurable effect on cation accession rate (2.5 mM hr^{-1}) within the limits of variability in these experiments.

Conclusion

1. The endolymph composition suggests (in the absence of knowledge of the electrical potential) active processes for accumulation of K^+, Cl^- and HCO_3^-.

2. The presence of carbonic anhydrase in the sacculus and canals, and the effect of acetazolamide upon HCO_3^- concentration and turnover in endolymph, show that the mechanism for HCO_3^- accumulation is that of hydroxylation of CO_2, similar to that in aqueous humor, cerebrospinal fluid, and pancreas.

3. The Na^+, K^+, and Cl^- turnover in endolymph is of the order of 100-150 hours while that for HCO_3^- is about eight hours. However the concentrations of ions that are being moved are such that the actual rates of accession or exit are roughly the same (within two-fold) for all four ions.

4. Whether these characteristics extend to other vertebrates remains to be found, but at least two elements are shared with mammals: High endolymph [K] and carbonic anhydrase in the secretory tissue.

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NATURAL LEVELS OF DDT-RELATED COMPOUNDS AND POLYCHLORINATED BIPHENYLS (PCB's) IN VARIOUS MARINE SPECIES

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Because of the widespread distribution of chlorinated hydrocarbon residues in the environment and because of the importance of these materials to many investigators of this and other marine laboratories, we have extended and continued the determination of these materials (The Bulletin MDIBL, 9, 2, 1969; Ibid 10, 1, 1970) in various marine animals. All of the results presented in this report were from specimens collected from Frenchman Bay, Maine, Specimens were wrapped in aluminum foil, frozen in individual plastic bags and maintained in a frozen (-40°) condition until they were assayed. The DDT and related compounds were analyzed as described previously (The