These observations suggest that we are dealing with the effects of an interaction of genuine catalytic nature between the enzyme present in the system, presumably adsorbed to the lipid-water interface (see *4* for evidence on absorption), and the added substrate molecules. It also appears that the enzymatic reaction is effective as a process and not as a source of active ions or molecules.

It should be emphasized that not all the phospholipid extracts produce films which show the impedance changes described. In fact, many of them yield films which are "inert" but otherwise indistinguishable from the "responsive" ones.

As yet we cannot explain such differences, although we have found that they depend on the particular brain material employed rather than on small variations in technique. It is, therefore, advisable to work with a number of brains at the same time, preparing and testing small amounts of chloroform-methanol extract from each. Larger volumes of solution can then be obtained from those brains which produce responsive membranes.

Both the quantity and quality of the cholesterol added to the phospholipid extracts are critical. Best results were obtained working with a mixture of 1 ml of extract, 150 mg of *dl*-α-tocopherol, and 30 mg of cholesterol; "99%+" cholesterol standard for chromatography (Sigma Chemical Company) was employed, since the products of oxidation of this compound, even in small amounts, block the responses of the lipid films (*5*).

It has been found possible, as well as convenient, to preserve the mixture of phospholipid extract, cholesterol, and tocopherol in sealed ampoules under a nitrogen atmosphere; these ampoules have been kept without appreciable deterioration for periods of up to 2 months at 5°C (*6*).

The appearance of the film and the absolute value of its transverse resistance are not critical for the occurrence of the impedance changes. Though most membranes show distinct black areas and their d-c resistances are of the order of 10^6 ohm cm^2, large responses have been observed both in relatively thick membranes where those areas could not be discerned and in films with resistances lower than 10^5 ohm cm^2. Therefore we are inclined to believe that such changes do not occur in the regions of the film where the phospholipid molecules form highly organized smectic structures, but rather at the level of more permeable areas with, perhaps, a predominantly micellar structure. However, no impedance changes were observed when specific antigens and substrates were added to the opposite side of the lipid film from that exposed to the corresponding antibody or enzyme.

Apart from the problems posed by the mechanisms responsible for such impedance changes, the effects are interesting for two main reasons. First, they can be used for extremely fast detection of antigen-antibody reactions and are already being used routinely in this laboratory for such purposes. Second, the fact that marked impedance changes are produced in enzyme-complexed membranes upon exposure to substrates of low molecular weight is interesting, since these systems may be regarded as analogs of chemical synaptic mechanisms in which the presynaptically liberated transmitter substance changes the impedance of the postsynaptic membrane upon its combination with receptor sites on its surface.

J. del Castillo
A. Rodriguez
C. A. Romero, V. Sanchez
Department of Pharmacology,
School of Medicine,
San Juan, Puerto Rico 00905

References and Notes

1. P. Mueller, D. O. Rudin, H. T. Tien, W. C. Wescott, *Circulation* **26**, 1167–1171 (1962); in *Recent Progress in Surface Science*, J. F. Danielli, K. G. A. Pankhurst, A. C. Riddiford, Eds. (Academic Press, New York, 1964), vol. 1, pp. 379–393.
2. J. H. Humphrey and R. R. Dourmashkin, in *Complement*, Ciba Foundation Symposium, G. E. W. Wolstholme and J. Knight, Eds. (Little, Brown, Boston, 1965), pp. 175–186.
3. A. L. Lehninger, *Physiol. Rev.* **42**, 467–517 (1962).
4. T. Hanai, D. A. Haydon, J. Taylor, *J. Theoret. Biol.* **9**, 422–432 (1965).
5. *Note added in proof.* Analysis by thin-layer and vapor-phase chromatography has shown that oxidation derivatives of tocopherol also block the film responses. Separate chromatographic analysis of the chloroform-methanol extract and the above-mentioned additives is strongly recommended.
6. We shall be glad to supply small amounts of this material to interested investigators.
7. Supported in part by PHS grant NB-05235.

28 February 1966

■

Diagenesis of Carbonate Sediments: Interaction of Magnesium in Sea Water with Mineral Grains

Abstract. *Samples of natural fine-grained carbonate sediment from Florida Bay, Florida, undergo mole-for-mole cation exchange with aqueous solutions of $MgCl_2$ and $CaCl_2$ in the laboratory. The exchange reaction, which involves the surface of the grains of sediment, can be essentially described by a simple mass action–law equation. Enrichment of Mg^{++} beyond the amounts found within particle interiors should take place on the surface of $CaCo_3$ sediments immersed in sea water; it may be on both exchangeable and unexchangeable sites.*

There is considerable evidence (*1, 2*) for the generalization that recrystallization of the common carbonate minerals of Recent marine sediments, aragonite and highly magnesian calcite, to the carbonate minerals characteristic of ancient rocks, calcite and dolomite, is not rapid in sea water of average salinity; in other words, carbonate diagenesis, with consequent formation of limestones and dolostones, is inhibited by sea water. This phenomenon cannot be explained simply by the bulk thermodynamic properties of the individual minerals. From the data of Lerman (*3*) and the thermodynamic data summarized by Langmuir (*4*) it can be shown that, in sea water of average Mg:Ca ratio, stoichiometrically ordered dolomite is more stable than low-magnesium calcite (calcite containing less than 4 mole percent $MgCO_3$ in solid substitution) and that low-magnesium calcite is more stable than aragonite or high-magnesium calcite (calcite containing more than 10 mole percent $MgCO_3$). Absence of recrystallization must result from surface effects that retard rates of solution of metastable phases or prevent nucleation and growth of low-magnesium calcite and dolomite.

The most common explanation of the absence of diagenetic formation of low-magnesium calcite in sea water is that Mg dissolved in the water acts as a specific inhibitor of crystallization. The role of Mg ion in retarding the rate of recrystallization of aragonite to calcite, even in the presence of calcite seeds, has been directly demonstrated in the laboratory (*5*). Other laboratory studies have shown that dissolved Mg: (i) severely inhibits precipitation of low-magnesium calcite from artificially

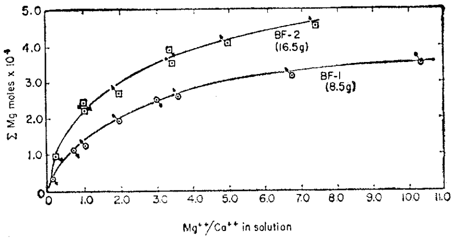

Fig. 1. Plot of ΣMg (total Mg on the grain surfaces) versus molar $Mg^{++}:Ca^{++}$ ratio in solution for samples BF-1 and BF-2. Surfaces of both samples were initially saturated with Ca^{++}. Each point represents at least 1 day of reaction. Arrows, attached to points, aim in the directions in which points would have moved if equilibrium had not been achieved; that is, $\nwarrow\odot$ indicates addition of $MgCl_2$ solution and $\odot\searrow$ indicates addition of $CaCl_2$ solution. Dry weights of the samples appear in parentheses.

supersaturated sea water, whereas the other major ions of sea water do not (*6*); and (ii) causes anomalous solubility behavior of natural carbonate minerals in sea water and in $MgCl_2$ solutions (*7*, *8*). Thus it is apparent that the surfaces of carbonate minerals placed in sea water must react with Mg^{++} dissolved in the water. I now demonstrate at least one phase of this reaction.

Samples of natural clay-sized carbonate sediment from Florida Bay, Florida, consisting almost entirely of aragonite and 15-mode mole percent Mg-calcite (*9*), were reacted at room temperature with aqueous solutions of $MgCl_2$ and $CaCl_2$. To enable measurement of large percentage changes in dissolved Mg and Ca, relatively small volumes of solution (10 to 20 ml), with concentrations ranging from 0.01 to 0.10*M*, were added to relatively large masses of solid sediment (10 to 20 g). The air-dried natural sediment was initially disaggregated with an ultrasonerator (Branson Instruments, Inc.) for 5 to 10 minutes and washed in distilled water to remove soluble sea salt.

Subsequent treatment varied according to the purpose of the experiment: the washed samples were either immediately reacted with small volumes of $MgCl_2$ and $CaCl_2$ solutions or first treated with a large excess of either 0.1*M* $MgCl_2$ or 0.1*M* $CaCl_2$ solution. The purpose of the latter procedure was to initially saturate the grain surfaces with either Mg or Ca; use of large excesses made possible high ion ratios of Mg:Ca or Ca:Mg ($\approx$ 50 to 1) in solution.

Treatment with small known amounts of dissolved $CaCl_2$ or $MgCl_2$ was used to delineate surface reaction. The measured gain or loss of Mg^{++} and Ca^{++} from solution was assumed to represent the loss or gain, respectively, of ions from the sediment particles. After addition of solution, each sample was placed in suspension with a common blender to form a thick slurry. For chemical analysis, clear water samples were collected by centrifugation, and by filtration of the supernatant through a $0.5\text{-}\mu$ Millipore filter. Chemical analysis was by ethylenediaminetetraacetic acid titration (*2*), precise within 1 percent.

In order to insure that steady state was achieved, reacting solutions were analyzed repeatedly at 1-day intervals until changes in concentration ceased; in the vast majority of samples, equilibrium was achieved during the 1st day. On the other hand, analyses conducted about 10 minutes after addition of solutions to the sample containers showed that equilibrium was not achieved on the time scale of minutes. When equilibrium of solution-plus-solid had been demonstrated, the weight of wet, centrifuged samples was recorded, and a new portion of $CaCl_2$ or $MgCl_2$ solution was added to the sediment. From the weights of dry solids and wet sediments and from the volumes and ionic concentrations of remaining and newly added solutions, subsequent gains or losses of Ca^{++} and Mg^{++} could be calculated. The procedure of solution addition, slow equilibration, and water analysis was repeated many times for each sediment sample in order to trace a long-term "titration" curve and to check for irreversible hysteresis effects during cycling between additions of $MgCl_2$ and $CaCl_2$ solutions.

Completion of several analyses showed that considerable reaction between $MgCl_2$ or $CaCl_2$ solutions and natural fine-grained carbonate sediments does take place in the form of mole-for-mole cation replacement. Initial reaction with sediments previously treated only with distilled water results in mole-for-mole exchange with the calcium and magnesium of the carbonate; this fact, along with limited reaction, indicates that Ca^{++} and Mg^{++} react directly (by replacement) with ions at or near the surface of the carbonate particles.

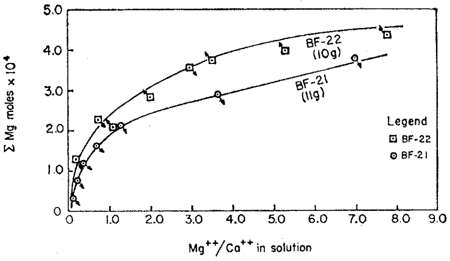

Fig. 2. Plot of ΣMg versus molar Mg^{++}: Ca^{++} ratio in solution for samples BF-21 and BF-22. Symbols as in Fig. 1. Surface of BF-21 was initially saturated with Mg^{++}; surface of BF-22, with Ca^{++}.

Figures 1 and 2 show the results for four "titration" runs. The value of ΣMg (total Mg added to the carbonate grains) was calculated from changes in concentration of dissolved Mg^{++} (or Ca^{++}) on the assumption that initially the surfaces of the grains were entirely in the calcium or magnesium form; thus pretreatment by soaking in a large excess of concentrated solution of $MgCl_2$ or $CaCl_2$ was employed for all runs shown. It is evident from Figs. 1 and 2 that the cation-replacement process is slowly reversible; in other words, no appreciable hysteresis was obtained if individual reaction periods were about 1 day.

As I have mentioned, in shorter runs on time scales of minutes equilibrium was not achieved, and a result was hysteresis effects, during cation cycling, with a separation of two curves: one for $CaCl_2$ addition and one for $MgCl_2$ addition. Reversibility was evident only if ΣMg was plotted against the ratio $Mg^{++}:Ca^{++}$ in solution. Plots of ΣMg versus concentration of Mg^{++} in solution were highly irregular and showed much point scatter. The relative smoothness of the curves in Figs. 1 and 2 indicates that arbitrary changes in total dissolved-cation concentrations ($Ca^{++} + Mg^{++}$) from point to point had no significant effect. Total cation concentrations ranged from 0.02 to 0.10*M*. It is quite probable, however, that if a broader range of concentration had been studied some dependence on total concentration would have been observed.

Total reversible Ca-Mg exchange capacity for BF-1, BF-2, and BF-22 was determined roughly by a method of simple linear extrapolation, which assumes ideal solution behavior for the grain surfaces (see legend to Fig. 3) (total capacity for BF-21 was measured directly). Because a wide range of Mg : Ca ratio was explored, extrapolated values are not far higher than

FIG. 3

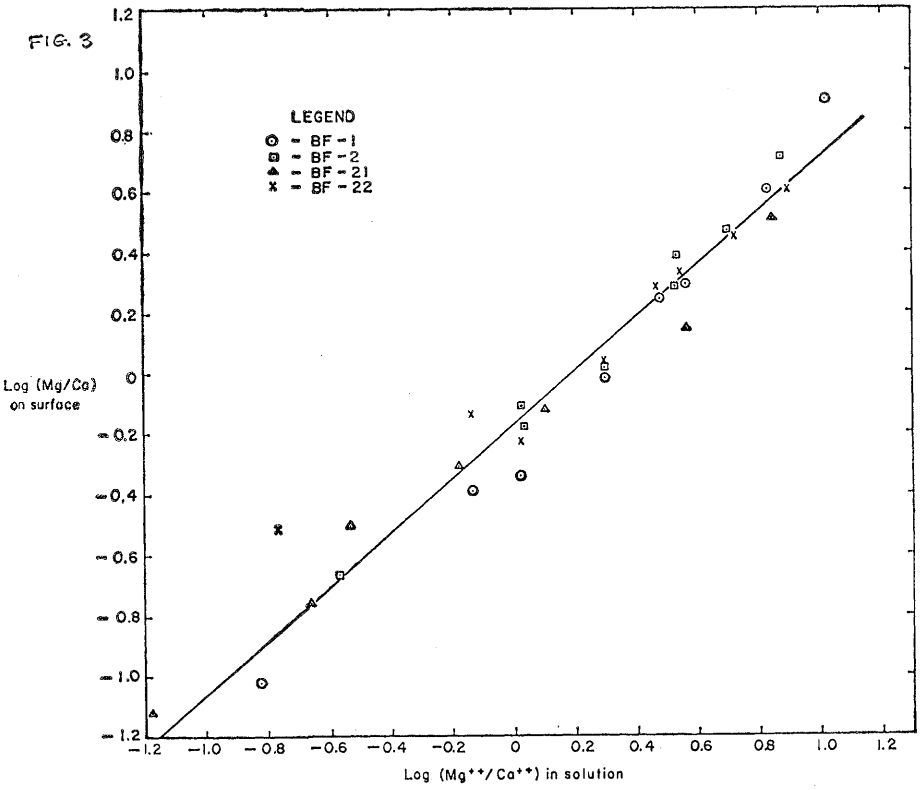

Fig. 3. Plot of log (Mg:Ca) on surface against log ($Mg^{++}:Ca^{++}$) in solution. Value of Mg:Ca on surface for BF-1, BF-2, and BF-22 assumed to equal $\Sigma Mg/(T - \Sigma Mg)$ where T is the extrapolated value for ΣMg at $Mg^{++}:Ca^{++} = \infty$ (T is obtained by plotting $Ca^{++}:Mg^{++}$ in solution against $1/\Sigma Mg$ and extrapolating to $Ca^{++}:Mg^{++} = 0$). Straight-line fit of points is by least squares. Values of T (in moles × 10^{-4}) are: BF-1, 4.0; BF-2, 5.5; BF-21, 5.0; BF-22, 5.5.

the nearest measured values. Total exchange capacities, in terms of units commonly employed by clay mineralogists, range from 6 to 12 meq/100 g—a rather high value for a natural substance, $CaCO_3$, not normally considered as an important ion exchanger (*10*); the high capacity most likely results from a very great specific surface area (*11*).

Centrifugation in distilled water of ultrasonically disaggregated sediment indicated that the median nominal grain radius of the samples studied was less than 2 μ. Electron micrographs at high magnification (*7*) show rough, irregular surfaces and microporosity in submicron grains, which features further contribute to a great specific surface area.

From knowledge of the total Mg-Ca ion-exchange capacity the molar ratio of exchangeable cations, Mg:Ca, on the grain surface can be readily calculated for each point of the curves. Figure 3 shows a log-log plot of this ratio against the corresponding Mg^{++} : Ca^{++} ratio in solution for each point. Because the points appears to crudely delineate a straight line, a line of best fit by least squares has been plotted; the equation of this line is:

$$\log (Mg : Ca)_{surf.} = -0.179 + 0.88 \log (Mg^{++} : Ca^{++})_{soln.} \quad (1)$$

which in antilogarithmic form is:

$$(Mg : Ca)_{surf.} = 0.66 \, (Mg^{++} : Ca^{++})_{soln.}^{\;0.88} \quad (2)$$

This exponential form of dependence is common and characteristic of many synthetic and natural ion-exchange materials (*12, 13*). The exponent 0.88 is close to 1, the value expected for an ideal ion exchanger (*13*). On the assumption that 0.88 is approximately 1 and reflects only experimental error, the Ca-Mg ion-exchange process on the surface of natural carbonate minerals, under the conditions of this study, can be described simply by the reaction:

$$Mg^{++}_{aq.} + Ca[surface] \rightleftarrows Ca^{++}_{aq.} + Mg[surface]$$

with K, the mass-action-law constant for this reaction, equalling 0.66. This fact indicates that there is a bonding preference for Ca, over Mg, on the grain surfaces and that the surface can be treated essentially as an "ideal" solution.

Because the total surface area of the sediment samples has not been determined, and because the exact nature of the exchange process (for example, monolayer, multilayer, and so on) is unknown, one cannot assume that the entire surfaces of both the Mg-calcite and the aragonite grains are implicated in reversible Ca-Mg exchange; it is quite probable that Mg exchange is restricted to only energetically favored sites; additional work suggests that only a portion of the grain surface is involved. When reacted with 0.1*M* $MgCl_2$ solution, reagent-grade calcite, thoroughly ground in a ball mill (Pitchford model 3800) to produce a strained surface and a large specific area, shows a significant irreversible exchange of Mg^{++} from solution for Ca (about 30 meq/100 g). Most of this Mg cannot be subsequently removed by exchange for Ca^{++} ion, and is taken up so slowly that equilibrium is not achieved within 10 days. Subsequent treatment with $CaCl_2$ solution, however, does result in limited ion exchange, and, upon continuous recycling between $CaCl_2$ solutions and $MgCl_2$ solutions, the rate of attainment of equilibrium becomes much faster, the total exchange capacity approaches a small reproducible value of about 3 meq/100 g, and a reversible-exchange curve similar to those in Figs. 1 and 2 is obtained. Therefore the exchangeable Mg on the calcite surface, for any Mg : Ca ratio in solution, represents only a small proportion of the total Mg present. This may be true for the natural sediment samples also; some irreversible, unexchangeable Mg may have been supplied by the original sea water from which the samples were taken.

High-magnesium calcite is the most abundant mineral in the samples studied; Mg is an important constituent of its crystal structure. It probably has a more reactive surface than aragonite, as shown by its preferential removal, relative to aragonite, during treatment with carbonic acid (*7*); and oolite aragonite ground in a ball mill shows considerably less exchange of Mg than similarly ground calcite. These observations suggest that much of the total Ca-Mg exchange may take place only on the surface of grains of high-magnesium calcite.

The molar Mg : Ca ratio of sea water is about 5 to 1. Assuming that

Eq. 2 represents cation-exchange behavior in sea water for aragonite and high-magnesium calcite, it is apparent that the outer surface of carbonate-sediment grains immersed in sea water should be enriched in Mg relative to the interior of the grains. Surface ratios of Mg : Ca in exchange sites, calculated from Eq. 2, should be approximately 3 in sea water, compared to bulk values of 0.16 and <0.01 for high-magnesium calcite and aragonite, respectively.

In sea water some of the exchange sites may be occupied by sodium as well as calcium and magnesium because of the high concentration of Na^+ in the water. Exchange experiments analogous to those described, involving Ca, Mg, and Na, indicate, however, that at the Na : (Ca+Mg) ratio of sea water no more than 20 percent of the total exchange sites are occupied by Na^+. This percentage must be a maximum because much of the exchange Na^+ may be associated with organic matter (see *11*). Because Na^+ is not a lattice ion in $CaCO_3$ and is univalent, it would not be expected in general to contribute heavily to exchange with Ca and Mg on $CaCO_3$ surfaces.

It is probable that Mg^{++} on or near the surfaces of particles of carbonate sediment immersed in sea water shows a range of reactivities from unexchangeable, strongly bonded Mg^{++} (incipient dolomite?), through slowly reversible Mg^{++} exchangeable only for Ca^{++}, to rapidly exchangeable, weakly bonded Mg entering readily into exchange not only with Ca^{++} but also with Na^+ and other cations of sea water. Which type of Mg may be able to inhibit carbonate grains from reacting to form low-magnesium calcite or dolomite remains unknown.

ROBERT A. BERNER
Department of Geology, Yale University, New Haven, Connecticut

References and Notes

1. K. E. Chave, *J. Geol.* **62**, 587 (1954); R. N. Ginsburg, *Soc. Econ. Paleontologists Mineralogists Spec. Publ. 5*, p. 80 (1957); S. O. Schlanger, *U.S. Geol. Surv. Profess. Papers 260-BB*, p. 991 (1963); W. H. Taft and J. W. Harbaugh, *Stanford Univ. Publ. Univ. Ser. Geol. Sci.* **8(1964)**, No. 2; O. H. Pilkey, *Bull. Marine Sci. Gulf Caribbean* **14**, 126 (1964); M. G. Gross and J. I. Tracey, Jr., *Science* **151**, 1082 (1966).
2. R. A. Berner, *Amer. J. Sci.* **264**, 1 (1966).
3. A. Lerman, *Geochim. Cosmochim. Acta* **29**, 947 (1965).
4. D. Langmuir, thesis, Harvard Univ. (1964).
5. W. H. Taft, *Physical Chemistry of Formation of Carbonates* (Elsevier, Amsterdam, 1966), chap. 5; W. S. Fyfe and J. L. Bischoff, *Soc. Econ. Paleontologists Mineralogists Spec. Publ. 13*, p. 1 (1965).
6. Y. Kitano and D. W. Hood, *J. Oceanog. Soc. Japan* **18**, 35 (1962); K. Simkiss, *Nature* **201**, 492 (1964); P. H. Monaghan and M. L. Lytle, *J. Sediment. Petrol.* **26**, 111 (1956).
7. P. K. Weyl, *EPR Publ. 428* (Shell Development Co., Houston, Texas).
8. R. A. Berner, unpublished.
9. No clay minerals are present in these samples; acid-insoluble residues ($\approx$ 3 percent by weight) consist of biogenic silica, organic matter, and a trace of quartz.
10. D. Carroll, *Bull. Geol. Soc. Amer.* **70**, 749 (1959).
11. A small portion of the total exchange capacity reflects noncarbonate material in the samples. The carbonate-free, acid insoluble (cold dilute HCl) residue of run BF-2 was titrated with $NaHCO_3$ (to exchange Na^+ for H^+ and neutralize acid), washed, and then reacted with $CaCl_2$ and $MgCl_2$ solutions in the same manner and at the same *p*H as the original sample. The total exchange capacity for the residue, due mainly to the organic matter, was about 5 to 10 percent of the total capacity of the original sediment.
12. H. F. Walton, in *Ion Exchange Theory and Practice* (Academic Press, New York, 1949), p. 3.
13. R. M. Garrels and C. L. Christ, *Solutions, Minerals and Equilibria* (Harper and Row, New York, 1965), p. 267.
14. I thank the Petroleum Research Fund, administered by the American Chemical Society, for support (grant 1937-A2). Most of the laboratory analyses were performed by Mary Sullivan.

16 March 1966

■

Foraminiferal Evidence of a Shallow Calcium Carbonate Solution Boundary, Ross Sea, Antarctica

Abstract. *In the Ross Sea, Antarctica, distributions of calcareous Foraminifera and other calcareous microorganisms are dominantly controlled by a shallow calcium carbonate solution boundary that occurs at depths of about 500 meters. Possible causes of substantial undersaturation of Ross Sea bottom waters in calcium carbonate are very low temperatures and high salinities, which favor the solution of calcium carbonate and, possibly, high concentrations of carbon dioxide.*

Foraminifera from 48 ocean-bottom samples collected within the Ross Sea between 1959 and 1961 by the New Zealand Oceanographic Institute during two cruises by H.M.N.Z.S. *Endeavour* have been studied. Of these samples, 37 were collected by orange-peel or Dietz-La Fonde grabs; 11, by Devonport dredge. No preservative was added to the foraminiferal samples and thus no post-collection solution of calcium carbonate could have occurred. Information from these samples has been supplemented by data from 60 earlier samplings by others (*1–3*). Ross Sea environmental factors and the distribution and foraminiferal content of Ross Sea samples studied by me are detailed elsewhere (*4*).

The presence of two greatly contrasting types of microfaunas are indicated: (i) Calcareous faunas made up of abundant calcareous Foraminifera, less-abundant Ostracoda, Bryozoa, and minute Mollusca, and other calcareous organisms, with relatively few arenaceous Foraminifera; (ii) dominantly noncalcareous and more-sparse faunas made up of arenaceous Foraminifera (> 85 percent benthonics), diatoms, and silicious sponge spicules (Fig. 1).

Most calcareous faunas are restricted to depths less than 550 m, while all the dominantly noncalcareous faunas occur deeper than 430 m. Abundant planktonic Foraminifera occur in all the calcareous faunas and generally constitute between 5 and 86 percent of the foraminiferal sample; they are generally absent from the arenaceous faunas and, in all but two samples, are very rare, constituting less than 3.2 percent of the foraminiferal sample (Fig. 2). The planktonics steadily increase in abundance to about 500 m; their sharp decrease at greater depths is associated with the appearance of dominant arenaceous faunas (Fig. 2).

The waters from which the samples were taken range in temperature as little as 2°C (+0.4 to −2°C) and in salinity less than 1 per mille (34.52 to 35.08 per mille); they are almost isothermal and isohaline, and the benthic faunas seem little affected by the small variations. There are no published data on the distribution of carbon dioxide in the Ross Sea.

Study of five cross sections across the Ross Sea (*4*) showed important relations between certain environmental factors and foraminiferal trends, of which the most striking is correlation between bathymetry and test (shell) composition; on the whole there is no definite relation between texture of bottom sediment and test composition. Although most calcareous faunas were associated with coarse sediment and most arenaceous faunas with fine sediment, the reverse was true for a reasonable number of stations—for example, stations A644 and A645, Mowbray Bay: A644 (530 m) is represented