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SPECIATION OF STRONTIUM IN HUMAN AND BOVINE MILK

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Abstract—The speciation of strontium in human and bovine milk has been computed using a chemical equilibrium model and the ECCLES program. The calculations show that the low-molecular-mass distribution of Sr(II) ions parallel that of Ca(II) ions. The analytical techniques used to remove strontium from milk include liquid–liquid extraction using dicyclohexyl-18-crown-6. This method relies on the selective complexation of Sr(II) ions by this ligand. The ability of dicyclohexyl-18-crown-6 to chelate both Sr(II) and Ca(II) ions in milk was investigated using the computer model.

The role of strontium in biological systems received but scant attention until the late 1940s, mainly because there were no reasons to suspect that the element was present in man and also, as with many ultra-trace elements, analytical methods were not available for detecting and measuring strontium. It is now known that strontium in mammals is inextricably linked with the biochemistry of calcium and that milk, being our principal source of calcium, also carries our main strontium-90 threat. This is not surprising since milk and dairy products are vitally important components of our diet, accounting for up to 90% of calcium intake.[1] Strontium, from group IIA of the Periodic Table, occurs with calcium and barium and has biological and physical properties similar to those for these elements.

Strontium presents a threat to man because of its radioactive isotopes. Strontium-90 is widely used in medical and research establishments and produced as a by-product of nuclear fission, 2 to 5 atoms of strontium-90 being produced for every 100 nuclei undergoing fission. Of the six strontium isotopes produced by nuclear fission, strontium-90 is the most important and the most prevalent. It is also the most persistent source of strontium radiation to man, since it has a half-life of 28 years.[2]

Inside the body, strontium-90 enters the blood by absorption from the gastrointestinal tract, from where it is continuously removed by incorporation into new bone or is lost from the blood by urinary and faecal excretion.[1,3] The distribution of strontium in the body parallels that of calcium, high levels being found in teeth, bones and other calcified tissues. Strontium's tendency to occur with calcium is also apparent in foodstuffs—those high in calcium also accumulating strontium. This was clearly demonstrated in the Windscale fire of 1957 in which iodine-131 and strontium-90 scattered over surrounding farmland resulted in the contamination of milk and dairy products. Strontium-90 reaches the milk within a few hours of ingestion by the cow, the maximum activity (which may be up to 4% of the total imbibed) occurring after about 2 days.

The majority of strontium in milk is bound to the protein casein. However, the smaller ultrafilterable portion is of nutritional importance, since the chemical speciation of strontium-90 in this fraction will dictate its bioavailability.

Although the total amount of strontium present in a given milk sample can now be accurately analysed (usually from analysis of the radioactive decay product, yttrium-90), its chemical speciation in such a biofluid is still beyond the reach of the most sophisticated analytical techniques. However, we have for many years used computer simulation models to evaluate the low-molecular-mass (LMM) metal-ion distributions in biofluids such as blood plasma. The approach relies on the assumption that many biological fluids approximate to a steady-state or equilibrium situation. It is thus possible to use thermodynamic chemical equilibrium models to compute the metal–ligand speciation of the biofluid based on analyses of total ligand and total metal ion concentration data and the relevant formation constants.

The ECCLES program has been used to establish

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simulation models for blood plasma, milk and other biofluids.[4,5] The milk model has now been extended to encompass the pollutant strontium. The total ligand concentrations in human[6–12] and bovine[6–13] milk are listed in Tables 1 and 2, respectively. Values for the total and free concentrations of the biologically essential metal ions are given in Tables 3 and 4.[3,13–15] Formation constants for these metal ions with the former ligands were as previously used in the blood plasma model.[4] The additional formation constants for the strontium system were extracted from literature compilations and corrected to blood plasma conditions of 37°C, I = 150 mmol dm^{-3} NaCl. Milk has an ionic strength of approximately 80 mmol dm^{-3} and the constants are not expected to differ much from those of blood plasma. The total ligand, free metal ion concentrations and formation constant data were used to compute the LMM metal-ion distribution of strontium in human and bovine milk at a pH of 6.8.

Table 1. Total ligand concentrations in human milk

Ligand	Concentration (mol dm^{-3})	Reference
Alanate	1.46×10^{-4}	6–8
Aminobutyrate	1.46×10^{-5}	6, 7
Arginate	1.01×10^{-5}	6–8
Aspartate	3.80×10^{-5}	6–8
Citrullinate	1.10×10^{-5}	6, 8
Cystinate	2.51×10^{-5}	6, 8
Glutaminate	1.72×10^{-4}	6–8
Glutamate	9.25×10^{-4}	6–8
Glycinate	8.09×10^{-5}	6–8
Histidinate	2.42×10^{-5}	6–8
Hydroxyprolinate	5.34×10^{-6}	6
Isoleucinate	1.04×10^{-5}	6–8
Leucinate	2.49×10^{-5}	6–8
Lysinate	2.45×10^{-5}	6–8
Methionate	4.37×10^{-6}	6–8
Ornithinate	3.18×10^{-5}	6–8
Phenylalanate	1.04×10^{-5}	6–8
Prolinate	5.04×10^{-5}	6–8
Serinate	8.07×10^{-5}	6–8
Taurinate	3.54×10^{-4}	6–8
Threoninate	6.67×10^{-5}	6–8
Tryptophanate	1.00×10^{-6}	9, 8
Tyrosinate	1.25×10^{-5}	6–8
Valinate	5.16×10^{-5}	6–8
Carbonate	4.00×10^{-3}	9
Phosphate	1.60×10^{-3}	9–11
Sulphate	4.37×10^{-4}	9, 11
Ammonia	1.17×10^{-4}	9
Citrate	1.00×10^{-3}	12
Lactate	6.30×10^{-4}	10
Pyruvate	5.60×10^{-5}	10
Histamine	3.60×10^{-6}	10
Thiocyanate	1.40×10^{-5}	*
Silicate	1.38×10^{-4}	*
Malate	3.50×10^{-5}	*
Oxalate	3.20×10^{-6}	*
Salicylate	5.00×10^{-6}	*
Succinate	4.20×10^{-5}	*
Ascorbate	4.30×10^{-5}	*

* Estimate based on concentrations in blood plasma (Ref. 4).

Table 2. Total ligand concentrations in bovine milk

Ligand	Concentration (mol dm^{-3})	Reference
Alanate	2.13×10^{-5}	6–8
Aminobutyrate	1.76×10^{-6}	6–8
Arginate	1.20×10^{-5}	6, 7
Aspartate	6.27×10^{-5}	6–8
Citrullinate	1.70×10^{-6}	8
Cystinate	9.24×10^{-6}	6–8
Glutaminate	8.93×10^{-5}	6–8
Glutamate	2.16×10^{-5}	6–8
Glycinate	1.44×10^{-4}	6–8
Histidinate	4.70×10^{-6}	6, 8
Hydroxyprolinate	1.27×10^{-6}	7, 8
Isoleucinate	4.03×10^{-6}	6–8
Leucinate	4.55×10^{-6}	6–8
Lysinate	2.67×10^{-5}	6–8
Methionate	9.00×10^{-7}	7
Ornithinate	1.04×10^{-5}	6–8
Phenylalanate	8.73×10^{-6}	6–8
Prolinate	3.05×10^{-5}	7, 8
Serinate	1.59×10^{-5}	6–8
Taurinate	6.23×10^{-5}	6–8
Threoninate	1.06×10^{-5}	6–8
Tryptophanate	1.00×10^{-6}	8
Tyrosinate	1.06×10^{-6}	6–8
Valinate	1.10×10^{-5}	6–8
Carbonate	4.40×10^{-4}	13
Phosphate	1.10×10^{-2}	10, 13
Sulphate	1.08×10^{-3}	10, 13
Ammonia	4.80×10^{-4}	10
Citrate	1.70×10^{-3}	12
Lactate	6.30×10^{-4}	10
Pyruvate	1.20×10^{-4}	10
Histamine	2.70×10^{-7}	10
Thiocyanate	1.70×10^{-4}	*
Silicate	1.30×10^{-4}	*
Malate	3.00×10^{-5}	*
Oxalate	1.00×10^{-5}	*
Salicylate	5.00×10^{-6}	*
Succinate	4.00×10^{-5}	*
Ascorbate	1.10×10^{-4}	*

* Estimate based on concentrations in blood plasma (Ref. 4).

Table 3. Metal ion concentrations in human milk

Metal ion	Free concentration (mol dm^{-3})	Total concentration (mol dm^{-3})	Reference
Ca(II)	2.52×10^{-3}	3.45×10^{-3}	14
Mg(II)	6.00×10^{-4}	9.28×10^{-4}	14
Cu(II)	1.00×10^{-14}	5.72×10^{-10}	15*
Zn(II)	2.00×10^{-10}	5.00×10^{-6}	15*
Fe(III)	1.00×10^{-20}	1.10×10^{-11}	15*
Sr(II)	1.01×10^{-6}	1.14×10^{-6}	3*

* Concentration in LMM fraction estimated from total concentration.

RESULTS AND DISCUSSION

The distribution of LMM strontium and calcium complexes in human milk are shown in Table 5. Those for bovine milk are given in Table 6. The similarities between the LMM distributions of calcium and strontium are clear. In human and bovine milk both of the metals are present mainly as the free metal ions, the major complexes present being citrate and phosphate species. Bovine milk has higher citrate and phosphate concentrations and this is reflected in the increased percentage of both calcium and strontium present complexed to these ligands. It is interesting to note that the strontium citrate complex is charged, whereas the strontium hydrogen phosphate species formed is neutral and thus more likely to cross cellular membranes and thus more bioavailable.

As a consequence of the chemical similarities between strontium and calcium, it is likely that analytical techniques used to remove Sr from milk will also deplete the biofluid of calcium. Analytical techniques have been developed which selectively remove strontium using ion exchange or nitrate

Table 4. Metal ion concentrations in bovine milk

Metal ion	Free concentration (mol dm^{-3})	Total concentration (mol dm^{-3})	Reference
Ca(II)	2.00×10^{-3}	3.76×10^{-3}	13
Mg(II)	8.10×10^{-4}	1.66×10^{-3}	13
Cu(II)	1.00×10^{-15}	5.72×10^{-10}	15*
Zn(II)	1.00×10^{-8}	1.55×10^{-4}	15*
Fe(III)	1.00×10^{-8}	1.00×10^{-15}	15*
Sr(II)	1.01×10^{-6}	1.34×10^{-6}	3*

* Concentration in LMM fraction estimated from total concentration.

Table 5. Predominating low-molecular-mass Sr(II) and Ca(II) complexes in human milk

Metal ion	Species formed*	log β	% metal in LMM fraction
Sr(II)	Sr^{2+}		87.8
	$Sr(CTA)^-$	2.8	8.0
	$Sr(PO_4)H^0$	12.71	1.8
	$Sr(LTA)^+$	0.90	0.4
	$Sr(SO_4)^0$	1.00	0.4
	$Sr(PO_4)H_2^+$	18.48	0.2
	$Sr(GLU)H^+$	9.50	0.1
	$Sr(MLA)^0$	1.60	0.1
Ca(II)	Ca^{2+}		73.0
	$Ca(CTA)^-$	3.26	19.0
	$Ca(PO_4)^-$	6.05	2.1
	$Ca(PO_4)H^0$	12.70	1.5
	$Ca(CO_3)H^+$	10.90	1.0
	$Ca(GLU)H^+$	10.40	0.9
	$Ca(LTA)^+$	1.30	0.9
	$Ca(SO_4)^0$	1.20	0.5
	$Ca(PO_4)H_2^+$	18.60	0.2

* Abbreviations used in Tables 5–8: CTA, citrate; PO_4, phosphate; LTA, lactate; CO_3, carbonate; GLU, glutamate; SO_4, sulphate; MLA, malate; CRE, dicyclohexyl-18-crown-6.

Table 6. Predominating low-molecular-mass Sr(II) and Ca(II) complexes in bovine milk

Metal ion	Species formed	log β	% metal in LMM fraction
Sr(II)	Sr^{2+}		74.7
	$Sr(CTA)^-$	2.80	12.3
	$Sr(PO_4)H^0$	12.71	10.2
	$Sr(PO_4)H_2^+$	18.48	1.0
	$Sr(LTA)^+$	0.90	0.4
	$Sr(PO_4)^-$	4.00	0.1
	$Sr(MLA)^0$	1.60	0.1
	$Sr(SO_4)^0$	1.00	0.1
Ca(II)	Ca^{2+}		53.2
	$Ca(CTA)^-$	3.26	25.0
	$Ca(PO_4)^-$	6.05	10.0
	$Ca(PO_4)H^0$	12.70	7.1
	$Ca(PO_4)_2H_3^-$	32.30	1.2
	$Ca(PO_4)H_2^+$	18.60	0.9
	$Ca(PO_4)_2H_2^{2-}$	25.30	0.7
	$Ca(LTA)^+$	1.30	0.6

Table 7. Predominating low-molecular-mass Sr(II) and Ca(II) complexes in human milk in the presence of 10^{-4} mol dm^{-3} dicyclohexyl-18-crown-6

Metal ion	Species formed	log β	% metal in LMM fraction
Sr(II)	$Sr(CRE)^{2+}$	5.0	82.2
	Sr^{2+}	—	15.8
	$Sr(CTA)^{-}$	2.8	1.4
Ca(II)	Ca^{2+}	—	72.2
	$Ca(CTA)^{-}$	3.26	18.8
	$CaPO_4^{-}$	6.05	2.0
	$CaHPO_4^{0}$	12.70	1.4
	$Ca(CRE)^{2+}$	2.51	1.2

Table 8. Predominating low-molecular-mass Sr(II) and Ca(II) complexes in bovine milk in the presence of 10^{-4} mol dm^{-3} dicyclohexyl-18-crown-6

Metal ion	Species formed	log β	% metal in LMM fraction
Sr(II)	$Sr(CRE)^{2+}$	5.00	84.5
	Sr^{2+}	—	11.7
	$Sr(CTA)^{-}$	2.80	1.9
	$SrHPO_4^{0}$	12.71	1.6
Ca(II)	Ca^{2+}	—	53.0
	$Ca(CTA)^{-}$	3.26	24.9
	$CaPO_4^{-}$	6.05	9.9
	$CaHPO_4^{0}$	12.70	7.0

precipitation. Kimura has developed a method of selectively removing strontium from milk involving liquid–liquid extraction using the macrocyclic ether dicyclohexyl-18-crown-6.[16] This method relies on the ability of the ligand to complex strontium more strongly than calcium. This was evaluated using the ECCLES program using formation constants for dicyclohexyl-18-crown-6 reported in the literature.[17] Tables 7 and 8 show the effect on the LMM distribution of Sr(II) and Ca(II) ions in human and bovine milk of the presence of 10^{-4} mol dm^{-3} of dicyclohexyl-18-crown-6. Clearly, the crown ether is selective for strontium ions mobilising some 80% of the strontium whilst the calcium distribution is virtually unaffected. This selectivity for Sr(II) ions is undoubtedly due to the difference in ionic radii between the Ca^{2+} and Sr^{2+} ions, Sr^{2+} being of optimum size to sit in the hole of the crown ether molecule. Hence, such liquid–liquid extraction techniques may successfully be used to selectively remove strontium from milk for analysis purposes.

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