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ELECTROLYTE PATTERNS OF DOGFISH CARTILAGE (Squalus acanthias)

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The possession of cartilage rather than bone in the skeleton represents a major and relatively unique feature of the elasmobranchs. However, little is known concerning the electrolyte composition of this tissue. Nor are there data concerning its role in electrolyte and acid-base metabolism.

Plasma and cartilage were obtained from nine adult female dogfish and analyzed for total water; Na^+, K^+, and Cl^- concentrations. In addition, sucrose was administered intravascularly and the magnitude of the sucrose space in cartilage determined. Finally, in five animals, analyses of plasma and cartilage electrolytes were performed four hours after the intravascular infusion of Na HCO_3.

The results are given in Table 1.

Table 1

	Plasma	Cartilage	$\frac{\text{Cartilage}}{\text{Plasma}}$
Na^+ mEq/L H_2O	267	336	1.23
Cl^- mEq/L H_2O	278	234	0.84
K^+ mEq/L H_2O	4.6	9.2	2.00
Sucrose space	20%		

It appears that $\frac{(Na^+)c}{(Na^+)p1}$ is equal to $\frac{(Cl^-)\ P1}{(Cl^-)\ c}$ suggesting that these ions may be in electrochemical equilibrium across cartilage cell membranes. Assuming that this is true the calculated transmembrane potential of cartilage cells would be -4.2 millivolts.

Marked increases in plasma HCO_3^- produce no substantial change in cartilage Na^+ of K^+ concentration suggesting that this tissue is not a major site of buffering by ion exchange.

The electrolyte pattern of dogfish cartilage appears to differ markedly from that reported for mammalian cartilage and continued studies of the role of this tissue in the general electrolyte metabolism of the dogfish appear warranted.

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THE PHARMACOLOGY OF ETHYL m-AMINOBENZOATE (MS222) IN THE DOGFISH, Squalus acanthias

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The permeability of the gill membrane to various drugs was studied in 1966 by two groups, Maren et al. (Bull. MDIBL 6:25, 1966) and Rall et al. (Bull. MDIBL 6:31, 1966). A direct correlation exists between lipid solubility and gill clearance, the lipid soluble drugs being cleared

relatively rapidly. Maren et al. (ibid.) found that MS222, an extremely lipid soluble compound ($CHCl_3$/buffer partition coefficient of 312), was rapidly excreted across the gill. Renal excretion was negligible. It appeared possible that the gill clearance of MS222 could be a measure of cardiac output.

The pharmacology of MS222 was studied further in the dogfish and forms the substance of this report. MS222 (Sandoz) is the methyl sulfonic acid salt of ethyl m-aminobenzoate. Because it is produced and used as the salt, doses and concentrations refer to this salt (M.W. 261) rather than to the base itself (M.W. 165).

Gill clearance and cardiac output was measured using the divided box (MDIBL 6:25) and the decay from the body studied in the free swimming fish. Catheters were placed in the dorsal aorta and the duct of Cuvier. Doses of 25-50 mg/kg were given in the caudal artery. The LD-50 is about 100 mg/kg based on our observation of 14 fish. The data for gill clearance and cardiac output are presented in Table 1. Each period is five minutes in the box. Fish 14 was in the box from injection onwards. In the other experiments the data refer to the first (and in a few cases successive) five minutes after the fish was placed in the box, following 24-30 minutes of free swimming after injection. This time was found adequate for distribution of drug through the body (see below).

Table 1 shows that gill clearance of MS222 averages 10.2 ml/kg per min, and cardiac output averages 22.2 ml/kg per min. The latter figure agrees well with those of both Burger and Bradley (J. Cell. Comp. Physiol. 37:389, 1951) and Robin et al. (ibid. 67:93, 1966). These data show that almost 50% of the drug is extracted in one passage through the gill.

The plasma half-life of MS222 in the fish swimming freely is 56 minutes (n = 8; range 36-80 minutes). First order decay curves could be plotted from about 25 minutes onward; earlier the shifting blood levels reflected profound expansion of drug from plasma volume to that approaching the whole fish. In this situation the volume of distribution cannot be obtained in the conventional way, by extrapolating back to zero time along the decay curve after distribution has been achieved, since a very large proportion of injected drug is lost from high plasma concentrations before distribution occurs. The volume of distribution can, however, be obtained from the relation between clearance (C) and half-life (t 1/2) according to the expression (Butler, Fed. Proc. 17:1158, 1958):

$$\text{vol dist} = \frac{C \cdot t\,1/2}{\ln 2}$$

$$= \frac{10.2 \text{ ml/kg per min} \cdot 56 \text{ min}}{0.7}$$

$$= 820 \text{ ml/kg.}$$

Tissues of two fish were analyzed 60 minutes after injection of 30 mg/kg MS222. Most tissues had about the same drug concentration as plasma, reflecting the high volume of distribution. Exceptions were brain, CSF, extradural fluid and kidney. Almost complete exclusion of the drug from brain and CSF can partially, but not completely, be explained by its high clearance through the gill. We feel that there must be special features of the circulation between gill (including pseudobranch) and the brain which operate in such a fashion that efferent blood from the gill to the body contains more drug than efferent blood from the gill to the brain. The kidney had ten times the plasma concentration, an inexplicable finding at this time. In agreement with out

Table 1

GILL CLEARANCE FOLLOWING INJECTION OF MS222 IN THE DOGFISH (S. acanthias)

Fish no.	Weight (kg)	Drug dose (mg/kg)	Interval from injection to study (min)	Mean venous conc. (Plasma - μg/ml)	Mean arterial conc. (Plasma - μg/ml)	Drug in gill effluent (μg/min)	Gill clearance* (ml/kg per min)	Cardiac output† (ml/kg per min)
14	1.4	35	13	22.6	7.0	332	10.5	15.2
17	1.9	35	30	7.1	4.5	136	10.1	27.5
			35	5.6	4.4	84	7.9	--
			40	4.9	4.0	84	9.0	--
			45	4.2	3.0	92	11.5	--
18	1.7	50	30	24.8	21.6	124	(5.0)	--
19	1.7	30	30	7.2	5.0	520	8.4	27.2
38	1.6	30	24	6.5	2.7	144	13.9	24.0
			29	6.1	2.3	104	10.6	17.3
Mean							10.2	22.2

*Clearance = $\frac{\text{Rate drug excreted } (\mu g \text{ per min})}{\text{Venous conc. } (\mu g/ml)}$ per kg.

†Cardiac output = $\frac{\text{Rate drug excreted } (\mu g \text{ per min})}{\text{Mean central venous conc. } (\mu g/ml) - \text{Mean arterial conc. } (\mu g/ml)}$ per kg

-- Not entered where ΔA-V is < 2 μg/ml, since analytical uncertainty is large.

( ) Not used in calculating mean for reasons given in text.

Table 2

ENTRY OF MS222 INTO THE BLOOD AND CEREBROSPINAL FLUID OF THE DOGFISH (S. acanthias)

	Fish no.	Drug dose		Intervals to sampling (min)	Drug concentration (μg/ml)				Comment
		mg/kg	μg/ml sea water		Plasma		CSF	Brain	
					Artery	Vein			
I	31	30	-	20	13	31	1	-	No anesthesia
I	9 & 10 (avg)	30	-	60	2	3	1	1	No anesthesia
I	34	100	-	37	560	748	7	-	Moribund; gill function reduced (?)
II	25	-	50	5*	65	20	8	-	Light anesthesia
II	24a	-	100	2*	111	13	17	-	Moderate anesthesia
II	24b & 28 (avg)	-	100	5*	73	32	23	-	Deep anesthesia
III	29 & 48 (avg)	-	100	7†	6	10	28	32	All deeply anesthetized at 5 min. Nos. 29, 48, and 26 beginning to recover; others appeared completely recovered.
III	26	-	100	13†	6	13	26	-	
III	30	-	100	17†	5	9	14	-	
III	49	-	100	25†	6	7	15	20	
III	51	-	20	120‡	11	13	8	9	

*Time that fish's head immersed in sea water containing drug.

†Time fish swimming in fresh sea water following 5 minutes in sea water containing drug.

‡Represents 60 minutes in sea water with drug followed by 60 minutes in fresh sea water, in laboratory box.

work of 1966, renal clearance of MS222 was less than GFR; about 2% of drug appeared in urine in six hours.

MS222 does not produce anesthesia in fish following intravascular injection, in accord with low or absent concentration in brain (Table 2, Group I). When fish are immersed in sea water containing 100 μg/ml, they attain surgical anesthesia within five minutes. Table 2 shows the pharmacological basis for these observations. In the latter situation, drug reaches brain and CSF almost at once (Group II). Anesthesia lightens when the fish are returned to fresh sea water. The drug is then excreted through the gill causing venous, brain, and CSF concentration to decline (Group III). Not shown in Table 2 is the interesting sidelight that in the experiments of Group II, no drug was detected in the extradural fluid. Comparison of the concentrations of drug in CSF in Group III with those of Group II suggests that recovery from anesthesia occurs at somewhat higher concentration than onset of anesthesia. The same is true of alcohol intoxication (Mirsky et al., Quant. J. Stud. Alc. 2:35, 1941) and perhaps other anesthetics.

The ester linkage in MS222 appears to be critical for its diffusibility. The sodium salt of the acid homologue, m-aminobenzoic acid, was injected at 30 mg/kg; no drug was detected in the gill effluate and the plasma half-life was 37 hours. This is like other ionic lipid-insoluble drugs studied in 1966 (MDIBL 6:25). This experiment also shows that no major part of the ester of MS222 is hydrolyzed in vivo, since the analytical method (Bratton-Marshall) detects both arylamino compounds.

In summary, ethyl m-aminobenzoate (MS222) moves into or out of the fish via the gill, the direction of movement being toward that of the lower concentration. The movement in either direction is extremely rapid and would appear to be blood flow dependent. The half-life of this drug injected intravascularly is 56 minutes with a volume of distribution of about that of body water. The gill clearance is 10 ml/kg per min which represents about half of cardiac output. Cardiac output, as measured using the Fick principle, is the same as that previously reported for direct measurement and dye dilution methods.

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RENAL EXCRETION OF D- AND L-GLUCOSE IN THE GOOSEFISH

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The question of renal secretion of 3-0-methyl-glucose and L-sugars has been raised by Huang et al. as a result of studies in the dog (Proc. Soc. Exptl. Biol. Med. 124:20, 1967). These findings have prompted us to measure the excretion of L-glucose in comparison to D-glucose in the aglomerular teleost, Lophius americanus.

Experiments were performed 24 hours after capture, urine samples being taken from the cannulated ureters. The urine flow per minute and gram kidney was 7.7 μl ± 0.75 S.E. (n = 16). Measurements of the osmolarity showed no significant difference between plasma (333 m osmol/L ± 29.1 S.E.) and urine (306 m osmol/L ± 33.3 S.E.).

The average concentration of D-glucose (glucose oxidase method) in the urine was 6.06 mg% ± 2.3 S.E. (n = 14). The urine to plasma ratio was 0.11 ± 0.027 S.E. (n = 14).