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THE INCORPORATION OF MAGNESIUM INTO THE SKELETAL CALCITES OF ECHINODERMS

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ABSTRACT. Approximately 1800 magnesium analyses of echinoid, asteroid, ophiuroid, crinoid, and holothuroid skeltons show that the distribution of this element in echinoderm calcite is a function of both environmental and genetic factors. The analyzed specimens represent inhabitants of polar to equatorial seas, from littoral to abyssal depths. Systematic compositional variations occur at many levels: (1) within a single skeletal component, for example the echinoid tooth; (2) within a skeleton of a single animal, for example the coronal plates (10.4), spines (9.6), pyramids (11.8), teeth (8.0), rotulae (10.9), and epiphyses (12.1) of a single specimen of *Tripneustes gratilla* from New South Wales, Australia, all concentrations being expressed in wt percent $MgCO_3$; (3) within a single echinoderm population, for example homologous skeletal components of different echinoid genera from the same marine community, such as the coronal plates of *Heterocentrotus* and *Echinometra* (15.6), *Tripneustes* (11.7) and *Diadema* (14.8) from Singatoka, Fiji. The mean magnesium content of coronal plates from 235 echinoids is 11.55 wt percent $MgCO_3$; observed maximum and minimum values are 17.1 and 5.5 percent respectively. For 565 asteroids studied, mean, maximum, and minimum skeletal Mg concentrations are 14.0, 18.5, and 8.3 percent respectively. The observations are consistent with a model based on rate of calcification and amino acid compositional variations in the mineralizing organic matrix. Unlike their Recent counterparts whose skeletons consist of high-magnesium calcite, all but one (of Pleistocene age) of the fossil echinoids examined were found to contain only a few percent of magnesium in their skeletal calcite. Dolomitization of such skeletal matter appears to be a rare event; recrystallization to the more stable low-magnesium calcite is the most important diagenetic process.

INTRODUCTION

The distribution of magnesium in the carbonate skeletons of marine invertebrates has an important bearing on a number of problems in the earth sciences. These problems, together with the status of current research on skeletal chemistry, have been reviewed by Dodd (1967). Echinoderm calcites are particularly interesting, because the skeletal ossicles are essentially single crystals of thermodynamically-metastable high-magnesium calcite characterized by a pronounced fenestrate structure. Biochemically, the soft tissues in echinoderm skeletons are similar to those of the vertebrates (Travis and others, 1967) in that collagen is a primary component. Yet only in echinoderms has a mineral other than apatite been found associated with a collagenous matrix. Furthermore, in the case of echinoids the concentration of skeletal magnesium can, in a single animal, range in a systematic way between wide limits.

The mechanisms by which such high-magnesium calcites form are unknown. On the basis of a few analyses, Clarke and Wheeler (1917, 1922) reported a positive correlation between temperature and magnesium content. Chave (1954) published additional analyses which also demonstrated a relationship between these two variables. Furthermore, Chave showed that at a given temperature, the magnesium concentration in skeletal calcite was to some extent dependent on the phylogenetic level of the organism. Although the valuable analytical studies of Clarke and Wheeler, of Chave, and of Vinogradov (1953) established the foundation for research on biogenic magnesian calcites and revealed the most important trends in the distribution of magnesium in the skeletons of marine invertebrates, it is surprising that so many years have passed without much in the way of detailed investigation of echinoderms. Pilkey and Hower (1960) studied specimens of one species of *Dendraster,* and Raup (1966) provided data for different echinoids from Eniwetok Atoll. Otherwise our information is indeed meager, so much so in fact that meaningful comparisons of echinoderm skeletal compositions with those of other invertebrates are difficult to make. In figure 1 of Dodd (1967), for example, mean magnesium concentrations for algal, foraminiferal, sponge, bryozoan, brachiopod, molluscan, annelid, arthropod, and echinoderm calcites, taken from the literature, are plotted as a function of phylogenetic level. Although Dodd is well aware of the limitations of these data, the comparison is nevertheless of little value. The mean $MgCO_3$ content of echinoids is calculated from 12 analyses published by Clarke and Wheeler (1922) and 22 analyses made by Chave (1954). Yet, an average value obtained in such a manner could fall anywhere within a range from 3 to 17 wt percent $MgCO_3$ depending upon what parts of the sea urchin skeleton were analyzed, where the echinoids were collected, and what particular genera were selected.

The purpose of the investigation reported here is to determine how the magnesium content of echinoderm calcites is controlled by the interaction of these variables, which include both environmental and genetic factors. A theory to account for these observations is proposed, and some supporting arguments are discussed in detail. The quantity of data collected and analyzed in this study is too large to publish in the formal literature. Furthermore, as the concentration of skeletal magnesium is dependent upon type of skeletal element, taxa, and temperature, most of the information can be presented in the form of summary statistics which express the relationships between these variables. The individual data, however, are available to interested persons by personal communication. Analytical values for identified specimens from known localities are tabulated in Monograph 4 of the Materials Research Laboratory, copies of which can be obtained from the author free of charge until the supply is exhausted. Additional copies have been placed in the library of the College of Earth and Mineral Sciences, the Pennsylvania State University, for interlibrary loan or photocopy reproduction.

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THE NATURE OF ECHINODERM CALCITE:—ABUNDANCE, STRUCTURE, CHEMISTRY

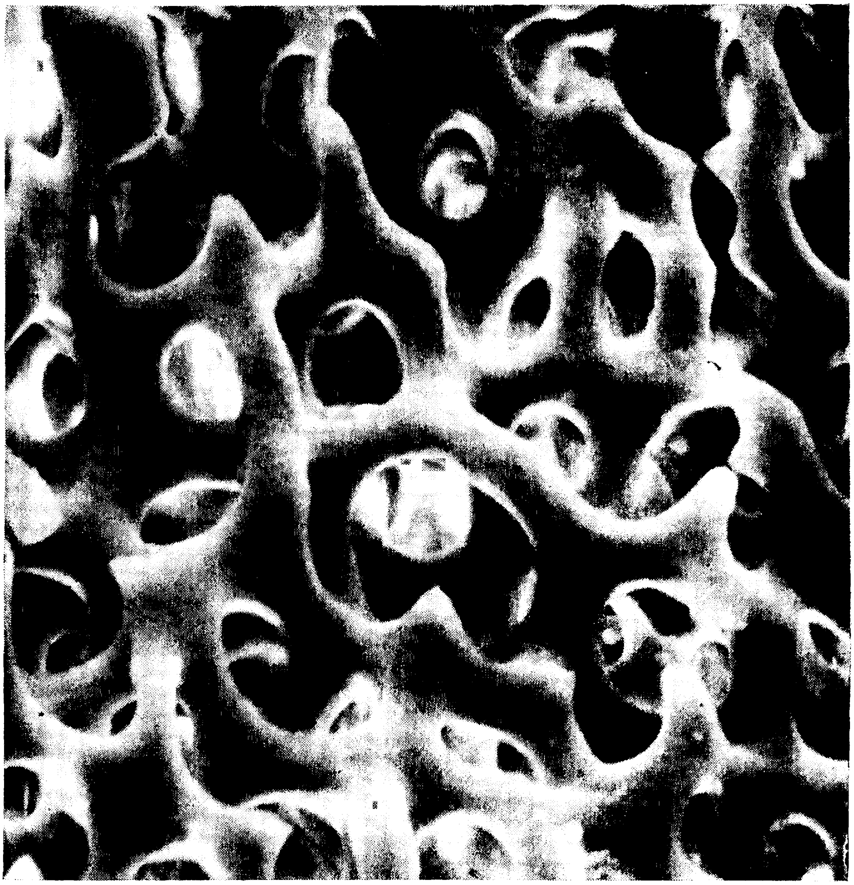
Living members of the phylum Echinodermata are referred to five classes: Echinoidea (sea urchins, heart urchins, sand dollars), Asteroidea (sea-stars), Ophiuroidea (brittle-stars), Crinoidea (sea-lilies, feather-stars), and Holothuroidea (sea cucumbers). Some of these animals are found on the ocean floor from the intertidal zone to abyssal depths, and they are widely distributed in polar to equatorial seas. Where environmental conditions are favorable, large populations of high density may be established; population density estimates for a variety of echinoderms have been reported by Fell and Pawson (1966), Zenkevitch (1963), Salsman and Tolbert (1965), Moore and others (1963), Vevers (1952), Thorson (1957), Fell (1961), Barham, Ayer, and Boyce (1967), Clarke, Flechsig, and Grigg (1967), Blegvad (1914), and others.

Echinoderms of each class precipitate a skeleton whose ossicles consist of high-magnesium calcite. The quantity of skeletal calcite in an animal of given size ranges widely from one genus to another. A specimen of the sea urchin *Heterocentrotus mammillatus* with a test 74 mm in diameter at the ambitus, for example, was found to contain a total of 247 g of calcite on a dry weight basis. Measurements of skeletal mass for echinoids, asteroids, and ophiuroids have been reported by Weber (1968).

Except for holothurians, in which the skeletal elements are usually microscopic sclerites in the form of plates, anchors, et cetera, the ossicles of an echinoderm skeleton are highly porous. Pronounced fenestrate structure, illustrated in plate 1, is evident in each of the major types of skeletal element in echinoids, asteroids, ophiuroids, and crinoids. Porosity determinations made by measuring the density of cylinders machined to a tolerance of 0.0001 in. from echinoid spines and by measuring the displacement of other skeletal elements of known mass, show that as little as 48 percent of the volume of an ossicle is accounted for by calcium carbonate. Porosity ranges widely from one type of ossicle to another and from one species to another; examples are given in table 1. Although there is some evidence that the exterior surface of echinoid coronal plates may be a polycrystalline aggregate with preferred orientation of the crystallites (Towe, 1967), most of the calcite of a skeletal ossicle appears to be precipitated in optical continuity such that, despite the fenestrate structure, the skeletal element is a single crystal (Raup, 1966; Currey and Nichols, 1967; Schroeder and others, 1968).

Echinoderm calcite contains few cations other than calcium and magnesium. Iron and strontium are minor elements (0.01 to 1 percent)

PLATE 1



Scanning electron photomicrograph showing the fenestrate structure of the single-crystal, high-magnesium calcite of which echinoderm skeletons are constructed. The pores are about 10 to 15 microns in diameter.

and Mn, Al, and Si are trace elements present at concentrations below 100 ppm. A variety of other elements, including Ba, Cr, Ni, V, Be, Ti, Sn, Pb, Zn, Cu, and Mo, were not detected by emission spectrography (N. Suhr, analyst) in a selection of the samples which were analyzed for magnesium.

SAMPLING AND ANALYTICAL TECHNIQUES

Collection of specimens.—The aim of the sampling plan was to obtain a collection of echinoderms that would adequately represent the present living population of Echinodermata. The term adequate is used in the sense that conclusions based on a study of the sample could reasonably be extended to the entire population. As far as limited resources permitted, broad taxonomic, geographic, and depth coverage was attempted. Many of the specimens were collected by the author while numerous others were donated by marine biologists, museums, and skin-diving clubs around the world.

TABLE 1

Porosity of echinoid skeletal elements

	Percent pore space (1)	Percent pore space (2)	Percent pore space (3)
Primary spines	52	45	13
Test (coronal plates)	28	28	30
Pyramids	17	16	11
Rotulae	16	15	11
Epiphyses	17	14	12

(1) *Heterocentrotus mammillatus,* Noumea, New Caledonia

(2) *H. mammillatus,* Singatoka, Fiji

(3) *Diadema setosum,* Singatoka, Fiji

The collection of analyzed echinoids comprises 292 specimens representing 28 families from 12 of the 15 orders containing existing species (McCormick and Moore, 1966). The temperature of the water in which these animals were living ranged from −1.9°C for a specimen of *Sterechinus neumayri* at McMurdo Sound in the Ross Sea, Antarctica, to over 28.3°C for specimens from the atoll reefs of the central Pacific. The depths at which the echinoids were collected ranged from intertidal to 2000 m.

Asteroids included in this investigation number 565 individual specimens from worldwide localities taken from the intertidal zone at one extreme to a depth of 5200 m at the other. All the Recent orders and 82 percent of the Recent families in the classification of Spencer and Wright (1966) are represented. The remaining families contain a total of 11 genera and quantitatively are unimportant.

Ophiuroids are represented in the present study by 154 specimens distributed among 11 of the 17 families (Spencer and Wright 1966). Brittle stars of the six unrepresented families are relatively uncommon. Geographic distribution extends from localities in Alaska, Siberia, and the Northwest Territories of Canada along polar seas to coral reefs in the tropical oceans, and the limits of the bathymetric range of the analyzed samples are M.S.L. (mean sea level) and 2086 m.

Existing crinoids are grouped into one order in Ubaghs' (1953) classification. The 43 specimens analyzed are distributed among 5 families in two suborders. The geographic and depth ranges are from Antarctic to Arctic and from intertidal to 370 m.

The sole holothurian whose skeletal calcite was analyzed for magnesium is *Psolidium granuliferum* from a depth of 35 m off Natividad Island, Mexico. This species secretes macroscopic skeletal components.

Sample preparation.—Specimens were preserved by a brief period of immersion in a solution of formaldehyde and were then air dried at room temperature. In the laboratory, care was taken to ensure that stomach contents such as molluscs, foraminifera, calcareous algae, bottom sediment, et cetera were removed before disarticulation of the skeleton in a 5 percent solution of sodium hypochlorite. Separation of organic matter from the skeletal calcite was completed in less than an hour for most crinoids and ophiuroids, but up to 30 hours were required for some large sea-stars and echinoids. Skeletal ossicles were washed briefly in distilled water, air dried at room temperature, and inspected again for possible contamination by other calcareous organisms.

Analysis for magnesium.—Magnesium was determined by powder X-ray diffraction using a technique similar to that described by Chave (1952) and since widely used by others (for example, Lowenstam, 1964). This method, based on the relationship between the size of the unit cell of calcite and the concentration of magnesium isomorphously substituting for calcium, has two advantages: (1) adequate accuracy and precision are obtained for magnesian calcites in which cations other than Mg and Ca are present only in very small amounts, and (2) magnesium possibly present in any form or compound other than the magnesian calcite is not included in the analytical result.

Calcium fluoride, serving as an internal standard, was admixed with powdered skeletal calcite, and the powder was mounted on a glass slide in the form of a layer of uniform thickness and density having a plane surface. The angular distance between the (111) diffraction maximum of CaF_2 and the (104) maximum of calcite was measured by scanning the spectrum at a rate of 1/8° per minute. The d spacing for the (104) planes of the magnesian calcite was related to the concentration of magnesium by a calibration curve constructed from a series of Mg-calcites analyzed in duplicate by emission spectrography (N. Suhr, analyst). Precision, calculated from replicate analyses, is expressed by a standard deviation of 0.27 wt percent $MgCO_3$. As discussed later, this value does not apply to the analysis of echinoid teeth. Unlike the narrow, symmetrical X-ray diffraction maxima provided by other echinoderm skeletal elements, the teeth of echinoids yielded a broad and somewhat irregular diffraction curve in almost all cases.

The electron microprobe, operated at 30kV, $0.02\mu A$ with a 10μ spot size, was used to examine the skeletal elements for the distribution of magnesium on a small scale. Ca and Mg were determined simultaneously, and the ratio of counts taken for a 10 second period was related to the concentration of Mg by a calibration curve constructed from analyzed standards ranging from essentially pure calcite to dolomite.

HOMOGENEITY OF SINGLE SKELETAL ELEMENTS

Although excellent correlation of unit cell size and Mg content determined by emission spectrography for a series of samples indicates that magnesium is uniformly distributed in these magnesian calcites on an atomic scale, isomorphously substituting for calcium more or less at random, it is possible that some portions of a given skeletal ossicle might contain more Mg than others. This possibility was investigated by determining simultaneously the concentrations of calcium and magnesium in areas about 10 microns in diameter by electron microprobe analysis. Between 30 and 50 such areas were examined for each major type of skeletal element of *Heterocentrotus mammillatus* (table 2). Analytical error, determined by repeated analysis of a single area of a Ca/Mg standard, is expressed as the standard deviation calculated from these replicate analyses (SD = 96 ppm Mg).

TABLE 2

Dispersion of magnesium in skeletal elements of *Heterocentrotus mammillatus*

Skeletal element	Mean Mg percent	Standard deviation ppm Mg	Number of microprobe analyses	Coefficient of variation
Primary spine	3.14	1070	30	3.4
Secondary spine	3.86	824	32	2.1
Pyramid	4.70	424	30	0.9
Epiphysis	4.44	415	30	0.9
Rotula	4.62	617	31	1.3
Tooth	2.86	8240	50	29.
Coronal plate at peristome	4.42	562	30	1.3

For each skeletal element, dispersion of the Ca to Mg ratios (recalculated to ppm Mg) is greater than analytical error. However, except for the tooth of this sea urchin, where the standard deviation is 8240 ppm, there appears to be no great inhomogeneity within skeletal parts. The non-uniform distribution of magnesium in echinoid teeth is apparent also in the X-ray diffraction maximum which is broader than the curve recorded for other skeletal elements and is often markedly asymmetric. As shown in figure 1, the Ca/Mg values determined by microprobe analysis are more or less uniformly distributed around the mean. The minimum and maximum concentrations of Mg in this single echinoid

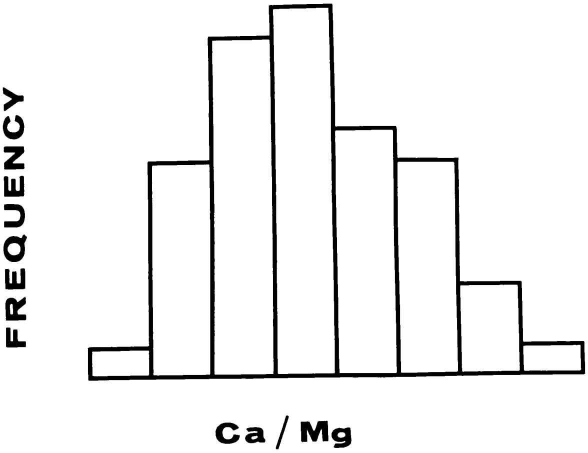


Fig. 1. Histogram constructed from 50 analyses of an echinoid tooth for Ca/Mg by electron microprobe.

tooth, determined by electron microprobe, are approximately 7 and 13 wt percent $MgCO_3$.

VARIATION WITHIN INDIVIDUAL ANIMALS:— THE EFFECT OF SKELETAL ANATOMY

Fairly large differences in magnesium concentration are found between various skeletal elements from an individual echinoid specimen. The interambulacral coronal plates of *Strongylocentrotus franciscanus*, for example, are more enriched in Mg at the peristome and periproct than at the ambitus (table 3), but variation along and between interambulacral and ambulacral columns is relatively small, that is, of the order of 1 or 2 percent $MgCO_3$. Much greater differences are observed between lantern parts (pyramids, teeth, rotulae, epiphyses), test, and spines as shown by examples in table 4. Epiphyses, pyramids, and coronal plates are generally most enriched in magnesium whereas teeth and spines exhibit the lowest Mg contents. The order most frequently observed is epiphysis > pyramid > test > rotula > tooth > spine.

TABLE 3

Variation of magnesium content of calcite skeletal elements of *Strongylocentrotus franciscanus*, California

	$MgCO_3$ wt percent
Ambulacral plate, peristome	9.3
Same, midway between peristome and ambitus	9.3
Same, ambitus	8.6
Same, midway between periproct and ambitus	7.7
Same, periproct	8.6
Interambulacral plate, peristome	10.2
Same, midway between peristome and ambitus	8.0
Same, ambitus	8.6
Same, midway between periproct and ambitus	8.6
Same, periproct	9.0
Pyramid	9.3
Tooth	6.4
Rotula	8.0
Epiphysis	9.6
Spines, primary	6.4
Spines, secondary	5.1

Coronal plates and spines are the skeletal elements that constitute the greater part of an echinoid skeleton, and for nine families, mean values of the difference in their magnesium contents are given in table 5. Paired test and spine samples were taken from 194 different echinoid specimens, including sand dollars and heart urchins together with the "regular" sea urchins, and in each case, the concentration of magnesium in the coronal plates exceeds that in the spines. The maximum difference, 8.7 percent $MgCO_3$, was found in a specimen of *Echinometra* from Tahiti; the minimum difference observed is 0.8 percent in a specimen of *Tripneustes*, from New South Wales, Australia.

TABLE 4

Magnesium concentrations in skeletal elements of echinoids

Species, locality	MgCO$_3$ wt percent Test	Spine	Pyramid	Tooth	Rotula	Epiphysis
Echinometra lucunter Bermuda	14.7	7.4	15.0	10.9	14.3	15.0
E. mathaei Lord Howe Island	14.3	8.6	14.0	10.2	13.4	14.7
Lytechinus variegatus Tampa Bay, Florida	12.1	7.4	11.8	10.2	10.2	12.1
L. variegatus Puerto Rico	13.1	7.4	11.5	8.3	11.2	13.4
Tripneustes gratilla Minnie Waters, N.S.W., Australia	10.4	9.6	11.8	8.0	10.9	12.1
Strongylocentrotus dröbachiensis Fox Islands, Maine	7.4	4.5	9.3	5.1	6.7	8.6
Evechinus chloroticus Kaikoura Peninsula, New Zealand	10.5	6.9	11.6	7.3	10.4	11.7
Heterocentrotus mammillatus Teahupoo, Tahiti	15.4	10.8	15.6	9.5	15.2	16.4
Echinothrix diadema Agana, Guam	13.5	9.7	11.6	9.8	12.9	14.9

Although compositional differences between one skeletal element and another, for example test and spine, of one individual are closely similar to those of other echinoids of the same species living together in the same population or community, there are large variations from species to species and from locality to locality for the same species. Both the group of urchins with a large difference between Mg_{test} and Mg_{spine} (table 5) and the group whose test and spine calcites are most similar in magnesium content include "regular" and "irregular" echinoids, littoral and sub-littoral species, and specimens from the warmest and the coldest seas sampled. Summary statistics pertaining to test-spine pairs for families (table 5) indicate that, in general, fractionation of Mg between coronal plates and spines is greater in cidarids and echinometrids than in species from other families, but the range of test-spine differences within any single family is large.

In contrast to echinoids, no significant variations were found in the magnesium concentrations of different skeletal parts of ophiuroids and asteroids. Spines, vertebrae, dorsal-, ventral-, and lateral-shields of brittle-stars, for example, provided identical $MgCO_3$ data within the limits of experimental error.

Maximum and minimum observed concentrations of magnesium in several different types of echinoderm calcite are given in table 6.

ENVIRONMENTAL AND GENETIC EFFECTS AT LOWER TAXONOMIC LEVELS

Statistics summarizing dispersion within a population consisting of one species from a given environment and locality (table 7) show that in most cases a single specimen extracted from a population adequately represents the population with respect to the magnesium concentration of the skeletal calcite. If homologous echinoid ossicles are compared, variation in Mg/Ca is found to be relatively small from specimen to specimen, but it exceeds dispersion resulting from analytical error alone by a factor between one and four. In each of the four echinoid populations investigated, the coefficient of variation (CV) for spines is considerably greater than that for coronal plates. The results for 16 different asteroid and ophiuroid populations indicate that variation among individuals from the same population is relatively small for these echinoderms also; CV in each case is less than 10.

Table 5

Compositional differences between echinoid spines and tests

	Test	Spines	Difference
	($MgCO_3$ wt percent)		
(1) Large difference			
Echinometra mathaei, Papeete, Tahiti	16.2	7.5	8.7
Laganum laganum, Noumea, New Caledonia	15.3	7.4	7.9
Echinostrephus aciculatus, Bikini Atoll	15.2	7.9	7.3
Goniocidaris umbraculum, Foveau Strait, New Zealand, 37 m	11.2	3.9	7.3
Echinometra mathaei, Bora Bora, French Polynesia	15.8	8.6	7.2
Salenia pattersoni, Puerto Rico	12.4	5.3	7.1
Echinometra viridis, Jamaica	15.2	8.2	7.0
(2) Small difference			
Tripneustes gratilla, Minnie Waters, New South Wales, Australia	10.4	9.6	0.8
T. gratilla, Noumea, New Caledonia	9.6	8.6	1.0
Diadema setosum, Agana, Guam	13.5	12.2	1.3
Stereocidaris japonica, Niigata Light, Sea of Japan, 110 m	5.5	3.9	1.6
Arbacia punctulata, Woods Hole, Mass.	9.3	7.7	1.6
A. punctulata, Woods Hole, Mass.	9.0	7.4	1.6
Cidaris cidaris, Banyuls, France, 70 m	6.7	5.0	1.7
Tripneustes ventricosus, Bermuda	11.5	9.6	1.9
Strongylocentrotus dröbachiensis, Waldoboro, Maine	6.7	4.8	1.9
Sterechinus neumayri, McMurdo Sound, Antarctica	7.8	5.9	1.9

	Mean difference (test—spines)	s.d.	N	CV
(3) Statistics for families				
Cidaridae	5.0	1.5	26	30
Diadematidae	4.4	1.4	12	32
Stomechinidae	3.4	0.54	5	16
Arbaciidae	2.9	1.1	16	37
Toxopneustidae	3.8	1.2	37	32
Echinometridae	5.3	1.3	38	24
Echinidae	3.8	1.5	14	40
Strongylocentrotidae	3.0	0.82	23	28
Echinarachniidae	3.6	0.59	5	17

Abbreviations:

s.d.—standard deviation
N—number of specimens
CV—coefficient of variation

TABLE 6

Minimum and maximum observed magnesium concentrations

	$MgCO_3$ wt percent Maximum	$MgCO_3$ wt percent Minimum
ECHINOID TEST		
Heterocentrotus mammillatus Noumea, New Caledonia	17.1	
Stereocidaris japonica Niigata Light, Sea of Japan, 110 m		5.5
ECHINOID SPINE		
Diadema setosum Agana, Guam	12.2	
Strongylocentrotus dröbachiensis Waldoboro, Maine		3.2
ASTEROID		
Linckia multifora Specimens from Pago Pago, Gilbert Islands, and Mauritius	18.5	
Asterias rubens Faxafloi, Iceland, 36 m		8.3
OPHIUROID		
Ophioplocus imbricatus Heron Island, Great Barrier Reef	17.2	
Ophiura sp. Amundsen Gulf, Northwest Territories, Canada, 25 m		6.7
CRINOID		
Comanthina belli Western Australia	16.6	
unidentified comatulid Dease Strait, Northwest Territories, Canada, 50 m		9.3

When the population is redefined to include all specimens collected at a specific locality belonging to one class, there is no significant increase in dispersion of magnesium concentrations except for echinoids. The coefficient of variation for the sample population consisting of 15 asteroids, each a different species, from Broome, Western Australia, is only 4.9 (table 8). Similar results were obtained for ophiuroid and asteroid populations from various world-wide localities, but the collection from Broome contained the greatest number of different species. Genetic effects are evident in some echinoid populations from single localities, the tests of irregular (sand dollar, heart urchin) echinoids usually containing less magnesium than the tests of the regular sea urchins. Similar findings for echinoids of Eniwetok Atoll are reported by Raup (1966). When *different classes* are compared, large differences in skeletal magnesium content between sea urchins, asteroids, et cetera from the same environment are readily apparent. Comparison of echinoderms at higher taxonomic levels can be made with greater reliability by increasing both the number of different specimens and the number of different localities in order to obtain better estimates of dispersion and the mean. As the quantity of magnesium in these calcites is dependent on temperature, it is necessary to allow for the effect of this variable by means of analysis of covariance.

TABLE 7

Variation within a single population

Different individuals of the same species from the same environment and locality

Species, locality		Mean $MgCO_3$	s.d.	CV
(1) Echinoids				
Strongylocentrotus dröbachiensis	(test)	7.5	0.50	6.7
Waldoboro, Maine	(spines)	4.4	0.66	15.0
Arbacia punctulata	(test)	9.6	0.47	4.9
Woods Hole, Mass.	(spines)	7.0	0.73	10.4
	(pyramids)	9.8	0.55	5.6
Podophora atrata	(test)	14.5	0.32	2.2
Oahu, Hawaii	(spines)	10.4	0.34	3.3
	(pyramids)	14.9	0.49	3.3
Echinometra mathaei	(test)	14.5	0.40	2.8
Lord Howe Island	(spines)	9.0	0.88	9.8
	(pyramid)	14.2	0.17	1.2
(2) Asteroids				
Asterias forbesi		10.2	0.82	8.0
Rhode Island				
A. forbesi		10.5	0.97	9.3
Maine				
A. rubens		9.3	0.56	6.0
Faxafloi, Iceland				
A. rubens		10.3	0.66	6.4
Roscoff, France				
Leptasterias tenera		11.1	1.02	9.2
Woods Hole, Mass.				
Mediaster aequalis		12.9	0.25	2.0
Santa Catalina Is., Calif.				
Asterina lorioli		16.3	0.38	2.3
Karachi, Pakistan				
Echinaster luzonicus		16.1	0.23	1.4
Heron Island, Australia				
Coscinasterias calamaria		14.6	0.23	1.6
Arrawara, Australia				
(3) Ophiuroids				
Ophioderma longicauda		15.7	0.54	3.4
St. George Bay, Lebanon				
Ophiomusium eburneum		12.8	0.31	2.4
Gulf of Mexico				
O. lymani		11.4	0.17	1.5
Pacific Ocean				
Macrophiothrix sp.		15.6	0.35	2.2
Monora Island, Pakistan				
Asteronyx loveni		11.3	0.12	1.02
Pacific Ocean				

COMBINED ENVIRONMENTAL AND GENETIC EFFECTS: TEMPERATURE AND TAXONOMIC LEVEL

Although the temperature dependence of Mg in echinoderm calcites has been known for some time (Clark, 1911; Clarke and Wheeler 1917, 1922; Chave, 1954), the number of published analyses is far too small to define adequately this relationship. Their data exhibited considerable variability which could not be explained solely by temperature. The mechanisms by which the thermodynamically metastable high-magnesium skeletal calcites originate are still poorly understood, and for biochemical calcification studies it is of interest to learn whether or not the temperature-magnesium relationship is the same for the different types of echinoderms. If not, at what taxonomic level do differences begin to appear?

TABLE 8

Dispersion in echinoderm populations consisting of different species from the same locality

Locality		Mean $MgCO_3$ wt percent	s.d.	CV
(1) Broome, Western Australia				
Stellaster princeps		15.9		
S. incei		14.3		
Anthenea conjugens		17.2		
Astropecten hatmeyeri		16.3		
Archaster laevis		17.8		
Goniodiscaster australiae		17.2		
G. acanthodes		16.9		
Gymnanthena globigera		16.9		
Tamaria tumescens		17.5		
Retaster insignia		16.6		
Echinaster varicolor		16.6		
Anseropoda rosacea		16.3		
Protoreaster nodulosus		16.6		
Metrodira subulata		16.9		
Nepanthia variabilis		16.9		
	TOTAL BROOME ASTEROIDS	16.7	0.81	4.9
(2) Friday Harbor, Wash.				
	Asteroidea	12.0	0.46	3.8
(3) Westernport, Australia				
	Asteroidea	14.3	0.56	3.9
(4) Bermuda				
	Echinoidea	12.9	1.65	12.8

Abbreviations:
s.d.—standard deviation
CV—coefficient of variation

While a correlation between temperature and Mg content is readily apparent from inspection of the data, determination of the parameters that precisely express this relationship is not *a priori* a simple matter. It is virtually impossible to use analysis of covariance at the species, genus, and sometimes family level as very few species or genera truly span a wide temperature range. Furthermore, the variables temperature and magnesium concentration are both subject to error (in a statistical sense), and despite excellent temperature records for many collecting localities, the greatest uncertainty is usually in the value of the temperature assigned to a given specimen. The reason for this difficulty is the fact that the water temperature of the environment in which a given specimen lives may vary between wide limits throughout the year. Skeletal accretion might be expected to be greatest during the warmer months of the year, but quantitative data concerning echinoderm growth rates as a function of annual temperature variation are lacking. Furthermore, there is some evidence that factors other than temperature (such as variations in food supply) may in part control the growth rate of echinoderms. Even negative growth rates are possible, probably by resorption of skeletal calcite under temporarily adverse environmental conditions (Ebert, 1967a).

"Growth" rings observed in echinoid spines, due to color bands of pigment such as echinochrome, appears to be analogous to the annual growth layers in woody perennial plants and hence related to the age of the animal. In order to determine the time of year when such a ring formed, Ebert (1967b) tagged 100 animals and removed a few spines from these echinoids throughout the year. The results were negative, and Ebert's detailed study of the spines demonstrated that, at least for the species studied *(Strongylocentrotus purpuratus)*, a new growth layer is secreted only when a spine is broken and regenerated, a process not necessarily restricted to any particular time of the year.

From this evidence it appears possible that skeletal calcite may be precipitated over a considerable temperature range in the case of echinoderms inhabiting shallow portions of temperate and polar seas, unlike those living in most tropical oceans where annual temperature fluctuations are relatively small. This was confirmed by measuring the half-height width of the (104) calcite X-ray diffraction maximum obtained from the three largest coronal plates near the ambitus of intertidal echinoids collected over a wide range of latitude. For example, the half-height width for *Strongylocentrotus dröbachiensis* from St. Pierre, Newfoundland (mean ann. temp 4.9°C, range 13.9°C), was about 15 percent greater on the average than the corresponding measurements made on *Echinometra mathaei* from Papeete, Tahiti (mean ann. temp 26.6°, range 2.2°C). Accordingly, it appears that the most reasonable temperature estimate to assign each echinoderm specimen is the mean annual temperature of the local water mass from which the animal was taken. This value was determined for samples when the collecting site and depth were accurately known.

Regression-correlation statistics for the different skeletal elements of echinoids as a group and for the tests and spines of the different echinoid orders are given in table 9. Statistics for the asteroid orders, suborders, families, and for the class Asteroidea are found in table 10, and similar data for the ophiuroids and crinoids are listed in table 11.

It is readily evident for the echinoids as a group that the relationship between temperature and the concentration of magnesium in the skeletal calcite is markedly different for most of the major skeletal elements, for example the tests and the spines (fig. 2). At a given temperature, echinoid coronal plates contain much more magnesium than do echinoid spines. Average Mg values at temperatures of 20, 25, and 30°C are listed at the right of table 9.

Although the temperature dependence of skeletal magnesium is obviously not the same for the different types of ossicles in the echinoid skeleton, it may be possible to use one set of constants defining the concentration of Mg as a function temperature for the entire class of

TABLE 9

Magnesium in echinoid skeletal calcite

	Determined			Regression-Correlation Statistics					Calculated $MgCO_3$		
	Mean $MgCO_3$	s.d.	N	Mean $MgCO_3$	Mean temp °C	b	r	N	20°C	25°C	30°C
SKELETAL ELEMENTS											
Test	11.55	2.55	235	11.54	19.53	2.15	0.74	223	11.8	14.1	16.4
Spine	7.25	2.02	222	7.28	20.03	2.52	0.70	217	7.27	9.25	11.2
Pyramid	11.92	2.17	175	11.91	20.16	2.16	0.66	172	11.8	14.2	16.5
Tooth	8.48	1.71	107	8.46	20.47	2.84	0.71	106	8.30	10.1	11.8
Rotula	11.65	2.30	101	11.64	20.96	1.86	0.68	100	11.1	13.8	16.5
Epiphysis	12.91	2.26	108	12.89	20.96	2.04	0.73	107	12.4	14.9	17.3
ORDERS (TEST)											
Cidaroida	10.5	2.09	26	10.61	16.05	2.12	0.62	24	12.5	14.8	17.2
Diadematoida	12.3	1.67	14	12.34	22.94	2.92	0.78	14	11.3	13.0	14.8
Salenioida	12.4	—	1	—	—	—	—	—	—	—	—
Phymosomatoida	14.5	0.34	5	—	—	—	—	—	—	—	—
Arbacioida	9.69	0.52	17	9.69	14.10	3.86	0.46	17	11.2	12.5	13.8
Temnopleuroida	11.7	1.00	40	11.68	24.20	1.81	0.53	40	9.4	12.1	14.9
Echinoida	11.5	3.42	76	11.52	17.98	2.00	0.88	76	12.5	15.0	17.5
Holectypoida	16.3	—	1	—	—	—	—	—	—	—	—
Clypeasteroida	12.4	2.63	33	12.36	21.42	2.12	0.69	26	11.7	14.1	16.4
Cassiduloida	12.4	—	2	—	—	—	—	—	—	—	—
Spatangoida	11.3	1.40	20	11.23	16.69	1.32	0.26	17	13.7	17.5	21.3
ORDERS (SPINES)											
Cidaroida	5.61	1.19	31	5.60	17.36	5.10	0.86	29	6.12	7.10	8.08
Diadematoida	8.31	1.48	17	8.31	24.41	1.69	0.57	17	5.71	8.66	11.6
Salenioida	5.3	—	1	—	—	—	—	—	—	—	—
Phymosomatoida	11.12	0.42	5	—	—	—	—	—	—	—	—
Arbacioida	6.96	1.02	18	6.96	14.78	0.26	0.05	18	—	—	—
Temnopleuroida	7.66	1.27	41	7.66	24.24	0.70	0.26	41	1.56	8.75	15.9
Echinoida	7.50	2.33	90	7.51	19.15	2.74	0.83	89	7.82	9.65	11.5
Clypeasteroida	6.30	1.51	13	6.32	18.06	4.19	0.68	12	6.78	7.98	9.17
Spatangoida	6.28	1.57	6	—	—	—	—	—	—	—	—

$MgCO_3$ in wt percent; s.d.—standard deviation; N—number of specimens; b—regression coefficient; r—correlation coefficient.

echinoids if homologous parts, such as spines alone or coronal plates alone, are considered separately. For different orders, the mean $MgCO_3$ contents at selected temperatures (table 9) are not identical, but these differences could be due to sampling. An F test at the 5 percent level of significance indicates heterogeneous variance, that is, the statistical samples (echinoid orders) are not from normal statistical populations having a common value of σ^2. If homogeneous variance *were assumed,* however, an F test for regression coefficients would indicate that differences in the slopes of the temperature-magnesium lines cannot be attributed solely to sampling variation. Plots of Mg versus temperature reveal that, for ecological reasons, certain orders are heavily biased with respect to temperature. Diadematoids for example prefer warm waters and arbacioids are concentrated in the temperate zone.

TABLE 10

Magnesium in asteroid calcites

	Determined			Regression-correlation statistics					Calculated $MgCO_3$		
	Mean $MgCO_3$	s.d.	N	Mean $MgCO_3$	Mean temp °C	b	r	N	20°C	25°C	30°C
Asteroidea	14.0	2.34	565	14.25	19.52	2.16	0.85	449	14.5	16.8	19.1
ORDERS											
Platyasterida	14.2	1.64	29	14.45	22.66	1.83	0.74	21	13.0	15.7	18.5
Paxillosida	13.4	1.52	79	13.76	20.97	1.92	0.65	54	13.2	15.9	18.5
Valvatida	15.8	1.78	153	16.41	24.52	2.07	0.74	120	14.2	16.6	19.0
Spinulosida	14.7	1.84	146	15.02	20.11	2.74	0.86	120	15.0	16.8	18.6
Forcipulatida	11.9	1.87	158	11.78	13.46	1.54	0.68	134	16.0	19.3	22.5
SUBORDERS											
Diplozonina	13.6	1.51	66	13.91	21.52	1.64	0.58	50	13.0	16.0	19.1
Cribellina	12.1	1.23	8	11.80	14.17	5.56	0.81	4	—	—	—
Notomyotina	13.2	1.07	5	—	—	—	—	—	—	—	—
Granulosina	15.8	1.78	153	16.41	24.52	2.07	0.74	120	14.2	16.6	19.0
Eugnathina	12.4	2.35	12	13.58	16.22	2.58	0.99	5	15.0	17.0	18.9
Leptognathina	14.9	1.65	134	15.09	20.28	2.76	0.84	115	15.0	16.8	18.6
Asteriadina	11.9	1.88	154	11.78	13.46	1.54	0.68	134	16.0	19.3	22.5
Brisingina	13.4	0.65	4	—	—	—	—	—	—	—	—
FAMILIES											
Luidiidae	14.2	1.64	29	14.45	22.66	1.83	0.74	21	13.0	15.7	18.5
Astropectinidae	13.6	1.51	66	13.91	21.52	1.64	0.58	50	13.0	16.0	19.1
Goniopectinidae	12.2	1.30	7	11.80	14.17	5.56	0.81	4	—	—	—
Porcellanasteridae	11.5	—	1	—	—	—	—	—	—	—	—
Benthopectinidae	13.2	1.07	5	—	—	—	—	—	—	—	—
Odontasteridae	12.0	1.10	4	—	—	—	—	—	—	—	—
Chaetasteridae	14.3	0.0	3	—	—	—	—	—	—	—	—
Archasteridae	17.4	0.38	8	—	—	—	—	—	—	—	—
Gonioasteridae	14.0	1.38	44	14.49	20.14	2.73	0.64	20	14.4	16.3	18.1
Oreasteridae	16.8	0.81	36	16.87	25.83	1.79	0.60	34	13.6	16.4	19.2
Ophidiasteridae	16.7	1.05	58	16.78	25.16	1.36	0.54	55	13.0	16.7	20.3
Solasteridae	11.6	0.72	8	—	—	—	—	—	—	—	—
Pterasteridae	14.0	3.74	4	—	—	—	—	—	—	—	—
Asterinidae	15.0	1.49	69	15.11	20.01	2.32	0.75	61	15.1	17.3	19.4
Ganeriidae	14.0	—	2	—	—	—	—	—	—	—	—
Poraniidae	14.2	1.90	10	14.85	19.40	2.88	0.96	6	15.1	16.8	18.5
Echinasteridae	14.9	1.86	47	14.95	20.55	2.92	0.93	40	14.8	16.5	18.2
Acanthasteridae	16.2	0.33	4	—	—	—	—	—	—	—	—
Mithrodiidae	16.3	—	1	—	—	—	—	—	—	—	—
Metrodiridae	16.9	—	1	—	—	—	—	—	—	—	—
Heliasteridae	15.3	1.00	3	—	—	—	—	—	—	—	—
Zoroasteridae	13.4	0.97	9	—	—	—	—	—	—	—	—
Asteriidae	11.7	1.83	142	11.72	13.28	1.44	0.65	132	16.4	19.8	23.3
Brisingidae	13.4	0.65	4	—	—	—	—	—	—	—	—

$MgCO_3$ in wt percent; s.d., standard deviation; N, number of samples; b, regression coefficient; r, correlation coefficient.

The condition of homogeneous variance is fulfilled in the case of the five orders of Asteroidea. At the 5 percent level of significance, an F test of regression coefficients indicates that the regression lines are not parallel (fig. 3). The same conclusion is reached by analysis of covariance for the two suborders of ophiuroids, the Gnathophiurina and the Chilophiurina.

TABLE 11

Magnesium in skeletal calcite: ophiuroids, crinoids, and one holothurian

	Determined			Regression-correlation statistics					Calculated $MgCO_3$		
	Mean $MgCO_3$	s.d.	N	Mean $MgCO_3$	Mean temp °C	b	r	N	20°C	25°C	30°C
Ophiuroidea	13.8	2.29	154	14.56	19.30	2.49	0.83	113	14.8	16.8	18.9
ORDERS											
Phrynophiurida	12.3	1.73	16	13.16	15.83	3.65	0.97	7	14.3	15.7	17.0
Ophiurida	13.9	2.29	138	14.65	19.53	2.40	0.81	106	14.8	16.9	19.0
SUBORDERS											
Ophiomyxina	11.0	0.35	4	—	—	—	—	—	—	—	—
Euryalina	12.7	1.81	12	13.98	19.50	3.22	0.98	5	14.1	15.7	17.2
Chilophiurina	14.0	2.39	101	14.83	20.02	2.21	0.81	76	14.8	17.1	19.3
Leamophiurina	10.5	0.85	3	—	—	—	—	—	—	—	—
Gnathophiurina	13.9	1.79	34	14.20	18.29	3.02	0.84	30	14.8	16.4	18.1
FAMILIES											
Ophiomyxidae	11.0	0.35	4	—	—	—	—	—	—	—	—
Asteronychidae	11.6	0.43	5	—	—	—	—	—	—	—	—
Gorgonocephalidae	13.5	2.01	7	13.98	19.50	3.22	0.98	5	14.1	15.7	17.2
Ophiuridae	12.8	2.62	52	13.89	17.44	1.89	0.77	29	15.2	17.9	20.5
Ophiocomidae	15.5	1.42	18	15.66	23.35	3.04	0.88	16	14.6	16.2	17.9
Ophionereididae	15.0	0.32	6	14.97	17.27	4.02	0.53	6	—	—	—
Ophiodermatidae	15.4	0.91	25	15.36	21.54	3.05	0.92	25	14.9	16.5	18.1
Ophiacanthidae	10.5	0.85	3	—	—	—	—	—	—	—	—
Ophiactidae	12.5	1.89	6	12.72	15.28	4.09	0.99	5	13.9	15.1	16.3
Amphiuridae	14.3	1.66	9	14.58	16.98	3.40	0.93	8	15.5	16.9	18.4
Ophiothricidae	14.2	1.68	19	14.45	19.79	2.78	0.78	17	14.5	16.3	18.1
Crinoidea	12.5	2.34	43	12.36	12.33	4.64	0.97	40	14.0	15.1	16.2
Holothuroidea	13.8	—	1	—	—	—	—	—	—	—	—

$MgCO_3$ in wt percent; s.d.—standard deviation; N—number of specimens; b—regression coefficient; r—correlation coefficient.

Although the data indicate that the temperature dependence of skeletal magnesium is slightly different for the different orders of asteroids and ophiuroids, it is possible that combining the data for orders into two classes, Asteroidea and Ophiuroidea, might result in an increase in dispersion such that one regression line might serve adequately for the combined "stelleroids" (fig. 2). Statistical analysis of the asteroid-ophiuroid data at the 5 percent level of significance indicates homogeneous variance. The hypothesis that the regression coefficients are equal is accepted at the 5 percent level, but the hypothesis that one regression line can adequately fit all of the data is rejected at the same level of significance. The analysis of covariance for asteroid orders is given in table 12.

Crinoids are much more difficult to obtain than are echinoderms from the other classes, their distribution in the world oceans being

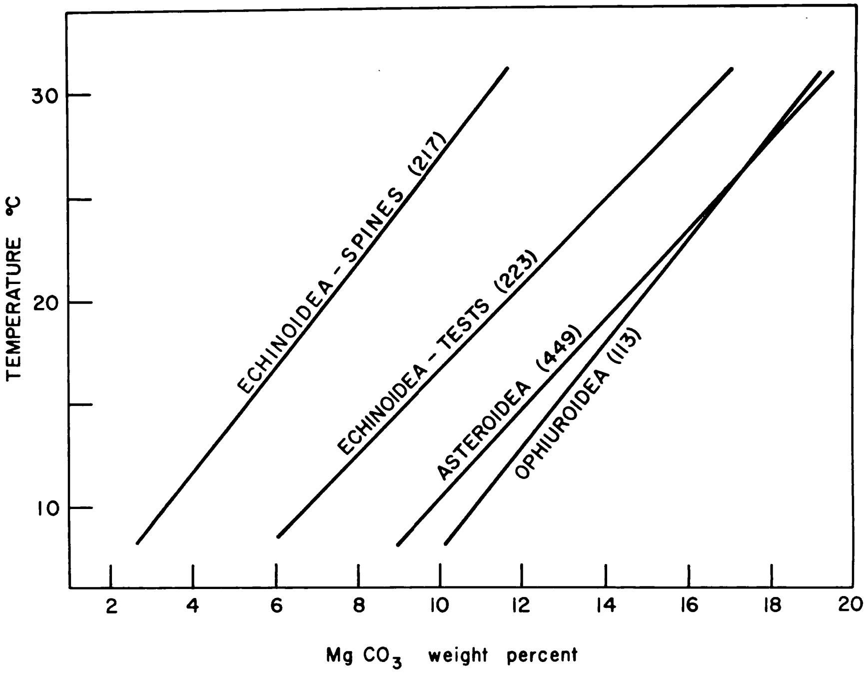


Fig. 2. Least squares regression lines for asteroids, ophiuroids, and echinoids. Numbers in parentheses indicate number of specimens.

severely limited by ecological restrictions (Fell, 1966). Accordingly, the magnesium and temperature data for the Crinoidea are presented (table 11) but are not compared statistically with those of the Echinoidea, Asteroidea, and Ophiuroidea.

In summary, differences in the temperature dependence of skeletal magnesium are apparent at taxonomic levels as low as family and perhaps genus. As a class, echinoids are distinctly different from "stelleroids" in skeletal magnesium content, being displaced toward lower Mg concentrations at a given temperature. Although the concentration of Mg in the skeletons of asteroids is similar to that in ophiuroid calcites at a given temperature, the small observed difference between the temperature-magnesium relationship of the two classes is statistically significant. Similarly, by analysis of covariance, differences at the level of order are shown to be statistically significant for both the Asteroidea and the Ophiuroidea. Rigorous analysis of the data for echinoid orders, however, is prevented by ecological restrictions on the distribution of these animals, such that adequate comparison of the orders cannot be made over the same temperature range. Nevertheless, it is evident that systematic Mg-temperature differences do occur at the family level and above. The echinoids *Echinometra* and *Heterocentrotus,* for example, invariably contain more magnesium than *Tripneustes* from the same collecting

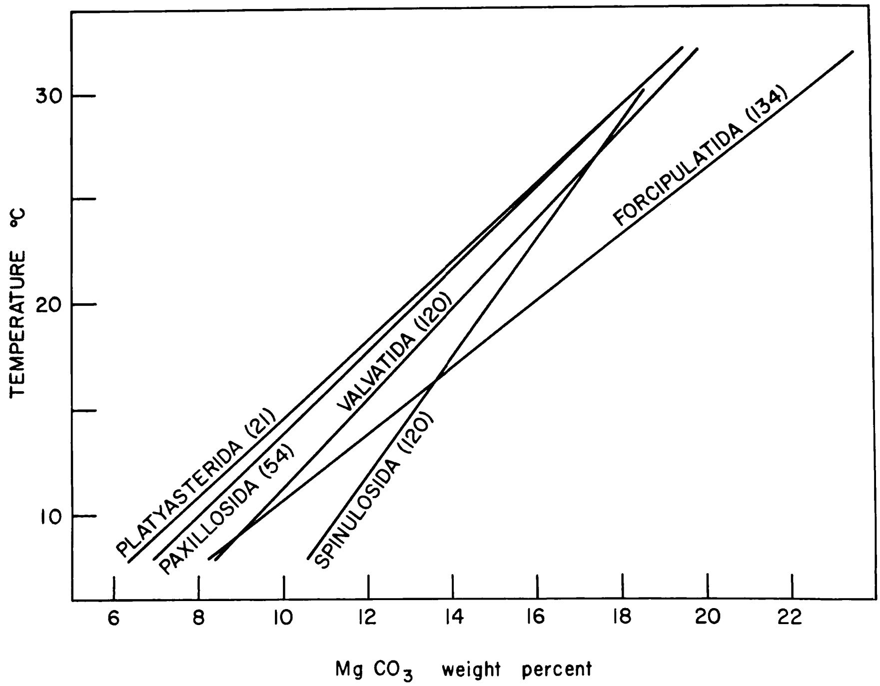


Fig. 3. Least squares regression lines for asteroid orders. Numbers in parentheses indicate number of specimens.

site. In terms of metabolic rates, the significance of these observations is considered later.

DIAGENESIS:—CHANGES IN SKELETAL CHEMISTRY AFTER DEATH

In all five classes of the phylum, the analyzed skeletons without exception were high-magnesium calcite, a thermodynamically metastable phase under earth surface conditions. In a collection of 83 fossil echinoids (including some spines) whose ages ranged from Mississippian to Pleistocene, Weber and Raup (1968) found only one specimen with a skeleton of high-magnesium calcite: a species of *Mellita* of Pleistocene age from Melbourne, Florida. The skeletal remains of the other 82 specimens consisted of low-magnesium calcite. As there is no evidence to suggest that the skeletal mineralogy of the ancient echinoids differed from that of their Recent counterparts, it is apparent that recrystallization with concurrent loss of some magnesium has taken place. Electron probe photomicrographs were taken of low-magnesium calcite fossil skeletons, and except for relatively rare cases where dolomitization has occurred (pl. 2; specimen not included in study of Weber and Raup, 1968), these showed that the distribution of magnesium was fairly uniform.

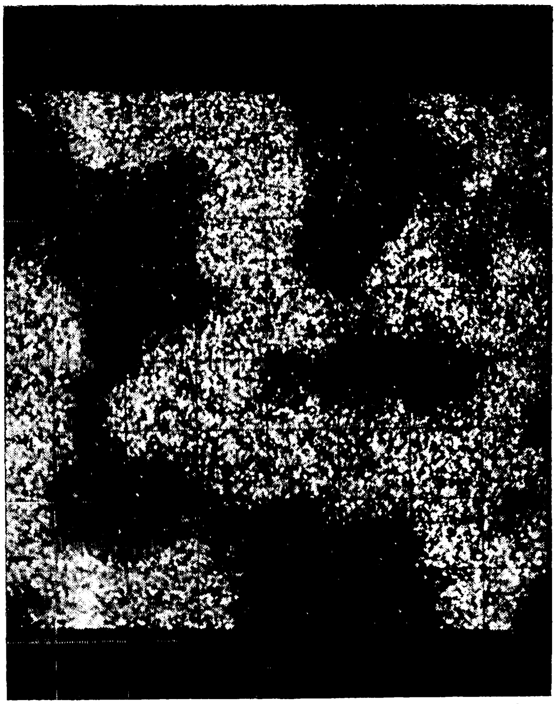
TABLE 12

Analysis of covariance–asteroid orders

	Deviations From Regression		
	f	$\Sigma y^2 - \frac{(\Sigma xy)^2}{\Sigma x^2}$	Mean square
Platyasterida	19	18.5	0.97
Paxillosida	52	67.9	1.31
Valvatida	118	98.0	0.83
Spinulosida	118	91.9	0.78
Forcipulatida	132	262.7	1.99
Within	439	539.0	1.23
Reg. Coef.	4	4.85	1.21
Common	443	543.85	1.23
Adjusted means	4	196.2	49.05
Total	447	740.0	

The skeleton of an echinoderm is usually disarticulated soon after death as the connective tissues decompose, and thus relative to the

PLATE 2



Electron probe photomicrograph of a portion of a coronal plate from the fossil echinoid *Duncaniaster australe* (Duncan) from the Upper Oligocene of Victoria, Australia, showing the two-phase intergrowth of dolomite and low-magnesium calcite. The area shown is about 150 x 150 microns.

abundance of living individuals, echinoderms are much less commonly preserved intact than are such organisms as molluscs and corals. The high-magnesium calcite of the individual ossicle, however, is unlikely to recrystallize to the more stable low-magnesium variety on the sea-floor or even during burial in the upper few feet or more of sediment. There is good evidence that the metastable phase assemblage consisting of biogenic aragonite and high-magnesium calcite, the dominant carbonate minerals in most shallow-water areas of carbonate sediment formation, are "stabilized" or prevented from recrystallization either by coatings of organic compounds or by Mg ions adsorbed on calcite. Berner (1966a) demonstrated adsorption of magnesium in an experimental study and suggested (Berner, 1966b) that inhibition of carbonate mineral recrystallization in sea water may be due to the formation of surface-protective or nucleation-inhibiting layers of Mg adsorbed on calcite. He reported that carbonate sediments that have been in contact with sea water throughout their history afford no evidence for the recrystallization of metastable high-magnesium calcite and aragonite to low-magnesium calcite and dolomite. The adsorption of Mg^{++} on the surface of calcite has been shown by Bischoff (1968), by de Groot and Duyvis (1966), and by Bischoff and Fyfe (1968). According to Bischoff (1968) the mechanism restricting recrystallization of the metastable carbonates in a mixture of such phases is the inhibiting effect of absorbed magnesium ions on the crystallization of calcite.

Recrystallization of the non-equilibrium carbonate phases in sea water is inhibited also by thin coatings of polar organic compounds (Chave and Suess, 1967; Chave and Schmalz, 1966; Chave, 1965). These coatings, until destroyed in one way or another, protect the individual grains from chemical interaction with sea water, and there is ample evidence that the recrystallization of carbonates during the diagenesis and lithification of sediments is a process in which water is required. Laboratory studies of the aragonite-calcite transformation, for example, have shown that in the presence of water and under near surface conditions aragonite would survive for about 100,000 years at 50°C or for a few million years at 10°C. In the absence of water, aragonite should remain unchanged almost indefinitely (Brown, Fyfe, and Turner, 1962). Unrecrystallized skeletal aragonite is occasionally found in the geologic record (Hall and Kennedy, 1967), preserved under conditions that protected the aragonite from water. The oldest known occurrence is in an Ordovician nautiloid (Grandjean, Grégoire, and Lutts, 1964).

If recrystallization of high-magnesium calcite is delayed until long after deposition and burial in bottom sediments, it is not unlikely that unrecrystallized echinoderm calcite will eventually be discovered in ancient rocks where in one way or another it has been protected from the presence of intrastratal water. There is little to suggest that the high-magnesium calcites found in nature, for echinoderms at least, may represent highly disordered protodolomites, which as suggested by Arntson (1967) would eventually form dolomite under certain conditions.

THE ORIGIN OF HIGH-MAGNESIUM CALCITE IN ECHINODERM SKELETONS: A WORKING HYPOTHESIS

The high-magnesium calcites secreted by echinoderms are thermodynamically stable at temperatures much higher than those of any seawater environment. The true solid solubility of magnesium in calcite increases with increasing temperature (Graf and Goldsmith, 1958; Goldsmith and Heard, 1961), but the positive correlation between these two variables in echinoderm calcites is not a consequence of this thermodynamic relation. The concentration of magnesium found in these skeletal calcites would be similar to equilibrium concentrations only in the temperature range of approximately 550 to 800°C.

Laboratory synthesis of high-magnesium calcite.—High-magnesium calcites have been synthesized at relatively low temperatures (0 to 40°C) and low pressures but only after a long history of experimental failures. McCauley and Roy (1966) prepared well-crystallized magnesian calcites by diffusing calcium and magnesium salts and Na_2CO_3 through silica gel, but the crystals were distributed in the low-magnesium portion of the known spectrum of natural magnesian calcites. Similar results with amorphous gels were obtained by Arntson (1967). Kitano and Kanamori (1966) were the first to report low-temperature, low-pressure laboratory synthesis of Mg calcites similar to those of natural biogenic origin. These calcites were precipitated from calcium bicarbonate solutions containing both magnesium ions and organic compounds such as the sodium or acid salts of citric, malic, pyruvic, succinic, and chondroitin sulfuric acids. The influence of citrate, pyruvate, and malate on the nature of the precipitate is marked, for in their absence, aragonite rather than magnesian calcite forms. By varying experimental conditions, Kitano and Kanamori obtained calcites with up to 12.5 percent $MgCO_3$ (determined by chemical analysis); corresponding changes in the size of the unit cell demonstrated that this quantity of magnesium was isomorphously substituting for calcium in the structure.

Glover and Sippel (1967) reported the synthesis of calcites containing up to 20 mole percent $MgCO_3$ in the temperature range 0 to 40°C by the simple addition of $NaHCO_3$ to seawater. These non-equilibrium preparations were difficult to reproduce and were demonstrably unstable in contact with the precipitating solutions, aragonite forming at the expense of high-magnesium calcite over a short period of time.

As aragonite and high-magnesium calcite are metastable carbonates in the sea water environment and yet are produced in great quantities by a large number of marine organisms, there has been considerable interest in accounting for the relative scarcity of the more stable low-Mg calcite as a skeletal material. The influence of various inorganic ions, especially magnesium and strontium, on the nucleation and growth of various calcium carbonate minerals in both natural and artificial seawater has been extensively investigated (for example Kitano, 1962; Simkiss, 1964; Pytkowicz, 1965), and a study by Okazaki (1956) specifically concerned the effect of calcium ion concentration on the development of skeletal spicules in sea urchin larvae. But the fact that in a given marine community, one type of organism invariably utilizes aragonite for its skeleton and another type precipitates only high-magnesium calcite, while yet another animal uses both aragonite and calcite in its shell, indicates that the most promising approach to the elucidation of calcification would include studies of the influence of organic compounds on carbonate crystallization.

Role of the organic matrix in calcification.—The effect of dissolved organic compounds in determining the polymorphic form of the carbonate precipitated from ocean water was shown by experiments described by Kitano and Hood (1965), Kitano (1964), Simkiss (1964), and others. Furthermore, comparative biochemical studies revealed that the organic matrix associated with calcite in the shell of a mollusc differed in amino acid composition from the organic matrix associated with aragonite layers in the same shell (Wada, 1966a). Despite differences between the amino acid patterns of non-mineralized and mineralized tissues of a given mollusc, Wada (1966b) found that any given species has a specific or characteristic amino acid pattern which appears to be unaffected by ecological conditions such as food habits or environment. Okazaki (1956, 1960) emphasized the importance of the organic matrix in the skeletal development of sea urchin larvae, and comparative studies such as those by Travis and others (1967) have now provided biochemical data for at least a few representatives of every major type of animal.

The amino (NH_2) and carboxyl (COOH) groups of amino acids comprising the animal protein are both polar, becoming positively and negatively charged respectively. Glimcher (1960) suggested that the charged amino acid side-chains of skeletal protein act as binding sites for oppositely charged inorganic ions, and that the nucleation of $CaCO_3$ crystals would depend on the distribution of such sites. In sulfur-containing amino acids, the sulfur atom is also a possible ligating site (McAuliffe, Quagliano, and Vallarino 1966). The manner in which anions and cations can associate themselves with protein and glycoprotein matrices, which in effect are mineralization templates, has been discussed by Matheja and Degens (1967).

Although Stevenson and Ufret (1966) found a relationship between the amount of iron in the skeletons of two sea urchins and the amount of iron in the algae eaten by these echinoids (suggesting that at least one trace element in the skeletal calcite may be derived from their food ?) it is likely that the Mg and Ca that form the high-magnesium calcite are taken as ions from seawater via perivisceral coelomic fluid. Magnesium ions are much more abundant than calcium ions in the oceans, and the writer believes that the incorporation of magnesium in calcite during skeletal formation is accidental or fortuitous, a consequence of both a rapid rate of calcification and magnesium complexing by the protein distributed throughout the porous ossicle.

The concentration of ionized calcium in seawater is 0.0102 m (Thompson and Ross, 1966); this is 84 percent of the total calcium, the remainder presumably being complexed with sulfate, carbonate, and bicarbonate. Ionized magnesium in seawater amounts to 0.048 m (Thompson, 1966), accounting for 90 percent of the total magnesium (Fisher, 1967). Variations in the concentration of magnesium in seawater are known (Fabricand and others, 1966), but these are relatively small. The magnesium ion is a major component of seawater everywhere, and no biochemical enrichment mechanism of the sort responsible for vanadium in some ascidians or copper in some molluscs needs be invoked to explain the presence of magnesium in calcite.

At any instant of time during the formation of mineralized tissue, a variety of elements present as ions in seawater would be temporarily "associated" or complexed with the amino acids of skeletal protein, that is, bonds of various kinds are continually forming, breaking, and reforming. Some elements such as Zn, Co, Cu, and Cd form relatively strong metal to amino acid bonds, and accordingly, such complexes have high stability constants. Calcium and magnesium, however, form rather weak complexes with such ligands, but the abundance of these alkaline earth elements is many orders of magnitude greater than the concentrations of trace elements which form much stronger metal-amino acid bonds. Stability constants for complexes with magnesium are greater than those for corresponding calcium complexes, and when the abundance of Mg^{++} in seawater is taken into account, appreciable association of magnesium with skeletal protein can be expected.

A crystal of calcite growing in seawater inorganically under ideal, equilibrium conditions would incorporate only a few of the magnesium ions that might strike a growth surface. On the other hand, skeletal calcite crystallizing rapidly on a "biological template" of the sort envisaged by Matheja and Degens (1967) would incorporate magnesium ions that happened to be associated with amino acids, albeit weakly, at the site of calcification. In this hypothesis, the magnesium in high-Mg calcites is considered to be an impurity accidentally incorporated in the crystal, because it happened to be captured or temporarily "detained" in the immediate environment of the growing crystal. Thus the amount of magnesium in a particular skeletal ossicle should be a function of the rate of crystallization, the concentration of magnesium ions in the biological fluids at the site of crystallization, and the amino acid composition of the biological substrate. Such a theory must then account for the observations that: (1) the temperature of the echinoderm's environment is positively correlated with the concentration of magnesium in its skeleton, (2) independent of temperature, there are appreciable variations in the magnesium content of homologous skeletal elements taken from different types of echinoderms (a genetic factor), and (3) within a single echinoid, magnesium is concentrated in some types of skeletal elements much more so than in others. In other words, the principal variables controlling the magnesium content of echinoderm calcites are temperature; type of echinoderm, and in the case of echinoids, type of skeletal ossicle.

Rate of calcification–dependence on temperature and genetic factors. —According to the working hypothesis, the fact that skeletal magnesium is positively correlated with environmental temperature when the two variables, type of ossicle and type of animal, are held constant is attributed to the rate of calcification. The assumptions inherent in this argument are: (1) calcification rates are greater at higher temperatures, and (2) the greater the rate of calcification the larger the quantity of magnesium captured by a crystal growing in a given organic matrix.

Although the argument is intuitively plausible, supporting evidence at the present time is meager and largely circumstantial. Moberly (1966) analyzed the skeletal calcite of Recent algae by electron microprobe and reported that the magnesium content is correlated with skeletal growth rate; the latter varies with temperature in a yearly cycle. These data cannot prove, however, that the relationship is causal rather than a result of one or more other variables which happen to be positively correlated with temperature and/or growth rate.

Further evidence consistent with the hypothesis is found in the few reported studies of echinoderm respiration rates. Determined by measuring the rate of oxygen consumption, respiratory rates of tropical echinoids have been shown to be higher than rates reported for urchins living in colder water (Lewis 1968), and there appears to be a positive correlation between rate of respiration and rate of growth. Of interest in the present argument is that in a comparative study of tropical echinoids from Barbados, Lewis found that epifaunal forms consume oxygen at a significantly higher rate than do the infaunal forms from the same community. The epifaunal forms are the "regular" echinoids (sea urchins) living on top of the sediment, whereas the infaunal forms are the "irregular" echinoids (sand dollars and heart urchins) which live below the sediment-seawater interface. The epifaunal genera studied by Lewis were *Eucidaris, Echinometra, Tripneustes,* and *Diadema.* The infaunal specimens belonged to species of *Mellita* and *Brissus.* Thus despite the general dependence of growth rate (and presumably rate of calcification) on temperature, Lewis showed that respiratory rates are also controlled by other factors. If skeletal magnesium is dependent on rate of calcification, the irregular infaunal echinoids should usually have less magnesium in their skeletons than regular epifaunal urchins from the same locality.

Inspection of the magnesium data, restricting the comparison to homologous skeletal parts, does support this argument. At Heron Island (Great Barrier Reef, Australia) magnesium in the coronal plates of *Laganum,* an infaunal (I) sand dollor, is between 1 and 2 percent lower than in coronal plates of the epifaunal (E) specimens of *Echinometra* and *Heterocentrotus.* The same relationship is found at other collecting sites where both regular and irregular echinoids were collected, for example New Caledonia, Eniwetok-Bikini, Florida, Jamaica, et cetera. The difference between *Maretia* (I) and *Echinostrephus* (E) at Eniwetok Atoll is 4.5 percent $MgCO_3$. Other examples are *Echinometra* (E) versus

Meoma (I) from Jamaica (difference 3.7 percent), and *Diadema* (E) versus *Echinarachnius* (I) from Florida (difference 5.0 percent). A striking exception is the genus *Tripneustes* (a regular epifaunal echinoid), specimens of which invariably contain less Mg in the coronal plates than other regular echinoids from the same community. Usually, the data for this genus fall within the range of magnesium concentrations for the infaunal urchins. At a number of localities where all the analyzed specimens were collected by the author, the exact collecting site was recorded. It is evident for these samples that echinoids taken from the high-energy zone at the reef edge (for example *Heterocentrotus, Echinometra*) contain more magnesium than do echinoids from shallower portions of the adjacent lagoon (for example, *Diadema, Eucidaris*) or quiet water bays (for example, *Lytechinus*) which in turn are enriched in magnesium with respect to the infaunal group. Analytical data for 15 echinoid species of Eniwetok (Raup, 1966) are consistent with these observations: the content of Mg in coronal plates of regular urchins is generally greater than for irregular echinoids, with *Echinometra* and *Heterocentrotus* near the top of the range for the regular forms and *Tripneustes* near the bottom.

Composition of the calcifying organic matrix.—Among calcareous marine invertebrates the echinoderms as a group are to some extent unusual in possessing certain biochemical features more similar to the chordates (which includes the vertebrates) than do other groups of the Bilateria (Travis and others, 1967). The organic matrix of echinoderm skeletons is largely collagenous (Travis and others, 1967) and except for echinoderms, the only mineral phase associated with collagen fibrils is apatite, for example, the calcium phosphate of vertebrate skeletons. The amino acid composition of echinoderm soft tissues is poorly known. Degens and Spencer (1967), however, reported data for *Arbacia punctulata* that indicate that the amino acid patterns for spines and coronal plates are different. From these data it is possible to make a crude calculation of the relative calcium and magnesium complexing power of spines and coronal plates.

Stability constants for Ca- and Mg-amino acid complexes were obtained in two ways: (1) experimentally determined values compiled by Bjerrum, Schwarzenbach, and Sillén (1957), and (2) values obtained by extrapolating experimental data for elements such as Fe++, Mn++, Zn++, et cetera as a function of ionization potential according to Ahrens (1966) (see fig. 4). The ratio calculated is:

$$\frac{\sum_{i=1}^{n}\left[\%A_i\,(K_{1(i)}{}^{Mg}/K_{1(i)}{}^{Ca})\right]_{\text{test}}}{\sum_{i=1}^{n}\left[\%A_i\,(K_{1(i)}{}^{Mg}/K_{1(i)}{}^{Ca})\right]_{\text{spine}}}$$

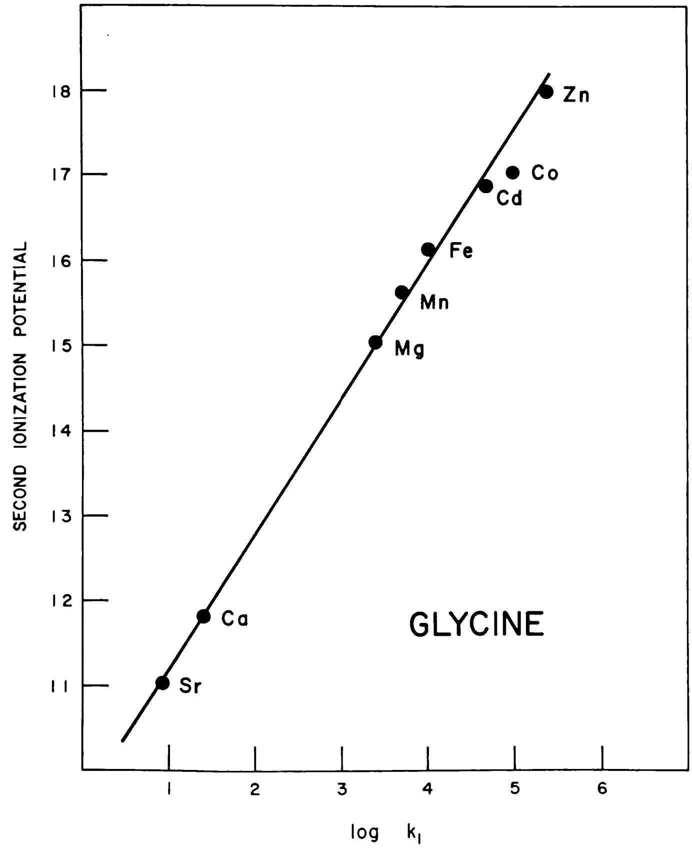


Fig. 4. Linear relationship between second ionization potential of divalent cations and the logarithm of the stability constant for complexes with the amino acid glycine.

where $K_{1(i)}{}^{mg}$ is the stability constant for amino acid *i* and Mg, and $\%A_i$ is the percentage of amino acid *i* in either spine or test. The calculated ratio is 1.38. The observed value of $(Mg/Ca)_{test}/(Mg/Ca)_{spine}$ based on magnesium analyses of 13 specimens is 1.36.

This approach deserves further experimental investigation along more rigorous lines. The complexing power of amino acids combined in the form of proteins, for example, will differ from the complexing power of the same free amino acids as some of the groups are used to form peptide bonds. Although the calculation shown above is based on stability constants for free amino acids, it is likely that differences in the complexing ability of free and combined amino acids will affect both the numerator and denominator of the test to spine ratio to a similar extent.

CONCLUSION

The concentration of magnesium in echinoderm calcites is a function of both environmental and genetic factors. Environmental temperature appears to be the most important variable, but systematic variation of Mg content within a single echinoid skeleton and significant compositional differences between homologous skeletal parts of different genera from the same environment indicate that genetic factors also control the distribution of skeletal magnesium. The rate of calcification is proposed to account for both the temperature effect and the "species effect", whereas within-skeleton variation is believed to arise from variations in the composition of the organic matrix which functions as a "mineralization template".

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