PERSPECTIVES: BIOGEOCHEMISTRY

You Are What You Eat

Matthew J. Kohn

The title of this commentary says it all, at least isotopically. First espoused 20 years ago for the stable-isotope ratio $^{13}C/^{12}C$ (*1*), this maxim now enjoys worldwide acceptance among biogeochemists. Numerous experimental and field studies document the strong correlation between stable-isotope ratios (such as $^{13}C/^{12}C$, but also D/H, $^{15}N/^{14}N$, $^{18}O/^{16}O$, and $^{34}S/^{32}S$) in vertebrates and that of their diets. These studies also elucidate how environment influences isotope compositions. Together with recent analytical advances, stable-isotope biogeochemistry is enjoying a dramatic expansion. The sharp increase in number and scope of studies and the promise of many more to come have prompted several international meetings on the use of stable-isotope compositions of animals, including symposia in Utah and Saskatchewan last year (*2*).

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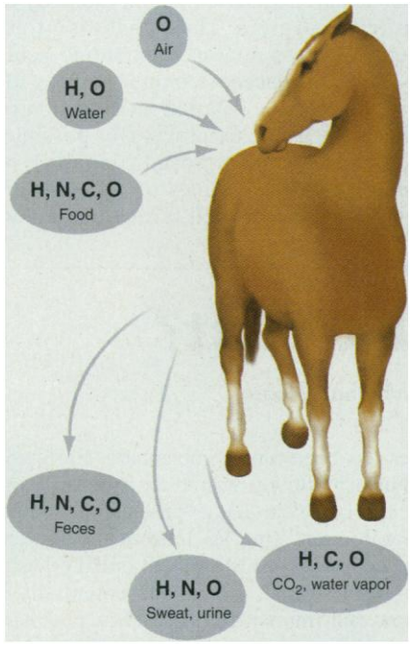
Various meteorological, geochemical, and biological processes cause foods in different environments (for example, marine versus terrestrial, nearshore versus open ocean, high-latitude versus low-latitude) to have characteristically different stable-isotope compositions. Because the isotope composition of an animal reflects what it consumes, these data tell us about an animal's occupancy of different environments. This in turn allows investigation of a wide range of ecological issues, including resource use, migration patterns, and so on. For example, foods at low latitudes generally have higher D/H, $^{13}C/^{12}C$, and $^{18}O/^{16}O$ than foods at high latitudes, because of differences in rainwater composition and in the proportions of plants that use C_4 versus C_3 photosynthetic pathways (C_4 plants have higher $^{13}C/^{12}C$ than C_3 plants). For animals that cannot be directly monitored, stable-isotope analysis of feathers, teeth, and other body parts provides new insights into migration distances and rates, seasonal variations in food sources, or changes in diet as an animal matures.

In concert with such modern ecological studies, an interest in past climates and processes is causing geologists to metamorphose the original paradigm. Applying the revised maxim "You were what you ate," they are now using compositions of ancient versus modern body parts of vertebrates to characterize paleodiets and paleoenvironments (*3*). For example, carbon isotope compositions of fossil herbivore bones between ~20 million years ago and the present reveal a rapid expansion of C_4 foods at ~7 million years ago, likely commensurate with a global decrease in atmospheric CO_2 concentrations at that time (*4*). Other geologists are studying evolutionary processes, because the physical changes evident in the fossil record often reflect adaptation of the evolving species to new habitats: habitats that likely have distinct isotope compositions. And anthropologists continue to use stable isotopes to investigate how changes in diet are related to social and physical change (*5*).



Isotopic diet. Generalized isotope mass balance of an animal. Food dominates over water and air as an input source of stable isotopes. Because food (and water) compositions depend on environment, animal isotope compositions reflect habitat.

As biogeochemists expand their investigations to older materials, however, preservation of original isotope compositions becomes increasingly problematic. The continuing search for the ultimate biological material reveals that $^{18}O/^{16}O$ in the PO_4 component of tooth enamel is extraordinarily well preserved, even in ancient samples. Biogenic phosphates are compositionally complex, and their chemical components do not all withstand isotopic alteration after burial. However, for enamel PO_4, the extremely strong P–O bond, the large crystal size of the mineral in teeth, and the nonporous structure of enamel all resist isotopic resetting through exchange and mineral recrystallization. Indeed, most fossil enamel retains an exquisite microstructure produced when the animal precipitated its tooth and is the material of choice for terrestrial studies.

Until recently, oxygen isotope compositions were difficult to relate quantitatively to the environment. Geochemists have long recognized that phosphate $^{18}O/^{16}O$ correlates with the $^{18}O/^{16}O$ of precipitation and that precipitation $^{18}O/^{16}O$ broadly correlates with air temperature (*6*). But the relation between animal and precipitation $^{18}O/^{16}O$ varies among genera, and other factors such as relative humidity also play a role. Some complications occur because oxygen is contained in every solid, liquid, and gas we excrete: Different genera have different proportions of inputs and outputs, and isotope compositions differ among input and output components. Other complications occur because plant $^{18}O/^{16}O$ responds to both water $^{18}O/^{16}O$ and humidity. Nonetheless, a broad spectrum of experiments and field studies, summarized and addressed in theoretical models (*7*), has clarified our understanding of how environment impacts isotope compositions. These refinements clearly identify food as the main control on compositions and more importantly now allow environmental signals to be decoded quantitatively from isotope measurements.

The oxygen isotope database for modern teeth is expanding concurrently with revised models, and these new data are revealing fascinating complexities that can in turn be used to refine investigations of past climates. Most important for tooth research is the recent discovery that enamel compositions provide a robust record of seasonal climate variations (*8*). Tooth enamel has a structure analogous to a carpet: Mineral fibers cluster into bundles that are highly elongate perpendicular to the surface of the tooth and that form an extremely compact, intergrown network. In a tooth, enamel mineralization first occurs at the crown and then sweeps toward the base, taking as much as a year to complete in the tooth of a large animal such as a horse. Each incre-

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ment of enamel records the isotopic composition of the animal at the time of precipitation, and because enamel is not replaced, the record is permanent. Seasonal, climatically driven changes in food $^{18}O/^{16}O$ cause the $^{18}O/^{16}O$ of an animal to vary during the year, and these seasonal changes are recorded in a crown-to-root isotope variation of the enamel. Thus, the oxygen isotope zoning of a tooth provides a record of seasonal climate variations at the time the animal lived.

Seasonal climate records are important for a variety of reasons. They are fundamental observations that must be explained by global climate models, which predict the worldwide impact of factors such as increasing greenhouse gas concentrations or sea-surface temperatures. Seasonal records can also address whether different climates in the past were associated with different seasonalities and whether global warming will likely increase or decrease seasonality. Increased seasonality may even have helped drive our own evolution in East Africa (*9*). Finally, teeth provide climate records from vitally important continental interiors. Continents respond to global change differently from oceans, but quantitative continental climate records are more sparse and difficult to obtain.

In some ways, the future of stable-isotope biogeochemistry remains obscure. There are so many recent revelations and developments that today's liberal speculations will seem conservative tomorrow. Nonetheless, one major development that is directing current research is the ability to rapidly separate and analyze specific compounds from a single sample (*10*). Different dietary compounds are processed within the body to different degrees. Thus, analysis of essential versus nonessential amino acids or nonproteinaceous compounds may allow much more detailed investigation of diet than has been previously possible. Ultimately, our improved capability of identifying the sources of different dietary components will further our understanding of resource use, animal behavior, and climate.

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PERSPECTIVES: HIV VACCINES

Magic of the Occult?

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How do we know when an experimental vaccine is working? In the case of human immunodeficiency virus type 1 (HIV-1), success has usually been gauged by the ability of candidate immunogens to generate measurable immune responses in human volunteers and animal models. The two responses to watch for have been virus-specific $CD8^+$ cytotoxic T lymphocytes (CTLs), which attack and destroy infected cells, and neutralizing antibodies, which bind to the virus and prevent infection of new cells. For HIV-1, a combination of both responses is likely to be more effective than either one alone. Recombinant poxviruses containing HIV-1 proteins have generated low numbers of HIV-specific $CD8^+$ CTLs in a subset of volunteers, and these CTLs can sometimes cross-react with genetically diverse HIV-1 strains—a potentially useful property (*1*). These prototype vaccines can and likely will be improved to generate more potent and consistent CTL responses. The induction of neutralizing antibodies has been more problematic. Early enthusiasm slowly waned as it became increasingly clear that a "principal neutralizing domain" (the V3 loop of the surface glycoprotein gp120) present on HIV-1 laboratory strains, which was potentially a powerful immunogen (*2*), was a poor target on virus recently isolated from patients (or "primary isolates") (*3*). These viral strains closely reflect the viruses a vaccine will encounter in the real world, but because the V3 loop is a poor target in these strains, antibodies generated by the current repertoire of candidate HIV-1 vaccines do not neutralize them efficiently (*4*). This failure is principally because the functional, oligomeric envelope glycoprotein complex of these strains has evolved structural features that reduce the exposure of antibody epitopes (*5*).

Because of this impasse, designing an immunogen that can generate an antibody response able to neutralize a broad spectrum of primary isolates has been a major goal. Now, on page 357 of this issue La-Casse *et al.* (*6*) may have identified a solution to this problem that could eventually be exploited for vaccine development. They proposed that epitopes with superior immunogenicity might be exposed or created as HIV-1 begins to fuse with cell membranes. Fusion complexes were, therefore, tested for their ability to induce neutralizing antibodies in mice. The complexes were created by taking simian fibroblasts engineered to express functional envelope glycoproteins from the primary HIV-1 isolate 168P (COS-Env cells) and mixing them with human neuroblastoma cells stably expressing the CD4 and CCR5 fusion receptors for HIV-1 (U87-CD4-CCR5 cells) (see the figure). The two cell types gradually fuse with one another because the HIV-1 envelope glycoproteins on the COS cells bind the fusion receptors on the U87 cells. During the fusion process, the conformations of the envelope glycoproteins change, leading to the exposure of previously occult epitopes or the de novo formation of neo-epitopes. Fixation of the fusing cells, and hence the fusing envelope-receptor complexes, with mild concentrations of formalin permit the immunogenicity of these epitopes to be evaluated. Immunizations were performed in mice engineered to be transgenic for human CD4 and CCR5 to reduce the possibility that antibodies would be raised to the human CD4 and CCR5 proteins and interfere with the interpretation of the neutralizing antibody assays performed on the immune sera.

Perhaps to the surprise of all involved, this ambitious experiment worked. The mouse sera, and antibodies purified from them, inhibited the infectivity of an impressive array of diverse HIV-1 primary isolates, including viruses from multiple genetic subtypes. Furthermore, the potency of

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