Table 2. Incorporation of 2-^{14}C-mevalonate into sterol fractions of mature human brain. Total amount of sterols per gram of brain tissue was 19.74 mg. Total disintegrations per minute per gram of tissue was 4476, as measured at the column effluent.

Sterols	Sterols in tissue (μg/g)	Radioactivity (%)
Δ^5 (cholesterol)	18,600	46.87
$\Delta^{8,24}$ ($C_{30,29,28,27}$)	95	12.00
Δ^8 ($C_{30,29,28,27}$)	255	5.10
$\Delta^{5,24}$ (desmosterol)	4	2.11
Δ^0 (cholestanol)	55	1.80
Other unsaponifiable components		32.12

spectrometry, by comparison with authentic standards (*10*). The column separation was performed in two steps, one column being used for separating the major portion of cholesterol and the remaining sterols being applied to a second column for further fractionation. All the sterols of the Δ^8 and $\Delta^{8,24}$ series with 30, 29, 28, and 27 carbon atoms were identified (Table 1). However, we cannot exclude the possibility that minute amounts of Δ^7-sterols were present and could not be detected under our experimental conditions. The C_{30} sterols, lanosterol ($\Delta^{8,24}$) and dihydrolanosterol (Δ^8), had not been previously detected in either adult rat brain or developing chick brain (*6*), and it is believed that improvement of the technique due to separation on two columns permitted the identification of these additional sterols in adult human brain. Another sterol not previously identified in nervous tissue was C_{27} (Δ^{14}) which may be suspected to take part in the pathway of cholesterol synthesis. The origin of 7-ketocholesterol, also detected by Fieser *et al.* (*5*), is not known.

Cholesterol accounts for about 99 percent of the total sterols in the analyzed brain; the second major sterol is cholestanol, C_{27} (Δ^0). Desmosterol, C_{27} ($\Delta^{5,24}$), which is quantitatively very important in developing human (*11*) and animal (*12*, *13*) brains, and represents 0.8 percent of the sterols in mature rat brain (*6*), is present in a very low concentration in adult human brain. The sterols of the Δ^8 series occurred in larger percentages than those of the $\Delta^{8,24}$ series.

No more than one-half of the radioactivity eluted from the column is attributable to cholesterol, and the specific activity is very low because the cholesterol pool is large (Table 2). The sterols of the $\Delta^{8,24}$ series, in spite of their lower concentration, show higher incorporation of mevalonate when compared with the Δ^8 series.

Although a precise calculation of specific activities is not always possible because separation of some of the homologous sterols is incomplete, it is clear that the specific activity of the $\Delta^{8,24}$ series is the highest. These data confirm our hypothesis that sterols with a double bond in the lateral chain are intermediates of cholesterol synthesis in the brain. The labeling of desmosterol is low, in contrast with the data obtained from developing brain (*6*, *12*) and brain tumors (*14*), further suggesting a special function for this sterol in immature and undifferentiated nervous tissue.

The identification of labeled intermediates of cholesterol biosynthesis together with the labeled cholesterol indicates that human brain retains the ability to synthesize cholesterol throughout life.

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Biologic Precipitation of Fluorite

Abstract. *X-ray diffraction patterns show that the statoliths of marine mysid crustaceans are composed of fluorite, and that this mineral is also a principal phase of the gizzard plates of some tectibranch gastropods. A phosphatic phase is also indicated by chemical analyses in the gizzard plates, but its crystallochemical characterization has not been feasible by x-ray diffraction. The occurrence of fluorite in mysid statoliths confirms the earlier interpretations based on insufficient documentation. Fixation of fluorine in hard tissues of marine invertebrates is extensive in the shelf seawaters and minor in the bathyal zone of the oceans.*

Information on the kinds of crystalline compounds precipitated by marine organisms is still very limited. This is borne out by discoveries of the minerals magnetite, goethite, lepidocrocite, and francolite in hard tissues of marine invertebrates, all previously thought to be solely of inorganic origin (*1*). Questions necessarily arise concerning the proper identification of some biologically precipitated minerals reported. One of these, fluorite (CaF_2), is stated to occur in the statoliths of marine mysid crustaceans (*2*, *3*) and in the spicules of a nudibranch (Mollusca) (*4*). The presence of fluorite was deduced initially from elemental determinations indicating that the mysid statoliths and nudibranch spicules were rich in calcium and fluorine (*2*, *4*). Microchemical tests subsequently performed on statoliths of another mysid species failed to detect fluorine, and the mineral matter was stated to consist of calcium carbonate (*5*). Fluorite was again reported from the statolith of still another mysid species (*3*). Identification of fluorite was obtained with the aid of x-ray diffraction, but no data on the *d*-spacings and line intensities of the diffraction were published to support the mineral determination. X-ray diffraction patterns obtained in a later study from spicules of the same nudibranch species failed to show the presence of a crystalline substance, thus excluding the possibility that it is fluorite (*6*).

We identified the mineral fluorite as one of the mineral constituents in the gizzard plates of some tectibranch gastropods. To determine whether this constitutes the first unquestionable occurrence of biologically precipitated

fluorite, we reinvestigated the mineral content of the statoliths of Mysidacea, including two of the species from which fluorite had been reported.

We investigated samples of the statoliths of the Mysidacea, *Praunus flexuosus* and *Neomysis intiger* from Isefjord, Vellerup Vig, Island of Zealand, Denmark; *Neomysis rayi* from off Orcas Island, San Juan Archipelago, Washington; and of the gizzard plates of the tectibranch gastropods, *Scaphander lignarius* dredged by the DANA near the Faeroes Islands (61°08'N; 8°47'W) at a depth of 60 m; and *Scaphander punctostriatus* from the "Thor" Station 1572 at a depth of 670 m (*7*). The statoliths and gizzard plates were mechanically removed from specimens preserved in a solution of 70 percent alcohol. One portion of the samples was used for elemental determinations with an electron-probe microanalyzer (Applied Research Laboratories); a second was finely ground, and the powders were investigated with a Debye-Scherrer camera and nickel-filtered copper radiation. A reference sample of fluorite was prepared for comparison.

The electron-probe analyses indicated that Ca and F are the major constituents; and Na, Cl, and P constitute trace constituents of the statoliths in the three mysid species (*8*). The electron-probe analyses of the gizzard plates of *Scaphander lignarius* and *S. punctostriatus* showed Ca, F, and P as the major and Mg, Sr, Na, K, Ba, Si, V, and Ti as minor constituents. The latter, when combined, make up 3.7 percent, of which Sr, Mg, and Na represent 3.5 percent.

The x-ray diffraction patterns (Fig. 1) of the statoliths from *Praunus flexuosus* (A) and of the gizzard plate of *Scaphander lignarius* (B) are similar to that of the reference fluorite sample (C) in both spacings and intensities. Similar powder diffraction patterns were obtained from the statoliths of *Neomysis intiger* and *N. rayi* and from the gizzard plates of *Scaphander punctostriatus*. The nature of the phosphatic component in the gizzard plates of the two *Scaphander* species is not revealed by the powder diffraction pattern, and it may be an "amorphous" calcium-phosphate hydrogel or an organic compound that contains significant phosphorus. It was not feasible to separate this second phase from the calcium fluoride; hence, the true nature of the phosphatic constituent remains an enigma.

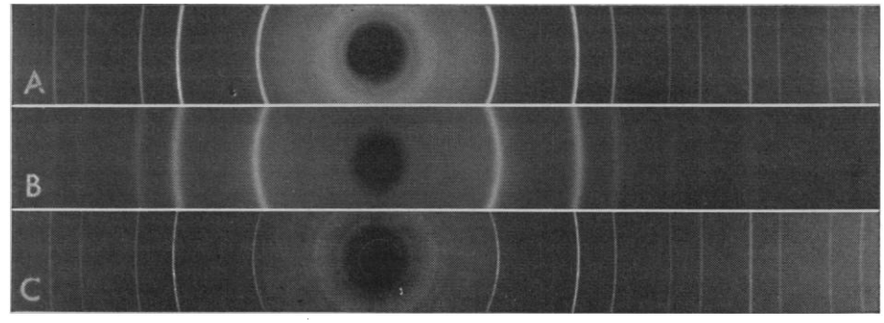

Fig. 1. X-ray diffraction patterns of (A) statolith of *Praunus flexuosus;* (B) gizzard plate of *Scaphander lignarius;* (C) reference fluorite.

Our data establish conclusively for the first time that biologic systems in the oceans precipitate the crystalline compound, fluorite, in their mineralized tissues. The agents involved in the biosynthesis of this mineral encompass species of two phyla, the Mollusca and Arthropoda. In the case of the mysid crustaceans our data confirm the mineralogic composition of the statoliths suggested earlier, but without proper documentation, for two marine species. The mysids which were reported in the literature to have statoliths composed of calcium carbonate came from the Caspian Sea (*5*). The chemistry of the Caspian water differs significantly from that of the oceanic reservoir (*9*), and this may account for the mineralogic difference of the statoliths in the local mysids from those in the marine environments.

Of the organisms indicated here to precipitate fluorite, the mysid crustaceans constitute a major segment of the marine biomass in the shelf seawater. They occur here seasonally in huge concentrations in localized areas where they serve as a major source of food for fish and invertebrates (*10*). The two statoliths of an adult individual contain on the average about 0.2 mg of fluorite. Molting occurs frequently, as often as 40 times during the life span of an individual, and the statoliths are shed with each molt (*2, 10*). A conservative estimate of the total amount of fluorite synthesized by a 1-year-old individual averages about 3 to 4 mg. We have seen that two gastropod species from neritic to bathyal depths in the Atlantic precipitate fluorite also. There are no data on their population size nor on the amount of fluorite synthesized by an individual, since we were unable to separate the mineral from the "amorphous" phase of the gizzard plates. Fluorine is further concentrated in large amounts in spicules of many nudibranchs in the form of an undefined mineral (*4, 6, 11*). These organisms occur in all shelf seas and are common locally in shallow waters. These data indicate that significant amounts of fluorine are fixed in the minerals of the hard tissues by biologic agents in the shelf seawaters and seemingly minor quantities in the bathyal zone of the oceans. Where huge mysid populations reside, there is the possibility that their large-scale fluorite precipitation may seasonally influence the fluoride concentrations of the seawater in the local environment.

Small numbers of mysid statoliths have been reported from the shelf sea deposits of the northeast Pacific (*3*). We have shown that fluorite occurs in statoliths of mysid species from the Atlantic and Pacific oceans. Hence, biologically precipitated fluorite in the form of mysid statoliths should be widely distributed in shelf sea sediments. Moreover, fluorite crystals should be freed from the organic matrices of statoliths by biomechanical and biochemical action of both scavengers and deposit feeders and become incorporated in the clay-sized fraction of the deposits. Sedimentary occurrences of fluorite were formerly attributed to inorganic, terrigenous sources. Therefore, it is necessary to distinguish in sedimentary fluorite between biologic and inorganically derived fractions.

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

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.

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 6*N* HCl) of the supernatant fluid and insoluble matter yielded significant amounts of combined acids (2 to 5 μmole 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.