Tracing Food Webs with Stable Hydrogen Isotopes

Abstract. *The hydrogen isotopic content of an animal's food, not water, determines that animal's hydrogen isotopic content. Liver and muscle tissue from mice reared on a diet such that the ratio of deuterium to hydrogen (D/H) of their food and water was kept constant, have the same average D/H ratio as the food source. In a simple, natural population of snails and their possible algal diets,* Littorina obtusata *(northern Atlantic intertidal snails that feed almost exclusively on the brown alga* Fucus vesiculosus*) has the same D/H ratio as* Fucus vesiculosis *and not that of the other algae available to the snails.*

The determination of food webs can be used to predict biochemical energy fluxes in ecosystems where a number of different organisms are interacting. Tracing natural food webs, however, is a time-consuming task that is subject to many observational biases. For example, the foods in the stomachs of many animals are partially digested and only sometimes are identifiable by the most experienced workers. A relatively objective method for examining the abundance of plants or animals of different species in an animal's diet is based on the distinctive carbon isotope ratios of plants and the fact that there is little isotope discrimination between an animal and its food source (*1*). Variations in the ratios of stable hydrogen isotopes may aid in tracing marine and terrestrial food webs and may make it possible to differentiate between species in the diet that have similar carbon isotope ratios.

The organically bonded hydrogen of plant tissues is derived from the surrounding water and has a reproducibly lower concentration of deuterium than the water (*2–4*). Plants collected from several ecosystems have characteristic hydrogen isotope fractionations of −90 to −110 per mil, but notable exceptions, such as certain micro- and macroalgae (for example, *Ulva lactuca* and blue-green algae), exist. Furthermore, as the hydrogen isotope ratios of environmental waters vary with salinity, latitude, and altitude, the organically bonded hydrogen in plants of the same species growing in different localities will reflect the differences in the hydrogen isotopic content of the environmental water (*3*). Because of variations in the hydrogen isotope ratios of plants, due to either metabolism or environmental influence, the hydrogen isotopic content of organically bonded hydrogen in an animal's tissue may indicate the presence of a particular plant in its diet or be useful in the investigation of environmental or migratory phenomena if the relationship between the hydrogen isotopes in an animal and its food source is known.

The hydrogen in animals is in the form of either water or organically bonded hydrogen. Most plants and animals are 80 to 90 percent water; this water is derived from either water ingested by the organisms (in the case of mammals) or water taken up from the environment by diffusion (in the case of plants and aquatic organisms). In this study, we were able to define the relationship between the organically bonded hydrogen isotopic content of an animal and its food source by studying laboratory-reared mice. We also give an example of the relationship between a simple, natural population consisting of snails and their possible algal food sources. The diets of both the snails and the mice had a ratio of hydrogen from the food source (percent by weight) to hydrogen from the water supply (percent by weight) of less than 1 and closer to 0.2.

Strain C3H mice were obtained as weanlings (9.5 to 11 g) from the Flow Laboratories, Dublin, Virginia, and were grown for 1 to 2 months by N. Zeller, University of Maryland, Baltimore, until

Table 1. Stable hydrogen isotope ratios of Boothbay Harbor samples.

Classification and species	δD (per mil)			Average δD (per mil)
	Site 1*	Site 2	Site 3	
Plants: Algae				
Ulva lactuca	−180	−166		−173
Enteromorpha clathrata			−174	−174
Fucus vesiculosus	−116	−102	−116	−111
Chondrus crispus	− 90	−103	− 84	− 92
Animals: Mollusks				
Littorina obtusata	−106 (I)†	−116 (C)†	−111 (C)	
Littorina obtusata	−119 (C)	−106 (C)	−113 (C)	
Littorina obtusata	−101 (I)	−115 (I)		
Littorina obtusata	−109 (I)	−107 (I)		
Average δD (site)	−109	−111	−112	−111

*The δD of water at sites 1, 2, and 3, respectively, are +12, +2, and 0. The δD of Atlantic Ocean water at this site is +10. †I, individual; C, composite.

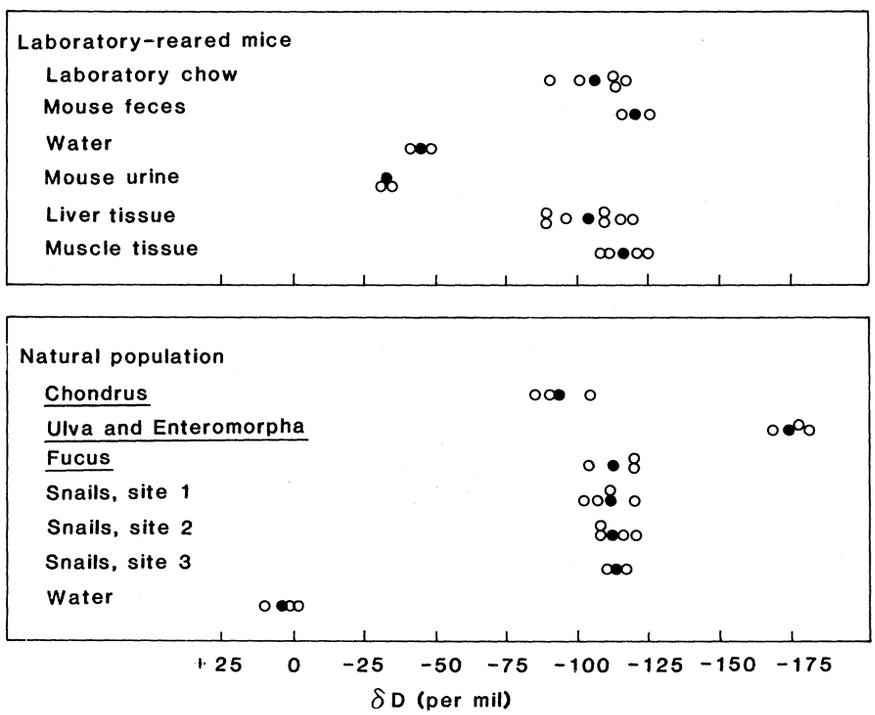

Fig. 1. Hydrogen isotope ratios of animals and their diets. Open symbols indicate individual values. Closed symbols indicate the average of individuals.

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they were killed. At this time, they weighed between 20 and 25 g. The mice were given a diet consisting of Purina laboratory chow and unlimited amounts of tap water. Both the food and water sources, the only sources of hydrogen, were characterized isotopically. Liver and muscle tissues from individual animals were dissected, frozen, dried, and analyzed.

A single species of snails and four species of algae were collected from three different tidal pools in Boothbay Harbor, Maine, by Drs. Wendy Harrison and Richard Wendlandt. The tidal pools were located at midtide level in a small harbor approximately 100 to 150 m from each other. The algal species were *Chondrus crispus* (Rhodophycophyta), *Fucus vesiculosus* (Phaeophycophyta), *Ulva lactuca* (Chlorophycophyta), and *Enteromorpha clathrata* (Chlorophycophyta). These algae were the dominant forms at this tidal level. The snails were of the species *Littorina obtusata* (*5*) and were collected attached to *Fucus* or browsing on the rocks beneath this algal species. Water samples were also collected from these pools and the Atlantic Ocean. The snails and the algae were kept frozen at −70°C for 2 months. The snail tissue was then separated from the shell. After freeze-drying, the samples were placed in a vacuum oven at 60°C over P_2O_5.

Six to ten milligrams of the dried sample were combusted in a platinum boat at 750°C in an atmosphere of oxygen. The water from the combustion was trapped with liquid nitrogen, converted to H_2, and analyzed with an isotope-ratio mass spectrometer (Nuclide Corporation model RMS-3-60) (*4*). The results are reported in terms of δD (per mil), which is defined as

$$\delta D = \left[\frac{(D/H)_{sample}}{(D/H)_{standard}} - 1\right]10^3$$

The hydrogen isotope standard was standard mean ocean water. This system for measuring hydrogen isotopes is routinely used on a variety of organic matter and typically yields results from triplicate analyses of these samples with a standard deviation of ± 5 per mil.

The hydrogen isotopic content of laboratory-reared mice (Fig. 1) is dependent on the isotopic content of their food source. The δD of mouse feces is related directly to that of the laboratory chow; the δD of urine is related to that of the available water. The small variation in δD among individuals, either mice or snails, corresponds with a similar variation in the δD of the food source and indicates that individual animals regulate hydrogen metabolism to a similar extent as the whole population.

The results of the hydrogen isotope analyses for ten samples of *L. obtusata* and ten samples of algae of four different species are given in Fig. 1 and Table 1. The average δD for the snails from sites 1, 2, and 3 are the same, as indication that they have the same diet. Furthermore, 80 percent of the individual δD values are within ± 5 of the average. Of the four species of algae, only for *F. vesiculosus* does the δD show any obvious relation to the δD of the snails. The δD of *Fucus* ranges from −102 to −116, or an average of −111, which compares with the δD of −111 for the snails at the three sites.

A combination of *Chondrus* (δD = −92) and *Ulva* (δD = −173) in the snails' diet could account for the δD of the snails. *Littorina obtusata*, however, has been observed in nature and also in controlled laboratory experiments. Not only do these snails live and breed on *Fucus*, but they also consume *Fucus* as the primary dietary source (*6*).

The δD of the food source, not the water, determines for the most part the δD of the organically bonded hydrogen in animals. This observation is supported by the fact that the δD of the snails and their food source (*Fucus*) are similar in a natural setting, even though the ambient water is more enriched in deuterium than the water in the laboratory study (*7*). Although there is greater variation in the δD of the diet and of the animal tissue than is seen in $\delta^{13}C$ measurements, the relationship that "you are what you eat" applies to hydrogen isotopes. In these simple cases, the conclusion from hydrogen isotope measurements is in agreement with direct observation.

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References and Notes

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7. The primary hydrogen isotope fractionation in living organisms occurs during photosynthesis in plants (*4*). The δD of algae that have been grown heterotrophically in the dark with either glucose or acetate is dependent on the δD of the organic food source rather than on the δD of the water.
8. We thank T. C. Hoering for invaluable assistance and discussions.

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Cell Lineage Analysis by Intracellular Injection of Fluorescent Tracers

Abstract. *Cell lineages during development of the leech are revealed by injection of a fluorescent peptide, rhodamine-D-peptide, into identified embryonic cells. Use of this peptide together with a nuclear stain showed a stereotypic cleavage pattern of stem cells and their progeny. Combined injection of rhodamine-D-peptide and pronase demonstrated the arrest of stem cell production in the pronase-injected teloblast.*

We reported previously that injection of horseradish peroxidase (HRP) as a tracer enzyme into identified cells of early embryos makes possible the determination of cell lineages during embryonic development (*1*, *2*). However, because the histochemical HRP reaction product is opaque, this method is unsuitable for experiments in which the mitotic state of tracer-labeled cells is to be examined in whole mount with nuclear staining. In addition, this method cannot be used to test the effectiveness of intracellular pronase injection as a means of ablating embryonic cells (*3*) because HRP is sensitive to proteolytic digestion. Fluorescent dye tracers would overcome both these limitations, but such small molecules (molecular weight on the order of 500) cannot be used directly as cell lineage tracers because upon injection they diffuse throughout the entire embryo (*1*), presumably via intercellular gap junctions (*4*). A fluorescent dye could be confined to the injected cell and its lineal descendants if attached to a larger carrier molecule, since it has been reported that the molecular weight limit for the permeation of insect salivary gland gap junctions by oligopeptide-fluorescent dye complexes is between 1200 and 1900 (*4*). To be suitable for cell lineage tracing, the carrier molecule should be of an appropriate size, have chemical sites to which the dye can be coupled, be reactive with histological fixatives, and

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