β angles can be seen in some crystals in this [010] direction (*11*).

Any non-[010] pattern that contains (101)* or $(10\bar{1})$* gives an image consisting of fringes of roughly 7-Å spacing. One such direction is shown for Swedish hollandite in Fig. 2b. There is no disordering evident; all the electron diffraction patterns and images of Stuor Njouskes hollandite are consistent with its sharp, readily interpreted x-ray pattern.

Viewing along the crystal lengths in the Priceless sample (Fig. 2c), we can see two types of tunnels—square and rectangular, corresponding, respectively, to hollandite and romanechite. Apparently the similarity of the double chains in both hollandite and romanechite permits coherent intergrowths of the two minerals as schematically illustrated in Fig. 1. No evidence of ordering of the intergrowths has been found; the streaking in the diffraction pattern reflects the degree of disorder. The same sorts of features occur in samples from the Rattlesnake mine; intergrowths of romanechite and hollandite are shown along the tunnel lengths in the image (Fig. 2d) of the Rattlesnake sample.

We can obtain some insight into the romanechite structure from the *b* axis images and diffraction patterns. An ambiguity in the literature on the crystal system of romanechite has led to its being reported as both monoclinic (*12*) and orthorhombic (*13*). All the romanechite in the romanechite-hollandite mixtures we have thus far observed appears to be monoclinic. Further HRTEM study of romanechite samples is necessary to confirm this deduction as it is possible that the hollandite influences the intergrown romanechite material.

Recently, silicate chains with widths greater than triple have been imaged by HRTEM (*14*). This is also the case with the octahedral chains of the Mn oxides. We interpret Fig. 3a as containing a quadruple chain associated with hollandite and romanechite, whereas Fig. 3b contains a septuple chain. The structures with greater width have thus far only been seen as isolated chains; corresponding minerals are as yet unknown. However, the crystal structure of todorokite, a Mn oxide found as a major constituent of deep-sea Mn nodules, has not yet been solved. Its structure is thought to be closely related to the hollandite and romanechite structures (*4*), and so it could possibly be similar to one of the greater-width structures reported here. HRTEM should be of aid in giving a general idea of the todorokite structure.

The known complexity of the Mn oxides derives from the small crystal size—in some cases samples are x-ray amorphous—and the potential for chemical solid solution and structural intergrowths. The present study has demonstrated that intergrowths are common between the hollandite and romanechite structures. Furthermore, HRTEM has been used to show that even wider tunnels can occur as coherent intergrowths. In light of these observations, it is not surprising that the mineralogy of the Mn oxides is complex and has been confusing. It may be expected that HRTEM will be a powerful technique for studying and helping to unravel the structural and chemical complexities of the Mn oxides.

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2 August 1978; revised 2 October 1978

Biological Bulldozers and the Evolution of Marine Benthic Communities

Abstract. *During the Phanerozoic, the diversity of immobile suspension feeders living on the surface of soft substrata (ISOSS) declined significantly. Immobile taxa on hard surfaces and mobile taxa diversified. Extinction rates of ISOSS were significantly greater than in other benthos. These changes in the structure of benthic communities are attributed to increased biological disturbance of the sediment (bioturbation) by diversifying deposit feeders.*

Most marine fossils are preserved in fine-grained, originally soft sediments. In the Paleozoic these habitats were dominated by immobile suspension feeders (*1, 2*): articulate brachiopods, dendroid graptolites, tabulate and rugose corals, bryozoa, cystoids, blastoids, and Archeocyatha (the only known extinct phylum). Physically equivalent environments are now occupied primarily by deposit feeders (*3, 4*), for example, protobranch bivalves, irregular echinoids, and certain crustacea, holothurians, and annelids. I suggest that newly evolved deposit feeders and other sediment-disturbing taxa displaced the immobile suspension feeders on soft substrata (ISOSS).

Deposit feeders "mine" organic particles from mud or sand (*5, 6*), whereas suspension feeders filter food particles from the water column. In Recent marine communities, deposit feeders may exclude suspension feeders by bioturbation (*7, 8*). This includes suspending sediment or feces that foul biological filters, fluidizing mud substrata (*9*), accidental ingestion (*10*), as well as bulldozing: overturning or burial of ISOSS. The effects of bulldozing will be greatest when the deposit feeders are much larger than the ISOSS; juvenile ISOSS will be especially susceptible (*3, 6*). If the ISOSS cannot complete their life cycles before the sediment is disturbed, they are unlikely to persist. Scavengers and predators may also act as bulldozers (*3, 11, 12*).

Modern bulldozers—holothurians, irregular echinoids (*13*), malacostracan crustacea—diversified after the Silurian (Table 1). They rework sediment an order of magnitude faster than do living taxa that were present in the early Paleozoic (annelids, protobranch bivalves) (*14-16*). Among the latter, ophiuroids have blind guts (*17*) and are virtually sedentary (*18*), while scaphopods are selective carnivores (*19*) and, by analogy with ecologically similar polychaetes (*20*), produce minimal bioturbation. Trilobites had small mouths, suggesting selective ingestion of small volumes. Their tracks indicate surface feeding (*21*) and minimal disturbance of sediment (*6, 22*). The feeding of *Limulus*, a modern trilobite analog, reinforces this inference (*12, 23*).

Thus rates of bioturbation probably increased several orders of magnitude since the Silurian, and the record of trace fossils supports this conclusion. Other factors being equal, the rate of bioturbation is proportional to the cross-sectional area of the burrower (*12, 24*). Throughout the Phanerozoic, the width (diameter) of trace fossils from shelf sediments increased, and systematic foraging became more efficient (*25*). Most important, bioturbation seems to have increased with time (*26*). Although the critical rate of bioturbation cannot be determined without knowing deposition rate, this uncertainty can be diminished by comparing sediments which accumulated in the same kind of environment. My observations of Devonian and Cretaceous deltaic deposits are consistent with the trend.

There are several possible explanations for the continued diversification of bulldozers. (i) Deposit feeders exploit a seasonally stable food source (*27, 28*). (ii) This resource probably increased through geologic time. Land plants now dominate global primary productivity (*29*) and contribute detritus that is an important food for marine deposit feeders (*30*). The diversification of both land plants and holothurians in the Devonian may be coupled. (iii) Many deposit feeders occupied a relatively stable environment within the sediment (*31*). None of the infaunal deposit feeding taxa in Table 1 have become extinct. (iv) Diversification of predators in the Mesozoic placed a premium on escape by "infaunalization" (*2, 32*). (v) Predators (such as brachyurans and naticids) "followed" prey into the sediment.

There has been a major reorganization of marine benthic communities over the span of the Phanerozoic (Fig. 1). All benthic groups show a statistically significant ($P < .05$) reduction in ISOSS since the Devonian and a corresponding increase in mobile taxa. Hard substrata are not bulldozed, and the occupants of this adaptive zone increased significantly. My conservative definition of ISOSS consigned many probable ISOSS to an intermediate adaptive zone (immobile on hard or soft substrata), which also experienced significant decline. When analysis is restricted to soft substrata, both brachiopods and bivalves as well as other benthos show a significant ($P < .001$) decline of ISOSS.

Articulate brachiopods are the dominant preserved macrobenthos of most marine communities in the Paleozoic, but during the late Paleozoic and early Mesozoic their abundance and diversity declined to present minor levels. All articulates were immobile suspension feeders (*33*) and many were ISOSS. The obvious early success of ISOSS brachio-

Table 1. First appearances of bioturbation agents (bulldozers). Only trilobites are extinct. Chronostratigraphic data from (*49*). Malacostracan crustacea italicized.

Age	Deposit feeders	Nondeposit feeders
Tertiary (T)	*Hippidea* *Amphipoda*	
Cretaceous (K)	Tellinacean bivalves	
Jurassic (J)	Irregular urchins *Thalassinidae*	*Brachyura*
Triassic (Tr)	*Mysidacea*	Burrowing suspension feeding bivalves
Permian (P)	*Leptostraca* *Isopoda* *Cumacea* *Tanaida*	
Carboniferous (C)		
Devonian (D)	Holothuroids (*50*)	
Silurian (S)		Scaphopods
Ordovician (O)	Protobranch bivalves (*51*) Ophiuroids	
Cambrian (Є)	Agnath fish (*52*) Trilobites	
Precambrian	Annelids	

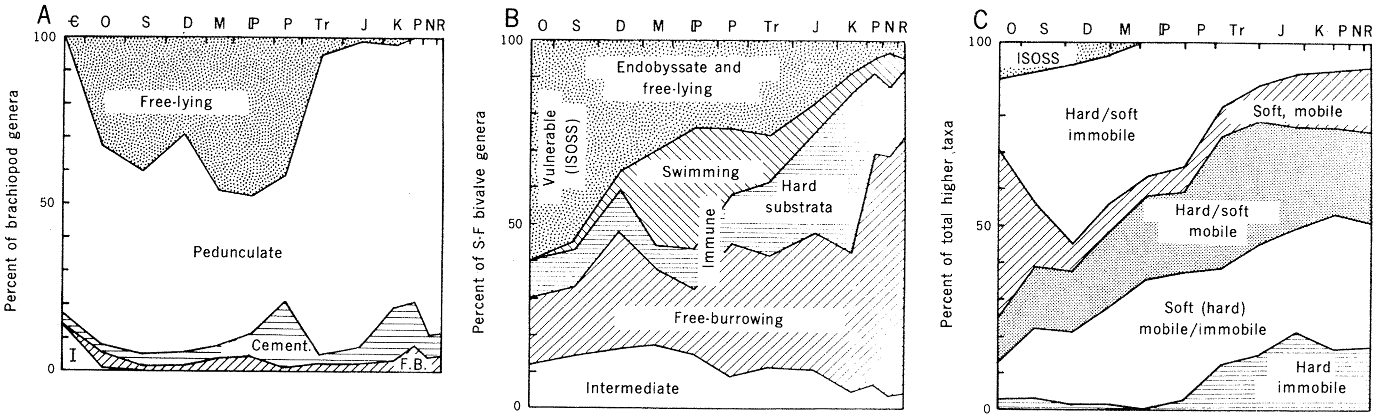

Fig. 1. Percentage of taxa in major adaptive zones as a function of geologic time. Brachiopods and bivalve mollusks are analyzed in detail because preserved, hard parts are reliable indicators of life habit. (A) Brachiopod genera. *Free-lying*, ISOSS; *I*, intermediate; *F.B.*, free-burrowing; *Cement.* and *Pedunculate*, animals attached to hard substrata. Chronostratigraphic data from (*53*). Life habits after (*53, 54*). (B) Genera of suspension feeding bivalves (*55*). Chronostratigraphic data from (*53*). Life habits after (*53, 56*). *Endobyssate*, byssally attached and partially buried in sediment. (C) Higher taxa (usually families) of well-skeletonized marine invertebrates (brachiopods and bivalves excluded). Chronostratigraphic data from (*49*). Twofold labels record hardness of substrate and mobility of organisms. Slashes represent intermediates: "hard/soft" inhabited soft bottoms but some attached to hard surfaces (such as dead shells). Often these objects were too small to stabilize the adults, which thus became functional ISOSS (for example, corals, bryozoa). "Soft (hard)" was predominantly soft.

pods posed an enigma that was compounded by the suggestion (*34*) that brachiopods are exceptionally tolerant of turbidity. If so, why do they no longer inhabit mud? Increased "bulldozing" may be responsible.

Stanley (*35, 36*) has described important evolutionary changes among the bivalves: a Mesozoic diversification of burrowing suspension feeders and a Paleozoic transition from partial burial and byssal attachment (ISOSS) to byssal attachment on hard substrata. Stanley attributed both radiations to the selective pressure of physically unstable sediments. But why did these changes occur relatively late in geologic time? In the early Paleozoic, bioturbation had not yet placed a premium on mobility or forced the ISOSS onto hard substrata.

The extinction at the end of the Permian was "one of the most striking events in the history of life"; a "crisis in earth history" (*37*), and the diversification of bulldozers probably contributed to it. In the brachiopods, bivalves, and other benthos (Fig. 1), the ISOSS suffered significantly ($P < .2$) greater extinction rates than non-ISOSS. For inhabitants of soft sediments only, P is $<.01$. Of course, bulldozing cannot explain the Permian extinction of pelagic taxa.

Living descendants of ISOSS occupy four types of refugia. (i) Articulate brachiopods are confined to hard substrates. Semi-infaunal byssate bivalves are abundant only in sediment which has been physically or biologically stabilized (*2*). Unlike Paleozoic corals, Recent scleractinians are more abundant and diverse on reefs as compared to soft bottoms. Perhaps their reef-forming ability (Triassic-Recent) was selected by the concurrent diversification of bulldozers. (ii) Mobile taxa such as gastropods, malacostraca, and regular echinoids were little affected by the Permo-Triassic "crisis." Originally immobile taxa have evolved mobility since the Paleozoic: comatulid crinoids (*38*), some ahermatypic corals (*39*), and lunulitiform bryozoa (*40*). Significantly, the most persistent "living fossils," *Lingula, Limulus*, and Monoplacophora, are mobile. (iii) The stalked crinoids typical of Paleozoic shelf seas are now concentrated in the deep sea (*38*) where bioturbation is extremely slow (*41*). Stromatolites survive where physical stress excludes burrowers and herbivores (*42*). (iv) Two bivalves, *Pinna* and *Placuna* persist as ISOSS on unstabilized sediment (*43*) and apparently grow rapidly to a "refuge-in-size."

Early deposit feeders (such as polychaetes and protobranchs) probably selected food particles from organic-rich sediments. After that adaptive zone had filled, a major resource remained unexploited: sediment with a low concentration of organic matter. Deposit feeders could not tap this food source until they evolved the capacity to process large volumes of sediment rapidly. This adaptive threshold delayed the development of holothurians, urchins, and thalassinids.

The diversification of these deposit feeders also may have been linked to the relatively late enrichment of marine sediments by land plants (*29, 30*). Infaunal suspension feeders did not diversify until bioturbation intensified. I attribute this to the energetics of infaunal as compared to epifaunal suspension feeders (ISOSS). Infaunal suspension feeders construct and maintain a conduit through which they pump large volumes of water. Furthermore, most are mobile. In contrast, ISOSS could deploy extensive passive filtration nets directly in the water column (for example, crinoids).

The Phanerozoic proliferation of bulldozers has other implications. The intensification of this relatively predictable disturbance (*44*) may have aided the increase in global diversity (taxon richness) of marine invertebrates (*26*). Regeneration (turnover rate) of nutrients from the sediment of the continental shelves was probably increased (*15, 29, 30, 45*), perhaps contributing to the Mesozoic diversification of phytoplankton (coccoliths, diatoms, dinoflagellates) and, via trophic linkage, zooplankton (radiolaria, foraminifera). Because of increased bioturbation, the temporal resolution of the stratigraphic record has probably decreased during the Phanerozoic. Biological mixing of strata obscures or obliterates the details of geologic history.

Bioturbation cannot be a panacea for extinction problems in general because there are as many possible extinction hypotheses as there are factors controlling the distributions of organisms. Complementary hypotheses invoke marine regression, plate tectonics, trophic resource regime (*46*), and increased predation (*2, 32*). However, modern predators appeared too late to contribute to the Permo-Triassic "crisis" and its long prelude (*47*). Nor does predation explain the differential success of immobile epifauna on hard versus soft substrata.

Unlike many extinction hypotheses, biological bulldozing offers testable corollaries. (i) Diversity and abundance of deposit feeders and other sediment reworkers increased; (ii) ISOSS decreased; (iii) ISOSS had greater extinction rates than non-ISOSS; (iv) among the immobile epifauna, diversity increased on hard substrata but decreased on soft; (v) rate of bioturbation increased with time; (vi) local extinctions of a given taxon were not synchronous (*37*); and (vii) relict ISOSS (such as endobyssate bivalves and stalked crinoids) occur in relatively nonbioturbated sediment.

Stochastic explanations have been offered for evolutionary patterns (*48*), but why be defeatist when there are credible deterministic alternatives? I have recorded some major evolutionary patterns and have suggested a specific cause: disturbance by deposit feeders and other bulldozers. This hypothesis is particulary appealing because it integrates a wide variety of data.

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12 May 1978; revised 26 September 1978

Chemotactic Factor–Induced Release of Membrane Calcium in Rabbit Neutrophils

Abstract. *The interaction of chemotactic factors (fMet-Leu-Phe and C5a) with rabbit neutrophils leads to rapid and specific release of membrane calcium, as evidenced by changes in the fluorescence of cell-associated chlorotetracycline. These two structurally different stimuli appear to interact with the same pool of membrane calcium.*

Chemotactic and secretory stimuli alter the ionic permeability of the plasma membrane and the intracellular concentrations of exchangeable calcium in neutrophils (*1*, *2*). The molecular mechanisms that cause these effects are of interest for an understanding of neutrophil physiology in particular and the contractile activities of nonmuscle cells in general. We reported recently that the interaction of chemotactic factors with neutrophil membranes leads to release of calcium from previously bound stores, and we postulated that the plasma membrane or other membranous cell components may act as such calcium stores (*2*). This tentative conclusion was based on indirect evidence derived from studies dealing with the effect of chemotactic factors on the movement of ^{45}Ca across rabbit neutrophil membranes. We report here the results of experiments that implicate membrane-associated calcium, and its release by chemotactic factors, in the initial events involved in neutrophil activation.

The fluorescence characteristics of the chelate probe chlorotetracycline are extremely sensitive to the concentrations of divalent cations within the hydrophobic environment into which it is preferentially partitioned. Chlorotetracycline fluoresces more intensely when complexed to divalent cations, and its emission and excitation spectra can be used to differentiate between its Ca^{2+} and Mg^{2+} chelates (*3*).

We took advantage of these fluorescence characteristics of chlorotetracycline, particularly the dependence on Ca^{2+}, to investigate the effects of chemotactic factors on the fluorescence of chlorotetracycline-loaded neutrophils. Rabbit peritoneal polymorphonuclear leukocytes (neutrophils) were incubated for 45 to 60 minutes at 37°C in the presence of 100 μM chlorotetracycline (Sigma Chemical Co., St. Louis, Mo.) in Hanks balanced salt solution containing 10 mM Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (pH, 7.3), 1.7 mM Ca^{2+}, and no Mg^{2+}, to which glucose and bovine serum albumin were added at 1 mg/ml each. The desired number of cells were then washed once, resuspended in Hanks balanced salt solution without Mg^{2+} and bovine serum albumin and with or without 0.5 mM Ca^{2+}, and transferred to a Perkin-Elmer MPF 2A fluorescence spectrophotometer equipped with a temperature control cuvette holder and stirrer. All the experiments were performed at 37°C with a cell density of 2.5×10^6 cells per milliliter. The viability of the cells, as measured by lactate dehydrogenase release, and their functional responsiveness, as indicated by their ability to release lysosomal enzymes in the presence of cytochalasin B and fMet-Leu-Phe (*4*), were found not to be affected by these experimental manipulations. Excitation and emission wavelengths were 390 and 520 nm, respectively. The excitation and emission slits were adjusted to maximize the signal-to-noise ratio. These experimental conditions are essentially the same as those described by other workers who used chlorotetracycline as a calcium probe (*3*). The synthetic chemotactic factor fMet-Leu-Phe was obtained as previously described (*5*). Its competitive antagonist Boc-Phe-Leu-Phe-Leu-Phe (*4*) was provided by R. J. Freer, Medical College of Virginia, Richmond. Partially purified C5a, the low-molecular-weight chemotactic fragment of the fifth component of complement, was generated by trypsin treatment of C5 as described by Cochrane and Müller-Eberhard (*6*). These preparations of C5a exhibited maximal biological activity (lysosomal enzyme release) at a dilution of 1 to 1000.

Immediately after the addition of either fMet-Leu-Phe or C5a, a rapid decrease in the fluorescence of chlorotetracycline-loaded neutrophils was observed (Fig. 1). Comparison of Fig. 1, A to C, shows that essentially similar results were obtained whether or not extracellular calcium was present at the time of stimulation. This indicates that the fluorescence signal reflects one of the initial molecular events that follows the binding of the chemotactic factor to its receptor rather than the net influx of calcium that occurs when chemotactic factors are added in the presence, but not in the absence, of extracellular Ca^{2+} (*1*, *2*). The dependence of the fluorescence changes on the concentration of the chemotactic factors is illustrated in Fig. 1B. Fluores-