DIVALENT ELECTROLYTE EXCRETION IN THE MARINE TELEOST, MYOXOCEPHALUS OCTODECIMSPINOSUS, WITH REFERENCE TO URINARY PRECIPITATES

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In the course of studies of the acid-base physiology of Myoxocephalus octodecimspinosus, the long-horn sculpin, we observed that some 20% of urines contained a fine granular precipitate (T.H. Maren, A. Fine, E.R. Swenson and D. Rothman, Am. J. Physiol. 263:F49, 1992). This had been noticed nearly 60 years ago, by both Pitts (J. Cell. Comp. Physiol. 4:389, 1934) and Grafflin (Biol. Bull. 71:360, 1936). Notably, concretions or obstruction to urine flow were never observed. The presence of these precipitates was not surprising, since we had found (vide supra) that urinary pH could vary in the marine teleost, normally from 6.3 to 7.2, and with acid or alkali load, from 6.1 - 7.8. Under these relatively alkaline conditions, Ca^{++} and Mg^{++} salts would be insoluble, and indeed Pitts (vide supra) had identified $MgHPO_4 \cdot 3\ H_2O$ in the gravelly residue.

The purpose of this work is two-fold: (1) to furnish basic data on divalent ion composition of teleost urine, in the context of the solubilities of the various salts of Ca^{++}, Mg^{++}, $SO_4^{=}$ and $HPO^{=}$; (2) to seek properties or constituents of urine that prevent concretions or precipitates when solubility products of these salts may be exceeded.

Techniques for the collection of urine and the administration of acid or base have been described (Maren et al., ibid). The urines were titrated with HCl or NaOH to yield titration curves in both acid and alkali regions. The electrolyte analysis was performed using a Synchron CX5 autoanalyzer (Beckman Instruments, Inc., Brea, CA). An iterative computer program, EQUIL, (C. Brown and D.L. Purich, Disorders of Bone and Mineral Metabolism, Eds. F.L. Coe and M.J. Favus, Raven Press, p. 613, 1992) was used to calculate the salt concentrations in the urine. The values are expressed as relative supersaturation ratios, the ion activity product of a salt in the solution divided by its solubility product. Protein from sculpin kidney was obtained by homogenizing tissue, the supernatant absorbed to DEAE-cellulose, and the batch eluted and fractionated by DEAE-cellulose column chromatography with a linear NaCl gradient from 0.1 - 0.5 M. Inhibition of crystal growth was measured using a calcium oxalate crystal growth system (Y. Nakagawa, M.A. Ahmed, L. Hall, S. Deganello and F.L. Coe, J.C.I. 79:1782-1787, 1987). A metastable solution of calcium oxalate was added to a quartz cuvette in a continuous recording spectrophotometer at wavelength 214 nm. Seed crystals were added to the solution and optical density continuously recorded to produce a time course of oxalate consumption. The assay was run with and without protein in an alternating fashion. A crystallization rate was determined in the presence of protein (v) and without protein (v_o). The crystal growth inhibition is expressed as the ratio of the reaction velocity in the presence of protein to the reaction velocity in the absence of protein, $(v/v_o) \times 100$.

Table 1 shows the urinary electrolytes in 30 long-horned sculpin, in normal acid urine and in fish given 12 meq/kg $NaHCO_3$ by intraperitoneal or intravenous injections. The latter treatment caused a significant ($p < 0.02$) increase in urinary pH, HCO_3^-, Na^+ and modest ($p = 0.05$) decrease in Mg^{++} and $SO_4^{=}$. Taking the proper valencies into account, there is an anion deficit of about 50 mM, so that there may be an unmeasured anion present to the extent of about 15%. However, this does not appear to be a buffer, since titration of the urine to pH 8 or to pH 4

showed a pattern similar to that of phosphate, suggesting that this may be the sole or major buffer.

TABLE 1. URINARY ELECTROLYTES IN LONG-HORN SCULPIN: EFFECT OF ALKALOSIS

					millimolar				
	pH	HCO_3^-	Na^+	K^+	Cl^-	Ca^{++}	Mg^{++}	PO_4	$SO_4^=$
Normal (21)	6.34	0.42	13	2	122	9	124	33	38
$\pm$ S.E.$_{mean}$	0.06	0.06	4	0.2	12	0.5	5	6	3
$NaHCO_3$ treated (9)	7.30	7	110	3	142	6	81	26	24
$\pm$ S.E.$_{mean}$	0.2	2	15	0.5	17	1.5	18	8	5

Table 2 gives the solubilities of the divalent salts which appear in teleost urine. It is clear that both phosphate salts, at pH about 6.5, have solubilities much below their concentration in urine. Ca and SO_4 concentration in urine are close to the solubility of the salt. Table 2 gives preliminary estimates of the relative supersaturation ratios for the relevant salts which appear in urine. The magnesium phosphate and calcium phosphate are in accord with our finding of solids that contain all three ions (see below). $CaSO_4 \cdot 2H_2O$ shows no supersaturation, but electron microprobe analysis reveals precipitates of calcium and sulfur. This remains to be resolved.

TABLE 2. SOLUBILITIES AND SUPERSATURATION RATIOS† FOR CALCIUM AND MAGNESIUM SALTS IN SCULPIN URINE

Compound	Solubility Product	Solubility H_2O	Relative Supersaturation Ratios in Sculpin Urine			
			Normal		Alkaline*	
	M^2	mM	Clear	Ppt	Clear	Ppt
$CaHPO_4 \cdot 2H_2O$ brushite	4×10^{-7}	2	2.9	4.6	1.4	4.3
$MgHPO_4 \cdot 3H_2O$ newberyite	1.5×10^{-6}	12	8	15	3	14
$CaSO_4 \cdot 2H_2O$	4.3×10^{-5}	15	0.3	0.2	0.14	0.9
			(9)	(12)	(1)	(8)

†Ion activity product/solubility product
*Fish that received $NaHCO_3$.
( ) gives number of fish in each group.

Of the 30 urines examined, 11 of 21 normal controls and 8 of 9 of the alkali treated fish had fine precipitates. There was no correlation between the urine pH of individual fish and the formation of the precipitates. This had also been observed by Pitts and Grafflin (vide supra).

When the data from individual fish are analyzed, the following relations obtained: Positive correlation between Ca^{++} and Mg^{++} concentrations in urine; negative correlation between Cl^- and $PO_4^=$ output; high PO_4 concentration (46 mM) in urine of fish with precipitate compared to 8 mM in clear urine. It is seen from Table 2 that supersaturation ratios are always higher in urine with precipitates than in clear urine.

A crystal growth inhibitor (Nephrocalcin, NC) has been isolated from kidneys of nine species of vertebrates, all mammals except chicken (Y. Nakagawa, C.L. Renz, M. Ahmed, and F.L. Coe. Am. J. Physiol. 260:F243, 1991). The protein now isolated from sculpin kidney by DEAE-cellulose chromatography was separated into four fractions by ionic strength required for elution (vide supra). The crystal inhibitory activity of each fraction was determined using 10 µg of protein. The (v/v_o) % for fraction A (eluting at 16 to 18 mS), fraction B (19-23 mS), fraction C (24-28 mS), and fraction D (29-36 mS) was 68.5%, 89.2%, 57.5% and 17.9% respectively. Human urine protein at 16 µg/ml has v/v_o% of 25-55; thus the protein from sculpin kidney is highly potent.

We attempted to analyze the precipitate from sculpin urine by x-ray crystallography, but the crystallinity of the sample was extremely poor, giving uninterpretable results. Electron microprobe analysis was used to define the elements present in the solid phase. There were three distinct solid phases seen with the electron microprobe. A bright lath-like crystal was seen which was composed of Ca and S in approximately a 1:1 ratio. The amorphous background material had a spectra showing P, S, Mg, Ca and trace K. There were rare well-formed crystals distinct from the bright lath-like crystals that contained Mg and P in a 1:2 ratio.

In conclusion, the composition of teleost urine, in neutral or alkaline pH, is conducive to formation of precipitates of calcium and magnesium phosphates and possibly calcium sulfate. The fine granular precipitates that are occasionally found never lead to frank concretions or obstruction, presumably because of protective effects of inhibitors. Clear urines are supersaturated with respect to crystallization of alkaline earth salts. Inhibition of crystallization in urine may be a common feature of vertebrate evolution.

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