

3. B. J. Enbysk and F. I. Linger, *J. Sediment. Petrol.* **36**, 839 (1966).
4. R. A. McCance and M. Masters, *J. Mar. Biol. Ass. U.K.* **22**, 273 (1937); D. A. Webb, *Sci. Proc. Roy. Dublin Soc.* **21**, 505 (1937).
5. A. P. Vinogradov, *The Elementary Chemical Composition of Marine Organisms* (Yale Univ. Press, New Haven, 1953), p. 411.
6. H. T. Odum, *Science* **114**, 2963 (1951). X-ray diffraction studies performed by us on spicules of several nudibranch species from the West Coast showed similarly that their inorganic content may be amorphous.
7. The statoliths in Mysidacea occur in the two endopodites. The samples studied were discoidal in shape, semitranslucent, and from 0.2 to 0.3 mm in diameter. The *Scaphander* species have three gizzard plates each, of which the two laterals are the larger. The lateral plates of *S. lignarius* used in the study were 13 mm long, 12 mm wide, and 2 mm thick; whereas, those of *S. punctostriatus* were 5 mm long, 4 mm wide, and 0.5 mm thick. The plates appear opaque and were ivory to light brown in color.
8. The Ca and F contents of the outer rim are higher than in the organic-rich center of the statoliths. The P contents show the reverse, amounting to 0.1 percent in the outer rim and 0.4 percent in the center of the statolith.
9. L. A. Zenkevich, *Mem. Geol. Soc. Amer.* **67**, 1, 891 (1957).
10. W. M. Tattersall and O. S. Tattersall, *The British Mysidacea* (Ray Society, London, 1961) p. 54.
11. Our unpublished data show that the spicules of 5 nudibranch species from the West Coast contain from 9 to 20 percent fluorine, presumably as an amorphous substance.
12. Contribution No. 1553 from the Division of the Geological Sciences, California Institute of Technology. Supported by NSF grant GB-6707 and NIH grant DE-01411. We thank H. Lemche for the *Scaphander* specimens, and M. Dekkers and A. Chodos for technical assistance.

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Amino Acid Composition of Organic Matrix in Calcareous Oolites

Abstract. Examination of the organic matter in some modern and fossil oolites has shown that it contains protein, with a high content of acidic amino acids. Artificial oolites from a water-processing plant contained no amino acids. The protein matrix may influence the formation of natural oolites by concentrating calcium ions.

The association of an organic matrix with mineralized structures in biological systems is well known. Recent studies have also indicated an association between organic and inorganic substances in the sedimentary environment. Calcium carbonate concretions may form as a result of decaying soft tissues (1), and carbonates and other minerals in seawater may have an organic coating (2). Organic matter has also been reported in manganese nodules (3) and oolites (4), indicating that these objects may form as a result of microbiologic activity.

Calcareous oolites are aragonitic, spheroidal, sand-sized, concretionary particles that form around a nucleus of

foreign material, usually quartz grains or shell fragments. They are interesting geologically because of the extensive deposits they now form in many parts of the world and because of their occurrence in rocks at least as old as Cambrian. Oolites generally form in shallow, agitated waters supersaturated with calcium carbonate, and they are used as environmental indicators.

Two major theories have been proposed for oolite formation. Proponents of an inorganic origin, currently the more popular theory, suggest that oolites are simply physicochemical precipitates formed as a result of supersaturation of the water with calcium carbonate (5). The presence of organic matter in oolites is well documented, however, and has been used as evidence of an origin by biochemical processes (4). The organic matrix, about 0.1 percent by weight, has been described by Newell *et al.* (5) as "a complex mesh of mucilaginous organic material" that is interlayered with the concentric aragonite laminations. Remains of boring algae are occasionally present in the oolites.

The composition of the organic matrix has heretofore been unknown. Thus, knowledge of the type of organic compounds in the matrix may provide useful information in determining the origin of oolites. The amino acid content (Table 1) was determined for modern oolites from two localities in the Bahamas and one locality in the Great Salt Lake and for fossil oolites from the Atlantic Continental Shelf (6, 7).

Oolitic sediments commonly consist, in addition to oolites, of calcified fecal pellets, tests of microorganisms, and shell fragments. Care must be taken in preparation of samples to avoid introducing these sources of extraneous organic matter. Typically, a sample of oolites, containing only spheroidal grains, separated from the raw sediment by sieving and hand picking, and weighing about 0.7 to 1.0 g was taken for analysis. The outer portion of the oolites was dissolved with dilute HCl to remove adhering contamination. To avoid sampling the nucleus, which may consist of shell fragments or fecal pellets, I sampled only the outer 15 to 20 percent of the grains by dissolving the calcium carbonate with dilute HCl. The supernatant and subsequent rinse water were poured off for analysis of free and soluble combined amino acids. Insoluble matter released from the oolites was isolated, washed, and hydrolyzed separately. Examination of the oolite grains

Table 1. Amino acid composition of insoluble organic matter in oolites in residues of amino acid per 1000 total residues. The Great Salt Lake and Bahamas oolites are modern, and the oolites from the Atlantic Shelf are approximately 26,000 years old.

Amino acid	Locality		
	Great Salt Lake	Bahamas	Atlantic Shelf
Aspartic acid	190	168	205
Threonine	42	60	59
Serine	52	50	51
Glutamic acid	168	86	105
Proline	48	43	49
Glycine	123	137	140
Alanine	90	115	99
Half-cystine*	16	12	tr†
Valine	62	79	65
Methionine*	10	6	tr
Alloisoleucine	—	—	2
Isoleucine	35	51	40
Leucine	63	66	53
Tyrosine	8	7	13
Phenylalanine	32	55	37
Ornithine	17	9	9
Lysine	17	26	36
Histidine	4	2	—
Arginine	23	28	37
Amide N	(183)	(103)	n.d.‡
Acidic residues §	175	150	—
Basic residues	66	64	82
Total number of micromoles per gram of oolite			
	3.6	4.5	2.3

* Includes oxidation product. † Trace. ‡ Not determined. § The acidic residues are aspartic acid plus glutamic acid, minus ammonia.

after the acid treatment revealed that only the concentric layers were dissolved.

Only very small amounts of free amino acids were detected in Bahamian and Great Salt Lake oolites (10^{-10} mole) and these may have been released during the dilute acid treatment. The hydrolyzates (in 6N HCl) of the supernatant fluid and insoluble matter yielded significant amounts of combined acids (2 to 5 μ mole of amino acid per gram of calcium carbonate). The release of amino acids by acid hydrolysis indicates that the organic substance in oolites is largely proteinaceous.

The amino acid composition of this protein (Table 1) is remarkably similar for all the samples studied, considering the diverse geographic localities in which the oolites formed. The two Bahamian samples, only one of which is listed in Table 1, give identical results. All of the oolites are characterized by a high content, approximately 175 residues per thousand, of the acidic amino acids aspartic acid and glutamic acid. The Great Salt Lake oolites have a higher content of glutamic acid than the others. The basic amino acids, ornithine, lysine, histidine, and arginine, are low, comprising about 65 residues.

Trace amounts of amino sugar were present in all the samples. In the fossil oolites, the nonprotein amino acid alloseucine was present. The ratio of alloseucine to isoleucine is about 0.05 which is approximately the same ratio as found in fossil shells of comparable age (8).

The Great Salt Lake oolites were serially dissolved to sample the outer and inner portions of the organic matrix. No significant differences in composition were detected. The second acid treatment of Great Salt Lake oolites exposed some nuclei, but they were composed entirely of mineral grains, mainly quartz.

In addition to natural oolites, artificial oolites, obtained from a water-processing plant in Homestead, Florida, were analyzed. In cross section they do not exhibit the concentric pattern of natural oolites and they do not contain any amino acids at the 10^{-10} mole level, either free or combined. Only natural oolites, therefore, appear to contain a proteinaceous matrix.

Several attempts have been made to grow oolites in the laboratory under a variety of conditions (9). Evaporation of seawater, addition of excess carbonate, and bacterial cultures caused precipitation of CaCO_3 . These precipitates, however, did not resemble natural oolites. Bacteria have also served as nuclei for precipitation of CaCO_3 by concentrating calcium ions on their cell surface (10). There is no evidence that natural oolites formed in this manner.

Most of the organic matrix in oolites consists of uncharacterized organic matter which appears to be distributed throughout the carbonate phase. Most investigators, whether supporting a physicochemical or biochemical origin, consider the matrix to be an algal product (4, 5), probably a cellular secretion which forms a coating on the oolite grain or nucleus. The presence of calcareous algae as one of the very few proliferating organisms, and the only carbonate-precipitating organism, in the Great Salt Lake supports the view that algae are the source of the organic matter in oolites. Proponents of an inorganic origin consider this organic matter to be simply occluded or trapped in the growing oolite (11). As Chave (2) has noted, organic coatings on mineral grains may be a common occurrence in the sedimentary environment.

The composition of the organic matrix in oolites, however, suggests that it may influence the deposition of

CaCO_3 . Molluscan shells, bones, and other biological hard parts contain protein as the substrate upon which the inorganic phase is formed (12). The organic matrix in these biochemical systems causes formation of the mineral either by concentrating the appropriate ions on charged sites on the protein or by providing a set of specific templates upon which the mineral nucleates. It has been suggested that the acidic amino acids in the protein of mineralized tissues are important in concentrating calcium because of their polar side groups (12).

Artificial oolites, formed by physicochemical precipitation, do not have an organic matrix. The presence in natural oolites of a proteinaceous matrix with a high concentration of acidic amino acids strongly suggests that the organic matter may influence the precipitation of CaCO_3 by concentrating calcium ions and it may be a prerequisite to natural oolite formation. Calcareous algae are a possible source of the protein. Natural oolites do not appear to form simply as physicochemical precipitates.

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References and Notes

1. R. A. Berner, *Science* **159**, 1965 (1968).
2. K. E. Chave, *ibid.* **148**, 1723 (1965).
3. J. W. Graham and S. C. Cooper, *Nature* **183**, 1050 (1959); J. W. Graham, *Science* **129**, 1428 (1959).
4. W. D. Nesterhoff, C. R. Hebd. *Seances Acad. Sci. Paris* **242**, 1047 (1956); A. Rothpletz, *Amer. Geol.* **10**, 279 (1892).
5. H. A. Lowenstam and S. Epstein, *J. Geol.* **65**, 364 (1957); N. D. Newell, E. G. Purdy, J. Imbrie, *ibid.* **68**, 481 (1960); R. N. Ginsburg, R. M. Lloyd, K. W. Stockman, in *The Sea*, M. N. Hill, Ed. (Wiley, New York, 1963), p. 554.
6. The oolites were collected off the coast of North Carolina and are similar to oolites dated at about 26,000 years described by O. H. Pilkey, D. Schnitker, D. R. Pevear, *J. Sediment. Petrology* **36**, 462 (1966).
7. The instrument is a custom-made amino acid analyzer based on modifications by P. E. Hare [*Fed. Proc.* **25**, 709 (1966)] of the system described by D. H. Spackman, W. H. Stein, S. Moore, *Anal. Chem.* **30**, 1190 (1958).
8. P. E. Hare and R. M. Mitterer, *Carnegie Inst. Wash. Year B.* **65**, 362 (1966).
9. P. H. Monaghan and M. L. Lytle, *J. Sediment. Petrology* **26**, 111 (1956); C. H. Oppenheimer, *Geochim. Cosmochim. Acta* **23**, 295 (1961).
10. L. J. Greenfield, *Ann. N.Y. Acad. Sci.* **109**, 23 (1963).
11. A. J. Eardley, *Amer. Ass. Petrol. Geol. Bull.* **22**, 1305 (1938).
12. P. E. Hare, *Science* **139**, 216 (1963); K. M. Wilbur, in *Physiology of Mollusca*, K. Wilbur and C. Yonge, Eds. (Academic Press, New York, 1964), vol. 1, p. 243; E. T. Degens, D. W. Spencer, R. H. Parker, *Comp. Biochem. Physiol.* **20**, 553 (1967).
13. Supported by NASA grant NSG 269-62. I thank M. L. Jensen, R. D. Perkins, O. H. Pilkey, and H. F. Nelson for providing samples; I thank S. E. Moiola and M. Bilelo for technical assistance. Contribution No. 80, Geosciences Division, Southwest Center for Advanced Studies.

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Receptors Sensitive to Carbon Dioxide in Lungs of Chicken

Abstract. *Receptors responsive to the removal of carbon dioxide from ventilatory gas were demonstrated in the lungs of the chicken. Apnea was induced in 0.64 second after rapid lowering of respired carbon dioxide in the absence of blood flow through the lungs. Afferent impulses from the receptors are conducted centrally in the vagi.*

Exact location of functional peripheral chemoreceptors that influence respiration in the bird is unknown despite the presence of tissue that appears to be similar to that in the mammalian carotid body (1). Chickens are, however, acutely sensitive to changes in ventilatory CO_2 (2), which suggests the

presence of CO_2 -sensitive chemoreceptive tissue. Furthermore, speed of the response suggests that the receptor site may be near or in the lungs. We, therefore, attempted to locate the rapidly responding CO_2 -sensitive receptors.

Mature, male, White Leghorn chickens, secured in dorsal recumbency, were

Table 1. Respiratory response to rapid reduction of P_{CO_2} in the respiratory system. The response time is a measure of the time between entry of CO_2 -free gas into the trachea and the first indication of an alteration in sternal movements.

Conditions	Response time (sec)	Birds (No.)	Trials (No.)
Control	$0.50 \pm 0.20^*$	34	276
Extracorporeal loops in brachiocephalic arteries	0.58 ± 0.22	6	127
Extracorporeal loops in aorta	0.52 ± 0.22	3	18
Lung circulation occluded	0.64 ± 0.22	10	27
Bronchi cannulated at lung hilus	0.47 ± 0.13	2	17
Unilateral thoracic vagotomy	0.78 ± 0.32	15	51
Bilateral thoracic vagotomy	4.5 ± 1.3	15	71

* Mean $\pm$ standard deviation.