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REVIEW

Natural Abundance Variations in Stable Isotopes and their Potential Uses in Animal Physiological Ecology

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ABSTRACT. Chemical, biological, and physical processes lead to distinctive "isotopic signatures" in biological materials that allow tracing of the origins of organic substances. Isotopic variation has been extensively used by plant physiological ecologists and by paleontologists, and recently ecologists have adopted the use of stable isotopes to measure ecosystem patterns and processes. To date, animal physiological ecologists have made minimal use of naturally occurring stable isotopes as tracers. Here we provide a review of the current and potential uses of naturally occurring stable isotopes in animal physiological ecology. We outline the physical and biological processes that lead to variation in isotopic abundance in plants and animals. We summarize current uses in animal physiological ecology (diet reconstruction and animal movement patterns), and suggest areas of research where the use of stable isotopes can be fruitful (protein balance and turnover and the allocation of dietary nutrients). We argue that animal physiological ecologists can benefit from including the measurement of naturally occurring stable isotopes in their battery of techniques. We also argue that animal physiologists can make an important contribution to the emerging field of stable isotopes in biology by testing experimentally the plethora of assumptions upon which the techniques rely. comp biochem physiol 119A;3:725–737, 1998. © 1998 Elsevier Science Inc.

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INTRODUCTION

Short-lived radiogenic isotopes of hydrogen (^{3}H), carbon (^{14}C), phosphorus (^{32}P), and sulfur (^{32}S) have been used extensively as tracers in animal physiology. For example, radio-labeled amino acids and proteins have been used to measure absorption, fates, and turnover of macro-molecules within animals (110). Experimentally-induced variations in the concentration of radiogenic isotopes are typically measured by scintillation counting. As the willingness to use radioisotopes in humans and other vertebrates has declined, metabolic and physiological research has increasingly relied on the use of substrates enriched in stable isotopes (^{2}H, ^{13}C, ^{15}N, ^{18}O, ^{34}S). For example, labeled water has been used to measure water turnover and metabolic rates in a wide range of animals (72). Isotopic measurements in experiments involving substrates enriched in stable isotopes are typically made on samples using relatively low resolution quadrupole mass filters or magnetic sector mass spectrometers. The chief limitations to the use of enriched stable isotope substrates are their cost and the difficulties in recapture when applied to wild animals.

Many light elements exhibit natural variations in the abundance of stable isotopes in the parts per thousand or even percent range. Following advances in the design of isotope ratio monitoring mass spectrometers that permit extremely precise analysis of these small differences (15,22, 25), there has been a tremendous increase in the use of these natural variations to trace biological, chemical, and physical processes, particularly within the earth, ocean, and environmental sciences. For example, plant physiologists have used natural variations in the abundance of stable isotopes in different biological materials to assess the photosynthetic mode and water use or nitrogen fixing efficiencies of plants (24,36). Paleontologists have used stable isotopes to study paleoclimate and the diets of extinct animals (57,86,108). Recently, ecosystem ecologists have begun to use stable isotopes to trace energy and nutrient flow through ecosystems (81,84), and stable isotopes are rapidly becoming a standard tool in the environmental sciences (60,64).

Although analysis of natural abundance variations in stable isotopes provides a potentially powerful tool for physiological research, physiological ecologists working with animals have not adopted it. Because most of the recent reviews are found far from the physiology literature, the pur-

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pose of this review is to introduce the use of natural abundance variations in stable isotopes to animal physiological ecologists and, hopefully, to encourage their use as tracers. There are a number of reviews of the use of stable isotopes in paleobiology (17,57,90,94,99,104), plant physiology (23,24,27,39,44,74,76), and ecology (60,81,84), consequently we do not intend to supply an exhaustive review of this literature here. Instead, we provide an introduction to stable isotopes and to the processes that lead to variations in their ratios in natural products. Then we summarize current uses of natural abundance variations in stable isotopes in animal ecology, focusing on a few key studies as examples and emphasizing research areas that would benefit from a greater input by animal physiological ecologists. Finally, we identify three key areas where stable isotopes may be applied to address important questions in animal physiological ecology. Throughout the review, we emphasize the use of the stable isotopes of carbon and nitrogen. Although other isotope systems are useful to physiologists (e.g., hydrogen, oxygen, or sulfur isotopes), carbon and nitrogen have received significantly more attention and hence, we devote most of this review to them.

We devote considerable attention to the processes that lead to variations in the stable isotope composition of plants. This attention to plants in a review devoted to animal physiology is justified for two reasons. First, plants are consumed by animals, thus processes that lead to distinct isotopic signatures in plants often find their way into animal tissues. Second, the processes of isotope fractionation have been well studied in plants. Thus plants provide clear examples of the processes that lead to natural abundance variations of stable isotopes in biological systems.

AN INTRODUCTION TO STABLE ISOTOPES

The tiny mass differences between isotopic forms of an element cause the isotopes to behave differently in both physical processes and chemical reactions. In general, the lighter isotope (e.g., ^{12}C or ^{14}N) tends to form weaker bonds and to react faster than the heavier isotope [e.g., ^{13}C or ^{15}N; (52)]. As a consequence of these bond energy and reaction rate differences, the abundance of stable isotopes of an element will vary between chemical species. The change in isotopic abundance between chemical species due to physical or chemical processes is termed fractionation.

The isotopic composition of a sample is measured as the ratio of one isotope to another (29,40). In most cases, the abundance of one isotope far exceeds the abundance of other isotopes(s). For example, in terrestrial materials, ^{12}C is ≈89 times more abundant than ^{13}C (22). Consequently, the ratio of ^{13}C to ^{12}C in most samples is a very small number. To make measurements of isotopic abundance of a manageable magnitude, the isotopic composition of most materials is expressed as the normalized ratio of the sample to a standard, in parts per thousand (per mil, ‰):

$$\delta X = [(R_{sample}/R_{standard}) - 1] * 1000$$

where R_{sample} and $R_{standard}$ are the ratios of heavy to light isotopes for the sample and the standard, respectively. A positive δX value means the sample has more of the heavier isotope than the standard. Atmospheric nitrogen and a marine carbonate from a belemnite from the Peedee Formation (PDB) are used as the standards for nitrogen and carbon, respectively.

Fractionation between chemical species A and B is described in terms of the difference in delta (δ) values between the species using the ϵ notation:

$$\epsilon_{A\text{-}B} \equiv \left(\frac{\delta X_A + 1000}{\delta X_B + 1000} - 1 \right) * 1000 \approx \delta X_A - \delta X_B$$

A negative ϵ value means that A has a relatively greater concentration of the isotope with a lower mass, and therefore has a lower isotopic value, than B. In enzymatically-catalyzed reactions, A is the reactant, B is the product, and ϵ values are almost always positive (i.e., the product is always enriched in the lower mass isotope, and therefore, has a lower isotope value, than the reactant).

Equilibrium isotope fractionation occurs among chemical species linked by equilibria as a result of bond strength differences between the isotopic species (25,44,52). For example, carbonate in bone and tooth apatite is probably derived from blood bicarbonate. Carbon and oxygen isotopes are rapidly exchanged among blood bicarbonate, dissolved blood carbon dioxide, and body water by the following equilibria: $CO_{2(aq)} + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$. The isotope composition of bone carbonate should be controlled by the composition of dissolved CO_2, which is produced by respiration, and fractionations associated with equilibrium exchange of carbon. At mammalian body temperatures, $\epsilon CO_{2(aq)} - HCO_3^-$ is ≈ −8‰ (71). Assuming that ϵ between dissolved bicarbonate and carbonate in apatite is ≈ −1 to −2‰ (the ϵ value for calcium carbonate), then apatite carbonate should have a $\delta^{13}C$ value ≈ 9 to 10‰ greater than respired CO_2. This result has been confirmed in the laboratory (101).

Kinetic isotope effects occur because of differences in the rate of transport or rate of reaction of isotopic species (24,44,52). For enzyme-catalyzed reactions, the magnitude of the fractionation can be used to approximate enzymatic affinity for an isotope. Also, for kinetic isotope effects to be expressed in biological materials, there must be more than one fate for the product downstream of the reaction that induces fractionation (44). If every atom transported by an enzyme ends up in a single product, the product must have the same isotope value as the reactant to preserve mass balance. As an example, consider the enzyme catalyst responsible for carbon fractionation in lipid synthesis, pyruvate dehydrogenase. The pyruvate dehydrogenase complex oxidizes pyruvate with a ^{12}C-carbonyl group faster than pyruvate with a ^{13}C-carbonyl group. The oxidation produces acetyl

coenzyme-A which is depleted of ^{13}C relative to its source, pyruvate (44). The magnitude of the fractionation effect is dependent on temperature and on the percent of the pyruvate pool entering this pathway versus other reaction pathways (44).

SOURCES OF ISOTOPIC SIGNATURES IN BIOLOGICAL MATERIALS

The use of natural abundance variations in stable isotopes as tracers relies on the fractionations that occur during chemical, physical, and biological processes. Differences in fractionation during these processes lead to distinct "isotopic signatures" for biological materials.

Carbon Fractionation during Photosynthesis

Perhaps the most intensively studied and certainly the most useful fractionating process to physiological ecologists is carbon fractionation during photosynthesis (78). Carbon isotope values can be used to differentiate among plants employing C3, C4, and CAM modes of photosynthesis (37). Carbon fractionation in C3 plants occurs at two steps in the photosynthetic process: diffusion/dissolution and carboxylation. CO_2 diffusion through the stomata and then dissolution into mesophyll water produces a slight fractionation of +4‰ and +0.7‰, respectively (75). The second fractionating step, carboxylation by the enzyme ribulose-1,5-bisphosphate carboxylase (RUBISCO) causes a much greater fractionation [ϵCO_2 − 3C sugar = +29‰; (74)], because RUBISCO has a much higher affinity for $^{12}CO_2$ than for $^{13}CO_2$. Fractionations during photosynthesis are not strictly additive, however; they depend on rate limiting steps (74). Carboxylation by RUBISCO is the rate limiting step in C3 photosynthesis, consequently C3 plant material is greatly depleted in heavy carbon [$\delta^{13}C = -34‰$ to $-22‰$; (76)] relative to atmospheric CO_2 [$\delta^{13}C = -8‰$; (74)]. C3 photosynthesis occurs in a relatively open system, and the ^{13}C-enriched CO_2 inside leaves, which is generated by the action of RUBISCO, can diffuse out of the photosynthetic system to the atmosphere.

Photosynthesis by C4 and CAM pathways leads to lower carbon isotope fractionation than C3 photosynthesis. The reason for this lower fractionation is that the action of RUBISCO occurs in relatively closed systems where the ^{13}C-enriched CO_2 cannot be liberated to the atmosphere and hence, has to be utilized. In both C4 and CAM plants, CO_2 is first carboxylated into organic acids by the enzyme phosphoenolpyruvate (PEP) carboxylase. Fractionation during this step is very slight [$\epsilon = +2‰$; (76)]. These organic acids are subsequently decarboxylated and the resulting CO_2 is utilized in the photosynthetic carbon reduction cycle (Calvin cycle) by RUBISCO.

In CAM and C4 plants, the acquisition of atmospheric CO_2 is separated from the Calvin cycle. In CAM plants, the separation is temporal; CO_2 is acquired during the night and the Calvin cycle takes place during the day when stomata are closed (23). In C4 plants, the separation is spatial; organic acids produced in mesophyll cells by the action of PEP-carboxylase are translocated into bundle sheath cells, where the Calvin cycle takes place (80). In both CAM and C4 plants, the action of RUBISCO takes place in relatively gas-tight compartments (mesenchyma cells with closed stomata or bundle sheath cells). Because the fractionation by PEP carboxylase and by diffusion/dissolution are modest, the tissues of obligate CAM and C4 plants are more similar to atmospheric CO_2 [$\delta^{13}C$ values range from −20 to −10‰; (76,77)], than those of C3 plants. Many plant species use the CAM photosynthetic pathway in a facultative fashion. Under drought conditions they have diurnal stomatal closing and nocturnal acid fixation, but they rely on C3 photosynthesis if water is available (42). The isotopic composition of the tissues of these facultative CAM species reflect the fraction of their carbon budget that is acquired by each photosynthetic mode (37).

Although marine plants fix carbon using the C3 metabolic pathway, marine plants can have distinct isotopic signatures from terrestrial C3 plants. The isotopic composition of aquatic plants falls in the range of terrestrial C4 plants (13,14), but isotopic composition of aquatic plants can vary greatly between sites and within individual plants (30). The causes of differences between aquatic and terrestrial plants is controversial and has been reviewed elsewhere (44,99).

Carbon Fractionation by Consumers

Because the isotopic ratio of a consumer's whole body generally resembles its diet (19,49,50), one of the major uses of stable isotopes in paleontology and anthropology is dietary reconstruction. For example, researchers have utilized the isotopic composition of animal remains to determine if the animal was a browser, and hence, ate primarily C3 plants, or a warm-climate grazer, and hence ate primarily C4 grasses (17,104).

Because diet is typically reconstructed using the isotopic composition of a specific tissue (e.g., bone collagen or bone apatite) and not the composition of the entire body, the fractionation associated with formation of the tissue must be understood for accurate dietary reconstruction. Specific body components exhibit characteristic fractionations relative to bulk diet. For example, the enzymatic fractionation of pyruvate dehydrogenase described above, produces lipids in plants and animals that tend to be enriched in ^{12}C (18,70). Fractionation values for tissues can be quantified by feeding animals diets of known and nonvarying isotopic value (3,19,22,41,47). Different amino acids show what appear to be characteristic isotopic values (1,41,54,63,103). Thus, the fractionation of a protein relative to bulk diet or dietary protein reflects, in part, its amino acid composition.

Isotopic values of specific tissue components may not al-

ways follow bulk diet values. The reason is that the carbon skeletons of different dietary constituents (protein, lipids, and carbohydrates) can be shunted to different tissue constituents. This effect has been termed "isotopic routing" (59,87). In following the sections, we will discuss the challenges that carbon isotope routing in animals present to dietary reconstruction.

Nitrogen Fractionation by Plants

Plant nitrogen resembles source nitrogen for two basic reasons (66). First, plants do not fractionate nitrogen when absorbing nitrate (33). Second, plants reuse their nitrogen by-products to a much larger degree than do animals (85). Although biosynthetic pathways in plants do not fractionate nitrogen, variation in source nitrogen composition can lead to plants with distinct isotopic signatures. For example, soil nitrogen contains more ^{15}N than the atmosphere. Therefore, plants that fix atmospheric nitrogen are ^{14}N-depleted relative to non-fixers at the same site (92). Furthermore, different soils (e.g., ridge tops and valley bottoms) have distinct nitrogen isotope signatures (33). Plant physiological ecologists have used plant $\delta^{15}N$ values to indicate nitrogen fixation and to estimate the importance of nitrogen fixation in ecosystems (36,93). The uses of nitrogen isotopes in plant physiology and ecology have been reviewed elsewhere (39).

Nitrogen Fractionation in Consumers

Nitrogen in the protein of consumers is generally enriched in ^{15}N by 3 to 5‰ relative to dietary nitrogen. This difference appears to be caused by fractionation during deamination and transamination (2,20). Deamination enzymes preferentially remove amine groups with ^{14}N. Consequently, excreted nitrogen (i.e., ammonia, uric acid, or urea) is ^{14}N-enriched relative to animal protein (96). In a similar fashion, transamination favors ^{14}N-containing amine groups (62). As a result, the nitrogen of glutamate, which is a frequent source for transamination to other amino acids and deamination in the urea cycle (9), is ^{15}N-enriched relative to nitrogen in dietary glutamate (31,41) and other amino acids (41,63). Animals at higher trophic levels (e.g., carnivores) have a higher $\delta^{15}N$ value than nitrogen in animals at lower trophic levels [e.g., herbivores, (2)]. Consequently, differences in $\delta^{15}N$ value have been used to study the trophic levels of animals in the field (2,7,69).

Starving animals literally "live on their own meat" (109). Therefore, the processes by which the tissues of animals at high trophic levels become enriched in ^{15}N probably apply to starving animals as well. Starving or protein-limited animals deaminate tissue protein to use as energy and amino acid sources (110). Because ^{14}N is excreted preferentially (96,97), the nitrogen remaining in animal protein becomes ^{15}N-enriched (47). As will be discussed later, the increase in ^{15}N in animal tissues can be used as an indicator of nitrogen balance and body condition.

USES IN ANIMAL ECOLOGY AND PHYSIOLOGY

The stable isotope methods used by animal ecologists and physiologists have been adapted from techniques used by paleontologists, geochemists, and plant physiologists. Animal physiologists and ecologists have just begun to realize and utilize the potential of stable isotope measurements in their fields (32). In this section, we review some of the uses that animal physiological ecologists have made of stable isotopes and present caveats associated with their use.

Dietary Reconstruction

Observational difficulties present a challenge for reconstructing diets of even the most observable animals. Often, visual observations are complemented by fecal analysis. The nutrients assimilated by animals on a diet may not match the bulk nutritional content of the diet, however (55). Stable isotopes provide an added tool for study of animal diets that avoids complications due to differential assimilation and observation, but they have their own limitations. Three caveats must be kept in mind when reconstructing animal diets from the isotopic composition of their tissues:

1. The dietary components of interest must have isotopically distinct signatures. For example, isotopic analyses of animal tissues can reveal the contribution of C3 vs C4/CAM plants, as well as marine vs C3 food sources to animal diets. Nitrogen isotope compositions can be used to discriminate among trophic levels (e.g., carnivory vs herbivory) and to differentiate between terrestrial and marine dietary sources. Given n different isotopes (e.g., C, N, S, O), the contributions of $n + 1$ food types can be distinguished provided that the signatures of these types are distinctive (87).
2. Researchers must be cautious to choose the appropriate animal tissue when attempting to reconstruct animal diets. Adopting the appropriate tissue or tissue component is important for two reasons. First, tissues and tissue components differ in turnover rate, and hence, the temporal resolution for dietary analyses differs among them. Second, as we will discuss in more detail below, the isotopic compositions of different tissues and tissue components depend-on how dietary nutrients are allocated.

   Because skeletal tissues are often well preserved in fossils, paleontologists have relied on the isotopic analysis of bone collagen and hydroxylapatite (bone mineral). Bone components have slow turnover and, consequently, the isotopic analyses of collagen and hydroxyl-

apatite provide lifetime averages of an animal's diet (49,100). Certain questions necessitate measuring short-term fluctuations (e.g., seasonal, weekly) in diet, as well as serial measurements on an individual. The most metabolically active tissues such as liver, blood, and brain, "turn over" (i.e., replace molecular components) faster than tissues such as bone (49,110). Other body components are laid down and remain biochemically unchanged (e.g., tooth and horn apatite, hair, feathers); tissues laid down by accretion that remain unchanged thereafter can be microsampled to give a time series of body isotope chemistry (58,88).

3. Different tissues may reflect the isotopic composition of different dietary constituents. This caveat is often ignored. Researchers often adopt a linear mixing model with the implicit assumption that the isotopic composition of a consumer's tissue is the arithmetic average of the isotopic compositions of its dietary constituents (87). This assumption is often untenable. The complications resulting from the differential allocation of nutrients to different tissues is best illustrated with the isotopic composition of carbon in tissue protein. The fate of carbon skeletons for omnivores has been modeled assuming two end-member states: "no mixing" and "complete mixing" of dietary carbon sources (59). If an animal catabolizes carbohydrates and lipids completely and uses dietary amino acids to synthesize protein ("no mixing"), the isotopic carbon composition of its body should resemble the isotopic value of dietary protein. In contrast, if carbon from dietary energy (i.e., lipid and carbohydrate) and protein sources are mixed in protein synthesis, the $\delta^{13}C$ value of animal protein should resemble bulk diet.

Two studies have elucidated some of the factors controlling the fates of carbon skeletons of different nutrients. Results from rats and mice fed artificial diets suggest that the degree of mixing depends on protein intake and relative nitrogen balance (3,101). The isotopic value of the protein of animals eating high-protein diets appears to resemble the isotopic value of dietary protein and not bulk diet (Fig. 1). Animals on low-protein diets, in contrast, apparently use carbon from non-protein components of the diet to synthesize new amino acid carbon backbones, because the $\delta^{13}C$ of their protein appears to more closely resemble bulk diet (3,101). Furthermore, the degree of mixing of dietary sources differs among tissues. The isotopic composition of collagen and muscle reflects dietary protein. In contrast, the isotopic composition of bone apatite more closely tracks that of bulk diet. Carbonate in apatite is a better predictor of the average isotopic composition of diet, perhaps because it is derived from circulating respiratory CO_2, which integrates the isotopic composition of the nutrient carbon used for energy metabolism (3,101).

A simple numerical example illustrates the dangers of

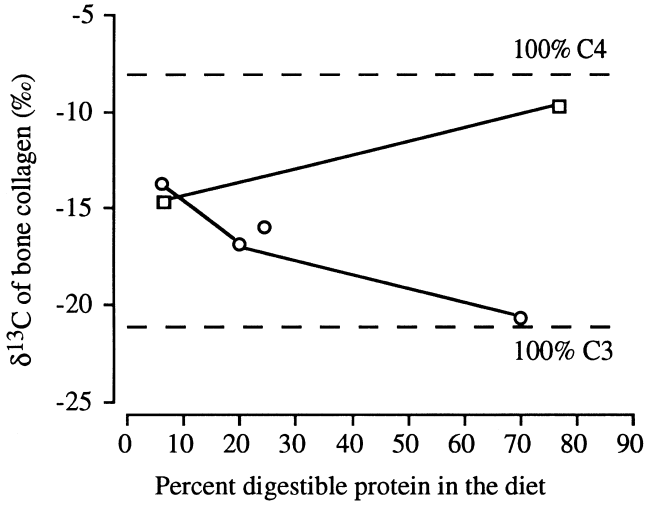

FIG. 1. Relationship between the percent digestible protein in the diet and the carbon isotopic composition of bone collagen. The carbon in bone collagen of lab rats fed high-protein diets is made almost entirely from dietary protein sources. Bone collagen from rats fed low-protein diets comes from carbon in both dietary carbohydrates and dietary proteins. Dashed lines are the bone collagen values of rats fed C3 or C4 only diets. Squares are bone collagen values for rats fed diets of C4 protein and C3 carbohydrates and circles are rats fed C3 protein and C4 carbohydrates (after 3).

isotopic routing for dietary reconstruction. The protein requirements of an active 70 kg human adult can be satisfied with only about 42 g/day (16). These 42 g contain approximately 756 kJ, which represent only about 6.3% of the total energy requirements of the individual (111). Assume that the protein source is isotopically distinct from the carbohydrate source [e.g., the human obtains protein from the meat of a C3 browser and carbohydrate from C4 maize, see (108)]. If all protein is directly routed into the replacement of lost protein, body protein will show a C3 isotopic signature even though only a minuscule fraction of the diet has a C3 origin. This example may seem extreme; on severely protein deficient diets the carbon skeletons of some amino acids have non-dietary protein origins. It must be remembered, however, that we still know little about the mechanisms and factors that influence the allocation of carbon skeletons in animals.

Even the isotopic composition of animals with apparently homogeneous diets, such as some herbivores, may strongly resemble dietary protein if animals mix two diets with contrasting protein contents [e.g., C3 plants contain more protein than C4 grasses; (11)]. The problems associated with varying contributions of different nutrients to specific tissues and tissue components are probably lessened in the analysis of the diets of ruminants. In ruminants, the isotopic composition of protein may

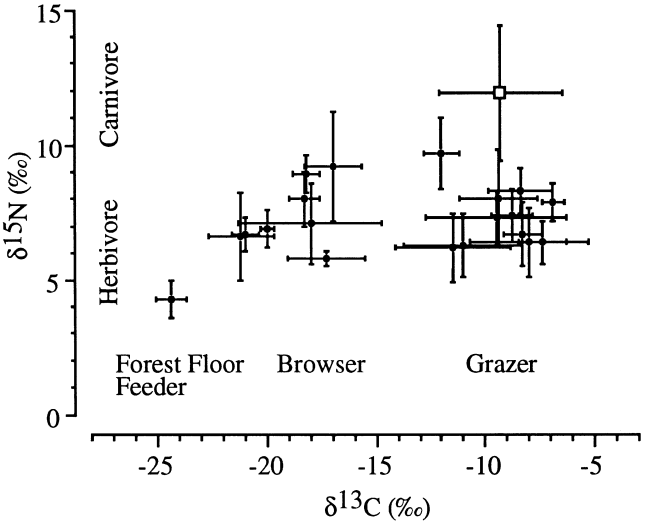

FIG. 2. Mean and standard deviations of $\delta^{13}C$ and $\delta^{15}N$ from skeletal collagen of east African mammals. Forest floor feeders, browsers and grazers are separated on the $\delta^{13}C$ axis and carnivores and herbivores are distinguishable on the $\delta^{15}N$ axis. The open square is the average value for 10 species of carnivores, and filled circles are average values of individual herbivore species (after 2).

accurately reflect bulk diet because gut symbionts produce most of the ruminant's protein from bulk diet (106). Urea recycling probably homogenizes the carbon skeletons from different dietary sources in body protein (65).

The use of stable isotopes has become a standard in the analysis of animal and human paleodiets (79,90,94). However, the effects of energy and protein balance on the fate of carbon skeletons in omnivores, carnivores, and perhaps in some herbivores, must be much better understood before we can fully interpret dietary construction using stable isotopes. The examples that follow illustrate the potential usefulness of isotopes in dietary reconstruction. The caveats outlined above must be kept in mind, however, when interpreting their results.

Although isotopic analysis may not always allow precise reconstruction of an animal's diet, it may allow discrimination among animals belonging to particular dietary guilds. The process of guild reconstruction is usually facilitated by the analysis of more than one element. A plethora of studies have investigated the relative importance of C3 vs C4/CAM plants in herbivore diets (57,60,81,84,90,104). An excellent example is the combined use of $\delta^{13}C$ and $\delta^{15}N$ values from bone collagen to characterize the distinctive feeding strategies of 43 species of African mammals (2). Carbon isotopes allowed discrimination between grazers and browsers, and between forest-floor and savanna grassland grazers (Fig. 2). Nitrogen isotopes allowed discrimination between carnivores and herbivores. Another example is a study of the diets of grasshoppers (*Arphia* sp.) from Wyoming grasslands (8). The grasshoppers consumed C3 plants in excess of their abundance at the site; the authors hypothesized that the grasshoppers prefer C3 plants because of their presumed higher nutritional content and digestibility (11). The higher digestibility of C3 plants complicates this analysis, however. Perhaps grasshoppers were not selectively feeding on C3 plants, but were merely preferentially assimilating the carbon of ingested C3 plants.

The relative importance of different food sources to secondary consumers (e.g., carnivores, insectivores) has been examined in a variety of studies (4,60,81,82,84,90,91,104). Carbon isotope analysis indicates that gulls (*Larus* spp.) have changed their diets over a short evolutionary time scale (46). *Larus* spp. from an approximately 2,000-year-old archaeological site depended almost exclusively on marine protein sources, whereas modern gulls (*Larus glaucescens* and *L. occidentalis*) consume ~30% terrestrial protein, much of which comes from human refuse. A recent study analyzed the diets of bears using both carbon and nitrogen isotopes (45). Brown bears (*Ursus arctos*) living on Chicagof and Admiralty Islands, Alaska, feed mostly on terrestrial plant matter during the early summer. Upon the arrival of migrating salmon, most brown bears eat less plant matter and more salmon, but a sub-population of the bears does not eat the seasonally abundant salmon.

The scale of dietary reconstruction can be enlarged to reconstruct the "diet" of the consumers of an entire ecosystem. Because of the astronomically increasing number of possible connections between members of an ecosystem as the number of members increases, only simple ecosystems have been analyzed. Isotopic analysis has revealed that in the Alaskan Arctic, terrestrial peat moss carbon provides a major fraction of the energy supplied to the coastal plain aquatic biota, compensating for the meager primary production of the ecosystem (89). Isotope analysis of food choice in a termite colony (*Macrotermes michaelseni*) traced carbon/energy flow from diet, to tended fungus gardens, to nonreproductive castes, and finally to reproductive castes (7). Isotopic analysis indicates that kelp is a major carbon source for nearshore consumers (21). Nearshore suspension feeders, detritivores, and vertebrate predators show a high percentage of kelp-derived carbon in their body tissues.

Animal Movement Patterns

Animal movement patterns can be difficult to trace, and both radio-telemetry and stable isotopes have shed light on movement patterns of many species. Under what conditions are stable isotopes a useful indicator of movement patterns in animals?

1. The geographic areas of interest must have distinct isotopic values. This requires an understanding of the elemental sources at each location (e.g., food, water, rock).
2. The best components for measuring movement patterns

are those laid down serially and not altered (e.g., baleen, nail/hooves/claws, horns, tooth enamel, hair, feathers). Furthermore, two body components can give values from temporally distinct periods. Analyzing tissues with high turnover (e.g., blood, or muscle protein) during growth should be avoided because the isotopic signature of interest may be diluted.

3. Researchers can increase the accuracy and specificity of the technique by combining analysis of several isotopes (12,58).

Stable isotopes can be extremely useful in tracing movement patterns in animals that are otherwise difficult to follow. By measuring stable carbon isotopes and radioactive carbon levels in baleen, one study traced and roughly dated the yearly migration patterns of bowhead whales [*Balaena mysticetus*; (88)]. The data contradict the conventional wisdom that bowhead whales feed during the summer in the eastern Beaufort sea and rely on lipid stores while overwintering in the Bering sea; the data indicates that the whales feed seasonally in the Chukchi, Bering and western Beaufort seas. Another study traced the migration of fish and shrimp in the Gulf of Mexico by measuring the signatures of carbon, nitrogen, and sulfur acquired by the tissues of these animals, while feeding in shallow and offshore feeding areas (28). Similarly, isotopes have been used to trace migration patterns of birds (12,51).

A combination of carbon, nitrogen, and strontium isotopes has been used to trace the changing movement patterns of elephants in response to changing vegetation conditions in Amboseli National Park, Kenya (58). Isotopic analysis of bone and molars have shown local migration between bushlands and the lake basin habitats in the park; however, most elephants in the park do not frequent the nearby Kilimanjaro forests. Although previous authors (105,107) have proposed using isotopic values to "fingerprint" source populations of ivory, variation in individual feeding and movement behavior complicates application of this method.

The applications of stable isotopes to trace diets and migration have recently been combined in studies of the effects of salmon spawning on freshwater ecosystems (5,56). Large marine carnivores have high $\delta^{13}C$ and $\delta^{15}N$ values because they are feeding at the top of relatively long trophic webs (30,69,81). In contrast, freshwater foodwebs are often shorter, and the carbon and nitrogen sources to these systems typically have lower isotope values than marine primary production (30,81). Consequently, the decomposition of salmon carcasses following spawning "pumps" a large amount of ^{15}N- and ^{13}C-enriched marine organic matter into freshwater ecosystems, which cascades up the trophic web to freshwater heterotrophs and autotrophs. In western Washington, salmon spawn in the fall, and carcasses are present in streams throughout the winter and spring (5). Winter $\delta^{15}N$ and $\delta^{13}C$ values are 3 to 4 per mil higher at all trophic levels. Furthermore, the growth rates of young salmon are higher in the winter, when carcasses are present in streams, than in warmer summer months. Estimates from isotope mass balance indicate that from 10 to 40% of the carbon and nitrogen in most heterotrophs is derived from marine sources (5). Marine nitrogen from rotting carcasses even contributes 17.5% of the nitrogen in riparian vegetation along the streams. These studies demonstrate the great importance of spawning to primary and secondary production in freshwater systems, and they imply that the dramatic decrease in spawning salmon in western streams may be self-perpetuating.

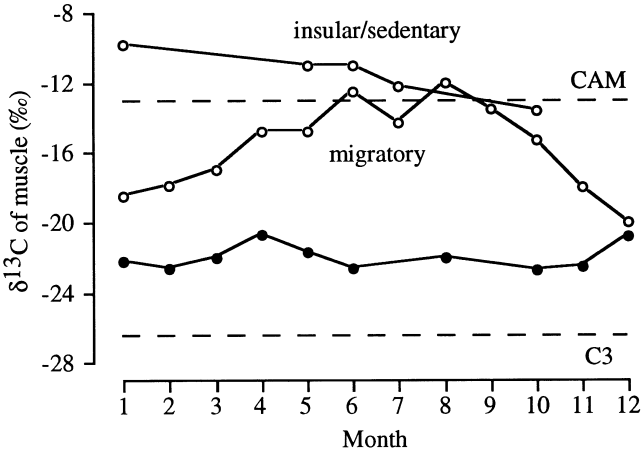

FIG. 3. Temporal variation in the carbon isotope composition of the muscle of North American flower-visiting bats. Open circles represent mean monthly values for *Leptonycteris curasoae*. The sedentary population feeds exclusively on the nectar and pollen of CAM plants (Agavaceae and Cactaceae). The migratory population feeds on CAM plants during the summer in its northern range and on a mixture of CAM and C3 plants in its wintering range. Closed circles represent mean monthly values for a sedentary population of *Glossophaga soricina* which feeds primarily on C3 plants. The mean isotopic composition of several species of C3 and CAM food plants is shown as a dashed line (after 26).

Diet changes and movement patterns of North American flower-visiting bats have been investigated using isotopic analysis (26). These bats feed on the pollen and nectar of either CAM (cacti and agave) or C3 plants, and different populations show contrasting migratory patterns. The isotopic composition of the tissues of migratory populations showed typical CAM values during the summer when bats inhabit arid and semi-arid areas in Northwestern Mexico and feed on the pollen and nectar of Agavaceae and Cactaceae. During migration and in their wintering grounds in Southern México migratory bats feed on a mixture of C3 and CAM plants. The isotopic composition of these migratory bats changed during the year and tracked the isotopic composition of the tissues of the plants that they consume (Fig. 3). In contrast, the tissues of sedentary bats show little annual variation in isotopic composition. The tissues of a

sedentary flower-visiting bat population inhabiting arid Baja California showed consistent CAM values throughout the year and the tissues of sedentary bats inhabiting tropical habitats show relatively constant C3 isotopic compositions (Fig. 3).

DIRECTIONS FOR FUTURE RESEARCH

The role of physiological ecologists in research involving naturally occurring stable isotopes is twofold. On the one hand, physiological ecologists can utilize the method to answer ecological and physiological questions. On the other hand, they can shore up the theoretical and experimental foundation for the application of stable isotopes in other areas. As mentioned above, stable isotopes are widely used in archaeology, paleontology, and ecology. The assumptions that underlie the use of stable isotopes, however, have not been fully examined. In this final section, we suggest a few areas where the use of naturally occurring stable isotopes can be applied. We also outline the areas where physiological research can contribute to clarify the limitations to the use of stable isotopes in biology.

Protein Balance

How well an animal is meeting its protein requirements can be estimated by measuring an animal's protein balance [the difference between nitrogen intake and protein losses; (83)]. Stable isotopes of nitrogen may provide an indicator of protein balance that can be used on free-ranging animals. As is the case for animals in nitrogen balance, protein limited and/or starving animals will preferentially excrete nitrogenous waste enriched in ^{14}N (69,96). Consequently, the isotopic composition of tissues remaining in the body should become progressively enriched in ^{15}N. A study of Ross' geese (*Chen rossii*) provides one of the few attempts to calibrate the ^{15}N-enrichment during food deprivation (47). Female Ross' geese lose significant body mass while incubating eggs in the Arctic. Concurrent with this mass loss is a significant ^{15}N-enrichment in liver and muscle tissue (Fig. 4). The only experimental studies of ^{15}N-enrichment under nutritional limitation, conducted on quail (*Coturnix japonica*) and crows (*Corvus brachyrhynchos*), showed similar results (47,50).

Although this method shows considerable promise as a diagnostic tool for nitrogen balance, many questions must be answered before its usefulness and reliability can be assessed. The demonstration of ^{15}N-enrichment during nitrogen limitation must be confirmed experimentally and in controlled free-range settings on animals other than birds, including ruminant and non-ruminant mammalian herbivores, and carnivorous, omnivorous, and insectivorous vertebrates. Furthermore, the rate at which an animals's tissue becomes enriched in ^{15}N during prolonged food and protein deprivation probably depends on several factors, including the rate at which protein is mobilized, and whether amino

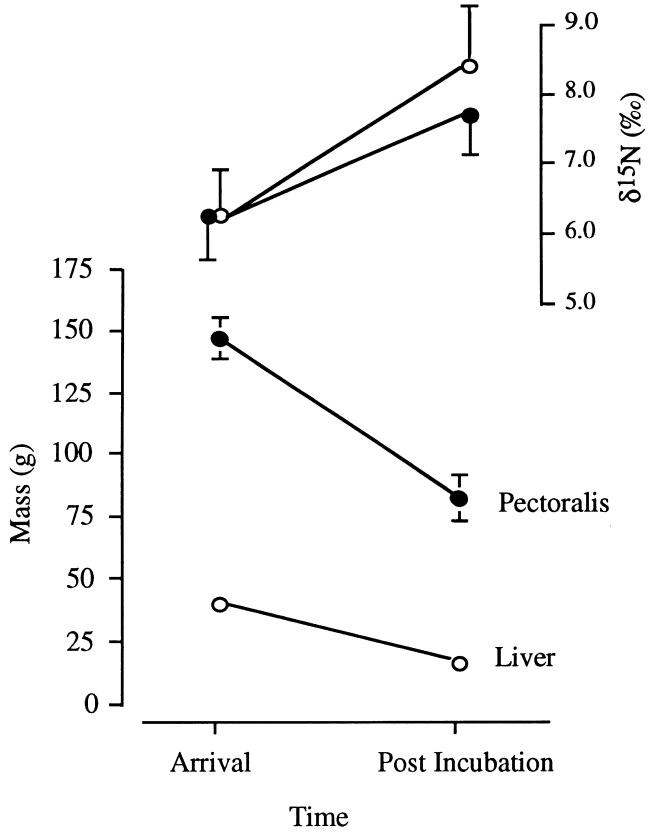

FIG. 4. Correlated changes in mass (±*SD*) and ^{15}N enrichment (±*SD*) in liver and pectoral muscle of female Ross' Geese (*Chen rosii*). Females fast during egg-laying and incubation and hence lose tissue mass. Tissue mass loss is accompanied by a significant increase in $\delta^{15}N$ (after 47).

acids are deaminated *in situ* or transferred to other tissues. The protein constituents of different tissues are mobilized at contrasting rates (35,73). For example, small passerine birds appear to deplete protein in flight muscle and liver during overnight fasts, but not to deplete protein in other tissues (98).

It is tempting to hypothesize that tissues that exhibit high rates of protein depletion will also show high rates of isotopic enrichment. The rate of protein depletion in a tissue, however, may not be correlated with its rate of ^{15}N-enrichment. The amino acids released from degraded proteins can be deaminated and oxidized directly by a tissue, deaminated and utilized for energy or gluconeogenesis in the liver, and/or used in the synthesis of other proteins (34). Tissues become enriched in ^{15}N by two of these processes: deamination *in situ* and synthesis of protein with the ^{15}N-enriched amino acids. Thus, ^{15}N-enrichment in a tissue depends on the intricacies of protein catabolism.

The physiological events accompanying starvation at the whole organism level are relatively well studied (10). However, data on the events at the tissue level that are likely to influence ^{15}N enrichment and that permit making predic-

tions on the relative rates of enrichment of different tissues are scanty. Physiologists can contribute to isotopic ecology by providing a quantitative description of the fate of amino acids in different tissues, by "calibrating" the isotopic enrichment of different tissues during starvation, and by illuminating the mechanisms that give rise to this enrichment (67). To our knowledge, no studies have calibrated the rate at which different tissues become enriched in ^{15}N as a function of their rates of mass loss, protein catabolism, and deamination. These studies are essential if stable isotopes can be used to assess body condition in both extinct and extant animals.

Nutrient Allocation

A number of studies have emphasized the problems that isotopic routing poses to the interpretation of isotopic data in diet reconstruction (3,59,79,90,94,101). However, differential routing of carbon skeletons in animals fed low versus high protein diets is exciting from a physiological standpoint because it may provide clues about how animals allocate nutrients to their tissues. So far, the pattern appears simple (Fig. 1); animals on high protein diets can rely almost entirely on dietary amino acids to synthesize protein carbon skeletons, but animals on low protein diets synthesize the carbon skeletons of amino acids from a combination of protein and carbohydrate carbon sources (3,101). The fate of dietary components was traced by feeding animals diets with components of contrasting isotopic values (e.g., C4 carbohydrates and C3 protein). The isotopic composition of different tissues and body components reflects how dietary nutrients are allocated to different tissues.

This simple scheme of the routing of carbon skeletons into protein is considerably more complex than it appears. We still know little about the mechanisms that lead to routing and how the magnitude of fluxes of carbon skeletons among dietary pools and the pools in different tissues vary. Understanding these mechanisms is quite important if we are to interpret isotopic data correctly, for example, to reconstruct the relative importance of protein versus carbohydrate sources in ancient diets.

The role of urea recycling in isotopic routing is a case in point. Urea recycling seems to take place in the vast majority of herbivores and in many omnivores, including humans (53,61). In this process, urea resulting from protein catabolism penetrates the gut and is hydrolyzed by gut bacteria (112). The resulting ammonia enters the portal circulation and can be utilized in the synthesis of non-essential amino acids. *De novo* amino acid production from recycled ammonia occurs via synthesis of glutamate and glutamine and the subsequent transfer of the amine groups in these compounds to α-keto acids (38). Because many α-keto acids can be obtained from the metabolism of carbohydrates, urea recycling can lead to significant routing of carbon from carbohydrate into protein.

Urea recycling has been implicated as a mechanism for nitrogen conservation (95). Hence, it can be predicted that the importance of urea recycling in nitrogen balance will increase in animals feeding on low protein diets (102). The magnitude of the effect of urea recycling on the mixing of carbohydrate and protein carbon sources in tissues, and hence, on isotopic routing, is still unclear, however. Urea recycling provides just one mechanism by which carbon skeletons from carbohydrates can be routed into protein. Other mechanisms are described elsewhere (68).

Physiologists can make a major contribution to isotopic ecology by disentangling the various effects on nutrient routing and allocation. Specifically, we can provide phenomenological accounts of the role of nitrogen balance on carbon mixing in animals. For example, animals with different dependence on dietary protein (e.g., ruminants vs carnivores) will probably route protein differently, but no such comparative studies have been undertaken. In addition, energy allocation in an animal may differ depending on season (e.g., before migration or hibernation) and these differences could affect isotopic values.

Tissue Turnover

The components of tissues (lipids, carbohydrates, and protein) have characteristic turnover rates that depend on their rates of acquisition, synthesis, and catabolism. Protein turnover, for example, depends on metabolic regulation, amino acid mobilization, elimination of non-functioning polypeptides and thermoregulation (6,43).

Purified diets with contrasting stable isotope signatures can be used to measure an animal's whole body and organ turnover of materials. After a diet switch, the change in isotopic composition of the tissue or body component depends on how fast these constituents are turned over (100). This approach has several advantages over more traditional radio-labeling studies. First, because the entire material is "marked" (has the same isotopic composition value), there are no problems of separating the dynamics of the marker and the actual material (110). Second, because stable isotopes do not pose health risks, they can be used on human subjects and can be fed in large doses to animal subjects. More than one element can be used and hence the turnover of parts of a material can be traced separately (e.g., the carbon skeleton vs the nitrogen in proteins). Similarly, turnover rates of tissue constituents can be estimated in free ranging animals that switch diets from isotopically distinct food sources (e.g., from C4 to C3). However, it is essential to characterize the composition of the body component prior to the switch, and the method assumes that the composition of the component was not undergoing change due to a natural change in diet prior to capture.

Several laboratory experiments have measured the turnover rate of animal tissues using stable isotopes (21,48,49, 50,100). Laboratory animals are fed an isotopically homoge-

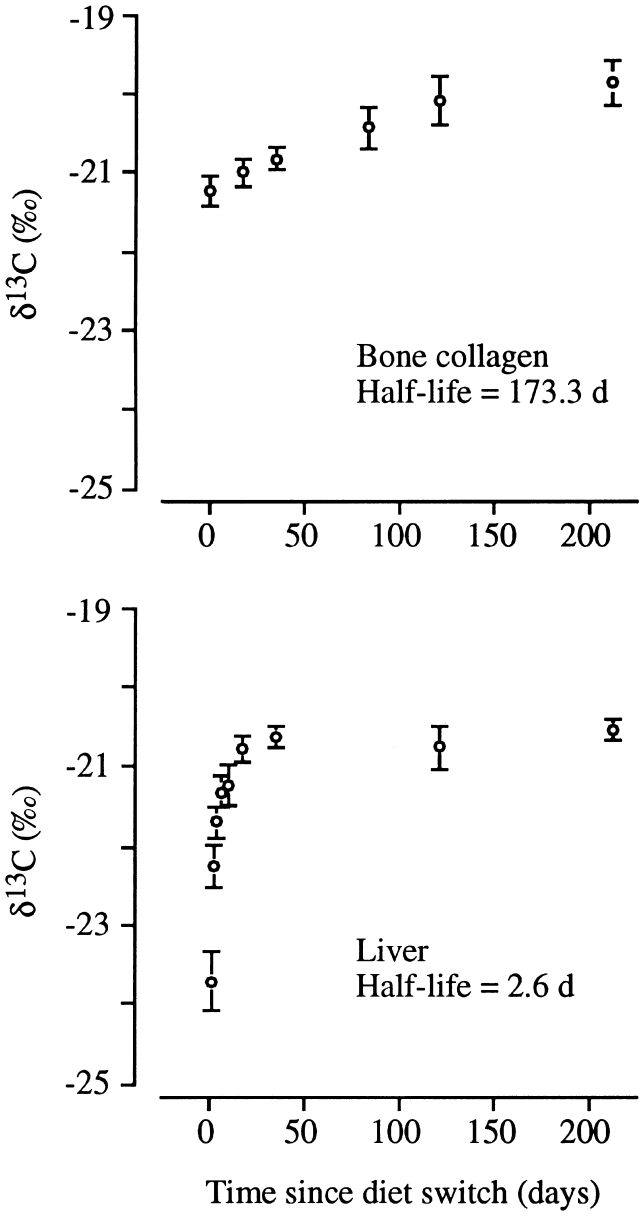

FIG. 5. Carbon isotope composition of liver and bone collagen of quail (*Coturnix japonica*). At day zero the quail were changed from a wheat-based diet to a corn-based diet and the curves show the turnover of carbon in the two tissues. Each point represents the mean (±*SD*) of three individuals (after 49).

neous diet and switched to a different isotopically distinct diet. As an example, quail (*Coturnix japonica*) were raised from hatching until adult size on a wheat-based (C3) diet, and upon reaching adult size quail were switched to a corn-based diet [C4; (49)]. The study measured the half-life of carbon in whole blood (11.4 d), pectoralis muscle (12.4 d), liver (2.6 d), and bone collagen (173.3 d) by measuring the change in the isotopic composition of each tissue (Fig. 5). Control birds ate a wheat-based diet throughout the experiment and the $\delta^{13}C$ values of their tissue did not change. A similar experiment (21) measured the turnover of both carbon and nitrogen molecules in American black bears (*Ursus americanus*) and found the half-lives of blood plasma and red blood cells to be ~4 and ~28 days, respectively.

CONCLUSIONS

The physical and biological processes that lead to variations in the natural abundance of stable isotopes provide a valuable tool for researchers interested in measuring the flow of materials and energy both within and among organisms. Plant physiologists, ecosystem ecologists, and paleontologists have used this technique with profit. The method holds promise for animal physiological ecologists because it can help to elucidate questions of energy flow, movement patterns, protein balance and body condition, and turnover of tissue constituents. Although the use of naturally occurring stable isotope variations is promising as a tool, it is not without challenges. Stable isotope techniques cannot be applied *carte blanche* to all animals and/or ecological systems. Their use requires knowledge of fractionation effects that we are just beginning to understand. Currently, most animal physiological ecologists use radiogenic isotopes in their research. We predict that in the near future the use of stable isotopes will be as common an ingredient in the animal physiologist's toolbox as their man-made radiogenic counterparts.

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