Table 2. Average weight ratios between various size fractions. Numbers of samples appear in parentheses.

Weight loss (%)	Size fractions (μ)			
	>250/<250	>177/<177	>125/<125	>250/<125
0–4 (4)	.184	.372	.847	.288
5–9 (3)	.185	.372	.830	.285
10–19 (2)	.175	.364	.795	.268
>20 (1)	.153	.297	.664	.220

Table 3. Rank-difference correlations between the percentages of broken specimens (A) and fragments (B) and weight loss by solution. Positive values indicate increasing amounts of damaged shells with progessive solution.

Case	Size fractions (μ)			
	>250	177–250	125–177	62–125
A	.90	.71	.29	.44
B	.19	−.01	.78	.51

Table 4. Rank-difference correlations between average weight of particles and weight loss by solution. Positive values indicate that the average weight decreases with progressive solution.

Size fractions (μ)			
>250	177–250	125–177	62–125
All particles			
.59	.39	.71	.90
Without fragments			
.60	.30	.38	.87

Preferential solution of large specimens and associated accumulation of fragments in the finer fractions are consistent with these findings.

It would be presumptuous to expect similar relations in all foraminiferal oozes that undergo solution. Fragments are expected to dissolve more quickly than whole tests because (i) the specimens producing them must have been less resistant to solution than the undamaged shells in the assemblage, and (ii) fragments have more effective surface area per unit weight than whole tests. Thus, the destruction of fragments may keep pace with their production at moderate rates of weight loss. This tendency should make it very difficult to recognize the effects of solution on the basis of damaged shells alone. The weight ratios, however, may still provide a clue if, indeed, large specimens dissolve more quickly than small ones.

The average particle weight in each size fraction was investigated also. The data (Table 4) demonstrate that particle weight decreases as solution progresses. That the effect is not due wholly to the increasing amounts of low-weight fragments is shown by the values in the second row. In many instances the effect of solution is a layer-by-layer removal of shell material, which makes tests lighter without leaving much evidence of damage; in this manner, solution should decrease the maximum particle weights for given sizes in each species.

There is evidence, from counting thin- and thick-shelled specimens separately, that thin-shelled varieties of a species dissolve much faster than thick-shelled ones. Thus the more delicate tests would be eliminated while the remaining thick tests were only somewhat thinned by the process just outlined, in foraminiferal ooze undergoing moderate solution. Ultimately, strong clustering of individual weights around a rather high value for each size bracket in each species should indicate the effects of selective solution.

My evidence indicates that *Globigerina* ooze is affected appreciably by the undersaturated ocean water well above the compensation depth, even over extremely short time spans. Some protection on the ocean floor therefore seems to be necessary, where exposure times are much longer than the duration of my experiment. Such protection may be provided by a semisaturated layer of bottom water. The end effect is adjustment of the assemblage to the surrounding ocean water.

The mechanism of adjustment seems to be selective solution. In the samples studied, the spinose species and the larger specimens of the various species appear to dissolve more rapidly. Solution tends to reduce the diversity of an assemblage, change the size distribution, and decrease the range of weights of single tests for each size in the various species.

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5. Set by M. N. A. Peterson at 18°49'N, 168°31'W; I was permitted to use it.
6. Weight loss and solution rate are related as follows: solution rate (percentage per day) = [4.60517—log (100—weight loss)]/120. For values lower than about 20 percent it is sufficient to divide the weight losses given in Fig. 1 by 120, for similar results. The two samples at 250 m show weight increases of 0.3 (untreated) and 2 percent (treated with hydrogen peroxide); this gain may be due to either precipitation of calcium carbonate or experimental error.
7. Comparison with the solution profile for calcite spheres on the same buoy (*4*) shows that the values at 4250 m are unrealistically low; for this reason they were omitted when the lines were drawn in Fig. 1. All other samples in this container (I have not reported on them) show the same effect. I suspect that the container did not turn freely into the current.
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Chemical Exfoliation of Vermiculite and the Production of Colloidal Dispersions

Abstract. *Chemical treatments involving ion exchange cause gross one-dimensional swelling in water of single crystals of vermiculite minerals. A delicate balance of forces holds the individual silicate layers (10 angstroms thick) parallel to one another although separated by several hundred angstroms. Colloidal dispersions produced from the swollen crystals are morphologically unique and show strong film-forming characteristics.*

Vermiculites are hydrated magnesian aluminosilicate minerals with a layer structure similar to that of the micas (*1*). Like the micas, they occur as plate-shaped crystals consisting of 10-Å-thick silicate layers superimposed as in a pack of playing cards. The silicate layers are negatively charged and electrical neutrality is preserved by the presence of cations between layers. In the case of micas, these interlayer ions are usually K^+, whereas in the vermiculites they are usually Mg^{2+} or Ca^{2+} and are associated with double layers of water molecules. Under appropriate conditions, vermiculites are able to im-

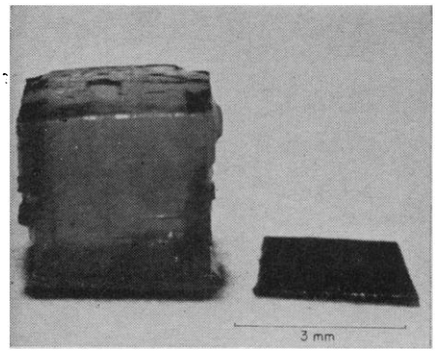

Fig. 1. Butylammonium-vermiculite crystals before and after swelling in water.

bibe much larger quantities of water and swell unidimensionally in the direction perpendicular to the silicate layer planes (Fig. 1).

The conditions under which this macroscopic swelling occurs involve the presence in the interlayer cation sites of specific cations which must be artificially introduced by ion exchange procedures. Among inorganic cations only Li^+ has been observed to produce this effect (*2*), while a number of organic cations, including *n*-propylammonium, *n*-butylammonium, *i*-amylammonium (*3, 4*) and certain amino acids (*5*) also initiate swelling.

The swelling mechanism resembles in important respects the swelling of Na-montmorillonite or Li-montmorillonite (*6*), in which hydration of the interlayer cations, by increasing the separation between successive silicate layers, weakens sufficiently the electrostatic attractive forces operating between them for osmotic repulsion to supervene (*7*). Swelling then proceeds in accordance with the theory of lyophobic colloids propounded by Verwey and Overbeek (*8*). In the case of a single macrocrystal of vermiculite, swelling does not proceed to complete dispersion of the silicate layers, and the swollen crystal remains coherent and jelly-like in consistency.

The negative charge on the silicate layers in vermiculites from different localities varies over a range but tends to be higher than that in montmorillonites and lower than that in micas. The amount of charge is an important factor in the ability of the various minerals to swell in the manner described. Thus, montmorillonites will swell with Na^+ and Li^+, while vermiculites do not swell with Na^+ but may do so with the more highly hydrated Li^+ provided their layer charge is not too high.

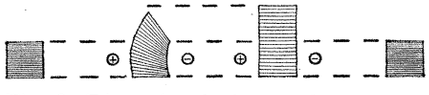

Fig. 2. Dimensional changes in a water-swollen butylammonium-vermiculite crystal immersed in dilute aqueous butylamine and placed in an electric field (schematic). From left to right the sketches are: before field applied; after 2 minutes; after 10 minutes; after removal of field.

With high-charge vermiculites and all the micas none of the inorganic or organic cations develop sufficient energy in the presence of water to overcome the attractive electrostatic forces and promote macroscopic swelling. In the case of the organic ions which activate the swelling process in vermiculites (*9*), only ions which form clathrate structures with water (*10*) are effective, and the step of ordinary ion hydration observed with the inorganic ions is replaced by one of clathrate formation (*4*).

According to theory, swelling is eventually limited by long-range attractive van der Waals forces which balance the osmotic repulsion. In a fully swollen crystal the silicate layers may be hundreds of angstroms apart, but remain essentially parallel to one another. This can be inferred from simple inspection (Fig. 1), but low-angle x-ray diffraction studies have confirmed that a considerable measure of regularity exists in the separation between successive layers (*11*). The structural picture therefore is one in which silicate layers approximating 10 Å in thickness but laterally very extensive are arranged in approximately parallel array and separated by hundreds of angstroms, the interlayer space being occupied by water molecules and a diffuse cloud of cations. The distance between silicate layers is reciprocally related to the concentration of electrolyte in the external solution, so that the swelling is maximal at infinite dilution.

Further swelling, electro-osmotic in origin and reversible, is induced if a swollen crystal is placed between electrodes so that the plane of the silicate layers lies in the direction of the field and a small potential is applied. As depicted in Fig. 2, the side proximate to the anode swells first and then the opposite side, till the crystal is perhaps twice as large as originally. When the field is removed the crystal slowly returns to its original size.

When a shearing force is applied to swollen crystals by means, for example, of a Waring blendor or colloid mill, the weak forces acting between layers break down and the individual layers disperse (*12*). In the shearing process, the silicate layers also tend to break across to some extent but even so the dispersed platelets may be of the order of tens of microns in lateral extent. The fact that a high proportion of these platelets exists in the form of single silicate layers can be demonstrated by means of shadowed electron micrographs.

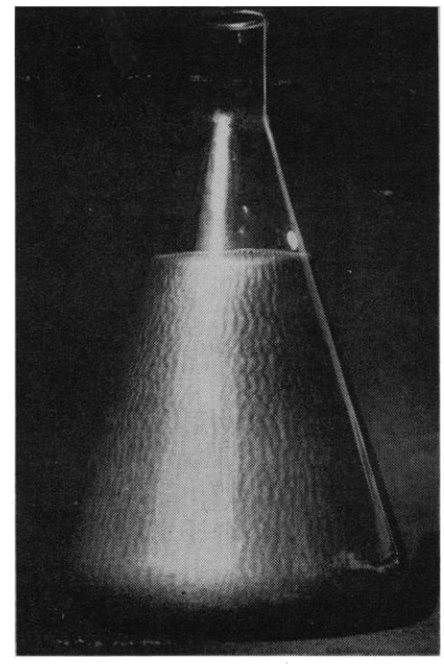

Fig. 3. Stable ripple structures produced by gentle vibration in aqueous butylammonium-vermiculite dispersion (2 percent solids).

The peculiar morphology of this system is reflected in its properties, the most obvious being the marked streaming birefringence of the dispersions. Moreover, in dispersions containing 2 or 3 percent, by weight, of solids, ripple structures produced by gentle vibration may remain stable for several days (Fig. 3). With certain vermiculites, light-scattering effects in the visible spectrum are also observed, colors ranging through violet, blue, green, yellow, and pink. This latter effect suggests that individual platelets adopt a rather regular inter-particle distance in the range 4000 to 7000 Å. The colors developed depend on the amount of shearing to which the dispersion has been subjected, that is, on the particle size, the color moving toward the violet end of the spectrum as the size is reduced. Dilution also affects the color, displacing it in this case toward the red end of the spectrum.

The dispersions are stable over long periods in distilled water and can be boiled vigorously without ill effect. They are, however, extremely sensitive to electrolytes; for example, the presence of $\sim 10^{-4}M$ Ca^{2+} causes flocculation. Even in distilled water they tend to deteriorate over a period of months owing to displacement of cations such as Mg^{2+} and Al^{3+} from the interior of the silicate layers by protons from the water. The presence of these ions in the "diffuse double layer" upsets the balance of forces operating between particles and leads to gradual flocculation of the system. Addition of acid produces a self-supporting gel at solids contents of the order of 0.25 percent. This gel is nonthixotropic and if broken up does not re-form. Alcohol and other organic liquids miscible with water can be added to lithium dispersions without causing flocculation, provided the dielectric constant of the medium does not fall below about 60. Butylammonium dispersions, however, are more sensitive to the addition of organic liquids and their behavior is less reproducible.

When an aqueous vermiculite dispersion is dried on a smooth surface, a coherent coating is deposited which can be subsequently stripped off the surface as a strong, flexible film. Films a few microns thick can be handled without difficulty. The marked film-forming property of the dispersions in clearly related to the morphology of the vermiculite platelets. When these are deposited by slow evaporation of the water they tend to lie flat on their basal surfaces and overlap with a number of other platelets, allowing strong electrostatic bonding to develop. In their original state the films are highly susceptible to moisture and rapidly disintegrate in water. However, they become insensitive to boiling water or organic solvents if the interlayer cations that produce macroscopic swelling are exchanged for "nonswelling" cations such as Mg^{2+}.

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21 February 1967

Amino Acid Coding in Sarcina lutea and Saccharomyces cerevisiae

Abstract. *Aminoacyl-tRNA's from* Sarcina lutea *were tested for incorporation into protein in a heterologous system from* Escherichia coli *or for binding in a homologous system from* Sarcina lutea. *Aminoacyl-tRNA's from* Saccharomyces cerevisiae *were tested for binding in a homologous* Saccharomyces cerevisiae *system. Synthetic polyribonucleotides were used as messengers. The code which exists in* Sarcina lutea *and* Saccharomyces cerevisiae *is the same as in* Escherichia coli.

Amino acid coding has been studied most extensively in vitro with systems derived from *Escherichia coli* (*1*), although a limited number of studies have also been performed on systems derived from other microbial (*2*), plant (*3*), and animal (*4*) cells. The number of different organisms examined is still relatively small and, in addition, most studies have been performed with relatively unpurified subcellular fractions. To evaluate further the universality of the amino acid code in microorganisms other than *E. coli*, systems were prepared from *Sarcina lutea* and *Saccharomyces cerevisiae* for study in vitro. These particular species were chosen because the base compositions of their respective DNA's differ from each other and differ from that of *Escherichia coli*: the content of guanine and cytosine in *E. coli* DNA is 50 percent of the total base content, whereas that of *Sarcina lutea* is 68 percent and that of *Saccharomyces cerevisiae* is 37 percent (*5*). Thus these species might be expected to have different genetic codes if such diversities do in fact exist in microorganisms.

In the first phase of our study amino acid incorporation as a function of various polyribonucleotides was examined in a heterologous system composed of aminoacyl-tRNA from *Sarcina lutea*, and transferase enzymes and washed ribosomes from *Esherichia coli*. To corroborate the data obtained, the binding assay of Nirenberg and Leder (*6*) was employed in a homologous *Sarcina lutea* system. In the second phase of the study the binding assay alone was used in a homologous system derived from *Saccharomyces cerevisiae*. In all cases synthetic polyribonucleotides, but not oligoribonucleotides or triplets, were used as messengers.

Sarcina lutea (American Type Culture Collection 10054) and *Saccharomyces cerevisiae* (ATCC 11795) cells were harvested and washed in Nirenberg's standard buffer (*7*); cell walls were broken in a French pressure cell. After the ruptured-cell suspension was centrifuged, the supernatant was incubated (*7*) and then centrifuged at 100,000*g*. A portion of the supernatant was passed through a Sephadex G-25 column, and fractions with high absorption at 280 mμ were stored in liquid nitrogen. The ribosomal pellets from this centrifugation were washed (*7*), dispersed in 0.01*M* tris-acetate buffer (*p*H 7.8) containing 0.001*M* magnesium acetate and 0.05*M* potassium chloride, and stored in liquid nitrogen. tRNA from *Sarcina lutea* was prepared according to Zubay's procedure (*8*). C^{14}-Aminoacyl-tRNA from this organism was prepared in the presence of 19 unlabeled amino acids by the method of Nirenberg *et al.* (*9*). tRNA from *Saccharomyces cerevisiae* (General Biochemicals, Inc.) was dissolved in 0.1*M* tris-hydrochloride buffer (*p*H 9.0) incubated for 2 hours at 37°C, and deproteinized with phenol. After ether extraction, the tRNA solution was dialyzed against distilled water and freeze-dried. Conditions for "charging" *S. cerevisiae* tRNA with C^{14}-amino acids were essentially the same as those for *Sarcina lutea*. *Escherichia coli* W3100 ribosomes were separated from the supernatant after centrifugation at 100,000g. The ribosomes were washed and the tRNA was prepared as out-