Survival of Amino Acids in Marine Sediments

Proteinaceous material is carried into marine sediments upon the death and burial of aquatic organisms. The fate of this material has been a subject of conjecture, most investigators believing that it is rapidly decomposed (*1*). Studies of the thermal stability of an amino acid have recently shown that these compounds may survive intact for a long time at ordinary temperatures (*2*). In addition, protective mechanisms may play a part in preserving protein from early microbiological decomposition in terrestrial and marine muds. Recent work (*3*) has demonstrated the formation of complexes of protein with lignins and with clays, which are resistant to attack by microorganisms. Traces of proteins or peptides have been detected in fossil clams from the Pleistocene (*4*), and amino acids have been found in invertebrate and vertebrate fossils as old as the Ordovician (*2*).

In the present work, the amino acid content was determined for a typical recent shallow-water, marine deposit and for a similar sediment laid down during the Oligocene, approximately 30 million years ago. The recent sample was collected by coring on the inner continental shelf of the Gulf of Mexico and represents a narrow sedimentary layer at a depth of approximately 120 cm; this is below the zone of major bacterial activity (*5*). The age of the sediment is probably not more than a few thousand years. The ancient, or Oligocene, sample comprised a section of a marine shale core that was cut from the lower Anahuac formation in Fort Bend County, Tex., at a depth of 5000 ft.

Table 1. Comparison of a Recent and an Oligocene marine mud.

Content	Recent	Oligocene
Carbonate carbon (%)	0.71	1.49
Organic carbon (%)	0.53	0.27
Organic nitrogen (%)	0.044	0.032
Amino acids (μ mole/g)*	3.0	0.51
Principal amino acids in order of decreasing abundance†		
Identification certain	valine +++	alanine +++
	leucines +++	glutamic acid ++
	alanine ++	glycine +
	glutamic acid ++	proline +
	aspartic acid ++	leucines +
	glycine ++	aspartic acid < +
	proline ++	
	tyrosine ++	
	phenylalanine +	
Identification tentative	arginine ++	valine +

* For an amino acid mixture, 1 μmole/g is approximately 0.01 percent.
† Order of abundance: +++, high; ++, medium; +, low.

Immediately upon collection, both sediment samples were frozen in dry ice. In the case of the shale sample, the outer portion of the core was removed by turning on a lathe while the core was still frozen; the center was then crushed, suspended in distilled water, and milled to yield a thin slurry. On freeze-drying, both the recent and ancient samples were reduced to a powder with a flourlike texture.

The two samples were hydrolyzed, and the solutions were freed of salts electrolytically and by passage through Dowex 50 cation-exchange resin. Amino acids in the salt-free concentrates were resolved by one- and two-dimensional paper chromatography, using t-butanol-water-formic acid and phenol-water-ammonia as irrigation liquids. Positive identifications of individual amino acids were obtained by comparison of R_f values, using pure amino acids for reference, and by means of specific color reagents—for example, ninhydrin, isatin, 1,2-naphthoquinone-4-sulfonic acid and 1-nitroso-2-naphthol.

Data in Table 1 show that the modern and ancient marine samples selected for study are quite similar with respect to concentrations of carbonate carbon, organic carbon, and nitrogen. In contrast, the concentrations of free or combined amino acids are less in the Oligocene sample by a factor of 6.

Despite the difference in concentration, the arrays of amino acids in the two samples, modern and ancient, are strikingly similar, with the exception of the absence of tyrosine, phenylalanine, and arginine in the latter. Tyrosine, containing a chemically reactive phenolic group, would not be expected to be as stable as the other amino acids under earth conditions. Phenylalanine and arginine, likewise, are among the less stable of these acids.

Hitherto the detection of amino acids in ancient material has been limited to fossil shells or bones. Little or nothing is known about finely grained marine sediments. Although shell fragments were not eliminated from the samples, amino acids were also obtained on alkaline hydrolysis, where the shell structure was not disintegrated. Using Abelson's values for ancient shells (*4*), it would appear that more than 80 percent of the amino acids are associated with the noncalcareous portion of the Oligocene sample. Thus the amino acids of living organisms, in addition to those in large fragments of bone and shell, also may survive for long periods of time.

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On the Presence of Free Sugars in Filtered Lake Water

It has almost universally been assumed that simple organic nutrients do not constitute more than a minute fraction of the "dissolved" organic matter of lake and ocean water. The main evidence for this assumption is the fact that fresh-water and marine bacteria, when they are cultured in the laboratory, are capable of utilizing extremely small concentrations of organic nutrients (*1*). The only low molecular weight organic compounds that have been demonstrated to occur in filtered lake water are the vitamins thiamin (*2*), niacin (*3*), and biotin (*3*), each of which is present in concentrations less than 1 mg/m³. There is also evidence that free amino acids may be present in lake water (*4*), but the concentrations and identities of the amino

Table 1. Concentrations of free sugars in samples of filtered lake water and filtered controls (double-distilled water). Trace (*t*) is less than 0.5 mg/m³.

Sample	Date	Depth (m)	Vol. of sample (lit)	Sucrose (mg/m³)	Glucose (mg/m³)
L. Opinicon	1/6/55	2	20	5	2
Little Round L.	1/8/55	1	4	2	1
Little Round L.	1/8/55	9	4	4	2
Little Round L.	1/8/55	14	4	10	5
Control			4	t	0
Control			2	1.5	0
Control			2	0	0

acids are uncertain. The purpose of the present work to test the truth of the aforementioned assumption as applied to free sugars in lake water (*5*).

Water samples were collected from two lakes in eastern Ontario: Lake Opinicon, located near Chaffey's Lock, and Little Round Lake, located near Maberly. The former is a shallow eutrophic lake with no thermocline and a maximum depth of 10 m. The latter is an oligotrophic lake with a well-developed thermocline and a maximum depth of 16 m. The water samples were collected with a Kemmerer water sampler and filtered through Millipore HA filters to remove the seston (plankton plus detritus).

Filtration was completed within 12 hours of collection for the Little Round Lake samples, and within 30 hours of collection for the Lake Opinicon sample. Before and after filtration the samples were stored at 4°C in the dark. Toluene was added as a preservative after filtration. It was not added before filtration because of the possibility that dying cells might liberate sugars to the water. The filtered samples were deionized with Amberlite IR-120 and IR-4B resins and then concentrated to dryness in a vacuum at temperatures below 45°C.

The dry residues were taken up in 1 ml of water, and amounts ranging from 5 to 300 μlit were spotted on sheets of Whatman No. 1 filter paper. Standard amounts of known sugars were spotted beside the unknowns, and the chromatograms were developed with a butanol-ethanol-water (45/5/50) solvent (*6*) for 3 days by descending chromatography. Chromatograms were run in duplicate. After being dried, one was sprayed with a benzidine spray (*7*) and the other with 2 percent orcinol in 2*N* hydrochloric acid. The amounts of the individual sugars were estimated by comparing the color intensities of standard spots with those of unknowns at the same chromatographic position. The comparisons were made in ultraviolet light. In order to determine the contamination level during analysis three separate samples of double-distilled water were subjected to exactly the same procedure: one 4-lit sample and two 2-lit samples.

The results are presented in Table 1. Sucrose and glucose were the only sugars detected in lake water. Both sugars occurred in low and approximately equimolar amounts. There was evidence in Little Round Lake that increased amounts of free sugars were present in the hypolimnetic water (below 8-m depth). On the other hand, the distilled water controls contained barely detectable amounts of sucrose and no glucose.

Under the conditions used in these analyses, about 25 percent of the sucrose and glucose initially present would have been removed by the ion-exchange columns (*8*). We are not prepared to make any statement about the exact amount of free sugar in the "dissolved" organic matter of lake water but feel justified only in stating that the concentrations are of the order of magnitude of *n* parts per billion parts of water. These low concentrations do not negate the assumption under test.

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Method for Predicting Amount of Strontium-89 in Marine Fishes by External Monitoring

If the total radioactivity in a whole fish could be determined by means of external monitoring, this information would be very useful to ecologists, fisheries biologists, and others. If such a method were found to be suitable for field work, it conceivably could eliminate much tedious dissecting, ashing, and counting procedures. During the course of some experiments on the uptake, accumulation, and loss of radiostrontium by marine fish, a record was kept of the counts per minute detectable at various external parts of the fish's body prior to dissection and analysis of the organs and tissues by conventional ashing and counting techniques. It was not known in advance whether or not these measurements would have any significance, but the possible occurrence of a direct relationship between some aspect of external monitoring and the total amount of radioactivity in the entire fish seemed worth investigating (*1*).

Several species of pelagic fish, including the black skipjack tuna (*Euthynnus yaito*), the yellowfin tuna (*Neothunnus macropterus*), and the dolphin (*Coryphaena hippurus*), were fed radioactive strontium-89 in gelatin capsules. The fish were kept in tanks with circulating sea water and were killed at intervals up to 27 days. A piece of pliofilm (Saran wrap) was used to cover the fish, and the probe of a count rate meter was used to determine the number of counts per minute on direct contact. The G-M tube used had a window thickness of 3.5 mg/cm², but it was protected by a screen that reduced its efficiency to some extent.

The various organ systems and tissues of the fish were subsequently analyzed for radioactivity. The total radioactivity recovered in each system—for example, the gills, axial skeleton, muscle—was then compared with the counts per minute (at 5-percent statistical error) that were previously found by monitoring the mouth, eye, operculum, gills, dorsal integument, and the pectoral, dorsal, and caudal fins. No apparent relationship was observed between any two parameters except that between the amount of radioactivity in the skeleton and the counts per minute found by monitoring the caudal fin. Figure 1 shows that the relationship between the number of microcuries of radiostrontium in the skeleton and the counts per minute in the caudal fin is approximately linear. Since about 30 percent of the radiostrontium found in the fishes after 7 hours occurs in the axial skeleton (*2*), it is therefore possible by measuring the counts per minute in the caudal fin to predict the total amount of radiostrontium in the entire fish.

This method proved successful only when the axial skeleton was found to have a radiostrontium content in excess of an undetermined threshold. Thus, a fish having 0.16 μc Sr^{89} in its skeleton showed only background radiation with the count rate meter, but a fish with 0.40 μc Sr^{89} had sufficient external radiation to be detected. The minimum Sr^{89} skeletal constant is therefore somewhere between

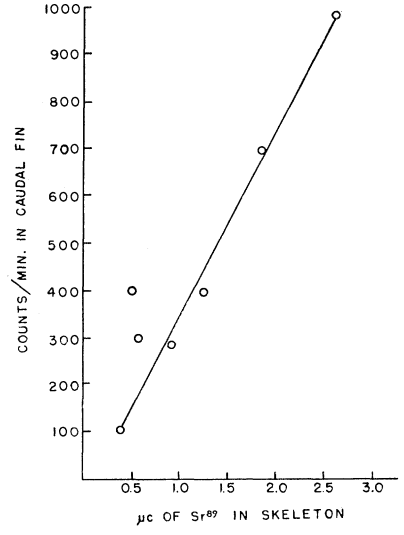

Fig. 1. Linear relationships between the counts per minute monitored in the caudal fin of tuna fed Sr^{89} and the microcuries of Sr^{89} found in the skeleton.