Table 1. Sources and physiographic provinces of cores sampled.

Core			Depth	
Province	Туре	Coordinates (N, W)	Water (m)	Sample (cm)
Abyssal plain	Box	23° 51′, 92° 27′	3358	32
Continental slope	Hydroplastic	27° 37′, 94° 47′	549	100
Continental shelf	Phleger	28° 42′, 90° 11′	36	4

The samples were quick-frozen by immersion in liquid nitrogen for several seconds and rapidly placed in a desiccator that was evacuated with a standard 1/3-hp pump; a gauge on the vacuum line showed a constant pressure of  $5 \times 10^{-2}$  mm-Hg. The samples were dried for 2 days at  $-23^{\circ}$ C.

With a sharp razor blade and binoculars, each dried sample was cut into several smaller samples (5 mm by 1.0 to 0.5 mm<sup>2</sup>); the smaller the final size, the more complete is impregnation of the pore space. The impregnating medium was Maraglas, an epoxy resin, thinned with propylene oxide, in which the samples soaked for 24 hours; the containers were sealed to prevent evaporation of the propylene oxide. The samples were then placed for 24 hours in beakers containing only Maraglas before they were placed in Beem capsules filled with Maraglas for curing in an oven at 60°C. Ultrathin sections of the samples were cut with a Porter-Blum MT-1 microtome using a diamond knife.

Figure 2A (10) shows the microstructure of an abyssal-plain sediment; the most striking feature is the loose, open, random arrangement of the particles. Figure 2B shows a continentalslope sediment at somewhat higher magnification; again the open nature of the microstructure is readily apparent. Clayey sediments commonly have porosities as great as 70 percent or greater, and this fact is well illustrated by the micrographs. Figure 2C shows the microstructure of a continentalshelf core; it too reveals an open, random arrangement of particles, but the particles appear more closely packed than in Fig. 2, A and B, showing dense clusters separated by considerably smaller areas of voids.

Many of the particles shown (Fig. 2) do not appear to be in contact with other particles, but seem to be "floating in space." One must remember that the microstructure is being viewed in only two dimensions and that in some plane, either above or below (or above and below) the plane of the ultrathin section, these "floating" particles certainly contact other particles.

Like all idealized representations, Fig. 1, A-C, unavoidably oversimplifies the true picture. Rather than being regular, well-defined plates or rods, the clay minerals shown have very irregular morphologies. Much of the material appears fluffy and diffuse and is probably montmorillonitic or illitic (11); there are indications (12) that sediments of the Gulf of Mexico are rich in montmorillonite and illite.

It is difficult (usually impossible) to tell where a single particle begins or ends. Thus it seems unreasonable to state flatly that the microstructure of undisturbed marine sediments resembles only a cardhouse structure or only a honeycomb structure. However, on the basis of only Fig. 2 the microstructure appears to resemble more closely Terzaghi's honeycomb idealization. It may be more accurate to say that the microstructure is characterized by a loose, open framework of randomly oriented particles.

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## **Radiolarian Skeletons:** Solution at Depths

Abstract. Radiolarian skeletons were placed at several depths on the taut mooring wire of a buoy in the central Pacific for 4 months. Recent radiolarian sediment dissolved at appreciable rates at depths shallower than 2000 meters; solution was greatest near the surface and decreased with depth. This pattern correlates with bathymetric distributions of dissolved silicon and of temperature. Siliceous Radiolaria from planktonic samples appeared to dissolve about eight times faster than those from sediment. Tripyleans seemed to be less resistant than polycystins. Acantharia dissolved completely at all depths.

Radiolaria are small shelled protozoans that live in the plankton of the open ocean. They comprise the groups Tripylea (Phaeodaria), Spumellaria and Nasselaria (polycystins), and Acantharia. Of these, the polycystins are important for reconstruction of the history of the oceans; they are the only ones preserved in the sediment over large areas of the present deep sea. The processes leading from shelled planktonic populations to sediment assemblages on the ocean floor are poorly understood. Recently the importance of solution of tests after death of the planktonic organisms has been stressed for Foraminifera (1). There is some circumstantial evidence that solution may also play an important part in the shaping of radiolarian assemblages. This evidence comes mainly from two kinds of observations: (i) undersaturation of the ocean water with respect to silicon (2), and (ii) differences between the amounts and kinds of shells observed in the plankton and on the ocean floor (3).

I now report direct evidence bearing on the solution of Radiolaria. Samples consisting entirely of polycystin skeletons were attached at various depths to the taut mooring wire of a buoy in the central Pacific close to Horizon Guyot (4). The samples were portions from Recent deep-sea sediment that had been acidified and washed in hydrogen peroxide solution; each weighed about 0.015 g. They were enclosed in small plastic tubes that were sealed with fine (openings smaller than 62  $\mu$ ) nylon gauze and placed within heavy pieces of plastic tubing, one at each depth selected. They were exposed for 4 months. Experimental procedure is given in the report on solution of Foraminifera (1).

An overall trend is visible in the weight losses (Fig. 1): there is decrease in loss with depth, with a maximum loss of 24 percent at 250 m and values of about 8 percent below 3000 m. This trend is statistically significant: the rankdifference correlation between the percentage loss and the depth of exposure yields  $r_d \sim .85$  (< P.01). A possible source of error (other than standard deviation) is loss of particles through the sealing screen during the experiment; it is difficult to evaluate because of the absence of zero weight losses. In the foraminiferal samples previously reported upon, which were about ten times heavier, such losses were entirely negligible (1). The samples below 3000 m all show the same weight loss of 7 to



Fig. 1. (A) Losses of weight by siliceous Radiolaria from Recent deep-sea sediment during 4 months at various depths in the central Pacific. Depths are in uncorrected meters; widths of the bars indicate standard errors in weighing. (B) Mean contents of dissolved silicate of several stations northeast of Hawaiian Islands (6).

8 percent. If one assumes that there was little solution at these depths, these losses must be ascribed to "washing out"; about 7.5 percent thus appears to be a reasonable upper limit for mechanical loss.

Recent work has shown that the solubility of silicon increases with increase in temperature and in pH (5). The pHdoes not seem to vary sufficiently in the ocean to influence markedly the solution of siliceous skeletons during 4 months. One expects, however, silicon-rich ocean water to be less aggressive toward siliceous skeletons than is water poorer in silicon, other factors being equal. An overall decrease in dissolution rate with depth is therefore in agreement with the increase in the silicon concentration (Fig. 1B) (6) and the decrease in temperature with depth. If these relations can be generalized, solution rates should be lowest in the Antarctic and Pacific oceans and highest in the Atlantic. The relatively greater abundance of siliceous skeletons on the bottom of the Antarctic and Pacific oceans is consistent with this hypothesis.

Dissolution of radiolarian skeletons apparently proceeds at different rates in different groups. In a typical radiolarian ooze there are at least as many Nasselaria as Spumellaria and often more (3,p. 205). In approximately 100 samples of shallow-water plankton from the northeast Pacific that I have studied there were many more Spumellaria than Nasselaria, however, and there is a decreasing ratio of Spumellaria to Nasselaria from progressively deeper tows. Apparently the bottom sediment is enriched in the skeletons of deeper-living forms having more robust skeletons.

There is also evidence that most Quaternary radiolarian skeletons are constructed of thinner elements than are most Tertiary forms, and that the Quaternary forms tend to dissolve more quickly (7). A reasonable explanation of this difference between radiolarian assemblages of different ages is that selective solution removed the more easily destroyed skeletons from the older assemblages.

Direct evidence of selective solution in Radiolaria comes from comparison of the sediment samples (Fig. 1A) with portions from a planktonic sample that were included in the same containers. The planktonic sample was washed in buffered hydrogen peroxide solution. Each portion weighed about 0.012 g and contained by weight about 5 percent Acantharia, 65 percent pteropods, and 30 percent Foraminifera. The fact that the Acantharia, whose skeletons consist of strontium sulfate, were completely dissolved at all depths demonstrates that ocean water is undersaturated with strontium sulfate. Between 15 and 40 specimens of siliceous Radiolaria (mostly larger than 100  $\mu$ ) also were found after the experiment in all planktonic portions except the shallowest one. The Radiolaria consisted of about 15 percent Tripylea, 5 percent Nasselaria, and 80 percent Spumellaria.

In the sample at 250 m, where the Radiolaria from the sediment show the greatest dissolution rate, only two cyclodiscid Spumellaria having dense skeletons were found in the planktonic portion; it is reasonable to assume that the sample initially contained between 15 and 40 specimens, as do the others. Thus the two specimens remaining constitute between 14 and 5 percent of the original amount present; thus about 90 percent of the assemblage of siliceous radiolarian plankton dissolved at the depth of 250 m. The corresponding daily solution rate is 19 per mille, which is eight times greater than the daily rate of 2.4 per mille of sedimentary Radiolaria at this depth (8).

The Tripylea appear to dissolve somewhat more quickly than the polycystins: on the average there were relatively fewer Tripyleans in planktonic portions that are associated with the higher weight losses in Fig. 1A (rank-correlation coefficient  $r_d$ , .54; P < .10).

My evidence indicates that opaline radiolarian skeletons dissolve relatively quickly at shallow depths in the central Pacific. The calcareous tests of Foraminifera, by comparison, dissolved most rapidly at the greatest depths. This inverse relation between opaline solution and calcium carbonate solution appears to hold true in all of the world oceans. Selective solution, which alters the composition of foraminiferal ooze, also is probably important in the shaping of radiolarian assemblages in sediment.

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## Chaetopterus Photoprotein: Crystallization and Cofactor Requirements for Bioluminescence

Abstract. The Chaetopterus photoprotein has been isolated in an amorphous form (molecular weight, 120,000) which in  $(NH_{4})_{2}SO_{4}$  solution converts to a crystalline form (molecular weight, 184,000) having the same specific lightemitting activity; quantum yields are 0.0093 and 0.0143, respectively. Two new cofactor requirements have been separated from impure extracts: a macromolecule resembling a nucleoprotein, and a lipid-like substance.

Bioluminescence of extracts of Chaetopterus variopedatus requires molecular oxygen, a hydroperoxide, ferrous iron, and a specific protein (1). Because the total light was proportional to the amount of the purified protein and because no diffusible substrate, in the usual sense, appeared to be involved, the general term photoprotein was suggested for this type of system, as distinct from the classical luciferin-luciferase type that characterizes most of the different bioluminescence systems yet successfully extracted (2). Two other photoprotein systems are now known: those of certain jellyfish (3) and of euphausid shrimps (4). Each of the three systems requires a specific protein to which the total light is related, but the specific proteins, as well as cofactors required for light emission, are different in all three.

Essentially final purification of the *Chaetopterus* photoprotein (CP) has shown that it can exist in at least two forms: one amorphous (CPA), which in solution of ammonium sulfate converts to crystalline (CPC). Two new cofactor requirements for luminescence have been separated from impure extracts: cofactors 1 and 2; the latter (or a substance with the same effect) occurs also in impure preparations of hyaluronidase. Our methods and results were as follows.

The 12th segment was cut from live specimens and immediately stored with dry ice. Batches of 100 g of the frozen material were homogenized for 4 minutes in a mason jar, the receptacle of a Teflon-bladed Omnimixer containing glass beads, 500 ml of saturated ammonium sulfate plus 40 g of undissolved ammonium sulfate, and a trace of NaHCO<sub>3</sub>. The homogenate was squeezed through rayon gauze and centrifuged, and the supernatant was discarded.

The precipitate and floating material were stirred into a 500-ml mixture of 0.0002M oxine (8-hydroxyquinoline) and 0.01M tris, pH 7.2, giving an extremely viscous mixture that was allowed to stand 1 hour, during which time the viscosity decreased. The mixture was centrifuged, the precipitate was discarded, and 200 g of ammonium sulfate was added to the supernatant. The precipitate that formed was removed by centrifugation and combined with similarly prepared precipitates from two to three additional batches.



Fig. 1. Ultracentrifugal patterns of (left) the amorphous form (CPA) of *Chaetopterus* photoprotein at two different concentrations at 4.5°C and (right) the crystalline form (CPC) at 2.4°C. Speed, 59,780 rev/min; time, 64 minutes (CPC), 80 minutes (CPA).

The combined precipitates were dissolved in a 200-ml mixture of 0.0002Moxine and 0.01M sodium phosphate, pH 7.0, and dialyzed for 1.5 hours against the same mixture of oxine and buffer.

The dialyzed material was centrifuged and the precipitate was discarded. The supernatant was filtered through a column of DEAE-cellulose (3.5 by 15 cm), and the filtrate was subjected to fractional precipitation by ammonium sulfate; the fraction that came down at 0.3- to 0.5-saturation was saved. After this step, all buffer solutions were mixtures of 0.0001M oxine and 0.01Msodium phosphate, pH 6.0, which we shall call "buffer."

The fractionally precipitated material was dissolved in 50 ml of buffer, dialyzed against buffer, and then adsorbed on a column of DEAE-cellulose (3.5 by 15 cm). Stepwise elution by graded concentrations of NaCl gave a CP fraction at about 0.2M NaCl and a cloudy cofactor fraction at about 0.5M NaCl. The latter was further purified by DEAE-cellulose chromatography with gradient NaCl elution, the active material (cofactor 1) being eluted at 0.3M NaCl. It proved to be undialyzable and heat-resistant (about 50 percent loss of activity in 1 minute at 100°C); it was almost colorless, with an ultravioletabsorption maximum at 278 mµ (optical density, 0.65), a minimum at 268  $m_{\mu}$  (O.D., 0.63), and a shoulder at 255  $m_{\mu}$  (O.D., 1.02), suggestive of a nucleoprotein. A slight blue fluorescence appeared under ultraviolet illumination. The active material is acidic, judged by its strong adsorption on DEAEcellulose. Relatively low concentrations sufficed for maximum activation of luminescence.

The eluate containing the photoprotein (CP) was further purified by Sephadex G-200 chromatography in buffer containing 0.5M NaCl, followed by saturation with ammonium sulfate and by centrifugation. The precipitate (CPA) was dissolved in a small amount of buffer, and the solution revealed a single peak in the analytical ultracentrifuge (Fig. 1). Ammonium sulfate was then added to produce slight cloudiness. In the course of several hours the crystalline photoprotein (CPC) formed (Fig. 2). After recrystallization this material also gave a single peak in the ultracentrifuge (Fig. 1). The  $s_{20}$  for CPA was calculated at 4.16  $\times$  10<sup>-13</sup>; for CPC,  $5.73 \times 10^{-13}$ . From the same centrifugal data,  $D_{20}$  was calculated, by

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