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 9. We thank R. Estes for permission to include his identifications of the nonmammalian vertebrates of Purgatory Hill and S. Babb for the drawings. Investigation supported in part by NSF grant GB-1798 to L.V. and by NSF grant GB-2213 to R.E.S.
 10. Use of minimum number of individuals always tends to overrepresent the relative frequency of species with one or a few specimens; use of total number of specimens has a greater error variance only if elements of the same individual are prevalent. Such overrepresentation of single individuals can be tested by a comparison of the calculated minimum number of individuals for each species with that expected on the basis of random sampling of the same number of elements each from a different individual, and then combining the probabilities. In the present case no overrepresentation is shown but the numbers are small. The number of identifiable parts is comparable among the mammals except for the multituberculates, where it is smaller. Minimum number was estimated using tooth homology, side of mouth and attrition.
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 13. This genus also occurs in the early Tiffanian of the Bison basin, Wyoming.
 14. *Chriacus pugnax* is referable to *Oxycloenus* by parts now known, as is *C. antiquus*.
 15. *Tricentes crassicolleus*, the type species of *Tricentes*, is a synonym of "*Chriacus*" *truncatus*. The other species of *Tricentes* are referable to *Mimotricentes*, and *Tricentes* is a genus distinct from *Chriacus* and is a senior synonym of *Epichriacus*, *Metachriacus*, and possibly *Prothryptacodon*.
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Strontium and Magnesium in Water and in *Crassostrea* Calcite

Abstract. *Distribution of magnesium and strontium was determined between waters and calcites secreted by the oyster species Crassostrea virginica and C. rhizophorae in natural habitats at eight localities, from Maine to Puerto Rico. The concentration of strontium in the calcite shells increases with increasing temperature in the range 13° to 25°C, and also with increasing Sr⁺⁺/Ca⁺⁺ molal ratio in the water. The concentration of magnesium in the shells increases irregularly with temperature, and it is apparently independent of the Mg⁺⁺/Ca⁺⁺ ratio in the water. The greater variation with temperature in the distribution factor for magnesium may be related to genetic differences between semi-isolated populations.*

A number of investigators in recent years studied various aspects of the occurrence of minor elements in the calcareous skeletons of marine invertebrates, such as general patterns of the distribution of minor elements in Recent and fossil phyla, and correlation of the concentration of minor elements in the skeletons with taxonomic level, geologic age, mean annual temperatures, and salinities (1). I report here my study of the composition of a biogenic carbonate as a function of more narrowly defined environmental conditions.

In order to relate a biogenic calcite of composition (Ca_{1-x-y}Mg_xSr_y)CO₃ to the environmental conditions under which it was formed, at least the following factors must be considered: (i) location of magnesium and strontium in calcite; (ii) change in the composition of calcite, in terms of the mole fractions N_{MgCO₃} and N_{SrCO₃}, with changes in temperature and water composition; (iii) possible effects of such inherent genetic differences as may exist between animal groups (such as between individuals or between subspecies) which secrete shells of comparable composition.

X-ray diffraction measurements (2-4) show that in biogenic carbonates MgCO₃ and SrCO₃ are in solid solution with CaCO₃. However, up to approximately 10 percent of the total MgCO₃ may be present in noncrystalline form in some high-magnesian algal and echinoid calcites (4), and some strontium has been reported to occur in a similar state in an aragonitic gastropod shell (3).

In order to determine the distribution of strontium and magnesium between biogenic calcite and water under natural conditions, the following has been done: water samples and temperature readings were taken in the upper 0.6 m of water for 5 to 10 months at approximately monthly intervals at the localities listed in Table 1 (5). At the end of the sampling period I collected live specimens of the American oyster, *Crassostrea virginica*, at seven North American localities, and *C. rhizophorae* in Puerto Rico. The linear growth of the shell takes place by increments at the ventral margin; therefore, the ventral portions of the shells, secreted within the sampling period, were treated with Clorox to remove organic matter, and were subsequently analyzed for magnesium and strontium (calcium was found by subtraction). The bulk of the secreted material is the inner calcitic layer of the shell, insofar as the outer prismatic layer in oysters is developed only discontinuously and very poorly (6). However, since the rates of growth under natural conditions are not known accurately, the correspondence between the last grown increment and the entire sampling time may not be exact. This uncertainty is likely to be reflected in some of the scatter of the points in Figs. 2 and 3.

Calcium and magnesium in sea water were determined by volumetric titration with disodium dihydrogen(ethylenedinitrilo)tetraacetate, with Metab (Fisher Scientific Co.) as indicator. Strontium was determined by x-ray fluorescence spectrometry, with reproducibility of about ±6 percent, in evaporated residues of 50 ml of the

Table 1. Sampling localities, water and temperature sampling periods, size of *Crassostrea* samples used for analysis, and depth of sample collection.

Locality, lat. N, long. W	Sampling period	Sample size (specimens)	Water depth (m)
Boothbay Harbor, 43°50.6', 69°38.5'	May to Sept. 1964	8	1
Great Bay, 43°07.8', 70°53.1'	June 1963	10	2
Pond Point, 41°11.7', 73°01.2'	May to Sept. 1964	3	8
Franklin City, 38°00.4', 75°23.2'	May to Oct. 1964	6	Intertidal
Beaufort Inlet, 34°43.4', 76°40.8'	May to Oct. 1964	5	<1
Newport River, 34°45.1', 76°41.4'	May to Oct. 1964	4	<1
Pass Marianne, 30°14', 89°15'	July 1964 to March 1965	8	3
Aransas Bay, 28°03.5', 96°57.5'	May 1964 to Feb. 1965	6	1
Magueyes Island, 17°58', 67°03'	June 1964 to March 1965	8	Intertidal mangrove

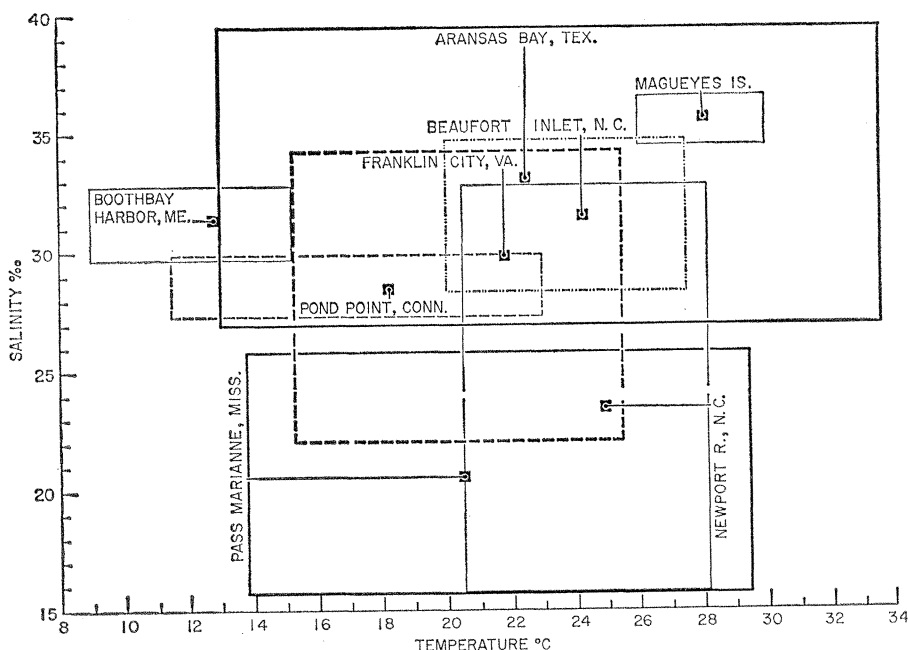


Fig. 1. Temperatures and salinities during the sampling period. Observed ranges at each locality shown by rectangles; means shown by heavy dot in each rectangle.

sample water to which 0.4 mg of yttrium in 2 ml of aqueous solution was added as an internal standard. In calcites, the precision of the strontium determinations by x-ray fluorescence is ± 3 percent; for the magnesium analyses, done spectrophotometrically with thiazole yellow as the indicator, the precision is approximately ± 5 percent. Chlorinities of the water samples were determined by volumetric titration with silver nitrate and fluorescein indicator. Salinities were calculated from the chlorinity values.

In general, the variation in temperature and salinity for most of the localities is great: the total ranges of the temperature and salinity values and their means for the sampling periods are shown in Fig. 1. Variation in salinity is small in relatively large bodies of water open to the sea, whether they receive river flow (Boothbay Harbor, Maine; Pond Point, Long Island

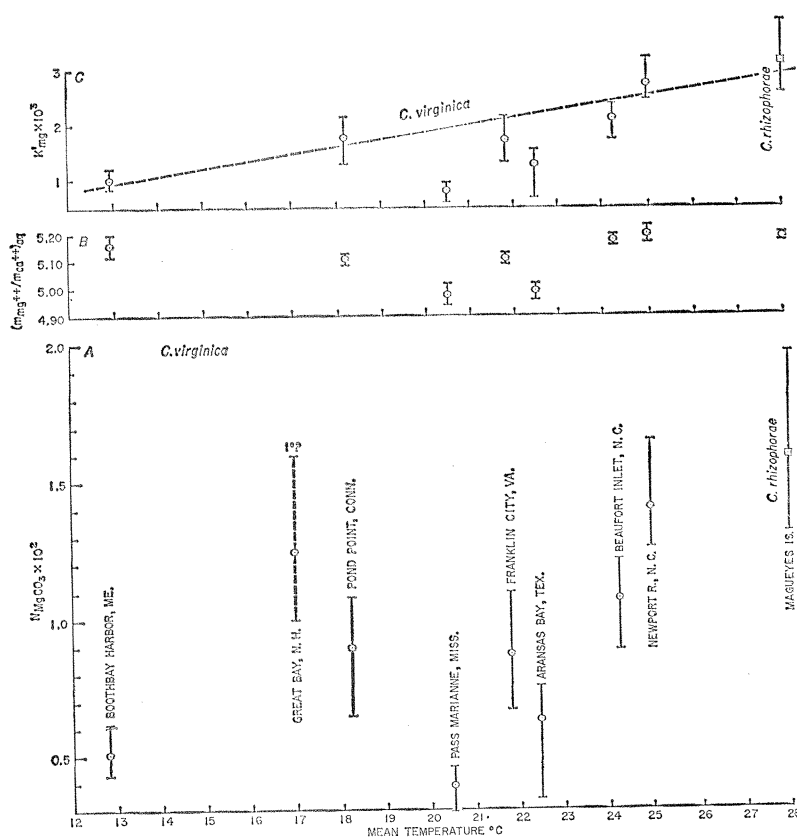
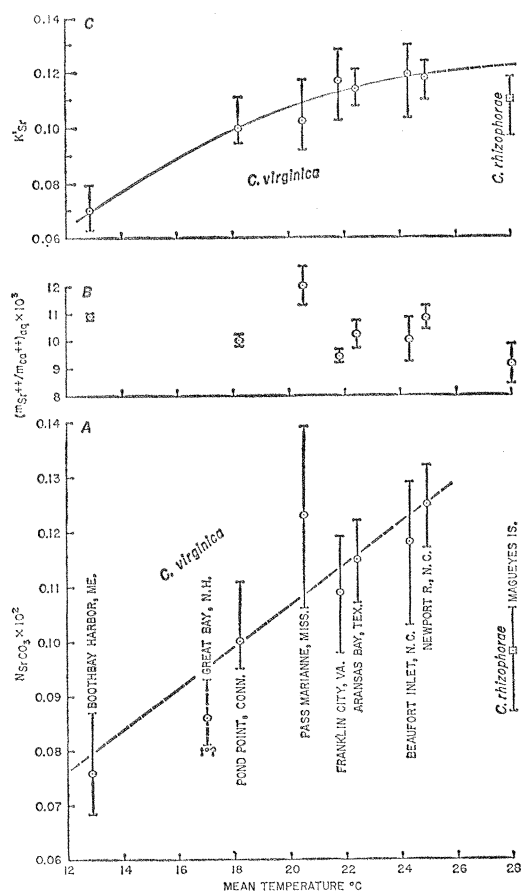


Fig. 2 (left). Strontium in *Crassostrea* calcites and water. (A) Mole percent ($N_{SrCO_3} \times 10^2$) strontium in the shell as a function of the mean temperature of sampling period; dots (in circles), sample means; vertical bars, range of N_{SrCO_3} in each sample; dashed line, least-squares regression to the *C. virginica* means ($N_{SrCO_3} \times 10^2 = 0.0307 + 0.0038t^\circ C$). (B) Sr^{++}/Ca^{++} molal ratio in waters; dots indicate means; vertical bars are $\pm$ standard error. (C) Distribution factor K'_{Sr} for strontium in calcite and water as a function of temperature; dots indicate means; vertical bars, range of K'_{Sr} values for each sample; curve, least-squares regression, $K'_{Sr} = -0.0513 + 0.0122t - 0.000215t^2$, to the *C. virginica* means. Fig. 3 (right). Magnesium in *Crassostrea* calcites and waters. Notation as in Fig. 2. (A) Mole percent $MgCO_3$ in the shell as a function of the mean temperature of sampling period. (B) Mg^{++}/Ca^{++} molal ratio in waters. (C) Distribution factor K'_{Mg} as a function of temperature; positive correlation for all eight points significant at .025 level; dashed line is the least squares fit to the upper six points: $K'_{Mg} = -0.79 + 0.1326t$.

Sound) or not (Magueyes Island, Puerto Rico). Much greater variation in salinity occurs in bodies of water with restricted sea connections, as at Long Reef in Aransas Bay, Texas, Franklin City on Chincoteague Bay, Virginia, and at Crab Point in the Newport River, North Carolina. Stations located landward of the offshore barrier islands but close to ocean inlets are intermediate in their salinity variation (Radio Island, Beaufort Inlet, North Carolina; Pass Marianne in Mississippi Sound). The large temperature spread for Aransas Bay and Mississippi Sound (Fig. 1) is due to long water-sampling periods. Sampling periods longer than about 6 months at the Atlantic Coast localities would give misleading data as shell secretion is usually interrupted by the cold season.

The strontium concentration in the shells of *C. virginica* increases with increasing mean temperature of the sampling period, as shown in Fig. 2A. The dashed line, fitted by least squares, passes through all the observed ranges of the strontium values (7). The Caribbean species, *C. rhizophorae*, is somewhat off the trend displayed by *C. virginica*.

The concentration of magnesium in the grown portions of *Crassostrea* shells also shows an increase with increasing temperature (Fig. 3A), although it is less well pronounced. In particular, the two samples from the Gulf Coast deviate from the more general trend.

From the analytical data, the coefficients of distribution of strontium and magnesium, respectively, between calcites and waters have been computed:

$$K'_{Sr} = \frac{(N_{SrCO_3}/N_{CaCO_3})_{ca}}{(m_{Sr^{++}}/m_{Ca^{++}})_{aq}}$$

and

$$K'_{Mg} = \frac{(N_{MgCO_3}/N_{CaCO_3})_{ca}}{(m_{Mg^{++}}/m_{Ca^{++}})_{aq}}$$

where K' is the distribution coefficient, N is the mole fraction of a component in calcite, and m is the molal concentration of the cation in the water. Plot of K'_{Sr} against temperature in Fig. 2C shows that the distribution coefficient changes with temperature rather than with the Sr^{++}/Ca^{++} ratio in solution. It may be noted that the high strontium concentration in the sample from Pass Marianne, Mississippi, is probably due to the higher value of the Sr^{++}/Ca^{++} ratio in the water (Fig. 2B), as the value of K'_{Sr} for that sample is fairly consistent with other values.

If the distribution of strontium between water and live shell of *C. virginica* is primarily a function of the water temperature and the ratio $m_{Sr^{++}}/m_{Ca^{++}}$, then it may be compared with an equilibrium distribution in an inorganic system. At 25°C the value of K'_{Sr} in the system strontian calcite-aqueous solution is about 0.14 ± 0.02 (8), which is close to $K'_{Sr} \cong 0.12$ for the *C. virginica* calcite at the same temperature. The dependence of K'_{Sr} in inorganic systems on temperature above and below 25°C is not yet completely understood (9), and consequently the correspondence between biogenic and inorganic calcites at other temperatures is not certain.

The distribution coefficient for magnesium (Fig. 3C) retains the spread of the magnesium concentrations in the samples. However, K'_{Mg} correlates positively with temperature ($r = .771$, significant at the .025 level). Concentrations in the range 0.5 to 1.5 mole percent $MgCO_3$ in calcitic shells (Fig. 3A) are lower than the calculated concentrations in calcite at equilibrium with solution in which $m_{Mg^{++}}/m_{Ca^{++}}$ is about 5, as in Fig. 2B (10). The non-equilibrium and a greater variation in the concentration of magnesium in shells may be due to its greater participation in the physiological functions of animals, in general, and marine invertebrates in particular (11). The samples in Fig. 3A which pronouncedly deviate from the general trend are from endemic populations in the Middle Oyster River, Great Bay, New Hampshire, and in Aransas Bay; the Pass Marianne sample is also of local stock. As opposed to these occurrences, many oysters have been artificially transported between the Middle and North Atlantic sections of the coast, which could have obliterated some of the original differences between the populations. The dependence of magnesium concentrations on the geographic location of the sample is compatible with the reported genetic differences between the temperature races of *C. virginica* (12).

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Immunoelectrophoresis Reveals Collagen Solubility in Human Serum

Abstract. *A fraction of human-serum proteins which contains hydroxyproline migrates, on electrophoresis, with the α_2 - β -globulins. Immunoelectrophoresis with rabbit anti-serum to soluble human collagen reveals an immunologically collagen-like protein in the human serum.*

Le Roy et al. (1) have shown that a protein containing hydroxyproline exists in human plasma. The protein, termed "hypro-protein," is presumed to be collagen or one of its precursors; it has been identified by Sephadex-gel filtration as either an