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CALCIUM, STRONTIUM AND RADIUM CONTENT OF MOLLUSCAN SHELLS

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INTRODUCTION

SHELL formation in molluscs is intimately associated with the metabolism of calcium (Wilbur and Yonge, 1964). Strontium and radium are also taken up by molluscan shells because of their chemical similarity to calcium. Strontium/calcium ratios in shells of molluscs have been shown to depend both on strontium/calcium ratios in water and on the relative proportions of calcium carbonate polymorphs, viz., calcite, aragonite and vaterite, in the shells (Thompson and Chow, 1955). Likewise, radium/calcium ratios in shells have been discussed in relation to the corresponding ratios in sea water (Koczy and Titze, 1958).

In continuation of work on trace element distribution in marine invertebrates (Bhatt *et al.*, in press), data are presented in this paper on the strontium, radium and calcium contents of shells of molluscan species from Indian coastal waters.

METHODS

Molluscan shells were collected during 1964-65 ; details of locality, dates and the radium content of sea water of the localities from where shells were collected are given in Table 1.

Determination of calcium and strontium

After being washed and freed of epiphytic matter, the shells were dried in an air oven at 100-110°C. 1 g. of oven-dried shell was dissolved in 60 ml. hydrochloric acid (1N). The solution was evaporated to dryness and the residue taken up in 50 ml. of acetic acid (2%). The solution was freed of phosphate by passing through a column of Dowex 1×8, 50-100 mesh anion exchanger (Hinson, 1962). Calcium and strontium were determined in phosphate free effluent by atomic absorption spectrophotometry using Perkin-Elmer Model 303 instrument (Panday *et al.*, 1966).

Determination of radium

Radium was coprecipitated with barium as sulphate (Goldin, 1961) and the sulphates dissolved in ammonical ethylene diaminetetra acetate (EDTA) solution. To effect a separation of calcium, radium and barium, Weiss and Lai (1961) passed a solution of their EDTA complexes through an ion-exchanger at controlled pH. From an inspection of the stability constants of the EDTA complexes of the various elements (Table 2), it was considered feasible in the present instance to use magnesium salts to effect an analogous separation and selective precipitation of barium.

Five grams of washed and oven-dried shell were dissolved in the minimum amount of nitric acid (1 : 4). Insoluble matter was separated on the centrifuge and treated with 5 ml. of hydrofluoric acid (40%), followed by fuming with three 5 ml. lots of perchloric acid (60%). The acid extract of the residue was transferred to the original solution with a few ml. of hydrochloric acid (1N). 100 ml. of amonium citrate (3N) were added to complex the metal ions, followed by the addition of 2 ml. of lead nitrate solution (100 mg. Pb/ml.) and 1 ml. of barium nitrate solution (6.7 mg. Ba/ml.). The solution was heated to 90°C. and the sulphates precipitated by the addition of sulphuric acid (1 : 1). The mixed precipitate of sulphate was let to settle overnight, removed on the centrifuge, washed twice with concenrated nitric acid and then dissolved in ammoniacal EDTA (0.25 M). The solution was diluted to 500 ml. and treated with a few drops of Eriochrome Black T indicator solution (1 mg. Eriochrome Black T in 1 ml. of methyl cellosolve). The solution was heated to 60°C. and magnesium sulphate solution (1N) added dropwise till the solution changed in colour from blue to distinct pink.

The precipitate of barium sulphate was dissolved in ammoniacal EDTA, the solution diluted to 500 ml. and heated to 60°C. Acetic acid (1 : 1) was added dropwise till a pH of 4.0 was attained. Addition of a few ml. of ammonium sulphate (1N) facilitated the complete precipitation of barium sulphate. The precipitate was let settle overnight, removed on the centrifuge, dissolved in ammoniacal EDTA and reprecipitated as before. The strontium-free precipitate was washed with water twice and transferred to a tared stainless steel planchette, dried under infrared lamp and aged for 70 days for decay of radium-223 and radium-224. Before counting, the planchette was flamed at 800°C. and kept aside for 30 minutes for decay of interfering alpha-emitters. The alpha activity of the sulphate precipitate was determined in a low-background zinc sulphide (silver activated) scintillation counter with an average efficiency of 16%. In the calculation of radium-226 activity, appropriate corrections were applied (FAO-WHO, 1959).

Known amounts of radium-226 were coprecipitated with barium in the presence of 25 mg. strontium and 3 g. calcium. Results of these experiments are given in Table 3.

Results and Discussion

Data on calcium, strontium and radium contents of molluscan shells are shown in Table 4.

In the molluscan shells investigated, the calcium content is in the narrow range 0.33-0.40 g. Ca/g. shell and constitutes on an average 37% by weight of the dry shell. The strontium content is highest in *Sepia*.

The phylogenetic aspects of variations is strontium/calcium ratios in shells of marine organisms were studied by Lowenstam (1964). The ratios were higher in the lower phyla such as the Bryozoa in which the mineralogy of shell is a more decisive factor than physiological control by the organism. In the advanced Mollusca the organisms actively discriminate against strontium with resultant low strontium/calcium ratios in shell. A special case is found in *Sepia* and this is attributed by Lowenstam to the shell being imbedded deep in tissue. The strontium/calcium ratios of 4.8×10^{-3} and 5.2×10^{-3} observed in *Sepia* shell in the present study (Table 5a) are similar to the ratios reported in the same genus by Lowenstam (viz.,

$4.6\text{-}4.8 \times 10^{-3}$). The low values in *Crassostrea* are comparable to those reported in *Ostrea* by Thompson and Chow (1955).

TABLE 1

Details of collection of molluscan shells

Sample No.	Species	Location		Date of collection	Radium-226 (seawater) ppm $\times 10^{10}$
1	*Anadara granosa*	Kalpakam	Aug.	1964	
2	*Anadara granosa*	Sewree (Bombay)	Nov.	1964	1.40
3	*Anadara granosa*	Sewree (Bombay)	Apr.	1965	1.40
4	*Crassostrea gryphoides*	Mahim (Bombay)	Nov.	1964	1.30
5	*Crassostrea gryphoides*	Mahim (Bombay)	Dec.	1964	1.30
6	*Crassostrea gryphoides*	Mahim (Bombay)	Feb.	1965	1.30
7	Cuttlebone of *Sepia*	Juhu (Bombay)	Oct.	1964	1.01
8	Cuttlebone of *Sepia*	Afghan Church (Bombay)	Oct.	1964	1.01
9	Cuttlebone of *Sepia*	Afghan Church	Dec.	1964	1.01
10	Cuttlebone of *Sepia*	Madras	Mar.	1965	
11	Cuttlebone of *Sepia*	Kovalam	Mar.	1965	
12	*Mytilus* sp.	Kalpakam	Aug.	1964	
13	*Mytilus* sp. (1″ length)	Trivandrum	Mar.	1965	
14	*Mytilus* sp. (2″ length)	Kovalam	Mar.	1965	
15	*Mytilus* sp.	Manavalakurichi	Mar.	1965	
16	*Donax scortum*	Kalpakam	Aug.	1964	
17	*Donax* sp.	Neendakarai	Jan.	1964	
18	*Donax* sp.	Neendakarai	Nov.	1964	
19	*Katelysia marmorata*	Sewree (Bombay)	Jan.	1965	1.40
20	*Katelysia marmorata*	Sewree (Bombay)	Feb.	1965	1.40
21	Veneridae sp.	Kalpakam	Aug.	1964	
22	*Chione* sp.	Kalpakam	Aug.	1964	
23	*Cardium* sp.	Kalpakam	Aug.	1964	
24	*Erachyodontes karachiensis*	Sassoon Dock (Bombay)	Nov.	1964	1.40

Differences in strontium/calcium ratios are also caused by factors other than phylogeny and shell mineralogy. These include temperature and salinity of water and organic processes (cf. Harris, 1965). To study the differential behaviour of strontium, Comar *et al* (1957) introduced the term Observed Ratio (OR) defining it as follows :

$$\text{OR sample-precursor} = \frac{\text{Strontium/calcium content of sample}}{\text{Strontium/calcium content of precursor}}$$

On the basis of an average strontium/calcium atom ratio of 8.72×10^{-3} in sea water (Cf. Viswanathan *et al.*, in press), OR values for strontium in sample Nos. 1-24 range from 0.14-0.59 and indicate discrimination against strontium relative to calcium.

Strontium/calcium atom ratios reported by Thompson & Chow (1955) are compared with our values in Table 5b. In the determination of strontium and calcium by flame-photometry, Thompson & Chow have not indicated clearly the efforts made to remove the interfering phosphate. In the present study, however, care has been taken to eliminate the phosphate.

TABLE 2

Stability constants of EDTA complexes of metals (Schwarzenbach, 1960)

Element	Stability constant
Barium^{+2}	7.76
Strontium^{+2}	8.63
Magnesium^{+2}	8.69
Calcium^{+2}	10.7
Lead^{+2}	18.04

TABLE 3

Coprecipitation of radium-226 with barium in the presence of calcium (3 g.) and strontium (25 mg.)

Radium-226
(disintegrations in 4 hours)

Added	Recovered	Percentage recovery
6468	6348	97.95
6336	6252	98.68
3012	2934	97.40
6600	6420	97.27

TABLE 4

Strontium, calcium and radium-226 contents of molluscan shells

Sample No.	Calcium g/g	Strontium ppm	Radium $\times 10^7$ ppm
1	0.354	1950	..
2	0.375	1000	0.40
3	0.368	1400	0.65
4	0.375	1250	0.27
5	0.377	1350	0.30
6	0.380	1000	0.40
7	0.375	4250	0.60
8	0.325	3400	0.85
9	0.377	2350	0.62
10	0.352	2200	0.92
11	0.350	1700	1.20
12	0.360	2500	..
13	0.336	2100	1.30
14	0.379	1350	1.00
15	0.385	1400	0.80
16	0.350	1950	..
17	0.375	1560	0.71
18	0.375	1330	..
19	0.380	2000	0.42
20	0.380	2500	0.51
21	0.350	1830	..
22	0.400	2700	..
23	0.367	1400	..
24	0.375	2870	..

The higher radium contents are observed in *Mytilus* shells from the vicinity of the high radiation 'Monazite belt' in Kerala. A similar association between the radioactivity of marine organism (alga) and that of the environment has been observed by Unni and Viswanathan (in press) in their studies on the gamma spectra of marine algae. Amongst samples from the non-monazite areas with

TABLE 5a

Strontium/calcium and radium/calcium atom ratios in molluscan shells

Sample No.	Atom ratios	
	$Sr/Ca \times 10^3$	$Ra\text{-}226/Ca \times 10^{14}$
1	2.14	..
2	1.26	1.89
3	1.74	3.13
4	1.52	1.28
5	1.64	1.41
6	1.20	1.86
7	5.18	2.83
8	4.78	4.63
9	2.85	2.90
10	2.85	4.62
11	2.22	6.07
12	3.16	..
13	2.85	6.84
14	1.62	4.67
15	1.66	3.68
16	2.55	..
17	1.90	3.35
18	1.62	..
19	2.40	1.95
20	3.00	2.37
21	2.39	..
22	3.08	..
23	1.74	..
24	3.49	..

TABLE 5b

Average Sr/Ca atom ratios in molluscan shells: Comparative study

Group	*Strontium/Calcium*	
	Thompson & Chow (1955)	Present studies
Ostreidae	1.22	1.45
Mytilidae	1.56	2.32
Veneridae	1.86	2.30

normal natural radiation background, the highest radium/calcium ratios are observed in *Sepia* shell and the lowest in *Crassostrea* (Table 5a). Koczy and Titze (1958) have given radium/calcium atom ratio varying from $2.35\text{-}4.43 \times 10^{-14}$. In the present studies data on the radium content in sea water is not available for all the regions from where the biological samples were collected. Wherever possible, the values obtained for radium content in sea water from the sampling regions are given in Table 1. From that it is seen that radium content varies from $1.01\text{-}1.40 \times 10^{-10}$ ppm, with corresponding radium/calcium atom ratio varying from $4.47\text{-}5.20 \times 10^{-14}$. OR values calculated vary from 0.22-1.04. Amongst, the calculated OR values the lowest is found in *Crassostrea*, and highest in *Sepia*.

Summary

Molluscan shells from Indian coastal waters have been examined for strontium, radium, calcium contents and strontium/calcium and radium/calcium ratios. In the 24 samples analysed, the strontium/calcium atom ratio ranged from $1.2\text{-}5.2 \times 10^{-3}$. The highest strontium/calcium ratio was observed in Cuttlefish (*Sepia*) and the lowest in Oyster (*Crassostrea*). Radium/calcium atom ratios varied from $1.4\text{-}6.8 \times 10^{-14}$.

A method has been standardised for the determination of radium in calcareous shells.

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References

Bhatt, Y. M., Sastry, V. N., Shah, S. M. and Krishnamoorthy, T. M. Zinc, manganese and cobalt contents of some marine bivalves from Bombay. *J. Nat. Inst. Sci. India* (in press).

Comar, A. L., Russell, R. S. and Wasserman, R. H. 1957. Strontium/calcium movements from soil to man. *Science*, **126** : 485.

FAO-WHO. 1959. Methods of radiochemical analysis. *Report of joint WHO/FAO Expert Committee, Atomic Energy Series*, **1**.

Goldin, A. S. 1961. Determination of dissolved radium. *Anal. Chem.* **33** : 406.

Hinson, W. H. 1962. An ion exchange treatment of plant ash extracts for/removal of interfering anions in the determination of calcium by atomic absorption. *Spectrochim. Acta*. **18** : 427.

Koczy, F. F. and Titze, H. 1958. Radium content of carbonate shells. *J. Mar. Res.*, **17** : 302-311.

Lowenstam, H. A. 1964 ed. Strontium/calcium ratio of skeletal aragonite from the recent marine biota at Palau and from Fossil Gastropods. *Isotopic and cosmic chemistry*, North Holland Pub. Co., Amsterdam.

Panday, V. K., Joseph, K. T. and Soman, S. D. 1966. Studies on atomic absorption spectrophotometry and its application in Health Physics works. *Rpt. AEET/HP/TM-13, Bhabha Atomic Research Centre, Bombay.*

SCHWARZENBACH, G. 1960 ed. *Complexometric titrations*, Mathew and Co. Ltd., pp. 8.

THOMPSON, T. G. AND CHOW, T. J. 1955. The strontium/calcium atom ratio in carbonate secreting marine organisms. *Deep Sea Research, Suppl.* 3 : 20.

UNNI, C. K. AND VISWANATHAN, R. 1968. Gammaspectrometric analysis of marine algae from West Coast of India. *Health Physics* (in press).

VISWANATHAN, R., SHAH, S. M. AND UNNI, C. K. 1968. Atomic absorption and fluorometric analysis of sea water in the Indian Ocean. *J. Nat. Inst. Sci. India* (in press).

WEISS, H. V. AND LAI, M. G. 1961. Radiochemical determination of radium in urine. *Anal. Chem.*, 33 : 39.

WILBUR, K. M. AND YONGE, C. M. 1964 ed. *Physiology of Mollusca*, 1, Academic Press, New York.