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

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

The isotopic composition of the nitrogen in an animal reflects the nitrogen isotopic composition of its diet. The $\delta^{15}N$ values of the whole bodies of animals are usually more positive than those of their diets. Different individuals of a species raised on the same diet can have significantly different $\delta^{15}N$ values. The variability of the relationship between the $\delta^{15}N$ values of animals and their diets is greater for different species raised on the same diet than for the same species raised on different diets. Different tissues of mice are also enriched in ^{15}N relative to the diet, with the difference between the $\delta^{15}N$ values of a tissue and the diet depending on both the kind of tissue and the diet involved. The $\delta^{15}N$ values of collagen and chitin, biochemical components that are often preserved in fossil animal remains, are also related to the $\delta^{15}N$ value of the diet.

The dependence of the $\delta^{15}N$ values of whole animals and their tissues and biochemical components on the $\delta^{15}N$ value of diet indicates that the isotopic composition of animal nitrogen can be used to obtain information about an animal's diet if its potential food sources had different $\delta^{15}N$ values. The nitrogen isotopic method of dietary analysis probably can be used to estimate the relative use of legumes vs non-legumes or of aquatic vs terrestrial organisms as food sources for extant and fossil animals. However, the method probably will not be applicable in those modern ecosystems in which the use of chemical fertilizers has influenced the distribution of nitrogen isotopes in food sources.

The isotopic method of dietary analysis was used to reconstruct changes in the diet of the human population that occupied the Tehuacan Valley of Mexico over a 7000 yr span. Variations in the $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen suggest that C_4 and/or CAM plants (presumably mostly corn) and legumes (presumably mostly beans) were introduced into the diet much earlier than suggested by conventional archaeological analysis.

INTRODUCTION

THE STABLE isotopic composition of the carbon in an animal reflects the $^{13}C/^{12}C$ ratio of its diet (DENIRO and EPSTEIN, 1978; HAINES, 1976; MINSON *et al.*, 1975; TEERI and SCHOELLER, 1979). It follows from this observation that certain aspects of diet can be reconstructed from the isotopic ratios of animal carbon if an animal's potential food sources had different $^{13}C/^{12}C$ ratios. It might be possible to study other aspects of diet by isotopic analysis if it could be shown that the isotopic ratios of other elements that comprise animal tissue also reflect the isotopic composition of the diet.

The relationship between the nitrogen isotopic composition of an animal and that of its diet has not been determined previously. Analysis of the $^{15}N/^{14}N$ ratios of organisms occupying successive levels in marine and freshwater food chains (phytoplankton, zooplankton and fish) (MIYAKE and WADA, 1967; WADA and HATTORI, 1976; PANG and NRIAGU, 1977) suggests that the $\delta^{15}N$ values of animals reflect the $\delta^{15}N$ values of their diets, although animals appear to incorporate dietary ^{15}N preferentially over dietary ^{14}N. Analysis of blood collected from cows raised in the field (STEELE and DANIEL, 1978) also suggests that the nitrogen incorporated into animals is enriched in ^{15}N relative to diet nitrogen.

The isotopic relationship between an animal and its diet cannot be determined reliably from analysis of plants and animals collected in the field because neither the makeup of the diet nor its isotopic composition can be known accurately in such studies (DENIRO and EPSTEIN, 1978). Accordingly, we have determined this relationship directly by analyzing animals which have been raised in the laboratory on diets of known and constant $\delta^{15}N$ value. The results indicate that the $\delta^{15}N$ value of an animal's diet can be estimated from the $\delta^{15}N$ values of either its whole body or of its individual tissues and biochemical components. Isotopic analysis of animal nitrogen can thus be used to reconstruct aspects of diet when potential food sources had different $^{15}N/^{14}N$ ratios. In order to demonstrate the usefulness of the isotopic method of dietary reconstruction, we used the relationships derived in this study and a previous analysis of the

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Table 1. The animals and diets that were analyzed in this study. The $\delta^{15}N$ values of the diets are also given. The number of diet samples collected and analyzed is indicated for those diets that were sampled more than once

ANIMAL	DIET	DIET $\delta^{15}N_{AIR}$ (‰)
Artemia salina (brine shrimp)	Algae (*Dunaliella* sp.)	−8.5
Caenorhabditis elegans (nematode)	Bacteria (*Escherichia coli*)	+3.3
Calliphora vicina (blow fly)	Horsemeat (*Equus caballus*)	+5.2
	or	
	Pork (*Sus scrofa*)	+5.4
Desmia funeralis (moth)	Grape leaves (*Vitis vinifera*)	+2.2
Helix aspersa (snail)	Romaine lettuce leaves (*Lactuca sativa*)	+2.8 ± 1.1 (n=3)
Melanoplus sanguinipes (grasshopper)	Corn seedlings (*Zea mays*)	0.0 ± 1.4 (n=3)
	or	
	Wheat seedlings (*Triticum aestivum*)	+0.8 ± 2.4 (n=3)
Mus musculus, Strain AQR (mouse)	Purina Rat Chow mixture	+5.8 ± 0.3 (n=4)
Mus musculus, Strain BALB/c (mouse)	Wayne Lab-Blox F6 mixture	+5.1 ± 0.7 (n=4)
Mus musculus, Strain BALB/cJ (mouse)	JAX 911A mixture	+4.6 ± 0.3 (n=10)
Musca domestica (house fly)	Horsemeat (*Equus caballus*)	+5.2
	or	
	Pork (*Sus scrofa*)	+5.4
Oncopeltus fasciatus (milkweed bug)	Milkweed seeds (*Asclepias syriaca*)	+6.6
Sitophilus granarius (weevil) or *Sitophilus oryzae* (weevil)	Wheat seeds (*Triticum aestivum*)	+1.3

influence of diet on the distribution of carbon isotopes in animals (DENIRO and EPSTEIN, 1978) to interpret variations in the $\delta^{15}N$ and $\delta^{13}C$ values of collagen isolated from human bones deposited during a seven thousand year occupation of sites in the Tehuacan Valley of Mexico.

A preliminary report of this work was presented by DENIRO and EPSTEIN (1979).

EXPERIMENTAL METHODS

All animals analyzed in this study were raised from birth on diets of known and constant nitrogen isotopic composition. The animals and their diets are listed in Table 1. The animals were the same ones as those used in a study of the influence of diet on the distribution of carbon isotopes in animals (DENIRO and EPSTEIN, 1978). The techniques used in raising the animals, in sampling them and their diets, in separating individual tissues and biochemical components and in preparing samples for isotopic analysis were the same as those used in the carbon isotope study except for a modification made in the procedure used to isolate collagen from bone. After the bone was demineralized in 1 M HCl and filtered as described previously, the residue was soaked in 0.125 M aqueous sodium hydroxide for 20 hr, filtered on a glass-fiber filter, then flushed with water until the filtrate was neutral. The residue was then subjected to the previously described gelatinization step

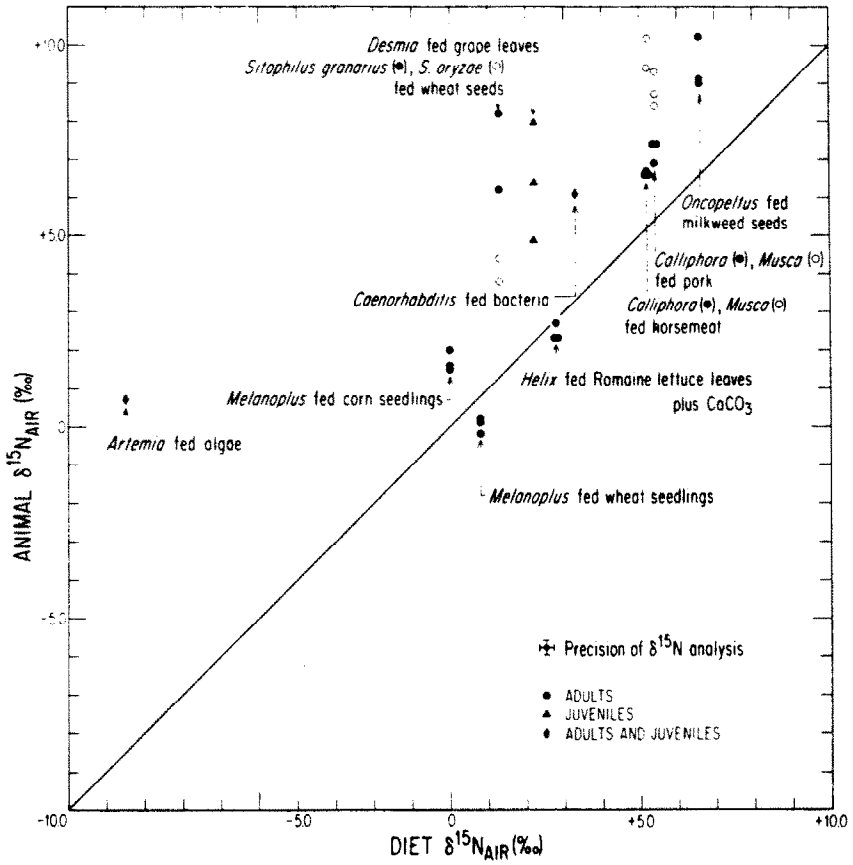

Fig. 1. $\delta^{15}N$ values of the whole bodies of animals and their diets. The whole body $\delta^{15}N$ values are for individual animals, except for the two *Sitophilus* species (three specimens were combusted together for each point) and for *Artemia* and *Caenorhabditis* (many animals were combusted together for each point).

that involved heating it in 10^{-3} M HCl. The sodium hydroxide soak was added to the procedure in order to prevent humic acids from contaminating the collagen isolated from fossil bone (HÅKANSSON, 1976).

The total carbon and nitrogen in the samples were converted to carbon dioxide and molecular nitrogen for isotopic analysis by the combustion method of STUMP and FRAZER (1973). The gas samples were analyzed in 60° sector, double-collecting mass spectrometers. The results are expressed in the δ notation

$$\delta^{*}X = \left[\frac{(^{*}X/X)_{\text{sample}}}{(^{*}X/X)_{\text{standard}}} - 1\right] \cdot 1000‰$$

where $^{*}X$ and X are the heavier and lighter stable isotopes of the element. For nitrogen, the standard is atmospheric nitrogen (AIR); for carbon, the standard is the Peedee belemnite (PDB) carbonate. The precisions of both the $\delta^{15}N$ and $\delta^{13}C$ analyses are $\pm 0.2‰$.

RESULTS AND DISCUSSION

Relationships between the $\delta^{15}N$ values of the whole bodies of animals and the $\delta^{15}N$ values of their diets

The $\delta^{15}N$ values of the whole bodies of animals and the $\delta^{15}N$ values of their diets are shown in Fig. 1. The following conclusions can be drawn from these data.

(1) The animal nitrogen is in most cases enriched in ^{15}N relative to the diet nitrogen. The $\Delta_{\text{animal-diet}}$ values ($\delta^{15}N_{\text{animal}}$-$\delta^{15}N_{\text{diet}}$) range from $-0.5‰$ to $+9.2‰$, averaging $+3.0 \pm 2.6‰$ ($n = 13$).

(2) The $\delta^{15}N$ values of different individuals of a species raised on the same diet can differ. The range in the $\delta^{15}N$ values of individuals varies from 0.1‰ for *Calliphora* (blow fly) raised on horsemeat ($n = 3$) to 3.1‰ for *Desmia* (moth) raised on grape leaves ($n = 3$).

(3) Two species fed the same diet can have large differences in their $\Delta_{\text{animal-diet}}$ values. The values for *Calliphora* (blow fly) and *Musca* (house fly) raised on horsemeat or *Sitophilus granarius* (weevil) and *S. oryzae* (weevil) raised on wheat seeds differ by about 3.2‰ for the two species in each case. The difference in $\Delta_{\text{animal-diet}}$ values between *Calliphora* and *Musca* raised on pork is 1.6‰.

(4) The $\Delta_{\text{animal-diet}}$ values for a species fed 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 and *Melanoplus* (grasshopper) raised on corn or wheat seedlings is about 1‰.

Relationships between the $\delta^{15}N$ values of the tissues of mice and the $\delta^{15}N$ values of their diets

In many cases, it is more practical to measure the $\delta^{15}N$ value of a part of an animal rather than its

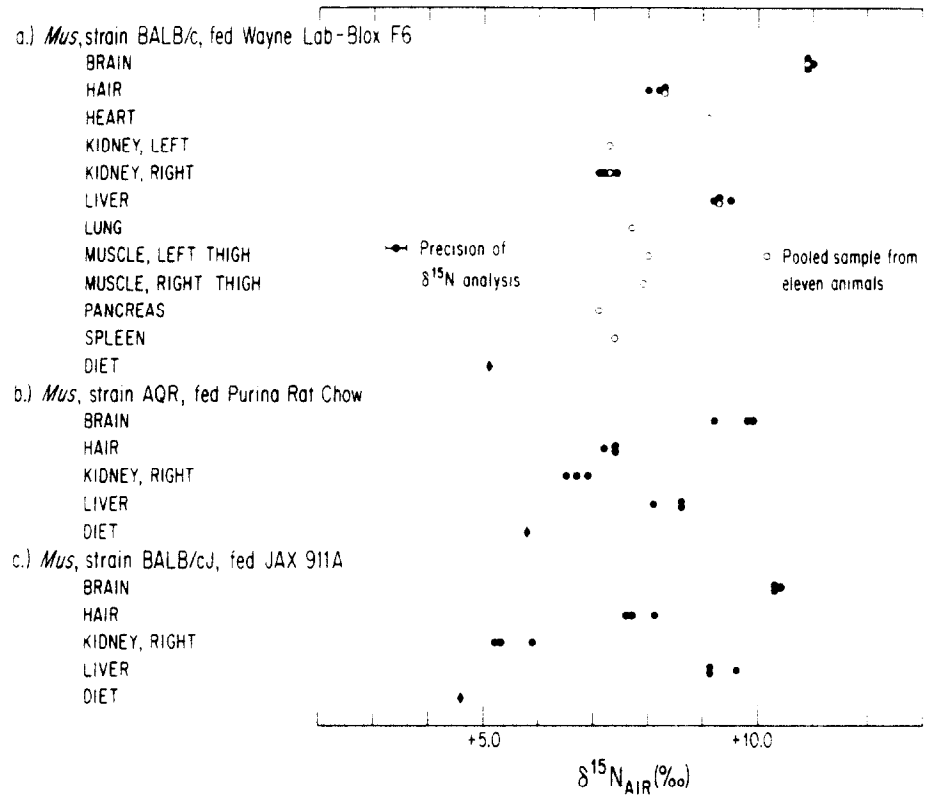

Fig. 2. $\delta^{15}N$ values of tissues of mice and their diets. Each point represents the analysis of tissue dissected from a single mouse, except as indicated.

whole body for dietary analysis. Consequently, the $\delta^{15}N$ value of various tissues of mice raised on diets of known nitrogen isotopic composition were compared to the $\delta^{15}N$ values of the diet.

The tissues from eleven mice raised on a diet of Wayne Lab-Blox F6 were pooled and homogenized prior to analysis to minimize the effects of differences between individuals. The $\delta^{15}N$ values of the tissues and the diet are shown in Fig. 2a. As is the case for whole animals, the tissues have $\delta^{15}N$ values which are more positive than that of the diet. The difference ranges from 2.0‰ for pancreas up to 5.8‰ for brain. The $\delta^{15}N$ values for tissues taken from individual mice raised on the same diet cluster around the $\delta^{15}N$ values of the pooled samples, indicating that there are only small differences between individuals. This observation is reinforced by analysis of tissues taken from individual mice raised on two other diets, shown in Fig. 2b and c.

The data shown in Fig. 2 demonstrate the similarity in the relationships of nitrogen isotope distribution among different tissues for mice raised on different diets. In all three cases, the $\delta^{15}N$ values increase progressively from kidney, to hair, to liver, to brain. This progressive increase was not observed in the $\delta^{15}N$ values of these four tissues from a single white rat analyzed by HOERING (1956). It is difficult to relate Hoering's results to those of the present study, since the dietary regime of his rat was not specified.

In summary, the data shown in Fig. 2 indicate that the relationship between the $\delta^{15}N$ value of a given tissue in an animal and the $\delta^{15}N$ value of the animal's diet depends both on the tissue being analyzed and on the nature of the diet. Thus, the estimation of the $\delta^{15}N$ value of diet should preferably be based on analysis of several types of tissue, if possible.

Relationships between the $\delta^{15}N$ values of some biochemical components of animals which are often preserved in fossil material and the $\delta^{15}N$ values of their diets

Information about the diet of a fossil animal can sometimes be obtained by isotopic analysis of a component whose original isotopic composition has not been altered by diagenetic processes. The isotopic relationship between the component and the diet must be known in order to perform this type of dietary reconstruction. Consequently, the $\delta^{15}N$ values of collagen, chitin and the insoluble organic fraction of shell, isolated from animals raised on diets of constant nitrogen isotopic composition, were analyzed. These components are often preserved in fossil material without having undergone apparent chemical alteration (e.g. ISAACS *et al.*, 1963; ROSENHEIM, 1905; WEINER *et al.*, 1976) and when such preservation occurs, are likely to retain their original $\delta^{15}N$ values.

The $\delta^{15}N$ values for chitin from insect exoskeletons, for collagen from mouse bones and for the in-

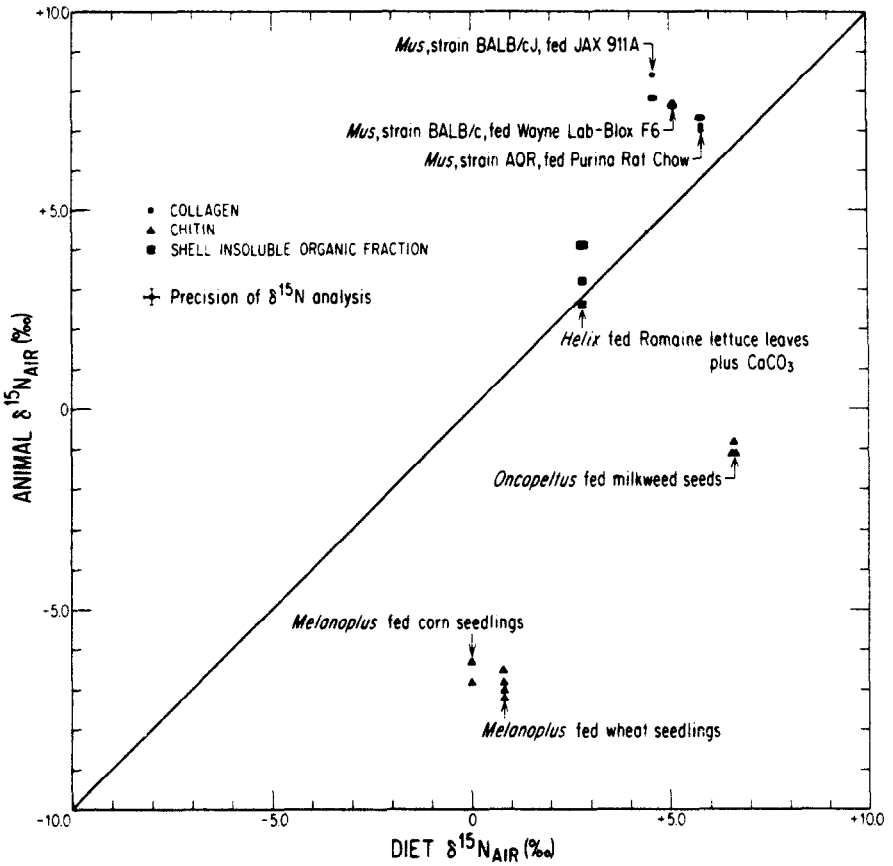

Fig. 3. $\delta^{15}N$ values of some biochemical components of animals which are often preserved in fossil material and their diets. Each point represents the analysis of the specified component isolated from a single animal.

soluble organic fraction of snail shells and the $\delta^{15}N$ values of the diets on which the animals were raised are shown in Fig. 3. The $\delta^{15}N$ values of chitin are more negative than the $\delta^{15}N$ values of the insects' diets. The average difference ranges from 6.6‰ for *Melanoplus* (grasshopper) raised on corn seedlings to 8.6‰ for *Oncopeltus* (milkweed bug) raised on milkweed seeds. The insoluble organic fraction of *Helix* (snail) shells is slightly enriched in ^{15}N relative to the diet. The average differences between the $\delta^{15}N$ values of mouse bone collagen and the $\delta^{15}N$ value of the diet is +1.4‰ for mice raised on Purina Rat Chow, +2.5‰ for mice raised on Wayne Lab-Blox F6, and +3.4‰ for those raised on JAX 911A.

The $\delta^{13}C$ values of collagen prepared from mouse bones according to the modified procedure outlined in this paper were also determined. The average difference between the $\delta^{13}C$ values of collagen and diet were +4.4‰, +3.8‰ and +3.5‰ for mice raised on JAX 911A, Wayne Lab-Blox F6, and Purina Rat Chow, respectively. For purposes of comparison, the corresponding values for collagen prepared according to the procedure outlined in DeNiro and Epstein (1978) were +3.7‰ and +2.8‰ for mice raised on JAX 911A and Wayne Lab-Blox F6. This observation indicates that the sodium hydroxide soak, whose primary purpose is to remove humic acid contaminants from collagen isolated from fossil bones, also removes a component of the collagen which is present in fresh uncontaminated bone. Removal of the sodium hydroxide extractable component causes the $\delta^{13}C$ value of the fraction of collagen which resists extraction to be increased by about 0.7‰.

DIETARY ANALYSIS BASED ON THE ISOTOPIC RATIOS OF ANIMAL NITROGEN

All applications of the isotopic method of dietary analysis to date have been based on determination of the isotopic ratios of animal carbon. The results of the present study indicate that additional information about an animal's diet can be obtained from the nitrogen isotopic composition of its whole body or of its tissues and biochemical components, provided its potential food sources had different $\delta^{15}N$ values.

Little is known about the nitrogen isotopic composition of different types of food. It has been suggested that plants which can fix molecular nitrogen (due to the presence of symbiotic bacteria) will have characteristically lower $^{15}N/^{14}N$ ratios than those which must assimilate other forms of inorganic nitrogen, such as ammonia or nitrate (Delwiche and Steyn, 1970; Delwiche *et al.*, 1979). This suggestion is based

on the assumption that the characteristic differences between the $\delta^{15}N$ values of the respective nitrogen sources are inherited by the two types of plants because of the presumed absence of any significant fractionation during either type of nitrogen incorporation (DELWICHE and STEYN, 1970; HOERING and FORD, 1960). The plants capable of nitrogen fixation in terrestrial ecosystems are almost exclusively members of the legume family; blue-green algae are responsible for most of the molecular nitrogen fixation that occurs in aquatic ecosystems (BURNS and HARDY, 1975). The data currently available indicate that legumes and blue-green algae usually have lower $\delta^{15}N$ values than plants which cannot fix molecular nitrogen. However, the differences between the $\delta^{15}N$ values of the two types of plants appear to vary depending on the location in which they grew and the time of year during which they were collected (BARDIN *et al.*, 1977; DELWICHE *et al.*, 1979; DELWICHE and STEYN, 1970; HOERING, 1955, 1956; MEINTS *et al.*, 1975; RENNIE *et al.*, 1976; WADA and HATTORI, 1976). The basis for geographical and temporal variability of plant $\delta^{15}N$ values must be resolved before dietary analysis based on the isotopic ratios of animal nitrogen can be exploited to its full potential.

The nitrogen isotopic method of dietary analysis will probably be of very limited usefulness in modern terrestrial ecosystems because of the extensive use of chemical fertilizers. The $\delta^{15}N$ values of many ammonia-containing and nitrate-containing fertilizers are similar to that of atmospheric molecular nitrogen (FREYER and ALY, 1974; SHEARER *et al.*, 1974). The difference between the $\delta^{15}N$ values of legumes and non-legumes will be reduced if the nitrogen used by non-legumes has a $\delta^{15}N$ value similar to that used by legumes. Thus, the optimal applications of the nitrogen isotopic method will probably be in reconstructing the diets of animals from remote modern ecosystems and from fossil situations.

It may also be possible to use the nitrogen isotopic method of dietary analysis to determine the relative amounts of terrestrial and aquatic food sources eaten by animals living in near-shore environments. The few available data suggest that the $\delta^{15}N$ values of aquatic organisms that are likely to be eaten, such as fish and shellfish, are more positive than the $\delta^{15}N$ values of most terrestrial food sources (HOERING, 1955; MIYAKE and WADA, 1967). More information on the distribution of ^{15}N in these two types of food sources is needed, however, before this application of the method can be made.

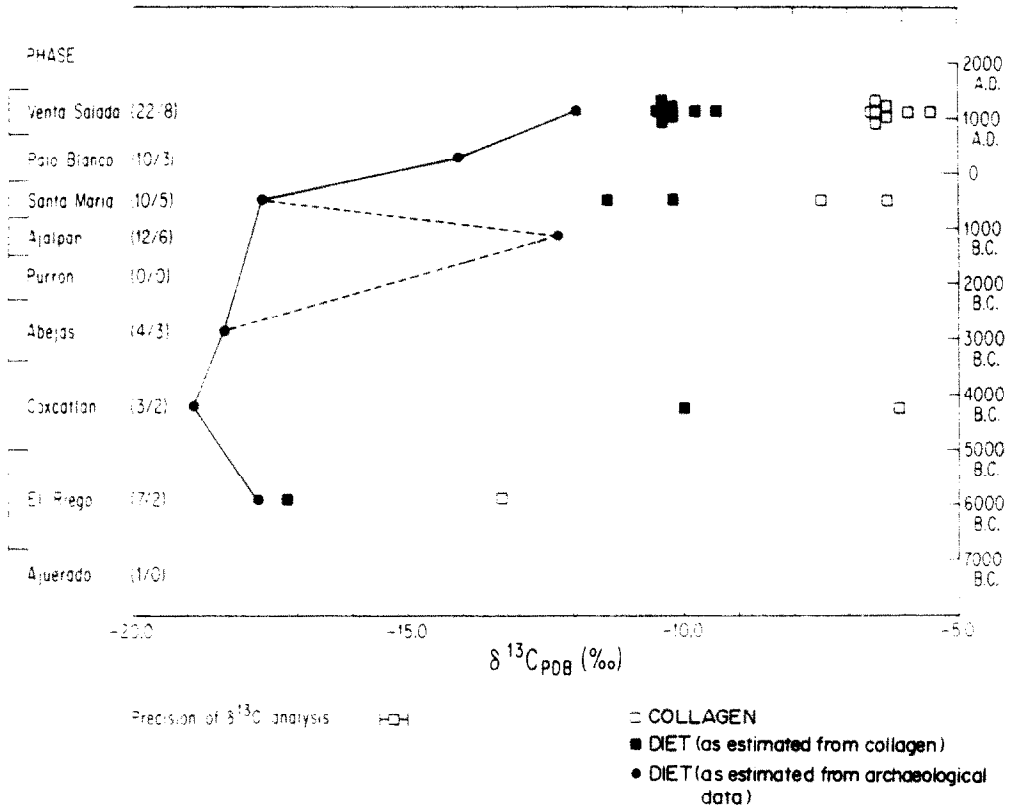

Fig. 4. $\delta^{13}C$ values of the diets of individuals who lived during the designated phases of the occupation of the Tehuacan Valley of Mexico, as estimated from the $\delta^{13}C$ values of their bone collagen. The $\delta^{13}C$ values of the diet for the entire population during each phase, calculated from the archaeological data of MACNEISH (1967), are also presented. The ratios of the number of skeletons that were excavated to the number that were made available for analysis are given for each phase; in some cases, the available bones contained no collagen.

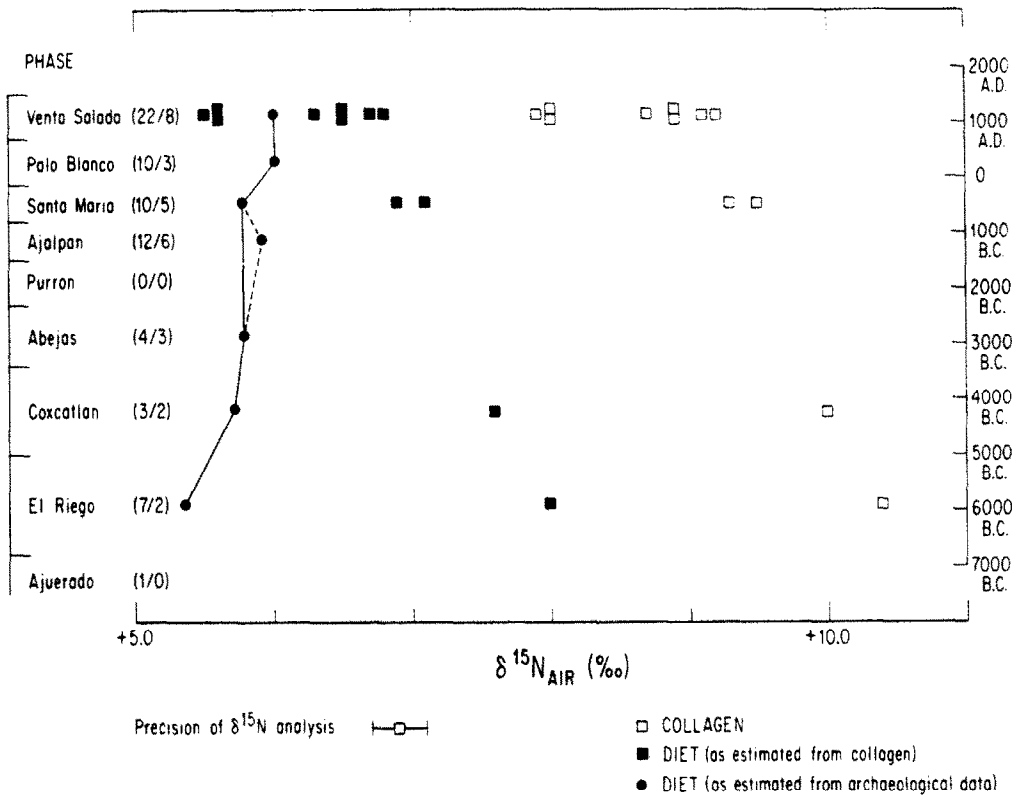

Fig. 5. $\delta^{15}N$ values of the diets of individuals who lived during the designated phases of the occupation of the Tehuacan Valley, as estimated from the $\delta^{15}N$ values of their bone collagen. The $\delta^{15}N$ values of the diet for the entire population during each phase, calculated from the archaeological data of MACNEISH (1967), are also presented. The ratios of the number of skeletons that were excavated to the number that were made available for analysis are given for each phase; in some cases, the available bones contained no collagen.

DIET RECONSTRUCTION BASED ON NITROGEN AND CARBON ISOTOPIC RATIOS OF COLLAGEN FROM HUMAN BONES EXCAVATED IN THE TEHUACAN VALLEY OF MEXICO

The isotopic method of dietary analysis was used to reconstruct the diets of members of the population that occupied sites in the Tehuacan Valley of Mexico over a seven thousand year span. This area is of critical importance to the rise of civilization in Mesoamerica, because corn, a major food source in the region, was probably first domesticated and cultivated extensively in the Tehuacan Valley (MACNEISH, 1967).

The $\delta^{13}C$ and $\delta^{15}N$ values of collagen extracted from human bones of known age excavated in the Tehuacan Valley (ANDERSON, 1967) and the corresponding isotopic ratios of the diets are shown in Figs 4 and 5. The isotopic compositions of the diets were calculated by subtracting 3.9‰ from the collagen $\delta^{13}C$ values and 2.4‰ from the collagen $\delta^{15}N$ values. These corrections represent the average isotopic fractionations associated with assimilation of the diet and collagen synthesis by the three strains of mice analyzed in this study.

MACNEISH (1967) summarized the results of a multidisciplinary investigation into the diet of the population that occupied the Tehuacan Valley sites. For each phase of the occupation, he estimated the percentage of the diet that was obtained by eating identified species of wild and domesticated animals and wild and cultivated plants. Using Macneish's reconstruction, we estimated the $\delta^{13}C$ values of the diet for each phase in the following way. First, the plants in the diet were classified according to the pathway they use to fix carbon dioxide (i.e. C_3, C_4, or CAM) (BLACK and WILLIAMS, 1976; DOWNTON, 1971; EVANS, 1971; SMITH and ROBBINS, 1974; SZARECK and TING, 1977). Second, the C_3 plants were assigned $\delta^{13}C$ values of $-25.0‰$, while the C_4 and CAM plants were assigned values of $-9.0‰$ (BENDER, 1968, 1971; BENDER *et al.*, 1973; LERMAN and RAYNAL, 1972; OSMOND *et al.*, 1973; SMITH and BROWN, 1973; SMITH and EPSTEIN, 1971). The $\delta^{13}C$ value of the total meat component of the diet was estimated by using an isotopic mass balance expression for the Venta Salada phase. This phase has more isotopic and archaeological data on the diet available than any other phase. The $\delta^{13}C$ value of meat was calculated to be $-18.5‰$ by this method, indicating that the animals' diets

favored C_3 plants. The $\delta^{13}C$ value of the diet for each phase was then estimated using an isotopic mass balance expression, utilizing MACNEISH's (1967) estimates of the composition of diet and the $\delta^{13}C$ values we assigned to or estimated for each diet component.

The $\delta^{15}N$ values of the diet for each phase were calculated from the archaeological data by the same method. The leguminous plants were assigned $\delta^{15}N$ values of 0.0‰, while the non-legumes were assigned values of +6.2‰ (BARDIN *et al.*, 1977; DELWICHE and STEYN, 1970; DELWICHE *et al.*, 1979; HOERING, 1955, 1956; MEINTS *et al.*, 1975; RENNIE *et al.*, 1976). The $\delta^{15}N$ value of meat was calculated to be +5.2‰ by solving the isotopic mass balance expression for the Venta Salada phase diet, indicating that the animals' diets were based primarily on non-legumes.

The $\delta^{13}C$ and $\delta^{15}N$ values of diet determined for individuals by analysis of collagen are compared with those calculated for the entire population from the archaeological data of MACNEISH (1967) in Figs 4 and 5. These comparisons indicate significant differences in the patterns through time. The $\delta^{13}C$ values of the diet as estimated from collagen display a significant shift to diet sources enriched in ^{13}C between the El Riego and the Coxcatlan phases, and a relative constancy thereafter. The diet $\delta^{13}C$ values that we reconstructed from the archaeological data, on the other hand, show only small changes from the El Riego up to the Santa Maria phases, followed by a rapid increase in $\delta^{13}C$ values during the Palo Blanco and Venta Salada phases. The diet $\delta^{15}N$ values as estimated from collagen decrease in going from the El Riego to the Venta Salada phases, whereas the archaeological data suggest that the diet $\delta^{15}N$ values increased slightly over the same period.

The $\delta^{13}C$ and $\delta^{15}N$ values estimated from the archaeological data for the diet of the Ajalpan phase are subject to more uncertainty than those for the other phases because the amount of fossil food recovered for this phase was approximately an order of magnitude less than that recovered for any other phase. Accordingly, we have connected the Ajalpan points to the curves in Figs 4 and 5 with dashed lines.

A shift towards higher $\delta^{13}C$ values for the diet would be caused by an increased utilization of C_4 and/or CAM plants (or of animals that fed on them) as diet sources. A shift towards lower diet $\delta^{15}N$ values would result if legumes (or animals that fed on them) made up an increasingly larger part of the diet. The most likely cause for these trends would be an increase in the use of corn, a C_4 plant, and beans, which are legumes, as food sources. The archaeological evidence suggests that utilization of these two types of food increased during the occupation of Tehuacan (KAPLAN, 1967; MACNEISH, 1967). However, the kinetics of the processes whereby these two food sources came to be used are different as judged by the isotopic and archaeological evidence.

The question arises as to which estimate of diet is more reliable. The archaeological reconstruction of diet done by MACNEISH (1967) is based on identification of plant and animal remains recovered, either free or bound in feces, in association with human artefacts and skeletons. The method involves estimating how much edible food a single fragment of a food source (e.g. a corn cob, a bird feather, etc.) represents and estimating how many specimens of that food source would be recovered if the total debris of the population that occupied the sites during a given phase could be excavated. After these estimates have been made for each type of food that was recovered, the percentage of diet derived from each food can be calculated. The method is subject to some uncertainty, since some diet components are more likely to be represented in the debris and feces than others, due to differences in resistance to disintegration during digestion and/or burial (MACNEISH, 1967). Additionally, this approach gives a better estimate of the food

Table 2. $\delta^{13}C$ and $\delta^{15}N$ values of fossil plants recovered in association with human bones in the Tehuacan Valley

PLANT	CARBON DIOXIDE FIXATION TYPE	NITROGEN INCORPORATION TYPE	$\delta^{13}C_{PDB}$ (‰)	$\delta^{15}N_{AIR}$ (‰)
Amaranth *(Amaranthus cruentus)*	C_4	non-legume	- 9.5	+11.2
Common bean *(Phaseolus vulgaris)*	C_3	legume	-24.0	+ 8.4
Corn *(Zea mays)*	C_4	non-legume	- 8.8	+13.1
Guaje *(Leucaena esculenta)*	C_3	legume	-21.2	+13.6
Soluchil *(Beaucarnea gracilis)*	C_4	non-legume	- 9.0	+26.1
Tepary bean *(Phaseolus acutifolius)*	C_3	legume	-23.5	+14.5

sources that were available to the population, rather than those that were actually eaten (SCHOENINGER, 1979).

The methods we used to estimate the isotopic ratios of the foods available in the Tehuacan Valley add uncertainty to the isotopic ratios calculated for the diets of each phase from MACNEISH's (1967) data. If the $\delta^{13}C$ and $\delta^{15}N$ values assigned to the various diet components differ from the actual values, the isotopic ratios we calculated will be in error. However, if the isotopic ratios of the food sources remained relatively constant over the lifetime of the population at Tehuacan, the general shapes of the two curves that trace shifts in the diet $\delta^{13}C$ and $\delta^{15}N$ values would be similar to those shown in Figs 4 and 5. The $\delta^{13}C$ values of the different plants eaten by the human population of the Tehuacan Valley and by the animals that they ate probably did not change considerably during the occupation, since plant $\delta^{13}C$ values are determined primarily by the well-buffered $\delta^{13}C$ value of atmospheric CO_2 (PARK and EPSTEIN, 1960). Any systematic change in the $\delta^{15}N$ values of the available plants would probably have been toward higher $\delta^{15}N$ values as time progressed (and hence in the opposite direction of the trend we observed in collagen $\delta^{15}N$ values), since both cultivation and fertilization with animal waste cause soil $\delta^{15}N$ values to increase (KREITLER, 1975).

The results of isotopic analysis of a few of the many plant specimens recovered in association with human skeletal material in the Tehuacan Valley, presented in Table 2, indicate that it may be possible to determine if there were temporal variations in the $\delta^{13}C$ values of different plants available during the occupation. The $\delta^{13}C$ values of the archaeological plant specimens are similar to those of modern C_3 and C_4 plants (BENDER, 1968, 1971; LERMAN and RAYNAL, 1972; SMITH and BROWN, 1973; SMITH and EPSTEIN, 1971), suggesting that these plants have retained their isotopic identities. On the other hand, the $\delta^{15}N$ values of the fossil plants, also given in Table 2, are much higher than observed for modern legumes and non-legumes (BARDIN *et al.*, 1977; DELWICHE and STEYN, 1970; DELWICHE *et al.*, 1979; HOERING, 1955, 1956; MEINTS *et al.*, 1975; RENNIE *et al.*, 1976). The relatively high $\delta^{15}N$ values for the archaeological specimens cannot be explained by the effects of chemical fertilizer on the $\delta^{15}N$ values of modern plants. Rather, it appears that the original $\delta^{15}N$ values of the archaeological specimens have been shifted during diagenesis. If this process affected all of the plant material excavated from the Tehuacan Valley, it will probably be impossible to determine the variability of the $\delta^{15}N$ values of the plants that were available during the occupation.

The estimates of diet based on isotopic analysis of bone collagen are also subject to uncertainty. First, the diet isotopic compositions were estimated from isotopic ratios of collagen from only one or two individuals for three of the four phases for which any data were obtained. However, for the one phase for which there is a reasonably large data base (the Venta Salada), the standard deviations about the means for both the $\delta^{13}C$ and $\delta^{15}N$ values of collagen are on the order of $\pm 0.5‰$. The changes in the diet isotopic ratios that are of significance in the arguments presented above are larger than this measure of the uncertainty in estimates of diet $\delta^{13}C$ and $\delta^{15}N$ values. Second, in order to estimate the isotopic ratios of diet from the collagen $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, we assumed that the isotopic fractionations between diet and collagen in humans are the same as those we observed in mice. If this assumption is not valid, the diet $\delta^{13}C$ and $\delta^{15}N$ values are in error, although probably by no more than 3‰. However, as long as the isotopic relationships between diet and collagen were the same for all individuals from the Tehuacan Valley, the diet isotopic ratios we calculated are all off by the same amount. Thus, although the estimates of diet $\delta^{13}C$ and $\delta^{15}N$ values based on isotopic analysis of collagen may be wrong by 2–3‰, the patterns of shifts in diet isotopic ratios are probably correct.

Finally, the isotopic method of dietary analysis involves the assumption that the $\delta^{13}C$ and $\delta^{15}N$ values measured for bone collagen are not significantly different from the values the collagen had when it was incorporated into the bone. The isotopic ratios of collagen might be altered by processes that occur during diagenesis, such as those that cause changes in the concentration of collagen in bone and in its amino acid composition (e.g. HO, 1967). The bones from the Tehuacan Valley apparently have undergone some diagenesis. The concentration of collagen in the bones, as shown in Fig. 6, varies over a wide range. For comparison, the fresh mouse bones we analyzed had $16.7 \pm 1.1\%$ collagen ($n = 10$). The carbon/nitrogen ratios of the fossil collagen, also shown in Fig. 6, are significantly higher than the ratios observed for collagen from the mouse bones, which averaged 2.9 ± 0.1 ($n = 10$). We do not have data on fresh human bone to determine the degree to which the discrepancies between the values observed for human and mouse bones reflect differences between species. However, the observation that the $\delta^{13}C$ and $\delta^{15}N$ values of collagen from the bones of the eight Venta Salada individuals agree to within $\pm 0.5‰$ in spite of the fact that the collagen concentrations range from 12.0% down to 5.4% suggests that diagenetic alteration did not have a significant effect on the isotopic composition of the collagen in these and the rest of the Tehuacan samples. We stress, however, that this argument is only suggestive of the conclusion we have drawn, and that rigorous analysis of the effects of diagenesis on the isotopic ratios of bone collagen is needed.

We conclude that the reconstruction of diet based on the isotopic ratios of bone collagen may be more reliable than that based on archaeological analysis of plant and animal remains from the deposits in the Tehuacan Valley. However, the reliability of the dietary reconstruction based on isotopic analysis will be

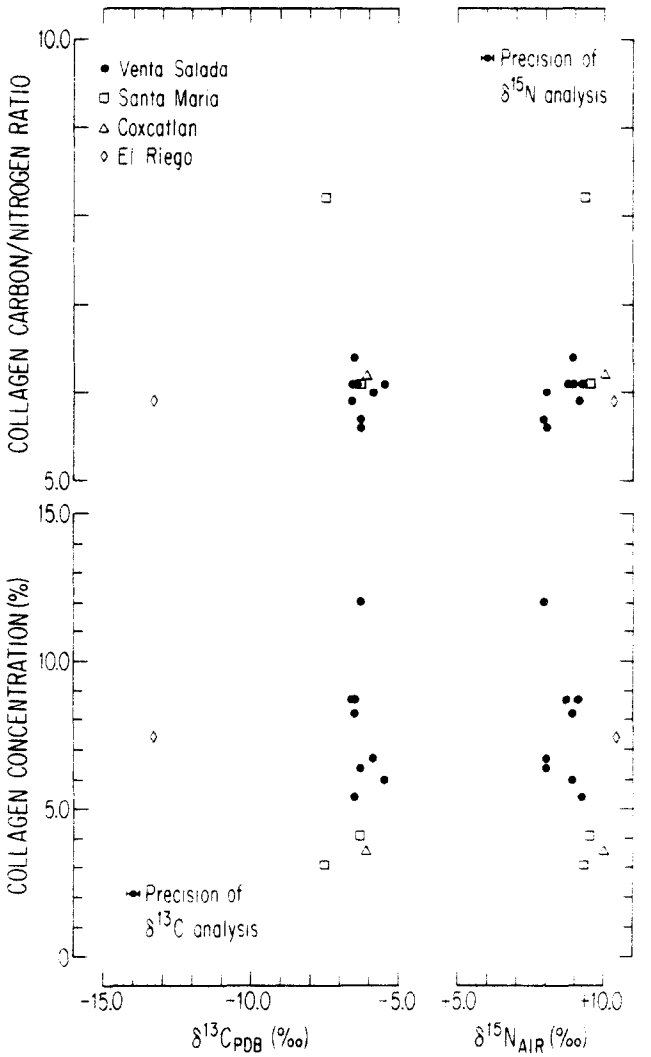

Fig. 6. Concentrations, carbon/nitrogen ratios, $\delta^{13}C$ values and $\delta^{15}N$ values of collagen isolated from bones of individuals who lived during the designated phases of the occupation of the Tehuacan Valley. The collagen concentrations are given as percentages of the dry bone weights. The carbon/nitrogen ratios were calculated from the elemental weight percentages of the collagen.

greatly increased when more fossil skeletal and plant samples are analyzed and when the effects of diagenesis on the isotopic composition of bone collagen are understood.

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