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Biogeochemistry of the Stable Isotopes of Hydrogen and Carbon in Salt Marsh Biota[1]

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ABSTRACT

Deuterium to hydrogen ratios of 14 plant species from a salt marsh and lagoon were 55‰ depleted in deuterium relative to the environmental water. Carbon tetrachloride-extractable material from these plants was another 92‰ depleted in deuterium. This gave a fractionation factor from water to CCl_4 extract of 1.147. This over-all fractionation was remarkably constant for all species analyzed. Plants also discriminate against ^{13}C, particularly in the lipid fraction. Data suggest that different mechanisms for carbon fixation result in different fractionations of the carbon isotopes. Herbivore tissues reflected the isotopic ratios of plants ingested. Apparently different metabolic processes are responsible for the different degrees of fractionation observed for hydrogen and carbon isotopes.

Plants remove hydrogen from wager and transfer it to organic compounds. Although many organic hydrogen atoms are exchangeable with environmental water, once a carbon-hydrogen bond is formed in an organism, the hydrogen is no longer readily exchanged. Early studies on the natural abundance of deuterium and hydrogen in organic matter made no attempt to relate the D/H ratios from organic matter to the hydrogen source, *i.e.*, environmental water (3, 18). Zborowski *et al.* (22) determined D/H ratios from fatty acids of fish and rats and marine sediments. They found the fractionation to be constant between fatty acids and water removed from the tissues and sediments by lyophilization. Epstein and Weiss (unpublished) collected plants at successive elevations in the Sierra Nevada. The D/H ratio of total organic matter varied with altitude, as did ground water at each collection site. Schiegl and Vogel (20) recently attempted to correlate organic D/H ratios with waters precipitated from the atmosphere in Europe and South Africa. We studied several species of plants and animals growing in close proximity in salt marsh, lagoon, and intertidal habitats utilizing the same water source. Since the D/H ratio in ocean waters from different geographic areas is nearly constant (9, 10) our samples were utilizing a common isotopic pool. Carbon isotope abundance in organisms is somewhat better understood (4, 16, 21) than hydrogen isotope abundance, so we have included carbon data for the same samples.

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MATERIALS AND METHODS

Sample Collection. Fourteen species of plants and six species of animals were collected in Southern California from three sites in a salt marsh and lagoon at Point Mugu Naval Air Base and from Leo Carillo State Beach on the open ocean. The organisms were collected from various habitats but utilized a common source of water. *Distichlis spicata* (L.) Greene, *Limonium commune* S. F. Gray, *Mesembryanthemum chilense* Mol., *Monthochloe littoralis* Engelm., and *Suaeda fruticosa* (L.) Forsk. were collected high in the marsh where the ground surface was dry. The low marsh site had a film of water on the ground. Collections included: *Entermorpha marginata* J. Agardh, *Frankenia grandifolia* Cham. & Schl., *Salicornia bigelovii* Torr., and the dominant (about 300 snails/m^2) *Cerithidia californica* (Haldeman). In the lagoon itself we collected: *Zostera marina* L., *Bulla gouldiana* (Pilsbry), *Dendroaster excentricus* (Escholtz), and *Navanax inermis* (Cooper). At Leo Carillo State Beach we obtained *Macrocystis pyrifera* (L.) C. A. Agardh, *Corallina chilensis* Descaisne, *Grateloupia setchellii* Kylin, *Gigartina cristata* (Setchell) Setchell and Gardner, *Phyllospadix torreyi* Wats., *Mytilus californianus* (Conrad), and *Cypraea spadicea* (Swainson). Samples of water were taken from each collection site.

Sample Preparation. In order to minimize possible exchange with atmospheric water vapor, all samples were prepared, combusted, and analyzed as soon as possible after collection. Since all samples were treated much the same, any exchange of hydrogen clearly does not affect the range of values obtained. *Phyllospadix* leaves and *Mytilus* flesh were lyophilized immediately upon return to the laboratory (2 hr after collection and stored in seawater until arrival in the laboratory). Water removed from the tissue was retained for analysis. Tissue samples were air-dried at room temperature and ground in a mortar. Portions of dried samples were refluxed 4 hr with CCl_4. After filtration, solvent was removed at room temperature in a rotary evaporator. The residue, a crude lipid fraction, was scraped from the flask. Plant material after extraction with CCl_4 gave essentially the same isotopic ratio in every case as whole, dry, unextracted plant material. Carbon tetrachloride (Baker, reagent grade) left a residue of 1.9 mg/liter of solvent ($\delta D = -126‰$; $\delta^{13}C = -27.7‰$). The ratio of solvent residue to total CCl_4-extractable material varied from 0.03 to 1.20% with an average for all samples of 0.36%. While this may have altered some values slightly, no correction was made for it.

Sample Combustion. Organic materials were combusted completely to CO_2 and H_2O over copper oxide in an oxygen atmosphere at 800 to 900 C as described by Craig (4). Water was reduced to hydrogen (10) by passing it slowly over uranium turnings at 600 to 700 C. Hydrogen gas evolving from the furnace was pushed into a sample tube with an automatic Toepler pump.

Mass Spectrometer Analysis. Hydrogen samples were analyzed in a Nier 60° sector type mass spectrometer as modified by McKinney *et al.* (13) but with an additional collector tube joined

Table I. *Hydrogen and Carbon Isotope Abundance Ratios of Plants and Their Respective Lipid Fractions*

	Place Collected	δD‰ SMOW		δ¹³C‰ PDB$_1$	
		Whole plant	Lipid	Whole plant	Lipid
1. Higher plants, high $\delta^{13}C$					
Zostera marina[1]	Lagoon	−76.0	−179.0	−10.0	−14.4
Monanthochloe littoralis	High marsh	−50.0	−149.0	−15.3	−22.2
Distichlis spicata	High marsh	−63.0	−164.0	−14.7	−23.2
Phyllospadix torreyi	Beach	−17.0	−158.0	−14.0	−25.5
2. Algae					
Enteromorpha marginata	Low marsh	−72.0	−161.0	−16.6	−22.9
Macrocystis pyrifera	Beach	−70.0	−159.0	−17.5	−24.0
Corallina chilense	Beach	−47.0	−156.0	−18.6	−24.9
Grateloupia setchellii	Beach	−94.0	−167.0	−22.7	−25.2
Gigartina cristata	Beach	−88.0	−148.0	−20.2	−27.2
3. Higher plants, low $\delta^{13}C$					
Limonium commune	High marsh	−67.0	−149.0	−23.2	−28.3
Salicornia bigelovii	Low marsh	−82.0	−169.0	−25.2	−29.8
Mesembryanthemum chilense	High marsh	−55.0	−146.0	−23.6	−30.1
Frankenia grandifolia	Low marsh	−61.0	−150.0	−26.4	−31.1
Suaeda fruticosa	High marsh	−64.0	−148.0	−26.5	−32.8
Average		−65.0	−157.0	−19.6	−25.8
Water from the lagoon			−10.0		
Water from low marsh			−12.0		
Ocean water, Leo Carillo Beach			−09.0		

[1] Plants listed in order of decreasing $\delta^{13}C$ of CCl_4-extractable material.

in such a way that it would collect the mass 3 ion beam simultaneously with the mass 2 ion beam (10). All samples were corrected for the appropriate machine background and for mixing of sample and standard gas. In addition, CO_2 samples were corrected for ^{17}O contribution to the mass 45 peak (5). Results are expressed in terms of a δ value:

$$\delta D‰ = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000$$

where $R_{standard}$ is the ratio of mass 3 to mass 2 of standard mean ocean water (6). With the SMOW[3] value taken as 0‰, the lagoon water is −9‰. In the case of CO_2 analysis,

$$\delta^{13}C‰ = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000$$

$R_{standard}$ is the ratio of mass 45 to mass 44 in CO_2 prepared from the fossil skeleton of a Cretaceous belemnite, *Belemnitella americana*, from the Peedee formation of South Carolina; this is known as the PDB standard (5). Thus, a sample with a $\delta^{13}C$ per mil of −10 has a $^{13}C/^{12}C$ ratio less than the standard by 10 per mil, or 1.0%. For example, the $^{13}C/^{12}C$ ratio of atmospheric CO_2 is smaller than that of the PDB standard by about 7 per mil. Therefore, the $\delta^{13}C$ per mil of this CO_2 is −7. Carbonate values (Table IV) were very close to the PDB standard. The precision of measurement is ±1.0‰ for δD and ±0.1‰ for $\delta^{13}C$.

The enrichment factor or fractionation factor between two compounds *A* and *B*, is defined (14) as:

$$\alpha = \frac{D/H_A}{D/H_B}$$

also

$$\alpha \simeq \frac{1 + \delta D_A/1000}{1 + \delta D_B/1000}$$

or

$$\alpha \simeq 1 + \frac{\delta D_A - \delta D_B}{1000}$$

Thus if $\delta D_A - \delta D_B = 100‰$ the fractionation factor between *A* and *B* ≃ 1.100.

RESULTS

Heavy isotopes of both hydrogen and carbon tend to be excluded from plant matter (Table I). Fractionation factors (α) from water hydrogen to whole plant hydrogen ranged from 1.008 to 1.085 with the average 1.055. That is, organic hydrogen from whole, dried plants had 55‰ less deuterium than the source water. No trend in fractionation factors from ocean and lagoon plants to high marsh plants was observed. A further discrimination of about 90‰ was observed between the whole plant and the CCl_4 extract of plant material. Fractionation factors from whole plant to lipid ranged from 1.060 to 1.141 with an average value for α of 1.092. The fractionation from water to lipid showed less variation (1.134 to 1.169, average 1.147) than either water to organic matter or organic matter to lipid. This consistency in δD values for lipids from land plants, intertidal forms, or plants always immersed is striking when you consider that these diverse organisms may differ substantially from one another in metabolic processes. The lightest δD value obtained was −179‰ for *Zostera* lipid.

Carbon isotopes appeared to experience a smaller difference than did hydrogen isotopes between the whole plant and the lipid fraction. The fractionation factor between whole plant carbon and lipid carbon was 1.0062 (range = 1.0025–1.0115). No trends were apparent when high marsh and lagoon plants were compared. However, even taking into account differences in measurement precision, the $^{13}C/^{12}C$ ratios of total plant tissue were quite variable in contrast with hydrogen values. Not only was the well documented difference between marine and land plants apparent (4, 15), but *Suaeda* and *Distichlis*, even though growing side by side in the upper marsh, differed in $\delta^{13}C$ by 12‰.

[3] Abbreviation: SMOW: standard mean ocean water.

It is, of course, possible that some plants accumulate ^{13}C-rich metabolites while others do not. The amount of lipid extracted with CCl_4 from whole, dry plants varied from 0.1% (weight lipid/dry weight whole plant) for *Grateloupia* to 3.2% for *Mesembryanthemum*, whereas the average lipid content for all plants was 1.1%. No correlation was noted between percentage of extractable lipid and variations of δD or $\delta^{13}C$ values. In contrast, Park and Epstein (15) did find a positive correlation between percentage of lipid and difference between $\delta^{13}C$ of the plant residue (after lipid extraction) and $\delta^{13}C$ of the lipid fraction. While it would be desirable to compare the same compounds in all plants, large differences (*e.g.*, 12‰) in $\delta^{13}C$ probably do not result from an accumulation of a particular compound. Bender (2) suggests that the ^{13}C differences may indicate different carbon fixation mechanisms for the two groups of plants. She has reported several panicoid grasses to have relatively high $\delta^{13}C$ values, while festucoid grasses have low $\delta^{13}C$ values.

If we assume that source carbon for all plants reported is atmospheric carbon dioxide, −7‰ relative to the PDB standard, the fractionation factors (atmospheric carbon dioxide to whole plant carbon) fall into three groups as indicated in Table I: 1. average = 1.0065 (range 1.0030–1.0083); 2. average = 1.0121 (range 1.0096–1.0157); 3. average = 1.0180 (range 1.0162–1.0195). Each group differs from the next by nearly 6‰. The second group is composed of algae. The first group is composed of two aquatic monocots and two salt marsh grasses. Since sympatric plants were very different in $\delta^{13}C$ values, it is likely that either Bender's suggestion of a different metabolic pathway is valid or that significant differences in rates of carbon fixation exist between species. Smith and Epstein (unpublished) confirm these results for a wide variety of species. The lightest $\delta^{13}C$ value obtained was −32.8‰ for *Suaeda fruticosa* lipid.

Fleshy green leaves of *Limonium* were separated from dead, brown, basal leaves, and the tap root was removed from both. Separate analysis after drying of different plant parts (Table II) showed no large isotopic discrimination between plant organs.

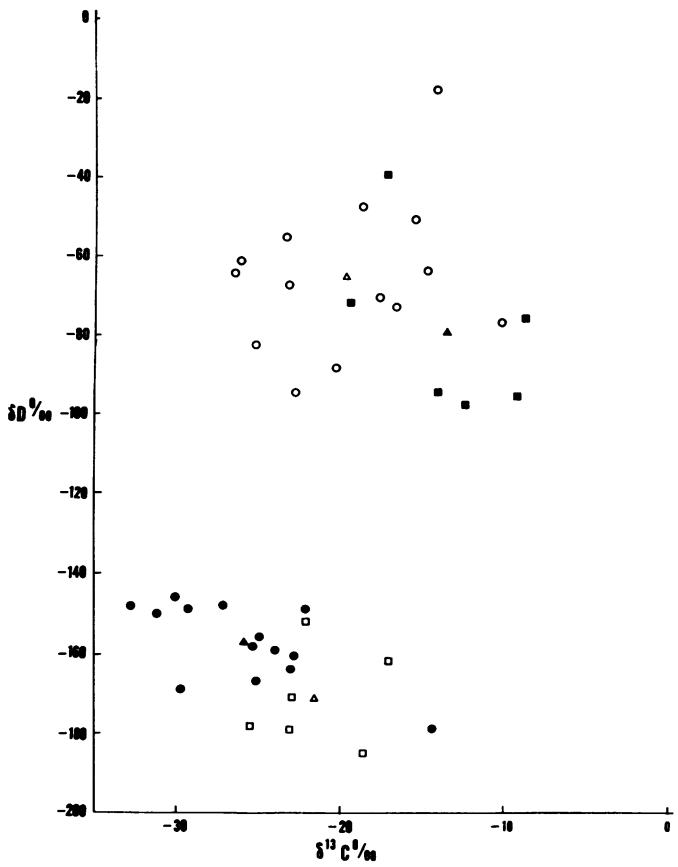

FIG. 1. Relationship between δD‰ and $\delta^{13}C$‰ for whole plant (○), whole animal (■), plant lipid (●), animal lipid (□), and average whole plant (△), average whole animal (▲), average plant lipid (▲), average animal lipid (△).

Table II. *Hydrogen and Carbon Isotope Abundance Ratios of Different Plant Parts*

	δD‰ SMOW		$\delta^{13}C$‰ PDB1	
	Whole organ	Lipid	Whole organ	Lipid
L. commune:				
Green leaves	−67.0	−149.0	−23.2	−28.3
Dead leaves	−62.0	−181.0	−23.1	−27.9
Roots	−67.0	−152.0	−24.1	−28.5
Average	−65.0	−161.0	−23.5	−28.2

Table III. *Hydrogen and Carbon Isotope Abundance Ratios of Animals and Their Respective Lipid Fractions*

	Place Collected	δD‰ SMOW		$\delta^{13}C$‰ PDB1	
		Whole animal	Lipid	Whole animal	Lipid
Cypraea spadicea	Beach	−71.0	−178.0	−19.3	−25.5
Navanax inermis	Lagoon	−94.0	−152.0	−14.0	−22.2
Mytilus californianus	Beach	−35.0	−178.0	−17.3	−22.9
Dendroaster excentricus	Lagoon	−75.0	−185.0	−8.6	−18.6
Bulla gouldiana	Lagoon	−95.0	−162.0	−9.1	−17.0
Cerithidia californica	Low marsh	−79.0	−171.0	−13.4	−21.6
Average		−79.0	−171.0	−13.4	−21.6

Table IV. *Carbon Isotope Abundance Ratios of Organic Carbonates*

	$\delta^{13}C$‰ PDB1
Corallina chilense	−1.4
Bulla gouldiana	+1.2
Cerithidia californa	−1.9
Cypraea spadicea	+1.3
Dendroaster excentricus	+0.3
Mytilus californianus	+0.6
Average	+0.02

This implies either good mixing and transport of carbon and hydrogen taken into the plant or a very constant source of isotopes.

Plants analyzed are the basis of an extensive food chain in marsh and marine environments. It would be interesting if each link in the chain were to perform a successive fractionation of hydrogen and carbon isotopes ingested. That such was not the case can be seen in Table III. Isotopically these animals nicely reflected their presumed diet. However, on the whole, animal hydrogen was lighter than plant hydrogen while animal carbon was heavier than plant carbon (Fig. 1). That carbon and hydrogen isotopic fractionation are dissimilar is not surprising, particularly in consideration of different probable modes of incorporation for the two elements.

Carbon was present not only in organic matter but also as calcium carbonate in shells of five animals and in concretions of

the calcareous red alga, *Corallina*. Carbon dioxide was liberated from carbonates with acid (8). Values in Table IV, from isotopic analysis of CO_2, show carbonate-carbon to be compatible with published values (16).

DISCUSSION

Water is absorbed from the soil and moves through the xylem in the transpiration stream to several possible fates. Water may be extruded from hydathodes as water of guttation, but most commonly is transpired through the stomates into the atmosphere as vapor. A small amount of water taken up by plant cells is either passed through the tonoplast membrane into the vacuole or retained in the cytoplasm, itself about 70% water. Some water in the cytoplasm becomes associated with the chloroplast. Light energy absorbed by photosystem II then splits the water molecule releasing gaseous oxygen (Fig. 2). Hydrogen is released to the medium from water split in photosynthesis. NADP then takes up hydrogen from the medium (1). Hydrogen involved in reduction of carbon dioxide comes from the NADPH. Thus, there is no direct link between hydrogen released in photosynthesis and the hydrogen atoms which end up in carbohydrate. Sugars and other early products of photosynthesis are rapidly transformed by various metabolic processes to all the different compounds of which living things are composed. One of the classes of compounds is lipid.

Hydrogen isotope fractionation factors are summarized in Table V. Our results indicate that membranes discriminate very little between the isotopes of hydrogen, since the fractionation measured between environmental water and water removed from tissue by lyophilization was about 1.011. This represents a small though significant fractionation and is the sum of all processes up to the splitting of water in photosynthesis. Aquatic plants do not seem to differ greatly from higher plants in the fractionation factor for this step. If water uptake is in a steady state and is not rate-limiting, then the fractionation factor should be the same regardless of the D/H ratio of the environmental water. Indeed, similar fractionation factors have been measured for intracellular water of aquatic animals kept in distilled water, which is isotopically different from sea water (Smith and Epstein, unpublished). That part of the hydrogen which reduces CO_2 is depleted in deuterium relative to the source (vacuolar and cytoplasmic water). Thus, the splitting of water in photosynthesis has a fractionation factor of 1.044. This indicates a difference in δD between water in the chloroplast and carbohydrate produced via photosynthesis. Transport of hydrogen within the chloroplast is actively being studied (7, 12, 19) as a vital part of the photosynthetic process. Key kinetic steps probably account for observed fractionations of the natural isotopes. Another large fractionation occurs during lipid synthesis. A fractionation of 1.092 occurs between carbohydrate and lipid. It has been suggested (11, 17) that NADPH contributes the large part of hydrogen during lipid synthesis. If our suggestion for isotope fractionation is correct, there could exist two completely different pools of NADPH—one associated with photosynthesis, the other produced metabolically and used in lipid synthesis. Thus, either the cofactor or the dehydrogenases would have to be strictly compartmentalized. Another possibility during lipid biosynthesis is that precursor molecules themselves contribute hydrogen depleted in deuterium. The fact that lipids have low $\delta^{13}C$ values as well as low δD values would strengthen this idea. The isotopic fractionation between total organic matter and fatty acids is considerably larger than the isotopic fractionation between whole tissue and the CCl_4 extract (Smith and Epstein, unpublished).

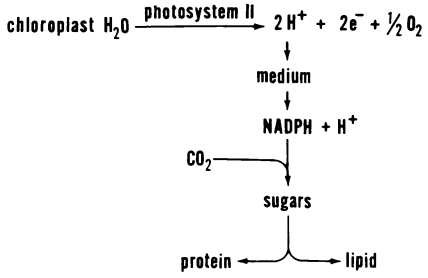

FIG. 2. Hydrogen metabolism in plants.

Table V. *Summary of Hydrogen Isotope Fractionation Factors for Salt Marsh and Marine Plants*

System	Fractionation Factor (α) ± 0.005
Extracellular water ⇌ intracellular water	1.011
Intracellular water ⇌ photosynthate	1.044
Photosynthate ⇌ lipid	1.092

The δD values reported here agree with values reported for natural organic substances (2, 10, 20, 21). The remarkable similarity in δD values indicate that both marine and salt marsh plants enjoyed a common water source. *Salicornia* collected in December were the dry, brown remains of annual plants dead since the previous fall. However, these plants were isotopically very similar to sympatric species alive at the time of collection. The isotopic record was bound into plant material with little apparent change by death and subsequent decay. This may mean that, as Wickman (21) suggested, isotopic analysis of plants long dead (*e.g.*, herbarium specimens) or even fossil organic matter may tell us something of the environment or metabolism of the organism.

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