calculated for calcium ion relative to magnesium, barium, sodium, potassium, and hydrogen ions.

The data in Table 1 indicate that the dioctylphosphate-collodion electrode has a favorable response for calcium ion over magnesium, barium, sodium, and potassium ions. The calcium selectivity relative to the alkaline earth ions is at least as great as that of previous solid ion-exchange electrodes, and, in the case of the alkali metal ions, it is significantly greater. In addition, the calcium-hydrogen selectivity coefficient provides a wide range of useful *p*H for this electrode. In $10^{-2}M$ calcium chloride, the electrode potential is independent of hydrogen ion concentration from *p*H 3.5 to *p*H 10. A decrease in potential is observed above *p*H 10 and an increase below *p*H 3.5. The behavior at high *p*H is attributed to the formation of $CaOH^+$, to which the electrode is insensitive, inasmuch as the solubility product of calcium hydroxide is not exceeded at *p*H 10.

The dynamic response of the electrode is very rapid. In solutions containing no interfering cations, potential equilibrium following a step-functional change in calcium ion concentration is attained in a matter of 2 to 3 seconds. The speed of response is very much greater than that of previous solid ion-exchange electrodes which exhibited sensitivity for divalent metal ions.

FRANKLIN A. SCHULTZ*
ARNE J. PETERSEN
CHARLES A. MASK
RICHARD P. BUCK†
Beckman Instruments, Inc.,
2500 Harbor Boulevard,
Fullerton, California 92634

References

1. R. M. Garrels, M. Sato, M. E. Thompson, A. H. Truesdell, *Science* **135**, 1045 (1962); A. H. Truesdell and A. M. Pommer, *ibid.* **142**, 1292 (1963); A. H. Truesdell and C. L. Christ, in *Glass Electrodes for Hydrogen and Other Cations: Principles and Practice*, G. Eisenman, Ed. (Dekker, New York, 1967), chap. 11.
2. C. E. Marshall and A. D. Ayers, *J. Amer. Chem. Soc.* **70**, 1297 (1948); C. E. Marshall and L. O. Eime, *ibid.* **70**, 1302 (1948).
3. K. Sollner and R. Neihof, *Arch. Biochem. Biophys.* **33**, 166 (1951); R. Neihof, *J. Phys. Chem.* **58**, 916 (1954); S. K. Sinha, *J. Indian Chem. Soc.* **31**, 572 (1954).
4. H. P. Gregor and H. Schonhorn, *J. Amer. Chem. Soc.* **79**, 1507 (1957); *ibid.* **81**, 3911 (1959); *ibid.* **83**, 3576 (1961).
5. J. W. Ross, *Science* **156**, 1378 (1967).
6. Flexible collodion; J. T. Baker.
7. L. Meites, in *Handbook of Analytical Chemistry*, L. Meites, Ed. (McGraw-Hill, New York, 1963), p. 1-8.

* Present address: Department of Chemistry, Florida Atlantic University, Boca Raton 33432.

† Present address: Department of Chemistry, University of North Carolina, Chapel Hill 27154.

21 August 1968

Lepidocrocite Iron Mineralization in Keratose Sponge Granules

Abstract. *Reddish-brown granules embedded in the spongin fibers of some keratose sponges consist of very fine crystallites of poorly organized lepidocrocite, γ FeOOH. This is the first occurrence of crystalline iron mineralization in the phylum Porifera and the first indication of hard tissue formation among the Keratosa.*

Crystalline iron compounds occur rarely as biologic precipitate except in the hydrous iron oxide making up the ferritin micelle. The most common and best documented of these occurrences are from the phylum Mollusca where radular teeth of certain chitons consist of magnetite (Fe_3O_4) (*1*) or magnetite plus lepidocrocite (γ FeOOH) (*2*). In addition the radular teeth of some groups of gastropods contain goethite (α FeOOH) (*3*). The sequence of iron mineralization in the magnetite radular teeth of *Cryptochiton stelleri* is the result of normal metabolic processes (*4*).

We report here on the occurrence of a crystalline iron mineral among Porifera and the first evidence of mineralization among the Keratosa (horny sponges). In certain species of keratose sponges the endosome is of a brick or rust-red color. This coloration varies in intensity from specimen to specimen and is caused by minute, strongly light-refracting particles attached to the fibers of the sponge skeleton (Fig. 1). The granules appear more or less spherical, are rusty brown, and vary in size between 0.5 and 2.5 μ (*5, 6*). These particles are intimately attached to and completely embedded in the spongin fibers.

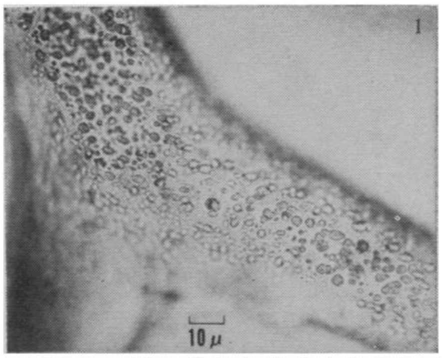
Similar particles have been observed only in the genera *Spongia, Hippospongia* (which is very close to *Spongia*), and *Ircinia* (order Keratosa, family Spongiidae). Because the abundance of red coloration in *Spongia* (and therefore of the particles along the fibers) is correlated with other qualities, especially elasticity and durability (*7*), the commercially useful species and varieties have been most thoroughly studied. The so-called iron mold "Eisenfleck" (*6*) is usually more common and intense at the "root" of the sponge, the area of attachment to the substrate where the fiber network is particularly dense.

The nature of the particles as an iron-containing compound was first recognized by Herberger (*8*) who determined 8.7 to 8.9 percent iron oxide in the ash of the sponge skeleton. Other quantitative values of iron oxide from the literature vary between 1.55 percent (*9*) and 5 to 10 percent (*10*) of the ash. Values of 0.01 to 1.51 percent of the spongin dry weight were also measured (*6, 11*). By its positive Prussian blue reaction and a less successful ferricyanide test (Turnbull blue), Sella (*5*) found that ferric oxide is a major constitutent of the granules. Because of their irregular shape and distribution he concluded that they were not organic formations but foreign mineral grains. This has since become the accepted interpretation.

Living specimens of *Ircinia fasciculata* (Pallas) from an iron rail of *Concrete Ship*, a half-submerged wreck southeast of Bimini, Bahamas, were collected for study. Fibers and filaments of the animals showed strong development of granules. The specimens were immediately fixed in 96 percent ethanol and then transferred to 70 percent ethanol. Small pieces were fixed for electron microscopy in 6.25 percent glutaraldehyde at *p*H 7.2, postfixed in 4 percent osmium tetroxide at *p*H 7.4,

Table 1. Interplanar spacings (*d*) and relative intensities (*I*) of x-ray and electron-diffraction data from sponge granules compared with reference lepidocrocite (*13*). Abbreviations: VB, very broad; D, diffuse; S, strong; M, medium; W, weak.

Sponge granules				Lepidocrocite (x-ray)	
X-ray		Electron diffraction			
d (Å)	*I*	*d* (Å)	*I*	*d* (Å)	*I*
6.85	VB, D			6.27	100
3.32	S	3.32	S	3.29	60
2.47	S	2.47	M	2.473	30
		2.39	W	2.362	15
				2.086	12
1.95	M	1.95	S	1.935	30
		1.86	VW	1.848	10
		1.74	VW	1.733	15
				1.567	8
1.53	M	1.53	S	1.532	7
				1.523	15
				1.493	2
				1.452	2
				1.436	7
				1.417	1
		1.39	W	1.391	3
				1.369	8
				1.264	1
		1.21	M	1.218	2
				1.201	2



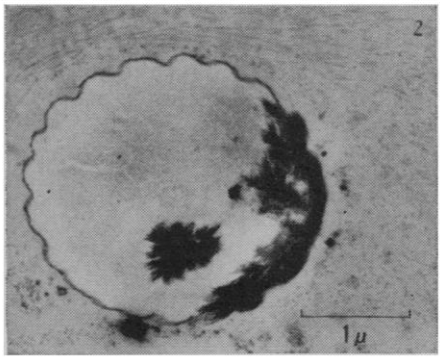


Fig. 1 (top). Reddish-brown granules and spongin fibers of *Spongia graminea*. Fig. 2 (bottom). Section through membrane-bound granule with incipient iron mineralization. The granule is surrounded by spongin (*Ircinia fasciculata*).

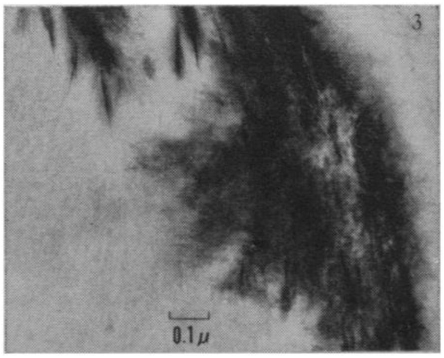
and stored in 70 percent ethanol before being dehydrated and then embedded in Epon. Ultrathin sections were cut on a Porter-Blum MT-2 microtome fitted with a diamond knife. Electron microscopy and electron diffraction were carried out with a Philips EM-200 at 80 kv. Dark reddish-brown skeletons of *Spongia graminea* Hyatt from the Florida Keys and of *Spongia officinalis* L. from the Mediterranean (uncatalogued specimens, U.S. National Museum) were also selected for x-ray and electron-diffraction studies.

Debye-Sherrer x-ray powder diffraction with the use of manganese-filtered iron radiation on isolated untreated reddish-brown fibers produced weak patterns exhibiting significant line broadening. Concentration of the granules through dissolution of the fibers with 5 percent sodium hypochlorite resulted in a somewhat stronger pattern. Five very broad lines could be detected (Table 1, column 1). These data compare favorably with the strongest lines of lepidocrocite (γ FeOOH) (Table 1, column 3) except for a shift toward larger d-spacings of several of the lines, particularly the large spacing at 6.85 Å. However, a similar phenomenon reportedly occurs in base-precipitated boehmite (γ AlOOH) (*12*) which is isostructural with lepidocrocite. The x-ray patterns of this so-called "pseudoboehmite" also exhibit broadening and displacement of the lines, especially the 020, from 6.11 Å to as much as 6.6 to 6.7 Å. These variations in spacing and intensity are considered due to disorder resulting from variable water content and foreign ion impurities.

Examination of the granules in the electron microscope and the line broadening of the x-ray patterns from the sponge granules indicate a very small crystallite size for the individual mineral constituents (Figs. 2 and 3). In addition to a finely fibrous ($<$ 50 Å) organic matrix, there are platy and lathshaped crystallites. Although their lengths are quite variable and difficult to measure, on the average these crystallites are less than 30 Å thick (Fig. 4). Electron-diffraction data from the granules are given in Table 1, column 2. Several weaker lines not observed in the x-ray patterns are measurable. The x-ray diffuse 020 spacing at 6.85 Å was not determined principally because of interference due to scattering from the primary beam. All spacings are correlatable with those of lepidocrocite. As with the x-ray data, there is some displacement of the lines toward smaller Bragg angles.

Prolonged exposure to an intense electron beam causes the ferric iron on the carbon-coated support film in the vacuum of the electron microscope to be reduced, changing it to wüstite (FeO). However, heating of the sponge granules in air to 300°C for 2 hours results in the formation of a material that is orange-red and ferromagnetic and has the x-ray and electron-diffraction patterns of maghemite (γ Fe_2O_3). This observation confirms the presence of lepidocrocite in the keratose sponge granules inasmuch as all other hydrated ferric oxides decompose in air to yield hematite (α Fe_2O_3).

The monomineralic nature of the granules in several species from varied geographic localities, the growth of the lepidocrocite in a very fine organic matrix, and the presence of the granules occurring embedded in the spongin fibers leave little doubt that the iron mineralization is the result of biological processes. The mechanisms involved in iron transport and mineralization together with the importance of ecological influences on the degree of development of this poorly crystalline product are not known. This occurrence in the sponges adds another group to those marine invertebrates known to be capable of iron mineralization. Such organisms may contribute small quantities of iron oxides and their diagenetic alteration products to the adjacent sediment.



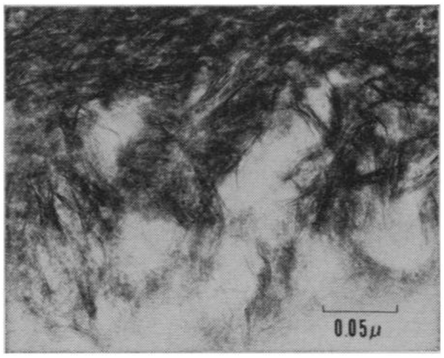


Fig. 3 (top). Enlargement showing lepidocrocite in close association with the very fine fibers of the organic matrix. Fig. 4 (bottom). Thin crystallites of γ FeOOH in section through unfixed, unstained granule of *Spongia officinalis*.

Kenneth M. Towe
Klaus Rützler
Departments of Paleobiology and
Invertebrate Zoology, Smithsonian
Institution, Washington, D.C. 20560

References and Notes

1. H. A. Lowenstam, *Bull. Geol. Soc. Amer.* **73**, 435 (1962).
2. ———, *Science* **156**, 1373 (1967).
3. ———, *ibid.* **137**, 279 (1962).
4. K. M. Towe and H. A. Lowenstam, *J. Ultrastruct. Res.* **17**, 1 (1967).
5. M. Sella, *Mem. R. Com. Talassografr. Ital.* **13**, 1 (1912).
6. W. Arndt, in *Die Rohstoffe des Tierreichs*, F. Pax and W. Arndt, Eds. (Gebrüder Borntraeger, Berlin, 1937), vol. 1, p. 1577.
7. H. F. Moore, *Bur. Comm. Fish. Bull.* **28**, 399 (1908).
8. F. E. Herberger, *Repert. Pharmac.* **65**, 60 (1836).
9. E. Richter, *Apothekerzeitg.* **26**, 317 (1911).
10. P. Siedler, in *Handworterbuch der Pharmazie*, A. Brestowski, Ed. (Vienna and Leipzig, 1896), vol. 2, p. 538.
11. P. Robin, *J. Pharmacie* **21**, 600 (1935).
12. E. Calvet *et al.*, *Bull. Soc. Chim. France* **1953**, 99 (1953); D. Papee, R. Tertian, R. Biais, *ibid.* **1958**, 1301 (1958); P. H. Hsu and T. F. Bates, *Mineral. Mag.* **33**, 749 (1964).
13. H. P. Rooksby, in *X-Ray Identification and Crystal Structures of Clay Minerals*, G. Brown, Ed. (Mineralogical Society, London, 1961).
14. Supported in part by Office of Naval Research contract NONR 552(07). We thank Mr. Robert Mathewson, director of the Lerner Marine Laboratory. We thank G. H. Hamilton for operating the ultramicrotome.

27 June 1968