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Influence of diet on the distribution of carbon isotopes in animals*

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Abstract—The influence of diet on the distribution of carbon isotopes in animals was investigated by analyzing animals grown in the laboratory on diets of constant carbon isotopic composition.

The isotopic composition of the whole body of an animal reflects the isotopic composition of its diet, but the animal is on average enriched in $\delta^{13}C$ by about 1‰ relative to the diet. In three of the four cases examined, the ^{13}C enrichment of the whole body relative to the diet is balanced by a ^{13}C depletion of the respired CO_2. The isotopic relationships between the whole bodies of animals and their diets are similar for different species raised on the same diet and for the same species raised on different diets. However, the $\delta^{13}C$ values of whole bodies of individuals of a species raised on the same diet may differ by up to 2‰. The relationship between the $^{13}C/^{12}C$ ratio of a tissue and the $^{13}C/^{12}C$ ratio of the diet depends both on the type of tissue and on the nature of the diet. Many of the isotopic relationships among the major biochemical fractions, namely the lipid, carbohydrate and protein fractions, are qualitatively preserved as diet carbon is incorporated into the animal. However, the difference between the $\delta^{13}C$ values of a biochemical fraction in an animal and in its diet may be as large as 3‰. The $\delta^{13}C$ values of the biochemical components collagen, chitin and the insoluble organic fraction of shells, all of which are often preserved in fossil material, are related to the isotopic composition of the diet.

These results indicate that it will be possible to perform dietary analysis based on the determination of the $^{13}C/^{12}C$ ratio of animal carbon. Analysis of the total animal carbon will in most cases provide a better measure of diet than the analysis of individual tissues, biochemical fractions, or biochemical components. The limits of accuracy of this method will generally restrict its application to situations in which the diet is derived from sources with relatively large differences in their $\delta^{13}C$ values, such as terrestrial vs aquatic organisms or C_3 vs C_4 plants. The method should be applicable to fossil as well as to living material.

INTRODUCTION

THE $\delta^{13}C$ values of the bodies of animals from marine, freshwater and terrestrial environments fall within the ranges of the $\delta^{13}C$ values of plants from the respective environments (CRAIG, 1953; DEGENS, 1969; DEGENS *et al.*, 1968; SACKETT *et al.*, 1965; SCHWARCZ, 1969; SMITH and EPSTEIN, 1970). Field studies have also shown that the large differences in $\delta^{13}C$ values characteristic of plants possessing either the C_3 or C_4 photosynthetic pathways (SMITH and EPSTEIN, 1971) are reflected in the carbon isotopic composition of animals which derive their carbon predominantly from plants of one or the other photosynthetic type (HAINES, 1976; MINSON *et al.*, 1975). It has been concluded from these observations that there is no large isotopic fractionation associated with the incorporation of carbon from the diet into an animal.

The accuracy with which the relationship between the carbon isotopic composition of an animal and its diet can be determined from the $\delta^{13}C$ values of animals and plants collected in the field is limited by two factors which cannot be adequately controlled in such studies. First, there may be seasonal variation in the carbon isotopic composition of the diet. For example, the $\delta^{13}C$ values of maple leaves and a grass species collected at a single locality can vary by more than 5‰ during the growing season (LOWDON and DYCK, 1974). Variations of similar magnitude probably exist for other plants. Unless recently synthesized animal components are analyzed or an integrated value for the isotopic composition of the diet is used, the relationship between the plant and animal isotopic composition cannot be accurately determined. Second, implicit in the design of most field studies is the assumption that herbivores will consume the available plants at random. However, animals may be selective in their choice of food plants and the $\delta^{13}C$ values of the plants eaten may differ from the average $\delta^{13}C$ value of the plants available to the animal. This assumption could lead to large errors if the potential food plants included both C_3 and C_4 plants, since there is some evidence that herbivores avoid eating C_4 plants (CASWELL *et al.*, 1973).

The first objective of this study was to determine the relationship between the carbon isotopic composition of an animal and its diet. This was done directly by analyzing animals which had been raised in the laboratory on diets of constant $\delta^{13}C$ value. A second objective of the study was to determine whether the dietary history of an animal could be reconstructed

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from the $\delta^{13}C$ value of its carbon. Since it may not always be possible or practical to measure the $\delta^{13}C$ value of a whole animal for this purpose, the relationships between the $\delta^{13}C$ value of the diet and the $\delta^{13}C$ values of several tissues, biochemical fractions and biochemical components of animals raised on diets of constant $\delta^{13}C$ value were also determined.

A preliminary report of this work was presented by DeNiro and Epstein (1976).

EXPERIMENTAL METHODS

Growth of animals

All animals were raised from birth on diets of constant carbon isotopic composition. The animals, their diets and

Table 1. Methods employed in raising animals on diets of constant $\delta^{13}C$ value

Animal	Diet	Animal growth technique
Artemia salina (brine shrimp)	Algae (Dunaliella sp.)	Hatchlings were inoculated into a liquid culture of algae. The animals were separated from the algae after the growth period by serial washing.
Caenorhabditis elegans (nematode)	Bacteria (Escherichia coli)	A small quantity of nematodes was inoculated into a liquid culture of bacteria. The animals were separated from the bacteria after the growth period by centrifugation (JOHNSON, 1977).
Calliphora vicina (blow fly)	Horsemeat (Equus caballus) or Pork (Sus scrofa)	Eggs were placed on the meat, which was then covered with sawdust. After 13 days, pupae were transferred to an empty container. Adults were collected within 12 hours of emergence from the pupal cases.
Desmia funeralis (moth)	Grape leaves (Vitis vinifera)	The animals had been maintained in the laboratory for several generations on the specified diet (ALINIAZEE and STAFFORD, 1973). They were provided with grape leaves and water ad libitum.
Helix aspersa (snail)	Romaine lettuce leaves (Lactuca sativa)	The animals had been maintained in the laboratory for several generations on the specified diet (CROWELL, 1973). They were provided with Romaine lettuce leaves and water ad libitum. The diet was supplemented with $CaCO_3$ or $Ca_3(PO_4)_2$.
Melanoplus sanguinipes (grasshopper)	Corn seedlings (Zea mays) or Wheat seedlings (Triticum aestivum)	The animals, starting with newly hatched nymphs, were provided with seedlings and water ad libitum.
Mus musculus, Strain AQR (mouse)	Purina Rat Chow mixture	The animals were obtained from the animal room of the California Institute of Technology, where they had been maintained for a number of generations on the specified diet. They were provided with the diet and water ad libitum.
Mus musculus, Strain BALB/c (mouse)	Wayne Lab-Blox F6 mixture	The animals were obtained from the L. C. Strong Company, San Diego, California, where they had been maintained for a number of generations on the specified diet. They were provided with the diet and water ad libitum.
Mus musculus, Stroin BALB/cJ (mouse)	JAX 911A mixture	The animals were obtained from The Jackson Laboratory, Bor Harbor, Maine, where they had been maintained for a number of generations on the specified diet. They were provided with the diet and water ad libitum.
Musca domestica (house fly)	Horsemeat (Equus caballus) or Pork (Sus scrofa)	Eggs were placed on the meat, which was then covered with sawdust. After 10 days, pupae were transferred to an empty container. Adults were collected within 12 hours of emergence from the pupal cases.
Oncopeltus fasciatus (milkweed bug)	Milkweed seeds (Asclepias syriaca)	The animals had been maintained in the laboratory for many generations on the specified diet (LA CHANCE and RIEMANN, 1973). They were provided with milkweed seeds and water ad libitum.
Sitophilus granarius (weevil) or Sitophilus oryzae (weevil)	Wheat seeds (Triticum aestivum)	The animals had been maintained in the laboratory for several generations on the specified diet (LUM and BAKER, 1973). They were provided with wheat seeds and water ad libitum.

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Mus musculus, Strain BALB/c (mouse)	Wayne Lab-Blox F6 mixture	The animals were obtained from the L. C. Strong Company, San Diego, California, where they had been maintained for a number of generations on the specified diet. They were provided with the diet and water ad libitum.
Mus musculus, Strain BALB/cJ (mouse)	JAX 911A mixture	The animals were obtained from The Jackson Laboratory, Bar Harbor, Maine, where they had been maintained for a number of generations on the specified diet. They were provided with the diet and water ad libitum.
Musca domestica (house fly)	Horsemeat (Equus caballus) or Pork (Sus scrofa)	Eggs were placed on the meat, which was then covered with sawdust. After 10 days, pupae were transferred to an empty container. Adults were collected within 12 hours of emergence from the pupal cases.
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Table 2. $\delta^{13}C$ values of the diets. The sampling methods are discussed in the text

Diet	Sampling method	$\delta^{13}C_{PDB}$ (‰)
Algae	A2	-20.4[a]
Bacteria	A2	-24.4[a]
Corn seedlings[b]	B	-22.6 ± 0.6 (n = 3)
Grape leaves	A1	-29.5[a]
Horsemeat	A2	-23.9[a]
JAX 911A mixture	B	-22.3 ± 0.5 (n = 10)
Milkweed seeds	A1	-27.1[a]
Pork	A2	-13.5[a]
Purina Rat Chow mixture	B	-18.3 ± 0.6 (n = 4)
Romaine lettuce leaves	B	-26.6 ± 0.3 (n = 3)
Wayne Lab-Blox F6 mixture	B	-19.3 ± 0.4 (n = 4)
Wheat seeds	A1	-25.0[a]
Wheat seedlings[b]	B	-40.2 ± 0.8 (n = 3)

[a] Duplicate analyses of the single sample of this diet differed by less than 0.2‰.

[b] The $\delta^{13}C$ values of these laboratory-grown plants are more negative than the $\delta^{13}C$ values for samples grown in the field (SMITH and EPSTEIN, 1971) because of differences in the $\delta^{13}C$ values of their respective sources of CO_2 (DENIRO, 1977).

the techniques used in raising the animals are indicated in Table 1. In those cases in which the animals had not been raised on the specified diet for at least several generations, the difference between the weights of new-born animals which were introduced to the diet and the animals which were analyzed was determined; the contribution of carbon from the maternal diet was found to be negligible.

Sampling procedures

The methods used in sampling the diets and the diet $\delta^{13}C$ values are listed in Table 2. In those cases in which the animal and its diet were supplied to us, a sample of the diet was taken immediately upon receipt (sampling technique A1). In these cases, the diet was drawn from a large homogeneous reservoir so that variation in the $\delta^{13}C$ value of the diet was negligible over the lifetime of the animal. In those cases in which the animal was given its entire lifetime supply of food at one time, a sample of the diet was taken when the animal was introduced to the diet (sampling technique A2). If several batches of food were required during the lifetime of the animal, at least one sample from each batch was taken (sampling technique B). The samples of the diets were stored at −20°C prior to analysis.

If the $\delta^{13}C$ value of a whole animal is to be determined, food in its gut represents contamination. This necessitated the adoption of several variations in the sampling procedures for the animals. All insects, except for *Calliphora* (blow fly) and *Musca* (house fly), were held in cages with water but no food for 24 hr prior to collection to permit them to empty their guts by the normal processes of excretion. This treatment was not necessary for *Calliphora* and *Musca*, since the food, which was available only to the juvenile larval stages, was not present in the gut of the adults which were analyzed. *Artemia* (brine shrimp) and *Caenorhabditis* (nematode) were maintained in liquid culture without food for 4 hr to permit them to purge their digestive tracts by excretion. The gut and its contents were removed from *Helix* (snail) specimens by dissection. Organs and tissues of *Mus* (mouse) exclusive of the alimentary canal, rather than the whole organism, were analyzed. All animals with the exception of mice were killed by immersion in liquid nitrogen and stored in their entirety at −20°C prior to analysis. Mice were killed by cervical dislocation and dissected; their tissues were stored at −20°C prior to analysis.

Preparation of biochemical fractions and components

Specimens from which the biochemical fractions lipid, carbohydrate (as glycogen) and soluble protein were to be isolated were first lyophilized (freeze-dried), then ground to a powder. The various fractions were isolated by the procedures outlined in the flow diagram of Fig. 1. These procedures are based on published techniques for the isolation of lipids (BLIGH and DYER, 1959), glycogen (JACOBSON *et al.*, 1972) and protein (MARROQUIN and FARBER, 1965).

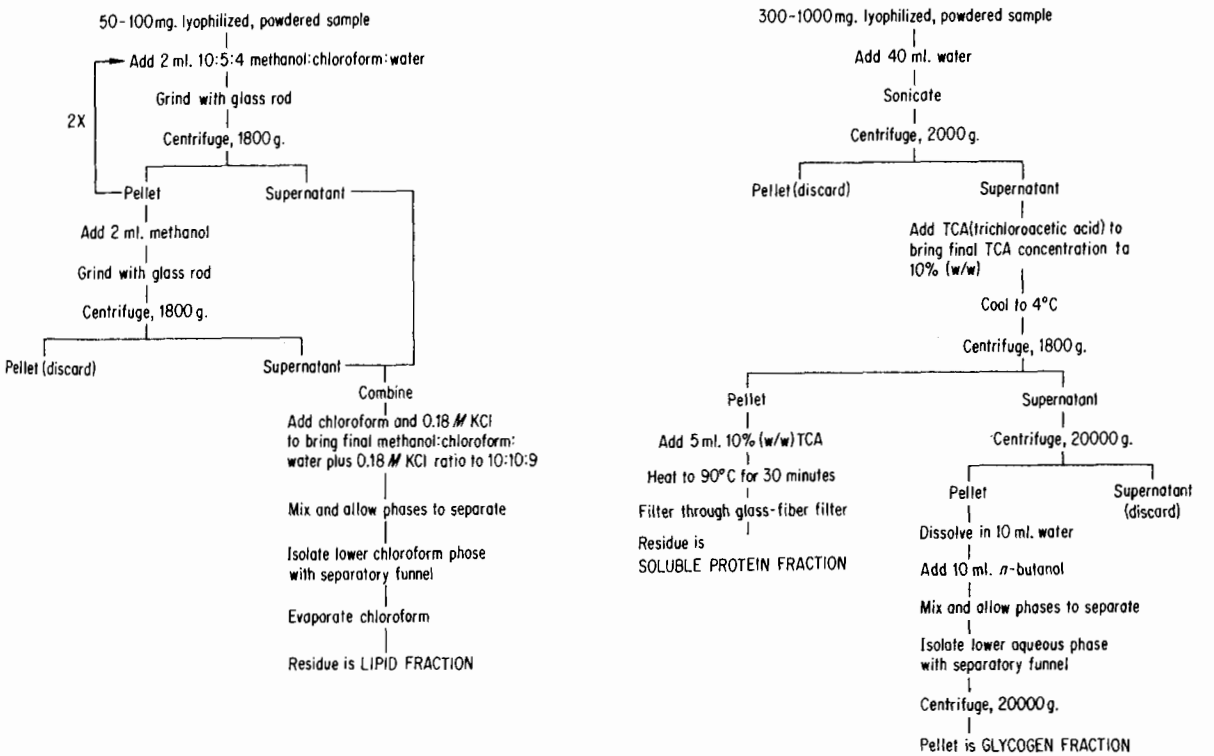

Fig. 1. Flow diagram for the isolation of the lipid, glycogen and soluble protein fractions.

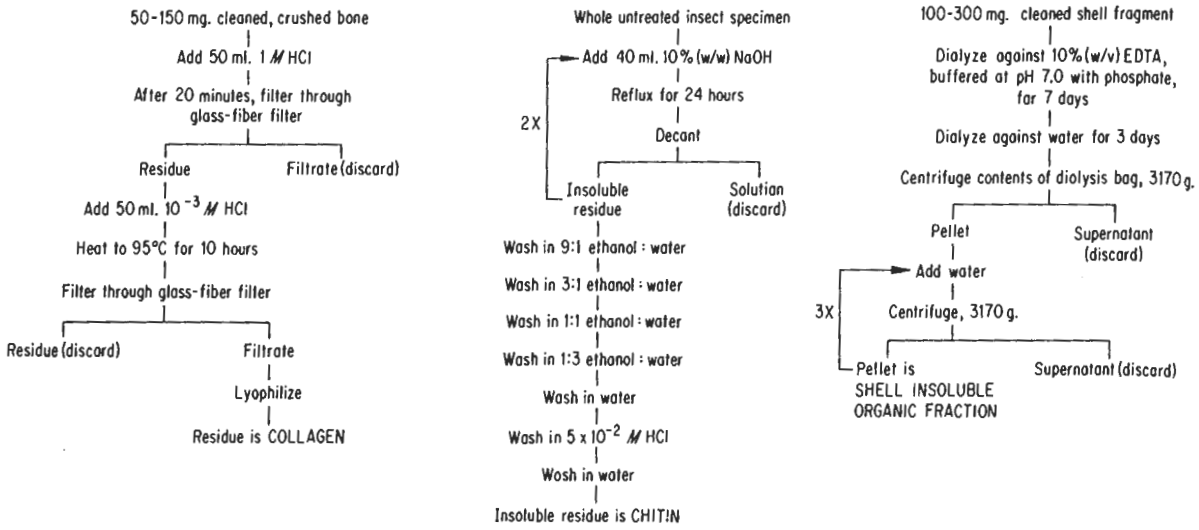

Fig. 2. Flow diagram for the isolation of collagen, chitin and the insoluble organic fraction of shells.

Mouse bones from which collagen was to be isolated were prepared by the following procedure. The flesh was removed from the bones by incubating them with dermestid beetle larvae for one month. Superficial debris was removed from the stripped bones using ultrasound. The bones were then lyophilized and any remaining debris was removed with forceps under a dissecting microscope. The cleaned bones were crushed in a steel piston. Snail shells from which the shell insoluble organic fraction was to be isolated were scrubbed in water to remove any superficial organic matter. No treatment was required to prepare insect specimens for the extraction of chitin. The procedures used to isolate collagen, chitin and the shell insoluble organic fraction are shown in the flow diagram of Fig. 2. These procedures are based on the published methods of Longin (1971), Tsao and Richards (1952) and Weiner *et al.* (1976).

Preparation of samples for isotopic analysis

Prior to combustion, samples of diets, whole bodies of animals, tissue specimens, biochemical fractions (excluding the lipid fractions) and biochemical components were lyophilized, then ground to a powder. Each lipid sample was taken up in 0.3 ml chloroform and transferred to a combustion boat. The chloroform was removed by evaporation and the sample was lyophilized prior to combustion.

Prior to isotopic analysis of their carbonate fractions, shell and bone samples were ground to a powder, then treated with a 50% aqueous solution of commercial Clorox to destroy organic matter (Lowenstam and Epstein, 1957).

Isotopic analysis

Calcium carbonate was converted to CO_2 for isotopic analysis by reaction with H_3PO_4 (McCrea, 1950). The precision of the carbonate analysis was $\pm 0.1‰$.

Organic carbon was converted to CO_2 for isotopic analysis by combustion in a stream of oxygen at 850°C (Craig, 1953). Water was removed from the combustion products by condensation in a dry ice bath. Oxides of nitrogen and sulfur were separated from the CO_2 by passage over Cu turnings and MnO_2 powder maintained at 450°C (Sackett and Thompson, 1963). The precision of the combustion procedure was $\pm 0.2‰$.

Respired CO_2 was collected for isotopic analysis in the line shown in Fig. 3. The line was evacuated, then filled with air scrubbed of CO_2 by passage over Ascarite and through a barium hydroxide bath. After the animals were introduced into the specimen bulb, trap I was immersed

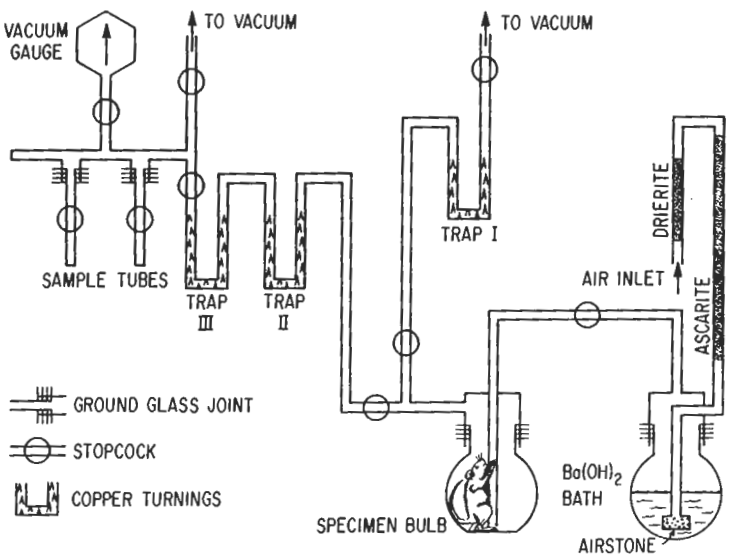

Fig. 3. High vacuum line for the collection of respiratory CO_2.

in liquid nitrogen. The resulting condensation of oxygen, carbon dioxide, and water caused the specimen bulb to be purged with CO_2-free air. The specimen bulb was isolated long enough to permit the animals to respire a CO_2 sample large enough for analysis, then swept with CO_2-free air by immersing trap II in a dry ice bath and trap III in liquid nitrogen. The respired CO_2 collected in trap III was purified from unidentified contaminants by passage over Cu turnings and MnO_2 powder maintained at 450°C (SACKETT and THOMPSON, 1963) prior to isotopic analysis. The precision of the respiratory CO_2 analysis was $\pm 0.3‰$.

The CO_2 samples were analyzed in a 60° sector, double-collecting mass spectrometer. The results are expressed in the usual $\delta^{13}C$ notation

$$\delta^{13}C(\text{in } ‰) = \left[\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{\text{standard}}} - 1\right] \cdot 1000.$$

The standard is the PDB carbonate.

RESULTS AND DISCUSSION

Relationships between the $\delta^{13}C$ values of the whole bodies of animals and the $\delta^{13}C$ values of their diets

The $\delta^{13}C$ values of the whole bodies of animals and the $\delta^{13}C$ values of their diets are shown in Fig. 4. The following conclusions can be drawn from these data.

(1) The animal carbon is in most cases enriched in ^{13}C relative to the diet carbon. In eight of the thirteen cases examined, the $\Delta_{\text{ANIMAL-DIET}}$ value ($\delta^{13}C_{\text{ANIMAL}} - \delta^{13}C_{\text{DIET}}$) is positive, while in only one case, *Caenorhabditis* (nematode) raised on bacteria, is it significantly negative. The average $\Delta_{\text{ANIMAL-DIET}}$ value is $+0.8 \pm 1.1‰$. Values range from $-0.6‰$ for *Caenorhabditis* raised on bacteria to $+2.7‰$ for *Desmia* (moth) raised on grape leaves and *Melanoplus* (grasshopper) raised on wheat seedlings.

(2) The $\delta^{13}C$ values of different individuals of a species raised on the same diet may differ. The range of $\Delta_{\text{ANIMAL-DIET}}$ values for individuals of a species varies from $0.2‰$ for *Calliphora* (blow fly) raised on pork ($n = 4$) to $1.8‰$ for *Melanoplus* (grasshopper) raised on wheat seedlings ($n = 4$).

(3) Two species fed the same diet have similar $\Delta_{\text{ANIMAL-DIET}}$ values. The largest difference in $\Delta_{\text{ANIMAL-DIET}}$ values for *Calliphora* (blow fly) and *Musca* (house fly) raised on horsemeat, *Calliphora* and *Musca* raised on pork, or *Sitophilus granarius* (weevil) and *S. oryzae* (weevil) raised on wheat seeds is $1‰$.

(4) The $\Delta_{\text{ANIMAL-DIET}}$ values for a species fed two different diets are similar. The largest difference in $\Delta_{\text{ANIMAL-DIET}}$ values for *Calliphora* (blow fly) raised on horsemeat or pork, *Musca* (house fly) raised on horsemeat or pork, or *Melanoplus* (grasshopper) raised on corn or wheat seedlings is $1‰$.

Carbon isotope mass balance between animals and their diets

The isotopic composition of carbon which an animal eats (input) must equal the integrated isotopic composition of the carbon which is incorporated into the body and that which is lost by respiration and excretion (output). Therefore, the ^{13}C enrichment of the whole animal relative to its diet must be balanced

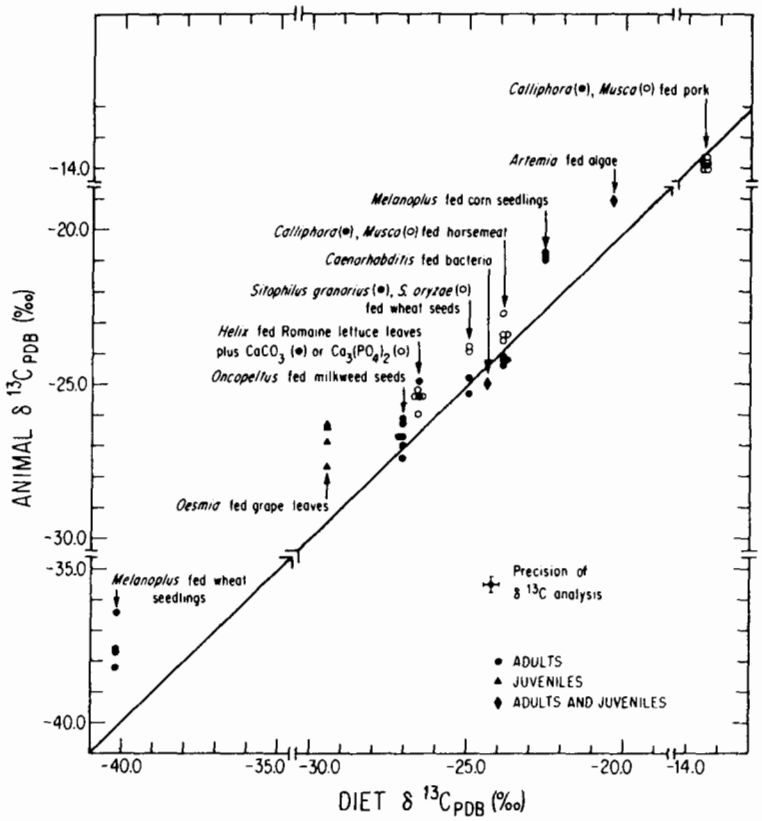

Fig. 4. $\delta^{13}C$ values of the whole bodies of animals and their diets. Each point represents the analysis of a single animal, except for the two *Sitophilus* species (five animals were combusted together for each point) and for *Artemia* and *Caenorhabditis* (many animals were combusted together for each point).

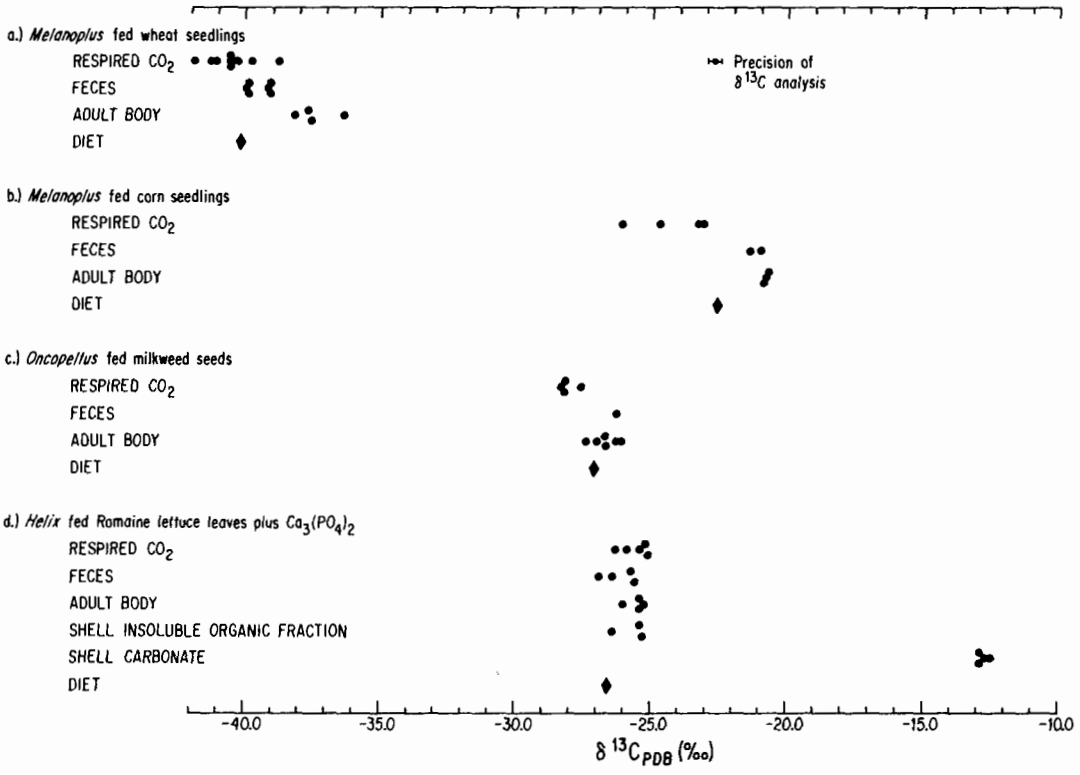

Fig. 5. $\delta^{13}C$ values of the input and analyzed output components of four animals. For *Helix*, uric acid and the slime laid down to aid in locomotion were not analyzed. Each point represents the analysis of the specified output component of a single animal, except for the feces (the output of several animals during one day was combined) and the respired CO_2 of *Oncopeltus* and *Helix* (the output of several animals was collected).

by a ^{13}C depletion, relative to the diet, of the respired CO_2, the excreted carbon, or both.

The $\delta^{13}C$ values of the input and output components of four animals are shown in Fig. 5. The same isotopic relationship among these components holds for the three insects analyzed (*Melanoplus* (grasshopper) fed wheat seedlings, *Melanoplus* fed corn seedlings, and *Oncopeltus* (milkweed bug) fed milkweed seeds): the respired CO_2 is depleted in ^{13}C relative to the diet, while the feces and the whole body carbon have more positive $\delta^{13}C$ values than the diet. It should be noted that in insects the nitrogenous waste products are incorporated into the feces (Wigglesworth, 1972), rather than excreted separately as in some higher animals, so that the feces in these cases represent all the output carbon lost through excretion.

The data needed to solve the isotopic mass balance expression for *Melanoplus* (grasshopper) raised on wheat seedlings are shown in Table 3. The sizes of the various input and output component pools, namely the weight of wheat seedlings eaten, the weight of feces produced, and the increase in body weight, over a five-day period for *Melanoplus sanguinipes*, the species used in this study, were estimated using the data of Smith (1959) for the closely related species *Melanoplus bilituratus*. The amount of carbon lost as respired CO_2, which Smith (1959) did not determine,

Table 3. Isotopic mass balance for *Melanoplus* fed wheat seedlings

Component	Total weight for five-day period[a] (in mg.)	Carbon concentration[b] (in μ moles CO_2 $mg.^{-1}$)	Total carbon for five-day period (in μ moles CO_2)	$\delta^{13}C_{PDB}$ (‰)
Input				
Food (wheat seedling)	148.0	34.5	5106	-40.2 ± 0.8
Output				
Respired (carbon dioxide)	not determined	---	1378[c]	-40.8 ± 0.6
Excreted (feces)	112.5	30.3	3409	-39.5 ± 0.4
Incorporated (whole body)	8.1	39.4	319	-37.5 ± 0.8
Total (calculated)	---	---	5106[c]	-39.7 ± 0.3

[a] Data of Smith (1959) for *Melanoplus bilituratus* adults.
[b] Average yield for samples from Fig. 5a.
[c] Calculated by assuming amounts of input and output carbon were equal.

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was assumed to make up the difference between the amounts of input and output carbon. The $\delta^{13}C$ value calculated for the output carbon, $-39.7 \pm 0.3‰$, agrees well with the $\delta^{13}C$ value of the input carbon, $-40.2 \pm 0.8‰$, indicating that the major output components have been identified and analyzed. The isotopic mass balance expressions for the other two insects could not be solved because data relating to the sizes of their input and output component pools were not available.

The apparent absence of isotopic mass balance between input and output components of the fourth animal reported in Fig. 5, *Helix* (snail) fed Romaine lettuce leaves plus $Ca_3(PO_4)_2$, is an artifact of the experimental design. It was not possible to collect either uric acid, which is excreted separately from the feces (POTTS, 1967), or the organic slime which is laid down by snails to aid in locomotion. The material balance suggests that at least one of these output components has a $\delta^{13}C$ value more negative than that of the diet. It is important to note that in this case the ^{13}C enrichment of the body relative to the diet is not balanced by a ^{13}C depletion of the respired CO_2, as was observed in the three other cases which were analyzed.

Relationships between the $\delta^{13}C$ values of the tissues of mice and the $\delta^{13}C$ values of their diets

It is impractical to determine the $\delta^{13}C$ value of the whole body of a large animal. An alternate approach useful for dietary analysis is to determine the isotopic composition of a part of the animal which reflects the $\delta^{13}C$ value of the diet. The $\delta^{13}C$ values of various tissues of mice raised on diets of constant carbon isotopic composition were measured in order to determine which tissues might be suitable for this purpose.

In the first experiment, tissues from eleven mice raised on a diet of Wayne Lab-Blox F6 were pooled and homogenized prior to analysis in order to minimize differences between individuals. The $\delta^{13}C$ values of the tissues and the diet are shown in Fig. 6a. Most of the pooled tissue samples have $\delta^{13}C$ values which are $\sim 1.5‰$ more negative than the $\delta^{13}C$ value of the diet. However, the $\delta^{13}C$ values of brain, hair and spleen are all more positive than that of the diet by up to 1‰. Analysis of tissues taken from individual mice raised on the same diet are also shown in Fig. 6a. The $\delta^{13}C$ values of brain, liver and kidney from individual animals do not differ from the $\delta^{13}C$ value of the pooled sample by more than 1‰. For some tissues, however, considerable variation among samples taken from individual mice was observed. Spleen and hair from individuals have $\delta^{13}C$ values which differ from the $\delta^{13}C$ value of the pooled sample by up to 3.6‰.

$\delta^{13}C$ values of tissues from individual mice raised on other diets and the $\delta^{13}C$ values of the diets are also shown in Fig. 6. Mice raised on a diet of Purina Rat Chow (Fig. 6b) have a distribution in the $\delta^{13}C$ values of their tissues similar to that observed for mice raised on Wayne Lab-Blox F6. Brain and hair generally have higher $\delta^{13}C$ values and the other organs have lower $\delta^{13}C$ values than the diet. The main difference between mice raised on the two diets is the absence of ^{13}C enrichment relative to organs other than brain and hair in the spleens of mice fed Purina Rat Chow. Mice raised on a diet of JAX 911A (Fig. 6c) differ in two features of the distribution of carbon isotopes in their tissues compared with mice raised on the other two diets. First, the liver and kidney of these mice have more positive $\delta^{13}C$ values than the diet. Second, the brain has a $\delta^{13}C$ value slightly lower than those of the other tissues analyzed. The basis for the non-uniform distribution of carbon isotopes among the various tissues of mice and the effect of diet on that distribution are being investigated.

In summary, the data shown in Fig. 6 indicate that no single tissue can be analyzed in order to determine the carbon isotopic relationship between the animal and its diet. For the purpose of dietary analysis, the determination of the $\delta^{13}C$ values of several tissues from an animal will allow for a better estimate of the $\delta^{13}C$ value of its diet than would the analysis of a single tissue.

Relationships between the $\delta^{13}C$ values of biochemical fractions in animals and in their diets

As discussed previously, different species raised on the same diet may have small differences in their $\Delta_{ANIMAL-DIET}$ values. Similarly, individuals of the same species raised on different diets may have slightly different $\Delta_{ANIMAL-DIET}$ values. Differences in the relative proportions of the major biochemical fractions incorporated from the diet into the animal could account for part of this variability in the isotopic relationships, because the major biochemical fractions have characteristically different $\delta^{13}C$ values (ABELSON and HOERING, 1961; PARK and EPSTEIN, 1961; PARKER, 1964). If this is so, comparison of the $\delta^{13}C$ values of biochemical fractions in an animal and in its potential diet sources might provide a more accurate measure of diet than would analysis of the total carbon. This approach assumes that there is little or no isotopic fractionation during the incorporation of the biochemical fraction by the animal.

Accordingly, the $\delta^{13}C$ values of the total organic matter and of the lipid, carbohydrate (as glycogen) and soluble protein fractions were determined for horsemeat and pork and for each of two species of flies, *Calliphora* and *Musca*, which were raised on each meat. The results of this analysis are shown in Fig. 7. The difference between the $\delta^{13}C$ value of the total organic matter of a fly and that of its diet is less than 0.5‰ for all four cases. However, the $\delta^{13}C$ value of a biochemical fraction of a fly may differ from the $\delta^{13}C$ value of the same fraction of the diet by up to 3.0‰.

The data shown in Fig. 7 indicate that the biochemical fractions of the diet are not incorporated

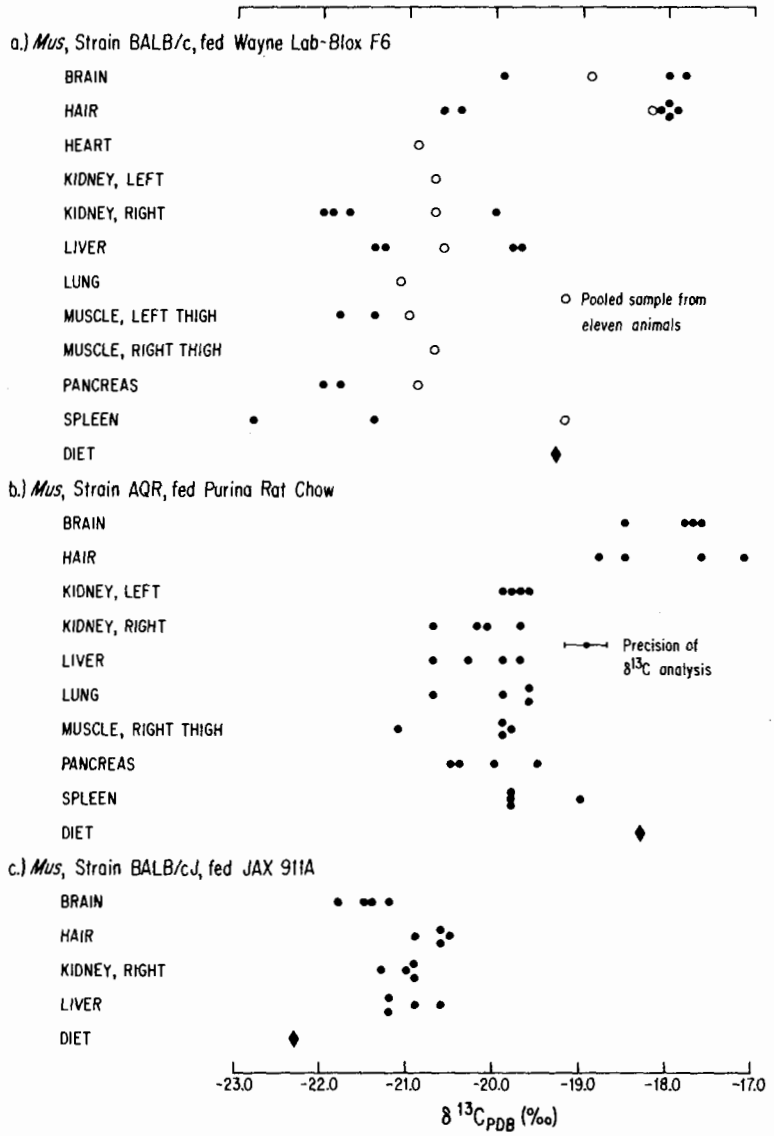

Fig. 6. $\delta^{13}C$ values of tissues of mice and their diets. Each point represents the analysis of tissue dissected from a single mouse, except as indicated.

into the animal without isotopic alteration. The difference between the $\delta^{13}C$ values of a fraction in an animal and the corresponding fraction in its diet could arise from isotope effects during incorporation of the fraction or during the *de novo* synthesis of some components of the fraction in the animal. The observation that these isotope effects tend to balance one another when averaged over the whole of an animal's metabo-

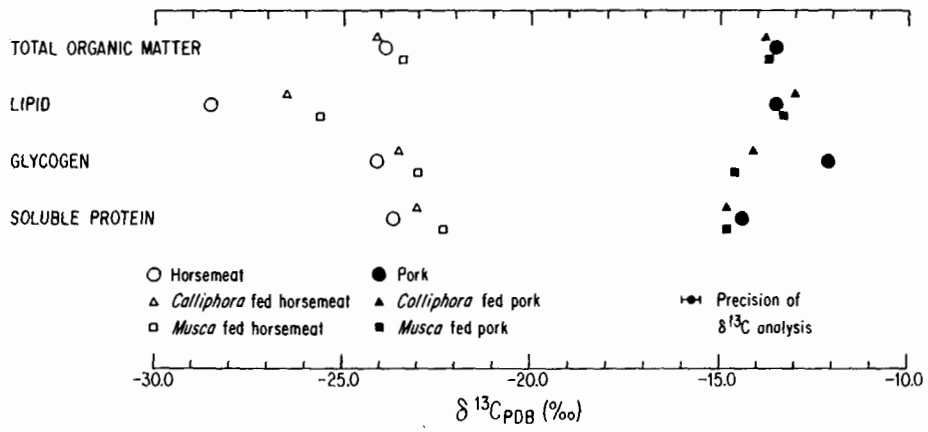

Fig. 7. $\delta^{13}C$ values of the total organic matter and the major biochemical fractions of two species of flies and their diets. Between 50 and 100 flies were combined to make the samples of total organic matter from which the biochemical fractions were isolated.

lism suggests that changes in the $\delta^{13}C$ value of one fraction occur at the expense of the isotopic composition of some other fraction, presumably by means of the numerous metabolic pathways which permit the conversion of carbon from one biochemical fraction to another (MAHLER and CORDES, 1971).

The isotopic relationships among the biochemical fractions of horsemeat, namely $\delta^{13}C_{LIPID} < \delta^{13}C_{TOTAL\ ORGANIC\ MATTER}$, $\delta^{13}C_{LIPID} < \delta^{13}C_{CARBOHYDRATE}$ and $\delta^{13}C_{LIPID} < \delta^{13}C_{PROTEIN}$, follow the pattern that has been observed in all animals, plants and microorganisms which have been analyzed (data compiled in DENIRO, 1977). The isotopic distribution among the biochemical fractions of pork is the first case in which all these relationships have not been observed. The different isotopic relationships among the biochemical fractions of the two meats probably reflect differences in the feeding regimes of the animals. Horses intended for slaughter are generally fed the same diet throughout their lives (CARR, 1977). On the other hand, the pork was obtained from corn-fed pigs (JOHN, 1977), which are fed mixed grains when they are young and, in order to fatten them for market, are fed a diet rich in corn during the second half of their lives (CARR, 1977). Most grains except for corn come from C_3 plants, and therefore have $\delta^{13}C$ values more negative than corn, which is a C_4 plant (SMITH and EPSTEIN, 1971). If the lipids and glycogen of the pork were formed predominantly during the fattening period, their $\delta^{13}C$ values would be more positive (corn-like) than that of the protein fraction. The lower $\delta^{13}C$ value of the pork lipid fraction relative to that of the carbohydrate (as glycogen) fraction is the usual relationship observed between the $\delta^{13}C$ values of these two fractions.

In spite of differences in the absolute $\delta^{13}C$ values of biochemical fractions in animals and in their diets, many of the isotopic relationships among the fractions appear to be inherited from the diet. The relationships $\delta^{13}C_{LIPID} < \delta^{13}C_{TOTAL\ ORGANIC\ MATTER}$, $\delta^{13}C_{LIPID} < \delta^{13}C_{CARBOHYDRATE}$ and $\delta^{13}C_{LIPID} < \delta^{13}C_{PROTEIN}$, which exist among the biochemical fractions of the horsemeat are also observed in the flies which were raised on it. The biochemical fractions of both species of flies raised on pork show the same anomalous isotopic relationships which exist among the corresponding fractions of the pork, namely $\delta^{13}C_{LIPID} \geq \delta^{13}C_{TOTAL\ ORGANIC\ MATTER}$ and $\delta^{13}C_{LIPID} > \delta^{13}C_{PROTEIN}$. In the instance of horsemeat and the flies which were raised on it, the similarities in the isotopic relationships among the biochemical fractions may arise from similarities in the biochemical pathways by which the fractions are synthesized. This possibility does not apply in the case of pork and the flies which were raised on it, since the irregular isotopic relationships among the biochemical fractions of the pork do not result from isotopic fractionation during synthesis. From these limited data, it appears that many of the isotopic relationships among the biochemical fractions at one trophic level will be inherited by the organisms at the next trophic level.

In summary, the data shown in Fig. 7 indicate that the $\delta^{13}C$ analysis of the total organic matter of animals results in a more accurate measure of the $\delta^{13}C$ value of the diet than does the determination of the $\delta^{13}C$ values of their biochemical fractions. Nevertheless, analysis of the isotopic distribution among the biochemical fractions of an animal may, in some cases, provide information about its dietary history.

Relationships between the $\delta^{13}C$ values of some biochemical components of animals which are often preserved in fossil material and the $\delta^{13}C$ values of their diets

Information about the $\delta^{13}C$ value of an animal's diet may be obtained from its fossil organic remains if two requirements are met. First, the $\delta^{13}C$ value of some component synthesized by the animal and preserved in the fossil material must remain unaltered by diagenetic processes. Second, the relationship between the $\delta^{13}C$ value of such a component and the $\delta^{13}C$ value of the diet of the animal in which it was synthesized must be known. This first requirement is often met by biochemical components of animals, such as collagen, chitin or the organic fraction of invertebrate shells, which retain their chemical identity in fossil material (ISAACS *et al.*, 1963; ROSENHEIM, 1905; WEINER *et al.*, 1976). Accordingly, the influence of diet on the $\delta^{13}C$ values of these components was determined.

The $\delta^{13}C$ values of chitin isolated from insect exoskeletons, collagen from the bones of mice, and the insoluble organic fraction of snail shells and the $\delta^{13}C$ values of the animals' diets are shown in Fig. 8. The average differences between the $\delta^{13}C$ values of chitin and the $\delta^{13}C$ value of the animal's diet for the four cases analyzed range from +0.1 to +1.7‰. There is considerable variability, ranging up to 3.5‰, in the $\delta^{13}C$ values of chitin isolated from individuals of a species raised on the same diet.

The differences between the $\delta^{13}C$ values of collagen samples and the $\delta^{13}C$ value of the diet for mice raised on diets of JAX 911A or Wayne Lab-Blox F6 are +3.7‰ and +2.8‰ respectively. The $\delta^{13}C$ values of collagen samples isolated from individuals raised on the same diet agree to within the precision of the analysis.

The $\delta^{13}C$ values for the insoluble organic fraction of *Helix* (snail) shells isolated from individual snails whose diet of Romaine lettuce leaves was supplemented with $Ca_3(PO_4)_2$ are more positive than that of the diet by up to 1.5‰. The corresponding $\delta^{13}C$ values for *Helix* individuals in which the same diet was supplemented with $CaCO_3$ (the $\delta^{13}C$ value of the carbonate being 13.2‰ more positive than that of the diet) are similar, indicating that the contribution of carbon from the $CaCO_3$ to the insoluble organic fraction of the shell was negligible.

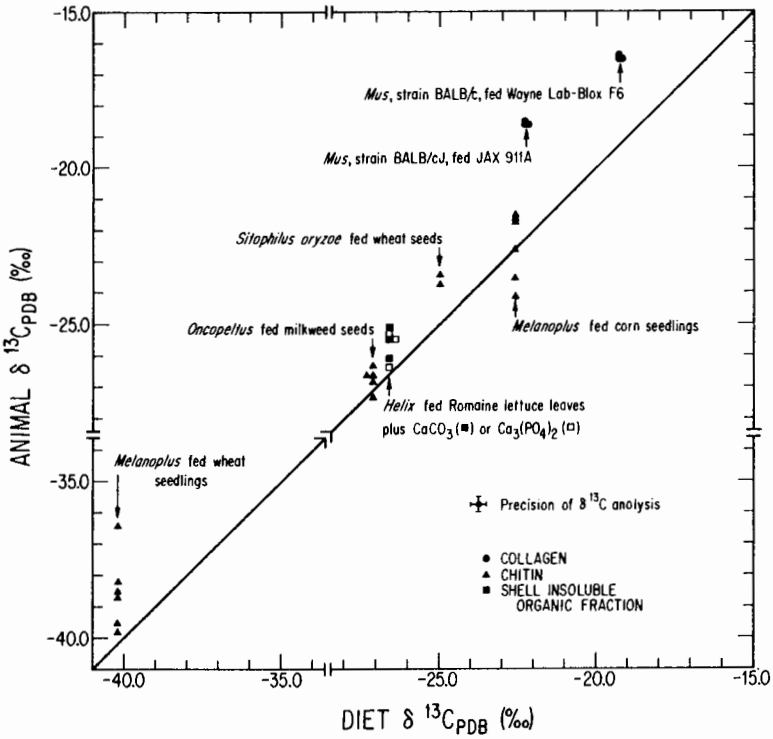

Fig. 8. $\delta^{13}C$ values of some biochemical components of animals which are often preserved in fossil material and their diets. Each point represents the analysis of the specified component isolated from a single animal, except for *Sitophilus oryzae* (chitin samples from ten animals were combusted together for each point).

DIETARY ANALYSIS BASED ON THE $\delta^{13}C$ VALUE OF ANIMAL CARBON

The methods commonly used to determine what an animal eats involve either visual observation or analysis of undigested food fragments in its stomach or feces. These methods are time consuming and are subject to considerable error, the first due to observer fatigue and the second due to differential digestability of different foods. Additionally, the first method does not lend itself to analysis of fossil situations, although the second can sometimes be applied if the feces are preserved.

Dietary analysis based on the determination of the $\delta^{13}C$ value of animal carbon offers a considerable improvement over these methods in some modern and fossil situations. The isotopic procedure consists of two steps: (1) estimating the $\delta^{13}C$ value of the diet from the $\delta^{13}C$ value of animal carbon; (2) determining the relative contribution of potential diet sources of known $\delta^{13}C$ values which would produce the $\delta^{13}C$ value for the diet estimated in the first step.

The isotopic composition of an animal's diet can be estimated from the $\delta^{13}C$ value of animal carbon by taking into account isotope effects during the incorporation of diet carbon into the animal. The magnitude of these isotope effects for carbon incorporated into whole animals and a number of suborganismic components has been discussed above. Analysis of the carbon of the whole animal will allow for the most accurate estimate of the $\delta^{13}C$ value of the diet, since this carbon serves as an integrator of dietary input. Analysis of a suborganismic component will generally provide a less accurate estimate of the $\delta^{13}C$ value of the diet because the isotopic composition of such carbon reflects various sources and isotopic fractionations during incorporation for which reliable corrections cannot always be made at present. Compounds such as vitamins or the essential amino acids, which cannot be synthesized by animals and therefore must be incorporated directly from the diet, could prove to be exceptions to this rule, since the $\delta^{13}C$ values of such compounds in an animal and in its diet should be identical, barring fractionation during incorporation or metabolism. Analysis of several specimens of an animal species taken from a locality should further improve the accuracy of the estimated diet $\delta^{13}C$ value by reducing the uncertainty caused by variations among individuals.

The isotopic method of dietary analysis can be applied to fossil material if the original $\delta^{13}C$ value of some component has been preserved. In many fossil situations, the best preserved animal carbon is contained in the carbonate fraction of invertebrate shells or vertebrate bones. The $\delta^{13}C$ values of the shell carbonates of marine and freshwater mollusks are known to reflect primarily the $\delta^{13}C$ values of CO_2 dissolved in the water (Keith *et al.*, 1964; Fritz and Poplawski, 1974) rather than the diet $\delta^{13}C$ values. Aquatic carbonate-depositing organisms of other phyla probably derive the carbon of their shell carbonates from the same source. The source of carbon of the shell carbonate fraction of terrestrial snails is

not known. However, even if it can be shown that there is some dietary influence on the isotopic composition of terrestrial snail shell carbonate, the prospects for using this value to estimate the $\delta^{13}C$ value of the diet appear dim, since relatively small contributions of carbon from atmospheric CO_2 or ingested $CaCO_3$ (RUBIN *et al.*, 1963), both of which have $\delta^{13}C$ values much more positive than diet sources, would have a large effect on the $\delta^{13}C$ value of the shell carbonate.

The source of carbon in the carbonate fraction of vertebrate bone has not been established. Preliminary results indicate that there is dietary influence on the $\delta^{13}C$ value of this fraction. The carbonate of bones taken from three mice raised on a diet of JAX 911A ($\delta^{13}C = -22.3 \pm 0.5‰$) have $\delta^{13}C$ values of -12.6, -12.6 and $-12.5‰$. Three mice raised on a diet of Wayne Lab-Blox F6 ($\delta^{13}C = -19.3 \pm 0.4‰$) have bones in which the $\delta^{13}C$ values of the carbonate fraction are -9.8, -9.8 and $-9.8‰$. The difference of 3‰ between the $\delta^{13}C$ values of the diets is reflected in the $\delta^{13}C$ values of the bone carbonates. However, the $\delta^{13}C$ values of the carbonate fraction of fossil bone probably cannot be used for dietary analysis. Measurements of ^{14}C in fossil bones have shown that the carbon in the carbonate fraction is exchangeable with CO_2 dissolved in groundwater (OLSON, 1963) and/or atmospheric CO_2 (TAMERS and PEARSON, 1965). Thus, the original $\delta^{13}C$ value of the carbonate fraction is not likely to be preserved in fossil bone.

The relative amounts of potential diet sources eaten by an animal can be determined from the $\delta^{13}C$ value of the animal's carbon if the diet sources have sufficiently different $\delta^{13}C$ values. In many situations, the small range and overlap of the $\delta^{13}C$ values of potential diet sources will not permit the determination of the contribution of each source to an animal's diet. In two general cases, however, the potential diet sources come from groups which differ sufficiently in $\delta^{13}C$ value so that the relative contribution of each group to the diet can be measured by determining the $\delta^{13}C$ value of animal carbon. The $\delta^{13}C$ values of aquatic plants and animals often do not overlap those of terrestrial organisms (CRAIG, 1953; DEGENS, 1969; SCHWARCZ, 1969). Thus, it should be possible to use this difference to determine the relative contribution of these two types of organisms to the diets of animals living in near-shore environments. The second case involves C_3 and C_4 plants as potential diet sources. The $\delta^{13}C$ values of most C_3 plants range from $-24‰$ to $-34‰$, while most C_4 plants have $\delta^{13}C$ values which lie between $-6‰$ and $-19‰$ (SMITH and EPSTEIN, 1971). This difference is large enough so that the relative amounts of C_3 and C_4 plants eaten by an animal can be determined from the $\delta^{13}C$ value of its carbon. One especially interesting application of the isotopic method of determining diet in this regard would be a field test of the hypothesis that herbivores are selective in avoiding C_4 plants in their diets (CASWELL *et al.*, 1973).

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