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Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet

L.L. Tieszen, T.W. Boutton*, K.G. Tesdahl, and N.A. Slade**
Department of Biology, Augustana College, Sioux Falls, 57197, USA

Abstract. The use of stable carbon isotopes as a means of studying energy flow is increasing in ecology and paleoecology. However, secondary fractionation and turnover of stable isotopes in animals are poorly understood processes. This study shows that tissues of the gerbil (*Meriones unguienlatus*) have different $\delta^{13}C$ values when equilibrated on corn (C_4) or wheat (C_3) diets with constant $^{13}C/^{12}C$ contents. Lipids were depleted $3.0^0/_{00}$ and hair was enriched $1.0^0/_{00}$ relative to the C_4 diet. Tissue $\delta^{13}C$ values were ranked hair > brain > muscle > liver > fat. After changing the gerbils to a wheat (C_3) diet, isotope ratios of the tissues shifted in the direction of the $\delta^{13}C$ value of the new diet. The rate at which carbon derived from the corn diet was replaced by carbon derived from the wheat diet was adequately described by a negative exponential decay model for all tissues examined. More metabolically active tissues such as liver and fat had more rapid turnover rates than less metabolically active tissues such as hair. The half-life for carbon ranged from 6.4 days in liver to 47.5 days in hair.

The results of this study have important implications for the use of $\delta^{13}C$ values as indicators of animal diet. Both fractionation and turnover of stable carbon isotopes in animal tissues may obscure the relative contributions of isotopically distinct dietary components (such as C_3 vs. C_4, or marine vs. terrestrial) if an animal's diet varies through time. These complications deserve attention in any study using stable isotope ratios of animal tissue as dietary indicators and might be minimized by analysis of several tissues or products covering a range of turnover times.

Introduction

The use of stable carbon isotopes in ecological and paleoecological studies of energy flow is becoming increasingly widespread (Jones et al. 1979; Tieszen et al. 1979; Boutton et al. 1980; Wing and Brown 1980). The utility of this method is based on the fact that C_3 and C_4 plants possess distinctly different $^{13}C/^{12}C$ ratios due to fractionation during photosynthetic carbon fixation (Smith and Epstein 1971; Vogel 1980; O'Leary 1981). In addition, aquatic and marine plants often possess stable carbon isotope ratios that are different than ratios for land plants (Rau 1978; Osmond et al. 1981; Fontugne and Duplessy 1981). Because animals do not substantially alter the carbon isotopic composition of their food (DeNiro and Epstein 1978), it is frequently possible to assess the relative dependence of animals on these isotopically distinct categories of primary producers. For example, Tieszen et al. (1979) were able to use $\delta^{13}C$ values of rumen contents to assess the dependence of ungulates on grass (all C_4 plants) or shrub/tree components (all C_3 plants) in East African savannas.

Information obtained from $\delta^{13}C$ values of animals will depend on the choice of animal tissue or product analyzed. Analysis of feces or gut contents would be indicative of the organism's diet during its recent past, ranging from a few hours for insects to several days for large mammalian herbivores. Animal tissues or biochemical fractions thereof would presumably have $\delta^{13}C$ values which would be an integration of dietary carbon over a longer time period. Because of significant variation in $\delta^{13}C$ values between biochemical fractions and between different tissues within an individual organism (Jacobson et al. 1972; DeNiro and Epstein 1978; Lyon and Baxter 1978; McConnaughey and McRoy 1979), the choice of what part of the animal to analyze may influence conclusions about diet. DeNiro and Epstein (1978) suggest that for small organisms an analysis of total animal carbon provides an accurate measure of diet.

An important complication with $\delta^{13}C$ analysis of animal diet is that each tissue and biochemical fraction can be expected to have an isotopic "memory," which would be a function of the $^{13}C/^{12}C$ ratio of the carbon in the food at the time of synthesis, $^{13}C/^{12}C$ ratios of subsequent foodstuffs, and the biochemical turnover rate of the component in question (Tieszen 1978). Presently, the length of time over which isotope ratios of different tissues and biochemical fractions indicate an animal's diet is poorly understood. It has been shown that tissue protein and other tissue components are in a state of dynamic equilibrium, with new components synthesized and older components being degraded continuously (Schoenheimer 1946; Bender 1975). In general, it appears that more metabolically active tissues (e.g., liver, pancreas, fat tissue) have faster turnover rates than less metabolically active tissues such as bone and connective tissue (Libby et al. 1964; Stenhouse and Baxter

* *Present Address:* Stable Isotope Laboratory, Children's Nutrition Research Center, Baylor College of Medicine, Houston, Texas 77030, USA
** Museum of Natural History and Department of Systematics and Ecology, University of Kansas, Lawrence, Kansas 66045, USA

Offprint requests to: L. Tieszen

1979; Thompson and Ballou 1956). It is also apparent that tissues may have carbon pools that turn over rapidly as well as carbon pools that turn over very slowly, with half-lives of several months to years, depending on the organism (Thompson and Ballou 1956; Stenhouse and Baxter 1979).

In order for $\delta^{13}C$ values of animal tissue to be more meaningful in ecological or paleoecological contexts, it is important to know the approximate turnover times of carbon in different tissues. We have initiated a series of these studies in large and small mammals. We now report on (1) the relationship between the carbon isotope ratio of the food source and the carbon isotope ratios of selected soft tissues of the gerbil, and (2) turnover times for carbon in different tissues. Since bone material is of greater interest for paleoecology and since there is some disagreement concerning the appropriateness of collagen or apatite for analysis (Sullivan and Krueger 1981; Land et al. 1980), we will describe these results in a separate more extensive communication.

Methods and materials

A sample population of gerbils (*Meriones unguiculatus*) was reared for two generations and equilibrated on a diet consisting of ground corn mixed with a vitamin and mineral supplement. $\delta^{13}C$ values of this diet were constant throughout the course of the study ($-12.2 \pm 0.37‰$) and characteristic of C_4 plants. The sample population was then randomly subdivided into a control group which remained on the corn diet, and an experimental group. Gerbils in the experimental group were shifted to a diet consisting of ground wheat supplemented with vitamins and minerals. $\delta^{13}C$ values of this diet were also constant throughout the study ($-21.8 \pm 0.25‰$) and characteristic of C_3 plants. Ages of gerbils were known within one week and indicated by toe clipping. Both groups were maintained at $25° \pm 3°$ C and at 80% relative humidity on 12-h photoperiods.

Prior to the formation of the two experimental groups, 5 adult males were randomly selected from the population and sacrificed. Tissue samples of liver, fat, muscle, brain and hair were taken from each individual and immediately frozen. $^{13}C/^{12}C$ ratios from these five individuals were taken to be representative of the population as a whole at the start of the experiment. After the formation of control and experimental groups, adult male gerbils from the experimental group were randomly selected and sacrificed at 2, 5, 15, 40, 75, and 155 days after the beginning of the experiment, and tissues were removed and frozen. Individuals from the control group were sacrificed 15 and 155 days after the start of the experiment. All tissues were dried at 60° C in a vacuum oven prior to isotopic analysis.

Samples of organic carbon were combusted to CO_2 for mass spectrometric analysis of $^{13}C/^{12}C$ according to the method of Buchanan and Corcoran (1959). Briefly, 5–10 mg of dried sample are placed in a baked out length (~ 16 cm) of 6 mm O.D. quartz or Vycor tubing and mixed with 0.5 g of oxidant ($CuO : MnO_2 : CuCl_3$ in a 5:1:1 ratio) and a 1 cm length of silver wire. Sample tubes are then attached to a vacuum manifold, evacuated to $< 10^{-2}$ mbar, and sealed with an oxygen-acetylene flame. Sealed sample tubes were then placed in a muffle furnace at 850° C for 1 h.

Carbon dioxide from the combustion was released from the sample tube and admitted to the evacuated inlet system of the mass spectrometer by means of a tube cracker (Des Marais and Hayes 1976). Water vapor was removed in a dry ice trap and CO_2 collected in a liquid nitrogen trap. Gases that were not condensed in the liquid nitrogen trap were pumped away. Purified CO_2 was then admitted to the mass spectrometer for $^{13}C/^{12}C$ determinations.

All analyses were performed on a Micromass 602E mass spectrometer. Results are expressed as:

$$\delta^{13}C‰ = \frac{\text{R sample} - \text{R standard}}{\text{R standard}} \times 1000$$

where R standard is the mass 45 to mass 44 ratio in CO_2 of carbonate from the fossil *Belemnitella americana* from the Peedee formation of South Carolina (Craig 1953, 1957). Values were corrected for errors from ^{17}O contribution to mass 45 abundance, switching valve leakage, and background. The standard error associated with our combustion procedure was determined to be $0.14‰$ for NBS-22 petroleum, while the standard error associated with machine error during analysis of NBS-22 petroleum was $0.05‰$, for an overall precision of $0.2‰$ on each determination.

Results

Stable carbon isotope ratios of different tissues of the gerbil prior to the establishment of experimental and control groups reflected the stable isotope ratio of the diet (Fig. 1). Fat tissue was $3.0‰$ more depleted in ^{13}C than the diet, and showed the largest departure from dietary ^{13}C. This is to be expected, since lipid synthesis discriminates against ^{13}C (DeNiro and Epstein 1977). Fat tissue was significantly more depleted in ^{13}C than all the other tissues during this initial analysis (Table 2). By contrast, hair was $1‰$ enriched in ^{13}C relative to the diet, and was significantly more enriched in ^{13}C than all the other tissues (Table 2). Brain, muscle, and liver differed from the diet by less than $1‰$ and appear to be the most direct indicators of gerbil diet. The general relationship between the stable carbon isotope ratios of the different tissues (i.e., $\delta^{13}C$ hair $> \delta^{13}C$ brain $> \delta^{13}C$ muscle $> \delta^{13}C$ liver $> \delta^{13}C$ fat) was preserved during all subsequent sample periods (Table 1). This same trend was also observed in $\delta^{13}C$ values of mice tissues by DeNiro and Epstein (1978).

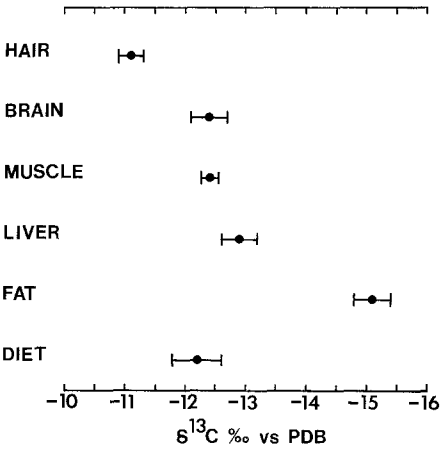

Fig. 1. Isotope ratios of selected gerbil tissues before start of experiment in relation to the isotope ratio of the diet. Values indicated are means ($N = 5$) $\pm$ standard errors

Table 1. $\delta^{13}C$ $^{0}/_{00}$ vs. PDB ($\bar{x} \pm$ std. error) for select gerbil tissues in control (corn) and experimental (wheat) groups. Time is in days from start of experiment

Time	N	Fat	Liver	Muscle	Brain	Hair
Wheat Diet						
T_0	5	-15.1 ± 0.28	-12.9 ± 0.28	-12.4 ± 0.13	-12.4 ± 0.26	-11.1 ± 0.15
T_2	4	-14.9 ± 0.15	-14.7 ± 0.30	-13.4 ± 0.62	-12.9 ± 0.17	-11.0 ± 0.09
T_5	4	-16.7 ± 0.59	-16.9 ± 0.59	-14.0 ± 0.26	-13.7 ± 0.21	-11.3 ± 0.11
T_{15}	4	-20.3 ± 0.38	-19.7 ± 0.35	-16.3 ± 0.70	-15.4 ± 0.28	-12.7 ± 0.46
T_{40}	4	-23.3 ± 0.50	-20.6 ± 0.40	-17.7 ± 0.47	-17.3 ± 0.42	-14.9 ± 0.79
T_{75}	4	-25.3 ± 0.24	-21.7 ± 0.34	-19.8 ± 0.56	-18.6 ± 0.16	-17.3 ± 0.38
T_{155}	3	-25.0 ± 0.43	-22.3 ± 0.20	-21.3 ± 0.50	-20.4 ± 0.14	-19.2 ± 0.05
Corn Diet						
T_0	5	-15.1 ± 0.28	-12.9 ± 0.28	-12.4 ± 0.13	-12.4 ± 0.26	-11.1 ± 0.15
T_{155}	4	-15.3 ± 0.25	-12.3 ± 0.30	-12.6 ± 0.68	-12.1 ± 0.24	-10.3 ± 0.13

Table 2. F-ratios from analysis of variance for differences in $\delta^{13}C$ values between tissues within sample periods in experimental animals. An asterisk indicates $p < 0.05$

Time	df		Fat	Liver	Muscle	Brain
T_0	1,4	Liver	135.36*			
		Muscle	196.57*	5.50		
		Brain	4,324.04*	6.04	0.001	
		Hair	169.53*	58.50*	43.81*	19.72*
T_2	1,3	Liver	0.14			
		Muscle	5.26	6.76		
		Brain	40.91*	44.56*	0.66	
		Hair	1,443.56*	127.14*	18.69*	53.73*
T_5	1,3	Liver	0.04			
		Muscle	16.45*	32.06*		
		Brain	23.76*	34.37*	1.97	
		Hair	68.48*	120.92*	273.94*	199.68*
T_{15}	1,3	Liver	1.04			
		Muscle	6.27	42.20*		
		Brain	8.31	180.04*	3.50	
		Hair	8.76	136.16*	34.44*	89.65*
T_{40}	1,3	Liver	6.89			
		Muscle	51.66*	8.04		
		Brain	51.77*	7.32	2.44	
		Hair	148.87*	8.31	20.88*	9.83
T_{75}	1,3	Liver	7.46			
		Muscle	176.58*	2.57		
		Brain	362.94*	8.87	3.11	
		Hair	253.80*	8.95	15.78*	15.53*
T_{155}	1,2	Liver	3.99			
		Muscle	3.51	2.40		
		Brain	3.97	201.97*	3.74	
		Hair	3.93	176.45*	21.03*	70.09*

Analysis of variance tests were conducted to determine differences between tissue types within each sample period (Table 2). In general, fat was significantly more depleted in ^{13}C than other tissue types throughout the course of the study. Hair was usually significantly more enriched in ^{13}C than the other tissues during each sample period. Liver, muscle, and brain were not significantly different in their $\delta^{13}C$ values during most sample periods.

Stable carbon isotope ratios of all gerbil tissues from the experimental group changed significantly during the period of measurement (Table 1). This shift in tissue $\delta^{13}C$ values was clearly towards the $\delta^{13}C$ value of the wheat diet, indicating that carbon previously assimilated while the gerbils were on the corn diet was being broken down and replaced with carbon derived from the wheat diet. Stable isotope ratios of tissues from the control group did not change significantly during the course of the study (Table 1), indicating that the $\delta^{13}C$ value of the diet was responsible for the observed changes in the experimental group.

The changes in $\delta^{13}C$ values of tissues from the experimental gerbils versus time are shown in Fig. 2. Because the data suggested a negative exponential change in the tissue $\delta^{13}C$ values, equations of the form $Y = P_3 + p_1 e\text{-}P_2^T$ were fitted to the data for each tissue. In this equation, Y is the $\delta^{13}C$ value of the tissue in question, P_1 is the total change in $\delta^{13}C$ when the tissue has changed from 0% wheat carbon to 100% wheat carbon, P_2 is the turnover rate of carbon in that tissue, P_3 is the asymptotic $\delta^{13}C$ value for that tissue on the wheat diet, and T is time in days since the switch from corn diet to wheat diet. Tests for lack of fit of the models to the observed data (Draper and Smith 1966) were not significant for any of the tissues (Fig. 2), indicating that the negative exponential model was appropriate for all tissues.

It is immediately apparent that the rate at which carbon derived from the wheat diet is incorporated is not the same for all tissues (Fig. 2). By rearrangement of the terms of the negative exponential model, halflives of carbon in the selected tissues can be calculated. In order to find the length of time required for α % turnover of carbon, the equation $T = \ln(1 - \alpha/100)/P2$ is solved, where T is time in days, α is some % turnover, and P_2 is the turnover rate for the tissue in question. To determine half-lives of tissue carbon, the equation is solved for $\alpha = 50\%$.

Carbon turnover was most rapid in liver tissue, with a half-life of 6.4 days (Fig. 2). This is consistent with the results of previous investigators who have found that the protein and fatty acid components of liver tissue are replaced at a much higher rate than in other body tissues (Schoenheimer 1946). Fat tissue also had a relatively short half-life of 15.6 days. Muscle and brain had very similar half-lives of 27.6 and 28.2 days, respectively. The slowest carbon turnover rate was found in the hair, with a half-life of 47.5 days. Thus, different tissues replace carbon at different rates. Thompson (1953), using tritium as a tracer, also found that half-lives of rat tissue increased in the sequence liver, fat, muscle, brain, and hair.

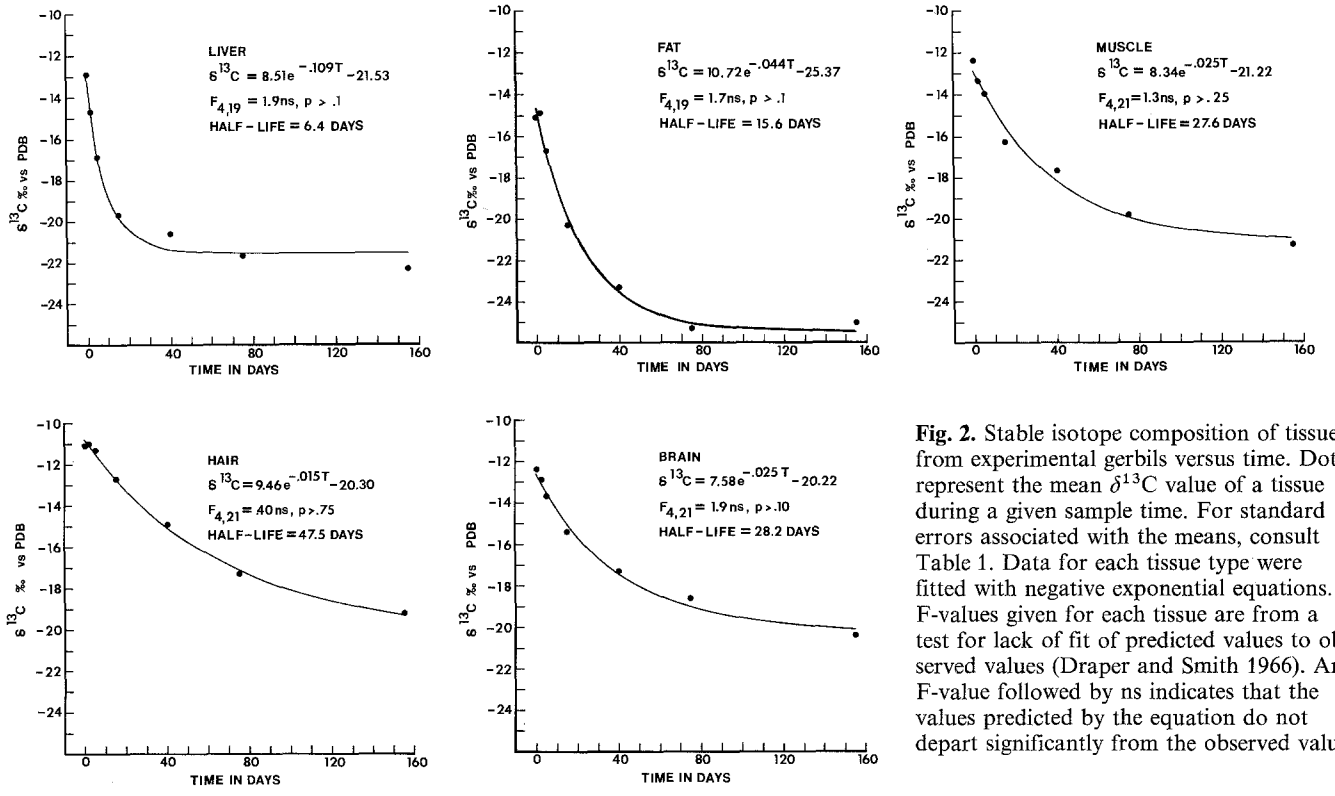

Fig. 2. Stable isotope composition of tissues from experimental gerbils versus time. Dots represent the mean $\delta^{13}C$ value of a tissue during a given sample time. For standard errors associated with the means, consult Table 1. Data for each tissue type were fitted with negative exponential equations. F-values given for each tissue are from a test for lack of fit of predicted values to observed values (Draper and Smith 1966). An F-value followed by ns indicates that the values predicted by the equation do not depart significantly from the observed values

Of the five tissues studied, only liver seemed to have had a complete turnover of carbon during the 155 day study period. By day 84, liver carbon derived from the corn diet was 99.99% replaced by carbon derived from the wheat diet. Fat tissue had the next highest turnover rate, but would have required 208 days to reach 99.99% turnover of carbon.

Discussion

The results of this study show that there are significant differences between $\delta^{13}C$ values of tissues from gerbils fed a diet with a constant carbon isotopic composition. Brain, muscle, and liver had $\delta^{13}C$ values which differed from the isotopic composition of the diet by less than $1^0/_{00}$, while hair and fat differed from the diet by $+1^0/_{00}$ and $-3^0/_{00}$, respectively. There were predictable relationships among $\delta^{13}C$ values of the different tissues with $\delta^{13}C$ hair > $\delta^{13}C$ brain > $\delta^{13}C$ muscle > $\delta^{13}C$ liver > $\delta^{13}C$ fat. DeNiro and Epstein (1978) showed a similar relationship in mice.

The cause of these differences between tissues is not presently known. However, since major biochemical fractions (e.g., proteins, carbohydrates, lipids, etc.) of organisms differ isotopically from each other (Jacobson et al. 1972; DeNiro and Epstein 1978), the isotopic differences between tissues may reflect variation in the biochemical composition of the tissues. For example, a tissue containing a high proportion of lipids would probably have a more negative $\delta^{13}C$ value than a tissue with a lower lipid content, since lipids are relatively depleted in ^{13}C. Although the secondary fractionation of carbon isotopes by animal tissues is relatively small, it must be taken into consideration in any study attempting to quantify dietary sources (e.g., proportion of C_3 vs. C_4 plants in diet) by using stable carbon isotope ratios of animal tissue. Failure to acknowledge the fractionation caused by a particular tissue could result in a serious under or overestimation of the isotopically different dietary sources.

Half-lives of carbon components in the tissues examined ranged from 6.4 days for liver to 47.5 days for hair, indicating that carbon turnover rate varies from tissue to tissue. For all tissues examined, the rate of replacement of carbon derived from the corn diet by carbon derived from the wheat diet could be described by negative exponential decay models. Similar models have been employed by other investigators to describe turnover rates of tissues and their biochemical components (Thompson and Ballou 1956; San Pietro and Rittenberg 1953).

It might be expected that more metabolically active tissues would have more rapid carbon turnover rates than less metabolically active tissues since they would be interacting to a greater extent with metabolites and nutrients derived from recently digested dietary components. To test this idea, carbon turnover rates of the tissues examined in this study were compared with known metabolic rates of rat tissues obtained in Altman and Dittmer (1968). Rates for rat tissues were used because no data were available for the gerbil. Assuming that metabolic rates of gerbil and rat tissues are similar, Fig. 3 indicates that there is a statistically significant relationship between the carbon turnover rate and the metabolic rate of the four tissues involved. Thompson (1953) also speculated that the turnover rate of a tissue was related to its metabolic activity. Since oxygen consumption declines as body size increases, we hypothesize that carbon turns over more slowly in tissues of animals with larger body size.

DeNiro and Epstein (1978) concluded that no single tissue was ideal for determining the relationship between the isotopic composition of an animal and its diet. In this study, we found that liver, muscle, and brain tissue differed

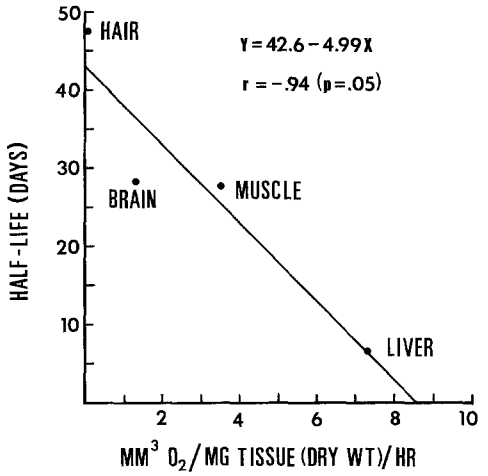

Fig. 3. Relationship between metabolic rates of rat tissue and half-lives of gerbil tissue. Metabolic rates of rat tissues were determined at 37° C in Krebs Ringer phosphate medium and were taken from Altman and Dittmer (1968). No data were available for fat tissue

from the corn diet by less than $1^0/_{00}$. In this particular situation, these tissues would certainly be adequate indicators of the isotopic composition of gerbil diet. However, DeNiro and Epstein (1978) have shown that the fractionation of ^{13}C from diet to tissue is not identical on different diets, possibly because of differential assimilation between major biochemical components of different diets. So, although liver, muscle and brain appear to be reliable indicators of the $\delta^{13}C$ value of the diet of gerbils, these tissues may not bear the same quantitative departures from diet in all circumstances. DeNiro and Epstein (1978) have suggested that whenever possible, the carbon of the whole animal should be used to estimate the $\delta^{13}C$ of the diet. Alternatively, we suggest that several tissues of known fractionation and turnover patterns should be used.

Analysis of animals or parts of animals for $\delta^{13}C$ in order to obtain information about diet clearly depends on the choice of what part of the animal is analyzed, as shown in this study and by DeNiro and Epstein (1978). The dietary information derived from the $\delta^{13}C$ value of an animal or part of an animal will further depend on the rate of turnover of carbon in the tissues of that animal. For example, gerbil liver tissue took 84 days to be completely replaced by carbon derived from the wheat diet. During this 84 day period, the $\delta^{13}C$ values of the liver tissue did not reflect the $\delta^{13}C$ value of the wheat diet, but were intermediate between values for corn and wheat. Thus, in a situation where an animal periodically changes from one isotopically distinct food source to another, stable carbon isotope ratios of that animal's tissues may provide only limited information concerning the relative importance of the two food sources due to complications introduced by carbon turnover.

If combinations of tissues or animal products were analyzed, greater information concerning the animal's diet might be obtained. For example, collagen turns over very slowly and has an extremely long half-life (Thompson and Ballou 1956; Libby et al. 1964; Stenhouse and Baxter 1979). Thus, we might expect that bone collagen $\delta^{13}C$ values would integrate the isotopic composition of dietary carbon over a much longer time period than other tissues. Analysis of animal feces or stomach contents for $\delta^{13}C$ should provide information concerning the immediate diet of the animal. To maximize the dietary information obtainable by $\delta^{13}C$ analysis of animals, the tissues or products analyzed ought to include a wide range of half-lives, such as bone, muscle, and feces. Comparison of muscle or some other soft tissue with fecal $\delta^{13}C$ values would indicate the present diet, and indicate if there have been any major dietary changes in the animal's recent past. Bone collagen values, as mentioned above, might average the animal's diet over a considerable time span. Together, the three values would provide a fairly complete dietary history of the animal being studied.

Based on the results of this study, we suggest that when possible, dietary analyses by means of $\delta^{13}C$ values should not be based on the analysis of a single tissue. Fractionation of an as yet unpredictable magnitude (apparently a function of diet) and the relatively rapid rate of carbon turnover in most soft tissues may obscure the relative importance of two isotopically distinct dietary components (such as C_3 vs. C_4, or marine vs. terrestrial) if an animal's diet varies through time. Information about an herbivore's food intake would best be maximized by an analysis of bone collagen, a soft tissue, and feces or stomach contents. This multiple analysis would reveal the animal's average long-term diet, its immediate diet, and whether or not any shifts in food habits had occurred recently. These recommendations should be carefully considered in future studies of animal food habits based on stable carbon isotope ratios.

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