

incoherent diffuse scattering spread widely over the hemisphere of sky.

7. A general expression for  $T_b$ , the apparent brightness temperature seen looking down at the planet from outside the atmosphere at radio wavelengths, may be written as

$$T_b = (1 - \alpha) \{eT_p + \rho'[\alpha T_a + (1 - \alpha)T_s]\} + \alpha T_a$$

where  $e$  is the surface emissivity,  $\rho'$  is the surface reflectivity (6),  $T_a$  is the effective physical temperature of the atmosphere, and  $T_s$  is the sky brightness temperature. The quantities  $\alpha$ ,  $e$ , and  $\rho'$  will depend on the angle at which the surface is being observed. This expression approximates the temperature-weighted integration of  $\sigma$  over the sky, as seen from the surface, by the simple product of  $\rho'$  and  $T_s$  in the direction corresponding to specular reflection. For Venus, this approximation should be adequate. The expression above may be further simplified by noting that, at wavelengths of interest here, the atmospheric interaction takes place almost entirely in the bottom scale height, where  $T_a \approx T_p$ . Furthermore,  $T_s$  is only about 4 K at decimetric wavelengths (barring an accidental alignment with the sun) and can be neglected to our level of accuracy. Combining these terms and using  $\rho' = 1 - e$ , we obtain Eq. 2.

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[*Science* **164**, 1059 (1969)] quote an empirical expression (modified slightly to better fit modern data)

$$\alpha = 1 - \exp(-15/\lambda^2)$$

where  $\lambda$  is the wavelength in centimeters, that specifies the one-way attenuation suffered by radio waves in penetrating the Venus atmosphere at normal incidence to the Venus surface lying at mean altitude.

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## Stable Nitrogen Isotope Ratios of Bone Collagen Reflect Marine and Terrestrial Components of Prehistoric Human Diet

**Abstract.** The  $\delta^{15}\text{N}$  values of bone collagen from Eskimos and from Northwest Coast Indians dependent on salmon fishing are about 10 per mil more positive than those from agriculturalists in historic times. Among prehistoric humans, two groups dependent on marine food sources show bone collagen  $\delta^{15}\text{N}$  values that are 4 to 6 per mil more positive than those from two agricultural groups. The nitrogen isotope ratios of bone collagen from prehistoric inhabitants of the Bahamas are anomalously low for reasons that relate to the biogeochemical cycle of nitrogen in coral reefs.

Knowledge of diet yields information about human social and economic organization, health, and way of life (1). Generally, prehistoric human diets have been reconstructed from determinations of the abundance of floral and faunal remains in archeological deposits (2). Recently, analyses of trace elements and stable isotopes in human bone have been applied to the problem (3-7). We have shown that there is a large difference in the  $^{15}\text{N}/^{14}\text{N}$  ratios of bone collagen between animals feeding in marine systems and those feeding solely on terrestrial foods (Table 1) (8). We now report that stable nitrogen isotope ratios of bone collagen can be used in reconstructing the relative amounts of marine and terrestrial food sources in diets of historic and prehistoric human populations.

A study (7) of animals raised on diets for which the nitrogen isotopic compositions were known showed that the  $^{15}\text{N}/^{14}\text{N}$  ratios of animal tissues are determined by the  $^{15}\text{N}/^{14}\text{N}$  ratios of their diets. Diet nitrogen isotope ratios ultimately depend on the  $^{15}\text{N}/^{14}\text{N}$  ratios of plants at the base of the food chain. Marine plants have higher  $^{15}\text{N}/^{14}\text{N}$  ratios

than terrestrial plants (9, 10), and this difference in  $^{15}\text{N}$  content is carried up food chains, causing marine animals to have higher  $^{15}\text{N}/^{14}\text{N}$  ratios than those of terrestrial animals (8, 10, 11). The  $^{15}\text{N}/^{14}\text{N}$  ratios of bone collagen of humans feeding on marine food sources should thus be higher than those of peoples

subsisting on terrestrial food sources.

We determined isotopic compositions of bone collagen of several individuals from each of four groups of historic human populations (12) known to have exploited primarily either marine or terrestrial food sources. These included (i) Alaskan Eskimos, whose diet was composed of nearly 85 percent marine mammals (13); (ii) Haida and Tlingit Indians from the Northwest Coast of the United States, who depended more on salmon fishing for food than on all other subsistence techniques combined (14); (iii) Havihuh agriculturalists from New Mexico; and (iv) manioc farmers from Colombia, South America. Agricultural products provided almost the entire diet for the latter two groups (14).

Prehistoric groups from North America, South America, and Europe were also studied. The inhabitants of the Mugu site just north of Los Angeles, California, lived on the coast all year and subsisted largely on marine fish and mammals with plants from the surrounding countryside as supplements (15). The Danish Mesolithic period people also lived on the coast and apparently used large quantities of marine foods, as suggested by archeological evidence and the stable carbon isotope ratios of bone collagen (6). The Neolithic period people from Europe depended largely on grains that they grew (6), while the agriculturalists from the site of Tehuacán in Mexico depended primarily on maize (2). The Bahamian peoples, from sites on several islands in the central Bahamas, exploited molluscs and fish in the reef system and also used some agricultural products, although there is disagreement whether these were primarily maize or manioc (16).

Table 1. The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (8, 19) of bone collagen from animals feeding exclusively on marine or terrestrial food sources. Two marine birds could not be classified as either fish or mollusc eaters. Abbreviation: S.D., standard deviation.

Animal	Sample (N)	$\delta^{15}\text{N}$ (per mil)			$\delta^{13}\text{C}$ (per mil)		
		Mean	S.D.	Range	Mean	S.D.	Range
<i>Terrestrial</i>							
Mammals and birds	25	+5.9	2.3	+1.9, +10.0	-18.6	3.1	-22.5, -11.9
Herbivores	19	+4.9	1.6	+1.9, +7.3	-19.3	3.1	-22.5, -11.9
Carnivores	6	+8.0	1.6	+5.9, +10.0	-18.4	2.1	-21.2, -15.8
<i>Marine</i>							
Mammals	41	+15.6	2.2	+11.7, +22.9	-13.1	1.6	-16.1, -9.6
Fish eaters	25	+16.7	1.8	+14.3, +22.9	-12.8	1.1	-15.2, -11.0
Plankton, mollusc, arthropod eaters	16	+13.8	1.5	+11.7, +16.6	-13.5	2.2	-16.1, -9.6
Birds	11	+13.0	2.8	+9.4, +17.9	-16.2	2.5	-19.6, -12.1
Fish eaters	4	+16.2	1.6	+14.2, +17.9	-15.2	2.3	-18.6, -13.6
Mollusc eaters	5	+10.9	1.3	+9.4, +13.0	-17.1	1.8	-19.0, -14.9
Fish	10	+13.8	1.6	+11.1, +16.0	-12.5	1.4	-14.4, -10.0

Bone samples were cleaned ultrasonically and ground to less than 0.71 mm before collagen was extracted as described previously (7). The collagen samples were combusted by a modified version of the Stump and Frazer method (17, 18), and  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  ratios of the resulting  $\text{N}_2$  and  $\text{CO}_2$  were determined by mass spectrometry (19).

The historic populations whose diets were primarily marine in origin (Eskimo, Haida, and Tlingit) show collagen  $\delta^{15}\text{N}$  values ranging from +17 to +20 per mil, whereas those representing agricultural-

ists range from +6 to +12 per mil (Fig. 1A). Thus the  $\delta^{15}\text{N}$  values of bone collagen are related to diet in these groups.

Marine and terrestrial feeding patterns could also be distinguished in most of the prehistoric populations (Fig. 1A). The Mesoamerican maize agriculturalists have a mean  $\delta^{15}\text{N}$  value of +9 per mil. Samples from European Neolithic period agriculturalists show similar values. In contrast, bone collagen samples from marine hunter-gatherers of Mugu have a mean  $\delta^{15}\text{N}$  value of +16 per mil, while those of the Danish Mesolithic period

fisher-gatherers have a mean  $\delta^{15}\text{N}$  value of +14 per mil. Among the Bahamian group, however, the  $\delta^{15}\text{N}$  values (mean +11 per mil) are lower than those of the other groups that ate large amounts of marine food. The larger amount of nitrogen fixation in coral reefs relative to that occurring in other parts of the oceans (20) might explain this result. Marine organisms in food chains that are based on nitrogen fixation show lower  $\delta^{15}\text{N}$  values than those of organisms that feed on plants that are not capable of nitrogen fixation (21). We would expect that animals around coral reefs would have lower  $\delta^{15}\text{N}$  values than those of animals in the open ocean. Indeed, fish from the Bahamian reef have bone collagen  $\delta^{15}\text{N}$  values as low as those of terrestrial mammals (8).

The  $\delta^{13}\text{C}$  values of bone collagen also vary depending on the use of marine and terrestrial foods (5, 6). The mean bone collagen  $\delta^{13}\text{C}$  values of the groups that subsisted on large amounts of marine foods in historic and prehistoric times all fall between -12.0 and -14.5 per mil (Fig. 1B). The bone collagen  $\delta^{13}\text{C}$  values of agriculturalists are either more positive or more negative than these values, depending on whether the crops grown were primarily  $\text{C}_4$  plants or  $\text{C}_3$  plants, respectively (2, 4, 6, 14, 22).

Although  $\delta^{15}\text{N}$  values of bone collagen can be used in estimating the marine and terrestrial components of human diet, distinguishing between freshwater and terrestrial food sources by nitrogen isotope analysis may be impossible. We found that the  $\delta^{15}\text{N}$  values of bone collagen of five freshwater fish ranged from +6.6 to +9.5 per mil (8). These values are at the high end of the range of values for terrestrial animals (Table 1). Analysis of phytoplankton and zooplankton also suggests that organisms in freshwater systems have  $\delta^{15}\text{N}$  values intermediate between those of terrestrial and marine organisms (11, 23, 24).

There is a progressive enrichment of  $^{15}\text{N}$  from lower to higher levels in food chains. Miyake and Wada (11) noted a  $^{15}\text{N}$  enrichment in the series: inorganic nitrogen, phytoplankton, algae, zooplankton, and fish. Our data (8) indicate that the enrichment occurs at the higher trophic levels as well, as suggested by Sweeney *et al.* (10). For terrestrial carnivores,  $\delta^{15}\text{N}$  values are higher, on average, than those of terrestrial herbivores (8) (Table 1). Among both marine mammals and marine birds, those that eat fish have higher bone collagen  $\delta^{15}\text{N}$  values than do their counterparts that feed on organisms lower in the food chain (that is, arthropods, molluscs, and plankton)

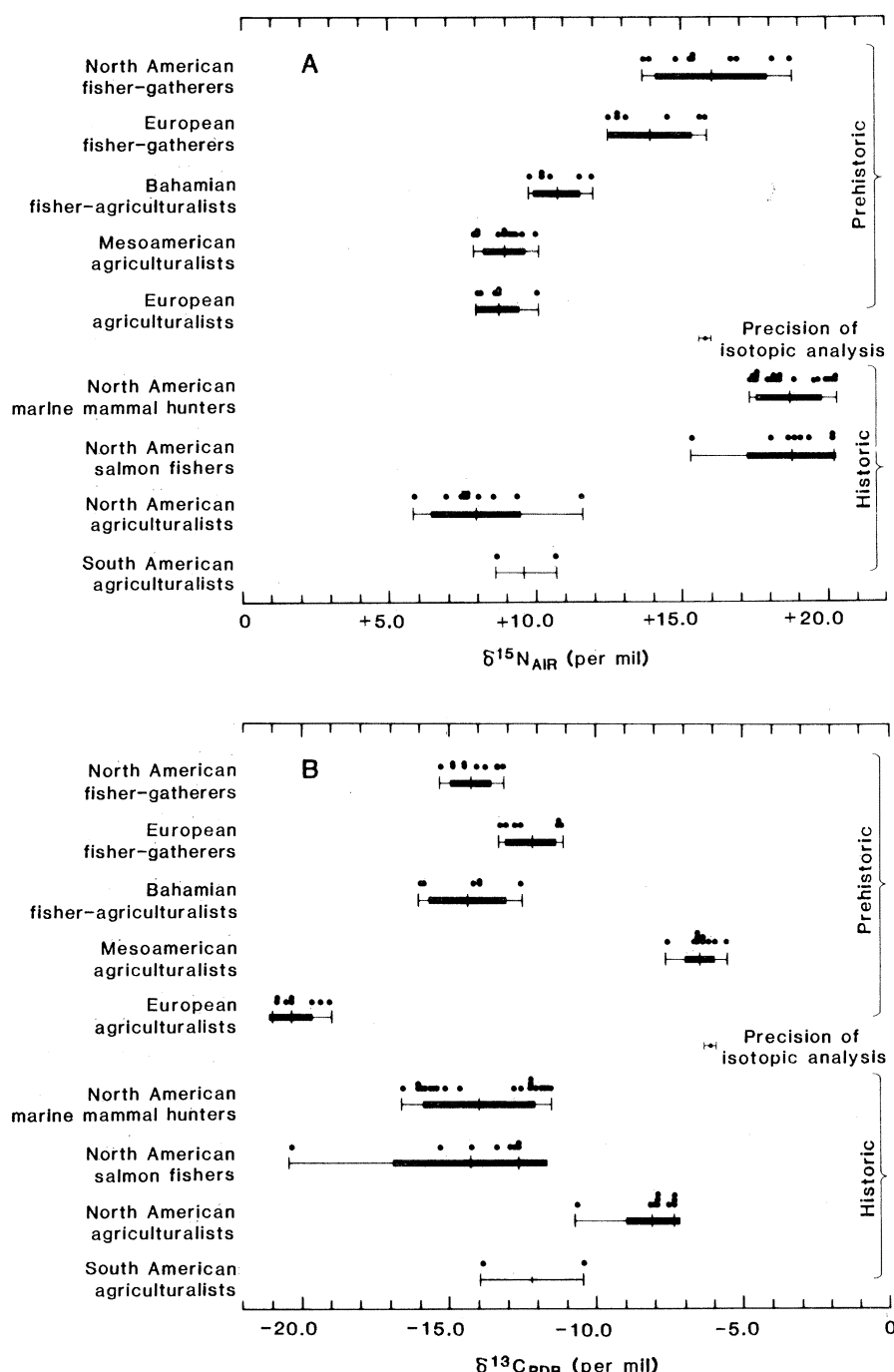


Fig. 1. (A) The  $\delta^{15}\text{N}$  and (B)  $\delta^{13}\text{C}$  values (19) of bone collagen from human groups in historic and prehistoric times. Each point represents the value for an individual; the range, mean, and standard deviation for each group are indicated.

(8) (Table 1). For the birds the average difference is 5 per mil, and for the marine mammals, 3 per mil. A  $^{15}\text{N}$  enrichment of this magnitude is consistent with the observation that the  $\delta^{15}\text{N}$  values of an animal's tissues are about 3 per mil more positive than that of its diet (7). In some cases, this trophic-level effect will have to be considered when human bone collagen  $\delta^{15}\text{N}$  values are used for dietary reconstruction.

We have shown that the  $\delta^{15}\text{N}$  values of bone collagen can be used to estimate the marine and terrestrial components of diets among historic and prehistoric human populations. In some cases, determination of both the  $\delta^{15}\text{N}$  values and the  $\delta^{13}\text{C}$  values of bone collagen will produce a more reliable reconstruction of this aspect of diet than analysis of only one isotope ratio. For example,  $\delta^{13}\text{C}$  values of bone collagen of humans whose diet consisted of equal amounts of  $\text{C}_4$  plants,  $\text{C}_3$  plants, and terrestrial mammals would mimic those resulting from a completely marine diet (4–7, 22), while  $\delta^{15}\text{N}$  values from humans subsisting on large amounts of tropical reef organisms, such as the Bahamian group, do not reflect the marine origins of their diet. Use of both the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of bone collagen to estimate the composition of the diet would clarify each of these situations.

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19. The results are expressed in  $\delta$  notation, where

$$\delta^{13}\text{C} = \left[ \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 1000 \text{ per mil}$$

$$\delta^{15}\text{N} = \left[ \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} - 1 \right] \times 1000 \text{ per mil}$$

The standards are the Pee Dee belemnite (PDB) carbonate for  $\delta^{13}\text{C}$  values and atmospheric nitrogen (AIR) for  $\delta^{15}\text{N}$  values.

20. Nitrogen fixation in coral reefs occurs at the rate of 25 g/m<sup>2</sup> per year, whereas in the shallow waters of the open ocean it occurs at 0.1 g/m<sup>2</sup> per year. Nitrogen fixation is also high in sea-grass meadows, salt marshes, and mangrove swamps [D. G. Capone and E. J. Carpenter, *Science* **217**, 1140 (1982)].
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## Atypical Pulmonary Thrombosis Caused by a Toxic Cyanobacterial Peptide

**Abstract.** Parenteral injection into mice of a toxic pentapeptide isolated from the cyanobacterium *Microcystis aeruginosa* induced thrombocytopenia, pulmonary thrombi, and hepatic congestion. The lethality of the toxin was unaffected by several anticoagulants. The acute liver damage that follows injection of the toxin has been attributed to direct action on liver cells but may be due to hypoxemia, heart failure, and shock.

The acute toxicity to mammals of the cosmopolitan freshwater cyanobacterium *Microcystis aeruginosa* (1–3) is attributed to a pentapeptide (4–6). A leucine (L)- and arginine (R)-containing pentapeptide (toxin-LR) occurs frequently in toxic strains of *M. aeruginosa* (7, 8). Extracts of *M. aeruginosa* parenterally injected into rodents elicit hepatotoxic effects, including sinusoidal congestion, hemorrhage, and necrosis (9–12). A lethal dose of purified toxin-LR induces multiple thrombi in the lung as well as hepatic changes. Liver toxicity observed on gross examination and by light microscopy may be the result of hypoxemia, heart failure, and shock, as expected from acute pulmonary vascular occlusion.

Studies were performed with 8- to 12-week-old female Swiss albino mice of the Hale-Stoner strain (13). The median lethal dose ( $\text{LD}_{50}$ ) of toxin-LR was about 0.06  $\mu\text{g}$  per gram of body weight. The route of injection, whether intravenous or intraperitoneal, did not substantially affect toxicity. Mice did not react to toxin-LR until 20 to 40 minutes after injection. Hunched posture, immobility, and piloerection were then observed during increasingly frequent and longer time intervals. This reduced activity was interrupted by apparently unprovoked

leaps. Such behavior was followed by lassitude, continued piloerection, tachypnea, and subcostal retraction. The ears and digits of affected mice became pale but not cyanotic. Mice surviving the injections by more than 2 hours usually lost all signs of toxicity within a few hours thereafter. If death ensued, it was preceded by pallor of eyes and tail, syncope, and then coma associated with occasional respiratory gasps. The usual time range between the injection of marginally lethal doses ( $\sim \text{LD}_{50}$ ) of toxin and death was not noticeably altered when substantially supralethal doses ( $\sim 4$  times  $\text{LD}_{50}$ ) were given.

Necropsies were performed immediately after the mice were killed by ether inhalation. Histological sections (5  $\mu\text{m}$ ) of formalin-fixed vital organs were stained with hematoxylin and eosin. Adjacent lung sections were stained by a fast phosphotungstic acid-hematoxylin method (PTAH) (14).

Livers appeared dark red and markedly enlarged ( $\sim 50$  percent increase in fresh weight) within 2 hours of injection with a lethal dose of toxin-LR. A thin film of pink ascitic fluid was sometimes noted. The cerebral cortex was slightly swollen and pale. No other abnormalities were observed on gross examination of the unfixed vital organs of mice given