crevices with their large primary spines and are not easily removed. Limited movements occur at night.

12. In five censuses taken in 1975–1976 at Onslow Island, the number of *Eucidaris* feeding on live *Pocillopora* ranged from 36 to 62 percent ($\bar{X}$ = 52 percent).
13. Sediment analysis was performed on gut contents dissected from urchins that were feeding and on fecal material collected from individuals held for 24 hours. Organic matter was removed by repeated treatments with dilute sodium hypochlorite. Particle identification was performed on grains of >0.85 to <2.00 mm, N = 150 to 200.
14. *Megabalanus galapaganus* (Pilsbry) is abundant on hard substrates surrounding the Onslow Island coral reef. Although *Eucidaris* commonly grazes on barnacle tests, no individuals were observed attaching live barnacles. Predators contributing toward the generation of barnacle skeletal grains are the asteroid, *Heliaster cumingii* (Gray) and several species of thaidid gastropods, especially *Thais planospira* (Lamarck).
15. Method 1 assumes that defecation and ingestion rates are equal, and that the mass defecated in 24 hours after removal from coral represents uninterrupted feeding. Defecation occurred continuously, and some material still remained in the gut after 24 hours. Method 2 assumes that the ingestion rate of urchins moved from coralline algae to coral is not interrupted and that coral in the gut was ingested during the 24-hour period. Coral ingested by urchins before their placement on coral (method 2) is probably insignificant because the urchins were collected from coralline algal pavement far removed (8 to 10 m) from live coral. Because feeding is interrupted in both methods, we believe that these estimates are minimal.
16. These results are based on 32 urchins recovered from 52 released; the mean test diameter was 4.98 cm (±0.11). The mass of coral present in individuals was determined as follows. (i) Organic matter was removed from gut contents by repeated treatments with dilute sodium hypochlorite. (ii) Contents were sieved and the four largest size grades (0.25 to ≥2.00 mm) were enumerated and weighed ($N \geq 200$ particles) to determine the percentage composition and the mass of coral (grain identification was based on coral skeletal ornamentation, crystalline texture, and hardness). (iii) The coral mass was regressed on particle size and showed a significant correlation ($P < .05$), that is, larger size grades contained progressively more coral, in 13 of 17 samples; the coral mass present in smaller size grades was extrapolated from the regression curves. Because of the small amount of coral in 15 urchins, these samples were pooled; the coral mass regressed on particle size again showed a significant correlation ($r = -.99$, $P < .01$). Total $CaCO_3$ represents mostly coral plus coralline algae (≥75 percent, numerically and by mass), echinoid, mollusk, barnacle and bryozoan fragments, unidentified calcareous material, and occasional rock fragments.
17. Data collected at Onslow Island and Point Cormorant, Floreana Island, 11 February to 22 March 1975; the mean test diameters ranged from 4.41 to 4.80 cm.
18. P. W. Glynn, G. M. Wellington, C. Birkeland, J. W. Wells, unpublished data.
19. Corals (three to four colonies per station) were stained with Alizarin Red S on the Onslow Island reef flat (1 to 2 m depth) and reef edge (3 to 4 m) during the warm and cool seasons, during 1975. Incremental growth (1- to 4-month periods) was determined from linear (branch-tip elongation) and mass (weight of branch tips) measurements of coral deposited after staining. Colonies and branches showing signs of grazing were excluded. Growth was equal in the two species tested and at both depths except in one of four comparisons, where *P. damicornis* showed 20 percent less growth on the reef edge as compared to the flat [N = 4 colonies, P (of difference) < .05]. The median growth of *P. damicornis* and *P. elegans*, the predominant species at Onslow, was 2.79 mm per month in the warm season (January to May) and 1.20 mm per month in the cool season (June to December). The adjusted annual rate is 2.24 cm/year.
20. Potential production is equal to the mass of *Pocillopora* produced per unit area of reef surface completely covered by this kind of coral. For converting linear growth into potential production see P. W. Glynn [*J. Mar. Res.* **35**, 567 (1977)].
21. R. Endean, *Biol. Geol. Coral Reefs* **2**, 389 (1973).
22. W. Beebe, *Galápagos: World's End* (Putnam, New York, 1924). The club-spined urchins referred to by Beebe are most probably *E. thouarsii*.
23. The only suspected fish predator found commonly along continental shores is the balistid *Balistes polylepis* Steindachner, which is rare or absent in the Galápagos (J. E. McCosker, personal communication).
24. Mean annual growth is 2.2 cm/year in the Galápagos and 3.1 to 3.9 cm/year in Panama.
25. P. W. Glynn and I. G. Macintyre, *Proc. Int. Coral Reef Symp. Miami* **2**, 251 (1977). One Galápagos coral reef (composed dominantly of *Pavona*) is comparable in thickness with Panamanian reefs, suggesting a similar age.
26. The Ecuadorian Ministerio de Agricultura y Ganadería and the Departamento de Parques Nacionales y Vida Silvestre, and the Charles Darwin Foundation (CDF) granted permission to carry out this study. Assistance in the Galápagos was provided by C. MacFarland, R. D. Sievers, and B. Schreyer of the Charles Darwin Research Station, and by T. J. Watson, Jr., and crew of the *Palawan*. Comments by P. Abrams, J. H. Connell, J. Cubit, A Ebeling, and H. Lessios helped to improve the manuscript. Corals were identified by J. W. Wells. Supported by the Smithsonian Institution and the U.S. Peace Corps Program. CDF contribution number 235.

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Pyrite: Its Rapid Formation in a Salt Marsh and Its Importance in Ecosystem Metabolism

Abstract. *Pyrite formation in salt-marsh peat occurs more rapidly than is generally thought for any natural system. Pyrite is the major end product of sulfate reduction, and sulfate reduction is the major form of respiration in the salt-marsh ecosystem. When the rapid formation of pyrite is ignored, the rates of sulfate reduction and ecosystem respiration may be grossly underestimated.*

The formation of pyrite (FeS_2) in nature is generally thought to be a very slow process, taking months, years, or decades as amorphous iron monosulfides (FeS) react with elemental sulfur (S^0) (*1*). In salt-marsh peat, pyrite can form in a day or less without iron monosulfides as intermediates. Measurements of sulfate reduction determined from the turnover of tracer amounts of $^{34}SO_4^{2-}$ (*2*) in the surface peat of a Cape Cod salt marsh show that pyrite is a major end product. Very little of the resulting ^{35}S label, at most 30 percent, ends up in soluble (H_2S, HS^-) or acid-volatile (FeS) pools (*3, 4*). If the ^{35}S in the pyrite fraction were not measured, the rate of sulfate reduction would be grossly underestimated. My measurements indicate that the rates of sulfate reduction are very high in the salt-marsh peat throughout much of the year and that the sulfate-reducing bacteria annually respire approximately 1800 g of carbon, an amount of organic carbon equivalent to the major fraction of net primary production in the marsh (*3*). Other terminal electron acceptors such as oxygen and nitrate are much less important in the total respiration of the salt-marsh ecosystem (*3*).

Pyrite is normally detected by x-ray diffraction and is quantified on the basis of the amount of sulfur released by digestion with aqua regia (*1, 5, 6*). Neither approach is sufficient to prove that the ^{35}S is being reduced and incorporated into pyrite in marsh peat. X-ray diffraction analysis of marsh peat has repeatedly demonstrated pyrite as a major mineral phase, but x-ray diffraction cannot show that the ^{35}S is associated with pyrite. The ^{35}S that remains in the sediment after acid treatment to free acid-volatile sulfides is not extracted by refluxing with 6N HCl but is extracted by aqua regia (boiling 1:1 HCl-HNO_3). That aqua regia but not refluxing HCl frees ^{35}S strongly suggests that it is in pyrite and proves that the ^{35}S is not in sulfate esters, amino acids, or proteins (*7*). However, refluxing with HCl may not extract ^{35}S from elemental sulfur or from humic or fulvic acids, and these possible sources must be examined by other approaches. Organic solvents such as CS_2 extract little or no ^{35}S, and thus no ^{35}S is in elemental sulfur. But some ^{35}S may be in fulvic acids (*8*): successive extractions with 0.1N NaOH release small but significant quantities of ^{35}S, approximately 5 percent of that extracted by aqua regia. Yet none of the ^{35}S is in humic acids (*8*), for when the alkaline extracts are acidified and centrifuged, all of the ^{35}S remains in solution (*8*). Since it seems unlikely that sulfur would be rapidly incorporated into fulvic acids but not humic acids, the labeled sulfur is probably incorporated into pyrite, which is then partially oxidized by the NaOH extraction procedure. Investigations with pyrite standards have confirmed that pyrite can be oxidized, although not quantitatively, by the extraction procedure (*9*).

Pyrite (specific gravity, 5.0) is considerably denser than most sediment materials. Separation of radiolabeled sediments by density in tetrabromomethane (specific gravity, 2.96) confirms that some pyrite is being formed rapidly in the marsh sediments. The denser pyrite-containing fraction (confirmed by x-ray diffraction) is virtually free of organic matter as shown by carbon-hydrogen-nitrogen analysis and has 10 percent of the ^{35}S. The lighter fraction, having 90 per-

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Table 1. Some attempted low-temperature syntheses of pyrite.

Reaction (partial pressure of H_2S)	pH	Time (days)	Temperature (°C)	Products	References
FeO · OH + H_2S (1 atm)	4	0.6	20 to 25	FeS_2, FeS	(*11*)
FeO · OH + H_2S*	3.8 to 6.5	1	25	FeS_2	(*12*)
FeO · OH + H_2S*	7	2.5	25	FeS (no FeS_2)	(*12*)
FeO · OH + H_2S (1 atm)	7.5	3	Room	FeS	This study
FeO · OH + H_2S (10^{-4} atm)	7.5	3	Room	FeS_2 (small yield)	This study
FeS + S^0	4.5	7	Room	No FeS_2	(*12*)
$FeSO_4(NH_4)_2SO_4$ + H_2S (1 atm)	3	6	20 to 25	FeS	(*11*)

*The partial pressure of H_2S was not reported.

Table 2. Solubility data for the salt-marsh pore waters and for the Teflon bag experiment.

pH	$[S^{2-}]$	$[Fe^{2+}]$	$[Fe^{2+}][S^{2-}]$	Interpretation
		Salt-marsh pore waters		
5.0 to 7.8 (usually 5 to 6.5)	Less than 10^{-14}*	2×10^{-4} to 2×10^{-5}	Usually much less than 2×10^{-18}	FeS undersaturated, FeS_2 supersaturated
		Teflon bag		
5.0	Less than 2×10^{-15}†	Less than 10^{-3}‡	Less than 2×10^{-18}	FeS undersaturated, FeS_2 supersaturated

*The S^{2-} activity varies greatly over time and space; 10^{-14} is a fairly high value. The activity is low even though the concentration of total soluble sulfides can be high because the pH is low (*3*). †The S^{2-} activity in the bag varies as the external partial pressure of H_2S varies; 2×10^{-15} is the highest value. ‡This was the starting concentration. Some Fe^{2+} was undoubtedly oxidized.

cent of the ^{35}S, is largely organic matter which probably has trapped some very fine-grained pyrite. The ^{35}S in this fraction is probably associated with such fine-grained pyrite, although a small percentage of it may be in fulvic acids (*10*).

To obtain additional evidence that pyrite can form rapidly under conditions such as those found in the marsh, I buried Teflon bags containing approximately 200 ml of 1 m*M* $FeSO_4$ (the pH was adjusted to 5.0 with citrate buffers) in the marsh sediments. The Teflon bags are permeable to gases but not to ions, and so the total sulfide concentrations and activities of S^{2-} were controlled by the external partial pressure of H_2S. No attempt was made to initially exclude air from the bags, and some ferrous iron was undoubtedly oxidized. Within 48 hours, pyrite, confirmed by x-ray diffraction, had formed in these bags. This pyrite was insoluble and stable in refluxing 6*N* HCl but was significantly oxidized in 0.1*N* NaOH.

A number of laboratory studies have demonstrated that pyrite can be synthesized rapidly, in 1 day to a few days, from inorganic solution under suitable conditions [see Table 1 and (*11, 12*)]. It is tempting to conclude from these studies that pyrite will form rapidly under suitable acidic conditions whereas iron monosulfides such as mackinawite or greigite will form under more alkaline conditions. This would explain the rapid formation of pyrite in the salt marsh where the pH is usually between 5.0 and 6.5. However, it can be easily demonstrated that pH itself is not the key variable. Roberts *et al.* (*12*) mixed FeO · OH and H_2S at pH 7 while vigorously excluding air and produced iron monosulfides but no pyrite. I repeated their experiment at pH 7.5 while maintaining the partial pressure of H_2S at 1 atm and achieved similar results. But, when the partial pressure of H_2S was maintained at 10^{-4} atm in another experiment, pyrite and not the iron monosulfides was the product (Table 1). The formation of mackinawite (and other iron monosulfides) is kinetically favored over the formation of the thermodynamically more stable pyrite, and, once iron monosulfides form, they are only very gradually converted to pyrite. My results support the hypothesis that, if the iron monosulfides are undersaturated, pyrite (which is still likely to be supersaturated because of its much lower solubility product) can precipitate rapidly, without competition from iron monosulfides (*5*). If this hypothesis is true, then the effect of changing the partial pressure of H_2S from 1 to 10^{-4} atm was to change the iron monosulfides from supersaturated to undersaturated.

The observed trend for pyrite to form at lower pH and iron monosulfides at higher pH may just reflect the effect of pH on S^{2-} activity. For a constant concentration of total soluble sulfides (H_2S, HS^-, S^{2-}), decreasing the pH will decrease the concentration and activity of S^{2-} and thus iron monosulfides are more likely to be undersaturated at lower pH.

Measurements of Fe^{2+} and of the S^{2-} activity in the pore waters of marsh peat indicate that the ion product $[Fe^{2+}][S^{2-}]$ is almost always less than the solubility product of mackinawite, 2.75×10^{-18} (*13*) (Table 2); that is, mackinawite is undersaturated. But the solubility product of pyrite is approximately 2.4×10^{-28} (*5, 14*), and so it can be assumed that pyrite is supersaturated. This finding is consistent with the hypothesis that pyrite forms rapidly at low temperatures only when iron monosulfides are undersaturated. Since mackinawite cannot form under the undersaturated conditions found in the marsh, pyrite forms quite rapidly, although not as rapidly as mackinawite or other iron monosulfides would form were the conditions suitable. Iron monosulfides were also undersaturated and pyrite supersaturated in the Teflon bag experiment (Table 2).

If pyrite can form rapidly whenever soluble sulfides are present but iron monosulfides are undersaturated, then it may be forming more rapidly than has been thought in some marine sediments other than salt marshes. Observations made in the Santa Catalina Basin and some other locations tend to support such a hypothesis. Sediments from these locations have significant concentrations of pyrite but not of iron monosulfides occurring in the surface sediments (*6*). This pattern is also found in marsh sediments, and, like the salt-marsh peat, these sediments have no major increase in pyrite concentration with depth. Such observations contrast with those made in sediments where pyrite forms slowly by conversion of iron monosulfides. There, the pyrite content increases with depth and the content of iron monosulfides decreases with depth, as in most anoxic marine sediments (*5*). The sediments from the Santa Catalina Basin are fairly oxidized, and sulfide concentrations are usually undetectably low (*6*). If sulfides were present only in very low concentrations, the iron monosulfides could be undersaturated and pyrite supersaturated even at the high pH found in these sediments. In light of the rapid formation of pyrite in salt marshes and its importance to ecosystem respiration there, the process should be more closely investigated in other likely systems.

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References and Notes

1. R. A. Berner, *Am. J. Sci.* **268**, 1 (1970).
2. The method is that of B. B. Jorgensen and T. Fenchel [*Mar. Biol.* **24**, 189 (1974)], modified as described in (*3*).
3. R. W. Howarth and J. M. Teal, in preparation.
4. In contrast to these marsh results, my measurements in anoxic subtidal marine sediments result in the recovery of roughly 80 to 100 percent of the reduced ^{35}S as water-soluble sulfides (H_2S, HS^-) and acid-soluble sulfides [presumed to be FeS (*1*, *5*)]. The rest is elemental $^{35}S^0$, recoverable by CS_2 extraction. The $^{35}S^0$ forms from $Fe^{35}S$ during acidification and is an artifact of the method [(*3*); R. A. Berner, *Mem. Am. Assoc. Pet. Geol. 20* (1974)].
5. M. B. Goldhaber and I. R. Kaplan, in *The Sea, Marine Chemistry*, E. O. Goldberg, Ed. (Wiley, New York, 1974), vol. 5, p. 569.
6. I. R. Kaplan, K. O. Emery, S. C. Rittenburg, *Geochim. Cosmochim. Acta* **27**, 297 (1963).
7. Proteins and sulfate esters are readily acid-hydrolyzed into water-soluble components [A. B. Roy and P. A. Trudinger, *The Biology of Inorganic Compounds of Sulfur* (Cambridge Univ. Press, Cambridge, England, 1970)].
8. A. Nissenbaum and I. R. Kaplan, *Limnol. Oceanogr.* **17**, 570 (1972).
9. The oxidation of both sulfides [M. Avrahami and R. M. Golding, *J. Chem. Soc.* **1968**, 647 (1968)] and ferrous ions [W. Stumm and J. H. Morgan, *Aquatic Chemistry* (Wiley-Interscience, New York, 1970)] is greatly increased with increasing *p*H. Since the oxidation of pyrite is rate-limited by the oxidation of ferrous ions and, perhaps under the conditions tested here, by the oxidation of sulfides, increasing the *p*H should increase the oxidation rate of pyrite.
10. Any ^{35}S incorporated into fulvic acids is almost certainly reduced from $^{35}SO_4^{2-}$ to $^{35}S^{2-}$ first and then incorporated as the sulfide, as Nissenbaum and Kaplan (*8*) have demonstrated for the introduction of sulfur into humic acids. Thus, even if some of the ^{35}S label in the "pyrite fraction" is not pyrite, it is reduced sulfur and must be considered when measuring sulfate reduction. For routine sulfate reduction measurements, there is no need to distinguish between pyrite sulfur and fulvic acid sulfur.
11. R. A. Berner, *J. Geol.* **72**, 293 (1964).
12. W. M. B. Roberts, A. L. Walker, A. S. Buchanan, *Miner. Deposita* **4**, 18 (1969).
13. R. A. Berner, *Am. J. Sci.* **265**, 773 (1967).
14. D. T. Rickard's finding [*ibid.* **275**, 636 (1975)] that pyrite can be formed rapidly through the direct reaction of aqueous ferrous ions and polysulfide ions strengthens my use of this theoretically calculated solubility product for pyrite if saturation with solid orthorhombic sulfur is assumed; elemental sulfur is abundant in marsh peat, and so saturation is likely.

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Diphytanyl and Dibiphytanyl Glycerol Ether Lipids of Methanogenic Archaebacteria

Abstract. *The lipids of nine different methanogenic bacterial strains are comprised of diphytanyl glycerol diethers, previously known only in extremely halophilic bacteria, as well as dibiphytanyl diglycerol tetraethers, known formerly only in the extremely thermoacidophilic bacteria* Thermoplasma *and* Sulfolobus. *Of the methanogens examined from four representative taxonomic groups,* Methanobacterium *and* Methanospirillum *contained both types of isopranyl ethers in nearly equal proportions, whereas the coccal forms,* Methanosarcina *and* Methanococcus, *possessed diphytanyl glycerol diethers, but with only a trace of or no dibiphytanyl diglycerol tetraethers. The occurrence of both types of isopranyl glycerol ethers in methanogenic bacteria supports the proposal that they have a close genealogical relationship to the extremely halophilic and thermoacidophilic bacteria.*

On the basis of 16*S* ribosomal RNA sequence analyses, Woese and Fox (*1*) and Fox *et al.* (*2*) proposed that the anaerobic methane-producing bacteria, which utilize hydrogen and carbon dioxide as energy and carbon sources, represent ancestral life-forms, designated as archaebacteria. The extremely halophilic bacteria and the thermoacidophilic organisms *Thermoplasma* and *Sulfolobus* also appeared to be closely related (*3*, *4*). As demonstrated elsewhere (*5*), the isopranyl ether lipids of the thermophilic methanogen *Methanobacterium thermoautotrophicum* are surprisingly similar to those of the halophile *Halobacterium cutirubrum* (*6*) and the thermoacidophile *Thermoplasma* (*7*, *8*) and *Sulfolobus* (*9*). The lipids were shown to contain isopranyl ethers of glycerol or its derivatives and a fraction of C_{20}, C_{25}, and C_{30} acyclic isoprenoid hydrocarbons (*5*). The results supported the concept that methanogens, halophiles, and thermoacidophiles share a common evolutionary episode. However, all of these bacteria whose lipids have been examined were originally isolated from extreme environments, having high temperature, low *p*H, high salinity, or some combination thereof. Because these ether lipids may reflect adaptation to an extreme environment rather than true genealogical relationships, it is necessary to determine whether mesophilic methanogens also possess the same unique composition of lipids. This report describes the basic component structure of the polar lipids from eight mesophilic methanogens relative to those of *M. thermoautotrophicum* and halophilic and thermoacidophilic microorganisms.

Lyophilized cells of *M. thermoautotrophicum, Methanobacterium* M.O.H., *Methanobacterium* strain AZ, *Methanobacterium ruminantium* PS, *Methanobacterium ruminantium* M-1, *Methanospirillum hungatii, Methanococcus vannielii, Methanococcus* strain PS, and *Methanosarcina barkeri* were prepared at the University of Illinois by W. E. Balch and were obtained from R. S. Wolfe. *Methanobacterium thermoautotrophicum* (*10*) and the remaining methanogens (*2*) were grown and harvested as previously described. One gram of lyophilized cells was suspended in a saline divalent solution (*11*) and lipids were extracted by the method of Bligh and Dyer (*12*). The residue was reextracted by an acid Bligh-Dyer procedure by replacing the water with 0.1*M* acetate buffer (*p*H 5.0). The twice-extracted residues were finally extracted a third time by refluxing in boiling methanol and chloroform (2:1) for 1 to 2 hours. Each of the three extracts was weighed and then combined to give total extractable lipids. The total lipids of all methanogens were fractionated on columns of silicic acid (Unisil, 325 mesh), with *n*-hexane, benzene, and finally chloroform to remove nonpolar lipids (20 to 30 percent of total lipids). The remaining polar glycolipids and phospholipids (70 to 80 percent of total lipids) were recovered by eluting with chloroform and methanol (2:1 by volume) and methanol and the eluates were pooled.

All studies described in this report were conducted on the pooled polar lipid fraction. In some instances, polar lipids contained a major component that was not soluble in chloroform. This insoluble material was acetylated and then dissolved in chloroform (*5*). Each of the lipid fractions was hydrolyzed in anhydrous 2.5 percent methanolic HCl and extracted with petroleum ether (*13*). The petroleum ether–soluble products were chromatographed on thin-layer chromatography (TLC) plates made with silica gel G and developed in solvents *n*-hexane, diethyl ether, and acetic acid (80:20:1) or chloroform and diethyl ether (9:1). Components were visualized with phosphate spray (*14*), periodate-Shiff reagent for vicinal glycols (*15*), acid charring, or by exposure to iodine vapors. Components were identified by comparing R_F values to those of both established and known standards. The relative intensities of components were recorded with a Zeineh soft laser densitometer. Components were scraped from the plates and eluted with chloroform and methanol (1:1). Portions of alkyl ether–containing components were further digested by refluxing in 47 percent HI for 12 hours to release alkyl iodides (*5*, *6*). The halides were either reduced to the alkane derivatives by refluxing in acetic acid and zinc (*16*) or converted to the alkyl acetates by refluxing with silver ace-

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