DEVELOPING BONE COLLAGEN STABLE HYDROGEN ISOTOPE ANALYSES FOR PALEOCLIMATE RESEARCH AND ENHANCING INTERPRETATIONS WITH BONE CARBON, NITROGEN AND OXYGEN ISOTOPES

by

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ABSTRACT

The hydrogen isotopic ratio (δD) of rain is known to vary according to climate and temperature. The hydrogen in plant tissue reflects both δD of rain and daytime relative humidity (RH) during the growing season. Since tissue from a herbivorous animal records the δD value of the plants in their diets, δD of herbivore bone collagen should ultimately reflect growing season δD of rain, and to a lesser extent, RH. Therefore, the measurement of δD of non-migratory herbivore bone collagen could have considerable potential for monitoring changes in climate through time and thus prove to be a valuable paleoclimatic tool.

In this study, methods for analyzing bone collagen for δD were tested and a means for correcting results for hydrogen exchange was devised. It was found that the δD of bone collagen of North American white tailed deer does indeed reflect both growing season rain δD and RH. With correction for the effects of RH the relationship of bone δD versus summer rain δD has a slope of 1.0 indicating a simple relationship and no unexplained sources of bias. It was also found that $\delta^{15}N$ can be used to correct for the effects

of RH so that bone δD can be used to estimate rain δD with good accuracy. The rain δD can then be used to estimate temperature. It was further found that relative humidity can be estimated using both bone collagen δD and bone phosphate $\delta^{18}O$.

Additional work found that the $\delta^{15}N$ of bone collagen of animals consuming some C_4 plants is related to local amount of precipitation whereas no such relationship exists for animals consuming only C_3 plants. $\delta^{15}N$ and $\delta^{13}C$ were also found to vary considerably across North America which could complicate their interpretation in paleodietary studies.

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CHAPTER ONE

POTENTIAL OF HYDROGEN ISOTOPES IN PALEOCLIMATIC RESEARCH -SCOPE AND RATIONALE OF THESIS-

1.1 Introduction:

Reconstruction of paleoclimates is a major task for the geologist while, for the archaeologist, the correlation of subsistence patterns and other cultural phenomena with climatic events is important. In April, 1982, I suggested to Dr. Henry Schwarcz that isotopic studies of hydrogen in bone collagen could provide a valuable paleoclimatic or paleoecological tool in both geology and archaeology and he agreed that this would be a suitable Ph.D. project. At that time it appeared that no δD work on bone collagen had been done.

1.2 Paleoclimatic Potential:

A thorough survey of the literature (Ch. 2) suggested that δD analysis of bone could provide a useful paleoclimatic indicator. The δD values of rain and environmental waters vary according to climate and geography and can be directly related to condensation temperature via a curvilinear relationship. A rain cloud, upon moving into higher altitudes and latitudes, cools and preferentially loses the heavier isotope (deuterium or ^{2}H) such that the rain becomes increasingly lighter in δD at cooler temperatures and at higher altitudes, latitudes, or in the interiors of continents (cf. Ch. 2, 7).

The hydrogen in plant tissue is derived from and reflects the δD value of leaf water (δDl) (Ch. 2). Animal tissue δD, in turn, reflects the δD composition of the plants (or animals) that comprise their diet. Leaf-H_2O hydrogen enters the biosphere during photosynthesis where hydrogen is removed from the H_2O molecule and incorporated into various carbohydrates which are then passed on through the food chain. All non-exchanged hydrogen in the food chain should, therefore ultimately reflect leaf-H_2O δD (δDl). Since all C-bonded hydrogens in plant and animal tissue are ultimately reduced during photosynthesis and, generally, do not undergo H-exchange (Ch. 3,6), it is expected that all C-bonded, non-exchangeable hydrogens in bone collagen directly represent photosynthetic-H, which, in turn, directly represent leaf-H_2O. Since leaf-H_2O comes from rain during the growing season, its isotopic value reflects that of the rain. However, average growing season relative humidity (RH), although a variable of secondary importance, also significantly affects the leaf-H_2O isotopic values via evapotranspiration effects at the leaf water-air interface. A decrease in RH causes an increase in the δDl as the lighter isotope (hydrogen or ^1H) is preferentially lost during evapotranspiration through the stomatal openings at the leaf surface. The δD of non-exchangeable hydrogens in bone collagen from a non-migratory herbivore should reflect the isotopic record of plants within a restricted area. These plants reflect mainly δD of growing season precipitation (δDw) with a smaller dependence on growing season daytime RH. Changes in the isotopic value of bone collagen through time should ultimately reflect changes in local climate.

A study of non-exchangeable hydrogens of nitrated tree ring cellulose (δD_{cel}) showed a strong relationship between δD_{cel} and δD of

local waters or yearly average precipitation δD (δDwy) (Yapp and Epstein 1982a). There was a significant, though lesser effect of the yearly average of daily (24h) relative humidity. Due to source water effects (below) some trees have been found to be biased towards winter rain (Gray and Song 1984) while others in well drained areas were biased towards growing season rain (White et al. 1985; White and Lawrence 1980; Edwards and Fritz 1986). Still others might have a positive precipitation amount effect so that as amount of rain decreases there is greater utilization of deeper ground-H_2O (see below).

The δD of nitrated insect chitin also reflected the δD of local water but was biased towards growing season rain δD (R. Miller 1984; Miller et al. 1988). As one moved into areas with short growing seasons, the δD of chitin became significantly more positive relative to the δDwy (of local yearly average of rain δD). In general, it is difficult to find terrestrial materials which commonly appear in association with archaeological materials, are widespread, well preserved, and which could provide a suitable record of δDw over time.

The possibility of a relationship between δD of herbivore bone collagen (δDb) and δD of rain was tested by using tibia samples of white-tailed deer (*Odocoileus virginiana*) from across North America. The use of bone collagen from a large non-migratory herbivore has a distinct advantage in that such animals are good samplers of plants and provide a time integrated average of the plant δD values in a restricted area over the animal's lifetime. This should reduce the variability in δD seen from plant to plant and year to year. The use of warm blooded animals would eliminate any isotopic temperature effects that could occur during the production of collagen. White-tailed deer seemed particularly

useful as they are non-migratory browsers with relatively long lifespans (15 to 20 years; Taylor 1956; Banfield 1974), are widely distributed across North America, are numerous in the wild, and appear in the faunal record for the last 2 to 3 million years (Hall 1981; Kurtén and Anderson 1980). Furthermore, since deer cannot reach the leaves of the larger trees in deep forests, they usually browse at the edges of forests where they consume a wide variety of smaller plants and fruits and leaves from smaller trees (Severinghous and Cheatum 1956; Hosley 1956). Whereas smaller plants have shallow root systems and depend on meteoric water during the summer growing season, larger trees have deeper root systems which can sample deeper H_2O having varied sources including the spring melt (Aravena 1982; White and Lawrence 1980; Gray and Song 1984; White et al. 1985; Edwards and Fritz 1986). Therefore, since deer eat only the smaller plants they should have δDb which forms a simple relationship with δDw. In contrast, δD_{cel} may be complicated by such things as source water effects and seasonality of deposition of cellulose in different portions of the rings (cf. Wilson and Grinsted 1975; Northfelt et al. 1981; Epstein and Yapp 1976; above refs.). Insect chitin results may also be complicated by as of yet incompletely understood factors of seasonality, variations in feeding patterns and temperature or interspecies, biological isotopic effects (cf. R. Miller 1984; Miller et al. 1988).

For archaeologists, an unambiguous correlation of prehistoric cultural patterns to changes in climate requires the use of materials typically found in archaeological sites. Bone, especially those of large herbivores, are the most commonly preserved organic material found in sites and can be reliably dated by ^{14}C if younger than ≈50,000 y.B.P.

Large herbivores often formed part of the human diet and should provide ample material for study. Where possible, one would also like to achieve a quantitative or semi-quantitative measure of change in climate such as can be provided through stable isotopic studies. Paleoclimatic interpretations arising from the analysis of other intra-site materials (i.e. faunal assemblages, pollen or stratigraphy) might also be greatly strengthened by the δD analysis of bone from these same sites.

1.3 <u>Work Presented in This Tesis:</u>

As this is the first study of δD of bone collagen in a first phase of work, techniques for extraction and δD analysis of bone collagen were developed to establish procedures allowing good reproducibility and accuracy (Ch. 3-6). The effects of H-exchange on results were evaluated and a calibration method for correcting δDb for exchange was developed (Ch. 3,6). A special extraction line was built to allow the simultaneous cryogenic collection of CO_2, N_2 and H_2O combustion products for determination of the N_2 and CO_2 gas yields and for C-, N- and H-isotopic analyses (Ch. 4). By supplementing the δD with C-, N-, and H-gas yields and with $\delta^{13}C$ and $\delta^{15}N$ measurements, it was possible to monitor effects on the δDb results of sample preparation, collagen denaturation, as well as effects from other variables including diet, local agricultural practices and climatic factors such as amount of rain (Ch. 2-6, 10).

The $\delta^{13}C$ and $\delta^{15}N$ isotopic results, by themselves, represent one of the few studies using a single species of a ruminant, browsing animal with a wide geographical distribution (Ch. 10). The $\delta^{13}C$ and $\delta^{15}N$ results presented here represent a valuable contribution to other

research using C- and N-isotopes of animals or humans for food chain or climatic studies.

In the second phase of this research, the δDb of w.t. deer tibia from 48 locations across North America were compared to precipitation δDw (growing season average) and δDwy (yearly average); to local RH and to theoretical estimates of δDl (leaf-H_2O) (Ch.8). Leaf-H_2O models attempt to account for how both δDw and effects of RH are reflected in the δDl and, ultimately, the δDb values. During biosynthesis in plants and incorporation of amino acids into bone collagen, no additional fractionation effects related to climate or environment are expected. An examination of the relationship between δDb of deer and theoretical estimates for δDl provided important information towards determining whether (a) deer bone δD conformed with theoretical expectations, (b) there were no additional fractionation effects related to climate or environment and (c) all the exchangeable hydrogens had been corrected for. Comparison of δDb results with δDl values calculated using leaf-H_2O models allowed a better understanding of the empirically observed relationships between both δDb and δDw and RH. Such relationships could then be examined for their conformation to theoretical expectations. Major biological effects resulting from photosynthesis in plants and from trophic level effects in deer should be reflected in the intercept of the δDb vs δDl relationship (Ch. 2). It was expected that δDb would form a linear relationship with δDl having a slope of 1.0 but an intercept which will reflect a relatively constant average offset in δDb from δDl arising from biological fractionation effects in both the plants and deer.

Rainwater uptake by plants should not result in any fractionations (see Ch.2). When RH=100 there should be no D-enrichments in the leaf-H_2O due to evaporation and the δDl should be equal δDw. Under such circumstances one would expect a 1:1 linear relationship between δDl and δDw with a zero intercept. Furthermore, there would be a linear relationship between δDb and δDw with a slope of 1.0 and an intercept expressing the biological fractionation effects already discussed. Since the δDw forms a non-linear relationship with average growing season temperature (T) at RH=100%, δDb would form a similar, curvilinear, relationship with T. If, in addition, the relationship between δDw and T were well understood, it might be possible to estimate the important climatic variable (T) directly from δDb.

In reality, δDb cannot be used to directly measure T due to the confounding effect that RH has on δDl (and hence on δDb) combined with the inherently curvilinear relationship between δDb and T. δDb will not prove useful for estimating RH due to the limited effect that RH has on δDl (Ch. 2, 8). Therefore, estimation of climate from δDb will depend entirely on how accurately it provides an estimate of δDw. In this sense, RH becomes a major unknown and an impediment in the use of δDb for estimating T.

Correcting for the effects of RH in a curvilinear relationship, such as between δDb and T, is difficult but it can be done by applying multilinear regression methods to an inherently linear relationship such as that expected to exist between δDb and δDw. Therefore, considerable use was made of multilinear regression techniques to quantify and correct for the effects of RH in order to use bone to provide a more accurate estimate of δDw (Ch. 8). In addition to δDb, laboratory

measurements of bone, such as $\delta^{15}Nb$, $\delta^{13}Cb$ and other variables related to sample preparation and H-exchange were tested in these regressions. Evaluation of the effects of these experimental variables was included because some might substitute for RH or calibrate for minor effects of sample preparation to allow a better estimate of δDw using bone data which would, in turn, provide a better estimate of T.

Once it is established that δDw can be estimated from δDb, an understanding of the relationship between δDw and T becomes crucial to any further interpretation of climate. There are two ways in which knowledge of δDw, as estimated from from δDb, could help in the evaluation of paleoclimate. One could compare δD results of numerous bone samples collected from different locations across North America for different time periods such as has been previously attempted using tree wood cellulose (Yapp and Epstein 1977). Alternatively, one could study the change in δD over time for a single location using a long, temporal sequence of bone from a single site which would parallel those studies using tree ring cellulose (i.e. Gray and Song 1984; Epstein and Yapp 1976; Ch. 2,8), peat deposits (Schiegl 1972; Brenninkmejer et al. 1982), glaciers (i.e. Dansgaard et al. 1971; Epstein et al. 1970; Benoist et al. 1982), or speleothem fluid inclusions (i.e. Harmon et al. 1979a,b; Schwarcz et al. 1976; Yonge 1982; Harmon and Schwarcz 1981). For paleoclimatic interpretation, both the intersite (transcontinental) and the intrasite (temporal) relationships between δDw and T must be understood. Although the main transcontinental relationship between δDw and T is distinctly non-linear, the literature does not contain any curvilinear relationships that simply relate δDw to T. For these reasons both the intersite and intrasite empirical relationships between δDw and δDwy

and T were re-examined in this work using data from IAEA stations in North America and empirical relationships were established relating δDw and δDwy to T (Ch. 2,7). Understanding seasonal variations in rain δD was also considered necessary as δDb is expected to reflect only growing season δDw rather than yearly δDwy.

Not only is relative humidity a useful climatic variable on its own but its evaluation allows a more accurate estimate of δDw and δDwy from δDb. A method for estimating the RH variable was established using the δDb (of bone collagen) and $\delta^{18}Ob$ (of bone phosphate) from a collaborative study headed by Dr. Boaz Luz of Hebrew University in Israel (Ch. 9).

Preliminary studies on the effects of diagenesis and contamination on δDb were conducted (Ch. 3,11). Seal was chosen for the study of bone weathering because ocean water (δD≈0‰) at the base of the marine food chain can be assumed to have remained fairly constant throughout recent time (Savin and Stehli 1977) in contrast to δD of leaf-H_2O in terrestrial mammalian bone collagen which can be greatly affected by changes in climate. Due to large differences in isotopic ratios in marine vs terrestrial environments (Ch. 2), H-exchange or contamination of the seal should also be readily recognizable, especially in arctic environments where local terrestrial waters, plants and animals are greatly depleted in deuterium.

A possible method of correcting results for diagenetic effects was further explored. This was done primarily by correlating changes in δDb with systematic changes in other variables which reflect bone weathering as determined from the literature (Ch. 3, 11). Finally, cooked bone is difficult to recognize but may alter isotopic signatures in

bone. Such effects of common forms of cooking were tested in this work (Ch. 11).

CHAPTER 2

ISOTOPES IN THE ENVIRONMENT AND HOW THEY MAY BE REFLECTED IN DEER BONE

2.1 Hydrogen:

It was suggested in Ch.1 that the δD of non-exchangeable C-bound hydrogens of bone collagen should directly reflect the δD of non-exchangeable photosynthetic hydrogen which, in turn, directly reflects the δD of leaf water (δDl). It was further explained that the δDl represented in the diets of the deer should derive directly from soil water which derives from rain during the growing season. This is because deer eat the leaves of small trees and plants with shallow root systems and there is expected to be no fractionation of H_2O in the soil (see below). Therefore, δDl represented by the deer should reflect the δD of growing season precipitation. It is primarily through the relationship between δD of rain and temperature that δD of deer will reflect climate and the ability of δD of bone to reflect climate will only be as good as our understanding of how δD of rain relates to T and climate. Relative humidity during the growing season (RH) is a variable of secondary importance through its effects on evapotranspiration in the leaves of plants producing an enrichment in δDl. Knowledge of potential factors affecting the δD values of all (1) meteoric water, (2) leaf-H_2O, (3) plant tissue and (4) animals is crucial to the understanding of how δD in bone will ultimately reflect climate. Therefore these will be discussed in detail below and may be referred to later, when results are interpreted (Ch.8).

2.1.1 Rain δD:

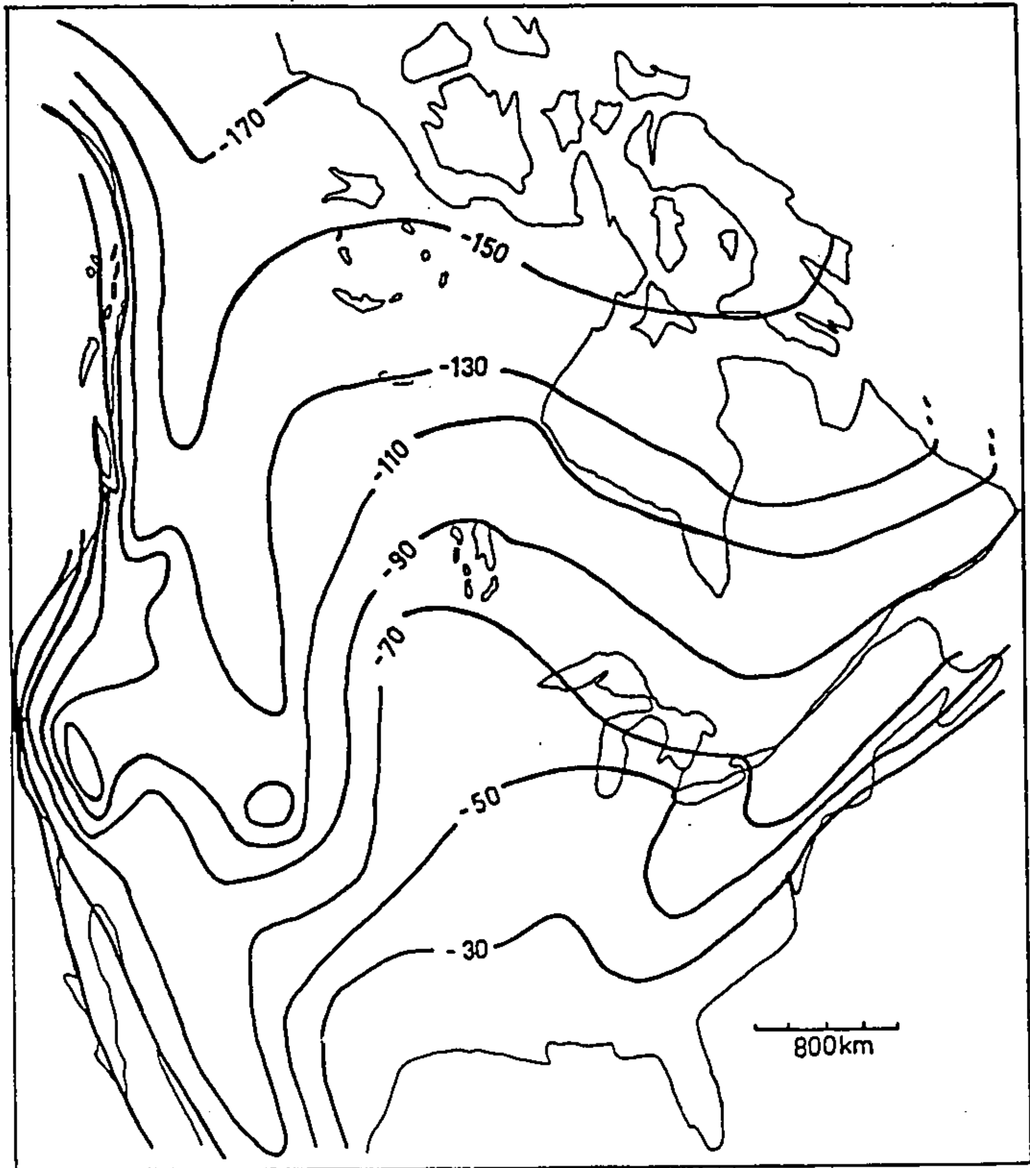
2.1.1.1 The Rayleigh Model:

Factors affecting precipitation δD ($‰D_{rain}$) are discussed in a number of sources referred to below (Dansgaard 1964; Yurtsever and Gat 1981; Friedman et al. 1964; Eriksson 1967; Joussaume et al. 1984; Hage et al. 1975). The δD of rain and environmental H_2O is known to vary according to climate and geography and is in a curvilinear relationship with the yearly average temperature, Ty (Yurtsever and Gat 1981).

Tropical sea surfaces are the main evaporative sources for the vapor clouds from which meteoric H_2O derives. The main forces which then determine the δD of continental rain can be accounted for by a Rayleigh distillation model with condensation under equilibrium conditions. Thus, in North America, the average annual global pattern would be of non-equilibrium evaporation at ≈20°C (Dansgaard 1964) and ≈80% relative humidity, rh (Jouzel et al. 1982) from the subtropical Atlantic Ocean and Gulf of Mexico areas followed by the gradual north-westerly poleward drift over North America of the marine vapor clouds with cooling and precipitation. The heavier hydrogen isotope (deuterium or ^2H) would be preferentially lost in rain producing increasingly lighter δD in rain at higher altitudes, latitudes, or in the interiors of continents. The expected isotopic variation in North American rain is illustrated in Fig. 2.1. During periods of global or hemispheric cooling we might expect thermal gradients to contract towards the equator and lower altitudes thus producing widespread decreases in the δD of continental rain.

Figure 2.1 North American Precipitation δD.

Contours Representing Average Annual δD of Precipitation Across North America with contours based on Sheppard et al. (1969) and Taylor (1974).


-170
-150
-130
-110
-90
-70
-50
-30
800km

On a global scale, continental, latitudinal and altitudinal effects can be modelled by Rayleigh conditions which, in turn, should show a good correlation of δD of rain with condensation temperature (T'). This is shown in Fig. 2.2 for a vapor cloud originally formed by evaporation from the ocean surface at 20°C (curve a) or at 30 °C (curve c). In nature, the curvature would probably be increased as shown in curve b where the equilibrium separation factor for ice ($\alpha_{I/V}$) is used in the Rayleigh equation at T'<5°C. For Fig. 2.2 the δD values of precipitation were calculated using the Rayleigh equation for 13 values of T'. Using the 13 theoretical data points, a curvilinear, best fit model relating rain δD to T' can then be calculated. The offset between curve a and c confirms that conditions at the source of a marine vapor cloud can also affect the δD of rain (cf. Siegenthaler and Oeschger 1980; Hage et al. 1975; Merlivat and Jouzel 1979; Jouzel et al. 1982).

2.1.1.2 <u>Empirical Rain δD vs T Relationship:</u>

In Fig. 2.3a is shown the δDw'y (yearly average of monthly δD_{rain}) data from all continental, coastal and island IAEA stations worldwide (Yurtsever and Gat 1981). Only continental rain from the interior, or colder areas of the continents can be adequately modeled by the Rayleigh equation (dashed line = curve b from Fig. 2.2). This is probably because only in interior continental areas is a vapor cloud sufficiently isolated from its coastal evaporative source, a necessary Rayleigh condition. Here it is assumed that T'=Ty (yearly average temperature at the earth's surface). Discrepancies between T' and Ty can account for some of the deviations from a Rayleigh curve described below.

Fig. 2.2 Rayleigh Precipitation δD.

Relationship Between Precipitation δD as Predicted from a Rayleigh Reverse Distillation Process and Condensation Temperature (T').

Rayleigh reverse distillation equation:

$$\left[\frac{(D/H)_v}{(D/H)_{vo}}\right] = f_v^{\,\alpha-1}$$

where: $\alpha = \alpha_{L/V}$ which is the equilibrium isotopic fractionation factor between the liquid (L) and vapor (V) phases in the vapor cloud; f_v is the fraction of the original vapor cloud remaining; $(D/H)_v/(D/H)_{vo}$ compares the D/H ratio of the current vapor (at time t) to that in the original vapor cloud (at time t_0)(Faure 1977).

Curve a: evaporation at 20°C;
$\delta D_{rain} = -118.6 + 7.793T(°C) - 0.09368T^2(°C)$; $r \approx 1$, $n=13$

Curve b: same as (a) except the isotopic fractionation factor between ice (I) and vapor (V) phases in the cloud, $\alpha_{I/V}$, is used below 5°C;
$\delta D_{rain} = -126.1 + 8.983T(°C) - 0.1370T^2(°C)$; $r \approx 1$, $n=13$

Curve c: evaporation at 30°C.

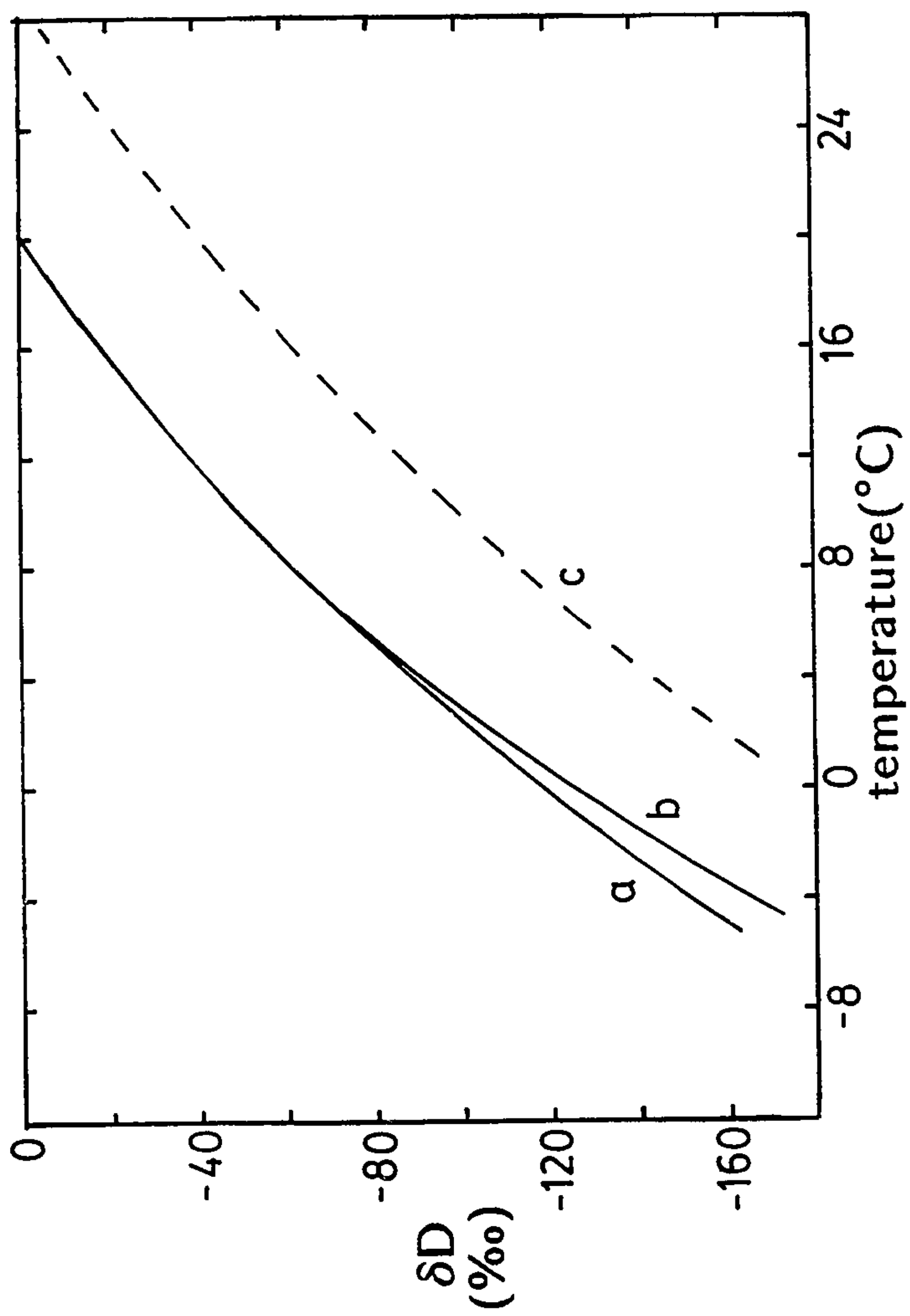

δD
(‰)
0
-40
-80
-120
-160
-8
0
8
16
24
temperature(°C)
a
b
c

Fig. 2.3 Precipitation δD vs Temperature.

Relationship between unweighted (a) and weighted (b) average annual precipitation ($\delta Dw'y$ and δDwy) from worldwide network of I.A.E.A. Locations and average annual temperature (Ty). Data are from Yurtsever and Gat (1981). The Rayleigh curve (Fig. 2.2b) is included for comparison (dashed line). Edmonton (#1 in Fig. 2.3b) was excluded from both unweighted and weighted continental calculations. Island locations are represented by open triangles, coastal by open circles and continental by closed circles.

The best fit relationships are:

(a) unweighted:

i island: $\delta Dw'y = -69 + 3.7Ty - 0.050Ty^2$; n=11, r=0.94

ii coastal: $\delta Dw'y = -76 + 4.7Ty - 0.086Ty^2$; n=25, r=0.96

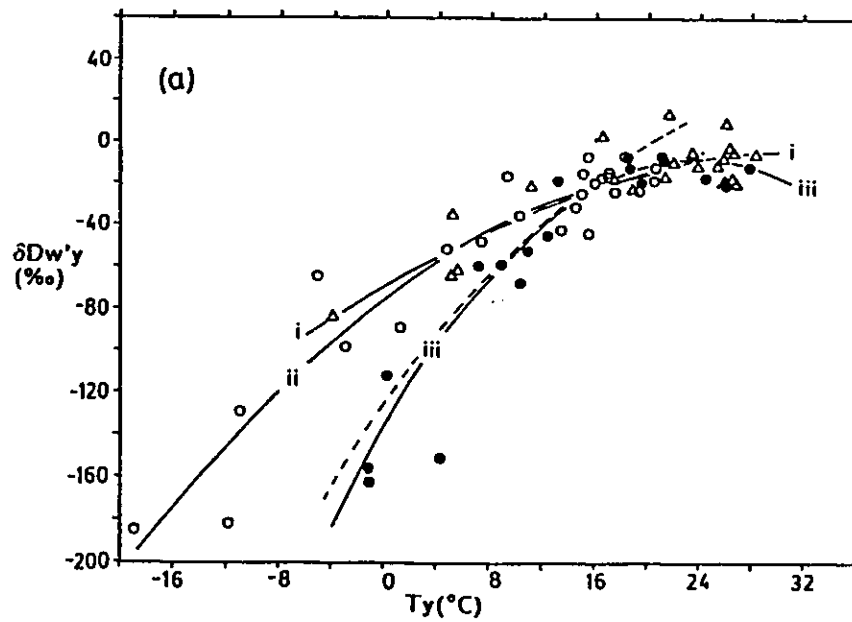
iii continental: $\delta Dw'y = -138 + 10.9Ty - 0.227Ty^2$; n=15, r=0.98

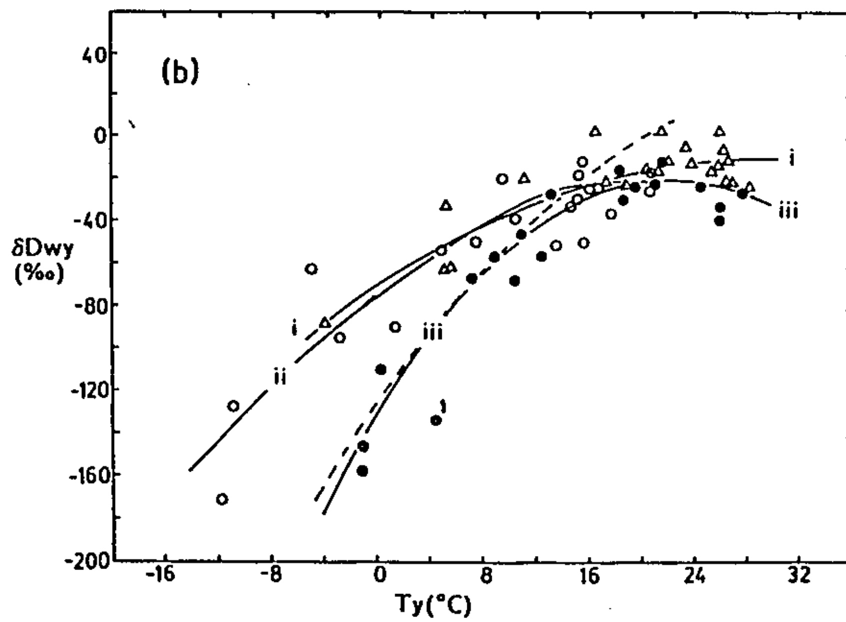
(b) weighted:

i island: $\delta Dwy = -71 + 4.2Ty - 0.700T^2$; n=11, r=0.94

ii coastal: $\delta Dwy = -75 + 4.5Ty - 0.094T^2$; n=25, r=0.95

iii continental: $\delta Dwy = -133 + 10.1Ty - 0.225T^2$; n=15, r=0.98


(a)
δDw'y
(‰)
40
0
-40
-80
-120
-160
-200
-16
-8
0
8
16
24
32
Ty(°C)
i
ii
iii


(b)
δDwy
(‰)
40
0
-40
-80
-120
-160
-200
-16
-8
0
8
16
24
32
Ty(°C)
i
ii
iii

A complex interaction of numerous regional, local, topographical, meteorological and climatic conditions can influence the measured δD composition of rain and are discussed in a number of references (Siegenthaler and Oeschger 1980; Jinsheng et al. 1984; Lawrence et al. 1982; Eriksson 1967; above refs.). Coastal and island locations may have shallower slopes due to (1) re-cycling of ocean water vapor thus decreasing the temperature gradient, (2) eddy diffusion (3) greater precipitation amounts and deep convection producing rain at a lower T' than Ty and (4) Exchange and re-equilibration with H_2O-vapor near the ocean under conditions of high surface humidity and mixing between upper and lower air masses over the ocean. In coastal and island areas, other conditions affecting rain may also dominate over Rayleigh effects such as "amount effects" including such factors as amount of rain, rh, exchange and evaporation from rain drops, size of raindrops and degree of cloud rainout. Here deep convection produces large amounts of rain at lower T' thus producing low δDw'y relative to Ty. High cloud rainout would also deplete the rain cloud in deuterium to produce a negative amount effect whereby an increase in amount of rain is accompanied by a decrease in the δD of rain. Coastal conditions may affect the warmer continental locations since most of these are also closer to the coastal areas of the continents.

Continental areas, on the other hand, seem to be dominated by Rayleigh effects but numerous additional phenomena can also affect the δD of rain such as topographical and meteorological effects as well as recycling of rain through re-evaporation from rain drops or water masses, exchange between rain and local H_2O-vapor, amount of precipitation in the form of snow or hail and bias due to seasonal shifts

in such factors as amount of rain and direction of rain cloud trajectories (above refs). In warm, arid areas at the interiors of continents, low amounts of rain and increased evaporation from rain drops may increase δDw'y to produce a negative precipitation amount effect.

2.1.1.3 Rain Record of Bone:

The δDw'y in Fig. 2.3a should most closely resemble Rayleigh processes which are a direct function of T' (and presumably Ty). On the other hand, weighted averages, whereby the monthly rain is weighted by the monthly amount of rain (i.e. δDwy in Fig. 2.3b), would more closely resemble the H_2O taken up by the plants and, hence, be represented by bone δD (δDb). In the case of the deer, δD of rain during the growing season weighted by the monthly amount of rain (δDw) and, hence, average growing season temperature (T) would be recorded in δDb. The two sets of data (Fig. 2.3a vs 2.3b) do not differ greatly. Nonetheless, rain δD vs T relationship (i.e. Fig. 2.3) is complex whereas that of δDb vs rain δD is expected to be relatively simple (see below). Therefore, the rain δD can first be estimated from δDb before attempting any interpretation of climate. Upon examination of rain δD vs T or Ty in North America (Ch. 7), it will be proposed that qualitative to semi-quantitative interpretations of climate should be possible from the estimates of rain δD obtained from δDb.

2.1.2 Leaf Water δD:

There is little or no isotopic effect on the δD of local input (environmental) H_2O to the plant (δDi) when it is adsorbed by and transported from the roots to the leaves of plants (Ziegler et al. 1976; Smith and Epstein 1970; Wershaw et al. 1966). Soil H_2O δD is independent of local rate of plant evapotranspiration through which H_2O is lost from the soil through leaves (Zimmerman et al. 1967). Therefore, in the case of the deer, δDi should equal δDw, the growing season average of monthly rain δD weighted by the monthly amount of rain.

2.1.2.1 Effects of Relative Humidity:

The effects of relative humidity (rh) on δDl are discussed in a number of sources referred to below (Schiegl 1974; Allison et al. 1985; Long et al. 1980; Edwards et al. 1985). At rh=100%, δDl should be identical to δDi and, for deer, to δDw. When rh<100%, significant fractionation can occur in the leaves prior to photosynthesis when evaporative loss of H_2O increases as rh decreases producing a positive offset between δDl and δDi. Under steady state conditions (cf. Dongman et al. 1974; Forstel 1978):

(eq. 2.1) $\delta Dl = \delta Di(1-h) + (\delta Dv-{}^2\epsilon k)h + {}^2\epsilon k + {}^2\epsilon e$

where: δDv is the local atmospheric H_2O-vapor δD and h is the local daytime (daylight) rh. ${}^2\epsilon k$ and ${}^2\epsilon e$ are the isotopic separation factors (in ‰) between the liquid and vapor phase of H_2O due to kinetic and equilibrium isotopic effects respectively as a result of diffusion, evaporation of H_2O from the leaf and exchange between leaf-H_2O and

atmospheric H_2O-vapor (cf. above refs; App. A). At 100% rh (h=1), the leaf-H_2O would be in equilibrium with atmospheric H_2O-vapor and δDl would be controlled by δDv (in turn, controlled by δDw). At low rh, δDl would mainly be determined by δDi but with considerable enrichments due to kinetic and equilibrium effects during evaporation of H_2O from the leaf.

The values for $^2\epsilon k$ depend on the boundary conditions at the liquid surface within the stomata. These range from fully developed static boundary conditions ($^2\epsilon k$=25; Merlivat 1978) resulting from diffusional transport in the air space within the stomata to turbulent conditions ($^2\epsilon k$=12) at the air/liquid interface (above refs.). Larger kinetic isotopic effects occur with static conditions while laminar flow conditions ($^2\epsilon k$=17) are intermediate between static and turbulent conditions. Most authors have found that a value of $^2\epsilon k$=25, indicative of stagnant boundary conditions, is most realistic (cf. Long et al. 1980; Yapp and Epstein 1982a; Edwards and Fritz 1986; Allison et al. 1985).

The main problem with applying the above equations is that there are several unknowns. During application to paleoclimate, where one attempts to determine δDi and, hence, T, one cannot at the same time know δDv, $^2\epsilon k$ and T. Knowledge of T, in turn, is needed to calculate $^2\epsilon e$ (cf. Majoube 1971, eq. A.9).

If it is assumed that (a) plants derive their water from rain such that δDi=δDw, as for deer, and (b) atmospheric H_2O vapor is in equilibrium with rain so that δDw−δDv=$^2\epsilon e$, then eq. 2.1 can be simplified as follows (cf. above refs.):

(eq. 2.2) $$\delta Dl = \delta Dw + (1-h)\cdot(^2\epsilon e + ^2\epsilon k)$$

Although the assumption of equilibrium between rain and vapor may not be accurate for some coastal, semi-arid or arid environments it should apply to most humid or temperate locations and to most of North America (cf. Allison et al. 1985). Therefore, it seems reasonable to assume that, for plants eaten by the deer of this study, $\delta Dw-\delta Dv \approx {}^2\epsilon e$.

2.1.2.2 Relative Importance of Relative Humidity:

Several studies have shown that both δDi (δDw for deer) and rh affect δDl but most studies suggest that δDi is significantly more important than rh (eg. Yapp and Epstein 1982a,b; Edwards and Fritz 1986; Epstein et al. 1977; above refs). However, the inclusion of this second unknown may hamper efforts to estimate δDw from deer δDb. The leaf-H_2O equations take into consideration the effects of rh on the δDl values. Given that the percent average rh during the growing season (RH) is $\approx h \cdot 100$, one can rearrange eq. 2.2 to show more clearly that as rh decreases there will be a positive offset between δDl and δDw which is directly related to the value of rh (cf. Edwards et al. 1985):

$$(\text{eq. } 2.3) \qquad RH = 100 + \left[\frac{100}{{}^2\epsilon e + {}^2\epsilon k}\right] \cdot (\delta Dl - \delta Dw)$$

Provided that ${}^2\epsilon e \approx \delta Dw-\delta Dv$ and ${}^2\epsilon k$ are roughly the same for all plants, the term $(100/{}^2\epsilon e+{}^2\epsilon k)$ may be quite constant since ${}^2\epsilon e$ is not very sensitive to changes in T (cf. Majoube 1971; Edwards et al. 1985; Long et al. 1980). If this is the case, then eq. 2.3 shows that RH may be

linearly related to $\delta Dl-\delta Dw$. If RH=100, then there would be no effects due to RH, and there would be no isotopic enrichment of δDl so that $\delta Dl=\delta Dw$.

2.1.3 Plant δD:

The deer δDb value should equal $\delta Dl+{}^2\epsilon_B$ where ${}^2\epsilon_B$ is the offset from δDl due to biological fractionation factors occurring in plants (${}^2\epsilon_{Bp}$) and in animals (${}^2\epsilon_{Ba}$) so that ${}^2\epsilon_B={}^2\epsilon_{Bp}+{}^2\epsilon_{Ba}$. In plants, ${}^2\epsilon_{Bp}$ arises when hydrogen is reduced during photosynthesis and becomes incorporated into various compounds making up the whole plant tissue and is discussed below. It is expected that the δDb of deer will reflect only the non-exchangeable, C-attached hydrogens of all the compounds comprising whole plant tissues.

2.1.3.1 Whole Plant Studies:

The biological separation factor (${}^2\epsilon_{Bp}$) between whole plant tissue δD and leaf-H_2O (${}^2\epsilon_{Bp}\approx\delta Dp-\delta Dl$) can be almost entirely attributed to the first steps of photosynthesis (Estep 1979). All plants discriminate, due to kinetic isotopic effects, against the heavier isotopes (^{13}C and D) of carbon and hydrogen which they obtain from atmospheric CO_2 and leaf H_2O, respectively. Most values of ${}^2\epsilon_{Bp}$ for whole plant tissues have been estimated from studies comparing δDp to δDl (thus accounting for the effects of rh) or by using aquatic photosynthetic organisms such as algae, phytoplankton and vascular plants where rh is essentially 100%. It has been estimated to be ≈-90‰ to -120‰ (i.e. Leaney et al. 1985; Estep and Hoering 1980, 1981; Schiegl and Vogel 1970; Ziegler et al. 1976; Keeley et al. 1986). However, ${}^2\epsilon_B$ has not been

quantified for non-exchangeable hydrogens of whole plant tissue, since whole plant studies (i.e. Strauch et al. 1982; Estep 1981a 1982; Estep and Dabrowski 1980; above refs.) do not take into account the effects of H-exchange (Sepal and Mason 1961; DeNiro 1981; Epstein et al. 1976). Evaluating H-exchange by soaking samples in heavy water (Estep and Hoering 1980) is unlikely to be successful in retaining a measure of H-exchange since re-equilibration of exchangeable hydrogens with atmospheric moisture occurs rapidly (cf. Friedman and Gleason 1980; Chs. 3,6). Other studies comparing leaf δDp to δDi have not taken into consideration the effects of rh (i.e. Ziegler et al. 1981). Thus, there is not yet an estimate for $^{2}\epsilon_{Bp}$ affecting deer δDb.

2.1.3.2 Non-Exchangeable Hydrogen of Single Plant Compounds:

Estimates of the biological separation factor for plant cellulose ($^{2}\epsilon_{Bcel}$) based on average results of non-exchangeable hydrogens of nitrated cellulose while accounting for rh effects, have varied considerably with values between ≈-2 to -60‰ and show large interspecies standard deviations even within the same photosynthetic cycle (discussed below) i.e. ±10 to ±20‰ for C_3 aquatic vascular, to ±64‰ for non-CAM aquatic vascular plants and to ±71‰ for algae (cf. Epstein et al. 1976,1977; Yapp and Epstein 1982a; Long et al. 1980; DeNiro and Epstein 1981b). A value for $^{2}\epsilon_{Bcel}$ of -47‰ for tree ring cellulose corrected for RH appears in Edwards and Fritz (1986). The cause of most of the above variability is unspecified and other single metabolites, including lipids, show similar large interspecies variabilities (i.e. Smith and Epstein 1970; Estep and Hoering 1980). A negligible amount of this of variability is likely due to direct temperature effects on

photosynthesis or biosynthesis of cellulose (after: Yapp and Epstein 1982a; Gray and Song 1984; White and Lawrence 1980; Long et al. 1980; DeNiro and Epstein 1981b).

2.1.3.3 <u>Plant Variability:</u>

The δDp of different parts from the same plant can vary by as much as ≈±7‰ (i.e. Schiegl and Vogel 1970) possibly due to chemical heterogeneity since different metabolites vary considerably (i.e. Friedman and Gleason 1980). Lipids are considerably lighter (≈-100‰) than whole plant values, as are sterols, phytol and carotene (≈-150‰), and protein (≈-26‰) whereas amino acids are considerably heavier than whole plant values (≈+105‰) as are sugars, organic acids, and starch (≈+37 to +90‰) (Smith and Epstein 1970; Estep and Hoering 1978,1980,1981; Hoering 1974; Smith and Jacobson 1976). While sugars are roughly equal to δDl, sterols and fatty acids are lighter (≈-150‰) as is mannitol (≈-72‰; Bricout 1979).

The inter-species variabilities, using single metabolites such as in ${}^{2}\epsilon_{Bcel}$, are also large and one might worry that this would increase variability in the deer δDb. However, since there are large differences between single metabolites of a single plant, it is likely that inter-species variability of non-exchangeable hydrogens from single metabolites would appear much higher than would non-exchangeable hydrogens of whole plant tissue. This is because different plant species may produce different amounts of specific metabolites, relative to other metabolites, from the same, finite carbohydrate pool. This would lead to a different partitioning of the carbohydrate pool among the same metabolites for different species. This could increase the apparent

inter-species variability of single metabolites over variability measured on the entire carbohydrate pool. Deer eat whole leaves which include all metabolites and therefore represent the average δD of the entire carbohydrate pool of a plant. Furthermore, they represent a time integrated average of many carbohydrate pools from individual plants of an area. Therefore, variability of deer δDb should be much lower than intra- and inter-species variability seen among plants using single metabolites.

2.1.3.4 Photosynthetic Cycle:

It is now well known that terrestrial plants can be grouped into three broad categories based on their $\delta^{13}C$ values with each category following a different photosynthetic cycle (i.e. Park and Epstein 1960, 1961; Bender 1971; Smith and Epstein 1971; Lerman and Queiroz 1974; Moore 1982). If the photosynthetic cycle affects $^{2}\epsilon_{sp}$ and if deer from different regions consumed different amounts of plants from different photosynthetic cycles, then the variability in δDb could be increased.

Most terrestrial plants, including all trees, most shrubs, and almost all plants in temperate climates of North America and Europe, follow the Calvin-Benson or C_3 cycle. Greater than 98% of all dicots in North America (Stowe and Teeri 1978) and ≈94% of all flora in British Columbia (Chisholm 1980) are C_3.

Plants using the Hatch-Slack, or C_4, photosynthetic cycle are best adapted to hot and dry climates or to life in salt marshes or along the oceans edge. Included are most tropical grasses, a few shrubs and some food plants such as amaranth, sorghum, millet, maize and sugar

cane. Many C_4 crop plants, such as maize, are non-indigenous to North America but were introduced via agriculture (Vogel and van der Merwe 1977; Van der Merwe and Vogel 1978; Bender et al. 1981; Schwarcz et al. 1985). The percentage of C_4 plants among grasses in North America varies from 0 to ≈82‰ with a higher percentage of C_4 among plants in in areas with high summer temperatures (Teeri and Stowe 1976). The %C_4's among dicots increases to a maximum of ≈4% in areas with high pan evaporation in the summer months (Stowe and Terri 1978) and, therefore, in areas of low rh.

A third group of plants have Crassulacean Acid Metabolism (CAM) which, depending on environmental conditions, allows them to switch temporally from a C_3- to a C_4-like photosynthetic cycle (i.e. Bender et al. 1973; Ting 1971; Neales et al. 1968). Many of these drought adapted plants, including tropical succulents, pineapples and cacti, are adapted to desert life and a few to alpine environments.

Browsers such as the deer should have a diet composed primarily of C_3 dicots. Deer rarely consume grasses except during periods of population stress and starvation and they have poor resistance to parasites found on grasses (cf. Hosley 1956; Severinghaus and Cheatum 1956). In agricultural areas, deer will eat corn and monitoring the $\delta^{13}C$ values of the deer bone collagen should help identify individuals consuming C_4 plants in the form of corn or through limited grazing in warm areas (cf. Vogel 1978a; Ambrose and DeNiro 1986a; DeNiro and Epstein 1978a; Tieszen et al. 1979a,b, below). However, it appears that consumption of C_4 plants should not alter δDb other than through effects already accounted for via RH effects on the rate of evapotranspiration. This is because there are no differences in δD_{cel} of

C_3 and C_4 plants as a result of differing ${}^2\epsilon_{scel}$ (Sternberg et al. 1984a,b, 1986b). C_3 and C_4 plants appear to have different evapotranspiration strategies, with C_4 plants able to continue to transpire and photosynthesize under dry conditions with low rh. This accounts for any observed differences in δD_{cel} between C_3 and C_4 plants but such differences have generally been found to be small due to the relatively low sensitivity of δDl to the effects of rh.

Although the δD_{cel} of grasses averaged -74‰ relative to other C_3 plants from nearby localities (Sternberg and DeNiro 1983), such differences might be due to different δDi from seasonal differences in growth patterns. Furthermore, grass values should not affect δDb since deer rarely graze.

In contrast to C_3 and C_4 plants, the δD_{cel} values of CAM plants are considerably higher (≈+100‰) than C_3 and C_4 plants due to biological fractionation factors and differences in ${}^2\epsilon_{scel}$ (cf. Sternberg et al. 1984a,b,c, 1986b; Keeley et al. 1986). However, CAM plants are unlikely to have entered the deer food chain in significant quantities so should not affect δDb.

2.1.4 Animal δD:

2.1.4.1 Whole Animal Studies:

Most studies confirm that whole animal isotopic values reflect the isotopic composition of their diets (Estep and Dabrowski 1980; Estep and Hoering 1980; Smith and Epstein 1970; Stiller and Nissenbaum 1980) while the water that an animal drinks, lives in, or body H_2O, which is mainly derived from drinking and metabolically released H_2O (Gleason and Friedman 1970), is not directly recorded in the dried tissue values

(Estep and Dabrowski 1980; Macko et al. 1982b; Estep 1981b). However, most experiments conducted on whole animal tissues suffer from lack of evaluation of, or correction for, H-exchange (DeNiro and Epstein 1981c).

2.1.4.2 <u>Non-Exchangeable Hydrogen of Single Animal Compounds:</u>

Previous studies using non-exchangeable hydrogens of single compounds (rather than whole animals) have been accomplished using nitrated chitin from terrestrial insects (Miller et al. 1988) and using chitin isolates, (hydrophobic derivative of D-glucosamine) which contain almost no exchangeable hydrogens, from marine and fresh water amphipods (Schimmelmann and DeNiro 1986). Good correlation was found between δD of nitrated chitin from terrestrial insects and local δDw. On the other hand, δD of the amphipod chitin isolates exhibited large inter- and intra-species variability in δD, with overlap of values from marine and fresh water species. This suggests that chitin macromolecules may better reflect an average value of the plant and animal H-pools than do single isolates thus show reduced variability.

2.1.4.3 <u>Trophic Level Effects:</u>

A portion of the offset (${}^{2}\epsilon_{B}$) between deer δDb and δDl could be due to a biological isotopic separation factor in animals (${}^{2}\epsilon_{BA}$) arising from trophic level effects. Some studies suggest that whole animal tissue is not D-enriched relative to dietary δD (after: Estep and Dabrowski 1980). Amphipods showed no trophic level effects when feeding on Gelidium but were enriched (+60‰) on Ulva (Macko et al. 1982b; Macko and Estep 1983). Positive trophic level effects, with successive D-enrichment during each step in the food chain, seem

indicated by other results. Maggots were +31‰ relative to diet (Schimmelmann and DeNiro 1986) and whole cod +76‰ relative to sea urchin and mussel (Schiegl and Vogel 1970). δD of chitin isolates from amphipods were high (≈+42‰ for herbivores and ≈+155‰ for carnivores) compared to ambient H_2O (Schimmelmann and DeNiro 1986). This suggests a large trophic level effect in animals since δDp of plants at the base of the food chain is generally lower than ambient or leaf-H_2O.

One might expect deer bone collagen to have high δD values relative to diet since plant amino acids, or amino acid precursors making up the collagen are considerably heavier than whole plant values. In this study, a comparison of δDb of seal bone to deer with respect to $^2\epsilon_s$ should further help determine whether there are trophic level effects (Ch. 8,11). Since the food chain is longer in the marine environment, one might expect that $^2\epsilon_s$ would be greater in seal compared to deer.

2.2 Oxygen:

The oxygens in bone phosphate are in equilibrium with body H_2O (i.e. Longinelli and Nuti 1973a,b; Kolodny et al. 1983). Body H_2O, in turn, is derived from drinking or leaf-H_2O. As discussed below, δ^{18}Ob and δDb are related and are affected by the same variables but to different degrees. This will provide a means of estimating RH using both δ^{18}Ob and δDb (Ch. 9). Since δ^{18}Ob is little affected by diagenesis (Shemesh et al. 1983), analysis of both δDb and δ^{18}Ob could also provide a means of checking the δDb results for change due to weathering (Ch. 11).

2.2.1 $\delta^{18}O$ vs δD Relationship of Precipitation:

Both hydrogen and oxygen are affected by the processes of condensation under equilibrium conditions, but oxygen proves to be less sensitive to Ty. This is evident from the slope of the meteoric water line for global precipitation (cf. Craig 1961):

(eq. 2.4) $$\delta D = 10 + 8 \cdot \delta^{18}O$$

Oxygen is more sensitive to isotopic effects during non-equilibrium evaporation. The less than 100% rh at the ocean surfaces leads to evaporation producing an original marine vapor cloud preferentially depleted in ^{18}O compared to D with an apparently high δD value and a positive intercept (d). As %rh approaches 100, d approaches zero (cf. Dansgaard 1964; Yurtsever and Gat 1981; Jouzel et al. 1982; Harmon and Schwarcz 1981; Sonntag et al. 1978). The δDw vs $\delta^{18}Ow$ of this study is expected to follow the meteoric water relationship established for North America (Yurtsever and Gat 1981):

(eq. 2.5) $$\delta D = 6.03 + 7.95 \cdot \delta^{18}O$$

2.2.2 $\delta^{18}O$ vs δD Relationship of Leaf Water:

Plant leaf water $\delta^{18}O$ ($\delta^{18}Ol$) is affected by the same variables and in the same manner as δD (above), so eq. 2.2 can be used to express the relationship of $\delta^{18}Ol$ to both $\delta^{18}Ow$ (weighted growing season average of monthly precipitation $\delta^{18}O$) and RH.

(eq. 2.6) $$\delta^{18}Ol = \delta^{18}Ow + (1-RH/100)\cdot(^{18}\epsilon e+^{18}\epsilon k)$$

Both δD and $\delta^{18}O$ increase during evaporation. For the plants of an area with a common H_2O source and temporal variations in RH, the relationship between δDl and $\delta^{18}Ol$ has been found to be linear with a slope between 2 and 5. The slopes of these evapotranspiration lines depend on T (through $^{18}\epsilon e$), boundary layer conditions (through $^{18}\epsilon k$), and the degree of equilibrium between atmospheric H_2O vapor and local H_2O (Lesaint et al. 1974; Long et al. 1980; Epstein et al. 1977; Allison et al. 1985).

2.2.3 Plant $\delta^{18}O$:

Only a relatively small proportion of oxygens in body H_2O and phosphate oxygen of the deer should derive from the non-H_2O component of plant tissue in the diet (Luz et al. 1984). The $\delta^{18}O$ of even this oxygen ($\delta^{18}Op$) will be related to $\delta^{18}Ol$. It is derived from dissolved CO_2 in the leaf which is either in equilibrium with leaf-H_2O (Burk and Stuiver 1981; Bricout 1979) or from 2/3 CO_2 (in equilibrium) and from 1/3 leaf H_2O (Epstein et al. 1977, model A; DeNiro and Epstein 1979; Edwards et al. 1985). The direct temperature effects on the cellulose (and leaf-H_2O) values, through $^{18}\epsilon e_{CO2}$, $^{18}\epsilon e_{H2O}$ and $^{18}\epsilon_{B}$, are very small (i.e. Ferhi and Letolle 1977,1979; Ferhi et al. 1977,1980; Long et al. 1980; DeNiro and Epstein 1979, 1981b).

Although complete equilibration between CO_2 and H_2O implies a relationship between $\delta^{18}O_{cel}$ (plant cellulose) and $\delta^{18}Ol$ with a slope of 1.0 (cf. Edwards et al. 1985, eq. 1; Burk and Stuiver 1980,1981), the measured slopes are <1 in natural samples, ranging from 0.91 to 0.86

(Edwards et al. 1985; Edwards and Fritz 1986; Edwards 1987; Burk and Stuiver 1981) to as low as 0.38 (Ferhi and Letolle 1979; Ferhi et al. 1980).

There is presently no satisfactory explanation for these <1 slopes. Either exchange of oxygens with, or addition of oxygens from a large reservoir of constant isotopic composition must be involved. This could occur in the plant either prior to or following cellulose formation or in the laboratory during extraction and analysis of cellulose (see also Wedeking and Hayes 1983; Berry et al. 1978; DeNiro and Epstein 1981b). An introduction of atmospheric O_2 into metabolic precursors of cellulose without additional exchange with plant H_2O could lower the $\delta^{18}O_{cel}$ vs $\delta^{18}Ol$ slopes. However, these unanswered questions about the $\delta^{18}O_{cel}$ and $\delta^{18}Ol$ relationship should not detract from our understanding of the $\delta^{18}Ob$ values which are believed to be mainly a function of $\delta^{18}Ol$ rather than of $\delta^{18}Op$.

2.2.4 Bone Phosphate $\delta^{18}O$:

The factors affecting the $\delta^{18}Ob$ value of body water ($\delta^{18}Obw$) have been discussed in Luz et al. (1984) and are briefly presented here. The $\delta^{18}Obw$ is at a steady state value and reflects the $\delta^{18}O$ value of two primary sources but appears to be dominated by the value of drinking water ($\delta^{18}Odw$) or leaf-H_2O ($\delta^{18}Ol$) (cf. Luz et al. 1984; Longinelli 1984; Pflug et al. 1979; Lifson et al. 1975; Longinelli and Padalino 1980). In white tailed deer this input or environmental H_2O is thought to derive mainly from leaf-H_2O contained in dietary plants rather than from drinking water (Luz et al. 1990). There should be only a negligible

contribution to body water from inspired atmospheric water vapor (Luz et al. 1984).

A second source of oxygen is metabolic H_2O and CO_2 from the oxidation of food carbohydrates from plants. The oxygens of this H_2O would derive mostly from atmospheric O_2 having a constant isotopic value (+23‰) and there would be negligible contributions from the non-H_2O component of plant tissue. The oxygens of metabolic CO_2 rapidly exchange with body H_2O. The introduction to total body H_2O of a substantial amount of atmospheric O_2 of constant isotopic value would lower the slope between $\delta^{18}Obw$ and input water ($\delta^{18}Ol$ or $\delta^{18}Odw$).

There are steady state losses of body H_2O as urine, perspiration or expired H_2O and these waters should all have $\delta^{18}O$ values identical to $\delta^{18}Obw$. The expired CO_2 should have a value of $\delta^{18}Obw$+38, due to its equilibrium with body H_2O at 37°C in most mammals (cf. Pflug et al. 1979; Luz et al. 1984).

The final slope between $\delta^{18}O$ of body H_2O and $\delta^{18}O$ of drinking water can be related to three main O-fluxes as follows (Luz et al. 1984):

(eq. 2.7) $$\delta^{18}Obw = [F1/(F1+F4+F5)] \cdot \delta^{18}Odw$$

where F1 is oxygen from environmental H_2O and F4 and F5 are from metabolic H_2O and CO_2 respectively. This slope is necessarily <1, but is quite constant for a given species and probably reflects the average metabolic rate of the species (cf. Luz et al. 1984; Longinelli and Padalino 1980; Longinelli 1984).

2.2.5 Environmental Record in Bone:

It is clear that $\delta^{18}Ob$ should be related to environmental H_2O and RH. Ayliffe and Chivas (1990) found that bone $\delta^{18}Ob$ of kangaroos and wallabies from areas of similar $\delta^{18}Ow$ does indeed reflect local rh. It is thought that these animals obtain most body water from leaf-H_2O. If leaf-H_2O were the dominant source of body H_2O for w.t. deer, then $\delta^{18}Ob$ should mainly reflect $\delta^{18}Ow$ (growing season) and RH in a manner similar to δDb. On the other hand, if drinking water were the dominant source of body H_2O, then $\delta^{18}Ob$ could reflect average yearly $\delta^{18}Owy$ and RHy (yearly average of average daily rh) since H_2O ingested from ponds or streams should reflect weighted yearly average of rain $\delta^{18}O$ and will have undergone evaporation in a manner similar to leaf-H_2O. It is not possible from studies such as Luz et al. (1990) to determine which is the dominant source of body H_2O due to metabolic considerations given above and the unknown slope between $\delta^{18}Ob$ and $\delta^{18}Oi$. In this study it will be initially assumed that leaf-H_2O is the dominant source of input H_2O.

From the above, it is clear that δDb and $\delta^{18}Ob$ should be linearly related, a relationship which will be empirically tested in this work. Numerous other studies also suggest that evaporative transpiration and RH produce a relatively large enrichment in $\delta^{18}Ol$ over the input value of $\delta^{18}Ow$ which may account for as little as 15% of the variation in $\delta^{18}Ol$ (cf. Ferhi and Letolle 1977,1979; Ferhi et al. 1977,1980). Thus, while δDb might provide a paleothermometer, $\delta^{18}Ob$ might be useful as a paleohygrometer since δDb is predominantly a function of δDw (T-effect) whereas $\delta^{18}Ob$ is considerably more sensitive to RH (cf. Allison et al. 1985; Epstein et al. 1977; Long et al. 1978,1980). It is this

difference between the effects of RH on $\delta^{18}Ob$ compared to δDb which will allow the estimation of RH using both $\delta^{18}Ob$ and δDb results (Ch. 9).

2.3 Carbon:

The systematics of carbon isotopes in bone and the environment are discussed in a number of general sources referred to again below (DeNiro and Epstein 1978a,b; Vogel 1978a; Van der Merwe 1982; Chisolm et al. 1982). Herbivore bone gelatin $\delta^{13}C$ ($\delta^{13}Cg$) reflects an accumulated, time integrated average lifetime value of the $\delta^{13}C$ values of the plants in the animals diet (after: Stenhouse and Baxter 1976; Stockwell 1983; above refs.). It is offset from dietary values by ≈3-5‰ (with larger offsets for larger species) and there is a <1‰ increase with each trophic level (cf. Schoeninger 1985; Rau et al. 1983; McConnaughly and McRoy 1979; above refs). Factors affecting plant $\delta^{13}Cp$ will therefore be reflected in bone $\delta^{13}Cb$.

2.3.1 Plant $\delta^{13}C$:

The plants in the deer diets can be subdivided according to photosynthetic cycle with average $\delta^{13}C$ values (and ranges) ≈-27‰ (-21 to -35‰) for C_3, ≈-13‰ (-6 to -19‰) for C_4 and a wide range of -11 to -31‰ for CAM. Only C_3 plants are expected to constitute a significant portion of the deer's diet.

Some authors have observed that some of the above variability in $\delta^{13}C$ of C_3 plants appears to be related to such things as light intensity, O-content, T, salinity, rh, growth rates and inter- and intra-species effects (i.e. Neales et al. 1968; Smith et al. 1976,1979; Winter et al. 1982). However, most of the variation may actually be related to

differences in stomatal resistance, diffusion of CO_2 into the leaves and the partial pressures of CO_2 in leaves. A decrease in $p(CO_2)$ causes an increase in $\delta^{13}C$ due to greater utilization of the C-pool within the plant and thus reduces the observed isotopic separation factor due to kinetic isotopic effects (cf. Troughton 1972; O'Leary 1981; Farquhar et al. 1982a,b). Among trees (all C_3 plants) and within a single species of trees, higher $\delta^{13}C$ values (to ≈-18‰) were found in areas of low average summer RH and high summer T indicating a direct effect of climate on plants (Stuiver and Braziunas 1987). Values may also be ≈3‰ higher for conifers compared to deciduous trees.

Although the $\delta^{13}C$ of atmospheric CO_2 is relatively constant (≈-7‰), temporal and geographical variations in local CO_2 $\delta^{13}C$ may be responsible for other diurnal, seasonal and canopy effects (i.e. Freyer and Wiseberg 1974; Lerman and Long 1978; Francey and Farquhar 1982; Farmer and Baxter 1974; Lowdon and Dyck 1974). A decrease in $\delta^{13}C$ in plants of up to 8‰ at the floor level in dense forests from the canopy effect is due to recycling of isotopically light CO_2 from plant respiration and decomposition of soil litter (cf. Vogel 1978a,b; Medina and Minchin 1980). Finally, deviations among plant parts or between individuals is likely due to chemical heterogeneity since metabolites vary in $\delta^{13}C$ as do individual amino acids (by up to 18‰) (i.e. Degens 1969; Abelson and Hoering 1961; DeNiro and Epstein 1977; Macko et al. 1983; Smith and Jacobson 1976).

2.3.2 $\delta^{13}C$ Record in Bone:

Animals must provide an average of local plant values since natural variation among individuals of specific mammalian groups in

restricted areas is only ±0.3 to ±0.6‰ (Hobson and Schwarcz 1986; Chisholm et al. 1983; Lovell et al. 1986a,b, Walker and DeNiro 1986) which is only a little larger than for mammals raised in th· laboratory on monotonous diets (±0.2‰ DeNiro and Schoeninger 1983). The end point δ^{13}Cg values for a large browsing mammal would be δ^{13}Cg≈-21.5‰ (range -17‰ to -24‰) if it ate 100% C_3 plants and ≈-7.5‰ if it ate 100% C_4 plants (after: Hobson and Schwarcz 1986; Walker and DeNiro 1986; above refs.). A low value of -24.4‰ was recorded in a grazer from the floor of a deep forest due to the canopy effect (Ambrose and DeNiro 1986a).

The δ^{13}Cg of deer in this study are expected to reflect the C_3 plants of their diet and to resemble other North American deer: -20.6±0.5‰ for a population of 55 mule deer from Colorado (Hobson and Schwarcz 1986), -20.8 for a deer from Okanagan region in B.C. (Chisholm 1980); -19.8±0.4‰ (n=7) for mule deer from coastal California (Walker and DeNiro 1986) and -22.0‰ for a deer from Florida (Schoeninger and DeNiro 1984).

2.4 Nitrogen:

Factors affecting the δ^{15}N of bone gelatin (δ^{15}Ng) are discussed in a number of sources referred to below (DeNiro and Epstein 1981a; DeNiro and Schoeninger 1983; Ambrose and DeNiro 1986a,b; Schoeninger and DeNiro 1984; Schoeninger et al. 1983). δ^{15}Ng is primarily influenced by the plant δ^{15}Np in the animal's diet. It is offset from dietary δ^{15}N values by ≈+3‰ (DeNiro and Epstein 1981a) with a 1 to 4‰ stepwise increase per trophic level (after: Minagawa and Wada 1984; Schoeninger 1985; Miyake and Wada 1967; Macko et al. 1982c). Trophic level effects may be due to a combination of ^{15}N-enrichment during

transamination and mechanisms of N-excretion and balance in the body (Macko et al. 1982a; below). Relatively low $\delta^{15}Ng$ variability was reported for herbivores from a single species from Peru (llamas ≈±1‰, n=5) and coastal California (mule deer ≈±0.8‰, n=7) (Schoeninger and DeNiro 1984; Walker and DeNiro 1986) which compares favorably with the very low variability of a single species raised on a monotonous diet (σ_p=±0.4‰, n=81; after: DeNiro and Schoeninger 1983). Therefore, factors influencing $\delta^{15}Np$ will be reflected in $\delta^{15}Ng$.

2.4.1 Plant $\delta^{15}N$:

The $\delta^{15}N$ of plants in a herbivore's diet depend on their N-sources, atmospheric N_2 (≈0‰) from symbiotic N_2-fixing bacteria for leguminous plants and soil-N (NO_3^- or NH_4^+) from bacterially recycled organic material for non-leguminous plants. The $\delta^{15}N$ of total soil nitrogen is similar to that of its NO_3^- and NH_4^+ components (Sweeney et al. 1978; Cheng et al. 1964; Rennie et al. 1976; Letolle 1980) and there is a ≈-3‰ offset between plant $\delta^{15}N$ and total soil-N $\delta^{15}N$ (DeNiro and Epstein 1981a; Delwiche et al. 1979; Peters et al. 1978; Virginia and Delwiche 1982; Kohl and Shearer 1980; Wada et al. 1981).

Average values for legumes have been measured at 0.81±1.6‰ (n=34) and non-legumes at 2.7±3‰ (n=142) (Virginia and Delwiche 1982). Thus, leguminous vs non-leguminous differences in diet of the deer could not be distinguished if modern-day averages for non-legumes were assumed for most of North America. Due to widespread use of artificial fertilizer ($\delta^{15}N$≈0‰), non-leguminous plant values (and soils) may currently be lower than in the past.

There are differences in $\delta^{15}N$ related to herbaceous vs woody growth forms with annual herbs having the highest $\delta^{15}N$ (≈6‰) and trees the lowest (≈0‰) (Virginia and Delwiche 1982). Since trees can be isotopically indistinguishable from most N_2 fixing plants, deer could have low $\delta^{15}N$ without consuming legumes.

Local variability in plant $\delta^{15}N$ relative to soil could also relate to root depth since $\delta^{15}N_{soil}$ varies with depth as well as to seasonal influences on biological processes in soils and plants (Virginia and Delwiche 1982; Delwiche et al. 1979; below). Amino acids have a range of ≈13‰ and some variability between plant parts might be expected due to chemical heterogeneity (Rennie et al. 1976).

2.4.2 <u>Soil $\delta^{15}N$:</u>

Since deer $\delta^{15}Ng$ should be ≈+3‰ relative to plants and plants ≈-3‰ relative to soil, deer $\delta^{15}Ng \approx \delta^{15}N_{soil}$ if legumes were not consumed. However, $\delta^{15}N_{soil}$ can be highly variable due to factors described in sources referred to below (Letolle 1980; Shearer et al. 1978; Rennie et al. 1976; Broadbent et al. 1980; Delwiche and Steyn 1970; Wada et al. 1981). A steady state soil-N cycle is completed through bacterial denitrification of soil nitrate which returns ^{15}N-depleted N_2 to the atmosphere (Sweeney et al. 1978; Wada et al. 1975). Some researchers place current average soil values at ≈+6‰ (Sweeney et al. 1978; Wada et al. 1975) and others at ≈+9‰ (Shearer et al. 1978; Rennie et al. 1976) with a higher average value (+6.4‰) for cultivated soils and lower one (+3.2‰) for forest soils (Wada et al. 1981).

Large variations in the $\delta^{15}N$ of deer from different locations should not be surprising as soil-N $\delta^{15}N$ is known to vary from -7‰ to

+18‰ (Cheng et al. 1964; Wada et al. 1975; above refs.) likely arising from the climatically sensitive processes of bacterial N_2-fixation, nitrification and denitrification (Ambrose and DeNiro 1986a; Letolle 1980; Wada et al. 1975). N_2-fixation is inhibited by high temperatures and soil dryness which increases the soil and plant $\delta^{15}N$ in savannah type habitats (Ambrose and DeNiro 1986a; Virginia and Delwiche 1982). Higher $\delta^{15}N$ are also found in moist tropical forest soils due to increased denitrification (Ambrose and DeNiro 1986a). Green manure and forest litter tend towards lower $\delta^{15}N$ whereas animal manure and crop pastures are more positive (DeNiro and Epstein 1981a,b; above refs.).

Additional sources of variability could arise from nitrogen in sea spray ($\delta^{15}N \approx +7‰$, Virginia and Delwiche 1982), in rain (≈-7 to -49‰ depending on latitude), or due to soil drainage patterns, availability of nutrients, soil type and the temperature dependence of NH3 volatilization from soil NH_4^+ (above refs.).

2.4.3 Direct Climatic Effects on $\delta^{15}Ng$:

Interpretation of $\delta^{15}Ng$ is complicated by possible direct climatic effects on animal $\delta^{15}N$. In Southern Africa, a negative correlation was found between the $\delta^{15}Ng$ and the annual amount of precipitation (PPTy) both for a single species of mixed feeders (elephants) and for herbivores in general (Heaton et al. 1986). Such observations are possibly the result of water conservation and nitrogen mass balance mechanisms in animals (Ambrose and DeNiro 1986a,1987; Sealy et al. 1987; Schoeninger and DeNiro 1984). Interspecies effects were also observed where drought tolerant species (mostly browsers) were found to have $\delta^{15}Ng$ values 2 to 4‰ higher than obligate drinkers

(mostly grazers and ungulates) (Ambrose and DeNiro 1986a, 1987). Here, high concentrations of ^{15}N-depleted urea in urine may be used as a strategy of water conservation among water independent animals which are able to maintain high protein diets through browsing. Human $\delta^{15}Ng$ values followed the effects of PPTy of the mammals in their diet (Heaton et al. 1986).

Ruminants and other herbivores eating foods with low amounts of bioavailable protein (mostly grazers) can also have high $\delta^{15}Ng$ values from increased microbial food processing in the digestive tract (especially in the rumen) and through internal recycling and conservation of nitrogen from urea (Sealy et al. 1987). Protein content and quality is lower in plants from arid areas, in dry grass compared to dicot leaves during the dry season (Sealy et al. 1987) and may be lower in C_4 plants adapted to such dry areas. In regions of Africa having <400mm/yr rainfall (<3.3 cm/mo), animals consuming high protein foods (mostly browsers) were found to have $\delta^{15}Ng$ values 1 to 2‰ lower than animals consuming low protein foods (mostly grazers) (Sealy et al. 1987). Therefore, low protein amounts in foods could produce interspecies effects on $\delta^{15}Ng$ in arid areas opposite to those produced by low PPTy.

In this study, any such browsing/grazing or ruminant/non-ruminant interspecies effects are avoided by use of a single species. This study of deer should provide a valuable means of examining both soil variations in $\delta^{15}N$ and the direct effects of climate on the $\delta^{15}Ng$ of individual animals (Ch.10). This, in turn, should provide valuable information to those using $\delta^{15}Ng$ in food web, and paleodietary studies.

2.5 Isotopes In Marine vs Terrestrial Environments:

In the above, factors affecting the isotopic values in the terrestrial environment were described. In Ch.11, isotopic results on seal bone collagen will be compared to those of deer and other terrestrial mammals for diagenetic effects but their baseline modern isotopic values will be found to be considerably different from terrestrial values. The factors affecting isotopic values in the marine environment and their expected values will therefore be given below.

The δDb of seal should to be significantly higher than any terrestrial mammal because the δD of ocean water (≈0‰) at the base of the marine food chain is significantly higher than the δD of terrestrial rain. There are also a potentially large D-enrichment in marine mammals due to a large trophic level effect in the marine environment where the food chains are longer.

δ^{13}Cg and δ^{15}Ng of marine mammals are generally high (δ^{13}Cg≈-13.1±1.5‰, δ^{15}Ng≈15.9±2.1‰, n=49; after: Schoeninger and DeNiro 1984; Schoeninger et al. 1983; Walker and DeNiro 1986) compared to terrestrial mammalian herbivores. Mammals feeding in arctic locations will likely have lower δ^{13}Cg compared those for warm locations mirroring similar variations in plankton (Wong and Sackett 1978a,b; Fontugne and Duplessy 1981; Sackett et al. 1965). Since the CO_{2aq} source for plankton photosynthesis has a constant, HCO_3^- buffered value of ≈-7‰, increased δ^{13}C values probably occur in warmer latitudes due to limited CO_2-pool effects as these are areas with high plankton growth rates and limited CO_{2aq} supply (cf. Calder and Parker 1973; Smith and Walker 1980; Deuser 1970; Degens et al. 1968a). A small amount of the increase in δ^{13}Cg may also be due to an integration of trophic level effects.

The marine N-cycle is similar to the terrestrial one with $\delta^{15}N \approx +7‰$ for $NO_3^-{}_{aq}$ near the surface and increasing to $\approx +18‰$ at greater depths ($\approx$300–1000m) where denitrification is greatest. Phytoplankton values reflect the $\delta^{15}N$ values of their $NO_3^-{}_{aq}$ source except in tropical seas where N_2–fixing phytoplankton dominate (i.e. Miyake and Wada 1967; Wada and Hattori 1976). Continuous influx of ^{15}N–enriched terrestrial-N from runoff (Peters et al. 1978; Sweeney et al. 1978; Wada et al. 1975) plus N-contributions to the marine food chain from greater depths combined with trophic level effects could explain the higher $\delta^{15}N$ of marine mammals, including seal, compared to terrestrial mammals.

CHAPTER 3

SPECIAL CONSIDERATIONS FOR PREPARING WHOLE BONE AND BONE COLLAGEN FOR HYDROGEN ISOTOPIC ANALYSIS

3.1 Standard Methods For Isotopic Work:

Several steps in sample preparation are necessary to produce gases from bone collagen for isotopic analyses and these have been standardized for $\delta^{13}C$ and $\delta^{15}N$ isotopic work. Initial preparation includes pre-cleaning and reduction of bone to bone powder followed by removal of lipids using organic solvents. In this thesis, collagen (col) denotes the material obtained through bone demineralization in a strong acid. This is generally followed by gelatinization in hot (90°C) acidified H_2O (pH=3) for 20h (cf. Longin 1971; Chisholm et al. 1983a). The gelatin (gel) produced by this method is generally filtered to remove humic acids before drying. Whole bone powders (w.b.) were also investigated in this study as an alternative method of sample preparation and became the method of choice.

The sample material must be outgassed and combusted to produce suitable gases for isotopic work. The Sofer (1980) combustion technique has been widely used for $\delta^{13}C$ and $\delta^{15}N$ work and has been applied (at 900°C) to δD analysis of some organic materials (Becker and Epstein 1982). In this method, organic samples are outgassed then sealed in evacuated Pyrex, quartz or Vycor tubes containing an excess

of cupric oxide as the O_2 donor. Combustion is at 900°C (in quartz or Vycor) or at 500°C (in Pyrex) which should quantitatively convert all organics to H_2O, N_2 and CO_2. The CO_2 and N_2 combustion products are then cryogenically isolated on a vacuum line, purified and, if possible, their yields determined. The separated gases (N_2 or CO_2) are used for isotopic analyses using a mass-spectrometer.

3.2 Additional Considerations For δD Work:

In this work the H_2O produced by the combustion reaction is also cyrogenically purified and an additional step reduces the H_2O to H_2 gas for δD analysis. Since this is the first study using the δD composition of bone collagen, several potential problems had to be investigated before adapting the methods to δD work. These will be considered below while details of the methods will be given in Ch. 4.

3.2.1 Non-Collagen Hydrogens:

A certain proportion of hydrogens in the H_2O combustion product are problematic in that they may not be photosynthetic-H. These include the possibility of exchangeble hydrogens (H_{EX}) in the collagen molecule and the presence of adsorbed H_2O or other H-containing impurities. Outgassing, in which adsorbed-H_2O is removed at elevated temperatures under vacuum before combustion, is a crucial step in sample preparation. Since δD analysis was found to be very sensitive to outgassing conditions, this step required a considerable amount of development and testing (Ch. 5).

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3.2.2 The Problem of Hydrogen Exchange:

While outgassing can remove adsorbed-H_2O, there is no simple method for removing exchangeable hydrogens (H_{EX}) bound in the dried gelatin. Therefore, H-exchange represents a major problem facing hydrogen isotopic work on collagen. The final δDx value of a sample prepared by a given method (x) should represent a simple, linear mixing function of the δD value of H_{EX} (δD_{EX}) and the δD value of the non-exchangeable hydrogens ($\delta Dx_c'$) in the sample:

(eq. 3.1) $$\delta Dx = p_{EX} \cdot \delta D_{EX} + (1-p_{EX}) \cdot \delta Dx_c'$$

Here: p_{EX} is the proportion of H_{EX} in the sample and $(1-p_{EX})$ is the proportion of non-exchangeable hydrogens in the sample. The proportion of H_{EX} could be different for different preparation methods depending on whether the material is whole bone powders (w.b.), x=b, dried intact collagen extracted from bone (col), x=co, or dried gelatinized collagen or gelatin (gel), x=g. The effect that the H_{EX} have on the final δD of the sample will depend on the values of p_{EX} and δD_{EX}. The value of δD_{EX}, in turn, will depend on the rate of H-exchange for each class of hydrogens in the molecule, the value of ambient H_2O with which the H_{EX} exchanges and the isotopic separation factor (ϵ) between H_{EX} and the ambient-H_2O.

As will be discussed in Ch. 6, H-exchange on the collagen molecule is sufficiently rapid that, for mammal bone samples stored in our laboratory, the H_{EX} would be expected to equilibrate with the layers of H_2O adsorbed to the bone sample. This water in turn would be in equilibrium with lab atmospheric moisture. Therefore δD_{EX} would

ultimately reflect δD of lab H_2O-vapor (δDv) but be be offset from it by an equilibrium isotopic separation factor (ϵe_{B-V}) which is temperature dependent.

Outgassing to remove adsorbed-H_2O might produce an additional isotopic separation factor between H_{EX} and H_2O-vapor (ϵo_{B-V}). During evaporation there may be D-enrichment of the H_2O remaining with the sample due to kinetic and equilibrium isotopic effects. If any exchange between H_{EX} and this water were to occur prior to its complete removal, such D-enrichment would be reflected in δD_{EX}. Therefore δD_{EX} would reflect δDv but be offset from it by an isotopic separation factor (ϵ_{B-V}) so that $\delta D_{EX}=\delta Dv+\epsilon_{B-V}$ and $\epsilon_{B-V}=\epsilon e_{B-V}+\epsilon o_{B-V}$.

The values of both ϵe_{B-V} and ϵo_{B-V} are likely to be positive and some variability in ϵe_{B-V} could occur due to variation in room temperature while that of ϵo_{B-V} due to variation in room relative humidity (rh) since different amounts of H_2O adsorbed to the bone could produce variations in the time required for its complete removal during outgassing. This latter could not only produce variations in amount of D-enrichment that occurs prior to the complete removal of this H_2O but also in the amount of time in which H_{EX} is allowed to exchange with this H_2O. Both sources of variability would be included in the overall variability of δDx for any given δDv. For most samples stored in the laboratory at relatively stable room temperatures and humidities and outgassed using standardized procedures, the values of both ϵe_{B-V} and ϵo_{B-V} should be almost constant as empirical results (Ch. 5) do indeed show.

It further seems possible that the contribution of ϵo_{B-V} to ϵ_{B-V} would be small when outgassing is conducted under good vacuum with

high outgassing temperatures. Increasing the rate of evaporation would reduce the amount of exchange between the vapor removed and water remaining thus reduce the equilibrium separation factor in $\epsilon_{O_{B}-V}$. It would also produce a poorly developed diffusional boundary layer and thus reduce the kinetic separation factor in $\epsilon_{O_{B}-V}$. Rapidly lowered amounts of adsorbed H2O in the vicinity of H_{EX} and shortened outgassing times would also discourage further exchange of H_{EX}. Finally, when samples are initially placed under vacuum, excess H_2O is removed essentially through freeze drying and the drop of temperatures in the vicinity of H_{EX} could lower its rate of exchange. This further suggests that the contribution of $\epsilon_{O_{B}-V}$ to ϵ_{B-V} is likely to be small compared to that of $\epsilon_{e_{B}-V}$.

Although gel preparations have been standardized for $\delta^{13}C$ and $\delta^{15}N$ analysis, alternative preparations (w.b. and col) were considered for δD analysis since H_{EX} can vary with preparation methods. In the beginning, whole bone powders were selected for the bulk of the work on modern deer on the assumption that intact collagen bonded to mineral would have fewer exchangeable and already exchanged-H than would denatured collagen extracted from bone and gelatinized. At that time there was also considerable concern that the standard method of extracting collagen from bone followed by gelatinization could produce irreversible H-exchange between the gel and the water/acid solutions. It was realized that H_{EX} could increase in w.b. samples if there were contributions from the bone mineral itself or from other non-collagen organic compounds in the bone. Studies of tooth enamel proved useful

for testing this since it closely resembles the mineral portion of bone but has <1% organic material.

3.2.3 Potential Problems of Sample Combustions:

Results may be further affected if during combustion there is incomplete combustion, if any H-containing compounds other than H_2O were formed or if hydrogen is otherwise lost or exchanged with any hydrogens remaining in the sample tubes or glass. Testing the combustion technique and selection of an appropriate combustion temperature was also a major consideration in the modification of established techniques to the δD analysis of bone. Monitoring the yields of H_2, CO_2 and N_2 provided a valuable technique for identifying some of the effects of sample preparation.

3.3 The Physical And Chemical Nature of Bone:

Following a general description of bone and bone collagen, the potential value of p_{EX} of bone collagen and the thermal properties of bone will be introduced as background information necessary for solving the problem of H-exchange and for determining the sample outgassing or preheating temperature (Tp) and combustion temperature (Tc).

The shaft (diaphysis) of a mammal's long bone is composed of an outer cylinder of hard, dense compact (cortical) bone and an inner medullary cavity containing the bone marrow (i.e. Wasserman 1970). By weight, fresh, cortical bone is composed of 69% inorganic material, 22% organic material and 9% H_2O (Triffitt 1980:48).

3.3.1 Inorganic Components of Bone:

The inorganic or mineral portion of bone is mainly hydroxyapatite (HAP) which has the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ (Posner 1969; Wasserman 1970; Dallemagne and Richelle 1973; Neuman 1980). A small portion of bone mineral may be a solid solution series of (1) dicalcium phosphate dihydrate (DCPD or brushite), $Ca(HPO_4) \cdot 2H_2O$ (2) Octacalcium phosphate (OCP), $Ca_4H(PO_4)_3$, (3) Amorphous calcium phosphate (ACP) $Ca_9(PO_4)_2$, (4) Tricalcium phosphate (TCP) $Ca_3(PO_4)_2$ and (5) HAP (Neuman 1980). As bone matures, the latter members of the series become predominant (Neuman 1980).

Carbonate (CO_3^{2-}) substitutes for PO_4^{3-} to ≈3.5% by weight in modern, dry, fat-free cortical bone (Neuman 1980; Dallemagne and Richelle 1973) and has a $\delta^{13}C$ value of ≈+6 to +9‰ relative to collagen (after: DeNiro and Epstein 1978c; Sullivan and Krueger 1981; Schoeninger and DeNiro 1982). An additional 2.5% CO_3^{2-} may be adsorbed on mineral surfaces (cf. Hassan et al. 1977). Other impurities include lattice substitutions of F for OH and heterionic exchanges for phosphate at the crystal surfaces of CO_3^{2-}, citrate, phosphate esters, diphosphonates, pyrophosphate, and amino acids (Neuman 1980).

Since tooth enamel is almost entirely HAP, it resembles the mineral portion of bone. It contains <1% organic material, 1 to 4% CO_3^{2-} and <2% total DCPD, OCP, and ACP by weight (Weatherell and Robinson 1973).

3.3.2 Organic Components of Bone:

The organic portion of bone is 95-99% collagen on a dry weight basis and the remainder consists of 2/3 soluble and 1/3 insoluble

glycoproteins with traces of proteoglycans and lipids (Wasserman 1970:696; Triffitt 1980). Lipids comprise only ≈0.04–0.20% of the organic portion in compact bone (Triffitt 1980). Lipids contain no nitrogen but have $\delta^{13}C$ ≈-2 to -8‰ relative to collagen (Vogel 1978a) and δD ≈-100‰ relative to whole animal tissue (Smith and Epstein 1970).

3.3.2.1 Bone Collagen:

The collagen molecule is made up of three long protein chains (α-chains) bonded to each other and coiled into a triple helical formation. Each α-chain, in turn, is composed of long sequences of amino acids. Each molecule is arranged through additional bonding into microfibrils. There is a close inter-relation between the organic and inorganic phases of bone that make up cortical bone tissue with 80-90% of the HAP crystals located within the collagen sub-fibrils.

The types of amino acids and nature of bonding will affect the classes of hydrogens existing in bone. These will be discussed in detail below since some of the classes likely represent photosynthetic-H and others H_{EX}. Knowledge of collagen composition will also affect the interpretation of the yields of gases evolved following combustion.

A number of sources referred to below give the conformational details of the collagen molecule, types of inter-chain bonding, types on inter-molecular bonding and arrangement of the collagen molecule into microfibrils along with bonding between mineral and collagen (Triffitt 1980; A. Miller 1984; Tanzer 1985). The amino acid composition of ox-bone collagen appears in Eastoe and Leach (1977), amino acid sequences in Piez (1976) and chemical configuration of each amino acid in Lehninger (1975).

The collagen protein is made up of two imino acids (proline and hydroxyproline) and 16 amino acids. Through the removal of one molecule of H_2O, 1055 amino acid residues (HN-CHR-CO) are covalently bonded to form long α-protein chains with a molecular weight of ≈95,000. Three of these chains are coiled into the tropocollagen molecule to produce a total of 3165 amino acid residues per molecule. There are 1014 amino acids within the helical portion of each α-chain and a tripeptide periodicity containing glycine in every third position (GLY-X-Y) giving 338 tripeptides per chain. A short sequence of 9 to 25 amino acids at the ends of each α-chain (telopeptides) do not participate in the helical formation.

A majority of the inter-α-chain bonds are believed to be H-bonds, possibly involving glycine and peptide amide-H with 1 to 1.6 of these bonds per tripeptide (Tishchenko et al. 1978; Segal 1969; McBride and Harrington 1967; above refs.). Hydroxyproline may further stabilize the molecule through additional H-bonding. Some authors have argued for the presence of covalent inter-chain bonds but their existence has not been conclusively proven (Light and Bailey 1981).

The arrangement of tropocollagen molecules into microfibrils is not fully resolved but the inter-molecular bonds are believed to be covalent involving mainly the lysine and hydroxylysine residues. As an animal matures some of the more labile covalent bonds may be replaced by more permanent multivalent cross linking (cf. Piez et al. 1968; Light and Bailey 1981; above refs.). Covalent (Neuman 1980) or both complexation and electrostatic (Gorbunoff 1984a,b; Gorbunoff and Timosheff 1984) linkages between collagen and inorganic phosphate may

provide a nucleating mechanism for mineralization and bonding between mineral and collagen subfibrils.

3.4 Exchangeable Hydrogens:

3.4.1 Non-Carbon-Bound Exchangeable Hydrogens:

In Table 3.1, the numbers of hydrogens of one molecule of collagen existing in various classes are itemized based on the amino acid composition of ox-bone collagen. Here, the molecule is assumed to contain three α-chains of 1055 amino acid residues each. With the exception of the N-terminal amino and imino acids, there are two hydrogens attached to the backbone of the peptide (as opposed to side chain) for each amino acid. One is the C-attached α-hydrogen and the other is the N-attached peptide amide-H. For the three N-terminal amino acids there are no peptide imide-N but there are nine terminal-H for each collagen molecule (Kingham and Brisbin 1968).

Most of the C-bound hydrogens are expected to be non-exchangeable (Kingham and Brisbin 1968). It is these C-bound hydrogens which are of prime interest in this work since they were attached to carbon during photosynthesis and therefore directly represent the δD of leaf-H_2O.

If all the non-C-bound hydrogens were exchangeable, then $p_{EX} \approx 0.22$. Covalent bonding could lower this number. For instance, if all the lysine and hydroxylysine in the molecule were involved in inter-molecular covalent bonding there could be ≈1% fewer exchangeables since each covalent link removes four exchangeable-H (cf. Triffitt 1980; Piez 1976). However, $\%p_{EX}$ could be increased by ≈2% for every 1% of

Table 3.1 Numbers of Hydrogens in Various Classes on One Molecule of Collagen[a].

	Class	#H	%H	
C-attached	side chain	12,015	64	
	α-hydrogen	2,477	13	
N-attached	side chain	823	4	22[b]
	peptide-amide	2,474	13	
other	side chain carboxyl+hydroxyl	853	5	
	N-and C-terminal	9	0	
		18,615	99	

a based on amino acid composition of modern ox-bone collagen (Eastoe and Leach 1977).

b total $\%H_{EX}$ (percent of exchangeable hydrogens) expected for one molecule of collagen.

the protein which has become hydrolyzed into free amino acids. Modern bone is essentially free of unbound amino acids, but bone that is millions of years old can contain as much as 10 to 20% free amino acids (cf. Schroeder and Bada 1976). These amino acids and many short peptide chains are soluble so natural in situ leaching and the demineralization treatments in the laboratory should remove most free amino acids from fossil bone gel.

For each tripeptide unit, there may be 1.0 very slowly exchanging and 0.6 slowly exchanging-H giving ≈5% very slowly and ≈3% slowly exchanging-H per molecule (Tishchenko et al. 1978; Segal 1969; McBride and Harrington 1967). Tishchenko exchanged these hydrogens within 24h at room temperature in solutions of acid soluble collagen at pH 3.7. However, other exchange-in and exchange-out experiments on denatured, native and reconstituted collagen suggest that anywhere from <1 day to a maximum of 30d may be required for total exchange of all slowly exchanging-H involved in inter-chain H-bonding (cf. Hvidt and Nielsen 1966; Englander et al. 1972; above refs.). Since the gelatinization procedure breaks the inter-chain bonds, there may be fewer slowly exchanging-H in gelatin.

3.4.2 Carbon-Bound Exchangeable Hydrogens:

The α-hydrogens constitute an additional group of extremely slowly exchanging-H making up 13% of the total. They exchange in the presence of H_2O during amino acid racemization with rates on the order of thousands of years but exact rates are poorly known (inter alia Dungworth et al. 1976; Bada et al. 1973; and below).

3.4.2.1 Racemization From Gelatin Extraction:

Although there should be no racemized amino acids in fresh, modern bone, gel extraction procedures could greatly increase racemization rates. Rates are accelerated by the presence of H_2O, by an increase in temperature and by extreme pH (pH<5, pH>8) (i.e. Bada 1985; Armstrong et al. 1983). They double for every increase in temperature of ≈5°C (Schroeder and Bada 1976). They are ≈13x faster at pH=3 than at neutral pH (cf. Bada 1972b) and ≈3x faster in bound amino acids compared to free amino acids (cf. Bada and Schroeder 1975; Schroeder and Bada 1976).

To estimate the maximum amount of racemization which might occur during gelatinization, data from Williams and Smith (1977) were used to calculate the racemization rate constants (k) for six amino acids at 90°C. These k-values were multiplied by 39 (3x for bound amino acids, and 13x for pH=3) and a simple rate equation (eq. 3.2) was used to estimate the proportion of each of the six amino acids that would have exchanged within 20h due to racemization:

(eq. 3.2) $$p_{EX}^{R} = 1-e^{-39kt}$$

where: p_{EX}^{R} is the proportion of H_{EX} due to racemization, k is the racemization rate constant for the free amino acid in aqueous solution and t is the time allowed for racemization. Since the unweighted average of the percentages for the 6 amino acids and the weighted average in which the proportions of each amino acid in bone collagen are used as a weighing factors, both give ≈4% in $\%p_{EX}^{R}$, the amount of amino acid racemized during gelatinization was estimated to be ≈4%. Since α-

hydrogens constitute 13% of all hydrogens this gives an increment of 0.5% in $\%p_{EX}$ in gels.

3.4.2.2 Racemization During Weathering:

Dried bone generally contains sufficient indigenous H_2O for racemization to occur slowly over time in fossil bone (Bada 1985). Racemization rates can be expected to be very slow relative to that occurring during gel-extraction since bone mineral buffers collagen against extreme pH values and rates of bound amino acids are not affected by pH in the 3 to 8 pH range (Armstrong et al. 1983; Bada 1985). Calculations similar to eq. 3.2 were used for to determine p_{EX}^{R} for bound amino acids but at neutral pH and t=10,000 years and with k-values determined for 20°C. An additional 3% in $\%p_{EX}^{R}$ was estimated for every 10,000 years of fossilization.

3.5 Selection of Outgassing Temperature:

Other sources of H_{EX} exist in H_2O loosely bound or adsorbed to bone. This water can be removed by preheating the sample under vacuum prior to sealing for combustion. Literature studies on the thermal stability of bone provide useful guidelines for selecting the outgassing temperature (Tp).

3.5.1 Collagen and Gelatin:

Since collagen is extremely hygroscopic it contains a great deal of adsorbed H_2O and will essentially dissolve itself if exposed to an atmosphere of >75% RH (cf. Ranganayaki et al. 1982; Bone and Pethig 1985; Bull 1944). This adsorbed H_2O may include both tightly bound (≈8%

by wt. or 1 or 2 molecules per tripeptide) and loosely bound molecules. The loosely bound H_2O molecules are probably in rapid exchange with the tightly bound H_2O while all adsorbed H_2O is likely in exchange with the H_{EX} in the collagen molecule (cf. Kuntz and Kauzmann 1974; Grigera and Bienkiewicz 1984; Chapman et al. 1971; Kleeberg and Luck 1984; Lalowicz and Remin 1972). If this H_2O is not removed by outgassing it could result in an increase in $\%p_{EX}$ from 22% to 31-34% if tightly bound H_2O were also included (after: Kuntz and Kauzmann 1974). The $\%p_{EX}$ could further increase to >50% if loosely bound H_2O were included.

Dried gelatin and collagen behave similarly when heated in the absence of O_2 and H_2O in being thermally stable to at least 150°C. Covalent cross-linking occurs in gels in the temperature range of ≈150 to 200°C followed by non-oxidative pyrolysis and melting (Yannas and Tobolsky 1968; Burdyginia et al. 1969). Under vacuum, the thermal denaturation of collagen in w.b. begins at ≈200°C (Liboff and Shamos 1973) indicating greater thermal stability for collagen when bonded to mineral. Nitrogen and hydrogen may be preferentially removed since elemental carbon or graphite is the residue of non-oxidative pyrolysis (Thompson and Gray 1977).

If moisture and O_2 are present, the thermal stability of extracted collagens decreases considerably. If moisture alone is present, heating to ≈120°C produces continuous weight loss and above 140 to 150°C there can be structural changes with melting (cf. Yannas and Tobolsky 1968; Burdyginia et al. 1969). If collagen is first dried at 105°C then heated in air which contains both H_2O and O_2, oxidative chemical degradation occurs by ≈120°C with continuous weight loss through loss of amino acids and release of ammonia. When collagen is

heated in air at 170°C, there is a 15% loss of N_2 by 10d and a 25% loss of sample weight by 20d. This is followed by losses of CO_2 at higher temperatures with rapid breakdown after ≈200°C (cf. Bowes and Taylor 1971; Finch and Jobling 1977; Takenovchi 1981). pH does not influence results.

Collagen is usually either heated to 105°C or placed under vacuum to remove adsorbed H_2O (cf. Kuntz and Kauzmann 1974; Bowes and Taylor 1971). Probably the best approach is to use both vacuum and heat since this combination removes more adsorbed H_2O and also prevents any oxidative decomposition of collagen (i.e. Bull 1944; Bone and Pethig 1985). Based on the above, outgassing conditions selected for this study consisted of heating col or gel preparations at Tp=100°C for 3 days under good vacuum prior to sealing for combustion. Since the accidental presence of air leads to oxidative decomposition, samples prepared under conditions of poor vacuum should be recognizable by an unusually high C/N ratio.

3.5.2 Bone Mineral and Whole Bone:

The selection of Tp for w.b. is complicated by the presence of bone mineral which contains ≈1 to 2% by wt. of adsorbed H_2O (Weatherell and Robinson 1973; Little and Casciani 1966). Based on studies of tooth enamel, it appears that this H_2O can be removed by heating in air to 140°C (LeGeros et al. 1978) or by heating under vacuum to 128°C (Skinner et al. 1975).

Thermogravimetric studies of whole bone, heated both in O_2 and under vacuum, show major H_2O losses (4 to 8.5% of initial weight) by Tp=150°C (Liboff and Shamos 1973; Haas and Banewicz 1980). Subsequent,

minor (1%) losses of weight, which occur between 150° and 225°C, could be attributed to some H_2O from bone mineral, some organic degradation through oxidation and, possibly, some CO_2 from adsorbed CO_3^{2-}. This weight loss probably does not include adsorbed H_2O. For this work on w.b., the conditions selected as being optimal for removal of adsorbed H_2O without collagen denaturation consisted of heating at T_P=150°C for 3 days under good vacuum (≈10^{-3} torr).

3.6 Selection of Combustion Temperature:

The temperature of Sofer-type combustion (Tc) must be re-evaluated for δD work. It must be high enough to ensure quantitative combustion of collagen, yet for w.b. samples it must be low enough not to cause the release of H_2O from bone mineral. It must also be low enough not to encourage any reactions that could involve loss or exchange of hydrogen such as between water and glass in the combustion tubes.

Whole bone powders should not be combusted at Tc=900°C due to the release, above ≈700°C, of H_2O from dehydroxylation of HAP and from H_2O-containing intermediates from OCP and DCPD (cf. Skinner et al. 1975; Liboff and Shamos 1973). Furthermore, devitrification of quartz, and to a lesser degree Vycor, can occur and is much accelerated at high temperatures, especially in the presence of alkali elements such as are contained in bone mineral (Holland 1964:130). Any attempts to combust w.b. at 900°C, in either Vycor or quartz tubing in this study, resulted in explosions of the tubes, most likely the result of a combination of high gas pressures and rapid degradation of the tubing.

Lower Tc in Pyrex tubing was also considered for δD work on gel and col samples since the devitrification reactions can involve both the formation of OH^- and the diffusion and exchange of H^+. Quartz and Vycor can also lose H_2 through diffusion at high T (Holland 1964). Although formation of H_2 seems unlikely, the exact intermediates that might be formed at high T and P in the vicinity of copper is uncertain. δD results could be adversely affected if devitrification occurred or if there were any transient formation of H_2.

Thermal studies of collagen and whole bone indicate that a relatively low Tc (450°C) should be sufficient for quantitatively converting all collagen to its H_2O, CO_2 and N_2 combustion products as all studies indicate rapid decomposition of gelatin and collagen above 200°C. Thermogravimetric analyses of whole bone in air show major weight losses between 225 and 500°C due to oxidative combustion of organic matter. When under vacuum, non-oxidative pyrolysis of collagen occurs between 225° and 500° with a peak at 341°C. Larger weight losses occur at lower temperatures when longer heating times are used (cf. Haas and Banewicz 1980; Liboff and Shamos 1973). Studies on w.b. suggest that <5% of the collagen remains after 12h of heating in air at 200°C and <2.5% collagen remains in intact bone maintained at ≈400°C for ≈20 min. (after DeNiro et al. 1985). Apparently 550°C is sufficient for combustions of gel samples with no advantages to be gained from combusting samples at 900°C since there are no significant improvements in CO_2 yields and no significant differences in $\delta^{13}C$ (Chisholm et al. 1983a). Tests described in this work (Ch. 5) also suggest that a lower Tc in Pyrex may be better for δD work. On the basis of the above, Tc=550°C was selected for all gel combustions.

Whole bone combustions are complicated by a possible contribution of H_2O which could arise from non-collagen sources not removed during outgassing. Water is irreversibly released from the DCPD and OCP, which make up <2% of the mineral portion, at a Tc in the 200 to 600°C range (cf. Weatherell and Robinson 1973; LeGeros et al. 1978; Dallemagne and Richelle 1973). With combustion of w.b., bone mineral could be the source of 3-4% of the total H_2O released at 450°C or 5-6% of that at 550°C (after LeGeros et al. 1978; Monma and Ueno 1979; Little and Casciani 1966; Smillie 1973; Triffitt 1980). Even though the H_2O released above 100°C appears to be either non-exchangeable or to have a very slow rate of exchange (cf. Little and Casciani 1966), selection of the lower (450°C) Tc might reduce by ≈2% that contribution to total H_2O released from bone mineral in the 450° to 550°C range.

Although thermogravimetric analysis of tooth enamel showed, via the nitrogen yields, that all protein was removed by 500°C, some residual, elemental carbon remained (Little and Casciani 1966). Some thermogravimetric studies of whole bone also reported weight losses between 450 and 550°C suggesting that ≈2.4% of the organic material might remain, probably as elemental carbon (after: Haas and Banewitz 1980; Little and Casciani 1966; LeGeros et al. 1978; Liboff and Shamos 1973; Triffitt 1980). However, since thermogravimetric studies use short heating times for each temperature, it is likely that extended heating times at 450°C would result in complete combustion of all organic residues. Furthermore, since both H and N are removed early during oxidative decomposition of collagen, shifts in $\delta^{13}C$ and CO_2 yields would be the main effects of incomplete combustion with few effects on the δD and $\delta^{15}N$ measurements and yields.

In considering the $\delta^{13}C$ data results from oxidation of w.b., potential effects from using the lower Tc (450°C) must be balanced against those resulting from a CO_2 contribution from bone CO_3^{2-} which could occur at Tc=550°C. Significant losses of bone carbonate and secondary CO_3^{2-} in fossil bone begin to increase by 500°C with large increases between 650 and 850°C (cf. Haas and Banewicz 1980; Liboff and Shamos 1973; LeGeros et al. 1978). Since one prefers to obtain only C from collagen, a Tc<500°C (i.e. 450°C) using extended heating times might be preferable for w.b. analysis which includes the interpretation of $\delta^{13}C$ and CO_2 yields. Since most thermal studies indicated that, with extended heating times, collagen should be quantitatively combusted at 450°C but with a lesser contribution of H_2O and CO_2 from bone mineral, Tc=450°C was chosen for all w.b. combustions. Monitoring all yields should further help identify any problems resulting from incomplete combustion.

3.7 Fossil Bone:

The use of δD of bone for paleoclimatic studies requires an examination of effects of weathering and contamination on bone chemistry and δD values. Bone from archaeological sites might also have been cooked or burned which could further affect results. There are four mechanisms that can alter δD results. (1) changing composition of bone collagen such as would result from preferential loss of specific amino acids or class of hydrogens such that the remaining hydrogens have isotopic values distinctly different from the hydrogens removed, (2) contamination with a substance having δD values distinctly different from the collagen, (3) change in p_{EX}, and (4) exchange among a class of

extremely slowly exchanging-H and/or change in the number of such hydrogens. Although this work represents the first study to examine diagenetic changes or effects of cooking on δD of bone (Ch. 11), the literature does provide some information on the probability that (1) or (2), above, might have occurred. Data from the literature, might also suggest systematic changes in chemistry against which changes in δD might be correlated so that monitoring or correcting δD results for effects of diagenesis or cooking might become possible. Alternative preparation methods to reduce contamination and the effects of diagenesis may further be suggested by a literature review.

3.7.1 Bone Weathering:

The effects of weathering on bone collagen and its amino acid content are discussed in a number of sources referred to below (Wyckoff 1972, 1980; Dennison 1980; Armstrong et al. 1983; Bada 1985; Tuross et al. 1980, [illegible]. Not only does δD of different amino acids from the same sample vary widely but due to problems of H-exchange and the fact that H (i.e. H_2O) is ubiquitous in the environment, δD could be more sensitive to diagenesis than $\delta^{13}C$ or $\delta^{15}N$. However, changes in δD should be paralleled by other indicators of chemical change. Most diagenetic change in bone collagen can be viewed as a slow hydrolysis of the collagen molecule with breakdown into smaller peptide units and amino acids. This is accompanied by groundwater leaching and a concommitant diffusion of the breakdown products to the outer layers of the bone (i.e. Hedges and Wallace 1980; Engle and Macko 1986; above refs.). Breakdown is greater at environmental extremes in pH (Bada et al. 1984; Bada 1975; above refs.) and removal of the breakdown products

reduces the overall protein and N-contents of bone. It also leads to larger amounts of original protein and larger peptide units in thicker, cortical bone than in cancellous bone and a gradient in peptide unit sizes from smaller to larger towards the center of bone fragments (Von Endt 1980; Von Endt and Ortner 1984; Schroeder and Bada 1976; Hare 1980; above refs.).

A review of the literature suggests that some variables, reflective of the chemical changes accompanying increased hydrolysis and leaching, vary in systematic ways such that covariance among some variables can be expected. Along with a decrease in collagen, nitrogen and total amino acid contents, the amino acid ratios are altered showing lower concentrations of hydroxyproline (HPRO), glycine (GLY) and other neutral amino acids relative to contents of aspartic acid (ASP), glutamic and other acidic amino acids (cf. Kessels and Dungworth 1980; Hassan and Hare 1978; DeNiro and Weiner 1988a; above refs.). The C/N yields of gel should increase with decreasing collagen content, N-content and GLY/ASP ratios since leaching preferentially removes GLY which has a low C/N yield relative to other amino acids, whereas the longer chained, heavier molecules, which are preferentially retained in bone, generally have higher C/N ratios (Ennis et al. 1986; Hassan and Hare 1978; DeNiro and Weiner 1988a; Masters 1987).

Preferential removal of GLY, which has high $\delta^{13}C$ and low $\delta^{15}N$ values relative to other amino acids, may produce higher $\delta^{15}N$ and lower $\delta^{13}C$ in leached bone (Tuross et al. 1988). Bone extracts with C/N yields between 2.9 and 3.6 (90% to 112%, this study) are relatively unweathered with their original $\delta^{15}N$ and $\delta^{13}C$ values. Bone gelatin with C/N yields outside this range generally have undergone considerable

leaching, containing <2% collagen and, in most cases, having high $\delta^{15}N$ and low $\delta^{13}C$. (after: DeNiro 1985; DeNiro and Weiner 1988a; Ambrose 1990).

3.7.2 Burning and Cooking:

Cooking and burning can also alter the isotopic signature of bone found in archaeological sites. Bone rapidly loses organic material with heating at T≥200°C, so low amounts of organic material can not be conclusively ascribed to leaching. For diagenetic studies, identifying changes arising from cooking or burning, as opposed to leaching, could be important if systematic changes in chemical indicators are to be used for correcting δD results for weathering effects (see Ch. 11).

Recognition of C/N values outside the 2.9 to 3.4 range (90% to 106%, this study) should effectively identify bone with isotopic signatures that have been altered due to cooking or burning (DeNiro et al. 1985). Physical features such as polygonal cracking and x-ray diffraction patterns, might be of further assistance for identifying bones heated at T≥200°C (Shipman et al. 1984), while calcined bone (T≥400°C) should be recognizable by its bluish-white, chalky appearance.

At T≥200°C, the $\delta^{15}N$ appears to increase, the $\delta^{13}C$ to decrease and the C/N yields either increase or decrease, depending on whether the bone is continuously heated at one temperature (200°C) or heated over a range of temperatures (170° to 420°C) (after: DeNiro et al. 1985). The GLY/ASP ratios similarly decrease or increase with continuous heating at 122°C or heating over a range of temperatures (122° to 132°C) (Wyckoff 1972). All results, except for heating over a range of temperatures, appear to be in the same sense as expected to result from leaching

during weathering. However, wet heating of bone with limited amounts of H_2O (150°C in sealed ampoules) can result in increased GLY/ASP ratios, opposite to those of leaching (after: Dungworth et al. 1976). Boiled and roasted bone retained their original C/N yields and their $\delta^{13}C$ and $\delta^{15}N$ to within 1% (DeNiro et al. 1985) with long bone exhibiting less alteration than ribs or radii. This indicates that, in contrast to burning, cooking may not alter results.

3.7.3 <u>Contamination:</u>

Humic acid, a common terrestrial contaminant, has relatively little hydrogen and nitrogen relative to carbon. It has $\delta^{13}C$ and δD values about the same as local terrestrial plants ($\delta^{13}C \approx -20$ to -30‰ and $\delta D \approx -50$ to -100‰; Nissenbaum 1974) but has C/N ratios between 10 and 20 (Masters 1987). Since $\delta^{13}C$ of plant material, from which humates predominantly derive, remains stable during degradation while $\delta^{15}N$ increases (DeNiro and Hastorf 1985; Turner et al. 1983), contamination of seal bone by such terrestrial material might be recognizable by low $\delta^{13}C$ and high C/N. However, it is not certain that contamination would be easily distinguishable from weathering, cooking or burning, so contamination may be best dealt with through improved sample preparation. It will be suggested in Ch. 11 that, whereas simple weathering by leaching should be recognizable by systematic covariation between some variables, other factors such as contamination or burning may be identified through data outliers relative to such trends observed for leaching.

3.7.4 Possible Preparation Improvements for Fossils:

In this work, w.b., col and gel preparations were all studied for use on modern or fossil bone. Use of only compact bone, removal of lipids, mechanical removal of surface stains and ultrasonic cleaning would probably considerably reduce potential contamination of fossil bone by fungus, algae, rootlets, charcoal or by severely degraded collagen (i.e. Piepenbrink 1986; Hassan and Ortner 1977; El-Daoushy et al. 1978; Bada 1985). Soluble free amino acids, some bacteria, small peptides, and acid soluble organic or mineral contaminants such as fulvic acids and carbonates, should also be removed during demineralization (cf. Nissenbaum 1974; Olsson et al. 1974).

Although the Longin (1971) method to gelatinize (solubilize) collagen should further remove acid insoluble, contaminants such as humates, roots, woods, saprophytes, and some inorganic contaminants such as sand silica or clay, it may not efficiently remove all contaminants including some humates and fulvic acids (cf. Chisholm et al. 1983a; Stafford et al. 1982; Kyle 1986; DeNiro and Weiner 1988a,b,c; above refs.). Although NaOH treatments seem effective for removing humic acids (Gurfinkel 1987; DeNiro and Epstein 1981a; Bada et al. 1974) others have noted loss of protein and minor changes in $\delta^{13}C$ and $\delta^{15}N$ values (Chisholm et al. 1983a; Olsson et al. 1974).

The undesirable breakdown of collagen into smaller peptides and potential biased amino acid compositions from poor collagen recovery may be reduced by gentler demineralization processes, including use of weaker acids, EDTA treatments or a combination of weaker acids and dialysis, followed by gelatinization in very weak acids or formic acid at lower temperatures (Jope 1980; Hassan and Hare 1978; Wyckoff 1980;

Hedges and Wallace 1980). Separation of gelatin extracts into larger size fractions to eliminate severely degraded collagen may also help (Wyckoff and Davidson 1976b, 1979; Tuross et al. 1988; Brown et al. 1988). Alternatively, extraction of collagenase reduced collagen could eliminate some forms of contamination (DeNiro and Weiner 1988b). Such improvements in sample preparation (eg. Brown et al. 1988) along with complete characterizations of the isolates, appears to be a very promising approach to understanding and controlling for the effects of diagenesis on the isotopic record in bone collagen. Use of alternative materials, such as dentine or organics from crystalline aggregates of bone, also needs to be explored (i.e. Smillie 1973; Masters 1987; Wyckoff 1972; DeNiro and Weiner 1988c).

CHAPTER 4

SAMPLES, PREPARATION, ANALYSIS AND YIELDS

It was indicated previously that several "a priori" considerations were necessary along with experimentation and testing before all methods were evolved and finalized for δD analysis of the deer. However, the effects of one step can only be studied when all other steps are held constant or are standardized. For this reason, a description of the samples, the final, standardized methods of sample preparation, and analytical methods are presented here for easier general reference and are to be assumed for all work except where variations in the procedures were tested as described later (Ch. 5).

4.1 The Samples:

In order to test the correlation of the δD of bone collagen (δDb) from w.t. deer (white tailed deer or *Odocoileus virginiana*) to precipitation δD (δDw) or other climatic variables, tibia specimens of adult animals were requested in writing or by phone from various wildlife agencies and universities across North America. Names, addresses and phone numbers were obtained from a number of published directories (Bracken 1984; Dresser and Hill 1983; USFWS 1983; ONMR 1983). Individuals who kindly contributed samples are listed in the acknowledgements. All sample information is given in App. B and

includes the sample location, age, sex, cause of death and any additional information on vegetation cover, agricultural practices in the area and possible diet of the deer. Tibia specimens from animals which were predominantly road-killed but also including hunted, or culled deer were collected by the wildlife personnel and were frozen. They arrived by courier and remained frozen until further sample preparation. Mule deer (*Odocoileus hemionus*) were obtained from the three sampling areas west of the Rocky Mtns. As indicated in the App. B, occasionally metatarsal was analyzed in place of tibia and one tibia sample was obtained from a fawn (ON-3). In all, 66 specimens were analyzed and their locations can be identified on maps showing areal distributions of δD, $\delta^{15}N$ or $\delta^{13}C$ results given in later chapters.

Tibia samples of a muskox (*Ovibos moschatus*) and a ringed seal (*Phoca hispida*) from a 100 year old site on Banks Island served as bone standards for a number of tests and are referred to as MUSK and SEAL. Also analyzed were fossil samples of bison (Bison sp.), ringed seal, harbor seal (*Phoca vitulina*), and human gelatins as well as modern flesh from marine and terrestrial animals. Additional tests were conducted on one fossil bison (Bison sp., ≈1200 y.B.P.) from south central Alberta designated as BIS. A commercially bought gelatin (GEL) was also used as a standard for testing sample preparation as was a sample of tooth enamel (ENAM) prepared from a modern bison (*Bison bison*) tooth.

4.2 Preparation of Whole Bone Powders:

For reasons outlined in Ch. 3 and 5, whole bone (w.b.) powders rather than gelatin (gel) or collagen (col) extracts were used for most of this work. Only cortical bone was used since it contains almost pure collagen and is most resistant to weathering and post burial contamination (inter alia Triffit 1980; Wasserman 1970; Lambert et al. 1982; Bada 1985; Ch. 3). The tibia shaft was cut into convenient pieces using a bone saw and the marrow, grease and blood of modern samples were removed. After this, care was taken not to contaminate samples with any organic materials, all of which contain hydrogen. For instance, samples never came into contact with hands, and metal tweezers, glass or foil containers, and Al-foil weighing papers were preferred over plastic or paper.

Bone fragments were soaked for 3 days in distilled H_2O to remove fresh blood and other solubles and the water was changed frequently during this time. Samples were then dried in air for 3 days then dried under vacuum for 24 hr at room temperature. After drying, samples were soaked in CCl_4 for 3 days to degrease them. At the end of 3 days the CCl_4 was removed by decanting and the bone was air dried at room temperature for another 3 days.

Samples of bone were mechanically cleaned by grinding away the outer surface with a diamond or stainless steel bit to remove any remaining areas showing stains, dried blood, grease or other insoluble surface contaminants. Samples were repeatedly washed in clean, cool distilled H_2O during this procedure. Mechanical removal of the surface is very important for fossil samples since molds, lichens, and organically stained material can be removed this way. Samples were then cleaned in

an ultrasonic bath for 3 periods of 10 minutes each in distilled H_2O to remove pulverized bone and remaining solubles. Fresh, distilled H_2O was used for each cleaning and additional cleanings, if necessary, were carried out until the supernatant remained clear. The cleaned samples were then air dried at room temperature for a minimum of 4 days.

A stainless steel mortar and pestle was used to break the dried bone pieces into small fragments. Slivers of bone were then ground for 10 minutes in a tungsten carbide mill. This was usually sufficient to reduce all the bone fragments to a fine powder. Any large fragments of bone were removed from the powder and reground. This powdered whole bone material is referred to as "w.b.".

4.3 Preparation of Collagen and Gelatin Extracts:

Methods for extracting collagen from bone for $\delta^{13}C$ and $\delta^{15}N$ analyses are well established (cf. Longin 1971; Chisholm et al. 1983a). Preliminary removal of lipids using chloroform (Bligh and Dyer 1959), was not necessary since most samples had been previously degreased with CCl_4. Bone powder was demineralized at room temperature for 20 to 30 min. using 6N HCl followed by filtration using #1 Watman paper. The procedure was repeated until completion of demineralization was verified by checking that the pH of the final wash was ≤1. The collagen was then gelatinized in hot (90°C) HCl-acidified water (pH=3) for 20 hr. This solubilized collagen was then filtered to remove any insoluble residue such as pieces of filter paper or the humic acids found in fossil bones. Finally, the gelatin was dried in air at 70-90°C. This material is referred to as "gel".

A limited number of deer collagen samples were not gelatinized but, rather, after demineralization the insoluble collagen was separated via centrifugation. These collagen samples were then dried and prepared in an identical manner to that of the gels. This material is referred to as "col".

4.4 Pre-Heating to Remove Adsorbed Water:

In preparation for combustion (Sofer 1980), ≈30mg of gel or col or ≈200mg of w.b. was placed at the bottom of a 9mm (o.d.) x 20 cm Pyrex breakseal. All breakseals were made from glass tubing with one end sealed using a torch. Prior to use, all material (glass tubing and CuO) were pre-cleaned by heating for 1 to 2 hrs in a muffle furnace. Pyrex breakseals were pre-heated at 550°C and quartz, Vycor and CuO at 900°C.

As discussed previously, it was necessary to pre-heat all samples under vacuum during the outgassing procedure to remove all adsorbed H_2O. Individual thermistor controlled furnaces, specially designed for this purpose and capable of maintaining Tp to ±1°C, were placed over the bottom tips of the Pyrex tubing to completely enclose the sample. To ensure that all the sample was heated, it was important that no powder remain on the sides of the breakseal. Therefore, when placing the sample into the breakseal, cut Pyrex tubing (6mm o.d.) was used as a funnel to ensure all powder reached the bottom. Cross-contamination was avoided by using a new funnel tube for each sample. An excess of CuO was then added to the tubes.

The breakseals containing the samples and CuO were then outgassed under vacuum at room temperature for ≈1hr. Clean glass wool

in the glass adaptor above each sample prevented any powder from entering the vacuum line. Each sample was then outgassed for 3 days using the thermistor controlled furnaces. For reasons discussed in Ch. 3 and 5, an outgassing temperature (Tp) of 150°C was selected for w.b. and 100°C for gel. When outgassing was complete, the top (open) end of each breakseal was sealed off with a torch and the samples were ready for the combustion procedure.

4.5 Combustion:

Three gaseous products (H_2O, CO_2, and N_2) were quantitatively produced from completely combusted bone collagen using the Sofer (1980) method in which organic samples are burned in 9 mm (o.d.), sealed, evacuated glass tubes containing an excess of CuO. Gelatin samples in Pyrex tubes were thoroughly mixed with the CuO and placed in a muffle furnace and combusted at 550°C (cf. Chisholm et al. 1983a). After 2 hours the samples were removed from the muffle furnace and inspected. Occasionally, sample material at some distance from the CuO did not combust completely as evidenced by graphite stains on the tubing. The CuO was redistributed to cover these stains and an additional 2 hours of heating usually ensured complete removal of the stains. An alternative combustion temperature (T_c) of 900°C for ≈2hr (Northfelt et al. 1981) was studied for samples of gel in Vycor or quartz tubing (see Ch. 5).

Modified Tc and combustion times were used for w.b. work of this study and were chosen on the basis of theoretical considerations given in Ch. 3 and on empirical studies of the methods presented in Ch. 5 and 6. The w.b. powders were combusted in Pyrex at 450°C. After 2-

3 hr the samples were removed from the muffle furnace, allowed to cool, remixed with the CuO by shaking, and replaced in the furnace for another 2-3 hr. This procedure was repeated 4x for 8-12 hr of total combustion time.

4.6 Extraction of Gases for Mass Spectrometry and Determination of Yields:

A vacuum line (Fig. 4.1) was specially designed to allow the simultaneous extraction of the CO_2, H_2O and N_2 combustion products and to allow determination of the N_2 and CO_2 yields. The 9mm breakseals containing the combusted bone powder were attached to the bottom portion of the line (B) where the sample gases were introduced using a tube cracker (DesMarais and Hayes 1976). Vacuum line cleanliness and H_2O transfers over short distances are essential for good δD preparatory work. Therefore, the evolved H_2O is transferred entirely within the bottom portion of the line which can easily be removed and cleaned. Cleanliness was further facilitated by use of greaseless stopcocks (Ace brand) and ball joints (Roulex) sealed by Viton O-rings which maintain an excellent static vacuum in the system.

For complete collection of H_2O, it is important to minimize the pressures of non-condensed gases in the system and to provide large surface areas for freezing down the H_2O in addition to keeping transfer distances short. As the volumes of sample gases in this work were large, a 500 ml bulb was used to increase the line volume and reduce gas pressures. This bulb could be easily cleaned and represented a large condensation area for H_2O and CO_2 without posing a problem for

Figure 4.1 H_2O, N_2 and CO_2 Extraction Line.

9mm sample tube in tube cracker (B).

500 ml bulb, H_2O, CO_2 trap (A).

U-tube, H_2O, CO_2 trap (D).

N2 manometer (F,G).

CO_2 manometer (E,G).

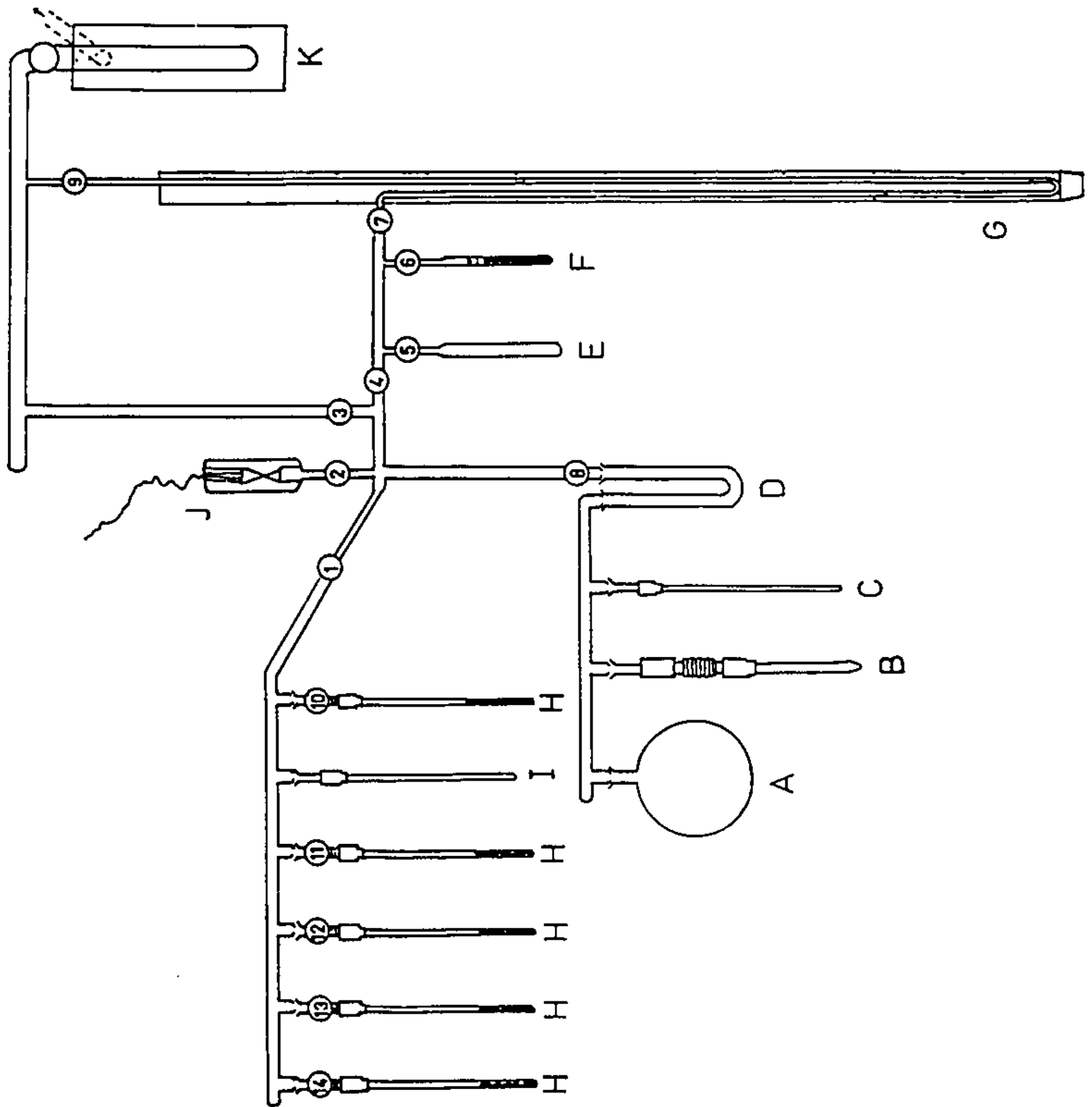
6mm, H_2O sample vessel (C).

6mm, CO_2 sample vessel (I).

6mm, N_2 sample vessels containing
outgassed activated charcoal (H).

Pressure gage (J) thermocouple 1946 tube.

To vacuum through cold trap and
Hg-diffusion pump (K).


K
9
G
7
6
F
5
E
4
3
2
J
8
D
1
C
B
10
H
I
A
11
H
12
H
13
H
14
H

subsequent transfer of H_2O out of the bulb (A) and into the Pyrex H_2O breakseal (6mm o.d. x 16 cm) (C).

The N_2 and CO_2 gases were cryogenically separated first by freezing the H_2O and CO_2 into the bulb trap (A), and then by separate cryogenic transfer through the U-tube trap (D) into the manometer (E,F,G). This manometer had two traps: one for CO_2 (E) and one for N_2 (F). The N_2 trap contained previously outgassed activated charcoal maintained at $N_2(l)$ temperature as a sponge for $N_2(g)$ (F).

Following separate manometric determination of their yields, each of the separate N_2 and CO_2 gases were cryogenically transferred and sealed within their respective 6 mm Pyrex breakseals in the upper portion of the line for later isotopic analyses. Five of the upper breakseals contained previously outgassed activated charcoal at $N_2(l)$ temperature for collection of $N_2(g)$ (H). A sixth breakseal, (I), was for collection of CO_2.

The H_2O from the sample which remained frozen in the 500 ml bulb until the CO_2 and N_2 gases were removed, was then transferred into the breakseal at (C) and sealed. All breakseals for individual H_2O, CO_2 and N_2 collection (C,H,I) had been previously outgassed at 550°C for 1-2hr and were attached to the line by glass joints and Ultra-torr vacuum seals. Additional details of this extraction procedure are given in App.C along with details of the manometer calibration for N_2 and CO_2 yield determinations.

4.7 Analytical:

The δ-notation (in permil or ‰) is standard for stable isotope work and compares the heavy (Z') to light (Z) isotope ratio in a sample gas to this same ratio in a standard gas:

$$\delta Z' = \left[\frac{(Z'/Z)_{SPL}}{(Z'/Z)_{STD}} - 1\right] \cdot 1000 \qquad \text{(eq. 4.1)}$$

where: $(Z'/Z)_{SPL}$ and $(Z'/Z)_{STD}$ are heavy to light isotope ratios in the sample and standard respectively.

The δ-values (δD, $\delta^{13}C$ and $\delta^{15}N$) were all determined by using a MICROMASS 602 or 602D mass spectrometer to measure the mass abundances of 3/2 (DH/HH), 45/44 ($^{13}C^{16}O^{16}O/^{12}C^{16}O^{16}O$) and 29/28 ($^{15}N^{14}N/^{14}N^{14}N$) of the gases H_2, CO_2, and N_2. All δ-values of this study were normalized, to the appropriate reference standard (1) Vienna Standard Mean Ocean Water (SMOW) for δD, (2) Peedee belemnite calcite (PDB) for $\delta^{13}C$ and (3) atmospheric N_2 (ATM) for $\delta^{15}N$. For practical reasons, a working reference gas of known composition is typically used for in-house δ-determinations and eq. A.2 or A.4 is used to convert results to conventionally normalized values. Details of specific analytical techniques used in this study and additional equations are given in App. C.

The CO_2 and N_2 gases were analyzed at McMaster University with analytical uncertainties of ±0.1‰ for both the $\delta^{13}C$ and $\delta^{15}N$ determinations. The standard deviation of replicate analyses of atmospheric N_2 using the charcoal sponge was ±0.15‰.

Water samples were transported to the University of Alberta where, prior to isotopic analysis, they were first converted to H_2 gas over uranium at ≈800°C (Bigeleisen et al. 1952) and the H_2 yield was determined manometrically. A limited number of samples were analyzed at the University of Waterloo where sample H_2O were also reduced to $H_2(g)$ over hot uranium.

As indicated in App. C, several precautions were taken to reduce errors in the δD determinations due to memory effects in the uranium furnace, loss of H_2 gas from sample vessels and H_3^+ contributions to the DH peak in the mass spectrometer. Residual errors due to over- or under-correction of the H_3^+ contribution, other machine errors, memory effects, or H_2 loss from sample vessels were also dealt with through additional monitoring and corrections using secondary calibrations. The pooled standard deviations (Johnston 1976:372) of the δD measurements are: (1) analytical, ±0.7‰ (n=221), and (2) replicate preparation and analysis of a H_2O standard, ±1.5‰ (n=48).

4.8 Calculation and Interpretation of Yields:

There is almost no interspecies or individual variation in the composition of mammalian bone collagen (Wyckoff 1972). Therefore, information on the amino acid composition of modern ox-bone collagen given by Eastoe and Leach (1977) was used to estimate the yields of CO_2, N_2, and H_2 per gram of quantitatively combusted gel. From the weighted average amino acid gram-molecular weight, using the proportions of each amino acid in 1000 residues as the weighing factors, and, assuming that there are 3165 amino acid residues per molecule of collagen, an estimated molecular weight for collagen of ≈290,000 was

determined. One gram of fully combusted collagen, would yield $4.209 \cdot 10^{-2}$ moles of CO_2, $0.654 \cdot 10^{-2}$ moles of N_2 and $3.253 \cdot 10^{-2}$ moles of H_2 and would give C/N', H/N' and H/C' elemental ratios of 3.2, 5.0 and 1.5, respectively.

In this work, two definitions of yields were calculated. One was the relative (%) yield (X/Y) based on what would be theoretically expected from the yields of either of the other two gases generated from the sample:

$$\text{(eq. 4.2)} \quad X/Y(\%) = \left[\frac{M^g(X) \cdot M^g(Y)'}{M^g(Y) \cdot M^g(X)'}\right] \cdot 100$$

where: $M^g(X)$ and $M^g(Y)$ are the mole amounts of the CO_2, N_2 or H_2 gases (g) released from the sample and $M^g(X)'$ and $M^g(Y)'$ are the mole amounts of each gas expected from one gram of fully combusted collagen defined above. The yield calculated on the basis of that expected from the sample weight (X/g) is:

$$\text{(eq. 4.3)} \quad X/g(\%) = \left[\frac{M^g(X)}{wt \cdot M^g(X)'}\right] \cdot 100$$

where: wt is the sample weight in grams.

The averages and standard deviations of the yields for several samples of gel and w.b. are summarized in Table 4.1. The measurement uncertainties, based on the manometer calibration statistics (App. C) are typically ±1.5 to 2.5%. Variability of the relative gas yields (X/Y) range from ±2 to ±6% and must reflect variability in replicate sample preparation since the standard deviation, σ, calculated on different

specimens of deer is only slightly larger than for the replicate samples of GEL, MUSK, or SEAL.

The percentage yields normalized to the sample weights (X/g) are useful for estimating the actual amount of gel undergoing combustion and, in fossil w.b., the amount of collagen remaining after weathering. However, effects of sample preparation are best monitored using the relative gas yields (X/Y). This is because both gel and w.b. are hygroscopic so final vacuum dried weights cannot be accurately determined. Losses on weighing paper and transfer funnels further impede accurate determination of weights. For w.b. analyses, the yields relative to N_2 might be most appropriate since N_2 derives almost exclusively from protein in bone whereas CO_2 could also be generated from bone carbonate.

The average relative yields from n=26 gel preparations are C/N=100±3%, H/N=93±4% and H/C=93±4% (Table 4.1). Those for the n=101 w.b. preparations are C/N=100±6%, H/N=91±4% and H/C=91±6%. Thus the H_2 yields are systematically lower than 100% (90 to 94%). This could be due to an initial inaccurate knowledge of the conformational details of collagen, its assumed composition and the true hydrogen content in bone. Alternately, during preheating there may be an alteration of collagen due to denaturation or crosslinking with the loss of some class of H_2O or hydrogen. Additional covalent crosslinking in gels may occur when they are incubated at 37°C or heated in N_2 to 160° or 170°C (cf. Burdygina et al. 1969; Bowes and Taylor 1971; Light and Bailey 1981). However, if there are losses of hydrogen due to outgassing, the

Table 4.1. Percentage Yields of Various Modern Gelatin and Whole Bone Samples.[a]

	n	C/N	H/N	H/C	H/g	N/g	C/g
Gelatin							
GEL[b]	6	98±3	92±3	94±4	77±5	84±6	82±7
Deer	14	99±3	93±5	94±5	76±5	82±3	81±3
MUSK	5	104±2	93±3	90±2	83±2	89±3	93±2
SEAL	1	102	94	92	76	81	74
Whole Bone							
Deer	74	100±6	90±4	90±6	68±6	75±8	75±7
MUSK	14	98±5	91±3	93±4	76±4	85±4	81±6
SEAL	13	101±5	98±2	97±5	70±6	71±6	72±6

a All samples prepared as described in text (prep. 5). See App. C, D and Ch. 5 for additional explanations, table notes, and raw data.

b Includes yield data from exchange experiments (see Ch. 6).

consistent H-yields and additional results presented in Ch. 5 suggest that the same classes of hydrogen are systematically removed. Therefore, there should not be any between-sample bias in isotopic results. Experimental results presented in Ch. 5 are not able to distinguish between the above two potential causes of low H_2 yields. Nonetheless, other causes of low H_2 yields, such as loss of H_2 during combustion, cryogenic extractions or reduction of H_2O or due to incomplete combustion, do not seem likely based on theoretical considerations (Ch. 3), on the precautions exercised and experimental observations (Ch. 5). Potential reasons for higher H_2 yields in SEAL w.b. will be discussed later (Ch. 5,6).

CHAPTER 5

DEVELOPMENT OF METHODS AND TESTS

In this chapter the results and interpretations from experiments used to develop and test methods for δD work on bone are presented. Special attention was paid to developing outgassing and combustion techniques and methods of preparing bone (w.b., gel or col) before standard techniques of isotopic analysis of bone collagen could be adapted for δD work. The MUSK, SEAL and GEL reference samples were used for much of this work as was ENAM (tooth enamel) which has very low quantities (<1%) of organic material and closely resembles the mineral portion of w.b. (App. B). Basic interpretation of the yields was given earlier and methods of preparation described in Ch. 4 are assumed for all work except where variations in procedures were tested as noted below.

5.1 Effects of Outgassing Conditions on Results

After the processes of combustion, cryogenic gas separation and isotopic analyses were eliminated as causes of variability, it was discovered that sample outgassing conditions could greatly affect the δD results. These underwent considerable changes before techniques were standardized.

Table 5.1 illustrates that results on samples prepared using different outgassing conditions can be quite different. The outgassing and preheating conditions were: (prep 1) In early experiments, samples were outgassed under vacuum using the "calcite line" which, in the McMaster lab had been specialized for converting calcite to CO_2 using phosphoric acid. Continuous pumping under good vacuum ($\approx 10^{-3}$ torr) was used to ensure evacuation of all evolved gases. Outgassing, however, was with heating tapes with poor control of outgassing temperature (Tp). There was also poor control of Tp along the length of the heating tape which was wrapped around several samples at a time. (prep 2) Samples were placed in a vacuum oven (100°C for gels or 150°C for w.b.) for 3d then dried under vacuum on the calcite line for 1d using heating tapes. The vacuum oven used a mechanical roughing pump which achieved only $\approx 10^{-1}$ torr. It did not hold a good static vacuum and had poor control of Tp with higher Tp on the floor where the samples were placed than elsewhere. Typically, the vacuum oven was evacuated with the samples inside then the pump was turned off so that the samples remained heated under static vacuum. During this time, however, air would gradually seep into the vacuum oven. (prep 3) Samples were placed in a vacuum oven for 3d as for prep 2, then dried under vacuum on the calcite line for 1d using voltage controlled furnaces. Although Tp varied by up to 10°C over time, it was considerably better than the heating tapes. Outgassings for some of the later samples were conducted using continuous pumping under good vacuum ($\approx 10^{-3}$ torr) on the new vacuum line described in Ch. 4.

Table 5.1 Summary Statistics for Various Outgassings, Tube Types and Combustion Conditions[a].

	prep	n	Isotopes			Yields						
			δD	$\delta^{13}C$	$\delta^{15}N^{G}$	C/N	H/N	H/C	N/g	C/g	H/g
GEL	1P	10	-51±6	-	-	-	-	89±8	-	91±2	81±7
	10900	9	-48±6	-	-	-	-	50±34[b]	-	96	46±33[b]
	2P	10	-63±6	-12.7±0.3	7.4±0.2	-	-	94±2	-	80±8	69±10
	2Q	3	-58±2	-12.6±0.3	7.5±0.1	-	-	99±1	-	77±0	75±0
	3P	16	-68±1	-12.7±0.4	7.5±0.2	97±1	88±1	91±5	96±1	91±2	84±4
	4P	8	-58±2	-12.6±0.5	7.7±0.1	98±1	89±2	90±3	94±2	93±2	84±2
	5P	1	-49	-11.8	7.2	102	91	89	89	91	81
MUSK gel	2P	4	-164±9	-22.3±1	3.8±0.2[M]		-	84±9	-	86±6	69±2
	2Q	1	-182	-21.9	4.7[M]	-	-	65	-	81	53
	5P	5	-145±2	-21.4±0.5	3.6±0.2	104±2	93±3	90±2	89±3	93±2	83±2
MUSK w.b.	2P	2	-171±2	-	-	-	-	82±6	-	79±8	64±2
	2Q	5	-166±5	-20.9±0.2	-		-	79±13	-	80±3	63±11
	4P	7	-151±7	-20.3±0.4	6.1±0.3	92±2	94±2	103±3	80±7	74±6	76±7
	5P	6	-141±3	-20.6±0.4	4.7±0.1	98±2	91±3	94±4	87±3	84±4	79±3
SEAL gel	5P	2	89±4	-16.0	18.3	103±0.7	94±0	92±0.7	83±2	84±3	78±2
SEAL w.b.	2P	1	-4	-15.9	-	-	-	79	-	68	53
	2Q	1	5	-15.9	-	-	-	78	-	67	52
	5P	6	61±3	-15.4±0.7	18.8±0.2	105±5	98±2	94±5	71±4	74±2	69±4

a All samples are defined in text and in App.D Additional notes designated by capital letters are given in App.D. Yields are relative to expected values as defined by eq. 4.2 and 4.3.

b Includes results for both high and low gas pressures.

This line was cleaner and held a better static vacuum than did the calcite line. Later extractions of gases were conducted on the new line which may have helped improve purification of gases, yields and reduce variability in isotopic results. Therefore, it is difficult to separate the improvements in results due to better cryogenic extraction procedures from those due to better outgassing procedures. (prep 4) Samples were placed in the vacuum oven for 1d (100°C for gel; 150°C for w.b.) then outgassed on the new vacuum line for varying lengths of time at various Tp using voltage controlled furnaces. (prep 5) All samples were placed directly on the new vacuum line and outgassed using continuous pumping and thermistor controlled furnaces. This vacuum line also held a good static vacuum and control of Tp was good (±1°C).

Also shown in Table 5.1 is some information on combustion conditions. The combustion tube types are Pyrex (P), quartz (Q) or Vycor (V). Unless a 900°C combustion temperature (Tc) is indicated (i.e. 1Q900), Tc=450°C for w.b. samples and Tc=550°C for gel and col samples. Since neither tube type nor Tc significantly affected results (below, App. D), almost all variations in Table 5.1 can be attributed to outgassing conditions. Results below will show that low δD of MUSK gel in Table 5.1 coincides with low H/C and indicate effects of outgassing conditions rather than tube type.

The above symbols are used throughout all tables under "prep" to indicate both outgassing and combustion conditions. All raw results on replicate preparations for which the summary statistics appear in various tables including 5.1, can be found in Tables D.1 through D.11 (App. D) and are arranged according to outgassing and combustion conditions. Any table notes indicated by capital letters, are

part of the table notes given in App. D. For reasons discussed later, no δD results were corrected for H-exchange.

It is clear that outgassing conditions greatly affect the δD results (Table 5.1). Poor control of Tp but good control of vacuum increased σ (the standard deviation) of δD but did not greatly alter its isotopic value (prep 1). Poor control of vacuum and poor control of Tp increased σ and decreased δD (prep 2). Poor control of vacuum invariably led to low δD and samples exposed to poor vacuum for the longest periods of time had the lowest δD (prep 2 to 4). The H- and C-content is also somewhat depleted in MUSK and SEAL w.b. and gel samples heated under poor vacuum, with poor control of Tp and especially when Tp is also poorly controlled during subsequent heating on the vacuum line (prep 2). The C/N yields are also slightly lower in both MUSK w.b. and GEL when heating is under poor vacuum with moderate control of Tp suggesting preferential loss of CO_2 when air is present. The $\delta^{15}N$ values appear slightly higher after heating under poor vacuum with no consistent change in $\delta^{13}C$. Evidently gels are more sensitive than w.b. to Tp and vacuum fluctuations since σ, especially for MUSK gels, is greater. The σ of $\delta^{15}N$ seems to also decrease with better control of Tp and vacuum.

There are three explanations of how variations in Tp along with degree of vacuum have affected the above results. Large changes in δD with minor changes in the other variables would suggest that heating under poor vacuum produces changes due to exchange of H_{EX} with lab atmospheric H_2O-vapor. Here, under high temperatures, the exchange rates will increase and ϵ_{eH-V} (the equilibrium isotopic separation factor between H_{EX} and atmospheric H_2O) will decrease so that

δD_{EX} will converge towards δDv (the value of atmospheric H_2O). δDv, in turn, is usually isotopically light compared to the δD of the non-exchangeable hydrogens (δD_c') of the sample so that most samples heated under poor vacuum may be expected to have a low δD due to H-exchange. A second source of change arising during heating under poor vacuum could be due to a slow oxidative combustion which, in addition to changes in δD, would change $\delta^{13}C$, $\delta^{15}N$ and the yields. The changes in these latter variables in Table 5.1 suggest a small amount of irreversible oxidative decomposition of bone collagen with heating under poor vacuum. Under good vacuum (preps 1,5), heating at high Tp could produce changes due to anoxic pyrolysis. However, any small releases of O_2 from the CuO at higher Tp could also result in some oxidative degradation.

A third source of difference could be through ϵ_{OH-V} (isotopic separation factor between H_{EX} and H_2O-vapor due to outgassing). With higher Tp, an increase in the evaporation rate of adsorbed-H_2O could reduce ϵ_{OH-V} and, hence, δD. Under poor vacuum, slower rates of evaporation could increase ϵ_{OH-V} through increase in exchange times for H_{EX} and the kinetic and equilibrium isotopic separation factors in ϵ_{OH-V} (Ch. 3). However, under poor vacuum δDx actually decreases indicating that contribution of ϵ_{OH-V} to overall separation factor ϵ_{H-V} is minor compared to that of ϵ_{eH-V}.

The above implies that only results of samples undergoing the same outgassing procedures can be directly compared. However, the following will suggest that results of samples undergoing different outgassing procedures will be offset from each other by a constant amount. Therefore, once this constant is determined for two outgassing

methods, it may be added to the results of one so that they become directly comparable to those of the other.

The basic equation for H-exchange (eq. 3.1) can be developed to derive one which will show how the variables for a given sample material (x) might be affected by two different outgassing procedures (i and j) to produce an offset in δD ($\delta Dx_i - \delta Dx_j$).

(eq. 5.1)
$$\delta Dx_i - \delta Dx_j = p_{EXi}(\delta Dv_i + \epsilon e_{H-vi} + \epsilon o_{H-vi}) \ldots$$
$$\ldots - p_{EXj}(\delta Dv_j + \epsilon e_{H-vj} + \epsilon o_{H-vj}) \ldots$$
$$\ldots + (1-p_{EXi})\delta Dx_c{}'_i - (1-p_{EXj})\delta Dx_c{}'_j$$

The first two terms relate to a shift in δD value of H_{EX} ($\delta D_{EX} = \delta Dv + \epsilon_{H-v}$) while the latter two terms relate to a shift in the non-exchanged hydrogens ($\delta Dx_c'$). All terms are defined as in eq. 3.1: δDv is of the lab H_2O vapor, ϵe_{H-v} is the equilibrium isotopic separation factor between H_{EX} and ambient H_2O vapor and ϵo_{H-v} is the separation factor produced during outgassing. x=b for w.b., x=co for col and x=g for gel preparations and p_{EX} is the proportion of H_{EX}. Both a shift in the first half or the second half of the equation could occur during a procedure such as oxidative combustion (as under poor vacuum) or anoxic pyrolysis (as under good vacuum but high Tp) if some fraction of hydrogen, either non-exchangeable or H_{EX} were preferentially removed thus changing the isotopic values of the hydrogens remaining (δD_{EX} or $\delta Dx_c'$) or their proportions (p_{EX}). However, the value of δD_{EX} could also be greatly altered without loss of hydrogen. Here, δDv and ϵo_{H-v} vary and, under poor vacuum, H-exchange with H_2O vapor at different temperatures produces changes in the value of ϵe_{H-v}. In contrast, shifts in p_{EX} or $\delta Dbx_c'$ would require a profound alteration of the nature

or composition of the collagen. In light of the relatively small changes in yields, $\delta^{15}N$ and $\delta^{13}C$ compared to the large negative shifts in δD (Table 5.1), most variability in δD appears to be the result of H-exchange rather than alteration of collagen.

If the nature of the collagen could be assumed to have remained constant, so that $\delta Dx_c'_i = \delta Dx_c'_j$ and $p_{EXi} = p_{EXj}$ then eq. 5.1 becomes:

$$\text{(eq. 5.2)} \quad \delta Dx_i - \delta Dx_j = p_{EX}(\delta Dv_i - \delta Dv_j + \epsilon e_{H-vi} - \epsilon e_{H-vj} + \epsilon o_{H-vi} - \epsilon o_{H-vj})$$

with most change occurring in the value of ϵe_{H-v} which varies according to Tp and lesser changes in ϵo_{H-v} which varies with amount of adsorbed water, Tp and degree of vacuum. Under such conditions, any group of similar types of samples outgassed together using prep i would all be offset by a constant amount from the same samples outgassed together using prep j.

The above results and discussion suggests that degree of vacuum is a primary reason for variation in δD during outgassing and maintaining good vacuum during this procedure is vital. In the following experiments, the effects of variation of Tp alone with the degree of vacuum held constant was tested (Tables 5.2 to 5.4). In addition to GEL and w.b., ENAM was also studied as representative of the mineral portion of w.b. Here, 200 mg samples of ENAM prepared according to App. B were outgassed under the same conditions as w.b. samples.

Results on GEL preheated at various Tp show decreasing δDg values with increasing Tp for the earlier (2Q and 1P) methods (Table 5.2). The lower H/C and C/g yields at 225°C and 210°C along with

Table 5.2 GEL Preheated at Various Temperatures.[a]

Outgassing			n	Isotopes			Yields						
°C	days	prep		δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g
≈100	4	2Q	3	-58±2	-12.6±0.3	7.4±0.1	-	-	99±1	-	77±0	75±0
≈130	4	2Q	6	-67±3	-13.0±0	-	-	-	85±3	-	92±1	79±3
150	4	2Q	1	-80	-	-	-	-	89	-	79	69
225	4	2Q	1	-77	-	-	-	-	62	-	71	44
≈100	3	1P	10	-51±6	-	-	-	-	89±8	-	91±2	81±7
≈130	3	1P	5	-60±6	-13.0±0.1	-	-	-	88±5	-	81±6	69±10
210	3	1P	6	-89±7	-12.8±0.2	-	-	-	56±9	-	73±8	41±7
94	5	4P	1	-59	-13.2	7.5	100	89	88	92	92	81
100	3	4P	1	-59	-11.8	7.7	97	90	93	95	92	85
104	1	4P	1	-59	-12.1	7.7	99	92	93	93	92	85
135	3	4P	1	-56	-12.9	7.6	97	88	91	-	-	-
143	1	4P	1	-56	-12.5	7.5	99	92	93	-	-	-
149	2	4P	1	-53	-12.9	7.7	97	86	88	-	-	-
152	4	4P	1	-58	-12.9	7.9	98	87	89	-	-	-
153	5	4P	1	-60	-	7.6	99	83	87	96	95	83
				-58±2	-12.6±0.5	7.7±0.1	98±1	89±2	90±3	94±2	93±2	84±2

a See Table 5.1 note a and text; all samples were combusted at 550°C

Table 5.3 Whole Bone Preheated at Various Temperatures.[a]

	Outgassing			n	Isotopes			Yields					
	°C	days	prep		δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g
MUSK	95	6	4P	1	-148	-19.7	5.9	91	98	107	85	78	83
	144	5	4P	1	-157	-20.9	5.7	96	95	99	64	61	60
	146	5	4P	1	-154	-20.2	6.4	92	93	101	83	77	77
	147	2	4P	1	-150	-20.0	6.0	90	94	106	83	75	79
	150	6	4P	1	-151	-	6.4	92	93	101	82	75	76
	156	4	4P	1	-149	-20.5	6.2	89	94	106	83	74	78
	160	3	4P	1	-151	-20.3	5.9	92	94	102	83	76	78
					-151±3	-20.3±0.4	6.1±0.3	92±2	94±2	103±3	80±7	74±6	76±7
	150	3	5P	1	-139	-20.7	4.5	100	89	89	83	83	73
	160	3	5P	1	-141	-20.2	4.8	101	92	91	81	82	75
	180	3	5P	1	-146	-20.9	5.3	104	87	83	85	89	74
	220	3	5P	1	-153	-20.5	5.7	111	88	80	74	82	65
SEAL	150	3	5P	1	59	-15.5	18.7	112	98	87	64	72	63
	160	3	5P	1	62	-	18.3	108	95	88	67	72	63
	180	3	5P	1	61	-15.6	18.5	111	96	87	74	82	71
	220	3	5P	1	63	-15.5	-	118	91	77	64	75	58

a See Table 5.1 note a and text; all samples were combusted at 450°C.

Table 5.4 Tooth Enamel (ENAM) Preheated at Various Temperatures.

T°C	prep[a]	Isotope			manometer reading for 138 mg enamel(cm)[b]			% Tot.[c]	% contribution to total w.b. yield[d]		
		δD	$\delta^{13}C$	$\delta^{15}N$	N_2	CO_2	H_2O	H_2O	N_2	CO_2	H_2O
150	5P	-192	-17.1	5.5	0	0	0.80	51	0	0	4.7
160	5P	-139	-16.8	9.0	0	0.20	1.03	57	0	0.4	6.1
180	5P	-160	-16.9	13.8	0	0	0.75	49	0	0	4.4
220	5P	13	-17.0	13.1	0	0	0.46	37	0	0	2.7

a See Table 5.1 note a and text. Samples were combusted at 450°C. ENAM is from modern Alberta bison (App.8)

b manometer reading of extracted gases following combustion minus contribution from 0.71% organics as: 0.35% protein, 0.03% citrate, 0.03% lactate and 0.3% lipids (after Little and Cascaini 1966; Smillie 1973; Triffit 1980)

c percent contribution of H_2O derived from mineral portion of ENAM relative to total H_2O from combustion of ENAM.

d percent manometer reading from 138 mg of ENAM relative to total manometer yields for a typical w.b. sample as calculated from 31 modern deer: 8.8±0.9 cm N_2, 52±5 cm CO_2 and 17±2 cm H_2.

lighter δDg results, suggest denaturation of GEL with preferential loss of hydrogen having a high isotopic value compared to the hydrogen remaining. These results also indicate that, at abnormally high Tp, low H/C yields correlate with low δD values which suggests low H/C yields may be useful for identifying samples which have been inadvertently over-heated during outgassing.

The results for prep 4 show that, when heating samples under good vacuum, no additional degradation of GEL appears to occur even with extended heating times at 150°C. Adsorbed H_2O is apparently removed after 1d at 100°C since the number of days of outgassing and Tp do not affect results. The $\delta^{13}C$ and $\delta^{15}N$ results do not appear to be consistently affected by Tp.

For the w.b. samples, using Tp≤160°C under good vacuum appears to have no significant effects on the δD, $\delta^{15}N$ or yields regardless of the length of heating time (Table 5.3). Other than for Tp=95°C, there are also no effects on $\delta^{13}C$ with Tp≤160°C. This indicates thermal stability of the collagen in the whole bone powders during outgassing at Tc≤160°C. Two or three days of heating at 150°C also appears to be sufficient for removing any adsorbed H_2O or gas.

Between 140°C and 160°C, the $\delta^{13}C$ values remain stable, but become ≈0.7‰ higher at Tp=95°C. Perhaps, a small amount of CO_2 from adsorbed CO_2 or CO_3^{2-} is released by preheating to 150°C.

At Tp>160°C there is an increase in the C/N and a decrease in the H/C yields suggesting a preferential loss of H and N over C due to anoxic pyrolysis. The $\delta^{15}N$ of MUSK increases with the higher Tp indicating loss of N_2 with low $\delta^{15}N$ values. The δD of MUSK decreased considerably with Tp≥160°C, while the δD of SEAL increased only

slightly. This indicates that the δD of the H removed at Tp≥160°C lies somewhere in the direction of SMOW relative to the H remaining in the samples but is closer in composition to the H remaining in SEAL. These results confirm results on the gels and indicate that thermal degradation of the bone collagen occurs above 160°C and that good control of Tp in addition to good vacuum is essential for δD isotopic work on w.b.

In Table 5.4 it is shown that, once a theoretical contribution to the yields of N_2, CO_2 and H_2O due to the organics in tooth enamel is subtracted from the total yields of gases evolved from ENAM, only H_2O is evolved from the mineral portion. This H_2O is probably non-exchangeable and likely arises from the DCPD and OCP in the mineral. At Tp=150°C this H_2O comprises ≈0.4% of the total weight of ENAM. In a typical 200 mg w.b. sample containing 138 mg mineral, the relative contribution that this H_2O would make to the total H_2O yields is ≈5%. This is a little higher than the theoretical estimate of 3-4% (Ch. 3). Lack of change in the H_2O contribution between 150°C and 180°C suggests that Tp=150°C is sufficient for removing all adsorbed H_2O. The results further indicate no release of CO_2 from adsorbed CO_3^{2-} in the 150 to 450°C range.

Examination of the isotopic results further suggests a predominantly organic origin for most combustion gases from ENAM. As ENAM is from a modern Alberta bison, its $\delta^{13}C$ and $\delta^{15}N$ values may be compared to BIS (-15.6‰ and 5.9‰, respectively) and its δD to modern Alberta deer (-85‰ to -97‰). About 40% of the organic material in tooth enamel is from lipids and ≈50% is protein (Table 5.4, notes). Considering that all the N_2 is from the protein while some CO_2 is from the lipids, the $\delta^{15}N$, $\delta^{13}C$ values and adjusted yields suggest that almost all the CO_2 and

N_2 arise from organic sources in ENAM. The δD values are quite low, probably due in part to the presence of lipids and non protein organics but, perhaps, also due to an ≈50% contribution of H_2O with an unknown δD value from the mineral portion of ENAM (col 9, Table 5.4). Significant removal of this H_2O does not occur before ≈220°C. However, significant changes in isotopic values of collagen also occur at this higher Tp suggesting loss of organic material during preheating. Therefore, it would not seem advisable to increase Tp beyond 150°C in an attempt to remove the undesirable contribution to total H_2O arising from the bone mineral.

The above shows that outgassing with Tp=150°C under good vacuum for 3d will remove all adsorbed H_2O from w.b. samples without degradation of the bone collagen. However, ≈5% of the total H_2O evolved from combustion will contain non-exchangeable hydrogen from the mineral phase of bone. Tests also show that Tp=100°C for 3d under good vacuum is a suitable outgassing strategy for gel and col samples.

5.2 Development and Testing of Sample Combustions at 900°C and 550°C in Quartz or PYREX Breakseals and Effects of Extraction Line Volume on H_2O Yields:

Some differences in δD of humans consuming marine vs terrestrial foods are expected and, in early combustion experiments, this was tested on prehistoric humans whose diets were known on the basis of *a priori* archaeological information and from $\delta^{13}C$ values. In these early tests, some marine-terrestrial differences observed at Tc=550°C were not preserved at Tc=900°C (Table 5.5). The δDg obtained on three

Table 5.5 "Marine" and "Terrestrial" Human Gelatins Combusted at 900°C and 550°C Using Early Methods.[a]

	prep		δD	$\delta^{13}C^{M}$	$\delta^{15}N^{M}$	H/C
Marine	1Q900	i	225	-13.9	24.1	68
	1Q900	ii	64	-13.0	26.7	74
	1Q900		60	-13.4	25.6	84
	1Q900		-9	-14.5	24.5	84
			85±99	-13.7±0.6	25.2±1	78±8
Terrestrial	1Q900		141	-15.1	25.8	72
	1Q900		96	-15.6	25.0	80
	1Q900	iii	91	-15.2	25.1	64
	1Q900		26	-17.7	23.5	87
	1Q900		-24	-18.2	18.2	66
			66±65	-16.4±1	23.5±1	74±10
Marine	1P		60	-12.9	25.0	71
	1P	ii	80	-13.0	26.7	57
	1P	i	32	-13.9	64.1	64
			64±28	-13.3±0.6	25.3±1	66±4
Terrestrial	1P		-26	-19.3	19.2	42
	1P		-85	-16.3	13.5	53
	1P	iii	-182[b]	-15.2	25.1	71
			-56±42 (n=2)	-16.9±2	19.3±6	55±15

a see Table 5.1 note a and text. P=Pyrex, Q=quartz tubing. 900 number indicates samples combusted at 900°C. All remaining samples combusted at 550°C.

b this sample was contaminated with atmospheric H_2O.

i, ii, iii see text.

samples combusted in Pyrex (i, ii, iii) also were not comparable to results on these same samples combusted at 900°C.

In later experiments on animal flesh, some marine and terrestrial differences were observed at 550°C and the two terrestrial samples from the same location had almost identical values as did the two marine samples (Table 5.6). Combustion tube type alone, whether quartz, Vycor or Pyrex did not affect results (Tables 5.6, D.1, D.3, D.4). The above results on humans at Tc=900°C, in addition to obvious devitrification of the insides of the quartz and Vycor tubes at 900°C, lead to an early assumption that a lower Tc would be best for δD work. However, since results were found to be greatly affected by outgassing conditions and gas pressures during the cryogenic extractions of combustion products, these early observations could not be conclusively attributed to Tc alone as outgassing and extraction procedures had not yet been standardized. Furthermore although it had been resolved that tube type does not affect results, it had not yet been determined whether or not exchange or loss of H_2O occurred during combustion. The evolution of some of the procedures is described below.

In the earliest experiments, when H_2O was extracted from combusted samples through cryogenic separation into small volumes, such as directly onto the cold finger of the H-line (Fig. C.1), some of the H_2-yields were so low that the samples could not be reliably analyzed. Increasing the surface area onto which the H_2O was frozen and reducing gas pressures by collecting H_2O in an adjacent vacuum line with larger volumes improved the H_2-yields somewhat so that the sample could, at the least, be analyzed (Tables 5.5, 5.6, D.1 (1P) and D.3). Under these

Table 5.6 Duplicates of Flesh from Marine and Terrestrial Animals Combusted at 550°C in Quartz (Q) or Pyrex (P) Breakseals.[a]

Flesh	prep	δD[I]	$\delta^{13}C$	$\delta^{15}N$[M]	Location
Marine					
Shrimp	2P	-98	-17.3	21.4	b
Herring	2P	-97	-18.6	19.6	b
Shrimp	2Q	-95	-17.3	21.4	b
Herring	2Q	-90	-18.6	69.6	b
		-94±4	-18.0±0.8	20.5±1	
Terrestrial					
Ptarmigan	2P	-139	-23.6	8.0	c
Snowshoe hare	2P	-141	-27.4	9.1	c
Ptarmigan	2Q	-147	-23.6	8.0	c
Snowshoe hare	2Q	-144	-27.4	6.1	c
		-143±4	-25.5±2.2	8.6±0.6	

a See Table 5.5 note a and text for explanations.

b Gerogia Straits, B.C.

c Telegraph Creek, B.C.

conditions, the human gels gave inconsistent results, especially at 900°C (Table 5.5) although results on GEL at Tc=900°C gave reasonable results despite the low H_2-yields (Table 5.1: 1P, 1Q900). Additional improvements in cryogenic extraction of H_2O were made in later experiments through addition of the 500ml bulb to further reduce gas pressures and increase the surface area for H_2O collection (Ch. 4). However, no additional improvements in H_2-yields resulted with the addition of still larger volumes such as one or two 1000 ml bulbs.

In order to test whether there was H-exchange or loss of H_2O during combustion and cryogenic extraction of gases, tests were conducted using water blanks. Here, a standard H_2O was sealed into previously evacuated quartz or Pyrex tubes with CuO, Cu_2O, Cu and a small amount of CO_2. These blanks were combusted at 550°C and gases were cryogenically extracted using standardized methods (Ch. 4). The H_2O yields were 100% and the δD values remained unaltered indicating quantitative collection of all H_2O and no H-exchange. Combustions and extractions of blanks comprised of cupric oxide alone sealed into quartz or Pyrex tubes produced minuscule amounts of CO_2 but no H_2O.

Once outgassing and cryogenic transfer methods were standardized, the two (550°C and 900°C) combustion temperatures were retested on MUSK and SEAL gels (Table 5.7). There were no significant differences in any of the isotopic results or relative yields as a result of the different Tc. However, the N/g, C/g and H/g yields were unexpectedly lower at Tc=900°C for both SEAL and MUSK. If combustion were more complete at Tc=900°C, then these yields should have been

Table 5.7 Samples Combusted at 900°C or 550°C.[a]

	°C	prep	n	Isotopes			Yields					
				δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g
MUSK	550	5P	5	-145±2	-21.4±0.5	3.6±0.1	104±2	93±3	90±2	89±3	93±2	83±2
gel	900	5Q	1	-149	-21.4	3.8	104	94	90	69	72	65
SEAL	550	5P	2	89±4	-16.0	18.3±0.1	103±1	94±0	92±1	83±2	84±2	78±2
gel	900	5Q	1	93	-16.1	18.2	104	97	93	66	69	64

a See Table 5.1 note a and text for explanations.

higher. The exact reasons for the poor yields at Tc=900°C remain unknown but results indicate that combustion at Tc=550°C should not adversely affect any results.

5.3 Tests on the 450°C Combustion Temperature:

In Tables 5.8 and 5.9 appear results comparing the two lower (450°C and 550°C) combustion temperatures. There were no significant differences seen in any isotopic results of gel or w.b. preparations of MUSK (Table 5.8). There were also no significant differences in the yields for MUSK gel and no improvements in the yields for MUSK w.b. at Tc=550°C. Higher CO_2 yields at Tc=550°C for MUSK w.b. could be due to both primary and secondary CO_3^{2-} since this sample had been buried for 100 years.

Results on GEL contrast with those of natural MUSK gel. The lower CO_2 yields of GEL at Tc=450°C may be due to residual, hard-to-combust graphite. Unlike the non-commercial gels, a graphitic appearing smear was often noticed on the insides of the combustion tubes of GEL samples, especially when they had been complexed with copper (Ch. 6) or contained salt. There were no improvements in the N- or H-yields at 550°C, nor were there any significant differences in isotopic results although $\delta^{13}C$ and $\delta^{15}N$ were slightly lower and higher respectively at Tc=450°C. Since the extraction and commercial preparation of GEL may be different from the Longin method used on non-commercial gelatins here, it is possible that the low C-yields at Tc=450°C pertain only to GEL and will not affect gels extracted by the Longin method.

Table 5.8 Samples Combusted at 450°C or 550°C.[a]

	°C	prep	n	Isotopes			Yields					
				δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g
5.8.1 Gelatins												
GEL	450	5P	1	-45	-12.2	7.8	93	88	95	88	81	77
	550	5P	1	-49	-11.8	7.2	102	91	89	89	91	81
MUSK	450	5P	1	-142	-21.2	3.3	101	90	89	90	91	81
	550	5P	5	-145±2	-21.4±0.5	3.6±0.2	104±2	93±3	90±2	89±3	93±2	83
5.8.2 Whole Bone												
MUSK	450	5P	6	-141±3	-20.6±0.4	4.7±0.1	98±3	91±3	83±5	94±4	83±5	79±3
	550	5P	1	-142	-20.8	4.8	109	84	86	77	86	66
5.8.3 Resealed Whole Bone[1]												
MUSK	450	2Q	1	-174	-21.0	-	-	-	79	-	82	64
	650	2Q	1	-860	-	-	-	-	16	-	2	0.4
SEAL	450	2Q	1	5	-15.9	-	-	-	78	-	67	52
	650	2Q	1	-147	-	-	-	-	9	-	8	0.7

a See Table 5.1 note a and text for explanations.

Table 5.9 Tooth Enamel (ENAM) Combusted at 450°C or 550°C.[a]

		Isotopes			manometer reading for 138 mg mineral (cm)			% Tot	% contribution to total yield		
T°C	prep	δD	$\delta^{13}C$	$\delta^{15}N$	N_2	CO_2	H_2O	H_2O	N_2	CO_2	H_2O
450	5P	-192	-17.1	5.5	0	0	0.80	51	0	0	4.7
550	5P	-133	-16.1	5.9	0	0.28	0.87	53	0	0.5	5.1

a samples were outgassed at 150°C. Organic contribution was subtracted from manometer readings and % contribution to total yield is based on the yield of a typical sized sample (see notes Table 5.4).

Previous isotopic results on ENAM (Table 5.4) combusted at 450°C indicated that most CO_2 and N_2 is primarily due to organics while in tooth enamel, ≈50% of H_2O is from organics and the rest from the mineral fraction. Using adjusted yields (minus the organics), releases of both H_2O and CO_2 from the non-organic, fraction are only slightly higher at 550°C than at 450°C (Table 5.9). Removal of all organic material from w.b. through combustion at 450°C with additional releases of H_2O from bone mineral seem to be also suggested by results on the resealed samples (Table 5.8.3). Here, following standard extraction of gases after a 450°C combustion, the w.b. samples of MUSK and SEAL were outgassed at 450°C, resealed then combusted at 650°C for several hours to determine if any additional gases would evolve between 450°C and 650°C. The 0.4% to 0.7% by weight of H_2O released between 450°C and 650°C can be attributed to theoretical yields of H_2O from the bone mineral. The low H/C yields also suggest a considerable excess of CO_2 from bone CO_3^{2-} between 450°C and 650°C.

The above results indicate slight advantages with the Tc=450°C for work on modern w.b. samples. There also appear to be no inherent disadvantages at Tc=450°C for natural gels extracted by the Longin method.

5.4 Pyrolysis as an Alternative Method:

In early experiments, the pyrolysis technique for simultaneous extractions of both hydrogen and oxygen for isotopic analyses was tested. It was quickly abandoned in favor of the Sofer (1980) method of combustion since it provided inconsistent results (Tables 5.10 and 5.11).

Table 5.10 GEL Pyrolyzed in Alberta.

δD	H/C[a]	Hours of Pyrolysis
-85	85	2
-85	85	2
-88	105	1.5
-92	98	1.5
-219	107	10.5
-97	113	10
-98	110	15
-101	61	1.5
-112	57	5
-114	86	4
-125	85	2
-126	90	5
-130	86	1
-143	88	1.5
-145	58	2.75
-160	90	30.5
-185	50	2
-207	37	3.25
-211	129	12
-230	103	12
-284	41	13
-310	39	0.5
-152±66	82±26	

a Moles H_2 obtained/moles H_2 expected on basis of sample weight x 100/0.92. Yields were estimated using the major ion beam (H_2) of the mass specrometer.

Table 5.11 Flesh from Marine and Terrestrial Animals Pyrolyzed in Alberta.[a]

	δDb[b]	δ^{13}C	δ^{15}N[M]
Marine			
herring	-98	-18.6	21.7
shrimp	-107	-17.3	21.4
"greasy" herring	-123	-20.9	22.3
	-109±13	-18.9±1.8	21.8±0.5
Terrestrial			
moose	-136	-24.7	9.1
beaver	-159	-24.7	9.1
mule deer	-206	-24.9	11.2
	-167±36	-25.7±1.5	10.1±1

a See Table 5.1 note a and text.

b +51‰ was added to all δD results in order to correct for a systematic offset produced by the pyrolysis method.

This method has been used at the University of Alberta for δD and $\delta^{18}O$ analysis of tree ring cellulose (Thompson et al. 1980). However, in this work, not only were the δDg measurements of GEL extremely variable (Table 5.10) but the presence of sample nitrogen thwarted any efforts to analyze the $\delta^{18}O$ of GEL because of the NO_2 (mass 46) interference with the CO_2 (mass 46) as well as incomplete collection of oxygen (see also Hoering and Estep 1981). Despite seemingly inconsistent results, the few δD results obtained on animal flesh (Table 5.11) did show some marine and terrestrial differences and "greasy herring" which presumably contained a high amount of lipids, had the lightest $\delta^{13}C$ and δD values.

5.5 <u>Comparison of Whole Bone, Gelatin and Collagen Results:</u>

The $\delta^{13}C$ and $\delta^{15}N$ results for the w.b., gel and col of 16 samples of w.t. deer are compared in Table 5.12. Since differences in δD between the three preparations could depend on the amount of H_{EX} in the three different products, the comparison for δD will be discussed in later sections dealing with H-exchange (Ch. 6). The comparison of all yields is given in Table 5.13.

One sample in Tables 5.12 and 5.13 (KS-1 w.b.) and, in all, six w.b. samples of the deer (TX-1, KS-1, NS-1, NB-1, ON-3 and ON-6) were pre-heated in the vacuum oven (prep 3P). This did not affect the $\delta^{13}C$ results but produced $\delta^{15}N$ values that were mostly too high. These raw results appear in Table D.12 (App. D) and are consistent with other results using the 3P method. A linear relationship between the biased $\delta^{15}N_0$ values (prep 3P) and the unbiased results (prep 5P) was used to

Table 5.12 Comparison of $\delta^{13}C$ and $\delta^{15}N$ Results of Whole Bone, Collagen and Gelatin Preparations of Modern Deer.

sample	$\delta^{13}C$			$\delta^{15}N$			Δ^{a}			
	$\delta^{13}Cb$	$\delta^{13}Cc$	$\delta^{13}Cg$	$\delta^{15}Nb$	$\delta^{15}Nc$	$\delta^{15}Ng$	$\Delta(\delta^{13}C-\delta^{13}Cg)$ w.b.	$\Delta(\delta^{13}C-\delta^{13}Cg)$ col	$\Delta(\delta^{15}N-\delta^{15}Ng)$ w.b.	$\Delta(\delta^{15}N-\delta^{15}Ng)$ col
TX-3]	-20.2	-21.3	-21.4	6.6	6.7	6.4	1.2	0.1	0.2	0.3
TX-5]	-21.2	-21.8	-21.2	6.2	-	6.6	0.0	-0.6	-0.4	-
KS-1[d]	-21.2	-22.3	-22.2	5.2[d]	5.2	5.2	1.0	-0.1	0.0	0.0
NE-2	-19.9	-20.0	-20.4	5.7	5.6	5.3	0.5	0.4	0.4	0.3
AB-4]	-18.3	-19.9	-20.0	8.7	8.9	8.8	1.7	0.1	-0.1	0.1
AB-5]	-18.5	-	-20.2	9.1	-	8.1	1.7	-	1.0[b]	-
AB-3]	-21.3	-22.9	-22.4	5.0	5.5	5.2	1.1	-0.5	-0.2	0.3
AB-2]	-22.3	-	-22.3	4.0	-	4.3	0.0	-	-0.3	-
AL-1	-22.2	-	-22.3	4.5	-	4.4	-0.1	-	0.1	-
MS-1	-23.1	-	-22.5	2.7	-	2.5	-0.6	-	0.2	-
WV-3	-17.5	-	-17.4	2.6	-	4.2	-0.1	-	-1.6[b]	-
QC-2	-22.9	-	-22.8	1.9	-	1.5	-0.1	-	0.4	-
WY-2	-20.5	-	-21.3	2.9	-	2.9	0.8	-	0.0	-
LA-2	-22.2	-	-23.3	5.7	-	6.2	1.1	-	0.5	-
LA-8	-	-	-24.5	-	-	4.6	-	-	-	-
ND-1	-	-	-20.6	-	-	9.1	-	-	-	-
						ave	0.6	-0.1	0.07[c]	0.2

Notes:

a Δ is the difference between the isotope result of w.b. or col and those of gel. Variations due to due to replicate preparation are ±0.4 for $\delta^{13}Cb$ (n=29), ±0.3 for $\delta^{15}Nb$ (n=17), ±0.4 for $\delta^{13}Cg$ (n=10) and ±0.2 for $\delta^{15}Ng$ (n=11).

b error is probably with the gelatin results.

c ave. does not include (b).

d corrected for bias due to sample preparation (Appendix D).

Table 5.13 Comparison of Yields of Whole Bone, Collagen and Gelatin Preparations of Modern Deer

	C/N			H/N			H/C			N/g			C/g			H/g		
sample	w.b.	col	gel	w.b.	col	gel	w.b.	col	gel	w.b.	col	gel	w.b.	col	gel	w.b.	col	gel
TX-3]	102	100	101	85	97	93	84	97	92	83	82	81	71	83	82	84	80	75
TX-5]	104	96	102	88	91	99	85	95	97	86	85	79	90	82	81	76	77	78
KS-1[d]	88x	181x	98	85x	165x	81	97x	92	83	76x	24x	86	67x	43x	84	65x	39x	70
WE-2	103	103	102	88	97	93	86	93	91	73	78	77	74	81	79	64	75	72
AB-4]	101	103	91	91	101	90	90	98	99	77	72	85	77	80	77	70	74	76
AB-5]	109	-	99	90	-	91	82	-	91	68	-	82	74	-	82	61	-	74
AB-3]	103	101	101	86	97	91	83	96	91	93	81	77	78	82	77	74	79	70
AB-2]	112	-	101	86	-	92	77	-	91	62	-	77	69	-	78	54	-	71
AL-1	102	-	15x	88	-	111x	86	-	106x	86	-	57x	87	-	60x	75	-	63x
MS-1	99	-	96	91	-	91	92	-	95	81	-	85	80	-	81	74	-	77
WV-3	100	-	101	94	-	92	94	-	91	68	-	81	68	-	82	64	-	75
QC-2	99	-	10	91	-	101	92	-	101	92	-	86	91	-	86	83	-	87
WY-2	108	-	101	90	-	97	83	-	96	71	-	81	77	-	82	64	-	79
LA-2	98	-	93	-	-	-	-	-	-	82	-	88	81	-	82	-	-	-
LA-8	-	-	101	-	-	100	-	-	99	-	-	85	-	-	85	-	-	85
ND-1	-	-	97	-	-	92	-	-	95	-	-	82	-	-	79	-	-	76
ave.	103±4	102±3	99±3	89±3	97±4	93±5	86±5	95±2	94±5	79±10	80±5	82±4	78±7	82±1	81±3	70±9	77±3	76±5
(n=74 deer w.b.)																		
ave.	100±6			90±4			90±6			75±8			75±7			68±6		

Notes:

d see Table 5.12 note d.

x not included in average.

correct the $\delta^{15}N$ results (App. D). Later analyses (Ch. 10) indicated that these corrected $\delta^{15}N$ values were comparable to $\delta^{15}N$ of deer from nearby locations.

The $\delta^{13}C$ of col and gel are the same within 2σ of each other where σ represents the overall standard deviation including replicate sample preparation ($\approx\pm0.4$ to ±0.8‰). A comparison of $\delta^{13}C$ of w.b. to that of gel shows that there is an average ($\delta^{13}Cb-\delta^{13}Cg$) offset of +0.6‰, probably due to a small contribution of CO_2 from bone CO_3^{2-} in w.b. The C/N yields of all preparations are within error of 100% but w.b. has the highest values. This, along with the slightly lower H/C yields, again suggests a small contribution of CO_2 from bone CO_3^{2-}.

For all but two results, the $\delta^{15}N$ values of the three preparations are within 2σ for replicate preparation ($\approx\pm0.2$ to ±0.3‰, see below). Therefore, there are no significant contributions from non-collagenous proteins in the w.b. The difference between $\delta^{15}Ng$ and $\delta^{15}Nb$ results for AB-5 and WV-3 probably represents a problem with the gel rather than w.b. results. AB-4 and AB-5 represent the right and left tibia of the same deer and are expected to be isotopically very similar. The $\delta^{15}Nb$ of AB-4 and AB-5 are, indeed, similar. However, the $\delta^{15}Ng$ of AB-5 is quite different from both the $\delta^{15}Ng$ of AB-4 and all $\delta^{15}Nb$ results suggesting that it is anomalous. Similarly, the WV samples are all from the same location and similar $\delta^{15}Nb$ and $\delta^{15}Ng$ results are expected. The difference between the $\delta^{15}Ng$ result of WV-3 and the remaining $\delta^{15}N$ values suggests that this result is also anomalous.

Whole bone and gelatin results of MUSK and SEAL (Table 5.1) may also be compared for their yields, $\delta^{13}C$ and $\delta^{15}N$. All relative yields are similar (within 2σ). For both SEAL and MUSK, after subtraction of

0.6‰ for CO_3^{2-} contribution, the $\delta^{13}Cb$ are identical to $\delta^{13}Cg$. The $\delta^{15}Nb$ of SEAL is within 2σ of its $\delta^{15}Ng$ whereas the $\delta^{15}Nb$ of MUSK (5P) is 1.1‰ heavier than its $\delta^{15}Ng$ (5P). All MUSK gels except one (2Q) were extracted from pulverized cancellous and stained bone whereas all MUSK w.b. samples are of well cleaned cortical bone. The $\delta^{15}Ng$ results of 2Q is similar to all w.b. results. This suggests that the gel extracted from the spongy bone may be isotopically different in $\delta^{15}N$ composition from compact bone, possibly due to contamination or denaturation of collagen in the spongy bone (see Ch. 11).

The above results indicate a slightly higher $\delta^{13}Cb$ compared to $\delta^{13}Cg$ due to a small contribution of CO_2 from bone CO_3^{2-} in w.b. The results for $\delta^{15}N$, on the other hand, are essentially the same whether combusting w.b. or its col or gel extracts.

5.6 Whole Bone Pre-Treatment With Degreasing Agents:

Due to the extremely low quantities of lipids in fresh compact bone (Ch. 3) only small shifts of <-0.02‰ in $\delta^{13}C$ and $\ll -0.2$‰ in δD would be expected if the lipids had not been removed. However, post mortem migration of lipids from the marrow into the outer layers of compact bone can occur (cf. Triffit 1980). This could explain why Chisholm et al. (1982) found that the $\delta^{13}Cg$ without lipid removal were, on average, -0.5‰ compared to the $\delta^{13}Cg$ following lipid removal.

The surfaces of the modern deer samples studied here were very greasy and CCl_4 was found to be a good degreasing reagent. Since MUSK had likely been naturally degreased, the chloroform and CCl_4 degreasing methods were tested on this standard and results compared to samples which had not been degreased (Table 5.14).

Table 5.14 Precleaning of Bone Samples.

Table 5.14.1 Pretreatment of MUSK Whole Bone with Chloroform or Carbon Tetrachloride Degreasing Agents.[a]

	prep[b]	n	Isotopes			Yields				
			δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g
not-degreased	4P	1	-158	-20.3	5.7	94	89	95	84	79
cloroform	4P	1	-158	-20.4	6.5	93	89	94	84	78
carbon-tet	4P	1	-159	-20.2	5.8	91	86	95	88	80

Table 5.14.2 Pretreatment of ON-6 Whole Bone with Soap.

	prep[b]	n	Isotopes			Yields				
			δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g
not treated	4P	1	-90	-22.5	3.2	95	90	94	76	79
soap	4P	1	-96	-22.7	3.6	105	87	84	72	75

Table 5.14.3 Pretreatment of ON-3 Whole Bone with NaOCl and HCl.

	prep[b]	n	Isotopes			Yields				
			δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g
not treated	4P	1	-89	-22.7	5.0	96	87	91	86	83
NaOCl	4P	1	-87	-	4.6	99	92	93	70	69
HCl	4P	1	-83	-22.7	4.4	95	90	95	75	71

a see Table 5.1 note a and text.

b lost record of time in oven, probably = 1 day with 3 days on line; voltage controlled furnaces.

Results show no significant differences at 2σ (see below) in any yields or isotopic results. This indicates that use of either reagent for degreasing should not adversely affect results. The use of a detergent for partial degreasing and removal of adsorbed surface contaminants was also tested on a modern w.t. deer sample (Table 5.14.2). The δDb values decreased but remained within 2σ and the δ^{15}Nb values increased slightly while the C/N_b yields increased. Also tested on modern deer was the use of HCl as a cleaning agent to remove surface contaminants including secondary CO_3^{2-} or free amino acids (i.e. Bada 1985) and the use of dilute NaOCl to remove some organic contaminants (Table 5.14.3). Here, one sub-sample was soaked for 10 min in 5% NaOCl and another for 5 min in 0.25N HCl followed by <1min in 1N HCl. Results show that none of the three alternative pre-cleaning methods produced statistically significant changes (Tables 5.12.2 and 5.12.3). However, the slight increases in δD and decreases in δ^{15}N (especially with HCl) and the lower N/g and C/g yields might suggest some denaturation, leaching, or, possibly, removal of some contaminant.

The above results indicate that either chloroform or CCl_4 can be used to degrease bone without adversely affecting results. The three alternative pre-cleaning methods (NaOCl, HCl and soap) need to be further investigated for future use but were not developed for use in this work.

5.7 Overall Variability in Isotopic Results Due to Replicate Preparation and Analysis.

The standard deviations in Table 5.1 and 5.15 calculated on replicate preparations and analyses of gels and w.b. of MUSK SEAL, and GEL may be examined in order to estimate the overall variability and pooled variability (σ_p, Table 5.15). Normally, one would expect that, if there were considerable variability in δDv (of lab atmospheric H_2O-vapor) then this would increase variability in the δD of replicate outgassings of standards prepared at different times, when δDv is different (see eq. 3.1). This variability may be compared to that of samples outgassed together on the vacuum line and exposed to the same δDv. The between-time variability may therefore be compared to the same-time variability to determine how widely δDv varies and how much effect H-exchange has on overall variability in δD results.
There is considerable reduction in σ(δD) for the later outgassing conditions (methods 3 to 5). The overall between variability in δD for prep 3 to 5 was ±3‰ for w.b. (n=19) and ±2‰ for gels (n=31) (Table 5.15). If GEL, which does not undergo gel extraction is excluded, the results for gels extracted by the Longin method becomes ±2.5‰ (n=7). The same-time variabilities are ±3‰ for w.b. (n=11), ±3‰ for gels extracted by the Longin method (n=2) and ±1.5‰ for GEL (n=26). The fact that between time variability does not exceed the same time variability, indicates that δDv in the McMaster lab does not change greatly over time and that H-exchange does not adversely affect reproducibility.

Table 5.15 Standard Deviations and Pooled Standard Deviations[a] of Isotope Results for Replicate Outgassings, Combustions and Cryogenic Extractions.[b]

	σ(δD)			$\sigma(\delta^{13}C)^c$	$\sigma(\delta^{15}N)^c$
	overall		same time[F]		
	prep	σ(δD)(n)	σ(δD)(n)		
GEL	1P	6(10)	-	-	-
	1Q900	6(9)	-	-	-
	2P		-	-	0.1(5)[M]
	2P	6(10)	-	0.3(4)	0.2(3)
	2Q[I]	3(6)	-	0.0(2)	-
	2Q	2(3)	-	0.3(2)	0.1(2)
	3P	1.4(16)	1(5)	0.4(13)	0.2(9)
	3P	-	2(5)	-	-
	3P	-	1(6)	-	-
	4P	2(8)	1(4)	0.5(7)	0.1(8)
	4P	-	1(4)	-	-
MUSK gel	2P	9(4)	-	0.1(3)	0.2(4)[M]
	5P	2(5)	3(2)	0.5(4)	0.2(5)
SEAL gel	5P	4(2)	-	0.0(2)	0.1(3)
MUSK	2Q	5(5)	-	0.2(5)	0.1(6)
	2P	2(2)	-		
	4P	3(7)	3(6)	0.4(6)	0.3(8)
	5P	3(6)	1(3)	0.4(6)	0.1(6)
	5P	-	4(3)	-	-
SEAL w.b.	5P	3(6)	-	0.3(5)	0.2(6)

σ_p ±S.E.				
all (prep 3 to 5):	2.3±0.2(50)	2.0±0.2(37)	0.38±0.04(59)[c]	0.18±0.02(65)[c]
gel (prep 3 to 5):	1.8±0.2(31)	1.4±0.2(26)		
w.b.(prep 3 to 5):	3.0±0.5(19)	3.1±0.5(11)		

a Pooled standard deviation(σ_p): $\sigma_p=\{\Sigma[(n-1)\sigma^2]/\Sigma(n-1)\}^{1/2}$; (Johnston 1976:372)
Standard Error of Pooled variability: S.E. $=\sigma/[2(n-1)]^{1/2}$.

b Refer to Table 5.1 note a and App.D for additional notes and raw data.

c Outgassing conditions and gel extractions do not affect variability so all results are included in σ_p.

The overall variabilities in the remaining isotopes (Table 5.15), including variability from samples used in the H-exchange experiments (Ch. 6) plus additional $\delta^{13}C$ and $\delta^{15}N$ analyses (data not shown), are given here. For $\delta^{13}C$ these are: ±0.5‰ for w.b. (n=50), ±0.4‰ for gel (n=12). For $\delta^{15}N$ these are: ±0.2‰ for w.b. (n=45) and ±0.2‰ for gel (n=14). Here, gel refers to gelatin extracted by the Longin method and N_2 was collected onto activated charcoal. Unlike δD, the variability in $\delta^{13}C$ and $\delta^{15}N$ was found not to be affected by outgassing conditions or by the nature of the preparation (i.e. w.b. or gel) nor was it increased in gels extracted by the Longin method when compared to GEL which is not affected by gel extraction.

The above indicates that H-exchange does not affect the variability in δD. Therefore, for most in-house bone work it may not be necessary to correct for H-exchange (see Ch. 6). Variability in $\delta^{13}C$ and $\delta^{15}N$ due to replicate preparation is reasonably low.

CHAPTER 6

HYDROGEN EXCHANGE

-EXPERIMENTAL RESULTS AND DISCUSSION-

It was shown previously that the presence of exchangeable hydrogens (H_{EX}) in a sample will alter its δD value. As indicated by eq. 3.1, the amount of alteration in δD will depend on the proportion of H_{EX} among the total hydrogens in a sample (p_{EX}) and the δD value of the H_{EX} (δD_{EX}). Results below will show that H-exchange is sufficiently rapid that, for bone stored in the laboratory, all H_{EX} are expected to equilibrate with lab atmospheric moisture and thus lose the record of ambient H_2O at the collection site. In this case, δD_{EX} is equal to $\delta Dv+\epsilon_{H-v}$, the δD value of lab atmospheric H_2O-vapor plus the isotopic separation factor between H_{EX} and H_2O vapor.

The process of gelatinization could also alter δDb if δD_{EX} and p_{EX} changed or if there were exchange of some slowly exchanging hydrogens. A number of experiments described below determine p_{EX}, whether H-exchange is rapid and whether variations in gelatinization procedures or other environmental conditions might alter p_{EX}, δD_{EX} or affect amounts and rates of slowly exchanging hydrogens.

6.1 Effects of Gelatin Extraction Procedures on H-Exchange:

In Expt. 1, GEL samples were incubated in isotopically heavy (δD_{EQ}≈100‰) HCl acidified water (pH=3) at 90°C to determine how gelatinization might affect δDg whether through a change in p_{EX} or through the exchange of hydrogens having very slow rates of exchange so that re-equilibration with lab H_2O vapor does not occur (see also eq. 3.1 and 5.1). Incubation times varied from 5 to 50 hr and samples were placed in an open beaker and oven dried in air at 60°C, 70°C, 90°C and 100°C. During drying the isotopic value of the ambient H_2O of the gelatin solutions (δD_{EQ}) would increase so that δD_{EQ}>100‰. The dried samples were then cooled, scraped from the bottom of the beaker using a metal spatula, placed in glass vials with screw-on lids, and stored over silica gel in a desiccator. These samples were outgassed ≈2 mo. later using prep 1P (Ch. 5). The δDg of the incubated and non-incubated samples were indistinguishable (Expt. 1, Table 6.1), indicating that all H_{EX} in the incubated samples had re-equilibrated with air H_2O-vapor during drying, storage and transfer of the samples. Further, lack of change in δDg indicated that there had been no significant change in p_{EX}.

This experiment was repeated for other pH values (pH=3, 6.65 and 11) using NaOH and HCl. A neutral salt (NaCl) was added to one of the two samples at pH=6.65 while one each of the pH=3, pH=6.65 and pH=11 samples were neutralized with NaOH or HCl before final drying (Expt. 2, Table 6.1). All samples were incubated for 20h at 90°C, dried in an open beaker in air at 70°C for ≈24h then cooled, transferred and stored as above. These were then outgassed at ≈130°C within ≈24h.

Table 6.1 Tests of Isotopic Exchange During GEL Preparation.[a]

	Outgassing		n	Isotopes			Yields						Preparation Notes		
	°C	prep		δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	incubation time (h)	drying T°C	
Expt 1.															
non-incubated		1P	10	-51±6	-	-	-	-	89±8	-	91±2	81±7			
incubated	≈100	1P	1	-48	-	-	-	-	88	-	89	78	20	90	
	≈100	1P	1	-45	-	-	-	-	92	-	90	82	30	60	
	≈100	1P	1	-50	-	-	-	-	91	-	87	80	40	60	
	≈100	1P	1	-50	-	-	-	-	89	-	91	81	13	60	
	≈100	1P	1	-50	-	-	-	-	85	-	93	79	20	100	
	≈100	1P	1	-55	-	-	-	-	88	-	93	82	20	70	
	≈100	1P	1	-58	-	-	-	-	87	-	91	80	20	60	
	≈100	1P	1	-61	-	-	-	-	85	-	84	71	5	60	
	≈100	1P	1	67	-	-	-	-	80	-	90	72	50	60	
				-54±7					87±4		90±3	78±4			
Expt 2.													pH		
non-incubated[b]	≈130	1P	1	-60	-	-	-	-	≈85±3	-	≈92±1	≈79±3			
													start	end	adjusted
incubated	≈130	1P	1	-53	-13.2	-	-	-	81	-	20[x]	16[x]	6.65	4.5	(salt)
	≈130	1P	1	-57	-12.8	-	-	-	86	-	85	74	11.0	7.5	7.0
	≈130	1P	1	-59	-13.1	-	-	-	91	-	70	64	3.0	3.0	7.0
	≈130	1P	1	-61	-12.9	-	-	-	87	-	82	71	11.0	7.5	-
	≈130	1P	1	-69	-13.0	-	-	-	94	-	86	81	6.65	4.5	7.0
	≈130	1P	1	-80[c]	-13.0	-	-	-	67[c]	-	80	54	3.0	3.0	-
				-63±10(6)	-13.0±0.1				84±10(6)		81±6	69±10			
				-60±6 (5)					88±5 (5)						

Table 6.1 (cont)

	Outgassing °C	prep	n	Isotopes δD	δ¹³C	δ¹⁵N	Yields C/N	H/N	H/C	N/g	C/g	H/g	pH start	end	adjusted
Expt 3.															
	210	1P	1	-93	-13.1	-	-	-	51	-	16[x]	8[x]	6.65	4.5	(salt)
	210	1P	1	-92	-12.7	-	-	-	54	-	71	38	11.0	7.5	7.0
	210	1P	1	-89	-12.8	-	-	-	61	-	62	37	3.0	3.0	7.0
	210	1P	1	-74	-12.7	-	-	-	68	-	73	50	11.0	7.5	-
	210	1P	1	-89	-2.9	-	-	-	61	-	79	48	6.65	4.5	7.0
	210	1P	1	-94	-12.9	-	-	-	41	-	82	34	3.0	4.5	-
				-89±7	-12.9±0.2				56±9		73±8	41±7			
Expt 4.															
GEL	100	4P	8	-58±2	-12.6±0.5	7.7±0.1	98±1	89±2	90±3	94±2	93±2	84±2			
Cu-GEL	100	4P	1	-57	-12.9	7.5	104	88	85	71	74	63			
GEL	100	5P	1	-44	-13.0	7.1	98	87	89	93	91	81			
Cu-GEL	100	5P	1	-47	-12.8	7.4	106	86	81	72	77	62			

a See Table 5.1 note a.
b Rough estimate based on GEL result of 2Q100, 130°C and 1P (δD:-51-9:-60)
c May have been overheated
x Not included in average. Yields are low due to salt content

Sub-samples from Expt. 2 were outgassed at ≈210°C to determine whether δDg might be affected (Expt. 3, Table 6.1).

Results of both Expt. 2 and 3 show no consistent relationship between δDg and pH or salt content. Since results of incubated samples (Expt. 2) are not significantly different from non-incubated samples, there has been no change in p_{EX} and all H_{EX} must have re-equilibrated with lab H_2O-vapor during the 24h of drying at 70°C, and then again during sample transfers and the 24h of storage in the desiccator at room temperature (T_{room}). Furthermore, lack of change in δDg in incubated samples indicates that the value of ϵ_{eH-v} must be that for T_{room} (≈24°C). Evidently, sufficient H_2O-vapor is present in the vicinity of the samples in the desiccator to ensure that re-equilibration can occur. The low δD value (-80‰) in Expt. 2 accompanied by low H/C yields are probably due to an abnormally high Tp. The high variability in results from Expts. 1, 2, and 3 is due to poor control of Tp (prep 1P, see Ch. 5).

Both δDg and H/C of incubated sub-samples in Expt. 3, outgassed at high Tp, are lower than those of Expt. 1 and 2 (including non-incubated samples). These observations are consistent with expected changes due to use of the higher Tp and do not necessarily indicate additional exchange effects or loss of H_{EX} compared to samples heated at lower Tp (Expts 1 or 2).

Since amino acid racemization rates are affected by ionic strength and the presence of metal ions (Williams and Smith 1977; Dungworth 1976; Armstrong et al. 1983), one might expect p_{EX} to be affected by the presence of Cu^{2+}. In a modification of Gelis et al. (1971), Cu^{2+} was complexed with GEL (Expt. 4). GEL (1 gm) was

dissolved in 100 ml of isotopically heavy H_2O (≈+100‰) containing 0.0509mg of Cu^{2+} from $CuCl_2 \cdot 2H_2O$. The pH was adjusted to 7 with $NaCO_3$ and the mixture evaporated in an open beaker to dryness at 70°C, transferred to sample vials and stored as above. In addition to an increase in the δD of ambient H_2O during drying, the pH of the Cu-GEL would also increase which should favor the binding of Cu^{2+} to the gelatin (cf. Gelis et al. 1971). The resulting dried compound was a blue-colored Cu-gelatin residue.

Results for two outgassing conditions show no significant differences in δDg between the Cu-GEL complex and untreated GEL. Evidently, Cu^{2+} is chelated with the gelatin without significant change either in p_{EX} or in irreversibly exchanged hydrogens (due to racemization). The C/N yield is greater in the Cu-GEL due to the presence of $NaCO_3$ but the H/N values are the same.

The above experiments suggest that p_{EX}, ϵ_{s-v} and $\delta D_c'$ are not significantly altered by pH, salt content, presence of metal ion (Cu+) or by the normal methods of gelatinization. All H_{EX} appear to re-equilibrate rapidly with H_2O-vapor in air even at T_{room}.

6.2 Preliminary Evaluation of The Existence of p_{EX} And Prospects for H_{EX} Removal:

Results given in Ch. 5 showed that p_{EX} must be relatively low. SEAL preserved its high δDb and δDg values (due to high δDi and trophic level effects, see Ch. 11) despite 100 yr of storage on land along side MUSK (Table 5.1). Nonetheless, the theoretical estimate of p_{EX} in collagen was 0.22. Although H-exchange was suspected, none of the earlier experiments described above were capable of distinguishing

whether the lack of change in δD results between incubated and non-incubated GEL samples was the result of rapid exchange or no exchange at all (p_{EX}=0). Expt. 5 was a crude attempt to detect whether a significant number of H_{EX} existed and whether any H_{EX} could be preferentially removed by varying Tp. Three sub-samples of MUSK w.b. were soaked in isotopically heavy H_2O (+147‰) directly on the vacuum line to prevent re-equilibration of H_{EX} with lab δDv prior to the outgassing procedure. Four samples of MUSK in 9 mm (o.d.) breakseals containing CuO were each attached to a glass adapter and then attached to the upper portion of the vacuum line (Fig. 4.1). Glass wool and a stopcock separated the samples from the line. A fifth position on the vacuum line contained a glass adapter with a rubber septum for introducing, via syringe, H_2O of desired isotopic value and a sixth position contained a 6 mm o.d. breakseal for collecting excess H_2O at the end of the exchange experiment.

The samples were allowed to outgas under vacuum at T_{room} for 2d then the stopcocks above all but one of the samples were closed. Twenty ml of isotopically heavy H_2O (+147‰) were cryogenically transferred onto the sample and this sample isolated from the line by the stopcock. The vacuum line above the samples was again evacuated and 20 ml of H_2O (+147‰) was cryogenically transferred onto each of the remaining samples, as above. The samples were allowed to exchange with the H_2O on the samples at T_{room} for 5 h.

Following exchange, excess H_2O was removed from the sample tubes and transferred cryogenically (10 min) into the 6mm breakseals. The samples were then outgassed at ≈10^{-3} torr for ≈10 min at T_{room}. The exchanged samples were then outgassed at 3 different temperatures (150,

160, and 220°C) for 3d prior to sealing for combustion. Results were compared to four non-exchanged control samples outgassed at Tp=150, 160, 180, and 220°C (Table 6.2, Fig. 6.1).

Results (Fig. 6.1) clearly show that the exchanged, MUSK-H samples (triangles) are ≈40‰ heavier than non-exchanged MUSK-O samples (circles). The main difference can be attributed to a difference in the δD of the H_2O with which H_{EX} were allowed to exchange. In the case of the untreated (O) samples we know that this was adsorbed-H_2O that was in equilibrium with δDv. For the treated (H) samples, the term $\delta Dv'_H$ will be used to represent air H_2O-vapor with which the adsorbed-H_2O on the bone would have been in equilibrium had it been stored under normal conditions in the laboratory. During the 10 min. cryogenic removal of excess H_2O and subsequent outgassing of the saturated H-samples, there may have been a greater amount of D-enrichment in the H_2O remaining thus increasing ϵ_{OH-V} compared to the O-samples. In all other respects the two sample sets can be considered to be identical so that eq. 5.2 can be simplified to represent the difference between MUSK-H and MUSK-O for a given Tp:

(eq. 6.1) $$\delta Db_H - \delta Db_O = p_{EX}(\delta Dv'_H - \delta Dv_O + \epsilon_{OH-V}(H) - \epsilon_{OH-V}(O))$$

If H_{EX} were preferentially removed at higher Tp then p_{EX}->0 and both δDb_H and δDb_O would converge towards $\delta Db_C'$, the δD of non-exchanged hydrogens. However, $\delta Db_H - \delta Db_O$ remains constant (within 2σ) for all values of Tp; $\delta Db_H - \delta Db_O$ equals 39‰ at Tp=150°C; 41‰ at Tp=160°C and

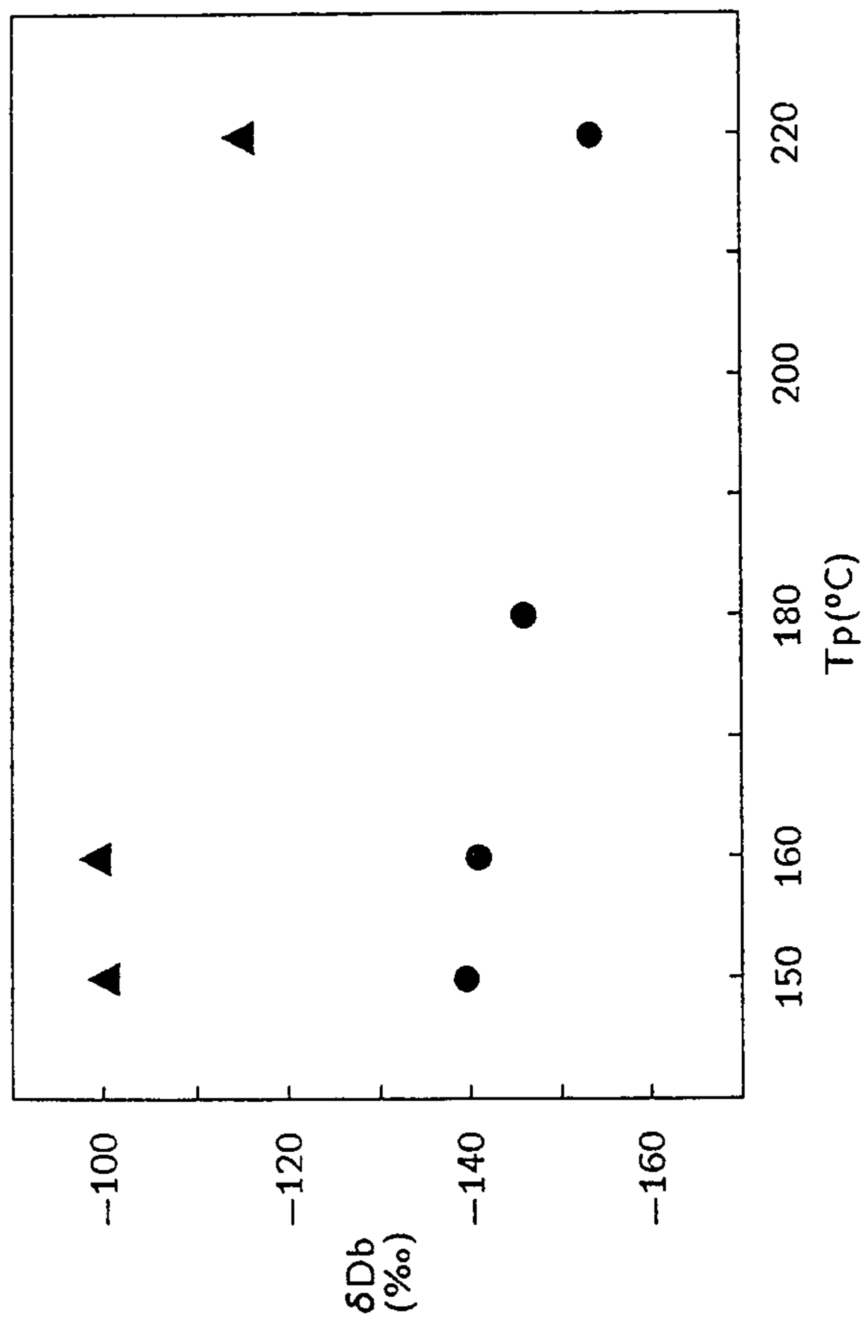
Table 6.2 MUSK Exchanged with Isotopically Heavy Water (MUSK-H) Compared to Untreated Controls (MUSK-O)[a].

	Tp(°C)	Prep	n	δD_{eq}	Isotopes			Yields					
					δDb	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g
MUSK-H	150	5P	1	147	-100	-20.6	4.6	105	91	87	81	85	74
	160	5P	1	147	-100	-	4.3	104	92	89	79	82	73
	220	5P	1	147	-115	-20.5	4.8	110	88	80	71	78	62
MUSK-O	150	5P	1	-	-139	-20.7	4.5	100	89	89	83	83	73
	160	5P	1	-	-141	-20.2	4.8	101	92	91	81	82	75
	180	5P	1	-	-146	-20.9	5.3	104	87	83	85	89	74
	220	5P	1	-	-153	-20.5	5.7	111	88	80	74	82	65

a Expt. 5. See Table 5.1 note a for additional explanations.

Figure 6.1 Exchanged MUSK Compared to Controls.

The δDb values of MUSK exchanged with isotopically heavy water (+147‰)(triangles) are compared to MUSK controls (circles). Samples were outgassed at four different outgassing temperatures (T_D)(Expt. 5)


δDb
(‰)
−100
−120
−140
−160
150
160
180
200
220
Tp(°C)

38‰ at Tp=220°C). This indicates that there is no change in p_{EX} such as preferential removal of H_{EX} at higher Tp. Therefore, H_{EX} is tightly bound to collagen even though it is readily exchanged.

6.3 Empirical Estimate Of p_{EX}:

In Expt. 6, p_{EX} was accurately determined using MUSK, BIS, SEAL and GEL as internal reference standards (R). This was done by assuming that all H_{EX} are rapidly exchanging and would equilibrate with surrounding H_2O-vapor within 48h as the earlier experiments had suggested. The equilibration temperature (T_{EQ}) was monitored throughout Expt. 6 and remained quite constant at ≈24°C and standardized outgassing conditions were used so that $\delta D_{EX}=\delta Dv_{EQ}+\epsilon_{H-V}$ would vary only according to the δD of water vapor used in the experiments (δDv^{EQ}).

From an examination of eq. 3.1, it can now be seen that under these experimental conditions a linear relationship will form between δDx(R) and δDv_{EQ} and the slope (m_R) will provide an estimate of p_{EX} of the reference such that $m_R=p_{EX}(R)$:

(eq. 6.2) $\delta Dx(R) = m_R \cdot \delta Dv_{EQ} + b_R'$

Here x indicates method of sample preparation whether w.b. (x=b), gel (x=g) or col (x=co). A slope of 0.0 would indicate no H-exchange ($p_{EX}(R)=0$) whereas a slope of 1.0 would indicate total exchange ($p_{EX}(R)=1$). A comparison of eq. 3.1 and 6.2 also shows that the intercept of eq. 6.2 (b_R') is related to the δD value of the non-exchanged hydrogens of each internal standard or $\delta Dx_c'(R)$. It will be shown later how b_R' can be used to derive $\delta Dx_c'(R)$.

In Expt. 6 the H_2O-vapor in each exchange experiment was maintained at a constant value through equilibrium with liquid H_2O of known isotopic value (δD_{EQ}) so that δDv_{EQ} = (δD_{EQ}+ϵ_{eV-L}). Here, ϵ_{eV-L} is the T_{EQ} related equilibrium isotopic separation factor between vapor and liquid (App.A). Therefore, the regression slope of eq. 6.2 may be determined directly from the relationship between $\delta Dx(R)$ and δD_{EQ}:

$$\text{(eq. 6.3)} \quad \delta Dx(R) = m_R \cdot \delta D_{EQ} + b_R$$

where: $b_R' = -\epsilon_{eV-L} \cdot m_R + b_R$. At 24°C, $\epsilon_{eV-L} \approx -78‰$ (Majoube 1971; eq. A.9, App. A) with an ≈1‰/°C variation in ϵ_{eV-L} in the room temperature range. Any small variations in T_{EQ} will be included in the regression uncertainties.

In Expt. 6 the above set of regressions were determined for each reference material. A sample each of BIS, SEAL, MUSK and GEL in 9 mm o.d. breakseals containing CuO were placed on the upper portion of the vacuum line (Fig. 4.1). Each sample was attached via a glass adapter containing a stopcock and glass wool as described in Expt. 5. A fifth position contained a 9 mm empty breakseal for removing excess H_2O-vapor from the line at the end of the exchange experiment. A sixth position contained a glass 100 ml bulb with a buret-style stopcock at the bottom. There was also a stopcock at the top of the bulb to isolate it from the vacuum line. This bulb contained H_2O of the desired isotopic value (δD_{EQ}) for the exchange procedures.

When the line was assembled, air was evacuated from the bulb and 60 ml of H_2O of the selected isotopic value (δD_{EQ}) was introduced into the evacuated bulb through the lower stopcock. The bulb remained isolated from the vacuum line by the upper stopcock while the samples were outgassed overnight at ≈24°C.

Following outgassing the stopcock to the bulb containing the H_2O was opened and the air in the upper part of the bulb briefly pumped away. The vacuum line was then isolated from the vacuum pump to allow H_2O-vapor to fill the line and to reach the samples while remaining in equilibrium with the H_2O in the bulb. The samples and vapor were allowed to equilibrate for 48h at $T_{EQ}\approx24^{\circ}C$.

Following equilibration, the bulb containing the H_2O and the individual samples were isolated from the vacuum line and each other. Liquid N_2 was then placed on the empty 9 mm o.d. breakseal and excess H_2O-vapor from the vacuum line was cryogenically transferred into the 9 mm trap. This breakseal and the bulb containing the H_2O were then removed from the vacuum line and a 6 mm o.d. breakseal was placed on to the line which was then thoroughly evacuated (with the samples still isolated). The line was isolated from further pumping action and the samples were then opened to the static vacuum-line. Excess H_2O-vapor from the four samples was immediately transferred cryogenically into the 6 mm breakseal. Five minutes were allowed for removal of this excess H_2O from the sample breakseals, then the line was opened to the vacuum pump in order to completely outgas the samples. The H_2O in the breakseal was sealed off with a torch and removed.

An additional 24 hr was allowed for complete outgassing of the samples at T_{room}. The samples were then outgassed at $Tp=150^{\circ}C$ for w.b. and $100^{\circ}C$ for GEL for an additional 3d, then combusted using standardized (5P) methods. This exchange experiment was repeated 7 times for H_2O values covering a range over 800‰ and each of the 7 experiments took 7d to complete.

During each exchange experiment an estimated 80μl of H_2O was adsorbed by the previously desiccated bone samples. At 100% RH, another estimated 4μl of H_2O as vapor would fill the vacuum line but a certain amount was also observed to exist as condensate on the insides of the glass tubing of the vacuum line and sample containers. However, since the amount of the H_2O-vapor and condensate in the line was small compared to the amount of H_2O contained in the bulb (60ml), its isotopic value would be controlled by the isotopic value of the H_2O in the bulb (δD_{EQ}).

Results of Expt. S appear in Table 6.3. In Fig. 6.2 appear linear regression plots which relate the δD of each reference material (R) [δDx(R)] to the δD of the H_2O used in the equilibration experiment (δD_{EQ}). The regression equations (6.4 to 6.7) appear in the caption for Fig. 6.2. The slopes of the equations vary between 0.204 and 0.231 and the uncertainties in these slopes are ≈0.005. All r-values are 0.999.

There are no differences in the slopes of the two terrestrial mammals whether modern or fossil, so these can be averaged to give 0.205. The m_R of the equilibration experiments can now be used to estimate a representative value of p_{EX} for each w.b. or gel preparation type. To this end, $\%p_{EX}$=20.5 for w.b. of terrestrial mammals compared to 23.1 for GEL and 22.0 for SEAL.

All p_{EX} values are close to the 22% theoretical value calculated earlier suggesting that all H_{EX} are rapidly exchanging and exchange is completed within 48h. It does seem that w.b. preparation of terrestrial mammal bone has a slight advantage over gel preparation with regards

Table 6.3 Reference Samples Equilibrated with Water Vapor in Equilibrium With Waters of Known Isotopic Value at 24°C.[a]

	Prep	δD_{eq}	Isotopes				Yields					
			δDx^b	$\delta Dx_c{}^b$	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g
MUSK	5P	372	-45	-130	-20.8	4.7	94	87	92	81	76	71
	5P	190	-88	-137	-20.3	4.8	99	91	92	85	84	77
	5P	140	-100	-140	-20.1	5.3	108[x]	92	85[x]	57[x]	61[x]	52[x]
	5P	-58	-132	-130	-20.0	4.5	95	91	96	85	81	77
	5P	-60	-136	-135	-	-	95	89	94	80	76	71
	5P	-222	-169	-135	-18.3	-	90	92	101	77	69	70
	5P	-391	-207	-139	-20.0	4.9	93	86	92	90	84	77
				-135±4	-19.1±0.8	4.8±0.3	94±3	90±2	95±4	83±5	78±6	74±3
SEAL	5P	372	161	125	-16.0	18.7	96	94	98	74	71	69
	5P	190	118	122	-15.4	18.7	93	98	105	87	81	85
	5P	140	103	116	-14.8	18.5	95	97	103	69	65	67
	5P	-58	66	123	-15.4	18.9	100	98	98	73	74	72
	5P	-60	67	125	-15.9	18.4	98	97	99	64	63	62
	5P	-222	24	116	-14.8	18.6	96	98	103	63	60	61
	5P	-391	-8	123	-15.3	18.7	104	102	98	72	75	74
				121±4	-15.4±0.5	18.6±0.2	97±4	98±2	97±5	71±6	72±6	70±6

Table 6.3 (cont)

	Prep	δD_{eq}	Isotopes				Yields					
			δDx	δDx_c	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g
BIS	5P	372	24	-43	-13.7	6.0	98	-	-	54	53	-
(fossil)	5P	190	-13	-44	-13.7	6.0	103	90	87	61	63	55
	5P	140	-25	-46	-13.9	6.1	101	93	92	54	55	50
	5P	-58	-60	-40	-14.4	5.7	102	97	96	50	50	48
	5P	-60	-67	-48	-12.7	6.1	83	79	96	60	55	52
	5P	-222	-	-	-14.7	5.8	94	-	-	54	51	-
	5P	-391	-133	-45	-14.0	5.9	104	108	104	59	61	63
				-44±3	-13.4±0.6	5.9±0.2	98±7	93±1	95±6	56±4	55±5	54±6
GEL	5P	372	68	3	-11.7	-	98	87	89	88	86	76
	5P	190	13	-7	-	-	100	92	93	76	76	70
	5P	140	11	-3	-	-	94	91	97	89	83	81
	5P	-58	-36	-6	-11.5	7.2	96	95	99	76	73	73
	5P	-60	-	-	-12.3	-	-	-	-	-	-	-
	5P	-222	-72	-3	-12.3	-	148[x]	140[x]	94	60	89	84
	5P	-391	-111	-3	-12.2	-	96	94	98	85	82	80
				-3±4	-12.0±0.4	7.2	98±3	93±3	94±4	84±6	82±7	77±5

a Expt. 6. See Table 5.1 note a for additional explanations

b x:g for gel, c for col and b for w.b. preparations

Figure 6.2 Value of p_{EX} Determined through Equilibration.

The $\delta Dx(R)_{EQ}$ of the four reference materials (R) SEAL, GEL, BIS and MUSK (y-axis) following equilibration with water vapours of varying isotopic values are regressed against δD_{EQ} (x-axis) of the equilibration H_2O used in the experiment. The water vapour with which H_{EX} exchanges was maintained in equilibrium at 24°C with the equilibration waters. The slope of each curve given below indicates the proportion of exchangeable hydrogens [$p_{EX}(R)$] for each standard while the isotopic value of the non-exchanged hydrogens [$\delta Dx_c(R)_{EQ}$] can be estimated from the intercept (b_R). A steeper slope indicates a greater $p_{EX}(R)$ (Expt. 6).

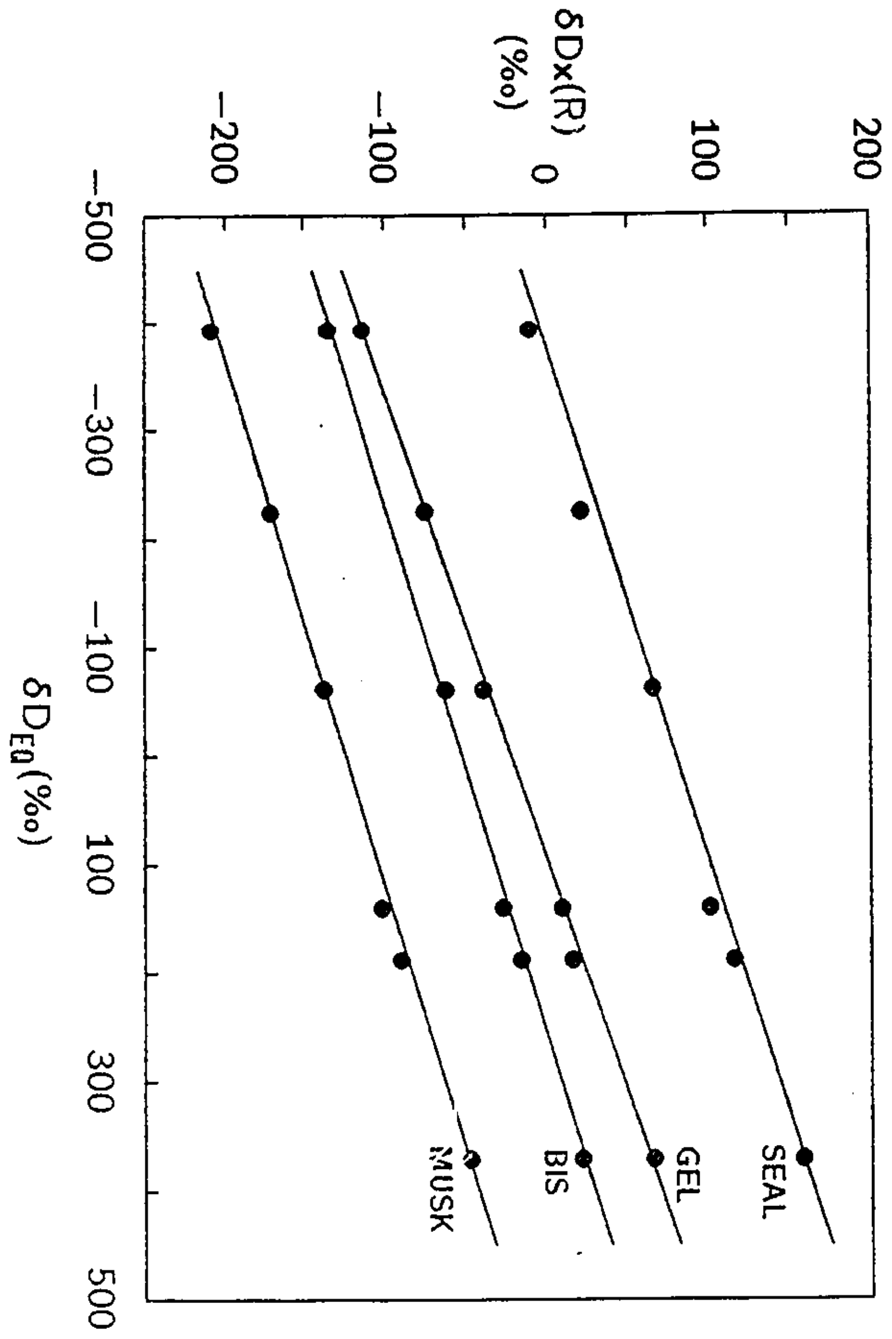
Regression Statistics:

(eq. 6.4) $\delta Db(MUSK)_{EQ} = -124 + 0.206\delta D_{EQ}$; $\delta Db_c(MUSK)_{EQ} = -136‰$

(eq. 6.5) $\delta Db(BIS)_{EQ} = -52 + 0.204\delta D_{EQ}$; $\delta Db_c(BIS)_{EQ} = -45‰$

(eq. 6.6) $\delta Db(SEAL)_{EQ} = +77 + 0.220\delta D_{EQ}$; $\delta Db_c(SEAL)_{EQ} = +121‰$

(eq. 6.7) $\delta Dg(GEL)_{EQ} = -21 + 0.231\delta D_{EQ}$; $\delta Dg_c(GEL)_{EQ} = -4‰$



δDx(R)
(‰)
200
100
0
−100
−200
−500
−300
−100
100
300
500
δD$_{EQ}$(‰)
SEAL
GEL
BIS
MUSK

to lowering H_{EX}. The slightly higher p_{EX} in SEAL compared to the terrestrial w.b. samples suggests that there may be either an intrinsic difference in this sample or some small amount of contamination.

6.4 Correcting for H-Exchange:

Substituting the above information into eq. 3.1 and solving for $\delta Dx_c'$ gives an equation for determining the δD values of any w.b. or gel sample corrected for exchange:

$$\text{(eq. 6.8)} \qquad \delta Dx_c' = \left[\frac{\delta Dx - m_R \cdot \delta Dv}{1-m_R}\right] - \left[\frac{m_R \cdot \epsilon_{B-V}}{1-m_R}\right]$$

It is not possible from these experiments to estimate the values of ϵ_{B-V} (between H_{EX} and H_2O-vapor). But it is not necessary to know the value of ϵ_{B-V} to determine p_{EX} since ϵ_{B-V} is a constant for a given reference sample (MUSK, BIS, SEAL or GEL) at T_{EQ}=24°C and when using standardized outgassing techniques. For any sample of a given type (w.b., gel, col) stored at a fairly constant T_{room} and then prepared according to standardized methods (Ch. 4), the value of p_{EX} and ϵ_{B-V} can be assumed to be constant and the second term of eq. 6.8 to also be a constant. In order to correct the δD values only for the effects of varying δDv, ϵ_{B-V} will be given a zero value which eliminates the second term of eq. 6.8.

$$\text{(eq. 6.9)} \qquad \delta Dx_c = \left[\frac{\delta Dx - m_R \cdot \delta Dv}{1-m_R}\right]$$

Here, $\delta Dx_c = \delta Dx_c' + [m_R/(1-m_R)]\cdot\epsilon_{B-V}$ where $[m_R/(1-m_R)]\cdot\epsilon_{B-V}$ represent the essentially constant offset between δDx_c and the true δD value of

the non-exchanged hydrogens in the sample ($\delta Dx_c'$). Once δDv is determined and δDx_c calculated, the empirical variability in δDx_c will include what should be a small variability in ϵ_{H-V} arising from variability in T_{EQ} and in lab humidity. Assuming that p_{EX}=0.205 for w.b., there could be a 0.8‰ shift in δDx_c for every 1‰ shift in ϵ_{H-V}. However, variability in δDx_c due to ϵ_{H-V} should be negligible for samples undergoing standardized preparation (Ch. 5). Therefore, in the case of samples stored at T_{room} with normal humidity and prepared by standardized methods, it should be possible to directly compare the δDx_c values calculated at different labs appearing in the literature.

Since (b_R) is the isotopic value of the standard sample when δD_{EQ}=0‰, and δDv_{EQ}=-78‰, substitution into eq. 6.9 gives:

$$\text{(eq. 6.10)} \quad \delta Dx_c(R)_{EQ} = \left[\frac{b_R + 78m_R}{1-m_R}\right]$$

Where subscript EQ denotes calculated results from Expt. 6. $\delta Dx_c(R)_{EQ}$ will also be offset from $\delta Dx_c'(R)$ by the essentially constant value $[m_R/(1-m_R)]\cdot\epsilon_{H-V(EQ)}]$.

The value of $\delta Dx_c(R)_{EQ}$ for each of the four internal standards (MUSK, SEAL, BIS, GEL) appears in the caption of Fig. 6.2. It is possible that these $\delta Dx_c(R)_{EQ}$ results could be offset slightly from $\delta Dx_c(R)$ values for these same reference samples had they been stored under normal conditions in the laboratory. This is because rh during Expt. 6 was 100% whereas that in the laboratory is lower. Thus, there may have been more adsorbed-H_2O on the samples of Expt. 6 which could prolong evaporation times and increase $\epsilon_{OH-V(EQ)}$. In this way,

$\delta Dx_c(R)_{EQ} - \delta Dx_c(R) = [m_R/(1-m_R)] \cdot (\epsilon_{OH-V(EQ)} - \epsilon_{OH-V})$. However, not only is any offset due to this difference likely to be very small but, as discussed below, it will not adversely affect interpretations of δDx_c.

6.4.1 Determination of δDv:

If the value of δDv were known then eq. 6.9 could be used to correct δDb for H-exchange. This value could be known if procedures such as Expt. 6 were used so that all samples were equilibrated with H_2O-vapor having a known δDv. Further if constant values for δD_{EQ} and T_{EQ} were maintained during such equilibrations then variability in δDb due to H-exchange would be eliminated.

Alternatively, the H_2O-vapor in air could be collected at the time a group of samples is outgassed and its δDv value directly measured. In order to estimate the laboratory value of δDv, H_2O-vapor was collected by placing 1000 ml bulb on a vacuum line in an arrangement similar to that of Fig. 4.1. Air was allowed to fill the vacuum line to which the bulb, a 6 mm o.d. breakseal (for H_2O collection), and a U-tube were connected. The line was then sealed and $N_{2(l)}$ placed on the bulb for 30 min in order to freeze down the H_2O and CO_2 in the air. Liquid nitrogen was then placed on the U-tube and the N_2 in the air was pumped away through the U-tube. The CO_2 was similarly pumped away by placing a dry-ice-isopropyl alcohol bath on both the bulb and U-tube. This left only the H_2O from air which was cryogenically transferred into the 6 mm breakseal for later isotopic analysis. The δDv of the lab H_2O-vapor collected on July 30, 1983 by this method was -122‰. This does not greatly differ from the average yearly lab δDv which can be estimated for Hamilton (≈-142±42‰) from the

1977 and 1978 δD_{wy} measured at Simcoe, Ontario (IAEA 1983) and assuming $\epsilon_{ev-L} \approx -78‰$ at 24°C.

Another measurement of lab δDv can be obtained by using a calibration bone standard which has equilibrated with the lab H_2O-vapor and is then attached to the vacuum line, outgassed and sealed using the same procedures and at the same time as a group of samples to be analyzed. $\delta Dx(R)$ could then be used for monitoring changes in δDv over time and for correcting or calibrating samples for H-exchange. Using any of the internal standards from Expt. 6 and by knowing $\delta Dx_c(R)$ and m_R, eq. 6.9 can be rearranged to give δDv for any given experimental time period:

$$\text{(eq. 6.11)} \quad \delta Dv = (1/m_R)\cdot[\delta Dx(R) - (1-m_R)\cdot\delta Dx_c(R)_{EQ}]$$

and this can be substituted into eq. 6.9 to give:

$$\text{(eq. 6.12)} \quad \delta Dx_c = \delta Dx_c(R)_{EQ} + \left[\frac{\delta Dx - \delta Dx(R)}{1-m_R}\right]$$

All δDx_c results in addition to being offset from $\delta Dxc'$ by $[m_R/(1-m_R)]\cdot\epsilon_{B-V}$ could also be offset by the practically constant $-[m_R/(1-m_R)]\cdot(\epsilon o_{B-V(EQ)}-\epsilon o_{B-V})$ due to use of $\delta Dx_c(R)_{EQ}$ results from Expt. 6. (above). All δDb_c of the deer samples would also be offset by this amount which could decrease the intercept of the δDx_c vs δDw (of growing season rain) relationship. However, δDx_c is already offset from $\delta Dx_c'$ by an unknown quantity, and the intercept of δDb_c vs δDw relationship contains the same unknowns (Ch. 8). Therefore, adding another unknown is of no consequence when interpreting δDx_c. In Ch. 8, it will be further shown using plots of δDb_c vs δDw that $\delta Db_c(MUSK)_{EQ}$ conforms well with trends established for deer. This indicates that any offset in ϵo_{B-V} contained in the EQ-results of Expt. 6 is negligible.

6.4.2 Relative Advantages of Different δDv Measures for H-exchange Correction:

The calibration bone method is a considerably less tedious method for determining δDv than is the equilibration method (Expt. 6) or direct collection of lab H_2O-vapor. Due to the high pressures of non-condensable gases in air, it is also not certain that there is quantitative collection of all lab H_2O-vapor so that the accuracy of δDv determined by this method is in question. An additional advantage of using a calibration bone standard is that effects of sample preparation can be monitored and correction for H-exchange may also provide correction for these effects.

The value of equilibration or calibration methods must also be examined in terms of the ability of each to reduce variability in δDb_c.

It might seem that equilibration would produce a lower variability in δDb_c than would bone calibration. This is primarily due to better accuracy of the δDv determination compared to $\delta Dx(R)$. In Table 6.3, δD values were corrected for H-exchange using $\delta Dv = \delta D_{EQ} - 78‰$. For each reference sample, the averages and standard deviations (σ) on δD_c were calculated. The pooled standard deviation (σ_p) is ±3.7‰. This is the overall variability following equilibration of samples by methods of Expt. 6. Knowing that the uncertainty in the uncorrected δDb measurement is lower, ≈±3‰, as determined from replicate preparations, the isolated error due to equilibration and correction alone is ≈±2‰. The uncertainty in the δDv estimate used in eq. 6.9 includes uncertainty in measures of δD_{EQ} (≈±1-1.5‰), T_{EQ} and ϵ_{V-L}. The overall uncertainty in equilibrated samples could be reduced slightly, to ≈±3.6‰, by equilibrating samples with vapor from a single H_2O standard of well known isotopic composition. In this manner, the uncertainty in the H_2O determination ($\sigma/\sqrt{n}$) is reduced by increasing n, the number of determinations of the standard H_2O. Additional reductions in variability might be accomplished by accurately controlling T_{EQ}.

There is no real opportunity to reduce uncertainty in the δDc value to less than that associated with the equilibration method through direct measurement of δDv of lab H_2O-vapor. This is because the measurement uncertainty of this δDv value will also be ≈±1-1.5%, the same as that of δD_{EQ} used in equilibration. In addition, there would be variability associated with sample preparation and possible unknown errors mentioned above.

The calibration bone method of correcting for H-exchange was used in this work as it also allows effects of sample preparation to be

monitored. Theoretically, the uncertainty of δDb_c in samples corrected using calibration bone standards could increase to ≈±4 to 4.5‰, largely due to a greater uncertainty in the $\delta Dx(R)$ measurement (≈±2-3‰) used to determine δDv. This would represent an increase over the equilibration method but does not take account of the potential for the calibration bone method to decrease variability through correction for effects of sample preparation. Such a correction was, in fact, apparent when studying the δDb_c of deer vs δDw relationship. Correction for H-exchange was found not to increase variability in δDb_c over non-correction (see Ch. 8).

6.4.3 Relative Advantages of Correction vs Non-Correction:

Previous results (Ch. 5) showed that the overall between-time variability of ±3‰ in δDb not corrected for H-exchange is no greater than same-time variability. This indicates that most variability in the δDb is not due to variability in δDv and its effects on H-exchange but arises from replicate sample preparation. With p_{EX}=0.205, variability in δDv over time would have to have been less than ±14‰ during the four years of experiments. Using the calibration bone standard to estimate δDv in the lab via eq. 6.11 also shows that from Dec. '83 through Nov. '85, there was low variability in δDv (Table 6.4). This explains the negligible effects that H-exchange has on variability of δDx.

The above indicates that both equilibration and calibration bone methods of correcting for H-exchange produce δDb_c measurements with higher variabilities (±3.5-4.5‰) than are associated with the uncorrected δDb values (±3‰). Any increase in variability of δDb_c

Table 6.4 Estimates of Lab Water Vapor (δDv) Derived from Results on Calibration Bone Standards[a].

Date		δDv	n
January 14, 1984		-153	
		-162	
February 8-18, 1984		-159	
		-150	
May 2, 1985		-123	
		-159	
Early June, 1985		-119	
		-159	
		-171	
July 27, 1985		-171±5	2
August 8, 1985		-160	
		-149	
Mid September, 1985		-159±18	3
October, 1985		-168	
Mid November, 1985		-139	
	ave.	-153±16	15

a δDv defined by eq. 6.11.

would increase the variability about the regression curve of δDb_c vs δDw. Therefore it may be advantageous to use uncorrected δDb values for most in-house work in which the main goal is to provide the most accurate estimate of δDw from bone δD. Nonetheless, a number of theoretical considerations regarding the deer need to be investigated which require correction for H-exchange. For instance, it is important to verify that, when the δDbc results are corrected for effects of RH (δDb_{c100}, see Ch. 8), the δDb_{c10c} vs δDw relationship has a slope of 1.0, thus confirming theoretical expectations and indicating no unexpected isotopic effects on the deer. Given that p_{EX}=0.205, if there were correction for RH effects but no correction for H-exchange (δDb_{100}) then this slope (δDb_{100} vs δDw) would be 20.5% lower than 1.0, i.e. 0.795.

The slope of this relationship between δDb_c and δDw can also be used to evaluate whether there are any slowly exchanging hydrogens in w.b. samples. It was not practical to do this using equilibration experiments such as Expt. 6 since such equilibrations could take many months. Any discrepancy between the actual δDb_{c100} vs δDw slope and 1.0 could indicate the presence of slowly exchanging hydrogens. For instance, if there were 8% slowly exchanging hydrogens, this slope would be 0.92 assuming that any slowly exchanging hydrogens would likely have equilibrated with lab H_2O-vapor during the typical bone storage period of several months before outgassing. Calculation of the δDb_c values has the additional advantage of allowing other researchers to compare their bone δDb_c to the δDb_c results presented here.

6.5 Comparisons of δD of Gelatins, Whole Bone and Collagens:

In Expt. 7, the δD results of w.b., gel and col preparations of 16 samples of modern w.t. deer were compared in order to evaluate their relative proportions of H_{EX} (Table 6.5). The trends in the δDx results were also compared to δDw. Results were not corrected for H-exchange and methods for determining δDw are given in Ch. 7.

Using the methodology given in Ch. 5 and App. D, the δDb of one sample in Table 6.5 and in all 6 deer samples were corrected for the effects of an earlier (3P) outgassing method by adding the constant, +23.5‰. Later analyses (Ch. 8) indicate that the six calibrated δDb_c results agreed well with the δDb_c results on the remaining 70 samples of deer.

The important question of whether the δD of w.t. deer contained a sufficient proportion of non-exchangeable hydrogen to provide a record of δDw is partly answered by the data in Table 6.5. All results of the three preparations (w.b., col and gel) exhibit trends similar to those observed in the local δDw. However, as one scans each column, it is apparent that the δD of bone decreases less rapidly than does that of rain. This is due to the existence of H_{EX} in the bone which produces a <1.0 slope between bone δD and rain δD. This relationship to rain δD will be shown using more samples in Ch. 8.

Results from regressions of δDco (col or demineralized bone) vs δDb (w.b. or powdered whole bone) appear in eq. 6.12 in the notes of Table 6.5. Here, a 1.0 slope within 2σ (σ=±0.02) indicates no difference in p_{EX} between col and w.b. preparations. Apparently, there is no major contribution to H_{EX} from bone mineral or non-collagen components in w.b.

Table 6.5 Comparisons of δD Results for Whole Bone (δDb), Collagen (δDco) and Gelatin (δDg) of Modern Deer.[a]

sample	δDb	δDco	δDg	δDw
TX-3[b]	-21	-24	-23	-20
TX-5[b]	-25	-30	-20	-20
KS-1	-40[e]	-41	-27	-38
NE-2	-53	-54	-37	-70
AB-4[c]	-90	-93	-78	-112
AB-5[c]	-85	-	-74	-112
AB-3[d]	-95	-96	-84	-112
AB-2[d]	-93	-	-82	-112
AL-1	-34	-	-13[x]	-22
MS-1	-28	-	-20	-23
WV-3	-40	-	-33	-36
QC-2	-64	-	-53	-63
WY-2	-80	-	-72	-91
LA-8	-34[f]	-	-27	-23
ND-1	-68[f]	-	-58	-75

a Expt. 7. Results are not corrected for H-exchange.
b,c,d samples from the same location.
e δDb corrected for effects of sample preparation as described in App.D.
f values estimated from δDg using eq. 6.13.
x outlier result excluded from regression.

Regression equations:

(eq. 6.12) $\delta Dco = -4 + 0.98\delta Db$; $n=6$, $\sigma=\pm 2$, $r=0.999$

(eq. 6.13) $\delta Dg = 4 + 0.91\delta Db$; $n=12$, $\sigma=\pm 4$, $r=0.989$

The intercept is also within 2σ of 0 ($\sigma=\pm2$). Lack of significant increase in relative H-yields in w.b. (Ch. 5) also indicates no significant additional contributions of H_2O in the w.b. samples, so analysis of w.b. apparently gives the same results as analysis of col.

The regression of δDg (gel or gelatin) vs δDb results for 12 samples (excluding AL-1) is given in eq. 6.13 in the notes of Table 6.5 and has a slope of 0.91 which is less than 1.0, probably due to the larger value of p_{EX} in gels (0.231, Fig. 6.2). However, the intercept is still within 2σ of 0 ($\sigma=\pm0.04$ for the slope and ±3 for the intercept). The results of AL-1 were eliminated from this regression because its δDg value and gel yield were anomalous. From the regression equation of δDg vs δDb (eq. 6.13), it was possible to estimate δDb values for LA-8 and ND-1 from their δDg (eq. 6.13, Table 6.5). Results agreed well with other δDb results of deer when compared to δDw (Ch. 8).

Gel vs w.b. results from Table 5.1 may also be compared. Results on MUSK contradict those of the deer in that the p_{EX} of gel and w.b. do not appear to be significantly different. Use of eq. 6.13 (Table 6.5) predicts a value of -124‰ for δDg of MUSK which is different from its measured value of -145‰ and 60‰ for δDg of SEAL instead of its actual value of 89‰. Whereas MUSK gel values are identical to MUSK w.b., SEAL gel is +28‰ relative to SEAL w.b. Reasons for these discrepancies are not known. Results appearing in Ch. 8 will show that the estimate of p_{EX} derived from MUSK w.b. is likely to be an accurate estimate of p_{EX} for modern deer w.b. Perhaps some component of the MUSK gel has altered in 100 yr so that a group of H_{EX} existing in fresh gel does not exist in the MUSK gel. The results on SEAL, on the other hand, suggest that SEAL w.b. may be different in composition from w.b.

of terrestrial mammals or that there may be some component or contaminant affecting its w.b. results. Since the $\delta^{13}C$ and $\delta^{15}N$ of SEAL w.b. and gel are not statistically different, it is unlikely to be significantly contaminated by lipids, amino acid or other organic contaminants of terrestrial origin. Nonetheless, DeNiro and Weiner (1988c) propose that the crystalline aggregates in whole bone material from marine mammals contain significant quantities of non-collagen protein (NCP) whereas those of terrestrial mammals contain little. NCP's have higher C/N and $\delta^{13}C$ ≈6–7‰ lower than gel. The C/N yield of seal w.b. is 2% higher than than of its gel extracts and its $\delta^{13}C$ value 0.6‰ lower. This suggests that perhaps the presence of NCP in SEAL w.b. compared to gel can account for their discrepancy in δD.

The above results indicate that δD_b, δD_{co} and δD_g all have relatively low p_{EX} and should provide a good record of δD_w. Col and w.b. analyses appear to have a slight advantage over gel analysis since gel preparations appear to have a higher proportion of H_{EX}. Nonetheless, col or gel would likely provide valuable preparation alternatives for fossil samples where removal of contaminants is often very important.

CHAPTER 7

THE ISOTOPE DATA AND DETERMINATION OF THE δD_w AND CLIMATIC VARIABLES

It was suggested previously that climate should be reflected in bone through the effects of T on the δD of rain and RH on the δD of leaf water which, in turn, is recorded in the δD of bone. The yearly average of monthly δD of rain appearing in bone would also be weighted by the monthly amount of rain. Amount of rain might also have a direct effect on the $\delta^{15}N$ of bone collagen (Ch. 2). The means of determining the climatic variables and the rain δD for each deer sampling location are given below. The relationship between climate and δD of rain will also be discussed. At the end of this chapter all the isotopic, climatic and rain δD data will be listed (Tables 7.3, 7.4, 7.5).

7.1 Determination of Climatic Variables for Each Sampling Location:

The average monthly rh, amount of rain and temperature (RH_m, PPT_m, T_m) were compiled for weather stations near each sampling location. Each monthly value had been averaged over a variable number of years. These were averaged together over the 12 months of the year or over the growing season months to provide 5 to 30-y yearly (y) or growing season averages of temperature (Ty and T), monthly amounts of rain (PPTy and PPT) and relative humidities (RHy and RH). Here, the

growing season is defined as the months of the year in which T_m (the average temperature over the month) is above 0°C (Miller et al. 1988). All climatic parameters not specifically identified as being yearly RHy, PPTy or Ty) are for the growing season (RH, PPT, T).

7.1.1 United States:

T_m, and PPT_m, averaged for a 30 y period ending in 1982, were obtained from the U.S. National Oceanic and Atmospheric Administration (NOAA 1983) publications. These 30 y average T_m and PPT_m were then averaged as above to provide the Ty, T, PPTy and PPT values for each U.S. weather station.

The RH_m data of NOAA had been calculated over a variable number of years (from 16 to 43) for two observation periods per day, morning and afternoon. The two observation periods were averaged for each month to provide an estimate of the daily (24h) RH_m. The 24h RH_m were then averaged over the 12 months of the year or over the growing season months to provide the RHy and RH values.

The observation periods for the U.S. locations were based on Greenwich Mean Time (GMT) (06h,12h,18h,00h) which meant that Local Standard Time (LST) varied across the continent. For instance, in the Atlantic Time Zone, the observation periods were 8 A.M. and 2 P.M. whereas in the Pacific Time Zone, these were 4 A.M. and 4 P.M. This could mean that in the eastern areas, RH could more closely resemble daytime (daylight) RH, whereas in the western areas it could more closely resemble average daily (24h) RH. Theoretically, it would be the daytime RH for the growing season that is reflected in δDb since this is when photosynthesis occurs. However, such daytime RH were not

available for the U.S. locations of this study. It was found (below) that the RH data for most locations in the U.S. should most closely resemble 24h RH. Results were not greatly biased when 24 hr RH was used instead of daytime RH.

7.1.2 Canada:

Canadian T_m, PPT_m and vapor pressure data were compiled from Environment Canada (1979 to 1983, inclusive). The T_m and PPT_m were averaged over the 12 months of the years and over the growing season months, then over the 5 years to produce the 5-y average Ty, T, PPTy and PPT values.

Water vapor pressures (P_v) in kilopascals were compiled for the four GMT observation periods (early morning, late morning, afternoon, and evening). The Tetens formula was used to convert the average monthly vapor pressures to RH_m for each daily observation period (Anayadike 1984):

$$\text{(eq. 7.1)} \quad RH_m(\%) = \left[\frac{1000 \cdot P_v}{6.11 \times 10^{[7.5T/(T+237.3)]}} \right]$$

Here, the denominator is the saturation vapor pressure in millibars (mb) at T(°C) during the observation period. The RH_m for each observation period were then averaged over 12 months, and then over the 5 years (or over the growing season months then over the 5 years) to give 5-y average rh data for each of the four daily observation periods. Then the 4 observation periods were averaged to give 24h RHy

(% yearly average 24h rh) and RH (% growing season average 24h rh) values.

7.1.3 Potential Bias in the RH Data:

The LST at each Canadian weather station also varied across the continent from 2 A.M., 8 A.M., 2 P.M., 8 P.M. in the Atlantic Time Zone to 10 P.M., 4 A.M., 10 A.M. and 4 P.M. in the Pacific Time Zone. Different calculations of rh for Canadian weather stations were examined in order to identify whether there was bias in the RH measures due to the use of 24hr calculations based on 4 observation periods. These RH calculations were compared to those using the same two observation periods as used by NOAA in the U.S. (RH_2, RHy_2) and also to an alternative (daylight) measure of RH (RH_{day}, RHy_{day}) which was derived from an average of two LST daytime observation periods (7AM, 1PM) in each location. The latter were obtained from Environment Canada (1984) which consisted of RH_m values averaged over 28 y for each of the two LST observation periods. The three alternative measures of rh for Canadian weather stations are compared in Table 7.1. The differences between the different measures of RH (big δ) are also given for each location.

The RH and RHy calculations are very similar to RH_2 and RHy_2 in all areas west of Quebec. This indicates that RH_2 calculations from all U.S. locations should approximate 24h rh since all U.S. sampling locations lie in time zones west of the sampling locations in Quebec. However, the calculation of RH and RHy values for Canadian stations is likely to

Table 7.1 Comparison of the Relative Humidity Tabulations from Across Canada

			RH						Δ					
			growing season			yearly			growing season			yearly		
	Standard Time Zone[a]	Observation Period (h)	RH^c	RH_2^d	RH_{day}^e	RHy^c	RHy_2^d	RHy_{day}^e	Δ (RH_2-RH)	Δ (RH_2-RH_{day})	Δ (RH-RH_{day})	Δ (RHy_2-RHy)	Δ (RHy-RHy_{day})	Δ (RHy-RHy_{day})
Kamloops	P	4[b],10,16[b],22	60	60	60	64	64	63	0	0	0	0	1	1
Sandspit	P	4[b],10,16[b],22	84	83	83	84	83	83	-3	0	1	-1	0	1
Calgary	M	5[b],11,17[b],23	56	57	59	62	62	63	1	-2	-3	0	-1	-1
Edmonton	M	5[b],11,17[b],23	69	69	67	75	75	70	0	2	2	0	5	5
Lethbridge	M	5[b],11,17[b],23	56	57	57	65	65	62	1	0	-1	0	3	3
Medicine Hat	M	5[b],11,17[b],23	58	59	57	66	67	62	1	2	1	1	5	4
Red Deer	M	5[b],11,17[b],23	66	69	66	73	74	69	3	3	0	1	5	4
Inuvik	M	5[b],11,17[b],23	69	69	71	81	81	73	0	-2	-2	0	8	8
Sachs Harbour	M	5[b],11,17[b],23	85	84	84	94	93	83	-1	0	1	-1	10	11
Saskatoon	C	0,6[b],12[b],18	61	66	63	71	75	68	5	3	-2	4	7	3
Kenora	C	0,6[b],12[b],18	66	69	69	73	75	72	3	0	-3	2	3	1
Peterborough	E	1,7[b],13[b],19	77	75	75[f]	81	78	76[f]	-2	0[f]	2	-3	2[f]	5
Sudbury	E	1,7[b],13[b],19	72	71	70[f]	79	79	74[f]	-1	1[f]	2	0	5[f]	5
Trenton	E	1,7[b],13[b],19	78	76	73[f]	81	79	74[f]	-2	3[f]	5	-2	5[f]	7
London	E	1,7[b],13[b],19	77	77	75[f]	81	81	77[f]	0	2[f]	2	0	4[f]	4
Ottawa	E	1,7[b],13[b],19	73	73	69[f]	77	77	70[f]	0	4[f]	4	0	7[f]	7
Mirabel	E	1,7[b],13[b],19	77	74	72[f]	79	77	72[f]	-3	2[f]	5	-2	5[f]	7
Baie Comeau	E	1,7[b],13[b],19	80	76	74[f]	82	79	74[f]	-4	2[f]	6	-3	5[f]	8
Fredricton	A	2,8[b],14[b],20	75	71	72	77	74	72	-4	-1	3	-3	2	5
Yarmouth	A	2,8[b],14[b],20	83	79	82	83	80	82	-4	-3	1	-3	-2	1
Sydney	A	2,8[b],14[b],20	83	77	79	84	79	80	-6	-2	4	-5	1	4
								ave.	-0.8	0.7	1.3	-0.7	3.7	4.4

a P=Pacific, M=Mountain, C=Central, E=Eastern and A=Atlantic Standard Times.
b Observation periods that match those of NOAA for the U.S. locations.
c Average of all four observation periods.
d Averages of 2 observation periods marked by b.
e Average data for 2 observation periods 7 AM and 1 PM LST (local standard time).
f Matches time zone of RH_2.

provide a more accurate estimate of 24h rh because it uses data from more observation periods compared to U.S. stations.

Since the RH_2 observation periods for 7 eastern locations are identical to those of RH_{day} (7AM, 1PM) their +2% to +7% higher RH_2, RHy_2 and RH, RHy values must be due to a difference in these values during the different years of observation rather than due to any trans-continental bias in the RH data. Normally, one would expect RH to be especially high relative to RH_{day} in the western and more interior and drier locations of Canada during the growing season. Nighttime RH in such locations should be considerably higher than in the daytime due to the decrease in nighttime temperature (cf. Edwards and Fritz 1986). This should produce a positive bias in RH as one moves from east to west across Canada. The RH-RH_{day} trend (Table 7.1) is the reverse of that expected, especially during the growing season. This suggests that use of the RH_{day} calculation available for Canadian sites will not correct the Canadian results in a coherent fashion for an expected bias produced by including 24h RH in the calculations presented later (Ch. 8,9,10). Comparisons of some of these calculations for the Canadian locations using the three different measures of RH suggest few adverse effects from using the 24h RH calculations for all Canadian and U.S. sampling locations.

7.2 Relationships Among Predictor Climatic Variables:

Plots of RH vs the T and PPT variables and PPT vs T were visually examined for any correlations (plots not shown). Similar plots for yearly average data were also examined. In this way it was possible to check for any redundancy among the climatic variables before

including them as predictor variables in multilinear regressions presented later (Ch. 8,9,10). It was also possible to observe whether a transformation of certain data might be appropriate if they showed curvilinearity. Finally, the δDb (of deer bone) is expected to be related to both T (through its relationship with δDw) and RH (through its relationship with δDl). If RH were related to T, then the δD data could be systematically biased as one moves from areas of warm to cold temperatures. For instance RH should decrease in warmer areas of low rainfall so it might be redundant to include all three as predictor variables in the same multilinear regression model. Multilinear stepwise regression conducted using methods given in App. E, did indeed show some correlation as well as curvilinearity in the relationship between RHy and both PPTy and Ty of this study. RH was found to decrease as one moves into warmer areas with lower rainfall ($n=51$, $\sigma=\pm0.94$, $R=0.761$).

(eq. 7.2) $\ln(RHy) = 28.5 + 0.205\cdot\ln(PPTy) - 4.36\cdot\ln[Ty(^{\circ}K)]$

Similar relationships were found for the growing season data.

An examination of the climatic data further showed that there is a general bias in climatic data across North America. As one moves into the interiors of the continents one also moves into areas of lower PPTy and lower Ty giving a positive relationship between Ty and PPTy. In addition, results appearing in Ch. 8 show that rh also tends to decrease in the interiors of continents, probably due to its dependence on amount of rain which is lower in these areas.

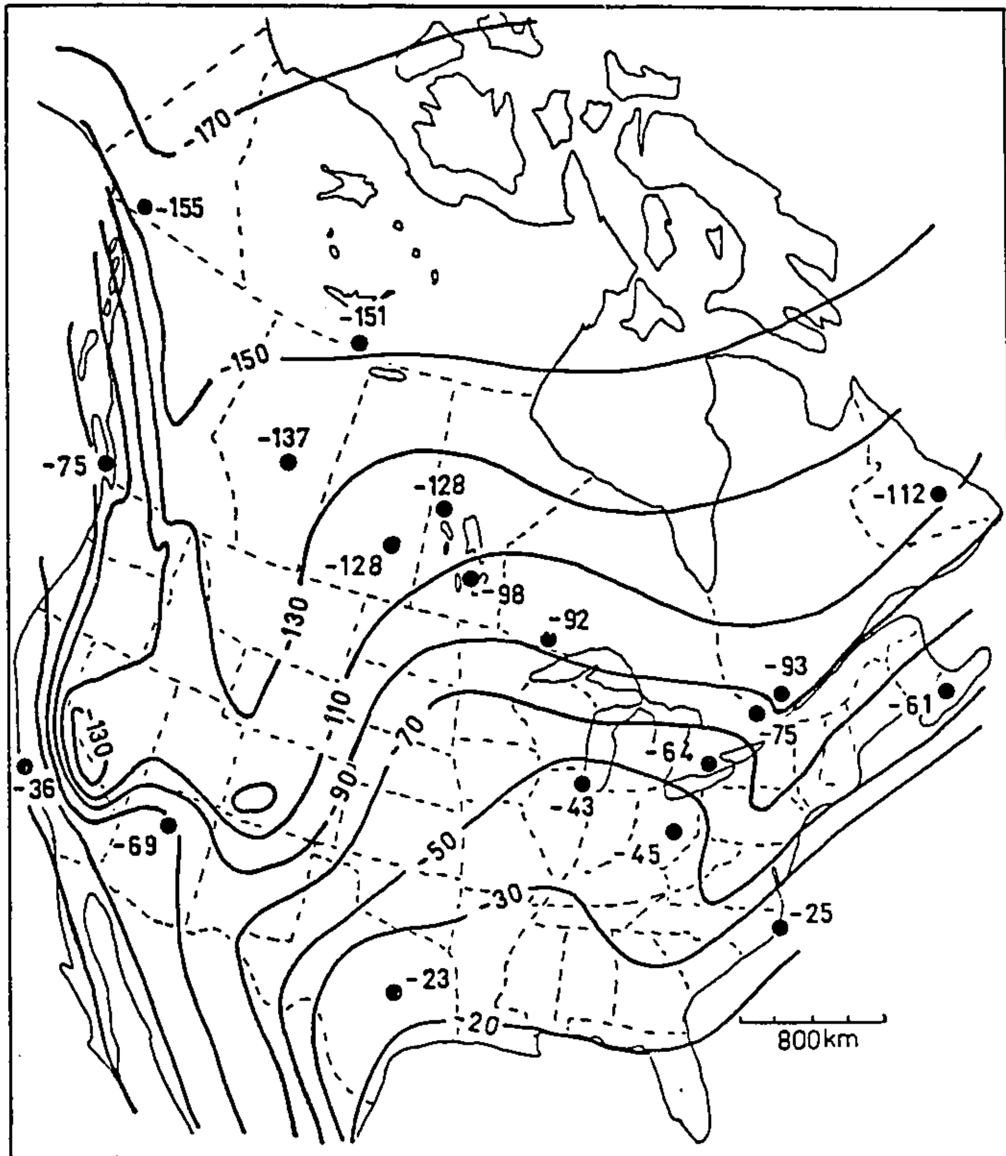
7.3 Determination of δDw and δDwy for Each Sampling Location:

The δDwy value represents the weighted average monthly precipitation δD calculated over 12 months of the year. The δDw values represent the weighted average monthly rain δD calculated over the growing season months. These were determined for each sampling location through interpolation between contours of rain δD similar to those constructed by Sheppard et al. (1969) and Taylor (1974). Included in the contours used here (Fig. 7.1, 7.2) are rain δD data compiled for 19 International Atomic Energy Association (IAEA) stations spread across North America for the years 1962 through 1979 (IAEA 1969, 1970, 1971, 1973, 1977, 1981, 1983). Theoretically, it is the δD of the rain produced during the growing season (δDw) which should be recorded in the deer.

For each IAEA location in North America, the δDwy were calculated using monthly rain δD measurements weighted by PPT_m' (monthly amount of precipitation at each IAEA location). In order to eliminate seasonal bias in the average annual or growing season rain δD measurements, data were compiled only for the years that had complete sets (i.e. 12 months) of both rain δD and PPT_m observations. The yearly average δD values were again averaged over 1 to 7 years and weighted by the yearly average of monthly amount of rain to produce the δDwy values for each IAEA location. These IAEA δDwy computations differ from those calculated by Yurtsever and Gat (1981) in that they include data from more recent years, and exclude data from years with missing monthly data.

Figure 7.1 Contours of Annual Weighted Average of Precipitation δD (δDwy).

The δDwy calculated for each IAEA station are shown. δDwy represents the 12 month average of monthly rain δD weighted by the monthly amount of precipitation at the IAEA station (PPTm'). Contour shapes are based on Sheppard et al. (1969) and Taylor (1974).


170
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-150
-137
-75
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-128
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-93
-61
110
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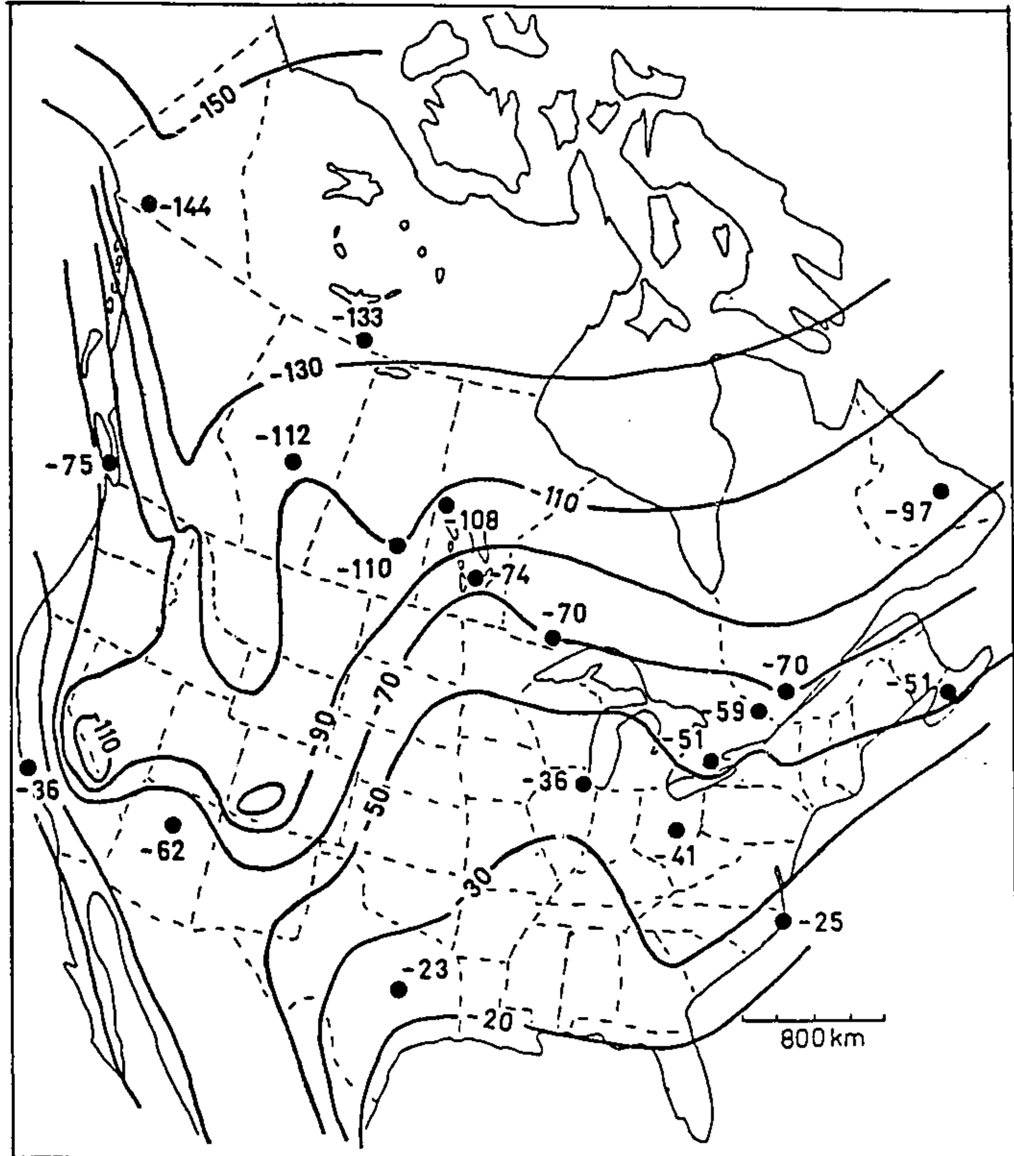
-75
-64
-36
90
-43
-69
-45
50
30
-25
-23
20
800km

Figure 7.2 Contours of Growing Season Weighted Average of Precipitation δD (δDw).

The δDw values calculated for each IAEA location are shown. δDw represents the weighted average of monthly precipitation δD calculated over the growing season months. The monthly amount of precipitation at the IAEA station (PPTm') was used as the weighing factor. The growing season is defined as the months of the year in which the monthly average temperature at each IAEA station (Tm') is greater than 0°C. Contour shapes are based on Sheppard et al. (1969) and Taylor (1974).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-133
-130
-112
-75
-110
110
-108
-74
-70
-97
-70
-59
-51
-51
110
-90
-70
-36
-50
-36
-62
-41
30
-25
-23
-20
800 km

In order to calculate δDw, the monthly δD values weighted by PPT_m' were averaged over only the growing season months for each IAEA location. These results were then averaged over all the years for which complete summer growing season data existed.

Both δDwy and δDw for the IAEA locations are shown in Fig. 7.1 and 7.2 respectively. Since there were few sites for which IAEA data were available, it was decided to complement these data with a set of δD contours so that δDwy and δDw values for each deer sampling location could be determined via interpolation between contours. It was found that some of the contours of Taylor (1974) or Sheppard et al. (1969) did not match the the IAEA δDwy values very well. Therefore the contours of Sheppard et al. and Taylor were re-drawn and shifted to pass through the known IAEA δDwy data (Fig. 7.1). Interpolation between these contours provided the δDwy estimates for each deer sampling location. Similarly, δDw contours were drawn that would pass through the IAEA values, and interpolation provided the δDw values for each sampling location (Fig. 7.2). Both δDw and δDwy values for each deer sampling location appear in Table 7.3 for comparison with the δDb values of this study. There is likely more uncertainty in the δDw determinations because they are averaged over a smaller number of months and there is considerable uncertainty in applying contour shapes to the growing season data.

7.4 Relationship Between Rain δD and Temperature in North America:

As discussed earlier, the δD of rain vs temperature relationship is an important one as it is through this relationship that δDb of bone is expected to reflect climate. In the following, this relationship for North American IAEA locations and for each sampling location will be examined.

The compilation of δDwy vs Ty for North American IAEA stations appears in Fig. 7.3a. In Fig. 7.3b are the values for δDwy interpolated from Fig. 7.1 for each deer sampling location. The Rayleigh curve (b) from Fig. 2.2 is included in Fig. 7.3a for comparison (dashed line) while the best fit relationship for the IAEA continental locations from Fig. 7.3a (solid line) is included in Fig. 7.3b for comparison. This best fit relationship for continental IAEA locations is given in the caption of Fig. 7.3a and excludes the outlier results for Edmonton. Plots of unweighted IAEA data (δDw'y, not shown) were similar to the weighted data of Fig. 7.3a. In Fig. 7.3a coastal data (open circles) is compared to interior continental locations (closed circles) while in Fig. 7.3b data from locations west of the Rocky Mountains (squares) and east of but near the Rockies (triangles) are compared to that from remaining locations (closed circles).

In Fig. 7.4, the weighted growing season (curve a) and winter (curve b) δD of rain is plotted vs temperature. The winter months δDw_6 and T_6 represent averages of IAEA monthly data from October through March. Plots using unweighted data were very similar to those using weighted data in curves a and b. Best fit regressions using both weighted and unweighted data from interior continental areas (excluding Edmonton) appear in the figure caption.

Figure 7.3a δDwy vs Ty Relationship for North American

IAEA Locations.

Relationship between weighted average annual precipitation δD (δDwy) for each IAEA location in North America and Average Annual Temperature (Ty). Total monthly amount of precipitation at the IAEA station (PPTm') was used as the weighing factor. Coastal locations are identified by open circles and interior, continental locations by closed circles. The Rayleigh curve (b) from Fig. 2.2 is included for comparison (dashed line).

Regression equations for continental locations (excluding Edmonton) are:

(eq. 7.3) $\delta Dwy = -120.7 + 9.204Ty(^{\circ}C) - 0.2113Ty^2(^{\circ}C)$; $n=14$, $r=0.952$

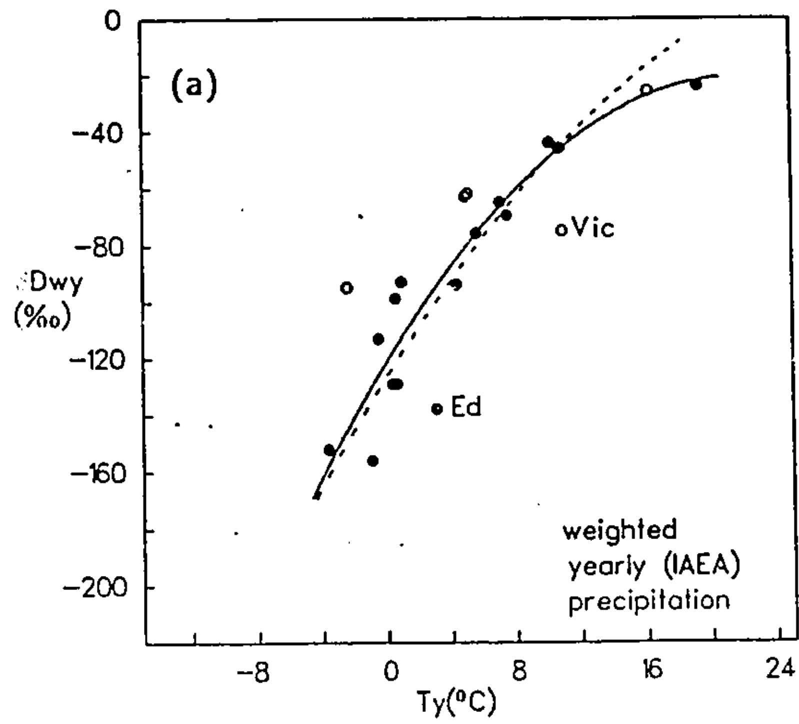
Plots of average unweighted data (δDw'y) vs Ty were similar (data not shown) and gave a best fit relationship of:

$\delta Dw'y = -132.0 + 10.85Ty(^{\circ}C) - 0.2595Ty^2(^{\circ}C)$; $n=14$, $r=0.97$

Figure 7.3b δDwy vs Ty Relationship for North American

Deer Sampling Locations.

Relationship between weighted average annual precipitation δD (δDwy) as determined from contours for each sampling location (Fig. 7.1) vs average yearly temperature (Ty) as determined from nearby weather stations. Areas from west of Rocky Mountains are identified by open squares, and east of but close to the Rockies by open triangles. Areas intermediate between the Rockies and east coast (i.e. SA, MI, NE) which may experience some influence from air masses originating west of the Rockies are identified by closed triangles. Precipitation in most interior continental sampling locations (with the exception of MUSK and OLD-C) likely derives from vapor clouds originating from subtropical Atlantic Ocean, Gulf of Mexico areas and data are identified by closed circles. The best fit relationship for the North American IAEA data from Fig. 7.3a (solid line eq. 7.3) is included for comparison.


(a)
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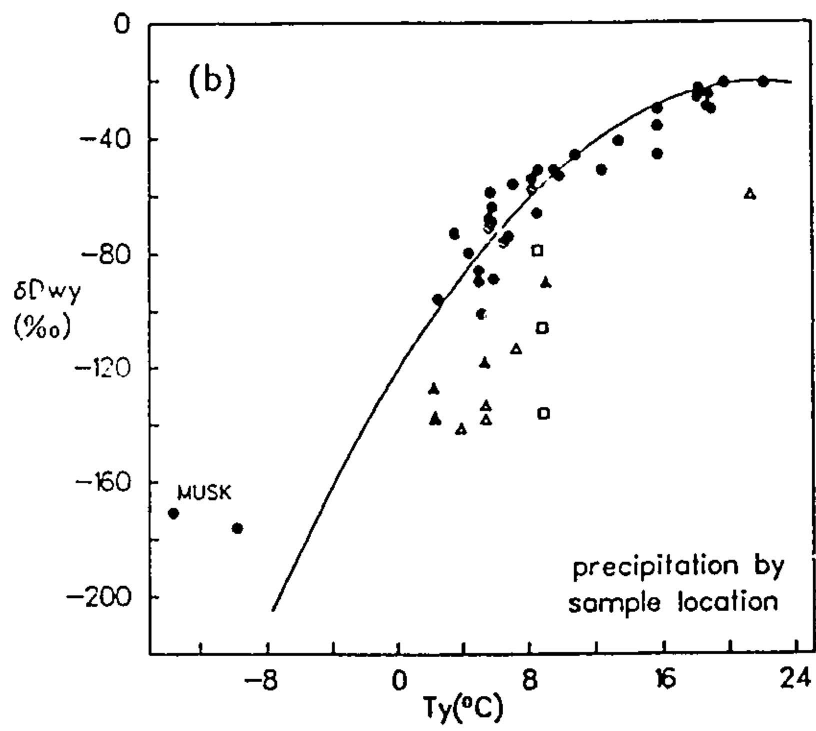
(b)
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-160
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δDwy
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precipitation by
sample location
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0
8
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24
Ty(°C)

Figure 7.4 Relationship Between Weighted Average of Growing Season (a) and Winter (b) Precipitation δD and Average Growing Season and Winter Temperatures from North American I.A.E.A. Stations.

The growing season data include the months of the year in which $T_m' > 0^{\circ}C$. October through March defines the winter months. Coastal and interior locations are indicated by open and filled circles respectively. The temperature is in degrees Celsius.

Regression Statistics:

Best fit relationships for continental locations excluding Edmonton are:

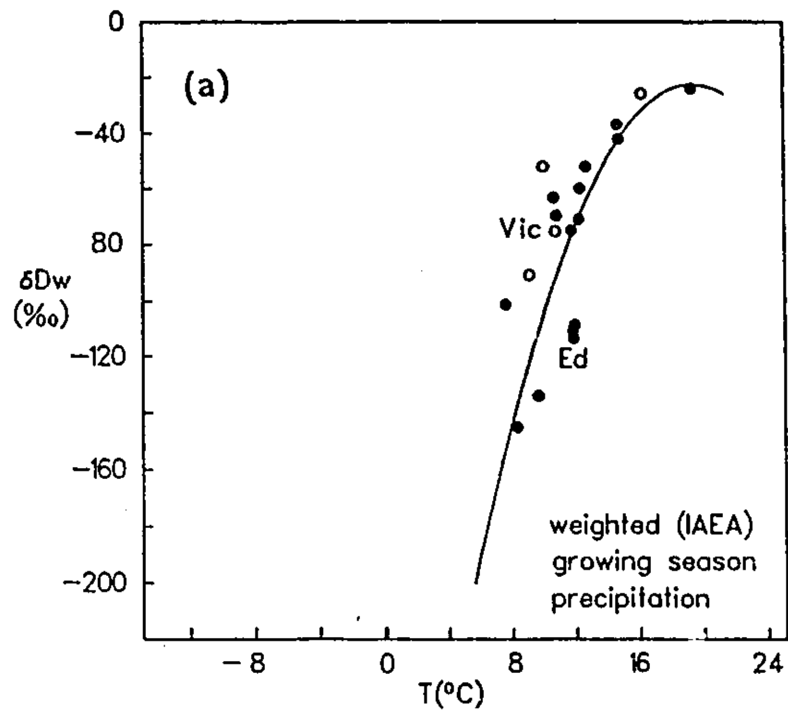
(a) growing season months (weighted):
$\delta D_w = -370 + 36.0T - 0.931T^2$; n=14, r=0.84

(b) winter months (weighted):
$\delta D_{w6} = -80 + 5.9T_6 - 0.115T_6^2$; n=14, r=0.98

Additional fits for the unweighted data were:

(c) growing season months (unweighted):
$\delta D_w' = -347 + 32.7T - 0.815T^2$; n=14, r=0.82

(d) winter months (unweighted):
$\delta D_{w'6} = -81 + 6.2T_6 - 0.123T_6^2$; n=14, r=0.91


(a)
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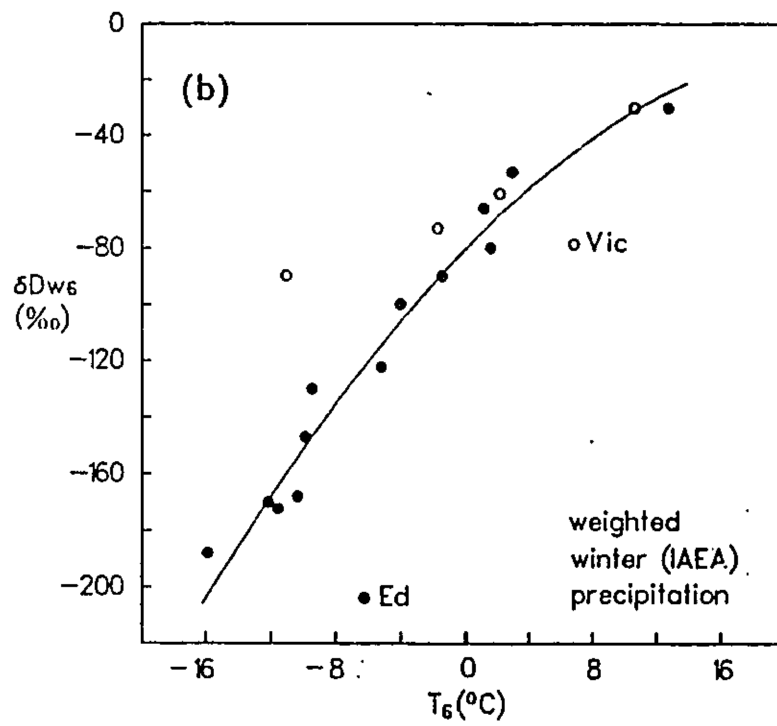
weighted (IAEA)
growing season
precipitation


(b)
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− 8
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16
T6(°C)
weighted
winter (IAEA)
precipitation

A number of generalizations may be made regarding these figures. The δDwy data of most interior continental locations agree well with values predicted by the Rayleigh curve (Fig. 7.3a). The MUSK location shows trends appropriate to its northern coastal location while OLD-C (near MUSK location) should also experience some coastal influences.

In the seasonal data of Fig. 7.4a and b, the inter-site slopes of rain δD vs temperature are considerably higher for the growing season and lower for the winter months compared to the yearly data. Both Edmonton (Ed) and Victoria (Vic) fall below the curve in the winter but are close to the curve during the growing season. Bethel, Alaska, a cold coastal location, also appears closer to the curve during the growing season while coastal Vic has moved from below the best fit curve (Fig. 7.4b) to above it (Fig. 7.4a).

The δDwy of North American areas both west and east of but close to the Rocky Mountains plot below the best fit curve calculated from the remaining North American locations (Fig. 7.3a,b, Fig. 7.4b). Most points east of, but close to, the Rockies plot between those west of the Rockies and the curve. This indicates different isotopic histories for air masses formed east and west of the Rockies and that mixing of rain derived from these two sources is a factor in areas east of but close to the Rockies (see also Hage et al. 1975; Friedman et al. 1964).

A number of factors could explain the phenomenon of lower δD_{wy} near the Rockies. In water vapor moving from the Pacific region over the Rocky Mountains, deep convection, altitude and rain shadow effects producing rain at cooler condensation temperatures (T') than surface temperatures (T_y, T, or T_6) and from clouds experiencing a high degree of rainout could produce the low δD values east of but close to the Rockies. Re-evaporation and recycling of continental rain, especially during the summer months and in air moving over the Rockies during the summer, could also produce low δD in Edmonton (Hage et al. 1975) and in other areas east of the Rockies (Siegenthaler and Oeschger 1980; Ch. 2). Mixing is also likely to occur between air masses originating from subtropical Atlantic and Pacific sources.

A summer maximum in convective rain could also produce low δD_{wy} in Edmonton (Hage et al. 1975). However, since Victoria and other areas in or west of the Rockies also have low δD_{wy} and these differences are most apparent during the winter (Fig. 7.4), it seems likely that vapor originating from the Pacific during the winter has a different isotopic history than vapor arising from the Atlantic. This difference may include evaporation of the original Pacific marine vapor at higher surface temperatures. There might also be a winter maximum in amount of rain, in deep convection and in cloud rainout with little re-equilibration or recycling of ocean water vapor in the Victoria or coastal areas and in vapor moving from the Pacific over the Rockies. Since snow likely records T' with little evaporation or equilibration with surface air, the lower winter δD values would remain unaltered. If amount of rain in the winter is low relative to summer months, this

could further produce a positive amount effect (see Ch. 2) in such cold interior areas as Edmonton and Chicago.

The discrepancies between yearly, summer and winter data (Figs. 7.3 and 7.4) could be caused by seasonal discrepancies between surface temperature and T' and a systematic bias in this discrepancy as one moves northward and into the interiors of the continents. Northern interior continental areas may have temperatures considerably hotter than T' and there may be greater amounts of convective rain in the summer. These same areas usually have T_6 values that are extremely cold relative to coastal locations. The upper air gradients in T' may not be as great between the coast and interior locations thus producing lesser amounts of rainout and less variation in δD of rain relative to that expected from the variation in T_6. Shifts in the curves could also be compounded by ocean surface evaporation at warmer temperatures in the summer and at cooler temperatures in the winter.

On the basis of the above, bone δDb_c (corrected for H-exchange and RH effects) could be used to interpret paleoclimate. First δDw or δDwy could be estimated from the bone and these values for a specific time period could be plotted vs modern T or Ty for each North American sampling location. Changes from modern patterns could be interpreted and plots for different time periods could be compared. This should provide a qualitative if not semi-quantitative evaluation of how global climate in North America has changed over time. For locations having δDwy falling along the best fit curve (eq. 7.3 of Fig. 7.3a) it may be possible to quantify changes in Ty that coincide with changes in δDwy between two time periods.

The yearly δDwy were examined for the intra-site relationship between rain δD and T over time since such a relationship would be pertinent when attempting to determine changes in climate from the δD record in bone for a long sequence within the same location. There are no locations in North America for which a sufficiently long record of IAEA δDwy exists to test the above. For the 12 locations with three to seven years of IAEA data, poor correlations were found between δDwy and Ty (data not shown). Slopes varied from -32.0 for Goose Bay to +9.4 for Ottawa and correlation coefficients varied from 0.01 for Waco to 0.788 for Ottawa and -0.981 for Goose Bay. Negative slopes may indicate areas where precipitation amount effects or seasonal biases of cloud trajectories dominate.

In contrast, the few examples in the literature of 5 year averages of tree ring δD_{cel} values vs measured Ty for single locations show rather high temperature coefficients and good linear relationships; in Scotland: m=22.8‰/°C, r=0.91 (Epstein and Yapp 1976) and in Edmonton: (1) m=7.3‰/°C, r=0.80 (2) m=13‰/°C, r=0.95 (3) m=15‰/°C, r=0.93 (Gray and Song 1984). In Edmonton the temperature coefficients were determined from three different trees and differed depending on where the tree grew, reflecting source water effects. Epstein and Yapp (1976) also found good qualitative agreement between δD_{cel} and Ty using 10-year intervals of a bristlecone pine from California. In none of these studies were the δD_{cel} values corrected for the effects of RH.

In Table 7.2 appear the δDwy vs Ty that might be expected at each IAEA location based on the Rayleigh relationship (Fig. 2.2 curve b)

Table 7.2 Intra-site δD of Rain vs Temperature Coefficients Determined from Monthly Empirical Data (IAEA) and from Inter-site δDwy vs Ty Relationships.

IAEA location	Ty (°C)	yearly theoretical intra-site Rayleigh[a] (δDwy/°C)	yearly theoretical intra-site IAEA[b] (δDwy/°C)	monthly empirical intra-site IAEA[c] (δDwm/°C)	r	n
Continental:						
Waco	19	3.7	1.1	0.8	0.37	84
Chicago	10	6.2	4.9	2.3	0.85	60
Flagstaff	7	6.9	6.1	2.7	0.69	24
Simcoe	7	7.0	6.2	3.3	0.82	24
Ottawa	6	7.4	6.8	2.5	0.82	60
Ste. Agathe	4	7.8	7.0	2.9	0.86	36
Edmonton	3	8.1	7.9	3.9	0.88	36
Atikokan	1	8.7	8.8	4.0	0.92	48
Gimli	1	8.8	9.0	3.8	0.96	12
Wynyard	0	8.9	9.0	2.9	0.85	24
Goose Bay	-1	9.1	9.4	1.9	0.78	36
The Pas	-1	9.2	9.5	3.4	0.94	12
Whitehorse	-1	9.2	9.5	1.4	0.54	48
Fort Smith	-4	10.0	10.7	2.6	0.90	24
Coastal:						
Hatteras	16	4.5	2.4	0.4	0.24	60
Victoria	11	6.0	4.6	0.9	0.34	36
Truro	5	7.6	7.0	2.4	0.78	24
Adak	5	7.6	7.1	-0.1	0.02	36
Bethel	-2	9.6	10.2	1.1	0.51	48

a estimates of intra-site temperature coefficients (δDwy/°C) using the Rayleigh relation (eq. for curve b, Fig. 2.2 caption)

b estimates of intra-site temperature coefficients (δDwy/°C) using the best fit relationship for North American IAEA precipitation data (Fig. 7.3a, eq. 7.3).

c empirical intra-site monthly temperature coefficient (δDwm/°C) for North American IAEA locations.

or from the best fit relationship to the IAEA continental data (Fig. 7.3a, eq. 7.3). As indicated previously, higher slopes are expected for colder areas and, within a restricted range of Ty, the relationships of δDwy to Ty should be approximately linear.

Due to the paucity of yearly intra-site data for each IAEA location, plots of the the monthly rain data (δDwm) vs Tm' were examined (plots not shown). The temperature coefficients of these relationships are given in Table 7.2 and were found to be approximately linear and to increase in colder areas. Precipitation amount effects were found to be negative in warm areas and positive in cold areas. However, all slopes relating δDwm to T_m' are low. A comparison of Fig. 7.4a to 7.4b illustrates why the slopes of the monthly data are lower than the δDwy vs Ty slopes predicted from the Rayleigh or IAEA yearly data which are also given in Table 7.2. A comparison of the growing season to the winter season curves shows that although there are large between season shifts in temperature there are only small changes in rain δD. Due to this seasonal bias, the monthly data are not particularly useful for predicting what an intra-site relationship between δDb_c and Ty over a number of years might look like.

A thorough study of intra-site δDwy vs Ty coefficients calculated over long periods of time for numerous locations using either tree ring cellulose or bone data corrected for the effects of RH would be useful prior to any future attempts at evaluating changes in climate over time for a given location. Use of a material such as bone might further reduce the problems of source water effects found in the tree ring data.

7.5 Analytical Data and Environmental Parameters for North American White Tailed Deer

In Tables 7.3, 7.4, 7.5 appear all the analytical data for 75 deer along with their associated climatic and δD of rain determinations used in this work. Results will be interpreted in Ch. 8 through 10. In Table 7.3 are given δDb, δDb_c, δDw, δDwy and the hydrogen yields. Averages of results for a given location are provided. Results are organized according to descending δDw values. Also included are the δDv values necessary for each δDb_c computation and averages of δDb and δDb_c for each sampling location. The δDb results of LA-8 and ND-1 were estimated from their δDg results using eq. 6.13 (Table 6.5). Also included in the table for comparison are results on MUSK, BIS and OLD-C.

Two additional calculated values appear in Table 7.3 (δDb_{c100}, δDwy^) which will be described fully in the next chapter. δDb_{c100} consists of the δDb_c results which have been corrected for the effects of RH at the growing site by subtracting the enrichment in δDb_c due to evaporation which occurs when RH<100. δDwy^ are the estimates of δDwy (yearly average of monthly δD of rain) derived from a multilinear regression model which includes δDb_c plus other laboratory measures of deer bone.

The $\delta^{13}Cb$ and $\delta^{15}Nb$ results are given in Table 7.4 along with the carbon and nitrogen yields. Averages of results for each location are also provided. The percentage of C_4 plants consumed by the deer have been calculated through linear extrapolation and assumes $\delta^{13}Cg$ (of gel) end point values of -21.5‰ for animals consuming 100% C_3 plants

Table 7.3 δDb of Modern Deer from Across North America and Estimated δDwy of Local Precipitation (δDwy^).

	Sample δD						Precipitation δD				Yields		
deer[a]	δDb	location average	δDv	$δDb_c$	location average	$δDb_{c100}$[f]	δDw	δDwy	δDwy^[g]	δDwy^[h]	H/N	H/C	H/g
TX-2	-14		-130	16		-2	-20	-20			89	96	67
TX-3(t)	-21	-19±6	-153	13	-13±4	-5	-20	-20	-17	-19	85	84	71
TX-5(m)	-25		-155	8		-10	-20	-20			88	85	76
FL-1	-29		-150	2		-18	-20	-20	-29	-28	90	88	74
AL-1	-34		-171	1		-21	-22	-22	-31	-	88	86	41
MS-1	-28		-168	8		-13	-23	-25	-23	-	91	92	74
LA-2	-33		-150	-3		-24	-24	-20	-37	-38	89	91	73
TX-6	-22		-150	12		-12	-28	-28	-24	-	90	86	69
OK-1	-23	-25±2	-153	10	8±3	-14	-29	-29	-16	-16	90	86	67
OK-2	-26		-153	6		-18	-29	-29			89	83	67
[c]TX-1	-36[c]		-153[c]	-6		-34	-29	-29	-30	-39	89	96	67
MO-4	-40		-153	-10		-33	-30	-40	-42	-42	89	88	73
OK-9	-21	-25±6	-130	7	3±6	-17	-32	-35	-27	-27	86	97	66
OK-10	-29		-139	-1		-25	-36	-35			81	85	58
WV-1	-43		-153	-15		-35	-36	-52			97	91	57
WV-2	-44	-42±2	-153	-16	-14±3	-36	-36	-52	-51	-47	93	88	62
WV-3	-40		-153	-11		-31	-36	-52			94	94	64
OK-5	-28		-130	-1		-25	-37	-45	-47	-45	90	93	64
[c]KS-1	-40[c]		-153[c]	-11		-33	-38	-50	-37	-37	85	97	65
OH-3	-34		-153	-4		-28	-40	-45			89	89	60
OH-2	-36	-36±3	-153	-6	-7±3	-30	-40	-45	-40	-38	90	88	63
OH-1	-39		-153	-10		-34	-40	-45			93	90	64
MI-11	-46	-48±2	-159	-18	-20±2	-39	-45	-50	-57	-	95	90	78
MI-5	-49		-159	-21		-42	-45	-50			86	94	64
MI-12	-54	-55±1	-159	-27	-28±1	-50	-47	-50	-66	-	92	92	69
MI-13	-55		-159	-28		-51	-47	-50			92	96	77
MI-1(t)	-42	-47±6	-123	-21	-27±8[i]	-44	-48	-53			76[i]	80	58[i]
MI-15(m)	-51		-123	-32		-55	-48	-53	-61	-	85	95	75
MI-2	-49		-123	-27		-48	-48	-65			88	93	66
MI-10	-54	-53±4	-159	-27	-29±2	-48	-48	-65	-66	-	93	94	74
MI-14	-51		-123	-29		-50	-48	-65			86	90	61
MI-8	-58		-159	-32		-53	-48	-65			93	90	65
[c]NS-1	-59[c]		-153[c]	-34		-47	-49	-55	-66	-65	94	92	58
MI-4	-54		-123	-36		-59	-49	-57	-73	-	86	90	65
MI-9	-53		-159	-26		-48	-50	-70	-65	-	83	91	68
MI-6	-51		-159	-23		-45	-50	-67	-66	-	88	93	58
NS-2	-56		-155	-31		-44	-53	-58	-70	-70	89	82	66
WI-1	-64		-159	-39		-60	-53	-75	-85	-81	93	84	54
MI-3	-62		-123	-46		-64	-53	-79	-86	-	88	92	60
ON-1	-59		-168	-31		-48	-55	-73	-71	-68	94	90	71
[c]NB-1	-61[c]		-153[c]	-37		-56	-55	-63	-66	-64	90	94	67
AZ-1	-33		-150	-3		-51	-58	-59	-46	-56	88	86	62
[c]ON-3	-66[c]		-153[c]	-43		-61	-58	-68	-69	-67	87	91	75
[c]ON-6c	-64[c]		-153[c]	-41		-62	-61	-72			89	94	67
[c]ON-6b	-66[c]	-66±2	-153[c]	-44	-43±2	-65	-61	-72	-73	-69	89	91	66
[c]ON-6a	-67[c]		-153[c]	-45		-66	-61	-72			92	100	73

Table 7.3 (cont)

	Sample δD						Precipitation δD				Yields		
deer[a]	δDb	location average	δDv	δDb_c	location average	δDb_{c100}[f]	δDw	δDwy	δDwy^[g]	δDwy^[h]	H/N	H/C	H/g
QC-2a	-63	-64±1	-123	-39	-40±1	-57	-63	-85	-77	-	91	92	83
QC-2b	-65		-159	-41		-59	-63	-85			91	96	76
QC-1a	-73	-75±2	-153	-52	-54±2	-70	-66	-89	-94	-95	77	77	57
QC-1b	-75		-159	-53		-71	-66	-89			97	99	60
QC-1c	-77		-159	-56		-75	-66	-89			90	90	78
QC-8	-62		-150	-39		-60	-67	-88	-85	-	105	107	63
ON-8	-75		-150	-56		-82	-69	-95	-98	-	88	98	67
NE-2	-53		-150	-28		-59	-70	-89	-70	-76	88	96	64
QC-3a	-39	-44±1	-130	-16	-18±3	-31	-73	-86	-	-	92	96	73
QC-3b	-49		-159	-20		-35	-73	-86	-	-	-	99	-
[b]BC-4	-71		-153	-50		-62	-77	-78	-99	-97	97	84	70
[b]OR-1	-72		-159	-49		-91	-97	-105	-90	-99	90	87	77
WT-2	-80		-159	-59		-91	-91	-112	-104	-105	90	83	64
MT-1	-74		-153	-54		-87	-96	-117	-130	-129	90	83	64
AB-6	-91		-130	-81		-113	-107	-132	-	-	88	91	75
SA-1	-72		-153	-51		-81	-110	-126	-110	-109	99	85	66
AB-5(R.t.)	-85	-87±3	-153	-68	-71±4	-102	-112	-137	-137	-133	90	82	61
AB-4(L.t.)	-89		-159	-71		-105	-112	-137			90	85	60
AB-4b(L.t.)	-90		-150	-74		-108	-112	-137			91	90	70
AB-2	-93	-95±2	-153	-78	-80±3	-102	-112	-137	-130	-131	96	77	54
AB-3	-95		-153	-80		-104	-112	-137			96	93	74
AB-1	-97		-153	-83		-107	-112	-137			98	90	65
AB-7b	-88	-92±5	-159	-70	-73±4	-104	-116	-140	-130	-120	93	100	54
AB-7a	-92		-171	-72		-106	-116	-140			88	92	62
AB-7c	-97		-171	-77		-111	-116	-140			92	88	66
[b]BC-3(L.t.)	-103	-105±2	-159	-88	-91±4	-119	-120	-135	-143	-146	89	97	70
[b]BC-2(R.t.)	-106		-159	-93		-124	-120	-135			92	88	66
[d]LA-8	-34[d]		-171	1		-20	-22	-24	-	-	100[d]	99[d]	95[d]
[d]ND-1	-68[d]		-171	-41		-68	-75	-100	-	-	92[d]	95[d]	76[d]
Others													
MUSK(n=7)	-139		-153	-136		-147	-153	-170			91±3	93±4	76±4
BIS(n=6)	-67		-153	-45		-71	-109	-136			93±10	98±7	54±6
[e]OLD-C(n=2)	-118[e]		-153[e]	-110[e]		-134	-153	-175	-	-	-	79±1	65±2

a t=tibia, m=metatarsal of same animal. Samples indicated by a, b, c are replicate preparations. All samples are of white tailed deer unless indicated otherwise. See text Ch. 8 for additional explanations.

b samples are of mule deer.

c samples were corrected for effects of sample preparation by adding +23‰ (Ch.6, App. D.)

d results were for gelatin samples. δDb results were estimated from δDg using eq.6.3 (Table 6.3).

e low δDv (-373‰) indicated sample outgassing problems (Prep 1P, 1Q, Ch. 5,6). The raw δDb value (-162‰) was corrected using eq. 5.2

f δDb corrected for H-exchange and effects of RH. $\delta Db_{c100} = \delta Db_c - 0.77\cdot(100 - RH)$

g $\delta Dwy\hat{} = -35.6205 + 1.5197\cdot\delta Db - 0.4968\cdot(\delta^{15}N)^2 - 0.1261\cdot\delta Dv + 0.3892\cdot(C/g) + 2.5241\cdot\delta^{15}N$

h $\delta Dwy\hat{} = -71.007 + 1.4551\cdot\delta Db + 0.6164\cdot RH\hat{} - 0.1168\cdot\delta Dv - 0.1960\cdot(\delta^{15}N)^2 + 0.3174\cdot(C/g)$; with:

$RH\hat{} = 83.4074 - 0.25153\cdot[-12.0077 + 0.975587\cdot\delta^{18}Ob - 0.1312\cdot\delta Db_c + 0.0266\cdot(\delta^{15}N)^2 + 0.0072\cdot\delta Dv]^2$

i low H-yield suggests faulty preparation for MI-1

Table 7.4 $\delta^{13}C$, $\delta^{15}N$ and Yields of Modern Deer from Across North America.

	Isotopes					Yields		
	$\delta^{13}C$			$\delta^{15}N$				
deer[a]	$\delta^{13}C_b$	location ave	$\%C_4$[f]	$\delta^{15}N_b$	location ave	C/N	C/g	N/g
TX-2	-20.0		6	4.4		92	69	75
TX-3(t)	-20.2	-20.4±0.5	5	6.6	5.4±1.4	102	84	83
TX-5(s)	-21.2		0	6.2		104	90	86
FL-1	-21.8		0	5.1		102	83	81
AL-1	-22.2		0	4.5		102	87	86
MS-1	-23.1		0	2.7		99	80	81
LA-2	-22.0		0	5.7		98	81	82
TX-6	-16.2		34	6.7		105	80	77
OK-1	-21.2		0	4.1	3.4±1.0	105	78	74
OK-2	-			2.6		107	81	75
[c]TX-1	-17.7		23	5.2[c]		93	70	75
MO-4	-17.7		23	3.0		101	83	82
OK-9	-22.4	-22.6±0.3	0	3.7	3.5±0.4	89	68	77
OK-10	-22.8		0	3.2		95	68	72
WV-1	-18.3		19	2.3		108	63	58
WV-2	-20.0	-18.6±1.3	6	3.6	2.8±0.7	106	70	66
WV-3	-17.5		24	2.6		100	68	68
OK-5	-20.7		1	8.0		97	69	71
[c]KS-1	-21.2		0	5.2[c]		88	67	76
OH-3	-25.9		0	2.6		103	70	68
[d]OH-2	-13.3[d]	-24.7±1.7	54[d]	-	2.2±0.6	103	72	69
OH-1	-23.5		0	1.8		103	71	69
MI-11	-17.2	-18.0±1.1	26	4.1	3.9±0.4	97	80	92
MI-5	-18.8		15	3.6		92	69	75
MI-12	-18.3	-19.9±2.2	19	3.5	3.0±0.7	100	75	75
MI-13	-21.4		0	2.5		96	80	84
MI-1(t)	-18.3	-18.2±0.1	19	4.1	4.2±0.1	95	72	76
MI-15(s)	-18.1		20	4.2		90	71	84
MI-2	-22.4		0	3.2		95	71	75
MI-10	-20.4	-21.8±1.0	4	3.5	3.7±0.6	99	79	80
MI-14	-21.6		0	4.5		97	68	71
MI-8	-22.7		0	3.5		103	72	71
[c]NS-1	-21.8		0	4.5[c]		102	63	62
MI-4	-18.6		16	4.5		95	72	76
MI-9	-21.4		0	4.0		92	75	81
MI-6	-20.8		1	3.1		95	62	66
NS-2	-22.3		0	5.2		108	81	74
WI-1	-19.9		7	1.6		111	64	58
MI-3	-21.9		0	3.9		96	66	69
ON-1	-21.3		0	3.6		104	79	76
[c]NB-1	-21.4		0	4.8[c]		96	71	74
AZ-1	-		-	-2.9		102	72	71

Table 7.4 (cont)

	--------Isotopes--------					--------Yields--------		
	$\delta^{13}C$			$\delta^{15}N$				
deer[a]	$\delta^{13}C_b$	location ave	$\%C_4$[f]	$\delta^{15}N_b$	location ave	C/N	C/g	N/g
[c]ON-3	-22.7		0	4.6[c]		96	83	86
[c]ON-6c	-		-	3.4[c]		94	71	75
[c]ON-6b	-22.5		0	3.5[c]	3.2±0.2	98	72	74
[c]ON-6a	-		-	3.0[c]		92	73	79
QC-2a	-22.9	-22.5±0.6	0	1.9	0.8±0.1	99	91	92
QC-2b	-22.1		0	1.7		95	79	94
QC-1a	-22.9		0	2.9		100	96	96
QC-1b	-24.3	-23.4±0.8	0	3.2	3.1±0.2	98	91	93
QC-1c	-23.1		0	-		100	96	86
QC-8	-18.7		16	1.7		98	59	60
ON-8	-22.4		0	2.0		100	76	76
NE-2	-19.9		7	5.7		103	74	73
QC-3a	-18.7	-18.6±0.2	16	5.7	5.8±0.1	85	76	89
QC-3b	-18.4		18	5.8		-	-	-
[b]BC-4	-21.0		0	2.0		104	84	81
[b]OR-1	-19.9		7	4.8		104	99	86
WY-2	-20.5		3	2.9		108	77	71
MT-1	-16.4		32	10.9		108	77	71
AB-6	-20.2		5	-		97	82	85
SA-1	-21.3		0	8.5		106	78	74
AB-5(R.t.)	-18.5		17	9.1		109	74	68
AB-4a(L.t.)	-	-18.4±0.1	-	9.0	9.0±0.1	106	70	67
AB-4b(L.t.)	-18.3		19	8.7		101	77	77
AB-2	-22.3		0	4.0		112	69	62
AB-3	-21.3	-21.8±0.5	0	5.0	4.8±0.8	103	96	93
AB-1	-21.9		0	5.5		110	81	74
AB-7b	-		-	5.6		93	53	58
AB-7a	-21.1	-21.0±0.2	0	5.4	5.5±0.1	96	68	71
AB-7c	-20.9		0	5.4		104	74	72
[b]BC-3(L.t.)	-19.9	-19.8±0.1	7	2.2	2.3±0.1	92	72	79
[b]BC-2(R.t.)	-19.7		9	2.3		104	75	72
[e]LA-8	-24.5[e]		0	4.6[e]		101[e]	85[e]	85[e]
[e]ND-1	-20.6[e]		6	9.1[e]		97[e]	79[e]	82[e]
others								
MUSK (n=11)	-21.7±0.8		0	4.8±0.3		104±2	93±2	89±3
BIS (n=1)	-15.6		42	5.9±0.2		99	72	73
OLD-C (n=2)	-19.4±0.1		11	-		-	82±3	-

a t=tibia, m=metatarsal of same animal. Samples indicated by a, b, c are replicate preparations. All samples are of white tailed deer unless indicated otherwise.

b samples are of mule deer

c $\delta^{15}N$ calibrated for effects of sample preparation (App. D).

d deer raised in captivity and fed corn (App.8).

e results are for gelatin samples.

f $\%C_4 = (\delta^{13}Cg' + 21.5)/0.14$; $\delta^{13}Cg' = \delta^{13}Cb - 0.6‰$

Table 7.5 Climatic Data for Each Sampling Location

	growing season			yearly			
deer	RH(%)	PPT(cm/mo)	T(°C)	RHy(%)	PPTy(cm/mo)	Ty(°C)	weather station
TX-2	77	6.04	22.2	77	6.04	22.2	Corpus Christi, TX
TX-3(t)	77	6.04	22.2	77	6.04	22.2	Corpus Christi, TX
TX-5(m)	77	6.04	22.2	77	6.04	22.2	Corpus Christi, TX
FL-1	74	13.03	19.8	74	13.03	19.8	Talahassee. FL
AL-1	71	10.55	18.2	71	10.55	18.2	Montgomery, AL
MS-1	72	10.92	18.1	72	10.92	18.1	Meridan, MS
LA-2	73	9.44	18.8	73	9.47	18.8	Shreveport, LA
TX-6	69	6.84	18.7	69	6.84	18.7	Dallas-Fort Worth. TX
OK-1	69	7.81	15.7	69	7.81	15.7	Tulsa. OK
OK-2	69	7.81	15.7	69	7.81	15.7	Tulsa. OK
TX-1	63	3.71	19.0	63	3.71	19.0	San Angelo. TX
MO-4	70	8.40	13.4	70	8.40	13.4	Springfield. MO
OK-9	69	7.81	15.7	69	7.81	15.7	Tulsa. OK
OK-10	69	7.81	15.7	69	7.81	15.7	Tulsa. OK
WV-1	74	9.53	13.2	74	9.15	9.8	Huntington. WV
WV-2	74	9.53	13.2	74	9.15	9.8	Huntington. WV
WV-3	74	9.53	13.2	74	9.15	9.8	Huntington, WV
OK-5	69	7.81	15.7	69	7.81	15.7	Tulsa. OK
KS-1	71	8.18	15.1	71	7.34	12.4	Topeka. KS.
OH-3	69	8.31	14.8	70	7.83	10.8	Columbus. OH
OH-2	69	8.31	14.8	70	7.83	10.8	Columbus, OH
OH-1	69	8.31	14.8	70	7.83	10.8	Columbus. OH
MI-11	73	7.01	12.8	75	6.43	8.6	Lansing, MI
MI-5	73	7.01	12.8	75	6.43	8.6	Lansing, MI
MI-12	70	7.26	13.7	71	6.71	9.5	Detroit. MI
MI-13	70	7.26	13.7	71	6.71	9.5	Detroit. MI
MI-1(t)	70	7.01	12.4	72	6.30	8.2	Flint, MI
MI-15(m)	70	7.01	12.4	72	6.30	8.2	Flint, MI
MI-2	72	7.21	12.5	73	6.67	8.5	Muskegon, MI
MI-10	72	7.21	12.5	73	6.67	8.5	Muskegon, MI
MI-14	72	7.21	12.5	73	6.67	8.5	Muskegon, MI
MI-8	72	7.21	12.5	73	6.67	8.5	Muskegon, MI
NS-1	83	4.99	9.2	83	5.08	7.1	Yarmouth. NS
MI-4	70	7.01	12.4	72	6.30	8.2	Flint. MI
MI-9	71	5.84	11.4	72	5.84	5.6	Alpena, MI
MI-6	71	6.63	11.4	72	5.84	5.6	Alpena, MI
NS-2	83	5.02	10.5	84	5.12	5.7	Sydney, NS
WI-1	72	6.99	12.9	73	5.72	6.5	Green Bay, WI
MI-3	76	7.70	10.5	76	6.70	4.4	Sault Ste. Marie. MI
ON-1	78	3.10	11.2	81	3.25	6.8	Trenton, ON
NB-1	73	4.79	11.7	77	4.49	5.8	Fredricton. NB
AZ-1	37	1.49	21.3	37	1.49	21.3	Phoenix. AZ

Table 7.5 (cont)

deer	growing season RH(%)	PPT(cm/mo)	T(°C)	yearly RHy(%)	PPTy(cm/mo)	Ty(°C)	weather station
ON-3	77	2.80	11.8	81	3.06	5.8	Peterborough, ON
ON-6c	72	3.97	11.9	79	3.64	3.5	Sudbury, ON
ON-6b	72	3.97	11.9	79	3.64	3.5	Sudbury, ON
ON-6a	72	3.97	11.9	79	3.64	3.5	Sudbury, ON
QC-2a	77	4.58	11.6	79	4.66	5	Mirabel. Montreal, QC
QC-2b	77	4.58	11.6	79	4.66	5	Mirabel. Montreal, QC
QC-1a	77	4.58	11.6	79	4.66	5	Mirabel. Montreal, QC
QC-1b	77	4.58	11.6	79	4.66	5	Mirabel. Montreal. QC
QC-1c	77	4.58	11.6	79	4.66	5	Mirabel. Montreal. QC
QC-8	73	3.41	12.5	77	3.63	5.9	Ottawa. ON
ON-8	66	3.98	12.2	73	3.69	2.5	Kenora. ON
NE-2	59	3.81	12.8	60	3.03	9	Scottebluff, NE
QC-3a	80	4.11	7.9	82	4.30	2	Baie Comeau, QC
QC-3b	80	4.11	7.9	82	4.30	2	Baie Comeau. QC
BC-4	84	7.11	8.5	84	7.11	8.5	Sandspit, BC
OR-1	45	1.68	10.9	49	1.79	8.8	Winnemucca. NV
WY-2	58	4.10	12.5	60	3.42	7.2	Sheridan. WY
MT-1	57	3.30	14.1	63	2.30	5.3	Glasgow, MT
AB-6	58	2.28	11.7	66	2.17	5.4	Medicine Hat. AB
SA-1	61	2.71	12.1	71	2.52	2.2	Saskatoon, SA
AB-5(R.t.)	56	3.31	12.5	65	2.81	5.4	Lethbridge, AB
AB-4a(L.t.)	56	3.31	12.5	65	2.81	5.4	Lethbridge, AB
AB-4b(L.t.)	56	3.31	12.5	65	2.81	5.4	Lethbridge, AB
AB-2	69	5.40	10.6	75	4.55	2.3	Edmonton, AB
AB-3	69	5.40	10.6	75	4.55	2.3	Edmonton. AB
AB-1	69	5.40	10.6	75	4.55	2.3	Edmonton, AB
AB-7b	56	3.10	11.0	62	2.96	3.9	Calgary, AB
AB-7a	56	3.10	11.0	62	2.96	3.9	Calgary, AB
AB-7c	56	3.10	11.0	62	2.96	3.9	Calgary, AB
BC-3(L.t.)	60	1.52	11.5	64	1.53	8.9	Kamloops, BC
BC-2(R.t.)	60	1.52	11.5	64	1.53	8.9	Kamloops, BC
LA-8	73	9.47	18.8	73	9.47	18.8	Shreveport, LA
ND-1	65	3.42	15.8	68	4.88	5.2	Bismark. ND
others:							
MUSK+SEAL	85	1.43	4.2	94	1.28	-13.5	Sachs Harbour. NWT
BIS	66	4.00	10.5	73	3.68	2.3	Red Deer. AB
OLD-C	69	1.72	9.8	81	1.32	-8.9	Inuvik. YK

and $-7.5‰$ for 100% C_4 plants. $\delta^{13}Cg'$ values used in the calculation represent $\delta^{13}Cb$ values adjusted for CO_2 contribution from CO_3^{2-} content ($\delta^{13}Cg'=\delta^{13}Cb-0.6‰$).

All the growing season and yearly climatic data are listed for each sample in Table 7.5. The weather stations from which the data were computed are also given.

CHAPTER 8

THE RELATIONSHIP OF δDb TO ENVIRONMENTAL PARAMETERS

In Ch. 1,2 and 7 it was shown that δDb_c is not likely to form a direct relationship with temperature due to the interfering effects of RH and the fact that the relationship between rain δD and temperature is curvilinear. For this reason rain δD is estimated from bone before making inferences about climate. In this chapter the relationship between δDb_c of deer w.b. samples (corrected for H-exchange) and δDw will be examined to determine whether δDw has a strong effect on bone δD so that rain δD can be estimated from bone δD. Next the relationship between δDb_c and δDl will be examined to determine whether, after RH effects are taken into account, the relationship is linear with a 1.0 slope in accordance with theoretical predictions. If it is, then a number of important generalizations can be made. First, the effect of RH on δDb_c is simple, it is accurately reflected in the leaf water model and there are no additional factors which produce inter-site bias in δDb_c. Second, several generalizations used in the leaf-H_2O models are likely to accurately reflect nature (a) $^2\epsilon k \approx$constant for all plants and locations (see Ch. 2), (b) rain water and atmospheric water vapor are in equilibrium so that $^2\epsilon e \approx (\delta Dw - \delta Dv)$ in each location and (c) $^2\epsilon e$ is relatively insensitive to T. Third, the assumption that deer eat small plants which reflect δDw rather than ground water δD values is

likely to be correct. Fourth, $^{2}\epsilon_{B}$ in plants and deer is approximately constant for all locations (see Ch. 2). Fifth, the p_{EX} value for w.b. samples has been accurately estimated and there are no additional slowly exchanging hydrogens. If the application of leaf-H_2O models to deer results shows that the above conditions applied during uptake and incorporation of hydrogen into bone collagen then the dependence of δDb_c on δDw and RH should be expressed by a simple trivariate linear regression relationship as predicted by eq. 2.3 and the δDw coefficient of this relationship should be ≈1.0.

Next, the relationship between δDb_c and both δDw and RH will be examined. If there is a good agreement between the experimental results and theoretical predictions, then the above assumptions will be further validated and RH should be a good estimate of growing season RH during the photosynthesizing hours (see Ch.7). Finally, using multilinear regression, it will be determined whether bone δD can be used to accurately estimate rain δD.

8.1 δDb_c Relationship to δDw:

It is evident from the isotopic results given in Table 7.3 (Ch. 7) that bone δD, with or without correction for H-exchange, follow the trends expected from δDw in that they also appear in the table ordered from highest to lowest values. In Table 8.1, the range in data for (a) different individual deer from the same area (≈5‰), (b) different bones from the same animal (≈5‰) and (c) replicate preparations of the same samples prepared at different times (≈4‰) indicates that most variability is due to replicate sample preparation, analysis, H-exchange and

Table 8.1 Spread in δDb$_c$ Analyses: (a) Same Location, (b) Same Animal and (c) Same Sample.

(a) same location

sample	δDb$_c$	range
TX-2	16	5
TX-3,5	11	
OK-1	10	4
OK-2	6	
OK-9	7	8
OK-10	1	
OH-3	-4	
OH-2	-6	6
OH-1	-10	
WV-3	-11	
WV-1	-15	5
WV-2	-16	
MI-11	-18	3
MI-5	-21	
MI-12	-27	1
MI-13	-28	
MI-2	-27	
MI-10	-27	5
MI-14	-29	
MI-8	-32	
AB-2	-78	
AB-3	-80	5
AB-1	-83	
	ave:	5

(b) same animal

sample	δDb$_c$	range
TX-3(tib)	13	5
TX-5(met)	8	
MI-1(tib)	-21[a]	11[x]
MI-15(met)	-32	
BC-3(L.tib)	-88	5
BC-2(R.tib)	-93	
AB-5(R.tib)	-68	5
AB-4(L.tib)	-73	
	ave:	5

(c) same sample
-replicate sample preparation-

sample	δDb$_c$	range
QC-3a	-16	4
QC-3b	-20	
QC-2a	-39	2
QC-2b	-41	
ON-6	-41	
ON-6	-44	4
ON-6	-45	
QC-1a	-52	
QC-1b	-53	4
QC-1c	-56	
AB-4a	-71	3
AB-4b	-74	
AB-7b	-70	
AB-7a	-72	7
AB-7c	-77	
	ave:	4

a probable sample preparation error (see text)
x not included in average

correction for H-exchange and that a deer's diet, within a restricted area is relatively homogeneous with respect to δD. This agrees well with observations based on $\delta^{13}C$ and $\delta^{15}N$ (Ch. 10). The disparate results for the MI-1 and MI-15 pair were excluded from this analysis as the low H-yields of MI-1 suggest faulty sample preparation.

The δDb_c results are plotted according to sampling location in Fig. 8.1 with averages appearing for locations represented by more than one analysis. Results for MUSK and the two fossil bison (BIS and OLD-C) are included for comparison. Again, it is clear that the δDb_c of the deer follow the trends expected from the δDw contours previously seen in Fig. 7.2 (Ch. 7) implying that variation in δDb_c is mainly dependent on variation in δDw. Ideally, if RH were 100% for all locations there would be no inter-site biases from varying RH and δDl would equal δDw. The δDb_c vs δDw relationship would be linear with a slope of 1.0. The δDb_c results for the 49 sampling locations (with averages representing locations with multiple analyses) are plotted against δDw in Fig. 8.2. MUSK is included for visual comparison only. Bivariate regression of these deer results confirms the strong effect that δDw has on δDb_c:

(eq. 8.1) $\delta Db_c = 21 + 0.901 \delta Dw$; $n=46$, $\sigma=\pm 9.8$, $r=0.932$

The uncertainty in the slope is ±0.05. For reasons discussed above, MI-1 was excluded from all regressions as were QC-3 and SA-1 for reasons given below.

In Fig. 8.2, samples from areas of specific ranges of RH are identified by separate symbols as indicated in the legend. The δDb_c from areas of lower RH appear higher in the Y-direction due to

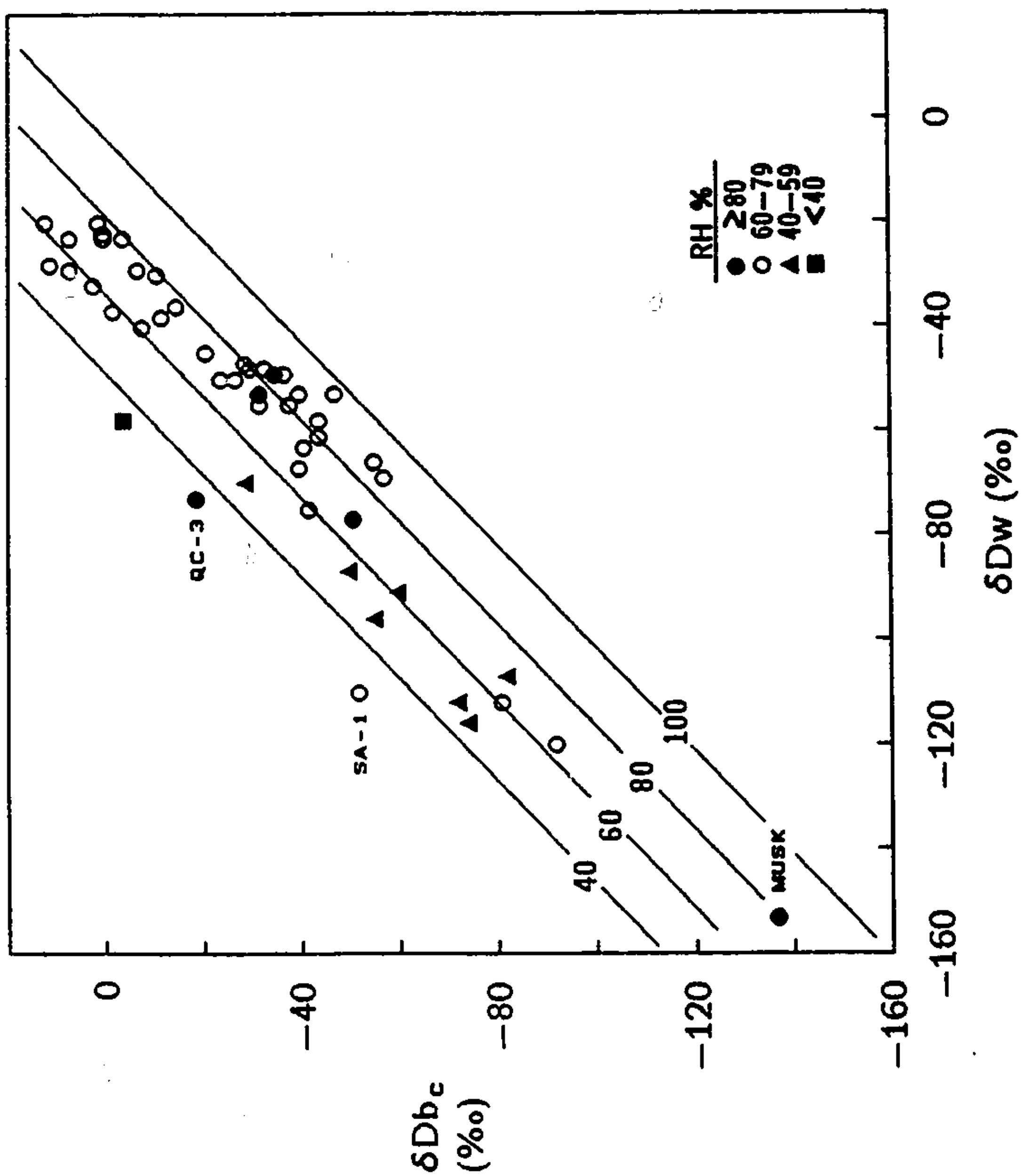
Figure 8.1. δDb_c of Deer Plotted by Sampling Location

The δDb_c (correctėd for H-exchange) of North American deer are plotted by sampling location. The white-tailed deer (*Odocoileus virginianius*) are represened by filled circles and muledeer (*Odocoileus hemionius*) by filled squares. Results for MUSK (filled triangles) and BIS and OLD-C (crosses) are included for comparison. Averages appear in locations where more than one analysis was available.

Figure 8.2. δDb_c vs δDw Relationship for North American Deer.

The relationship between deer bone δD corrected for H-exchange (δDb_c) and average weighted growing season precipitation δD (δDw) are presented. Samples from areas of specific ranges in growing season average 24 hr relative humidity (RH) are identified by symbols. Humidity contours are plotted using results of trivariate regression equation:

$$\delta Db_c = 80 + 1.02\delta Dw - 0.77RH \quad \text{(eq. 8.3)}.$$



RH %
≥80
60–79
40–59
<40
100
80
60
40
QC-3
SA-1
MUSK
0
−40
−80
−120
−160
δDw (‰)
0
−40
−80
−120
−160
δDb$_c$
(‰)

D-enrichment of leaf-H_2O during evapotranspiration. There is also a bias towards higher δDb_c values in colder, more interior areas of the continent due to a preponderance of site locations with low RH. This transcontinental bias in RH has produced a δDb_c vs δDw slope which is <1.0. Both QC-3 and SA-1 appear to be outliers with abnormally high δDb_c. The nature of the QC-3 sample is in question (App. B). SA-1 also has an abnormally high phosphate $\delta^{18}Ob$ (Ch. 9) suggesting that δDw or RH have not been accurately estimated for this location.

8.2 Relationship between δDb_c and Leaf Water δDl:

The relationship between δDb_c and δDl accounts for the positive bias in δDb_c with decreasing RH and the less than 1.0 δDb_c vs δDw slope. For each sampling location, δDl was estimated from eq. 2.2, using $\epsilon k=12$ for turbulent leaf-H_2O/air boundary conditions.

(eq. 8.2) $\delta Db_c = -4 + 1.07\delta Dl$; $n=46$, $\sigma=\pm8.2$, $r=0.952$

Substituting $\epsilon k=17$ (laminar) or $\epsilon k=25$ (static) increased the intercept from $\approx$-4‰ (turbulent) to $\approx$-8‰ (static), lowered the r-value ($r=0.946$, static) but did not affect the slope. The slope is higher than 1.0 possibly due to an over-correction for H-exchange or a deficiency in the leaf-H_2O model as applied (see also Ch. 9). Despite this, the slope is within 2σ of 1.0 ($\sigma=\pm0.05$) and when RH effects are accounted for, the r-value has been increased over that of eq. 8.1. This indicates that the leaf-H_2O model does provide correction for the effects of RH and can account for the <1.0 slope of eq. 8.1.

8.3 Relationship of δDb_c to Both δDw and RH:

It now appears that variation in the δDb_c about the δDb_c vs δDw regression curve can be adequately represented by the RH effects in leaf-H_2O models of plant leaves with $^2\epsilon e \approx \delta Dw - \delta Dv$; $^2\epsilon e$ and $^2\epsilon k \approx$constant. Since other generalizations (above) also appear to be correct, the offset between between δDb_c and δDw should be linearly related to RH as predicted by eq. 2.3. Therefore, a trivariate linear regression, relating δDb_c to both δDw and RH, is appropriate for data from the 46 deer sampling locations:

(eq. 8.3) $\delta Db_c = 80 + 1.02\delta Dw - 0.77RH$; $n=46$, $\sigma=\pm7.5$, $R=0.961$

The uncertainty in the δDw coefficient is $\approx\pm0.04$ indicating that, after correction for RH, δDb_c forms a linear relationship with δDw with a slope of 1.0. Clearly the deer δDb_c results reflect leaf-H_2O derived from rain during the growing season. The negative RH coefficient indicates that as RH decreases δDb_c increases in accordance with theoretical expectations. It can also be concluded that there are no significant inter-site biases due to any theoretical constants ($^2\epsilon e$, $^2\epsilon k$) affecting leaf-H_2O or due to any unknown factors in plants or deer. It is also apparent that $p_{EX} \approx 0.205$ and there are no additional slowly exchanging hydrogens.

Contour lines of constant RH calculated using eq. 8.3 are plotted in Fig. 8.2. Most δDb_c data from each humidity range plot within the appropriate RH contours and the bias towards low RH in cold climate areas is evident. The high multiple correlation coefficient (R) indicates that the trivariate linear regression model relating δDb_c to both δDw and RH (eq. 8.3) is appropriate and statistical results are

improved over the leaf-H_2O model. Additional advantages of the trivariate approach include simpler calculations and no need for additional assumptions about other variables affecting leaf-H_2O calculations such as ϵe, ϵk, and δDv.

It is now possible to correct the δDb_c data for humidity effects by considering the following. If RH were 100% in all locations there would be no RH effects and $\delta Dl=\delta Dw$. Under these circumstances, δDb_c would form a linear relationship with δDw with a slope of 1.0 as suggested by Fig. 8.2. This indicates that the RH regression coefficient of eq. 8.3 can be used to correct for the effects of RH by removing the enrichment in δDl due to evaporation when RH<100 by use of the following relationship:

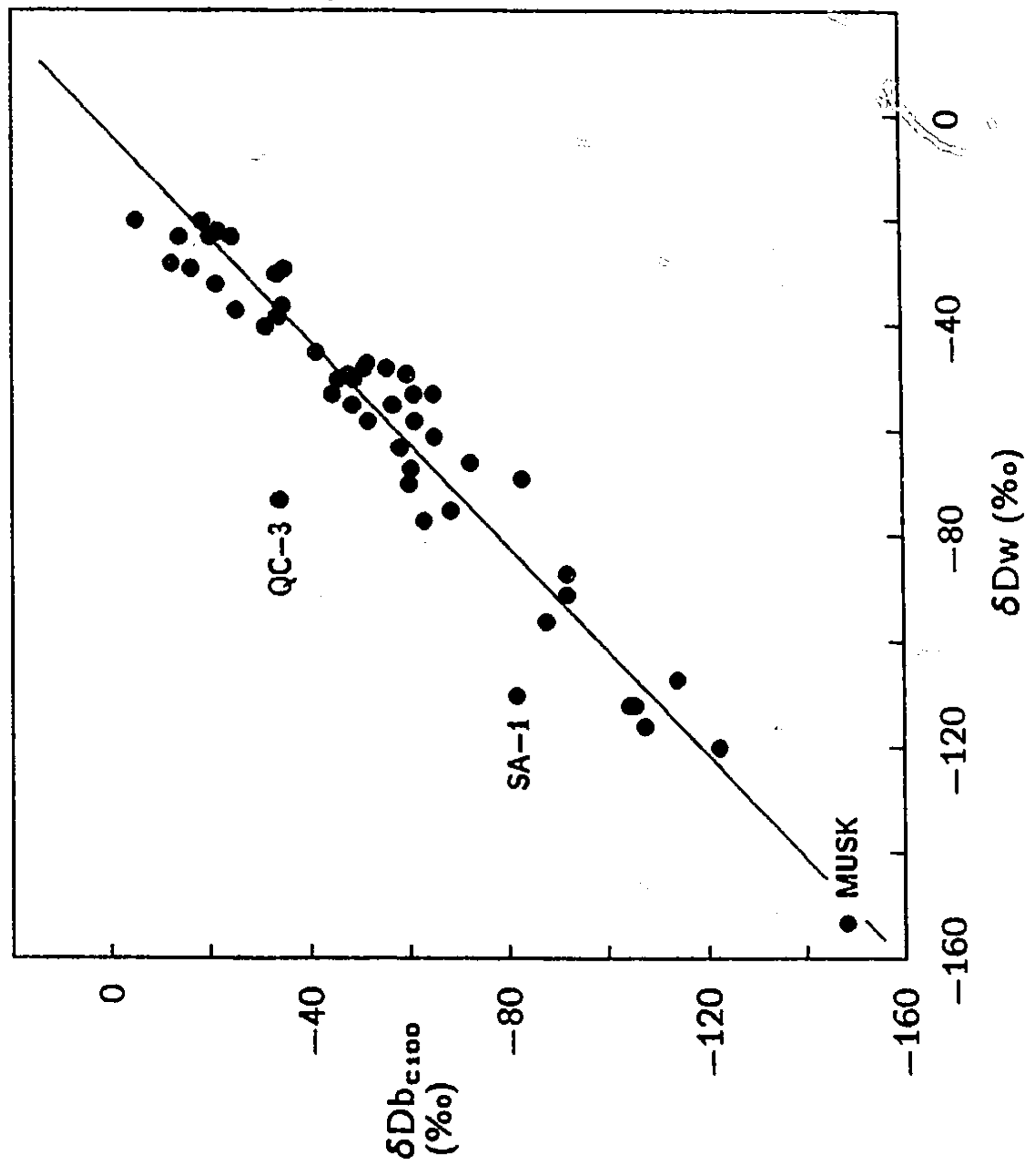
(eq. 8.4) $$\delta Db_{c100} = \delta Db_c - 0.77\cdot(100-RH)$$

Thus, through subtraction of the increase in δDb_c due to effects of RH in the leaf the data are corrected back to the 100% humidity line of Fig. 8.2. The humidity corrected data (δDb_{c100}) calculated from eq. 8.4, are listed in Table 7.3 and are plotted against δDw in Fig. 8.3. Regression of these data confirms that, after correction for RH, there is an excellent linear relationship between δDb_c and δDw with a slope of 1.0:

(eq. 8.5) $$\delta Db_{c100} = 4 + 1.02\delta Dw; \quad n=46,\ \sigma=\pm7.5,\ r=0.967$$

Figure 8.3. δDb_{c100} vs δDw Relationship for North American Deer.

The relationship between deer bone δD corrected for H-exchange and for RH-effects (δDb_{c100}) and average weighted growing season precipitation (δDw) is presented.
Here: $\delta Db_{c100} = \delta Db_c - 0.77(100-RH)$ (eq. 8.4).
The regression line on these data is defined by:
$\delta Db_{c100} = 3.8 + 1.02\delta Dw$, $n=46$, $\sigma = \pm 7.5$, $r=0.967$ (eq. 8.5).


QC−3
SA−1
MUSK
0
−40
−80
−120
−160
δDw (‰)
δDb$_{C100}$ (‰)

The uncertainty of the slope is ±0.04 and of the intercept ±4‰. QC-3 and SA-1 again appear as outliers which further justifies their and SA-1 again appear as outliers which further justifies their exclusion from the regression analysis. However, none of the above regressions were greatly altered when SA-1 results were included. Furthermore, none were improved and the δDw slope did not increase when RH_{day} was substituted for RH indicating that RH provides a reasonable estimate of relative humidity during plant photosynthesis (see Ch. 7). If RH (24 h) were too high in the interior continental areas compared to RH_{day}, the δDw slope would be too low due to undercorrection.

When effects of RH are accounted for, both intercepts of eq. 8.2 and 8.5 are close to zero. However $^{2}\epsilon_{B}$ cannot be directly inferred from the intercept due to lack of knowledge of ϵ_{H-V} (between H_{EX} and lab H_2O-vapor) which produces an unknown, constant offset in the δDb_c results. In addition, dear eat whole leaves and there are no satisfactory estimates of ϵ_B between non-exchanged hydrogens of whole plant material and leaf water (Ch. 2).

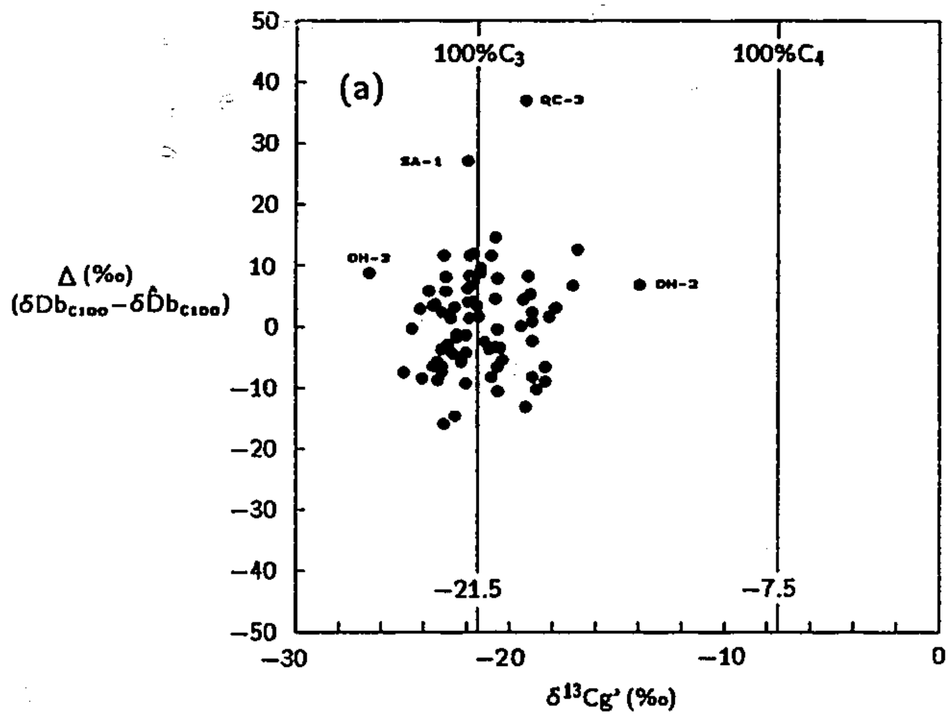
Most of the remaining variability about the regression curve of Fig. 8.3 likely arises from the uncertainty associated with the RH and, especially, δDw variables. In order to test whether a significant proportion of the residual variability about the δDb_{c100} vs δDw regression line could be due to deer diet or other climatic factors as measured by the $\delta^{13}C$ and $\delta^{15}N$ results, the residuals about the regression curve (δDb_{c100}-$\delta Db_{c100}\hat{}$) were plotted against $\delta^{13}Cb$ and $\delta^{15}Nb$ in Fig. 8.4a and 8.4b respectively. Diet or climate as measured by $\delta^{13}C$ or $\delta^{15}N$ does not seem to have any significant influence on remaining

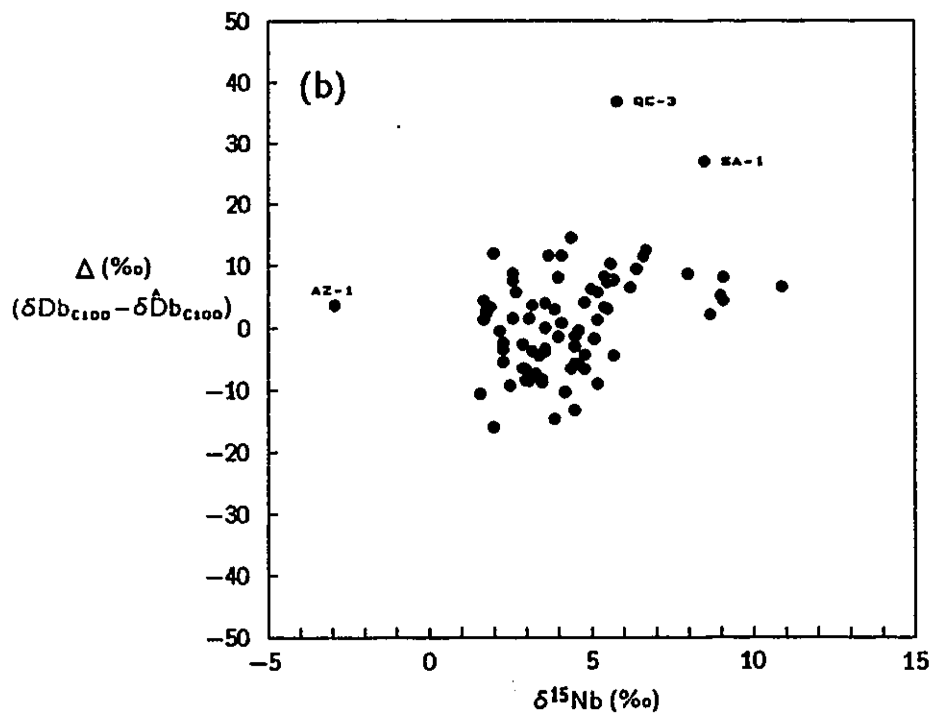
Figure 8.4a. Effects of Diet or Ecology on Variability in δDb_{c100} as Measured by $\delta^{13}C$.

The Y-axis contains the residuals of deer bone δD corrected for H-exchange and RH effects (δDb_{c100}) about the regression curve defined by: $\delta Db_{c100}\hat{} = 3.8 + 1.02\delta Dw$ (eq. 8.5).
The bone $\delta^{13}C$ values (X-axis) have been corrected for a contribution from bone carbonate ($\delta^{13}Cg' = \delta^{13}Cb - 0.6‰$) and reflect diet or ecology as described in the text (Ch. 10).

Figure 8.4b. Effects of Diet or Ecology on Variation in δDb_{c100} as Measured by $\delta^{15}N$.

The Y-axis contains the residuals of deer bone δD corrected for H-exchange and RH-effects (δDb_{c100}) about the regression curve defined by: $\delta Db_{c100}\hat{} = 3.8 + 1.02\delta Dw$ (eq. 8.5).
The bone $\delta^{15}N$ (X-axis) reflects diet or ecology as described in the text (Ch. 10).


(a)
100%C3
100%C4
QC-3
SA-1
OH-2
OH-2
−21.5
−7.5
Δ (‰)
(δDbC100 − δD̂bC100)
δ13Cg' (‰)
50
40
30
20
10
0
−10
−20
−30
−40
−50
−30
−20
−10
0


(b)
QC-3
SA-1
AZ-1
Δ (‰)
(δDbC100 − δD̂bC100)
δ15Nb (‰)
50
40
30
20
10
0
−10
−20
−30
−40
−50
−5
0
5
10
15

variability. From Fig. 8.4a it is clear that some animals have eaten C_4 plants but the presence of C_4 plants does not affect δDb_c This confirms previous observations on plants (Sternberg et al. 1984a,b; 1986b; Ch. 2).

8.4 The Estimation of Precipitation δD from Bone Data:

A more accurate estimate of rain δD could lead to better interpretations of temperature or climate. Since there are two unknowns in eq. 8.3, (δDw and RH), lack of knowledge of RH would impede the estimation of rain δD from δDb_c. However, an examination of the r-coefficients of eq. 8.1 and 8.3 confirms that, compared to δDw, RH has a relatively minor effect on δDb_c. This allows estimation of rain δD directly from δDb_c by ignoring the effects of RH as discussed below.

Although δDb_c is related to δDw, the climatic significance of δDw is less well understood than that of δDwy so it might be preferable to use bone δD to estimate δDwy. A comparison of the δDb_{c100} to the δDwy contours of Fig. 7.1 confirms that there is a bias in δDb_{c100} towards δDw of the growing season. The (δDb_{c100}–δDwy) difference increases from ≈5‰ to ≈40‰ when moving from areas of long growing seasons (i.e. Florida) to areas of short growing seasons (i.e. Alberta). This would produce a <1.0 slope between δDb_{c100} or δDb_c and δDwy. However, a good empirical relationship exists between δDb_c and δDwy, regardless of whether RH or RHy is used:

(eq. 8.6) $\delta Db_c = 68 + 0.79\delta Dwy - 0.63RH$; $n=46$, $\sigma=\pm7.5$, $r=0.961$

(eq. 8.7) $\delta Db_c = 68 + 0.75\delta Dwy - 0.65RHy$; $n=46$, $\sigma=\pm7.6$, $r=0.960$

This indicates that δDb_c can be used to estimate δDwy despite the bias of δDb_c towards δDw.

Finally, due to the low variability in δDv, δDb (not corrected for H-exchange) does not vary more than δDb_c. Therefore, substitution of δDb into the trivariate regression also produces good empirical results indicating that δDb can be used to estimate δDw or δDwy without correction for H-exchange:

(eq. 8.8) $\delta Db = 32 + 0.78\delta Dw - 0.61RH$; $n=46$, $\sigma=\pm 6.4$, $R=0.951$

As discussed in Ch. 6 the δDw slope will be less than 1.0 when the bone δD results are not corrected for H-exchange. Although the statistics for eq. 8.8 are good, the higher R-coefficient of eq. 8.3 suggests that, during correction for H-exchange, there is compensation for variability due to sample preparation. It will be shown below that the same correction occurs when δDv is inserted into the multilinear regression model containing δDb.

Using stepwise multiple linear regression analysis with the precautions outlined in App. E, the δDwy were regressed against 19 possible input predictor variables: δDb or δDb_c, RH, RH^2, ln(RH), $\delta^{15}N$, $(\delta^{15}N)^2$, $\ln|\delta^{15}N|$, PPT, $(PPT)^2$, ln(PPT), H/N_b, C/N_b, H/C_b, N/g_b, H/C_b, G/g_b, H/g_b, δDv, T(°C), T^2(°C), lnT(°K). Results of 67 individual deer were used since some of the input variables can reflect individual differences in the animals or in sample preparation. There were no $\delta^{13}C$ results for six of the samples. $\delta^{13}C$ and its transformations were excluded from the regression after it was discovered, using the remaining 61 samples that $\delta^{13}C$ was not a significant predictor variable.

The best estimate of δDwy (δDwy^) was provided by the following multilinear regression model:

$$\text{(eq. 8.9)}\quad Dwy\hat{} = -71.0070 + 1.4551\cdot\delta Db + 0.6164\cdot RH - 0.1168\cdot\delta Dv \ldots$$
$$\ldots - 0.1960\cdot\delta^{15}N^2 + 0.3174\cdot C/g_b;\; n=67,\; \sigma=\pm7.6,\; R=0.981$$

the variables are ordered according to the step in which they were selected by STATPRO. Use of δDb, δDv and RH as well as the two additional variables in eq. 8.9 (R=0.981) improved the results over use of δDb_c and RH alone (R=0.969). Since RH is the only predictor variable which cannot be measured in the laboratory, regressions were re-evaluated without it:

$$\text{(eq. 8.10)}\quad \delta Dwy\hat{} = -35.6205 + 1.5197\cdot\delta Db - 0.4968\cdot\delta^{15}N^2 - 0.1261\cdot\delta Dv \ldots$$
$$\ldots + 0.3892\cdot C/g_b + 2.5241\cdot\delta^{15}N;\quad n=67,\; \sigma=\pm8.6,\; R=0.975$$

Regression statistics for both models are good and the accuracy of the δDwy estimate is not greatly lessened when RH is ignored. All variance inflation factors (VIF, App.C) were <2 for non-quadratic terms indicating low levels of multicolinearity among predictor variables.

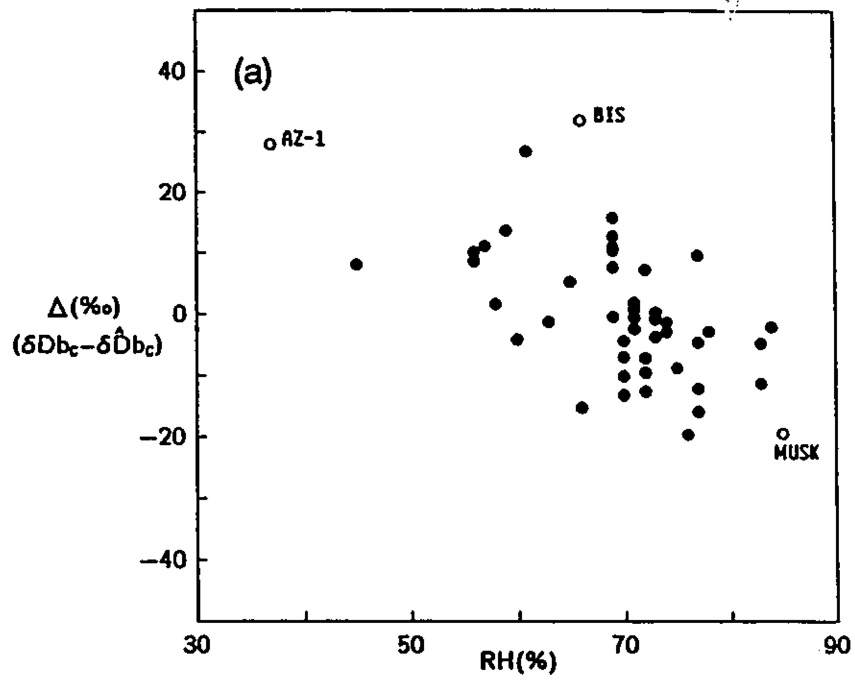
The ability to predict δDwy is improved in both equations when $\delta^{15}N$ is included as a predictor variable. This may be due to the relationship between $\delta^{15}N$ and other climatic or ecological phenomena and $\delta^{15}N$ may, in part substitute for RH since it correlates with RH (see Ch.10). In Fig. 8.5a it is seen that the residual variability in δDb_c about the regression curve defined by eq. 8.1 negatively correlates with RH (r=-0.498, n=45, p>0.98). Shown in Fig. 8.5b is the positive correlation between these same residuals and $\delta^{15}N$ (r=0.511, n=45). These

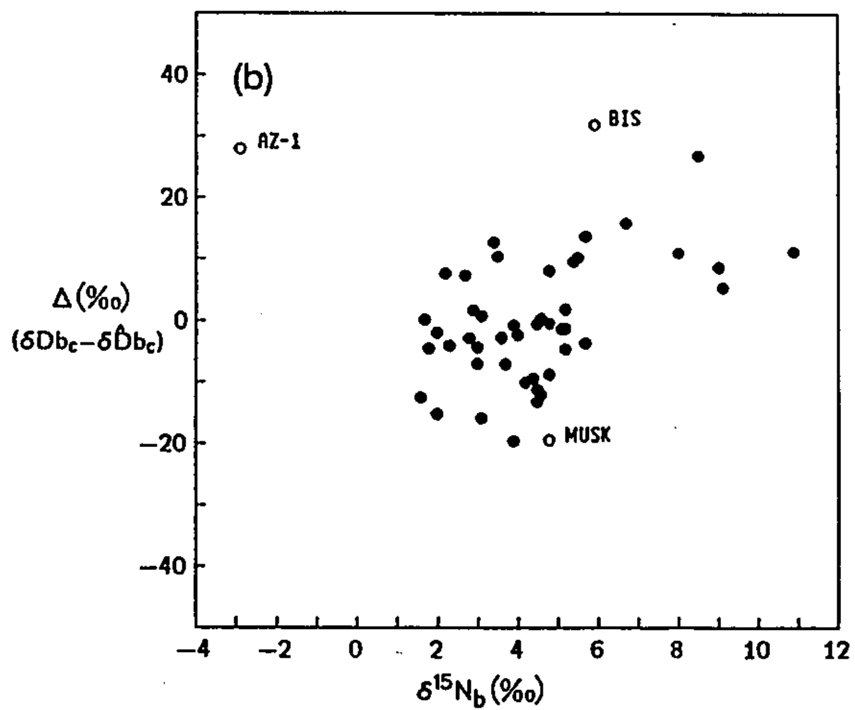
Figure 8.5a. Residual Variability in δDb_c vs RH.

The residual variability in δDb_c about the regression curve defined by: $\delta Db_c = 21 + 0.901\delta Dw$ (eq. 8.1) for n=45 deer sampling locations are plotted against growing season relative humdity (RH). Regressions were calculated on data represented by closed circles (see text) and include only deer results but excludes AZ-1 which has an outlier $\delta^{15}N$ value (see Fig. 8.5b). BIS and MUSK results were included in the figure for visual comparison only.

Figure 8.5b. Residual Variability in δDb_c vs $\delta^{15}Nb$.

The residual variability in δDb_c about the regression curve defined by: $\delta Db_c = 21 + 0.901\delta Dw$ (eq. 8.1) for n=45 deer sampling locations are plotted against $\delta^{15}Nb$. Non-deer and one outlier $\delta^{15}N$ result (AZ-1) are represented by open circles and were not included in the regressions discussed in the text.


(a)
AZ-1
BIS
MUSK
Δ(‰)
(δDbc−δD̂bc)
RH(%)
40
20
0
−20
−40
30
50
70
90


(b)
AZ-1
BIS
MUSK
Δ(‰)
(δDbc−δD̂bc)
δ15Nb (‰)
40
20
0
−20
−40
−4
−2
0
2
4
6
8
10
12

relationships are for 45 deer sampling locations excluding AZ-1 which has an anomalous $\delta^{15}N$ value. The curvilinear relationship apparent between the residuals and $\delta^{15}N$ explains the curvilinear transformation of $\delta^{15}N$ in eq. 8.9 and 8.10. Thus $\delta^{15}N$ may be a proxy variable for RH in eq. 8.10 but its inclusion in eq. 8.9 implies some additional correction, perhaps for other climatic or ecological influences on both δDb and $\delta^{15}N$.

Substituting δDb and δDv for δDb_c allows use of the regression equation to correct for between-sample variation due to H-exchange and other minor effects of sample preparation. The inclusion of the C/g_b variable in both regression equations suggests that δDb is slightly dependent on degree of combustion which, in some cases, may not be 100% (Ch.5). Since C/g_b may also be related to diagenesis (see Ch. 11), this variable may be excluded from the regression with little sacrifice in accuracy:

$$\text{(eq. 8.11)}\quad \delta Dwy\hat{} = -5.8184 + 1.5200\cdot\delta Db - 0.5164\cdot\delta^{15}N^2 - 0.1163\cdot\delta Dv \ldots$$
$$\ldots + 2.8476\cdot\delta^{15}N;\quad n=67,\ \sigma = \pm 9.1,\ R = 0.972$$

This form could be used for the interpretation of fossil samples.

It is now possible to compare the different calculations with respect to the uncertainty associated with the $\delta Dwy\hat{}$ estimate. Deer bone from 43 locations will be used and laboratory measurements, such as $\delta^{15}N$, will be substituted for RH. In these calculations QC-3, SA-1 and MI-1 are excluded for reasons indicated above. LA-8 and ND-1 which had no C/g_b and AB-6 which had no $\delta^{15}N$ results were also excluded. Phosphate $\delta^{18}Ob$ results were available for deer from 29 locations so the RH^, estimated using both δDb_c and $\delta^{18}Ob$ results (see

Ch. 9, eq. 9.12), was substituted into eq. 8.9 for RH. For deer from the remaining 15 locations eq. 8.10 was used in which $\delta^{15}N$ serves as a proxy variable for RH. Regression of δDwy vs $\delta Dwy\hat{}$ with this combined use of eq. 8.9 and 8.10 gave R=0.972. Use of eq. 8.10 alone for all 43 locations gave R=0.968. This indicates only slight improvement in R-value with use of $\delta^{18}Ob$ and $RH\hat{}$ from eq. 9.12. Finally, $\delta Dwy\hat{}$ can be estimated directly from δDb_c (R=0.934). Therefore, use of the four measurements (δDb, $\delta^{15}N$, δDv and C/g_b) greatly improves the estimate of δDwy when RH is ignored compared to this estimate from δDb_c alone.

Both the $\delta Dwy\hat{}$ results calculated using eq. 8.10 alone and using a combination of eq. 8.9 and 9.12 are listed in Table 7.3. Both estimates are closely comparable to δDwy as determined from IAEA data and contours. In Fig. 8.6a the δDwy, determined from contours, is plotted vs Ty. In Fig. 8.6b the $\delta Dwy\hat{}$, estimated using the combination of eq. 8.9 and 9.12 for samples with $\delta^{18}Ob$ results and using eq. 8.10 for the remaining samples, are plotted vs Ty. Clearly the $\delta Dwy\hat{}$ vs Ty relationship closely resembles that of δDwy vs T. This indicates that $\delta Dwy\hat{}$ estimated from bone data can be used to make the same climatic interpretations that are possible using δDwy.

In the above, it was possible to obtain an excellent estimate of δDwy while ignoring measured RH because bone is very sensitive to variations in rain δD but relatively insensitive to RH. A comparison using data from the literature of divariate (δD_{cel} vs δDi) to trivariate results (δD_{cel} vs δDi and RH) indicates a large improvement in statistics for nitrated tree wood cellulose when RH is included compared to those when RH is ignored (after: Yapp and Epstein 1982b):

Figure 8.6a. δDwy vs Ty for Each Deer Sampling Location.

This figure is a repeat of Fig. 7.3b for comparision to Fig. 8.6b. Relationship between weighted average annual precipitation δD (δDwy) as determined from contours for each deer sampling location vs average yearly temperature (Ty) as determined from nearby weather stations. Areas from west of Rocky Mountains are identified by open squares, and east of but close to the Rockies by open triangles. Areas intermediate between the Rockies and east coast are identified by closed triangles while those of the remaining continental sampling locations by closed circles. The best fit relationship for the North American IAEA data from Fig. 7.3a (solid line; eq. 7.3) is included for comparison. See Fig. 7.3b for additional explanations.

Figure 8.6b. $\delta Dwy\hat{}$ Estimated from Bone Data vs Ty for Each Deer Sampling Location.

See Fig. 8.6a caption for explanation of symbols. Averages of deer results were used for locations for which more than one result were available. The $\delta Dwy\hat{}$ are from Table 7.3.

For locations with deer having $\delta^{18}Ob$ results:

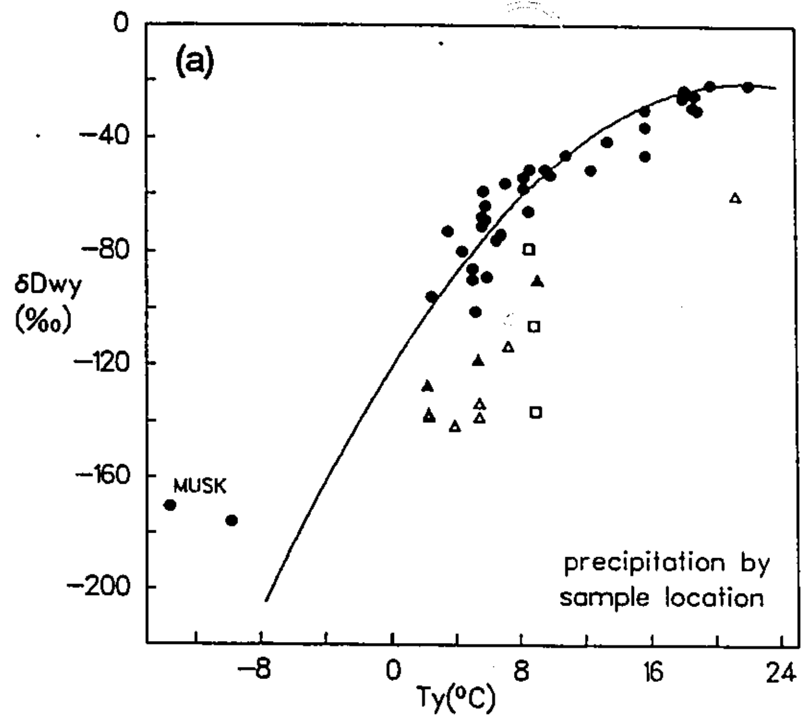
$$\delta Dwy\hat{} = -71.007 + 1.4551 \cdot \delta Db + 0.6164 \cdot RH\hat{} - 0.1168 \cdot \delta Dv \ldots$$
$$\ldots - 0.1960(\delta^{15}N)^2 + 0.3174(C/g) \quad \text{(eq. 8.9)}$$

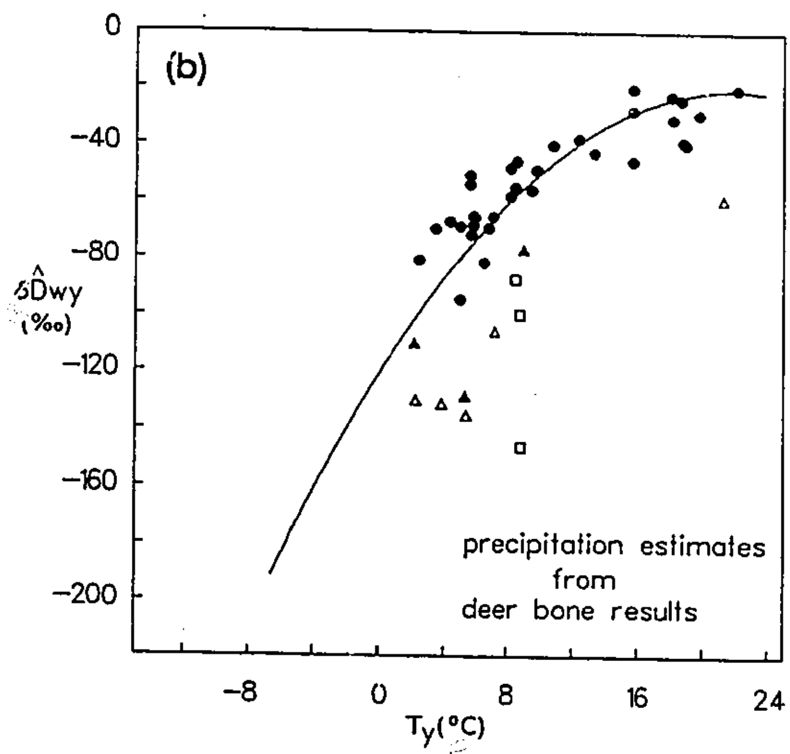
with:

$$RH\hat{} = 83.4074 - 0.25153 \cdot [-12.0077 + 0.975587 \cdot \delta^{18}Ob \ldots$$
$$\ldots - 0.1312 \cdot \delta Db_c + 0.0266 \cdot (\delta^{15}N)^2 + 0.0072 \cdot \delta Dv]^2 \quad \text{(eq.9.9)}.$$

For the remaining locations deer:

$$\delta Dwy\hat{} = -35.6205 + 1.5197 \cdot \delta Db - 0.4968 \cdot (\delta^{15}N)^2 - 0.1261 \cdot \delta Dv \ldots$$
$$\ldots + 0.3892 \cdot C/g + 2.5241 \cdot \delta^{15}N \quad \text{(eq. 8.10)}.$$


(a)
δDwy
(‰)
0
-40
-80
-120
-160
-200
MUSK
-8
0
8
16
24
Ty(°C)
precipitation by
sample location


(b)
δD̂wy
(‰)
0
-40
-80
-120
-160
-200
-8
0
8
16
24
Ty(°C)
precipitation estimates
from
deer bone results

(eq. 8.12) $\delta D_{cel} = -11 + 0.87\delta Di$; $n=33$, $\sigma=\pm22$, $r=0.888$

(eq. 8.13) $\delta D_{cel} = 93 + 1.05\delta Di - 1.25RH$; $n=33$, $\sigma=\pm13$, $R=0.961$

These equations for cellulose (eq. 8.1 and 8.3) parallel those given earlier for deer bone where r=0.932 w/o RH and R=0.961 w/RH. Not only do the cellulose results show larger changes in the correlation coefficient but the RH coefficient is also larger (1.25 for trees; 0.77 for deer). Tree cellulose appears to be more sensitive to RH than deer bone. This suggests that bone may have inherent advantages over trees for estimating δDwy^ and climate. Perhaps the RH effects are lessened in deer because of the large variety of small plants consumed. Some plant species may have different evapotranspiration strategies than trees and other species might be browsed from damp areas such as cedar swamps, where a proportion of plant material could have been produced in locally restricted humid conditions.

For reasons discussed above δDwy^ was estimated instead of δDw^. However, δDw^ can also be estimated using the same procedures:

(eq. 8.14) $\delta Dw\hat{} = -51.0729 + 1.1360\cdot\delta Db + 0.7180\cdot RH - 0.1651\cdot\delta^{15}N^2 \ldots$

$\ldots - 0.0604\cdot\delta Dv$; $n=67$, $\sigma=\pm6.8$, $R=0.976$

(eq. 8.15) $\delta Dw\hat{} = -3.6094 + 1.2111\cdot\delta Db - 0.5286\cdot\delta^{15}N^2 + 3.1093\cdot\delta^{15}N \ldots$

$\ldots -0.0687\delta Dv$; $n=67$, $\sigma=\pm8.4$, $R=0.964$

The above indicates that δDb_c is simply related to δDw and RH by a trivariate linear regression relationship. This relationship is in agreement with theoretical expectations based upon the leaf water models

of the effects of RH on δDl. In addition, either δDwy^ or δDw^ can be accurately estimated using four laboratory measures (δDb, δ^{15}N, δDv, C/gby PPTm'

CHAPTER 9

RELATIONSHIP BETWEEN THE HYDROGEN AND OXYGEN ISOTOPES OF BONE AND THE USE OF BOTH ISOTOPES FOR THE ESTIMATION OF RH

In this chapter the relationship of $\delta^{18}Ob$ to ingested water will first be discussed. The slope should reflect the metabolic processes in the deer (Ch. 2) and the intercept should reflect the equilibrium isotopic separation factor between bone phosphate and body water ($^{18}\epsilon e_{p-bw}$) at mammalian body temperature (37°C). These considerations for oxygen will become important when examining the bone $\delta^{18}Ob$ vs δDb relationship.

Next, the relationship between oxygen and hydrogen isotopes in bone will be evaluated. Once corrected for the effects of RH, it is assumed that the $\delta^{18}Ob$ vs δDb relationship will reflect that of meteoric water in North America (eq. 2.5).

The remainder of this chapter will discuss how RH can be estimated using oxygen and hydrogen isotopic data of bone and the leaf water model of isotopic fraction due effects of evapotranspiration.

9.1 The Relationship between Phosphate Oxygen and Ingested Water:

The relationship between $\delta^{18}O$ of body water ($\delta^{18}Obw$) and ingested water $\delta^{18}O$ ($\delta^{18}Oi$) for deer should reveal something about metabolic effects on oxygen isotopic fractionation in the deer (see Ch. 2). Although a less than 1.0 slope is assumed to arise from

incorporation of atmospheric O^2 into body-H_2O, it is not yet known what the slope of δD_{bw} vs δDi should be for deer. It is also difficult to assess how much body-H_2O is derived from drinking as opposed to leaf-H_2O. The $\delta^{18}O$ of bone phosphate ($\delta^{18}Ob$) is expected to reflect body water but should be offset from it by +17.3‰, the value of $\delta^{18}Ob-\delta^{18}Obw \approx {}^{18}\epsilon e_{p-bw}$ (cf. Longinelli and Nuti 1982; Kolodny et al. 1983).

In order to evaluate the relationship between $\delta^{18}Ob$ and $\delta^{18}Oi$, $\delta^{18}Ob$ values for 39 of the deer of this study were obtained in a collaborative study presented in Luz et al. (1990). Assuming that most body-H_2O derives from leaf-H_2O then the theoretical $\delta^{18}O$ value of leaf-H_2O ($\delta^{18}Ol$) can be calculated from values of local meteoric water $\delta^{18}Ow$ and RH using the leaf water model given earlier (eq. 2.6). Here, fixed constants for the values of the kinetic (k) and equilibrium (e) isotopic separation factors (${}^{18}\epsilon k$ and ${}^{18}\epsilon e$) were used for the growing season with ${}^{18}\epsilon k=14$ (turbulent conditions; Merlivat 1978; Edwards et al. 1985; Dongman et al. 1974). Since ${}^{18}\epsilon e$ varies little with temperature, a constant value was chosen based on the average growing season T of all samples of 13.9°C so that ${}^{18}\epsilon e=9.714$ (Baertschi and Thurkauf 1969). In all of the regressions discussed below, results of deer from 31 sampling locations (39 deer in all) were used with averages representing locations having more than one result:

(eq. 9.1) $\delta^{18}Ob = 16.9 + 0.881 \cdot \delta^{18}Ol$; $n=31$, $\sigma=\pm1.3$, $r=0.927$

Regression results agree with theoretical predictions in that the intercept is close to the $18\epsilon e_{p-bw}$ value of ≈17.3‰ and the slope is <1.0. The statistics are also reasonably good.

If a large proportion of body-H_2O came from drinking water (ponds, lakes and streams) then $\delta^{18}Ob$ could be biased towards yearly average $\delta^{18}Owy$ and RHy. Since this drinking water also undergoes evaporation similar to leaf-H_2O, eq. 2.6 can be used to calculate its theoretical value using $^{18}\epsilon e=10$ for the yearly average Ty of 9.9°C for all samples. The $\delta^{18}Ob$ regressed against this drinking-H_2O value ($\delta^{18}O_{dw}$) gives:

(eq. 9.2) $\delta^{18}Ob = 18.1 + 0.667 \cdot \delta^{18}O_{dw}$; n=31, σ=0.995, r=0.958

The value of $^{18}\epsilon e_{p-bw} \approx 17.3‰$ lies between the intercepts of eq. 9.1 and 9.2 but it being closer to that of eq. 9.1 suggests that both sources of H_2O are present but that leaf-H_2O might be more important. The higher r-value of eq. 9.2 is not particularly diagnostic since there is a greater uncertainty in the estimates of $\delta^{18}Ow$ and RH used to calculate δDl than there is in those of $\delta^{18}Owy$ and RHy used to calculate δD_{dw} (Ch. 7).

Luz et al. (1990) have shown that, by using trivariate linear regression on deer data from 35 locations, $\delta^{18}Ob$ can be directly related to $\delta^{18}Ow$ and RH or to $\delta^{18}Owy$ and RHy in the manner indicated for δDb:

(eq. 9.3) $\delta^{18}Ob = 35.9 + 0.881\delta^{18}Ow - 0.180RH$; n=35, R=0.93

(eq. 9.4) $\delta^{18}Ob = 34.6 + 0.650\delta^{18}Owy - 0.171RH$; n=35, R=0.95

The less than 1.0 $\delta^{18}Ow$ and $\delta^{18}Owy$ slopes are, again, due to metabolic effects in the deer. It cannot be determined from the above which source of water, leaf or drinking is the main source of body water.

9.2 The Relationship Between the Oxygen and Hydrogen Isotopes of Bone:

Both the oxygen and hydrogen isotopic signatures of bone should be strongly related to those of meteoric water. Furthermore, since $\delta^{18}Ob$ is related to leaf water as is δDb and since both $\delta^{18}Ob$ and δDb are affected by the same variables there should be a strong relationship between bone $\delta^{18}O$ and δD. When $\delta^{18}Ob$ and δDb are corrected for the effects of RH ($\delta^{18}Ob_{100}$ and δDb_{100}) the relationship between these two variables should reflect meteoric water.

The expected strong relationship between δDb and $\delta^{18}Ob$ is confirmed in Fig. 9.1a. MUSK, OLD-C and BIS are included for visual comparison but excluded from all regressions and the significance of their results will be discussed in Ch. 11. Regression of the δDb vs $\delta^{18}Ob$ data for the deer gives:

(eq. 9.5) $\delta Db_c = -160 + 7.8 \cdot \delta^{18}Ob$; $n=31$, $\sigma=\pm 12$, $r=0.917$

The slope of this relationship can not yet be unambiguously interpreted since both $\delta^{18}Ob$ and δDb may suffer a transcontinental bias in RH and require correction prior to meaningful interpretation (Ch. 8).

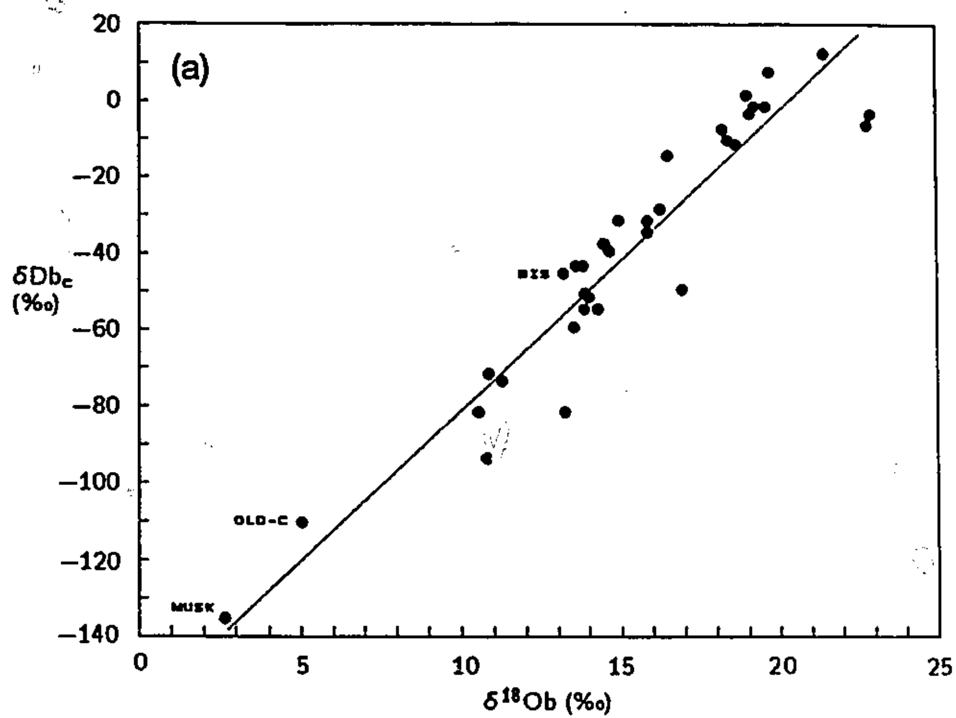
The $\delta^{18}Ob_{100}$ values can be calculated to subtract the effects of RH from $\delta^{18}Ob$ in a manner identical to that described for δD. Here, the RH regression coefficient of -0.180 from eq. 9.3 was used and the δDb_{c100} vs $\delta^{18}Ob_{100}$ for deer from 31 locations are plotted in Fig. 9.1b. MUSK, BIS and OLD-C are included for visual comparison only.

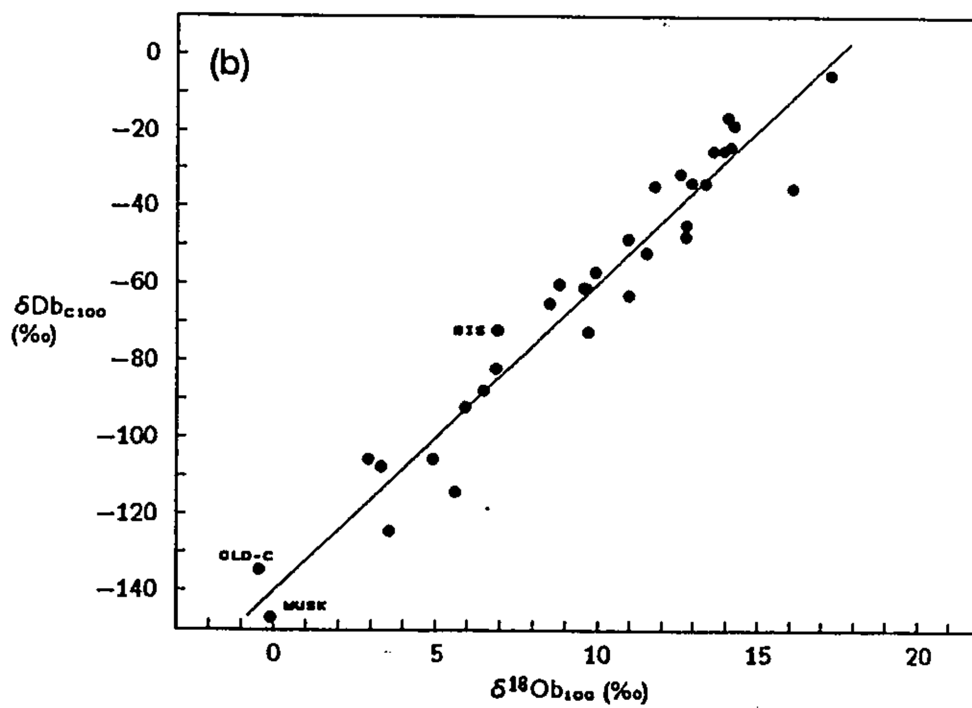
Figure 9.1a. Relationship Between δDb_c and $\delta^{18}Ob$ of Deer.

The collagen δD corrected for H-exchange (δDb_c) are plotted against $\delta^{18}O$ of bone phosphate ($\delta^{18}Ob$). The regression line is defined by: $\delta Db_c\hat{} = -160 + 7.8 \cdot \delta^{18}Ob$ (eq. 9.3).

Figure 9.1b. Relationship Between δDb_{c100} and $\delta^{18}Ob_{100}$ of Deer.

The collagen δD corrected for H-exchange and RH-effects (δDb_{c100}) are plotted against bone phosphate $\delta^{18}O$ corrected for RH-effects ($\delta^{18}Ob_{100}$). Here: $\delta^{18}Ob_{100} = \delta^{18}Ob - 0.180 \cdot (100-RH)$ using the RH-coefficient from Luz et al. (1990): $\delta^{18}Ob = 35.9 + 0.881 \cdot \delta Dw - 0.180 \cdot RH$. δDb_{c100} were calculated using eq. 8.4. The regression line is defined by: $\delta Db_{c100} = -140 + 8.0 \cdot \delta Db_{100}$ (eq. 9.4).


(a)
δDb_c (‰)
δ18Ob (‰)
BIS
OLD-C
MUSK
20
0
−20
−40
−60
−80
−100
−120
−140
0
5
10
15
20
25


(b)
δDb_c100 (‰)
δ18Ob_100 (‰)
BIS
OLD-C
MUSK
0
−20
−40
−60
−80
−100
−120
−140
0
5
10
15
20

If both δDb_{c100} and $\delta^{18}Ob_{100}$ formed linear relationships with δDw and $\delta^{18}Ow$ respectively, with slopes of 1.0, then the δDb_{c100} vs $\delta^{18}Ob_{100}$ slope should be identical to the meteoric water line (≈8). It was previously shown that the δDb_{c100} vs δDw relationship does indeed have a slope of 1.0 but the $\delta^{18}Ob_{100}$ vs $\delta^{18}Ow$ slope is only 0.95 (n=30, excluding SA-1, for reasons given in Ch. 8). This indicates that the theoretical δDb_{c100} vs $\delta^{18}Ob_{100}$ slope should be ≈8.3. Regression of the data for deer in Fig. 9.1b gives:

(eq. 9.6) $\delta Db_{c100} = -140 + 8.0\delta^{18}Ob_{100}$; $n=31$, $\sigma=\pm9$, $r=0.962$

Since the uncertainty of the slope is ±0.4, it is within 2σ of the theoretical prediction. This indicates that both δDb_c and $\delta^{18}Ob$ coherently reflect rain δD and $\delta^{18}O$. The high r-value for both eq. 9.5 and 9.6 and the small difference in the slopes indicates that δDb_c and $\delta^{18}Ob$ covary, with both isotopes being strongly influenced by growing season rain and weakly influenced by RH.

9.3 RH Estimation Using Bone δD and $\delta^{18}O$:

The leaf water model (eq. 2.3 and 2.6) predicts that one should be able to estimate RH from the isotopic offset between leaf water and meteoric water. This results since a decrease in RH produces an isotopic enrichment in leaf water so that RH becomes a simple linear function of this isotopic offset (see. eq. 2.3). All the preceding discussion in this work has shown that there are strong relationships between the isotopic values of both oxygen and hydrogen in bone and those of leaf water and meteoric water. Therefore, it should be possible to use bone isotopic data to estimate this offset. It has been found that

$\delta^{18}Ol$ is considerably more sensitive to RH than is δDl (Ch. 2). This greater sensitivity is also reflected in bone whereby the results on deer (Luz et al. 1990) show a much wider spacing between the lines of constant RH in the $\delta^{18}Ob$ vs $\delta^{18}Ow$ relationship than was found for the analogous relationship of δDb_c vs δDw in Fig. 8.2. For this reason the leaf water model (eq. 2.3) will be adapted for $\delta^{18}O$ so that RH is shown as being linearly related to the difference ($\delta^{18}Ol - \delta^{18}Ow$) as this should be the more sensitive measure of RH:

(eq. 9.7) $RH = \beta_0 + \beta_1 \cdot (\delta^{18}Ol - \delta^{18}Ow)$

where: $\beta_0 \approx 100$ and $\beta_1 \approx [-1/({}^{18}\epsilon e + {}^{18}\epsilon k)] \cdot 100 \approx [-1/(9.714+14)] \cdot 100 \approx -4.2$. This equation shows that, since the relationship is linear the coefficients of β_0 and β_1 may be evaluated through regression analyses thus eliminating the need to give theoretical consideration to the values of ϵe and ϵk.

Since $\delta^{18}Ob$ is a direct reflection of $\delta^{18}Ol$ (eq. 9.1) it is a simple matter to estimate $\delta^{18}Ol$ from $\delta^{18}Ob$. Estimating $\delta^{18}Ow$, however, is considerably more difficult. In wood cellulose work $\delta^{18}Ow$ is generally estimated from δD_{cel} (i.e. Edwards et al. 1985). The rationale for this is that $\delta^{18}Ow$ is related to δDw through the meteoric water relationship (eq. 2.5) Further, δD_{cel} can be used to estimate δDw (and $\delta^{18}Ow$) while ignoring the relatively minor effects of RH since RH effects on δDl (hence δD_{cel}) are considerably less than on $\delta^{18}Ol$ (hence $\delta^{18}O_{cel}$).

In a manner similar to cellulose work, $\delta^{18}Ob$ can be used to estimate $\delta^{18}Ol$ while δDb can be used to estimate $\delta^{18}Ow$. Ultimately, a linear regression containing $\delta^{18}Ob$ will be substituted for $\delta^{18}Ol$ while another linear regression containing δDb will be substituted for $\delta^{18}Ow$.

Eq. 9.7 may then be expanded to show that RH is a linear function of both $\delta^{18}Ob$ and δDb with $\delta^{18}Ob$ functioning as a proxy variable for $\delta^{18}Ol$ and δDb as a proxy variable for $\delta^{18}Ow$:

(eq. 9.8) $RH = \beta_0' + \beta_1' \cdot \delta^{18}Ob + \beta_2' \cdot \delta Db$

Here β_0', β_1' and β_2' could represent combinations of linear regression coefficients such as might be derived from the substitution of the two linear regressions into eq. 9.7 and through additional use of linear regression to determine the values of β_0 and β_1.

When the derivation of RH is viewed in light of eq. 9.8, a number of alternative approaches might also be considered for evaluating the best values for β_0', β_1' and β_2' in order to better estimate RH. For example, a simple regression of RH directly against $\delta^{18}Ob$ and δDb as for Model 4 could be considered. However, Models 3 through 5 all suffer from the same problem which is that, in essence, they are models in which $\delta^{18}Ow$ is estimated directly from δDb while ignoring the effects that RH when, in fact, δDb really does reflect the effects of RH. This means a less accurate estimate of $\delta^{18}Ow$ hence a limited the accuracy to which RH can be estimated. The accuracy with which RH can be estimated using Models 3 through 5 will now be discussed followed by a description of how the RH estimates can be improved through use of Model 1 or 2 which allow additional sample information to be used in order to improve the estimate of $\delta^{18}Ow$.

Model 3 uses $\delta^{18}Ob$ and δDb data on deer from 31 locations and follows the logic outlined above where linear regressions, which provide estimates for each of $\delta^{18}Ol$ and $\delta^{18}Ow$, are substituted into eq.

9.7. First, $\delta^{18}Ol$ is estimated from $\delta^{18}Ob$ using the linear regression relationship:

(eq. 9.9) $\delta^{18}Ol\hat{} = -16.6 + 0.976 \cdot \delta^{18}Ob$; $n=31$, $\sigma=\pm1.4$, $r=0.927$

It was shown previously (Ch. 8) that δDb could be related to δDw via simple linear relationships. Since δDw is also related to $\delta^{18}Ow$ via the linear, meteoric water relationship, it should now be possible to use linear regression to directly estimate $\delta^{18}Ow$ from δDb:

(eq. 9.10) $\delta^{18}Ow\hat{} = -4.3596 + 0.1279 \cdot \delta Db_c$; $n=31$, $\sigma=1.3$, $r=0.932$

Regression of RH vs ($\delta^{18}Ol\hat{}-\delta^{18}Ow\hat{}$) (eq. 9.7) using the two above substitutions for $\delta^{18}Ol\hat{}$ (eq. 9.9) and $\delta^{18}Ow\hat{}$ (eq. 9.10) gives an intercept (β_0) of 104% and slope (β_1) of -4.8 ± 1.0. These are quite close to the expected values (100% and -4.2) for eq. 9.7. Estimates of RH (RH^) provided by this means are compared to measured RH in Table 9.1 (Model-3). All final equations for estimating RH also appear in the table notes. The low correlation coefficient ($r=-0.659$) for Model 3 indicates that the RH^ estimate could be better.

The final equation given for Model 3 in the Table 9.1 notes is written in the form of eq. 9.7. If desired, it could be rewritten and presented in the form of eq. 9.8. This allows one to examine some alternative approaches for evaluating the coefficients of eq. 9.8. One could then start with a different set of initial regression equations which are empirical thus eliminate the need to calculate theoretical values fcr $\delta^{18}Ol$ as was the case in Model 3. One could start with (1) $\delta^{18}Ob$ vs $\delta^{18}Ow$ and RH (eq. 9.3) and (2) δDb vs δDw and RH (eq. 8.8)

Table 9.1 Tests of 5 Models for Estimation of RH

sample	RH (meas)	RH^ model 1[a]	model 2[b]	model 3[c]	model 4[d]	model 5[e]
NS-2	83	70	70	70	70	74
NS-1	83	73	72	68	68	70
NB-1	75	75	76	73	73	80
ON-3	77	75	76	72	73	79
ON-6	72	76	76	73	74	82
ON-1	78	75	74	74	75	83
QC-1	77	68	68	65	66	65
BC-4	84	71	71	68	68	70
SA-1	61	62	63	67	67	68
BC-2	60	60	60	56	57	49
AB-4,5	56	63	67	69	70	74
AB-1,2	69	66	68	64	65	65
AB-7	56	67	67	66	67	67
AB-6	58	57	62	52	52	41
FL-1	74	75	74	76	75	85
LA-2	73	72	71	72	71	77
MO-4	70	73	72	71	71	75
NE-2	59	61	59	70	70	74
WI-1	72	73	72	71	71	76
TX-1	63	59	55	53	51	37
KS-1	71	73	73	69	69	72
OK-1,2	69	76	75	76	75	85
WV-1,3	74	77	77	77	77	90
OH-1,3	69	75	75	74	73	81
TX-2,5	77	71	70	71	69	73
OK-5	69	69	71	73	72	78
OK-10	69	73	73	71	70	75
AZ-1	37	36	36	54	52	39
MT-1	57	52	54	63	64	61
WY-2	58	67	66	64	64	63
OR-1	45	56	53	54	54	43
R	1	0.824	0.824	0.655	0.659	0.658
±σ(%)	0	6.3	6.3	8.3	8.3	8.3

a (model 1) this study; $RH\hat{} = 83.41 - 0.2515 \cdot [-12.0070 + 0.9756 \cdot \delta^{18}Ob - 0.1312 \cdot \delta Db_c + 0.0266 \cdot \delta^{15}N^2 + 0.0072 \cdot \delta Dv]^2$ (eq. 9.12)

b (model 2) this study; $RH\hat{} = 81.36 - 0.3772 \cdot [-18.5828 + \delta^{18}Ob - 0.1548 \cdot \delta Db_c + 0.0204 \cdot \delta^{15}N^2 + 0.0075 \cdot \delta Dv]^2$

c (model 3) this study; $RH\hat{} = 104.34 - 4.7817 \cdot [-12.2887 + 0.9756 \cdot \delta^{18}Ob - 0.1279 \cdot \delta Db_c]$

d (model 4) this study; $RH\hat{} = 167.42 - 4.9246 \cdot \delta^{18}Ob + 0.6158 \cdot \delta Db_c$

e (model 5) Edwards (1987); $RH\hat{}/100 = 19.2275 \cdot h^2 + (0.944574 \cdot \delta^{18}Ob - 0.0242538 \cdot \delta Db_c - 125.79494) \cdot h$ ...
...$+ (1.041183 \cdot \delta Db_c - 8.822734 \cdot \delta^{18}Ob + 243.439333)$

using (i) ${}^{2}\alpha_b = 0.99096347$ (from intercept δDb vs δDl (static), n=44, r=0.950);
(ii) ${}^{18}\alpha_b = 1.01692895$ (from intercept $\delta^{18}Ob$ vs $\delta^{18}Ol$ (turbulent), n=31, r=0.9269;
(iii) ${}^{2}\alpha_e = 1.092689847$, (Majoube 1971); ${}^{18}\alpha_e = 1.00971412$, (Baertschi and Thurkauf 1969), T=13.8°C;
(iv) ${}^{2}\alpha_k = 1.0249$ (static); ${}^{18}\alpha_k = 1.014$ (turbulent), (Long et al. 1980; Merlivat 1978)

and then the meteoric water relationship (eq. 2.5) could be substituted for δDw. The equations are then be equated to eliminate $\delta^{18}Ow$. This gives RH vs $\delta^{18}Ob$ and δDb and it shows that RH is essentially related to $\delta^{18}Ob$ and δDb by regression coefficients in the form of eq. 9.8. This approach was tested (data not shown) and gave results almost the same as those given in Table 9.1 for Model 4. For Model 4 (Table 9.1) the coefficients of eq. 9.8 are evaluated through simple regression of RH directly against $\delta^{18}Ob$ and δDb. Here also, however, the low R-value (0.659) indicates relatively poor estimates of RH.

Model 5 (Table 9.1) is from Edwards (1987) and Edwards and Fritz (1986). It also provides similar results to those of Model-3 where leaf-H_2O models are used and $\delta^{18}Ow$ is estimated directly from δDb_c while ignoring RH. Results of Models 3, 5 (or Model 1, below) were not improved when values for ϵe, ϵk or ϵ_B were varied in the $\delta^{18}Ol$ or δDl calculations.

The low R-values of Models 3, 4, and 5 are due to the relatively poor accuracy of the $\delta^{18}Ow\hat{}$ estimate derived from δDb while ignoring the effects of RH on δDb. Models 1 and 2 allow improvements in RH^ by improving the estimate of the $\delta^{18}Ow\hat{}$ substitution into eq. 9.7.

It was found previously that multilinear regression using $\delta^{15}N$ and δDv in addition to δDb (or δDb_c) enhances the accuracy of the δDw estimates (eq. 8.15). Therefore, it would be expected to also improve the estimate of $\delta^{18}Ow$:

(eq. 9.11) $\delta^{18}Ow\hat{} = -4.6406 + 0.1312\cdot\delta Db_c - 0.0266\cdot(\delta^{15}N)^2 - 0.0072\cdot\delta Dv$;

$n=29, \quad \sigma=\pm 1.0, \quad R=0.969$

Substitution into eq. 9.7 of values for $\delta^{18}Ow^{\wedge}$ estimated using eq. 9.11, on instead of eq. 9.10, followed by additional regression to estimate values for β_0 and β_1 produces an intercept (β_0) of 101% and a slope (β_1) of -4.35 ± 0.58 ($\sigma=\pm6.5$). These regression coefficients (using the same results of the deer from 31 locations) are close to theoretical predictions. Use of eq. 9.11 instead of 9.10 for the $\delta^{18}Ow^{\wedge}$ substitution also considerably improved the R-coefficient (R=0.808 compared to R=0.659). The estimate of RH was further improved when the ($\delta^{18}Ol^{\wedge}$-$\delta^{18}Ow^{\wedge}$) term of eq. 9.7 was squared, possibly compensating for the introduction of a slight non-linearity into the predictor variable by use of $(\delta^{15}N)^2$ instead of RH to correct $\delta^{18}Ow^{\wedge}$ for RH-effects. RH is plotted against this squared term in Fig. 9.2 and regression of these data produces eq. 9.12, the final best fit equation (Model 1) for estimating RH^:

(eq. 9.12) $RH^{\wedge} = 83.4074 - 0.25153 \cdot (-12.007 + 0.975587 \cdot \delta^{18}Ob \ldots$

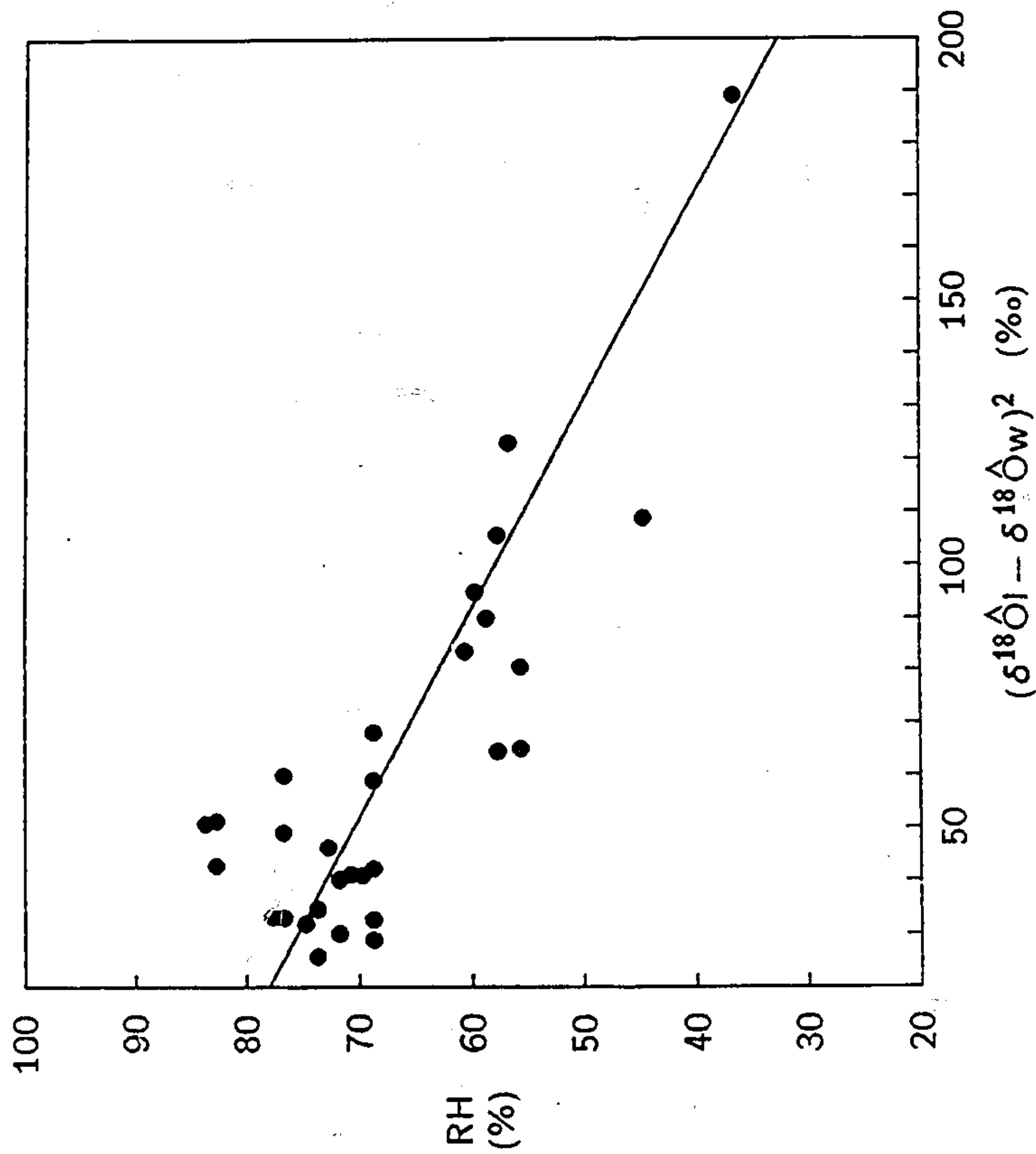
$\ldots - 0.1312 \cdot \delta Db_c + 0.0266 \cdot (\delta^{15}N)^2 + 0.0072 \cdot \delta Dv)^2;$

$n=31,\ \sigma=\pm6.2,\ R=-0.824$

This latter (squared) equation does not appear to make biological or physical sense since its intercept predicts no difference between $\delta^{18}Ol$ and $\delta^{18}Ob_{100}$ when RH>80%. However, since the goal here was to achieve the <u>best</u> estimate of RH, this equation was used to generate the RH^ estimates (Model-1, Table 9.1) which were used in eq. 8.9 of Ch. 8 to estimate $\delta Dwy^{\wedge}$ (Table 7.3).

Figure 9.2. Use of $(\delta^{18}Ol\hat{} - \delta^{18}Ow\hat{})^2$ to Estimate RH.

The regression of these data is defined by:
$RH\hat{} = 83.4074 - 0.25153 \cdot (\delta^{18}Ol\hat{} - \delta^{18}Ow\hat{})^2$ (r=0.824, eq. 9.9);
with: $\delta^{18}Ol\hat{} = -16.6 + 0.976 \cdot \delta^{18}Ob$ (eq. 9.6);
and: $\delta^{18}Ow\hat{} = -4.6406 + 0.1312 \cdot \delta Db_c - 0.0266 \cdot (\delta^{15}N)^2 - 0.0072 \cdot \delta Dv$ (eq. 9.3).


RH
(%)
100
90
80
70
60
50
40
30
20
50
100
150
200
$(\delta^{18}\hat{O}l - \delta^{18}\hat{O}w)^2$ (‰)

Further improvements in RH estimates occurred when only data from the 13 warm locations ($T \geq 13^{\circ}C$, using growing season temperature) were included in the above regression.

(eq. 9.13) $RH\hat{} = 81 - 0.22 \cdot (\delta^{18}Ol\hat{} - \delta^{18}Ow\hat{})^2$; $n=13$, $\sigma=\pm 3.3$, $R=-0.952$

As before, eq. 9.9 and 9.11 were used to estimate $\delta^{18}Ol\hat{}$ and $\delta^{18}Ow\hat{}$ respectively. A comparison of the R-coefficients of eqs. 9.6 and 9.11 indicates that most of the remaining uncertainty in ($\delta^{18}Ol\hat{}-\delta^{18}Ow\hat{}$) arises from the $\delta^{18}Ol\hat{}$ which was derived from regressions involving $\delta^{18}Ol$ calculated via eq. 2.6. Fig. 9.3a shows the comparison of ($\delta^{18}Ob$-$\delta^{18}Ol$) to T and indicates that a negative bias may exist for the cold climate locations (open circles) and a slight positive bias for the warm climate areas (closed circles). A direct temperature effect on either $\delta^{18}Ob$ or $\delta^{18}Ol$ in cold areas could explain why $\delta^{18}Ob$ provides a poor estimate of $\delta^{18}Ol$ in these areas ($r=0.77$, $n=18$). Results are particularly poor when only Canadian locations are considered ($r=0.63$, $n=14$). A poor estimate of $\delta^{18}Ol$ (and $\delta^{18}Ol\hat{}$) and a worsened RH estimate would result when the database includes data from the cold areas.

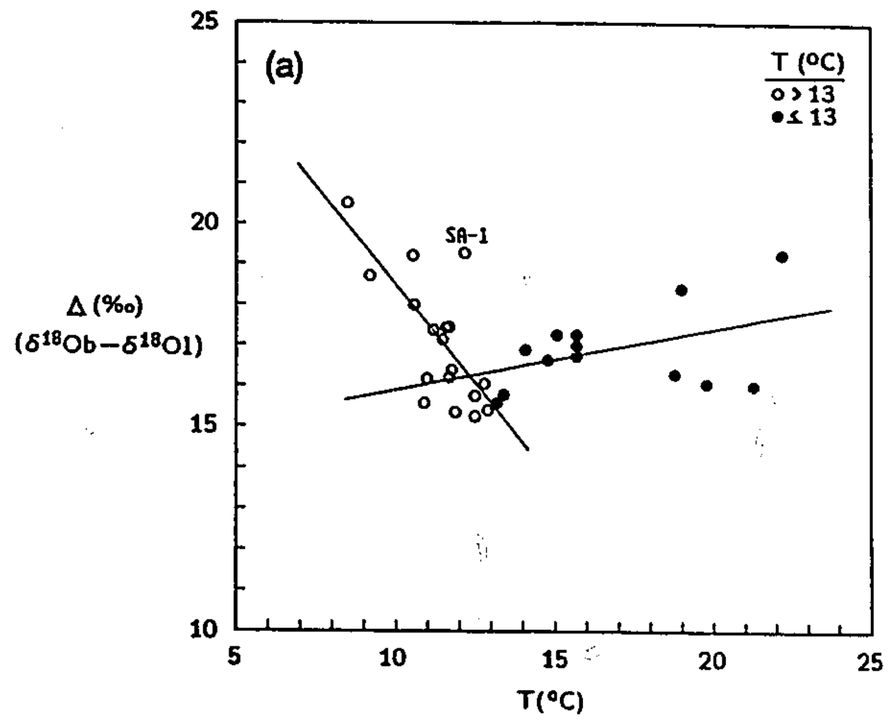
Fig. 9.3b shows that (δDb_c-δDl) also exhibits a T-dependent bias indicating that it must occur before hydrogen or oxygen is incorporated from leaf-H_2O into bone. Not only are hydrogen and oxygen in different components of the bone (hydrogen in organic collagen for δDb and oxygen in inorganic phosphate for $\delta^{18}Ob$) but their respective pathways for incorporation differ considerably. $\delta^{18}Ob$ represents body-H_2O derived from leaf or drinking-H_2O and δDb

Figure 9.3a. Direct Effects of T on $\delta^{18}Ob$.

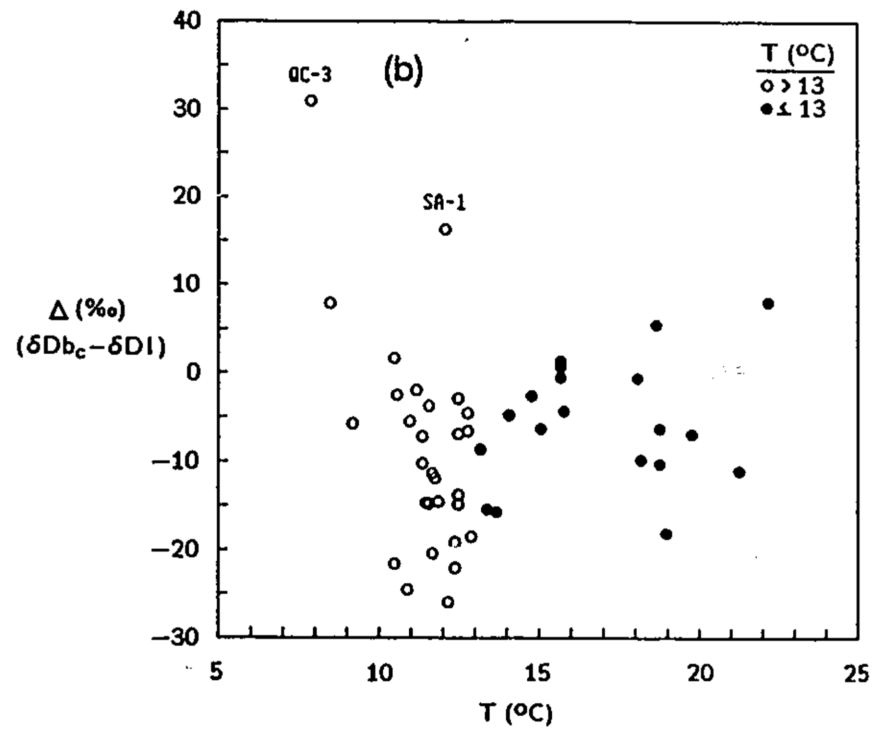
The Y-axis contains variability in $\delta^{18}Ob$ which is not directly explained by the variability in leaf water ($\delta^{18}Ol$) as calculated using eq. 2.6 (constants in text). Here the differences between bone phosphate and leaf water ($\delta^{18}Ob-\delta^{18}Ol$) are plotted against average growing season temperature (T). Data from cold climate areas ($T<13.0^{\circ}C$) are represented by open circles and those from warm climate areas ($T \geq 13.0^{\circ}C$) by closed circles.

Figure 9.3b. Direct Effects of T on δDb_c.

The Y-axis contains variability of deer bone δD corrected for H-exchange (δDb_c) which is not explained directly by the variability in the leaf water δD (δDl) as calculated using eq. 2.2 with $\epsilon k=12$. Here the differences between bone collagen and leaf water ($\delta Db_c-\delta Dl$) are plotted against average growing season temperature (T). See caption Fig. 9.3a for explanation of symbols.



(a)
T (°C)
○ > 13
● ≤ 13
SA-1
Δ (‰)
(δ18Ob−δ18Ol)
T(°C)
25
20
15
10
5
10
15
20
25



(b)
T (°C)
○ > 13
● ≤ 13
QC-3
SA-1
Δ (‰)
(δDbc−δDl)
T (°C)
40
30
20
10
0
−10
−20
−30
5
10
15
20
25

represents plant tissue derived from leaf-H_2O. Perhaps there is a bias in one of the variables in δDl or $\delta^{18}Ol$ ($\delta Z'l$) calculations for the cold or Canadian locations. Substituting RH_{day} in $\delta Z'l$ calculations for Canadian locations does not remove the T-bias. There does not appear to be bias in $\delta^{18}Ow$ since no systematic relationship between the residual variability ($\delta^{18}Ow-\delta^{18}Ow\hat{}$; eq. 9.11) and T was found. This could indicate that some biological or physical phenomena is operating in cold areas which affect $\delta Z'l$ values but are not accounted for by the leaf water model.

Model 2 eliminates the need to calculate $\delta^{18}Ol$ so only empirical regression coefficients are used to estimate RH. In this manner, any bias associated with the $\delta Z'l$ calculations would be eliminated. However, it produces RH^ values with the same accuracy as Model-1 and this suggests that a real physical phenomenon affecting isotopic fractionation in leaf-H_2O is producing a bias in results for cold climate areas.

Luz et al. (1990) showed that a contour relationship similar to Fig. 8.2 also exists for $\delta^{18}Ob$ vs $\delta^{18}Ow$. From Fig. 8.2 it is apparent that RH should be linearly related to the offset ($\delta Db_c-\delta Db_{c100}$). Therefore RH must also be linearly related to ($\delta^{18}Ob-\delta^{18}Ob_{100}$). Eq. 9.1 can now be adapted to show that $\delta^{18}Ob_{100} = 16.9 + 0.881\delta^{18}Ow$, since $\delta^{18}Ol=\delta^{18}Ow$ when RH=100. Therefore:

(eq. 9.14) $RH = \beta_0 + \beta_1(\delta^{18}Ob-\delta^{18}Ob_{100})$

for Model 2 with $\beta_0 \approx 100$ and $\beta_1 \approx (1/\beta_1') \approx (1/-0.180) \approx -5.56$ and where β_1' is the RH regression coefficient from eq. 9.3. Using the same arguments as above for $\delta^{18}Ow$, $\delta^{18}Ob_{100}$ can be estimated from δDb_c, $\delta^{15}N$, and δDv and then substituted into eq. 9.14. This was done here and further

regression analyses produced coefficients close to those expected (above) with an intercept (β_0) of 97% and slope (β_1) of -5.0±0.7 (n=31, σ=6.5, R=-0.808). As was the case for Model 1, further improvements occurred when the ($\delta^{18}Ob-\delta^{18}Ob_{100}$) term was squared giving the final equation to estimate RH^ that appears in the notes of Table 9.1. Regression statistics appearing in the table give: R=-0.824. Thus RH can be estimated using only empirical relationships and Model 2 with the same accuracy as Model 1.

Although RH can be estimated with considerable accuracy using Models 1 or 2 and only data from warm locations, when data from all locations are considered, this valuable climatic variable can only be estimated with moderate accuracy. Edwards and Fritz (1986) and Edwards (1987) had considerably more success in estimating RH using Model-5 on seven samples of nitrated wood cellulose. As indicated in Ch. 8, RH seems to have a clearer and more dramatic effect on δD_{cel} compared to δDb. If this were also true for $\delta^{18}O_{cel}$, it might explain why the $\delta^{18}O_{cel}$ data of Edwards appears to be better suited for estimating RH. In addition, the sensitivity of $\delta^{18}Ob$ to RH could be reduced over that of $\delta^{18}O$ due to the introduction of atmospheric O_2 to body-H_2O. This would reduce the slope between $\delta^{18}Ob$ and input H_2O ($\delta^{18}Ol$ or $\delta^{18}Odw$) and thus lessen the dependence of $\delta^{18}Ob$ on RH. Furthermore, it is presently unclear what proportion of the deer's body-H_2O derives from leaf-H_2O as opposed to environmental drinking-H_2O or whether the inter-site proportion of the water sources is constant. Nor is it known what proportion of the oxygens in body-H_2O might be introduced via O_2, H_2O and CO_2 in air or from dietary plant tissue. Water from more than one source and from sources other than leaf-H_2O could further weaken

the RH effects seen in $\delta^{18}Ob$. However, some theoretical uncertainties also persist in the $\delta^{18}O_{cel}$ work. These problems need to be resolved before there is a full understanding of how RH can be determined from δD_{cel} and $\delta^{18}O_{cel}$ and especially from $\delta^{18}Ob$ and δDb. Despite the above considerations, it seems possible that $\delta^{18}O_{cel}$ could provide a better estimate of RH than $\delta^{18}Ob$. This needs to be further tested for cellulose using a larger database than currently available in the literature.

CHAPTER 10

CARBON AND NITROGEN ISOTOPES OF DEER BONE COLLAGEN

Bone gelatin contains an accumulated lifetime average value of the $\delta^{15}N$ and $\delta^{13}C$ of an animals diet and under most circumstances both isotopic ratios appear to be quite stable during diagenesis (see Ch. 2,3,11). Most current applications of $\delta^{15}N$ or $\delta^{13}C$ to human paleodietary or food web studies require the additional assumptions that: (1) The isotopic values of the foods actually consumed are well known and that different foods are significantly different isotopically, (2) an average isotopic value for a food of interest can be applied globally and (3) the $\delta^{13}C$ and $\delta^{15}N$ of a food type remains stable over time. Other assumptions that (4) $^{13}\epsilon_B$ (the offset in $\delta^{13}C$ between animal collagen and diet) is a constant, known value and (5) $^{13}\epsilon_B$ does not vary according to the food type or physiological effects in animals seem to be valid for populations of deer (Ch. 2).

The discovery, in Africa, of direct climatic (Heaton et al. 1986; Ambrose and DeNiro 1986, 1987) and metabolic (Schoeninger and DeNiro 1984; Sealy et al. 1987) effects on $\delta^{15}N_g$ could complicate the interpretation of paleodietary or paleoecological studies (such as human paleodietary studies) based on the interpretations of amount of leguminous vs non-leguminous plants (i.e. DeNiro and Epstein 1981a), animal vs leguminous protein (i.e. Schwarcz et al. 1985; Ambrose and

DeNiro 1986b) or marine vs terrestrial protein (i.e. Schoeninger et al. 1983; Walker and DeNiro 1986) in diets (see Ch. 2). Such studies are based on the concept that leguminous plant protein is isotopically lower than that of other plants or animal protein and that terrestrial proteins will have lower $\delta^{15}N$ than marine ones. The African work suggests that any strong effects of amount of rain could lead to an over-estimate in humans of the amount of marine vs terrestrial protein in dry coastal areas and of animal vs plant protein in dry interior areas. Likewise, any strong metabolic effects could lead to over-estimates of animal or marine foods in hot dry areas where the local herbivores consume foods having low protein contents.

In this study, the evaluation of $\delta^{13}C$ and $\delta^{15}N$ of a single species of browsing ruminant herbivore from across North America provides a valuable means of evaluating the inter-site variations in environmental and food $\delta^{13}C$ and $\delta^{15}N$ which allows testing of assumptions 1 and 2, above. Knowledge of the isotopic values of the deer also provides information on a common dietary substance in human paleodiets of North America. In work presented below, the relationships between deer $\delta^{13}Cb$, $\delta^{15}Nb$ and climate will also be examined.

10.1 <u>$\delta^{13}C$ and $\delta^{15}N$ Results:</u>

The $\delta^{13}Cg'$ and $\delta^{15}Nb$ results for 75 deer from Table 7.4 are plotted according to sampling location in Fig. 10.1 and 10.2, respectively, with results for MUSK, BIS and OLD-C included for comparison. Here $\delta^{13}Cg' = \delta^{13}Cb - 0.6‰$ and $\delta^{15}Nb \approx \delta^{15}Ng$. Gelatin ($\delta^{13}Cg$) values were substituted where available. For both isotopes, all values discussed are

Figure 10.1 $\delta^{13}C$ of Modern Deer Plotted by Sampling Location.

$\delta^{13}Cg$ and $\delta^{13}Cg'$ of modern white tailed deer (*Odocoileus virginiana*; solid circles) and mule deer (*Odocoileus hemionus*; solid squares) from 47 locations across North America. $\delta^{13}Cg$ represent results on gelatins, whereas $\delta^{13}Cg'$ represent results on whole bone powders minus a contribution due to carbonate CO_2 ($\delta^{13}Cg'=\delta^{13}Cb-0.6‰$). Averages appear in locations for which there are more than one analyses or specimen. Included for comparison are a 100 y.B.P. muskox (MUSK) (*Ovibos moschatus*; solid triangles) and two bison (Bison sp.; crosses) from south central Alberta and the Yukon (BIS, 1500 y.B.P. and OLD-C, 12000 y.B.P., respectively).

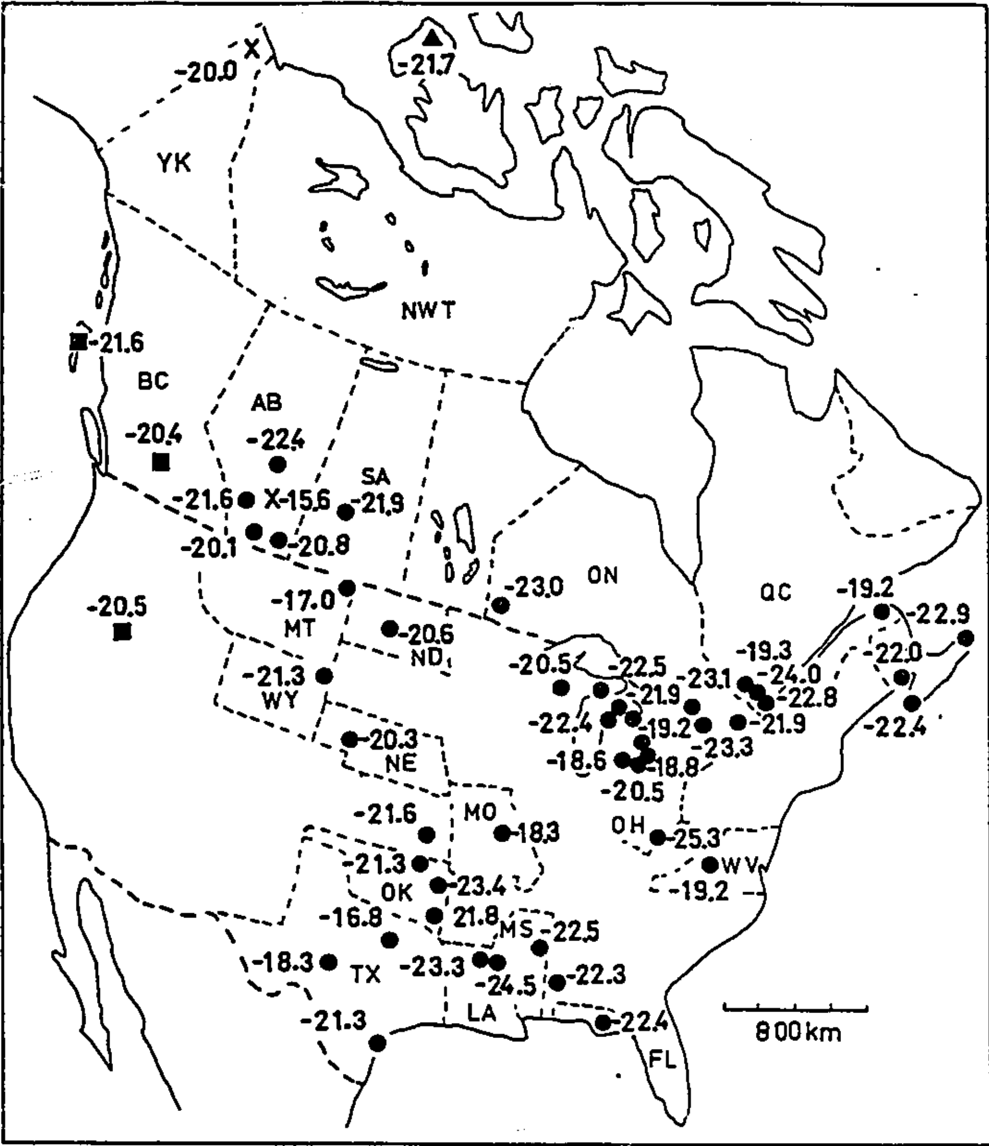

-20.0
X
-21.7
YK
NWT
-21.6
BC
AB
-20.4
-22.4
SA
-21.6
X-15.6
-21.9
-20.1
-20.8
-17.0
-23.0
ON
QC
-19.2
-22.9
-20.5
MT
-20.6
ND
-20.5
-22.5
-19.3
-24.0
-22.0
-21.3
WY
-23.1
-22.8
-21.9
-22.4
-19.2
-21.9
-22.4
-20.3
NE
-23.3
-18.6
-18.8
-20.5
-21.6
MO
-18.3
OH
-25.3
-21.3
WV
-23.4
-19.2
OK
-16.8
21.8
MS
-22.5
-18.3
TX
-23.3
-24.5
-22.3
LA
-22.4
-21.3
FL
800 km

Figure 10.2. $\delta^{15}N$ of Modern Deer Plotted by Sampling Location.

$\delta^{15}Nb$, $\delta^{15}Ng$ and $\delta^{15}Nc$ from modern white tailed deer (*Odocoileus virginiana*; solid circles) and mule deer (*Odocoileus hemionus*; solid squares) from 47 locations across North America. The $\delta^{15}Nb$ and $\delta^{15}Nco$ are assumed to be identical to $\delta^{15}Ng$ results. Averages appear in locations for which there are more than one analyses or specimen. Included for comparison are results on MUSK (*Ovibos moschatus*; solid triangle) and BIS (Bison sp.; crosses).

to be interpreted as representing gel preparations which can be directly compared to literature values. Average values appear for locations with more than one analysis. The δ^{13}Cg' and δ^{15}Nb values of FL-1 and MUSK are quite similar to results reported in the literature for a Florida deer (δ^{13}Cg=-22.0‰, δ^{15}Ng=5.8‰, Schoeninger and DeNiro 1984) and a 3870 y.B.P. Greenland muskox (δ^{13}Cg=-19.7‰, δ^{15}N=4.8‰, Nelson et al. 1986). All theoretical considerations and previous studies regarding interpretation of δ^{13}C and δ^{15}N results were discussed previously (Ch. 2).

For both isotopes, duplicate analyses are reproducible within error and results on different animals from nearby locations are very similar (Table 7.4) confirming previous studies and suggesting that diet within a restricted area is quite homogeneous. Exceptions are WV, OH and four locations in MI for δ^{13}C (σ=1.0 to 2.2‰) and OK-1,2 and TX-1,3,5 for δ^{15}N (σ=1.0 to 1.4‰). The reasons for this variability are not known but could include variations in diet of the individual deer (see below).

The average δ^{13}Cg' of all specimens (excluding OH-2, raised in captivity) was -21.3±2‰ (n=59). This is consistent with expectations for a browsing herbivore and is similar to δ^{13}Cg' values for MUSK and BC-4 which are from areas with no C_4 plants. In all, 75% of the animals had consumed less than 10% C_4 and 90% less than 20% C_4 plants (Table 7.4).

The generally low δ^{15}Nb values (ave. 4.2±2.0‰, n=60) may result from the deer browsing on woody plant forms or from browsing on agricultural lands treated with artificial fertilizers. However, fossil mule deer from California (δ^{15}N=4.9±0.8‰, n=7; Walker and DeNiro 1986), modern reindeer (*Rangifer tarandus*) from Greenland (δ^{15}N=2.9±0.6‰, n=8; Nelson et al. 1986), fossil muskox in the literature (above) and MUSK and BC-4 all have low δ^{15}N despite the fact that agriculture is unlikely to have

influenced any of these results. In addition, BIS from Alberta shows a high $\delta^{13}Cg'$ value indicative of grazing (Fig. 10.1) but a $\delta^{15}Nb$ value only slightly higher than modern deer from a nearby location (Fig. 10.2). This could suggest that grazing in this environment is not much different from browsing with respect to $\delta^{15}N$ and that soil-N values in Alberta have not changed greatly over time, despite present day agricultural practices.

10.2 Inter-Site Variation in $\delta^{13}C$ and $\delta^{15}N$:

The range in values is 9.7‰ for $\delta^{13}C$ and 13.8‰ for $\delta^{15}N$ indicating large intersite variations. There is a geographical clustering of both $\delta^{13}C$ and $\delta^{15}N$ results (Fig. 10.1 and 10.2). Information concerning local climatic variables, agricultural practices and vegetation for each area in conjunction with the recorded diets of some of the deer (Table 7.4 and App. B) suggests several reasons for the clustering.

10.2.1 Areas of High $\delta^{13}C$ and Low $\delta^{15}N$:

High $\delta^{13}C$ values in the central U.S. and Great Lakes regions indicate that deer have consumed as much as 34% C_4 plant material. A number of deer in Michigan had access to agricultural lands and some are known to have eaten some corn (MI-1,4,5,11,12,13,15). This explains their higher $\delta^{13}Cg'$ values (-17.8 to -19.4‰) compared with other nearby areas in Michigan and Ontario (-21.9 to -23.3‰). Dietary corn can also explain the higher results of MO-4 and WV-1,2,3. Probable consumption of conifers (MI-2,3,6,8,9,10,14) does not appear to greatly affect results. The unusual δDb and nature of sample for QC-3 (Ch. 8) could indicate that the relatively high $\delta^{13}C$ of this sample is also anomalous.

Low $\delta^{15}N$ values in the Great Lakes area could reflect: (1) increased amounts of fixed-N_2 in soils arising from high moisture content or other conditions favorable to N_2-fixation, (2) increased contribution to soils of fixed-N_2 from the cultivation of bean or soybean legumes, (3) agricultural use of artificial fertilizers and (4) likely inclusion of beans, soybeans or other legumes directly in the diets of several of the deer (MI-1,4,5,11,12,13,15). Thus low $\delta^{15}N$ associated with high $\delta^{13}C$ in the Great Lakes region as well as in West Virginia, Missouri and Central BC (where BC-2,3 ate alfalfa, a legume) could reflect the simultaneous effects of agriculture on both isotopes. Alfalfa was also consumed by WY-2 and accounts for its low $\delta^{15}N$.

10.2.2 <u>Areas of High $\delta^{13}C$ and High $\delta^{15}N$:</u>

Occasional grazing by the animals due to starvation or climatic effects (low RH, high T) directly on plants may produce the higher $\delta^{13}C$ in the drier areas with high T (i.e. TX-1, TX-6, MT-1, AB-4,5, AB-6,10, OR-1, BC-2,3, ND-1). Some animals such as WY-2 may have eaten some conifers but only extensive use of such browse would significantly alter the animal's $\delta^{13}C$. Therefore, consumption of C_4 plants, through grazing, would be a more probable explanation of higher $\delta^{13}C$. However, any consumption of corn among those exposed to agricultural crops (MT-1, WY-2) could produce results indistinguishable from direct climatic effects or occasional grazing.

Low contributions of fixed-N_2 to soils in warm dry areas, agricultural use of crop pastures and animal manures and direct effects on the deer of H_2O stress or low protein amount effects may account for the clustering of high $\delta^{15}N$ values in dry areas with high summer

temperatures such as northern Montana, North Dakota, Saskatchewan and southern Alberta. Therefore, moderately high to high $\delta^{15}N$ associated with high $\delta^{13}C$ values in southern Alberta, northern Montana, and central and northern Texas could indicate food and H_2O shortages as well as a combination of effects from low protein content of foods (C_4 plants in diet) and low amounts of rainfall (see below). High $\delta^{15}N$ values in Oklahoma compared to lower values elsewhere in this state illustrate that soil $\delta^{15}N$ and dietary conditions can change considerably over short distances.

10.2.3 Areas of Low $\delta^{13}C$ and Low $\delta^{15}N$:

The cluster of low $\delta^{13}C$ associated with low $\delta^{15}N$ values in Mississippi, Ohio, Western Ontario and the Great Lakes areas may be explained by deep forest effects such as a canopy effect on $\delta^{13}C$ along with an effect on $\delta^{15}N$ from increased N_2-fixation. Whereas sea spray effects do not appear to have greatly increased the $\delta^{15}N$ values along the coastal areas of North America, clusters of low $\delta^{13}C$ along the east coast of Canada and in the U.S. from Florida to Louisiana may be due to the canopy effect. The very low $\delta^{15}N$ for BC-4 from Queen Charlotte Island could be due to enhanced N_2-fixation in the soils of this temperate rain forest. The very low $\delta^{15}N$ for AZ-1 remains difficult to explain but could be due to unusual feeding patterns, low soil $\delta^{15}N$ or an undetected problem in N_2 sample preparation.

10.3 Relationships to Climatic Parameters:

Several studies have suggested that $\delta^{13}C$ or $\delta^{15}N$ might prove useful for determining paleoclimate or paleoecology (Ch. 2). The large number of analyses of deer in this study represents a robust data base for testing variability and climate effects on the isotopic results of a single species of mammal. Plots of $\delta^{13}C$ and $\delta^{15}N$ against all climatic variables were visually examined for any obvious trends, for curvilinearity and for outlying results. Using 55 deer results for which both $\delta^{13}Cb$ and $\delta^{15}Nb$ results were available, linear and multilinear regression analyses were conducted to search for any relationships between the isotopic results and climatic variables. Results on QC-3 and the gel results of LA-8 and ND-1 were excluded from all regressions. All previous studies used for interpreting the effects of climate on $\delta^{13}C$ and $\delta^{15}N$ are summarized in Ch. 2.

There were no significant ($p>0.98$) linear or curvilinear relationships between $\delta^{13}C$ and any climatic variable examined including PPTy, PPT, RHy, RH, Ty and T. Although the proportion of C_4 plants among grasses correlates with summer temperature, deer are browsers so no such correlation with T would be expected. Nonetheless, for a subgroup of 13 deer consuming more than 10% C_4 plants ($\delta^{13}Cg'>-20.1‰$), the highest correlation of $\delta^{13}C$ was with T ($r=0.568$). For the remaining 42 deer which had consumed less than 10% C_4 plants, the highest correlation of $\delta^{13}C$ was with RH ($r=-0.300$). Therefore, the $\delta^{13}C$ patterns in the deer consuming low amounts of C_4 plants seem to resemble those of $\%C_4$ found among dicots in North America whereas those consuming more C_4 plants ($\%C_4>10$) more closely reflect $\%C_4$ found among grasses. This suggests that the highest $\delta^{13}C$ values in deer reflect some grazing,

possibly the result of reduced browsing opportunities in areas with high T and low PPT.

Previous studies have found a negative correlation between $\delta^{15}N$ and annual amounts of rain (Heaton et al. 1986, Ch. 2). Therefore, plots of $\delta^{15}N$ vs PPTy for deer were examined here. In Fig. 10.3a, it is shown that the $\delta^{15}Nb$ results of the 42 deer consuming low amounts of C_4 plants (open circles) do not correlate with local amounts of rainfall ($r=-0.074$) despite a large range in PPTy from 1.5 cm/mo (180 mm/yr) to 13.0 cm/mo (1560 mm/yr). In contrast, a negative trend is noted between $\delta^{15}N$ and PPTy for the 13 animals having consumed more than 10% C_4 plants ($r=-0.669$, $p>0.98$). Thus most deer which consumed low amounts of C_4 plants have low $\delta^{15}N$ even in areas of low PPTy whereas deer consuming high amounts of C_4 plants show low $\delta^{15}N$ in areas with high PPTy; slightly higher $\delta^{15}N$ values in areas with PPTy<7.1cm/mo (<850mm/yr) and considerably higher $\delta^{15}N$ in areas with PPTy<3.3cm/mo (<400 mm/yr).

In Fig. 10.3b data extrapolated from Heaton et al. (1986) appears with the subset of 14 deer from this study which had consumed >10% C_4 (including QC-3). Both sets of data show similar trends. Therefore, only the deer consuming higher amounts of C_4 plants resemble the trends found for mammals in Africa.

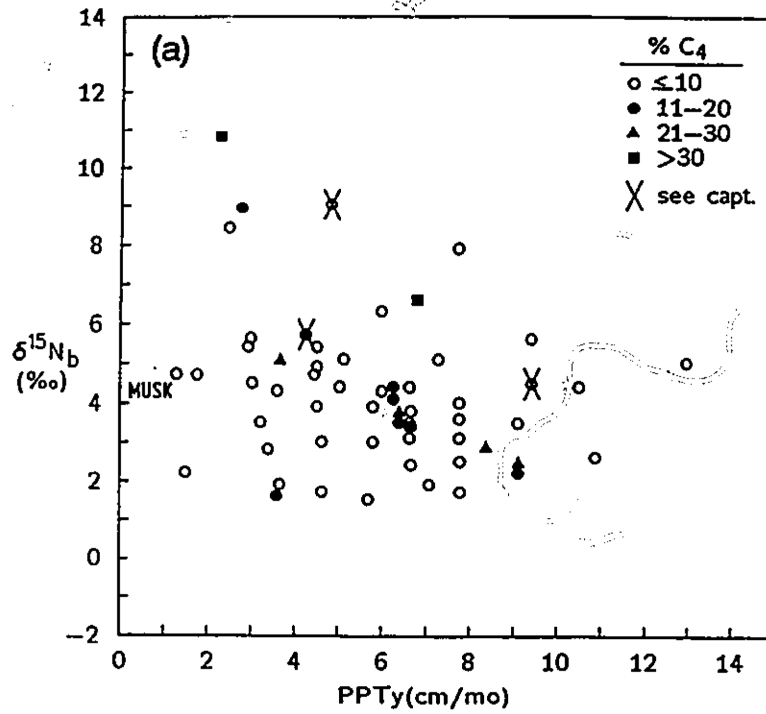
High $\delta^{15}N$ among animals consuming C_4 plants in areas of low PPTy may be accounted for by a combination of two explanations found in the literature (Ch. 2). In such areas, some animals may consume dry adapted plants including C_4 plants or grasses which may have lower protein content or quality and thus lower their intake of bioavailable

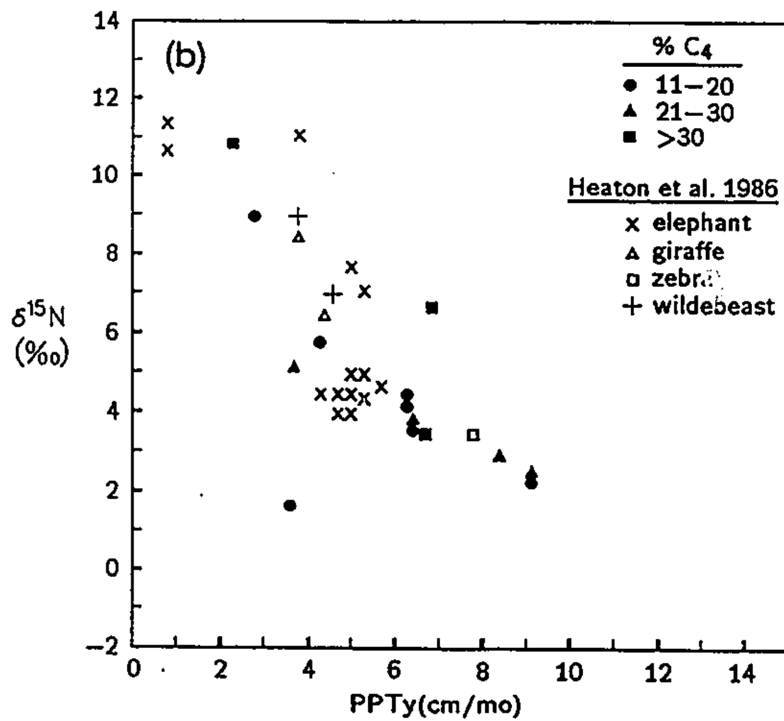
Figure 10.3a. $\delta^{15}Nb$ vs PPTy of Deer.

The $\delta^{15}Nb$ results of 56 specimens of deer are plotted against annual average of monthly amount of precipitation (PPTy). MUSK results are included for comparison. Symbols associated with X are of LA-8, ND-1, and QC-3, and are excluded from all regression statistics.

Figure 10.3b. $\delta^{15}Nb$ vs PPTy Results on African Animals Plotted With the Deer Consuming More Than 10% C4 Plants.

The $\delta^{15}Nb$ results of 14 specimens of deer consuming more than 10% C4 plants are plotted against annual average of monthly amount of precipitation (PPTy). Results for $\delta^{15}N$ and PPTy of African animals have been extrapolated from graphs presented in Heaton et al. (1986) and are included for comparison.


(a)
% C4
≤10
11–20
21–30
>30
see capt.
δ15Nb (‰)
MUSK
PPTy(cm/mo)


(b)
% C4
11–20
21–30
>30
Heaton et al. 1986
elephant
giraffe
zebra
wildebeast
δ15N (‰)
PPTy(cm/mo)

protein. A maximum of 4% of the dicots in an area would be C_4 plants. Therefore, >10% C_4 plants in the deers diet could indicate subtantial consumption of grasses since in most North American areas <50% of the grasses are C_4 plants (Teeri and Stowe 1976, Ch. 2). Some of the highest $\delta^{15}N$ occur in the MT-1, AB-4 and AB-5 animals which are from areas with <1% C^4 plants among dicots and <20% C^4 plants among grasses. Grass consumption, in turn, would indicate food shortages and environmental stress. These animals might conserve nitrogen through increased microbial food processing in the rumen including increased recycling of urea into the rumen and thus increase the trophic level of the protein (from digested bacteria) made available to the animal (Sealy et al. 1987). At the same time, lower amounts of moisture in such plants coupled with lower amounts of H_2O in the environment might produce H_2O-stress for these animals for which they compensate by excreting concentrated urine. With the increase in concentration of the urine there is a disproportionate increase in excretion of ^{15}N-depleted urea into the urine and an overall increase in the loss of nitrogen (Ambrose and DeNiro 1986a, 1987). Thus animals undergoing water stress which consume low amounts of protein may provide the additional nitrogen necessary through increased microbial recycling of nitrogen. Both H_2O-stress and low protein amount effects would increase $\delta^{15}N$ and both may need to operate simultaneously so that their combined effects can be seen above variability in $\delta^{15}N$ due to all other causes including variability in soil $\delta^{15}N$, or consumption of browse from N_2-fixing plants or woody vs non-woody growth forms (see Ch. 2). It is also possible that H_2O-stress may be the cause of or occur at the same time as food shortages so the animals are forced to graze during seasonally arid

conditions. This could produce a coincidental relationship between $\delta^{15}N$ and both PPTy and $\delta^{13}C$ in arid areas.

In contrast, variability dominates the subset of 42 animals consuming low amounts of C_4 plants. Browsers which consume C_3 plants may derive sufficient protein and water from the leaves they eat so that variation in $\delta^{15}N$ which is correlated to PPTy cannot be distinguished above variability arising from the other causes. The three animals with high $\delta^{15}N$ despite low consumption of C_4 plants are from areas of relatively low PPTy (SA-1, ND-1 and OK-5). Perhaps, a combination of consumption of some C_3 plants with poor quality protein, water stress, and high soil $\delta^{15}N$ can still affect a small minority of C_3 consumers without producing a distinct correlation for the group as a whole. It would be interesting to know if most herbivores in the African studies had $\delta^{13}Cg$ values greater than -20.1‰ as this might explain why there appears to be a relationship between $\delta^{15}N$ and PPTy in those studies but not for the North American deer of this study.

While the above combination of effects can explain the high $\delta^{15}N$ among C_4 consumers in low PPTy areas, it does not explain the apparently low $\delta^{15}N$ among C_4 consumers in high PPTy areas. Perhaps in the high PPTy areas of this study, results are dominated by agricultural effects which increase $\delta^{13}C$ while lowering $\delta^{15}N$. This could occur in agricultural areas of low soil $\delta^{15}N$ and where the deer might consume both corn and legumes. The one sample from an area of low PPTy with low $\delta^{15}N$ despite consumption of C_4 plants (QC-8) is from an area where other deer (QC-1, QC-2) have low $\delta^{15}N$ and have not consumed C_4 plants. Ample water supply, along with above mentioned

agricultural effects, are likely explanations for the low $\delta^{15}N$ and high $\delta^{13}C$ of this animal.

For both groups of animals (i.e. those consuming >10% and <10% C_4 plants) weak correlations of $\delta^{15}N$ with $\delta^{13}C$ were found, suggesting some protein amount effects may affect all animals (r=0.240, n=42 for $\%C_4<10$; r=0.519, n=13 for $\%C_4>10$). A significant (p>0.98) relationship was found between $\delta^{15}N$ and RH (r=-0.365, n=55) confirming previous observations in which $\delta^{15}N$ was found to be a proxy variable for RH (Ch. 8). However, while a significant relationship was found between $\delta^{15}N$ and RH for the 13 animals consuming more C_4 plants (r=-0.907), no such relationship was found for the 42 remaining deer consuming few C_4 plants (r=-0.080). Thus climatic effects, in general, seem only to dominate in animals consuming more C_4 plant foods.

Results on all 55 animals were also examined for relationships to climatic variables by using stepwise multiple linear regression. Three transformations of $\delta^{15}N$ ($\delta^{15}N$, $(\delta^{15}N)^2$, $\ln|\delta^{15}N|$) were regressed in turn against the following 18 climatic variables (including transformations): RH, ln(RH), RH^2, PPT, ln(PPT), PPT^2, T, ln(TK), T^2, RHy, ln(RHy), RHy^2, PPTy, ln(PPTy), $PPTy^2$, Ty, ln(TKy), Ty^2 as well as against three transformations of $\delta^{13}C$ ($\delta^{13}C$, $\delta^{13}C^2$, $\ln|\delta^{13}C|$). Best fit results given in eq. 10.1 confirmed above observations in that $\delta^{15}N$ increases in areas of low PPTy, high T and with increased consumption of C_4 plants having higher $\delta^{13}C$ (R=0.528, p>0.98):

(eq. 10.1) $$\delta^{15}N^2 = -3530 - 20.3\cdot\ln(PPTy) + 661\cdot\ln T(^{\circ}K) - 50.0\cdot\ln|\delta^{13}C|$$

All variance inflation factors (VIF, App.E) were less than 2, indicating negligible covariation among predictor variables.

The above suggests that a number of variables simultaneously affect the $\delta^{15}N$ results. The earlier noted correlation of $\delta^{15}N$ with RH is likely due to a decrease in RH with both decreasing PPTy and increasing T (Ch. 7), two variables used in the multilinear regression (eq. 10.1). Therefore, both the effects of water stress and low protein content in foods appear to be factors affecting $\delta^{15}N$ in addition to soil and plant $\delta^{15}N$ and to animal diet.

10.4 Implications for Paleodietary Research

From the above it appears that the proportion of C_4 plants in the deer's diet dominate the inter-site variability in $\delta^{13}C$ but considerable influences may also arise from the canopy effect. Markedly fewer influences could potentially arise from direct climatic effects on plants and, perhaps, from extensive consumption of conifers.

A combination of several factors appears to affect inter-site variability in $\delta^{15}N$. Soil nitrogen $\delta^{15}N$ probably dominates but significant influences may derive from low protein content of foods in drier, hotter areas and from direct climatic effects on the animals in such areas. Agricultural effects appear to dominate in areas of high rainfall. Additional effects may be due to consumption of browse from N_2-fixing plants or from woody vs non-woody growth forms.

The above observations have several implications for paleodietary studies which rely on the interpretation of $\delta^{15}N$. An average $\delta^{15}N$ value for plants or animals cannot be assumed to apply globally. Further, since soil and climatic conditions can change over

time, it cannot be assumed that the $\delta^{15}N$ in the food chain has remained constant over time. The low $\delta^{15}N$ of deer in this study and from non-agricultural areas and times also indicates that it cannot be assumed that there will be sufficient difference between some foods of interest to paleodietary studies such as legumes vs non-legumes.

It is proposed, here, that all human paleodietary studies using $\delta^{15}N$ should be accompanied by $\delta^{15}N$ values of a contemporaneous local herbivore to establish baseline estimates for local plants and terrestrial animals on which dietary interpretations of humans can be based. In most areas not affected by food or H_2O shortages, the deer $\delta^{15}Nb$ should provide an estimate of both average plant values and soil $\delta^{15}N$ values since the deer collagen $\delta^{15}N$ values are offset by ≈+3‰ relative to the average plants in an area which, in turn, are ≈-3‰ relative to soil $\delta^{15}N$. If additional studies confirm that animal flesh or muscle is ≈+2.5‰ higher than collagen (cf. DeNiro and Epstein 1981a) then humans consuming 100% deer flesh might be ≈+5.5‰ relative to those consuming 100% of most non-leguminous plant foods. The two end point values are within 4σ of each other ($\sigma \approx \pm 1.5$‰) indicating reduced ability to distinguish consumption of animal from plant foods. However, consumption of animal protein may be distinguishable from that of leguminous protein in areas where the deer (and soil) $\delta^{15}N$ values are above ≈4‰. In coastal areas of low soil $\delta^{15}N$, marine vs terrestrial differences should also be quite obvious since humans consuming only marine foods or anadromous fish should have $\delta^{15}N$ values greater than ≈12‰ (Sealy et al. 1987; Schoeninger et al. 1983; DeNiro 1985).

Although modern agriculture has undoubtedly altered deer diets and soil $\delta^{15}N$, soil values also reflect an interplay of numerous

other geographical, biological and climatic variables which, in turn, inevitably affect the $\delta^{15}N$ values of the entire terrestrial food chain. Without analysis of a contemporaneous herbivore, such as deer, along with the human analysis, there can be no reliable estimate of past soil, plant, or animal $\delta^{15}N$ nor would it be possible to chart $\delta^{15}N$ changes through time.

While $\delta^{13}C$ results seem to be more straight forward to interpret than $\delta^{15}N$, they can also vary considerably between locations due to factors such as canopy effects, or grazing which could well have existed in prehistoric times. This challenges the assumption that an average isotopic value for at least one important food item in paleodiets, specifically the deer, can be applied to all locations. For this reason paleodietary studies using $\delta^{13}C$ could also benefit from the $\delta^{13}C$ analysis of a contemporaneous, local herbivore such as the deer.

CHAPTER 11

FOSSIL BONE IN THE ARCHAEOLOGICAL SETTING

-EFFECTS OF WEATHERING AND COOKING-

Diagenetic change, contamination and cooking could alter the isotopic signature of archaeological bone. Before any application of δDb to paleoclimate can be initiated such effects must be evaluated and distinguished from changes due to climate. In this chapter, the effects of diagenesis and cooking on bone isotopic results will be examined. All theoretical considerations and previous studies were summarized in Chs. 1 and 3.

Effects of diagenesis were tested using modern and fossil seal. The 100 y.B.P. SEAL standard is of ringed seal (*Phoca hispida*) from Banks Island. It, plus a sub-sample of this standard, were used as a control for "modern" δD values. Two fossil ringed seal, one from Banks Island and one from Herschel Island and four fossil harbor seal (*Phoca vitulina*) from two separate locations in coastal British Columbia were also analyzed. Sample descriptions, locations and initial observations on preservational state are given in App. B and are summarized in Table 11.1. Initial observations on the state of the bone samples classified them as being either dense, well preserved with apparently good recovery of collagen or light weight, porous, apparently poorly preserved with low recovery of collagen. Color and staining were also noted.

Table 11.1 Summary of Initial Observations on MUSK, Fossil Seal and Bison Samples.[a]

	Stain	Species		Age	Location
		Harbour	Ringed	(y B.P.)	
DENSE					
Seal 1	none		X	100	Banks I., NWT
Seal 2	dk brown		X	2500	Banks I., NWT
Seal 5	light brown[b]		X	500-950	Herschel I., NWT
Seal 6	none[c]	X[d]		100-600	near Kwatna, BC
POROUS					
Seal 7	light buff	X[e]		200-1200	Kwatna, BC
Seal 4	buff	X		3400	Namu, BC
Seal 3	light buff	X		4500	Namu, BC
OTHERS					
BIS (porous)	light brown	*Bison sp.*		1500	Drumheller, AB
OLD-C	brown	*Bison sp.*[f]		12000	Old Crow, YK
MUSK	none	*Ovibos moschatus*		100	Banks I. NWT

a tibia unless indicated otherwise
b "washout site"
c waterlogged site
d humerus
e femur
f rib

A distinct disadvantage of using seal is that seal tibia bone does not resemble tibia of terrestrial mammals in its physical characteristics. While the long bones of large terrestrial mammals are composed mostly of thick, cortical bone, seal bone is porous and composed almost entirely of cancellous bone which is considerably less resistant to weathering. Stained areas are difficult to remove mechanically. A diagenetic study of bone from terrestrial mammals would be preferred and may be possible using both δD and $\delta^{18}O$ analyses.

An additional problem with the tests described here is that inferences about modern δD values for seal were made using only one modern control sample. Therefore, it must be assumed that all the seal fed at similar trophic levels, since there could be large trophic level effects on δD. It is also necessary to assume that there has not been a large introduction of terrestrial organics into the food chain at any particular location and that seal feeding patterns have not changed greatly over time. As discussed below, the isotopic results can be used to evaluate the correctness of above three assumptions. However, in the future, natural variability in modern seal should be evaluated using a larger number of samples. Results presented here are to be considered as only a preliminary evaluation of the potential effects of diagenesis.

11.1 Methods:

Bone samples of seal tibia, BIS and OLD-C were carefully precleaned and w.b. powders prepared or gels extracted as described in Ch. 4. After the cancellous bone was removed from the seal, only thin slices, (≈1-2mm thick), of more compact bone from the outer rim of the

tibia remained. It was not possible to mechanically remove all stained areas from such samples. A similar problem existed with OLD-C as rib has very little compact bone. Well preserved cancellous bone was included in the preparations of Seal-0 and Seal-1. For all samples, the degreasing step was eliminated since it was assumed that compact bone is essentially lipid-free and that any lipids would have been removed by natural processes following deposition.

Sub-samples of gels were sent to Simon Fraser University where they were analyzed for their amino acid content by Tom Brown using standard preparation procedures and high pressure liquid chromatography.

11.2 Amino Acid Analyses:

The amino acid contents are given in Table 11.2. The hydroxyproline (HPRO) content of all seals confirms that "gel" extracts are composed of collagen but there is no consistent relationship between HPRO content and "porous" vs "dense", categories of preservation despite the anticipation of diagenetic removal of HPRO for the "porous" group (Wyckoff 1972; Hare 1980; Hassan and Hare 1978). Valine (VAL) is lowest in the best preserved fossil sample (Seal-6) as might be expected from the literature (Nelson et al. 1986). Seal-2 initially noted for its dark brown stain (App. B), has the highest VAL content. However, the variability in amino acid concentrations is large (±5% to 10%; Dr E. Nelson, pers. comm.) indicating that only very large shifts in amino acid contents will be useful for identifying diagenesis compared to other indicators (below).

Table 11.2 Amino Acid Analyses of Seal Gelatins.[a]

Amino Acid	Human	Modern Seal[b]	Seal-2	Seal-5	Seal-6	Seal-7	Seal-4
HPRO .	10.3	9.8	6.37	6.16	6.61	7.06	6.42
ASP	4.3	4.9	3.96	3.53	3.43	4.57	3.9
THR	1.7	2.0	2.59	2.62	2.54	2.99	2.72
SER	3.3	4.3	4.29	4.62	4.15	4.93	4.33
GLU	7.1	8.1	5.53	6.42	4.65	5.13	6.05
PRO	12.0	11.7	10.46	10.44	10.25	11.4	11.04
GLY	33.5	32.5	28.17	29.3	29.68	30.79	29.46
ALA	11.1	11.4	8.51	8.89	8.83	9.54	8.93
VAL	2.6	1.8	9.58	8.51	4.65	5.62	8.62
MET	0.6	0.5	0.53	0.71	0.46	0.65	0.61
ILEU	0.9	0.7	1.21	1.22	0.84	1.39	1.29
LEU	2.3	2.6	3.13	2.94	2.12	3.12	2.97
TYR	0.2	0.4	0.39	0.41	0.34	0.43	0.15
PHALA	1.2	1.4	1.21	1.35	1.4	1.59	1.1
LYS	2.3	2.4	4.02	4.76	1.75	2.52	2.57
HIS	0.6	0.7	4.31	2.38	12.56	2.48	4.09
GLY/ASP	7.8	6.6 8.6[c]	7.1	8.3	8.7	6.7	7.6

a amino acid concentrations in mole%.

b modern values for harbour seal (*Phoca vitulina*) obtained from the literature (Nelson et al. 1986)

c value estimated from relationship shown in Fig. 11.5

In light of the potential for inter-laboratory differences in calibration, it can not be unambiguously determined whether low HPRO contents of all seals compared to modern harbor seal (Nelson et al. 1986) represents increased weathering. Comparison of seal HPRO to the modern human control (which has HPRO values close to modern harbor seal) might indicate diagenetic influences on all seal. However, all samples appear to have unusual contents of valine (VAL) and histadine (HIS) with results from "dense" samples or relatively unweathered sample (Seal-6) not approaching "modern" values in the literature. Although the uncertainty in the glycine to aspartic acid ratios (GLY/ASP) is likely to be high (±0.6 to ±1.2), ratios for all "dense" samples appear higher than those calculated for modern ox-bone collagen (GLY/ASP=7.2, Ch. 3) or those in the literature for gelatin extracts (GLY/ASP≈7.5; Ho 1965,1966; Wyckoff 1972; Matter and Miller 1972; Hedges and Wallace 1980; Nelson et al. 1986; DeNiro and Weiner 1988a). This suggests a systematic offset for all GYL/ASP values of this study and, possibly, an overall calibration problem for many amino acid concentrations. The significance of the high HIS of Seal-6 is not clear.

11.3 Isotope and Yield Results:

All isotope and yield results are given in Table 11.3. The seal δD are high relative to MUSK and other terrestrial mammals. Correcting for H-exchange and knowing that there are no RH effects on marine plants, it is possible to estimate the offset between the bone corrected for RH effects and source water: (δDb_{c100}-0‰) is 121‰ for Seal-1, (δDb_{c100}-δDw) is 6‰ for MUSK from the same site as Seal-1 and,

Table 11.3 Comparative Results On Modern and Fossil Seal, Bison, Deer and MUSK

	ISOTOPES				YIELDS					
Samples	δD	$\delta^{13}C$	$\delta^{15}N$	$\delta^{18}Ob$	C/N	H/N	H/C	N/g	C/g	H/g
GELATINS (g)[a]										
Seal-1 (n=3)	90±3	-16.0	18.3±0.1	-	103±1	94±0	92±1	83±2	84±3	78±2
Seal-2	44	-16.0	18.8	-	111	99	90	72	79	71
Seal-5	101	-14.2	18.0	-	101	89	88	76	77	68
Seal-6	85	-11.3	16.1	-	105	94	90	66	69	62
Seal-7	112	-13.1	16.6	-	101	97	96	77	78	75
Seal-4	121	-10.4	18.9	-	105	97	92	57	60	55
Seal-3	166	-10.4	19.3	-	106	98	93	72	76	71
ave:	103±37	-15.4±1.0[b] -11.3±1.3[c]	18.0±1.2		105±3.5	95±3.4	92±2.6	72±8.4	75±7.9	69±7.8
BIS	-69	-15.6	6.0	-	99	101	102	73	72	74
MUSK (n=5)	-145±2	-21.4±0.5	3.6±0.5	-	104±2	93±3	90±2	89±3	93±2	83±2
Modern Deer (n=14)	-	-	-	-	99±3	93±5	94±4	82±3	81±3	76±5
WHOLE BONE (b)[a]										
Seal-1	58	-15.8	18.4	15.3	107	99	92	69	74	68
Seal-2	36	-15.3	18.7	-	101	89	88	84	85	75
Seal-5	58	-14.6	18.4	-	104	90	87	71	74	64
Seal-6	88	-11.7	16.5	-	98	92	93	84	82	77
Seal-7	104	-15.7	17.2	-	109	102	94	52	56	53
Seal-4	93	-12.6	18.7	-	135	105	78	30	41	32
Seal-3	112	-11.1	18.5	-	104	91	88	65	67	59
ave:	[illegible]	-15.2±0.6[b] -12.8±2.0[c]	18.1±0.9		108±12	95±6	89±5	65±19	68±15	61±15
BIS (n=7)	-67±2	-13.9±0.6	5.9±0.2	13.1	98±7	93±10	95±6	57±5	55±5	54±6
AB-7 (n=3)[d]	-92	-21.0±0.2	5.5±0.1	11.5	98±6	91±3	93±6	61±6	65±11	67±8
OLD-C	-119±12	-19.4±0.1	-	4.9	-	-	79±1[e]	-	92±3	65±2
MUSK (n=6)	-141±3	-20.6±0.4	4.7±0.1	2.7	98±2	91±3	94±4	87±3	84±4	79±3
Modern Deer (n=75)	-	-	-	-	98±5	91±3	93±4	85±4	86±6	76±4

a not corrected for H-exchange; corrected δDb_c results for MUSK, BIS and OLD-C appear in Table 7.3. $\delta^{13}Cb$ results not corrected for carbonate contribution

b high latitude samples, n=3

c low latitude samples, n=4

d modern deer sample from location close to BIS for comparison

e low H-yield due to early sample preparation (prep 1) -see Table 7.3 notes and Ch. 5

on average, is −3.2‰ for 75 deer analyses (Table 7.3). The enrichment in seal is likely due to the large increase in δD with trophic level with marine mammals, such as the seal, representing higher trophic levels than terrestrial mammals.

The average amount of collagen present in the fossil seal bone, as estimated from H/g_b yield, is lower in the "porous" (48±14%) than in the "dense", well preserved (72±7%) samples and lower than modern deer (76±4%, n=75) and modern Seal-1 (68%). Clearly, diagenetic leaching of collagen has occurred in the seals labeled as "porous". Overall, there is a very large variation in the δD values (σ=±37‰ for δDg and σ=±28‰ for δDb) with ranges of 122‰ for δDg and 76‰ for δDb. This may be compared with the pooled standard deviation (σ_p) of δDb for modern deer from nearby locations of only ±3‰ (n=29, Table 7.3). Therefore, variability in δDg of the seal is more than 12x that of modern mammals from the same area. It will be argued that relatively little of this large variability can be attributed to either contamination or natural variation in seal diet but that diagenesis is the most plausible cause.

11.4 Use of $\delta^{13}C$ and $\delta^{15}N$ for Distinguishing Effects of Diet or Contamination on δD.

It must now be determined, on the basis of $\delta^{13}C$ and $\delta^{15}N$, whether dietary influences or contamination can account for a significant portion of variation in δD of the seal. A decrease in δD due to contamination or introduction of terrestrial materials into the seal diet would also cause a decrease in $\delta^{13}C$. A change in δD due to trophic level effects should also show a concomitant change in $\delta^{15}N$. Thus, all

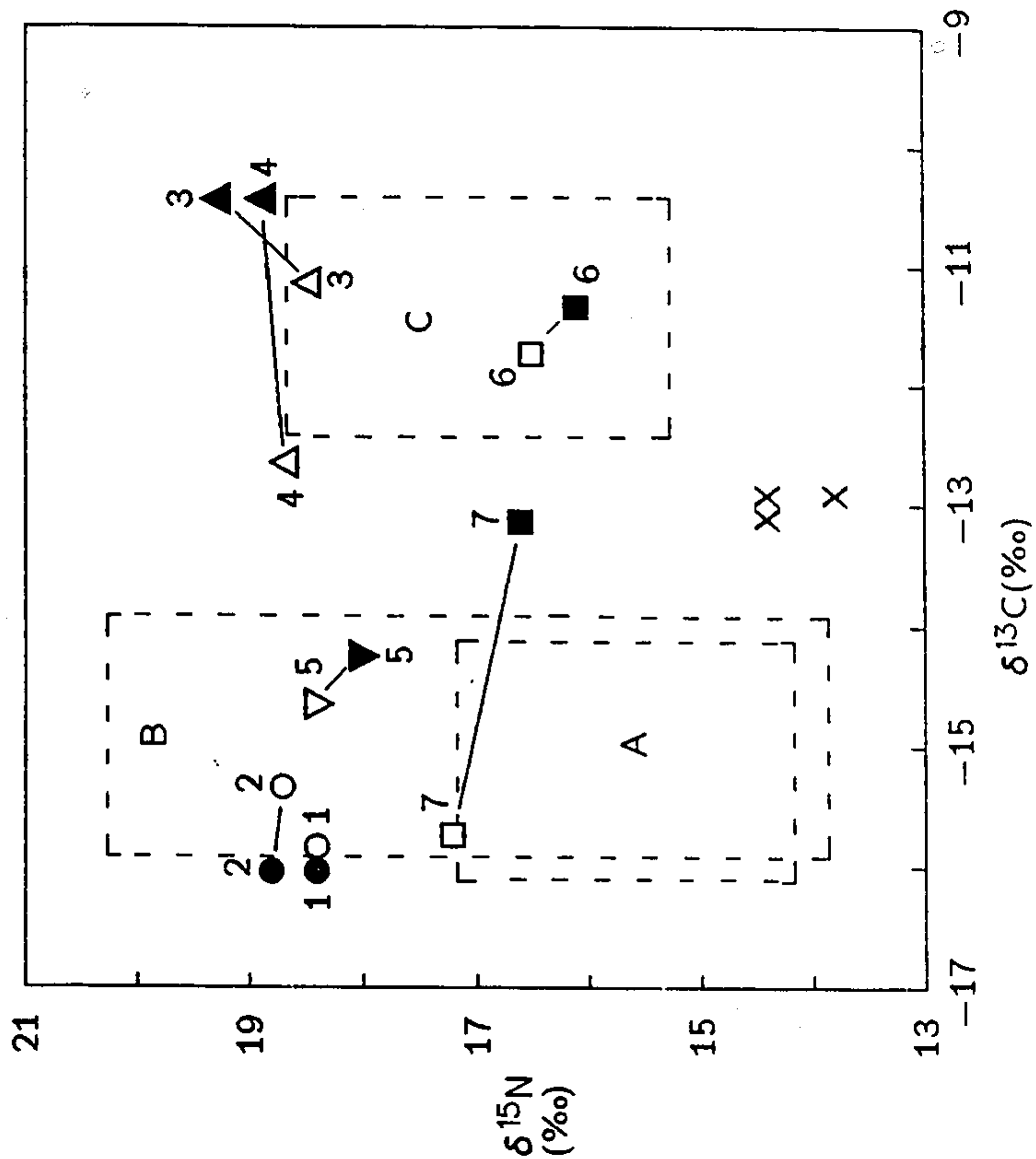
plausible non-diagenetic causes of changes in δD can be monitored by using $\delta^{13}C$ and $\delta^{15}N$ which, in turn, are relatively unaffected by diagenesis. From Table 11.3 it can be seen that variability in seal $\delta^{13}C$ for animals from similar latitudes (see below) and in $\delta^{15}N$ is only a little higher than that expected for animals from the same habitat as seen in the modern deer results (σ_p=±1.2, n=21, for $\delta^{13}Cb$; and σ_p=±0.8‰, n=22, for $\delta^{15}Nb$; Table 7.4). This low variability in $\delta^{13}C$ and $\delta^{15}N$ contrasts sharply with the high variability in δD.

The $\delta^{15}N$ vs $\delta^{13}C$ results of the seal are plotted in Fig. 11.1 with the four different locations of the samples represented by different symbols and each sample identified by number. Whole bone results are shown as open and gelatin extracts as closed symbols. Also shown are two standard deviation ranges of results on modern seals and three fossil results (crosses) from Greenland for comparison.

With the exception of Seal-2 the fossil gel vs w.b. results show that gels have on average higher $\delta^{13}C$ (+1.2‰), lower C/N (-6%) but with little difference in $\delta^{15}N$. This change in results with gel extraction is greatest for the "porous" samples considered to be poorly preserved (+1.8‰ for $\delta^{13}C$ and -12% for C/N). Gel results for samples from nearby locations appear closer to each other than do results on w.b.. The changes in $\delta^{13}C$ and C/N are in the opposite direction to that expected from the potentially higher proportion of NCP's in the more weather w.b. samples (see Ch. 6). This suggests some contamination of terrestrial origin, probably humates, is affecting the w.b. results so that only gel results may be considered reliable.

Figure 11.1 Relationship Between $\delta^{15}N$ and $\delta^{13}C$ of Fossil and Modern Seal.

Results of individual seal specimens are indicated by their sample numbers. Open symbols indicate whole bone results and closed symbols indicate gelatin results. Samples from nearby areas are indicated by the same symbols, Banks Island, NWT by circles, Kwatna by squares and Namu B.C. by triangles (see Table 11.1). Two standard deviation ranges for modern seal values from the literature are provided: (A) harp seal (*Pagophilus groenlandicus*) from Greenland and north-western Europe (n=4), (B) harbour seal (*Phoca vitulina*) from Greenland and north-western Europe (n=5), and (C) harbour seal from southern California (n=3). Three values taken from the literature for fossil seal (crosses) from Greenland are also included. All literature data are from Nelson et al. (1986) and Schoeninger and DeNiro (1984).


δ15N (‰)
21
19
17
15
13
−17
−15
−13
−11
−9
δ13C (‰)
A
B
C
1
2
3
4
5
6
7

Seal-2 is an exception as its gel vs w.b. results show a decrease in $\delta^{13}C$, and an increase in C/N indicating some atypical processes may have affected this sample. The C/N_g value of Seal-2 is also higher than the 106% value at which point effects of diagenesis or cooking on isotopic results may be considered significant. This indicates that all isotopic results on Seal-2 must be considered suspect. Seal-3 has a C/N yield of 106% so its results might be regarded with caution. The C/N_g values of the remaining samples are all within the appropriate range indicating $\delta^{13}C$ and $\delta^{15}N$ are not expected to have been significantly altered by cooking or diagenesis.

The gel $\delta^{13}Cg$ and $\delta^{15}Ng$ values of the arctic seal of this study (Fig. 11.1, Seals 1,2,5) fall within or near the appropriate range of values for modern seal from cold areas, whereas three of four samples from B.C. fall within or near the range for modern seal from warm areas. With the exception of Seal-7, gel results of samples from identical or nearby locations appear tightly clustered. Seal-7 has modern $\delta^{15}N$ values but $\delta^{13}C$ values outside the modern range and is somewhat different than Seal-6 from a nearby location even though its C/N_g value is normal. Apparently contamination rather than diagenesis or diet is affecting Seal-7 as (a) its $\delta^{15}N$ remains unaltered, (b) results on Seal-6 are not similarly affected, and (c) no similar dietary effects are seen in the modern ranges from the literature.

In spite of the fact that their C/N_g values are close to 100%, fossil seal from the literature have both $\delta^{13}C$ and $\delta^{15}N$ values outside the range for modern arctic samples (Fig. 11.1) indicating that C/N values may not be sufficiently sensitive for identifying some cases of diagenesis or contamination. However, the modern isotopic ranges for

seal from the literature are based on only a few samples and may be too narrow.

Seals-3 and 4 have $\delta^{15}N_g$ values ≈3‰ higher than Seals-6,7 even though Kwatna is near Namu. This is unlikely to represent effects on diet due to use of foods from different trophic levels, since δD is not similarly affected (see below).

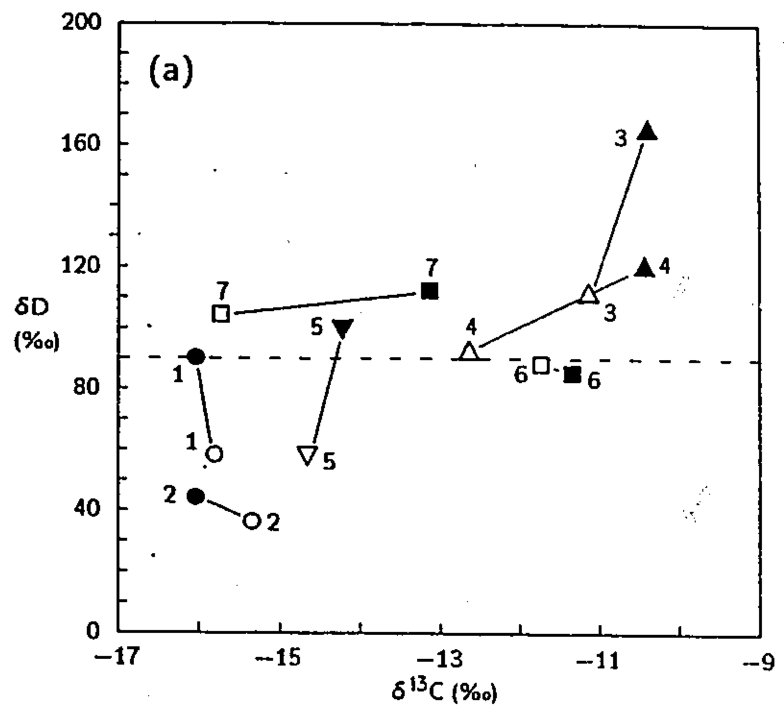
Most above results do not indicate significant changes in $\delta^{13}C$ or $\delta^{15}N$ due to diagenesis. Most $\delta^{13}Cg$ and $\delta^{15}Ng$ results are not affected by contamination and there do not appear to be any large variations due to animal diet such as introduction of a significant amount of terrestrial material or use of foods from different trophic levels. This suggests δD should also not be affected by diet or contamination. Therefore, it is likely that most of the large variation in δD discussed earlier is due to weathering.

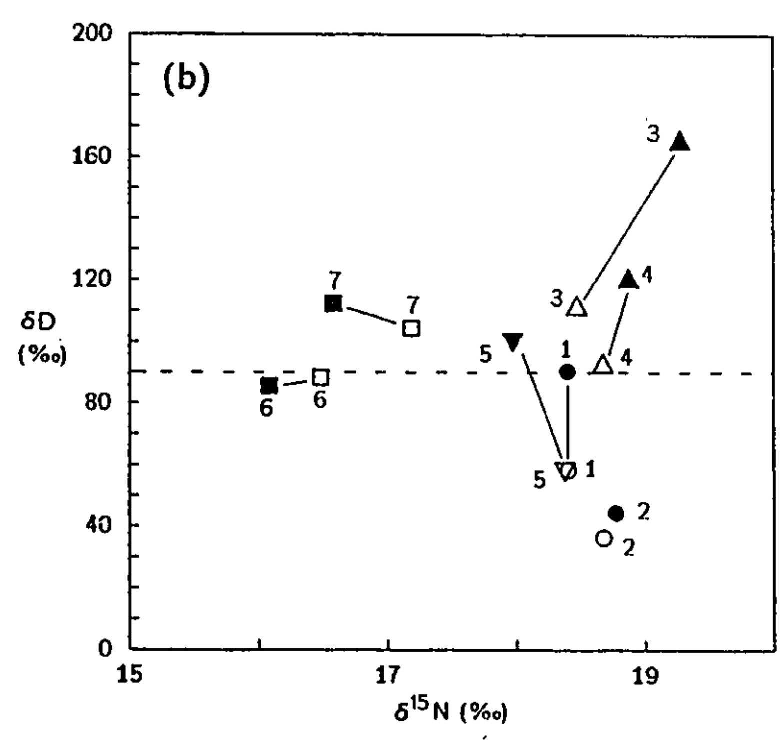
11.5 <u>δD Results and Weathering.</u>

The δD values of both w.b. (open symbols) and gels (closed symbols) are plotted against $\delta^{13}C$ and $\delta^{15}N$ in Fig. 11.2a and Fig. 11.2b, respectively. Also shown in both figures is a 90‰ reference line which represents the proposed value for modern, unweathered seal based on analyses of Seal-0 or Seal-1. The δDg values average +24‰ relative to δDb supporting previous suggestions that w.b. results have been contaminated by terrestrial organics, possibly humates. Low δDb of the "porous" samples (7,4,3) could result from a greater proportion of terrestrial contaminants with the most weathered sample exhibiting the greatest discrepancy between δDg and δDb (i.e. Seal-3). Although, any

Figure 11.2 Relationship of δD to (a) $\delta^{13}C$ and (b) $\delta^{15}N$ for Fossil Seal.

See Fig. 11.1 caption for explanation of symbols.


(a)
200
160
120
80
40
0
δD
(‰)
−17
−15
−13
−11
−9
δ13C (‰)
1
2
3
4
5
6
7


(b)
200
160
120
80
40
0
δD
(‰)
15
17
19
δ15N (‰)
1
2
3
4
5
6
7

terrestrial contaminant and any with higher p_{EX} would lower δDb, low δDb could also result even in less weathered samples if they are from arctic environments (i.e. Seals 1,5) as the δD of the terrestrial organic contaminants is especially low at high latitudes. This indicates that, for fossil samples, it is best to analyze only gel extracts.

The δDg results seem to display a pattern consistent with initial observations on preservational state with the exception of Seal-2. δDg results of the "dense" samples (Seals-5,6) lie close to Seal-1 and the 90‰ line. The δDg of all "porous" samples with low collagen content (Seals-3,4,7) lie above this line. The δDg value of Seal-2 is low and inconsistent with the pattern followed by the other samples supporting previous suggestions of an atypical diagenetic pattern.

The above indicates that diagenesis rather than contamination or diet appears to have affected the δD results of fossil seal. Following a description of expected trends from the literature, it will be determined below whether δD of the seal varies in a systematic manner with other measurements of bone which are known to be affected by weathering processes.

11.6 Empirical Relationships Between Variables from the Literature:

Co-variation among variables, following collagen leaching has been suggested in the literature as discussed in Ch. 3. Most notably, with a decrease in collagen content, a decrease in total amino acid content or a decrease in N-yields, there should be a corresponding decrease in the GLY/ASP ratio and an increase in the C/N yield. The examples of literature data shown in Fig. 11.3 confirm that, with leaching

Figure 11.3 Relationship Between GLY/ASP, C/N and Collagen Content of Fossil Bone.

(a) The GLY/ASP ratio of the amino acid extract decreases with decreasing nitrogen content of whole bone. Data are calculated from a linear regression relating the concentration of each amino acid to nitrogen content as given in Dennison (1980). Amino acid concentrations are in ppm and nitrogen content in g%. Possible contamination of bone collagen should be reduced by use of recent human bone <700 y.B.P.:

$GLY/ASP = -1.61 + 1.31 \cdot \ln(N\%)$; $r=0.990$, $n=7$

(b) The GLY/ASP ratio of the amino acid extract decreases with a decrease in amino acid (aa) content of whole bone. Data are from Kessels and Dungworth (1980) and amino acid concentrations are in mole%. Total % amino acids is given by $[(\mu mole/g_{dry\ bone})/(\mu mole/g_{dry\ bone})_{modern}] \cdot 100$:

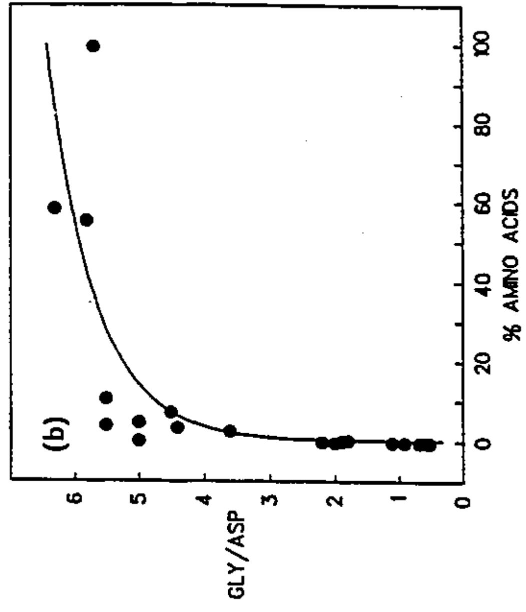
$GLY/ASP = 3.07 + 0.730 \cdot \ln(\%aa)$; $r=0.872$, $n=20$

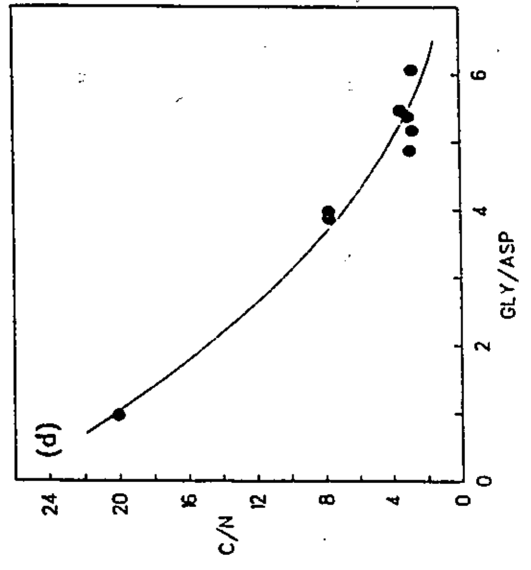
(c) The C/N ratio of the gelatin extract increases with a decrease in collagen content of whole bone. Data are from DeNiro and Weiner (1988a) and the collagen concentration is given as % dry weight bone. Contamination with humates unlikely given NaOH treatments and gelatin filtration:

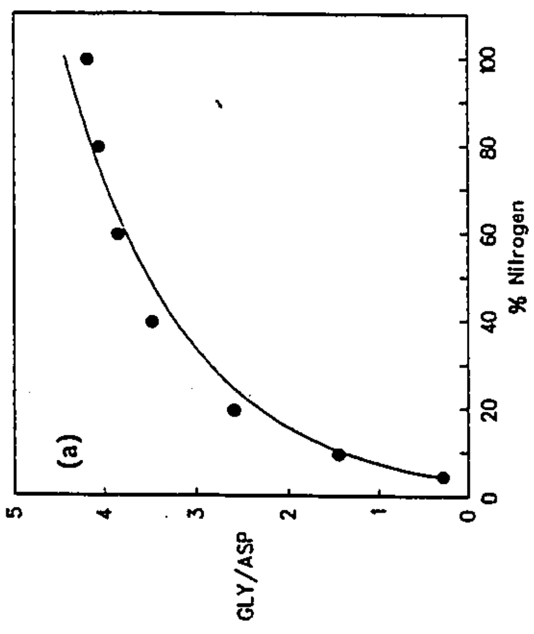
$C/N_g = 5.47 \cdot (col\%)^{-0.213}$; $r=-0.772$, $n=44$

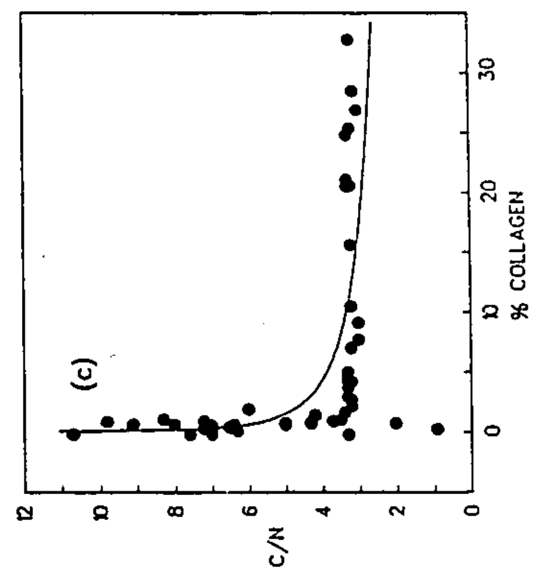
(d) The C/N ratio of gelatin increases with a decrease in the GLY/ASP ratio of amino acid extract. Data are from Hassan and Hare (1978) and amino acid concentration is given as residues/1000. Contamination with humates reduced by dialysis with 12,000 AMU membrane pore size:

$C/N_g = 26 - 6.56 \cdot (GLY/ASP) + 0.422 \cdot (GLY/ASP)^2$;
$r=-0.990$, $n=8$


(a)
GLY/ASP
% Nitrogen


(b)
GLY/ASP
% AMINO ACIDS


(c)
C/N
% COLLAGEN


(d)
C/N
GLY/ASP

of collagen, there are covariations among these variables. The data sources and best fit curvilinear relationships are given in the figure caption. Other plots of literature data were also examined (i.e. Hare 1980; McMenamin et al. 1982; Ennis et al. 1986; Nelson et al. 1986; Tuross et al. 1988; Masters 1987; other data from refs. given in Fig. 11.3 and 11.4 caption) and mostly support conclusions drawn here. Therefore, it is reasonable to expect that, if δDg changes as a result of weathering by leaching, it could co-vary with other variables.

Given the sample treatments used (Fig. 11.3 caption) it is unlikely that the increases in C/N observed in Fig. 11.3c and 11.3d are due to contamination with humates. However, since changes in C/N appear to accompany only severe loss of collagen, it may not prove to be a less sensitive indicator of diagenesis. In contrast, the GLY/ASP ratio and different measures of collagen content in fossil bone appear to be quite sensitive indicators of weathering.

Comparisons of the $\delta^{13}C$ and $\delta^{15}N$ vs collagen content give inconsistent results (Fig. 11.4). There are decreases in both isotope ratios in marine carnivores and increases in both isotope ratios in terrestrial herbivores. The sample treatment used (Fig. 11.4, caption) along with additional lines of evidence make it unlikely that contamination with humates has affected these results. First, the $\delta^{15}N$ appears to be affected to a similar degree as $\delta^{13}C$ whereas changes in mainly $\delta^{13}C$ would be expected since humates have $C/N \approx 10-20$ compared to collagen ($C/N=3.2$) (Ch. 3). Second, results of all samples within the same sub-category (marine or terrestrial) converge towards the same, common isotopic value with collagen removal, yet this common isotopic

Figure 11.4 $\delta^{13}C$ and $\delta^{15}N$ Versus Collagen Content of Fossil Bone.

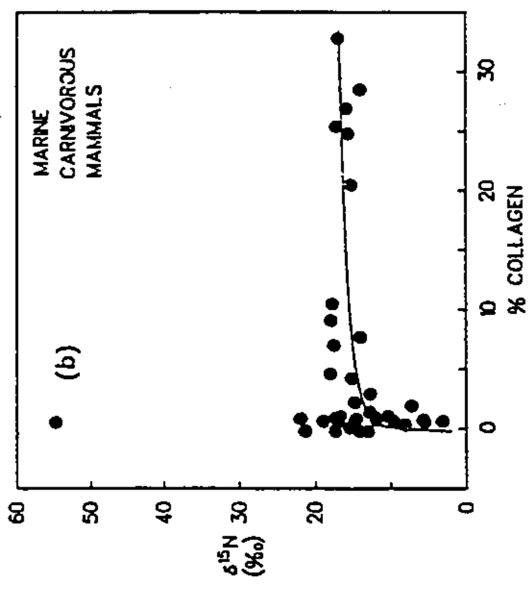
The relationships between $\delta^{13}C$ and $\delta^{15}N$ of gelatin and collagen content of whole bone from fossil mammals is plotted.
Data are from DeNiro and Weiner (1988a) and collagen content is given as %dry weight of bone. Isotopic values are of gelatin extracts. Contamination with humates is unlikely given NaOH treatments and gelatin filtration. The equations of the "best fit" plotted curves are given below:

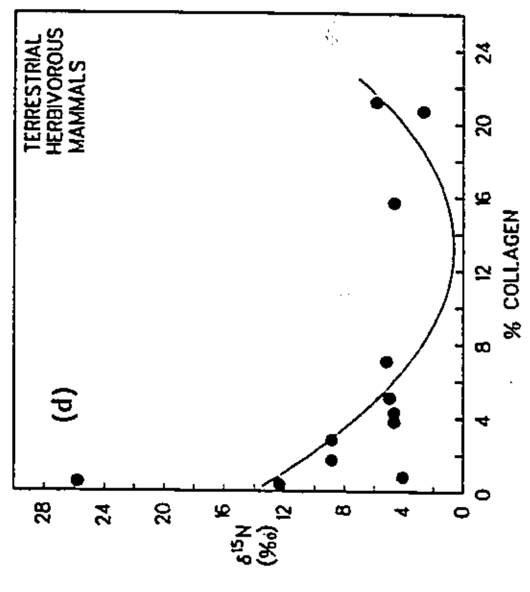
(a) $\delta^{13}C$ in marine carnivores:
$\delta^{13}C_g = -17.9 + 1.69 \cdot \ln(\%col)$; $r=0.652$, $n=32$

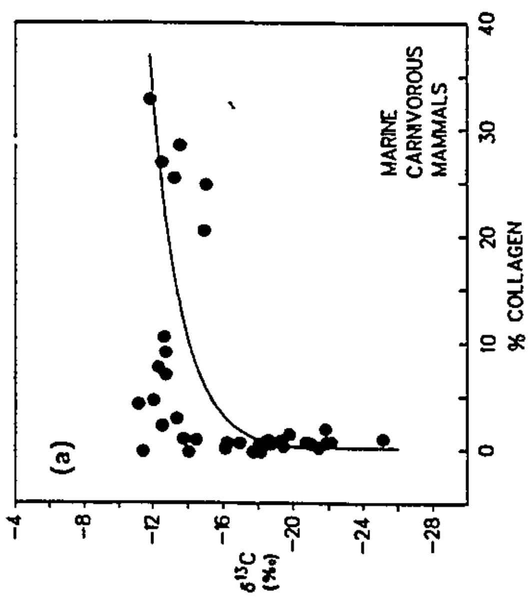
(b) $\delta^{15}N$ in marine carnivores:
$\delta^{15}N_g = 12.8 + 1.15 \cdot \ln(\%col)$; $r=0.372$, $n=31$

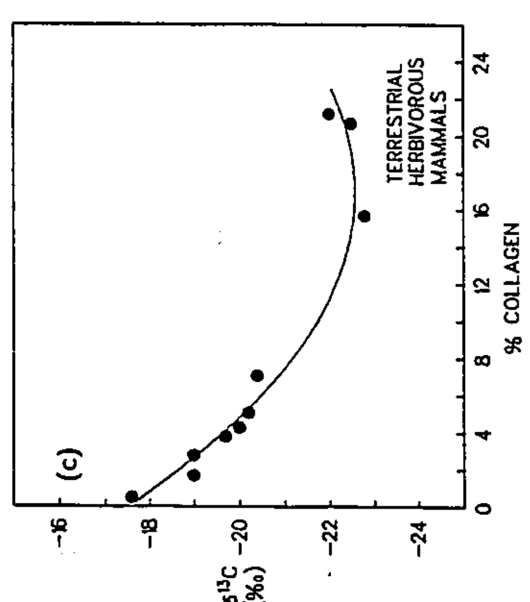
(c) $\delta^{13}C$ in terrestrial herbivores:
$\delta^{13}C_g = -17.5 - 0.594 \cdot (\%col) + 1.76 \cdot (\%col)^2$; $r=0.984$, $n=12$

(d) $\delta^{15}N$ in terrestrial herbivores:
$\delta^{15}N_g = 14.0 - 2.01 \cdot (\%col) + 7.56 \cdot (\%col)^2$; $r=0.615$, $n=12$


(a)
MARINE CARNIVOROUS MAMMALS
$\delta^{13}C$ (‰)
% COLLAGEN


(b)
MARINE CARNIVOROUS MAMMALS
$\delta^{15}N$ (‰)
% COLLAGEN


(c)
TERRESTRIAL HERBIVOROUS MAMMALS
$\delta^{13}C$ (‰)
% COLLAGEN


(d)
TERRESTRIAL HERBIVOROUS MAMMALS
$\delta^{15}N$ (‰)
% COLLAGEN

value appears to be different for all marine as opposed to all terrestrial samples. Fig. 11.4a and 11.4b further confirm that, at least for marine mammals, neither isotope is likely to provide a sensitive indicator of weathering. The fact that $\delta^{13}C$ and $\delta^{15}N$ can also be related to diet and climate, makes it further unlikely that $\delta^{13}C$ or $\delta^{15}N$ will prove useful for monitoring diagenetic change.

11.7 Relationship Between δD and Other Variables of This Study:

Using the data of the 7 seals (Tables 11.1, 11.2 and 11.3), individual plots of δD vs all variables, including the median of the age range from Table 11.1 were visually inspected. Plots between all predictor variables were also examined. In Fig. 11.5a through 11.5d, the more coherent relationships between variables are shown along with the best fit regression curves.

Whole bone C/N_b increases with decreasing N/g_b (Fig. 11.5a) which might be a result expected from diagenetic leaching (see Fig. 11.3c). However, closer examination shows that the results are not entirely coherent. Two samples (Seals 2 and 6), one of which is likely contaminated (Seal-2), show N/g_b yields greater than the modern example (Seal-1). The extremely high C/N_b value of Seal-4 is not repeated in the gel extracts which suggests contamination. None of the remaining variables using w.b. results, including δDb, when plotted against the other variables showed any consistent patterns. This is probably because most w.b. samples are contaminated. In contrast, results on gel extracts did show correlations between δDg and other variables (Fig. 11.5b to 11.5d). Previous work suggested that Seal-2 might have

Figure 11.5 Relationship Between Variables for Fossil Seal.

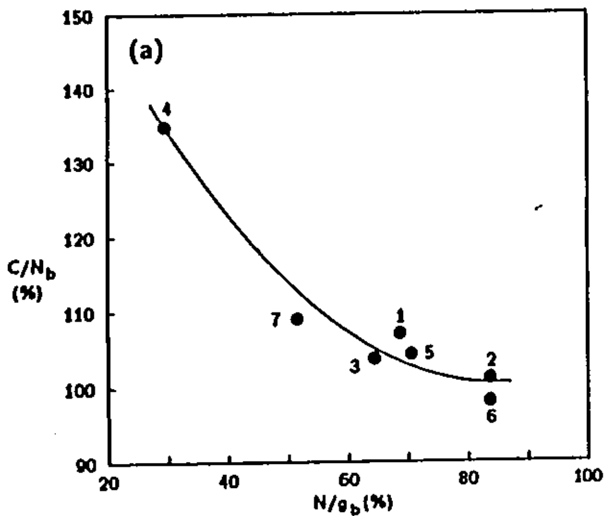
C/N_b is related to the collagen content of whole bone N/g_b in (a). δD_g is related to H/N_g, H/g_b and GLY/ASP in (b) through (d) respectively. All best fits in (a) through (d) are quadratic of the form:
$Y = \beta_0 + \beta_1 X + \beta_2 X^2$
with the following regression coefficients:

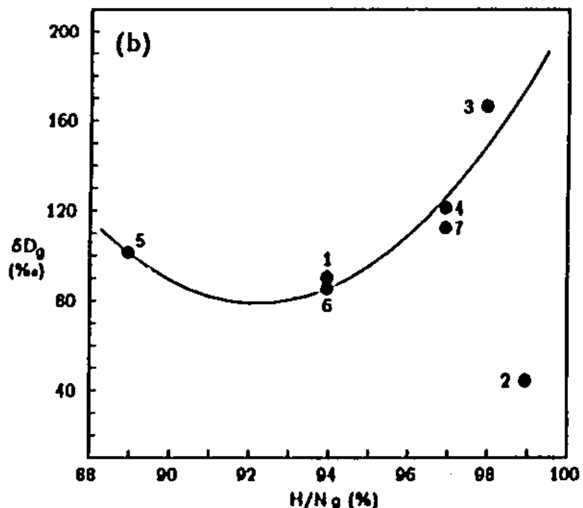
(a) C/N_b vs N/g_b:
$\beta_0 = 180.996$, $\beta_1 = -1.90110$, $\beta_2 = 0.0112159$; $r=0.980$, $n=7$

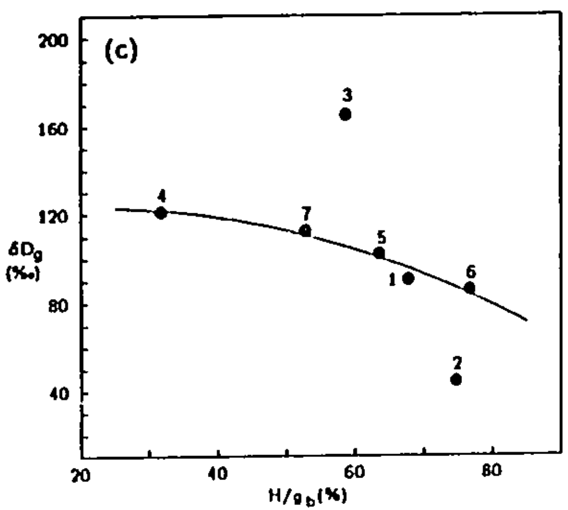
(b) δD_g vs H/N_g
$\beta_0 = 17964.5$, $\beta_1 = -387.954$, $\beta_2 = 2.10375$; $r=0.934$, $n=6$, $X_0=94$

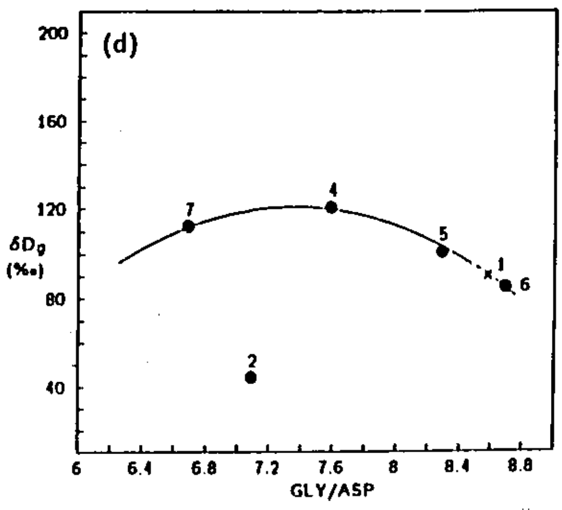
(c) δD_g vs H/g_b:
$\beta_0 = 116.907$, $\beta_1 = 0.557343$, $\beta_2 = -1.29528$; $r=0.971$, $n=5$, $X_0=68$

(d) δD_g vs GLY/ASP (amino acid concentrations in mole%):
The X_0 value (GLY/ASP_0) for Seal 1 was estimated from the best fit relationship in (d) and the value is indicated by a cross in that figure.
$\beta_0 = -983.942$, $\beta_1 = 300.478$, $\beta_2 = -20.4271$; $r=0.996$, $n=4$, $\hat{X_0}=8.6$


(a)
C/N_b (%)
150
140
130
120
110
100
90
20
40
60
80
100
N/g_b (%)
4
7
1
3
5
2
6


(b)
δD_g (‰)
200
160
120
80
40
88
90
92
94
96
98
100
H/N_g (%)
5
1
6
3
4
7
2


(c)
δD_g (‰)
200
160
120
80
40
20
40
60
80
H/g_b (%)
4
7
3
5
1
6
2


(d)
δD_g (‰)
200
160
120
80
40
6
6.4
6.8
7.2
7.6
8
8.4
8.8
GLY/ASP
7
4
5
1
6
2

undergone different diagenetic processes and in Fig. 11.5b to d, Seal-2 appears as a distinct outlier. The dark brown stain, low δD coupled with high C/Ng values suggests that Seal-2 might be contaminated with humic acids or was burned.

For the remaining seal samples, high H/Ng values appear in the most leached samples (Seals 4,7 and, especially, 3) which also have the highest δDg values (Fig. 11.5b). Low H/g_b are also associated with leached samples having high δDg values with the exception of Seal-3 (Fig. 11.5c) which was noted previously as having a suspect C/N_g value. Here, H/g_b is an alternative to N/g_b (or C/g_b) for measuring collagen content or degree of leaching but gives slightly better regression statistics. Finally, the high δDg values of the more leached samples also seem to correlate with lower GLY/ASP values. The trend toward lower GLY/ASP in more leached samples is also consistent with observations in the literature.

11.8 Potential for δDg Correction:

The above suggests that samples undergoing diagenetic alteration related to hydrolysis of collagen and leaching may follow a consistent pattern of increasing H/N_g, decreasing H/g_b, and decreasing GLY/ASP with a concomitant increase in δDg. The best fit regression equations for all these relationships (Fig. 11.5 caption) are quadratic of the form:

(eq. 11.1) $$\delta Dg = \beta_0 + \beta_1 X + \beta_2 X^2$$

where X represents H/N_g, H/g_b or GLY/ASP as the predictor variable. The regression coefficients appear in the caption of Fig. 11.5.

Assuming that there has been a minimal amount of contamination, that burned samples can be rejected on the basis of their C/N ratios, and that future work confirms systematic relationships between δDg and other variables, it is now possible to propose a means for assessing and correcting the δDg values for the effects of weathering by hydrolysis and leaching of collagen. This method should also help distinguish the δD of samples that do not undergo this mode of alteration and, hence, cannot be corrected by this method.

At the time of deposition (t_0), the δDg value of bone would be unaltered (δDg_0) as would the values for X (X_0). Eq. 11.1 can be used to express the relationship between δDg_0 and X_0 whereas the offset in δDg produced by diagenesis can be expressed as ($\delta Dg - \delta Dg_0$). An estimate for δDg_0 ($\delta Dg_0\hat{}$) can now be provided by subtracting equation 11.1 written for δDg_0 from that for δDg and solving for δDg_0:

(eq. 11.2) $$\delta Dg_0\hat{} = \delta Dg + \beta_1(X_0 - X) + \beta_2(X_0^2 - X^2)$$

Here, δDg_0 is estimated from measured values of δDg and X. Modern, unaltered values of X_0 (Fig. 11.5 caption) can be determined from fresh samples of bone represented by Seal-1. Due to an apparent calibration problem for all amino acid concentrations (above), X_0=8.6 was stipulated for GLY/ASP using the regression equation (Fig. 11.5d caption).

In Table 11.4 it is shown that Eq. 11.2 is solved using the three alternatives for X (H/N_g, H/g_g, GLY/ASP). Results were then averaged and standard deviations calculated for each column and each row. The between-column standard deviations are extremely high for

Table 11.4 Correction of Seal Gelatin Results for Effects of Diagenesis Through Estimates of Time Zero (t_0) Values.[a]

sample	$\delta Dg(t)$	$\delta Dg_0\hat{}(t_0)$[a] (H/Ng)	(H/g_b)	(GLY/ASP)	row ave
Seal 1[b]	90	90	90	90	90±0
Seal 2	44	-46	53	14	7±50[c]
Seal 5	101	86	96	88	93±7[d]
Seal 6	85	85	97	90	89±6[d]
Seal 7	112	70	97	89	92±17[e]
Seal 4	121	79	94	91	96±17[e]
Seal 3	166	102	156	-	129±38[c]
	(n=5)	82±8	95±3	90±1	
	(n=6)	85±11			

a $\delta Dg_0\hat{}$ values estimated using eq. 11.2. Variable used here for X given in parentheses at top of column.

b δDg value for Seal-1 (90‰) is assumed to be the unaltered value for all the seal at the time of original deposition (t_0).

c large σ for the row suggests samples are contaminated or bone has not undergone weathering by simple leaching. Results should be discarded.

d low σ indicates a low degree of weathering and a quite accurate estimate of δDg_0 at t_0.

e moderate σ suggests more advanced weathering than for Seals 5 and 6, but average value is still within 2σ (±6‰) of 90‰ indicating that corrected results would provide reasonable estimates of δDg_0 at t_0.

Seals 2 and 3 indicating that these samples do not follow the diagenetic pattern established by the formulae and results should be rejected. The standard deviations of the remaining younger samples, previously judged to be less weathered (Seals 5,6), also appear to be generally lower than those of the older and more weathered samples (Seals 4,7). The averages for these remaining 4 seals appear to be within 6‰ of the "modern" value of 90‰ indicating that the correction procedure for this set of samples appears to give acceptable results. The standard deviations on each method used (bottom of each column, Table 11.4) further suggest that the most valuable correction methods might be provided by (in descending order of accuracy) GLY/ASP, H/g_b then H/N_g.

The sample set used here is too small to propose a definitive method for correcting δD for diagenetic alteration. A great deal of additional study is needed using a complete data set on a much larger number of samples. Expanding the numbers of variables studied for X, including those related to the inorganic matrix of bone (cf. Parker et al. 1974; White and Hannus 1983; Kyle 1986), could also help strengthen the reliability of such a correction procedure. As shown below, a thorough study of bone from large terrestrial herbivores is also warranted.

11.9 Effects of Outgassing Temperature on Fossil Seal:

An understanding of the nature of the change in δDg of the seal could lead to alternative methods for dealing with diagenesis through such measures as preferential removal of the altered hydrogens on the gel molecules (below) or through improved sample preparation. It is most likely that changes in δDg relate to non-exchanged hydrogens

on the gel molecule rather than to H-exchange. Increased H-exchange should lower δDg of the seal but the most weathered samples have the highest δDg. The gels exchanged at different pH values in the presence of Cu+ (Ch. 6) further suggest that variables such pH and presence of metal ions in the depositional environment will not greatly change p_{EX}. Exchange experiments on BIS also did not indicate any increase in p_{EX} with fossilization.

Tests were conducted to determine whether increasing T_P (the outgassing temperature) could be used to preferentially remove exchanged or altered hydrogens from fossil Seal-2 which is from the same location as the modern Seal-0 (SEAL standard) (Table 11.5, Fig. 11.6). The fossil seal values do not converge towards those of modern seal with increased heating. Instead, at all Tp, δDb values remain separate by a relatively constant amount [(Seal-0 - Seal-2) = 23 to 25‰] indicating no benefits with higher Tp. The similarity of the curves suggests that most material in the fossil seal has physical properties similar to modern seal with isotopically light hydrogen removed from both samples at increased T_P.

The high C/N_b values in Seal-0 of this experiment are difficult to explain especially since these values were lower (<105%) in later experiments using the same preparation methods (Ch. 5). The increase in C/N_b value of Seal-2 heated at 220°C suggests that burned bone may be identified by high C/N values.

Table 11.5 Modern and Fossil Seal Whole-Bone from Nearby Locations Preheated at Various Temperatures.[a]

		ISOTOPES			YIELDS					
	Tp	δDb	δ^{13}Cb	δ^{15}Nb	C/N	H/N	H/C	N/g	C/g	H/g
Seal 0	150	59	-15.5	18.7	112	98	87	64	72	63
	160	62	-	18.3	108	95	88	67	72	63
	180	61	-15.6	18.5	111	96	87	74	82	71
	220	63	-15.5	-	118	91	77	64	75	58
Seal 2	150	36	-15.3	18.7	101	89	88	84	85	75
	160	38	-	18.4	103	89	87	87	89	77
	180	38	-15.7	18.9	104	89	86	88	92	79
	220	38	-15.4	17.7	111	87	78	72	81	63

a prep.5 used for all samples (see Ch. 5)

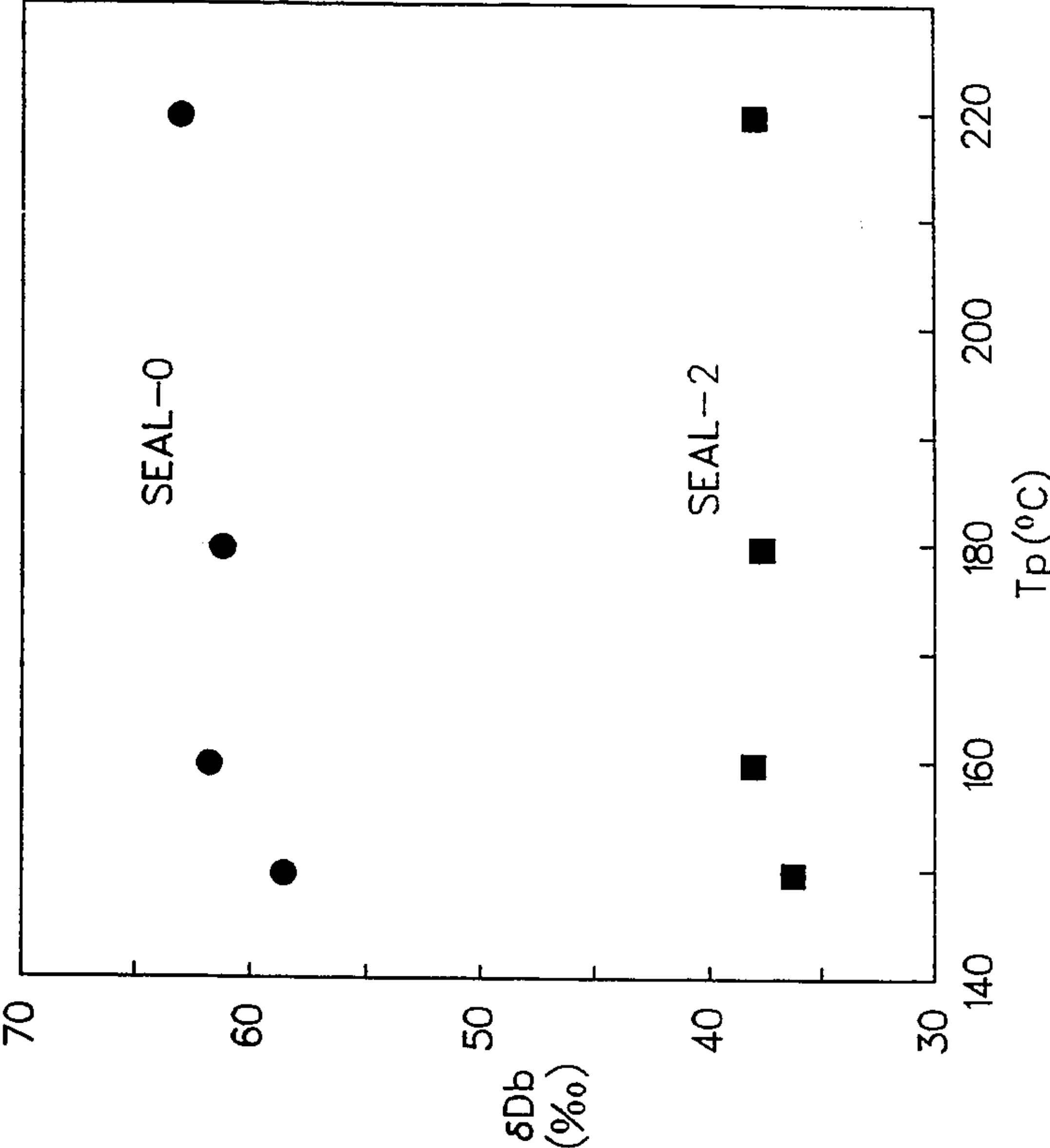
Table 11.6 Results of Cooking Experiment Using Beef Femur With Meat.[a]

	ISOTOPES					YIELDS					
Preparation	δDb	δDv	δDbc	δ^{13}C	δ^{15}N	C/N	H/N	H/C	N/g	C/g	H/g
uncooked	-76	-159	-55	-16.0	6.1	93	85	91	90	84	77
boiled	-72	-139	-55	-17.1	6.3	96	91	95	97	93	88
barbecued	-72	-139	-55	-17.0	-	96	94	98	90	86	84
roasted	-66	-130	-49	-17.2	6.3	90	86	96	92	83	79
steak	-65	-139	-49	-20.2	6.5	97	101	104	74	72	75

a prep. 5 used for all samples (see Ch. 5)

Figure 11.6 Effects of Outgassing Temperature on Modern and Fossil Seal.

The δDb of modern seal, Seal-0, and fossil seal, Seal-2, are plotted against outgassing temperature (Tp).



70
60
50
40
30
δDb
(‰)
140
160
180
200
220
Tp (°C)
SEAL-0
SEAL-2

11.10 Examples of Fossil Terrestrial Herbivores:

From Table 11.3 it appears that w.b. preparation of terrestrial mammals suffer a minimum of contamination compared to seal since both BIS and MUSK w.b. results resemble their gel results. Any diagenetic effects on seal are also likely to be greatly accelerated due to the porous physical nature of seal bone so a study of terrestrial mammalian bone is preferable. $\delta^{18}O$ is not affected by diagenesis and results showed that δD and $\delta^{18}O$ are well correlated in modern mammals (Fig. 9.1a). Therefore, $\delta^{18}O$ could be used to control for the effects of climate on bone from terrestrial environments thus allowing an examination of the diagenetic effects alone on δD.

An examination of two examples of fossil bone from terrestrial mammals does indeed suggest that such bone may be considerably more resistant to diagenesis than seal. Both BIS and OLD-C have high δD compared to modern herbivores from nearby locations (AB-7 for BIS and MUSK for OLD-C) and this could result from diagenesis. However, the $\delta^{18}Ob$ values of both BIS and OLD-C are also high relative to AB-7 and MUSK respectively. It can be further seen from Fig. 9.1a that relationships between the δDb_c and $\delta^{18}Ob$ of these fossil samples agrees well with those of modern deer and MUSK. This indicates that climate, rather than diagenesis has likely affected the δDb_c results of these samples.

Using equations developed previously we can estimate $\delta Dwy\hat{}=-91‰$, $\delta Dw\hat{}=-74‰$ and $RH\hat{}=73$ for BIS (eqs. 8.10, 8.15 and 9.12) which can be compared to modern values ($\delta Dwy=-136‰$, $\delta Dw=-109$, $RH=66$). Assuming a well understood δDw vs T relationship and a T coefficient of

15.5‰/°C (Edmonton, Fig. 7.4, eq. a) one might tentatively suggest that 1500 years ago in Alberta, T was 2°C higher than at present.

The above observations suggest that both BIS and OLD-C have preserved their original δD values. Post depositional weathering and contamination appear to have considerably less influence on bone δD from terrestrial mammals compared to seal.

11.11 Cooking and Burning - This Study

Since all the seal bone was collected from archaeological sites the possibility that their δD results may have been altered by cooking or burning cannot be ignored. Low δD might result from burning and might be diagnosed in gels by decreased C/g, H/g_g and unusual C/N_g yields. However, unlike burnt bone cooked bone cannot be distinguished from un-cooked bone on the basis of C/N yields, $\delta^{13}C$, $\delta^{15}N$ or physical appearance. Therefore, possible effects of cooking on δD must be examined before δD analysis of any bone derived from archaeological sites can be used to interpret climate.

In a cooking experiment, sub-samples of beef containing long bone (femur) were thoroughly cooked using the following four methods: (a) boiled in water, (b) barbecued above charcoal, (c) cooked as a steak in a frying pan, and (d) cooked as a roast in an oven. The cooked meat was then removed from each bone and from an uncooked control sample and each bone was prepared and analyzed by standard methods. These results appear in Table 11.6.

Although the δDb_c of steak and roast were 6‰ higher than the uncooked sample, in no samples did the δDb_c vary beyond 2σ (σ=±4‰). However, the δDb ranged by 11‰ apparently because the δDv

values were unusually variable. The $\delta^{15}N$ value did not change greatly with cooking but the $\delta^{13}C$ value was lowered by 1‰ in three cooked samples and by 4‰ in the steak. The lowest $\delta^{13}C$ values are also associated with the highest δD values indicating that cooked samples are not contaminated with unaltered lipids.

The C/N yields increased with cooking in most samples. All H/C yields increased slightly with cooking and were highest in the steak. Steak bone also had low C- and H-yields and low $\delta^{13}C$ suggesting contamination with a cooking by-product. Alteration of collagen does not seem as likely an explanation since heating of bone to a degree that alters collagen generally affects $\delta^{15}N$ more than $\delta^{13}C$ and would likely produce significantly lower δD. The samples cooked here, exhibit slightly increased C/N for most samples, lack of change in $\delta^{15}N$, with minor increases change in δD_{bc} and a negative correlation between δD and $\delta^{13}C$. This suggests that, rather than alteration of collagen with cooking, the samples may be contaminated with a cooking by-product which could be composed predominantly of C and H. Such cooking compounds may also be preferentially removed during diagenesis or with improved sample preparation. Such contamination appears to be less of a problem in samples not cooked in their own juices such as boiling and barbecuing) which would likely be the main prehistoric cooking techniques.

The above results indicate that cooking slightly alters bone $\delta^{13}C$ probably through contamination by a cooking by-product of some samples when the meat is cooked in its own juices. The effects of most forms of cooking in a prehistoric setting should not significantly alter bone δD or $\delta^{15}N$.

CHAPTER 12

SUMMARY, CONCLUSIONS AND FUTURE WORK

This work, originally proposed by the author, is the first to demonstrate that the δD of bone collagen from a large, non-migratory terrestrial herbivore has excellent potential as a valuable paleoclimatic or paleoecological tool. Using white tailed deer specimens from across North America it was found that with correction for H-exchange, the variation in the δD of bone collagen in whole bone powders (δDb_c) can be almost entirely explained by the variation in local δD of summer precipitation (δDw), with a lesser influence from relative humidity (RH), during the plant growing season. The interdependence of these variables is clearly represented by a simple trivariate linear relationship with a δDw coefficient of 1.0. The relationship between δD_{bone} and δD_{rain} may prove be be less complicated than that of δD_{cel} or δD of insect chitin to δD_{rain} making it a valuable addition to methods of determining climate from isotopic measurements of terrestrial materials.

The above observations suggest that δDb forms a linear relationship with the δD of plant tissue in the animal's diet with a slope of 1.0 and supports the contention that it represents the accumulated lifetime δD of all local plant tissue eaten by the animal. The δD of plant tissue reflects that of leaf-H_2O which, in turn, reflects δDw. RH has a much smaller effect on δDb and its effects are predicted by the leaf-H_2O

model of evapotranspiration. Due to the relative insensitivity of δDbc to RH, it can be ignored and δD_{rain} can be accurately estimated using data which can be gathered in the laboratory, δDb, δDv, $\delta^{15}N$ and C/g_b. Here, $\delta^{15}N$ appears to act as a proxy variable for RH. Although δDb_c is biased towards growing season δDw, it can be used to accurately estimate δDwy for which the climatic significance is better understood.

The δDwy^ estimates could be used to interpret climate using information from the δD_{rain} vs temperature relationships. Although the inter-site relationship between δDwy and T is complicated by other climatic phenomena, δDwy^ for different time periods vs modern Ty could be compared to provide qualitative if not semi-quantitative evaluation of how global climate patterns have changed over time. For most locations in eastern and central North America which follow a more general pattern of evaporation and precipitation, the best fit relationship for δDwy vs Ty might allow the quantitative estimate of change in Ty over time.

A thorough study of intra-site δDwy vs Ty coefficients calculated over long periods of time for numerous locations, using either tree ring cellulose or bone data corrected for the effects of RH, would be necessary to elucidate the temporal δDwy vs Ty relationship. Such studies would benefit any future attempts at evaluating changes in climate over time for a given location.

It was also shown in this work that δDb is well correlated with $\delta^{18}Ob$ of bone phosphate and, with correction for RH and the less than 1.0 slope between $\delta^{18}Ob$ and body water, the relationship between these two variables is consistent with that for meteoric water.

Due to the larger effect that RH has on $\delta^{18}Ob$ compared to δDb, RH may be estimated using both $\delta^{18}Ob$ and δDb provided that $\delta^{15}N$ and δDv are included in the regression equation for estimation of $\delta^{18}Ow$. Solving some of the following problems in the interpretation of $\delta^{18}Ol$, δDl and $\delta^{18}Ob$ may allow some improvement in the RH estimate. These include (a) an understanding of the apparent direct temperature effect on $\delta^{18}Ol$ and δDl, (b) the determination of the $\delta^{18}Ob$ vs $\delta^{18}Oi$ slope for the deer where $\delta^{18}Oi$ is of environmental water and (c) the determination of the proportion of input H_2O which derives from drinking as opposed to leaf-H_2O. Nonetheless, the $\delta^{18}O$ and δD of tree cellulose may be more sensitive to RH thus providing a more accurate estimate of RH than does bone.

A considerable amount of work went into development of sample preparation methods suitable for δD measurements of bone collagen. Outgassing conditions to remove adsorbed H_2O were found to greatly affect results and it was found necessary to considerably improve the control over vacuum and temperature. Tests of the final methods, preparation of w.b. powders or gel extracts, derivation of outgassing and combustion conditions, and methods for cryogenic separation of combustion products, indicated that such procedures allowed accurate determination of all isotopic results giving good reproducibility and reasonable yields. The gel and col preparations provide valuable options for analyses of fossil samples where it is important to remove contamination. Slightly higher (0.6‰) results for $\delta^{13}Cb$ compared to $\delta^{13}Cg$ were attributed to a small contribution of CO_2 from bone CO_3^{2-}.

There are 20.5% exchangeable hydrogens in w.b. powders and col of terrestrial mammals and 23% in modern gels. These empirical determinations are close to the theoretical estimate of 22% for collagen. It appears that all H-exchange occurs within 48 hours so a calibration bone standard can be outgassed with a series of samples to determine the δDv of lab atmospheric H_2O vapor. This allows correction of the δD results of w.b., col or gel for H-exchange. Such a calibration bone standard method would be implemented when comparison of results to other laboratories becomes important or when the study of the slope of the δDb vs δDw relationship requires an accounting of H-exchange. However, since δDv does not change greatly over time, H-exchange does not affect the reproducibility of the δD results and correction for exchange is not necessary for most in-house work. Including the δDv variable in multilinear regressions for estimating δDwy^ reduces variability due to varying δDv in the lab over time.

Changes in δDg of fossil seal bone compared to a modern counterpart suggest diagenetic alteration and a comparison of their δDb to δDg results suggests considerable contamination of w.b. preparations. Nonetheless tests conducted on terrestrial herbivore bone using δDb and $\delta^{18}Ob$ results showed that cortical bone from large terrestrial herbivores may well retain its original δD value. It may further be possible to monitor or correct δDg values for effects of weathering by hydrolysis and leaching of collagen through use of chemical indicators such as the C/N_g, H/N_g and H/g_b yields and the GLY/ASP amino acid ratio. Monitoring changes in $\delta^{13}C$ and $\delta^{15}N$ should be useful as a means of distinguishing changes in δDb of fossil specimens due to contamination or diet from those due to weathering. Future studies should include

the study of temporal sequences of bone from large terrestrial herbivores using δ^{18}Ob as a control for changes in δD due to climate. Other studies on marine mammals such as the seals could also benefit through use of a large number of modern control samples to better determine natural variability due to diet and environment and through broadening the base of variables used to detect chemical change.

In additional work, it was found that the low average δ^{13}C values of the deer bone is consistent with the deer's browsing habits. Nonetheless, some animals consumed more than 10% C_4 plants. The average δ^{15}N value is low, possibly as a result of agricultural use of artificial fertilizers or consumption of legumes, but most likely due to browsing on woody growth forms. Both δ^{13}C and δ^{15}N from across North America show significant intersite variations and distinct geographical patterns. Variations in δ^{13}C can be largely explained by variations in consumption of C_4 plants and the canopy effect. The simultaneous interplay of a number of variables affect δ^{15}N including variation in soil δ^{15}N, agricultural patterns and dietary habits of deer. Animals consuming more C_4 plants exhibit a negative δ^{15}N vs PPTy relationship and this adds an additional complication to its interpretation. Interpretation of human dietary studies using both δ^{15}N and δ^{13}C could benefit from analysis of an associated herbivore in order to establish baseline estimates for the local plants and terrestrial animals upon which dietary interpretations are based.

From the above, it is clear that a main limitation in applying δDb to the interpretation of climate is imprecision of our present knowledge of exactly how δD_{rain} relates to temperature or climate. The main limitation in this study for application to archaeological bone

results from the preliminary nature of its investigation into diagenetic effects on bone δD. More information about $\delta^{18}Ob$ in deer would be of benefit to any diagenetic or climatic study that includes this variable and might help improve the ability to use bone for estimating RH. It is towards filling these gaps in knowledge that the direction in future work should lie. The study of all isotope, yield and other chemical indicators on a long, temporal sequence of white tailed deer bone from a single location could go a long way towards further evaluating the use of isotopes in bone for detecting effects of diagenesis on δDb and its ultimate utility for interpreting paleoclimates.

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APPENDIX A

GLOSSARY OF SYMBOLS, ABBREVIATIONS AND ADDITIONAL ISOTOPE EQUATIONS

A.1 Definition of symbols used (in alphabetical order):

symbol:	meaning:
$\alpha_{A/B}$	Isotope fractionation factor defined by eq. A.6.
β_n	The regression coefficients from stepwise multilinear regression with n representing the order in which a variable was selected during each step in the program.
$\delta^{13}C$	Measured carbon isotopic ratio of a sample as defined by general eq. A.1 and normalized to PDB.
$\delta^{13}Cb$	Measured $\delta^{13}C$ value of collagen from combustion of w.b. powders.
$\delta^{13}Cco$	Measured $\delta^{13}C$ of col samples.
$\delta^{13}Cg$	Measured $\delta^{13}C$ of gel samples.
$\delta^{13}Cg'$	The $\delta^{13}Cg$ value as estimated from $\delta^{13}Cb$ corrected for the contribution of CO_2 from bone carbonate. $\delta^{13}Cg'=\delta^{13}Cb-0.6‰$.
δD	Measured hydrogen isotopic ratio of a sample as defined by general eq. A.1 and normalized to SMOW:
δDb	Measured δD value of w.b. samples not corrected for H-exchange.
δDb_c	Measured δDb corrected for H-exchange.
δDb_{c100}	Measured δDb corrected for H-exchange and corrected to 100% RH whereby the effects of RH on leaf δD are eliminated.
δDb_{c100y}	Measured δDb corrected for H-exchange and corrected to 100% RH. However, unlike δDb_{c100} the yearly (RHy) rather than growing season relative humidity is used in the correction procedure.
δDb_{100}	δDb results corrected for RH effects but not for H-exchange.

δDco Measured δD of col samples

δD_c Measured δD of a sample corrected for H-exchange.

$\delta D_c'$ The isotopic value of non-exchangeable hydrogens in a sample. This "true" value differs from the measured δDc value by $\delta D_c' = \delta D_c - [m_R \epsilon_{eH-v} / (1-m_R)]$ where ϵ_{eH-v} is the isotopic separation factor between H_{EX} and atmospheric H_2O-vapor.

δD_{cel} The δD value of non-exchangeable hydrogens in nitrated plant cellulose.

δD_{EQ} The δD values of the H_2O used in the equilibration experiments with which δDv_{EQ} were maintained in equilibrium.

δD_{EX} The δD of H_{EX} (exchangeable hydrogens).

δDg Measured δD of gel samples.

δDg_c δDg corrected for H-exchange.

δDi The δD of input, environmental-H_2O entering plants. For deer, $\delta Di = \delta Dw$.

δDl δD value of leaf-H_2O. In this work this value can be theoretically estimated using leaf-H_2O models from the literature.

$\delta Dl^\wedge$ Theoretical estimate of leaf-H_2O obtained using $\delta^{18}Ob$.

δDp The δD of plant tissue.

$\delta D(R)$ The measured δD of an internal bone reference standard.

δDv The δD value of the lab atmospheric H_2O-vapor. For H-exchange correction, δDv is that of atmospheric H_2O vapour in the laboratory. In leaf water models, δDv is the atmospheric H_2O vapour at the deer collection site at the time of plant growth.

δDv_{EQ} The δD value of the H_2O-vapors used in the equilibration experiments.

δDw Weighted growing season average of monthly δD of precipitation using PPTm as the weighing factor. The growing season months are defined as the months of the year in which Tm' is above 0°C. Values for each sampling location were determined by interpolation between contours.

The contours, in turn, were constructed using δDw values calculated for each IAEA location and contours shapes of Sheppard et al. (1969) and Taylor (1974).

$\delta Dw'$ Unweighted growing season average of monthly δD of precipitation for each IAEA location.

$\delta Dw\hat{}$ Estimate of δDw for each sampling location derived using δDb and other laboratory measures in multilinear regression models.

δDwy Weighted annual (12 mo.) average of monthly δD of precipitation using PPTm as the weighing factor. Values for each sampling location were determined by interpolation between contours. The contours, in turn were constructed using δDwy values calculated for each IAEA location and contour shapes of Sheppard et al. 1969 and Taylor 1974.

$\delta Dw'y$ Unweighted annual (12 mo.) average of monthly δD of precipitation for each IAEA location.

$\delta Dwy\hat{}$ Estimate of δDwy for each sampling location using δDb and other laboratory measures in a multilinear regression equation.

$\delta Dx(R)$ The δD of a bone reference material (R) where x represents the preparation method: co=col, g=gel, b=w.b.

$\delta Dx(R)_{EQ}$ The $\delta Dx(R)$ value measured from equilibrium Expt. #6.

$\delta Dx_c(R)$ The $\delta Dx(R)$ value corrected for H-exchange.

$\delta Dx_c(R)_{EQ}$ The $\delta Dx(R)$ value calculated using results from equilibrium Expt. #6.

$\delta^{15}N$ Measured nitrogen isotopic ratio of a sample as defined by general eq. A.1 and normalized to atmospheric N_2 (ATM).

$\delta^{15}Nb$ Measured $\delta^{15}N$ value of w.b. samples.

$\delta^{15}Nco$ Measured $\delta^{15}N$ of col samples.

$\delta^{15}Ng$ Measured $\delta^{15}N$ of gel samples.

$\delta^{18}O$ Measured oxygen isotopic ratio of a sample defined using general eq. A.1 and normalized to SMOW.

$\delta^{18}Ob$ Measured $\delta^{18}O$ of bone phosphate.

$\delta^{18}Obw$ The $\delta^{18}O$ value of body-H_2O.

$\delta^{18}O_{cel}$ $\delta^{18}O$ of wood cellulose.

$\delta^{18}O_{dw}$ The $\delta^{18}O$ value of deer drinking water.

$\delta^{18}O_i$ The $\delta^{18}O$ value of input or consumed water for the deer.

$\delta^{18}O_l$ The $\delta^{18}O$ value of leaf-H_2O. Here, $\delta^{18}O_l$ can be estimated using leaf-H_2O models.

$\delta^{18}O_l\hat{}$ The $\delta^{18}O_l$ value as estimated from $\delta^{18}O_b$.

$\delta^{18}O_w$ The growing season average of precipitation $\delta^{18}O$ calculated for each sampling location from δD_w using the meteoric water relationship (eq. 2.5).

$\delta^{18}O_w\hat{}$ The $\delta^{18}O_w$ value as estimated from δD_{bc} and other variables in multivariate regression equation.

$\delta^{18}O_{wy}$ The yearly average of precipitation $\delta^{18}O$ calculated for each sampling location from δD_{wy} using the meteoric water relationship (eq. 2.5).

$\delta Z'$ General expression for isotopic ratios as defined by eq. A.1.

Δe_{V-L} The equilibrium isotopic separation between vapor and liquid defined by eq. A.7.

ENAM A standard of tooth enamel from a modern bison from Alberta (App. B).

ϵe Equilibrium isotopic separation factor defined by eq. A.7.

ϵe_{H-V} The equilibrium isotopic separation factor between H_{EX} (exchangeable hydrogens) in a bone sample and atmospheric H_2O-vapor with which H_{EX} equilibrate.

ϵe_{V-L} Equilibrium isotopic separation factor between vapor and liquid phases of H_2O.

ϵ_{H-V} The overall isotopic separation factor between H_{EQ} and atmospheric H_2O vapour.

$\epsilon_{H-V(EQ)}$ ϵ_{H-V} for reference samples of Expt. #6.

ϵk Kinetic isotopic separation factor defined as in eq. A.7 using αk.

ϵ_B Isotopic separation factor due to biological fractionation effects defined as in eq. A.7.

$^{2}\epsilon_{B}$ Isotopic separation factor between δDb and δDl ($\approx \delta Db - \delta Dl$) due to biological fractionation factors.

$^{2}\epsilon_{Bp}$ Separation factor between δDp and δDl ($\approx \delta Dp - \delta Dl$) due to biological fractionation factors in plants.

$^{2}\epsilon_{Ba}$ Separation factor between between δDb and δDp ($\approx \delta Db - \delta Dp$) due to biological or trophic level effects in animals.

$^{13}\epsilon_{B}$ Separation factor between $\delta^{13}Cb$ and $\delta^{13}C$ of an animal's diet due to biological fractionation in the deer.

$^{18}\epsilon e_{p-bw}$ Equilibrium isotopic separation factor between bone phosphate $\delta^{18}O$ and body-H_2O $\delta^{18}O$ ($\approx \delta^{18}Ob - \delta^{18}Obw$).

ϵo_{H-V} Isotopic separation factor between H_{EX} and atmospheric H_2O vapour that occurs during outgassing due to D-enrichment of adsorbed water prior to removal.

$\epsilon o_{H-V(EQ)}$ ϵo_{H-V} for the reference samples of Expt. #6.

σ In this work, σ means standard deviation.

σ_{p} In this work, σ_{p} is the pooled standard deviation, or weighted average standard deviation where each standard deviation in the average is weighted by n, the number of samples on which each standard deviation had been calculated (cf. Johnson 1976:372 and Table 5.15 notes).

ACP Amorphous calcium phosphate

ATM Atmospheric N_2 used as the normalization standard for all $\delta^{15}N$ determinations.

b_R The intercept of regression equation which relates $\delta Dx(R)$ to the δD_{EQ} used in the equilibrium experiment. b_R is ultimately related to $\delta Dx_c(R)$ by: $\delta Dx_c(R)=(b_R - {}^{2}\epsilon e_{V-L} \cdot m_R)/(1-m_R)$

b_R' The intercept of a regression equation which relates $\delta Dx_c(R)$ to the δDv_{EQ} used in an equilibration experiment. The H_2O-vapors, in turn, are maintained in equilibrium at room temperature with liquid H_2O of known isotopic value (δDv_{EQ}) so that: $b_R' = ({}^{2}\epsilon e_{V-L} \cdot mR) + b_R$

BIS Bone standard: powdered fossil tibia of bison from Alberta (App. B.)

col Intact collagen extracted from bone by demineralization. These samples differ from gels in that they have not been gelatinized.

C/N — Relative percent carbon to nitrogen yield as defined by general eq. 4.2. C/N_g is of gel and C/N_b is of w.b. preparations.

C/g — Percent carbon yield per gram collagen in a sample as defined by general eq. 4.3. C/g_g is of gel and C/g_b is of w.b. preparations.

DCPD — The mineral dicalcium phosphate dihydrate $[Ca(HPO_4) \cdot 2H_2O]$.

gel — Denoting collagen extracted from bone and gelatinized.

GEL — A standard of thoroughly mixed commercial gelatin (App. B).

HAP — The mineral hydroxy apatite $[Ca_{10}(PO_4)_6(OH)_2]$.

H_{EX} — Abbreviation for the words "exchangeable hydrogens" or "exchanged hydrogens".

H/C — Percent relative yield of hydrogen to carbon as defined by general eq. 4.2. H/C_g is of gel and H/C_b is of w.b. preparations.

H/g — Percent yield of hydrogen per gram of collagen as defined by general eq. 4.3. H/g_g is of gel and H/g_b is of w.b. preparations.

H/N — Percent relative yield of hydrogen to nitrogen as defined by general eq. 4.2. H/N_g is of gel and H/N_b is of w.b. preparations.

m — The regression slope derived from equilibration experiments denoting the proportion of exchangeable hydrogens (p_{EX}) in a specific type of sample (w.b. col, gel). This value is used to correct for H-exchange.

m_R — The regression slope of the relationship between $\delta Dx(R)$ and δDv_{EQ} from equilibration experiments.

MUSK — A bone standard: powdered compact bone of muskox from a single site on Banks Island, NWT (App. B). Same site as SEAL.

N/g — The percent yield of nitrogen per gram of collagen as described by general eq. 4.3. N/g_g is of gel and N/g_b is of w.b. preparations.

OCP — The mineral octacalcium phosphate, $[Ca_4H(PO_4)_3]$

OLD-C — A bone sample: powdered rib of a fossil bison from the Old Crow region in the Yukon (App. B).

PDB — Peedee Belemnite. The carbonate normalization standard used for all $\delta^{13}C$ determinations.

p_{EX} — The proportion of exchangeable hydrogens among the total number of hydrogens existing in a sample.

$\%p_{EX}$ — $\%p_{EX}=100 \cdot p_{EX}$

PPT — Growing season average of PPTm for each sampling location.

PPTm — Total monthly amount of precipitation for each sampling location obtained from NOAA and Environment Canada Weather Stations.

PPTm' — Total monthly amount of precipitation for each IAEA location used as weighing factors in weighted average δ D.

PPTy — Same as PPT except annual (12 mo.) average.

r — Linear correlation coefficient.

R — Multiple linear correlation coefficient. $R^2=1/100 \cdot \%VAR$ where: %VAR is the percentage of variation of the dependent variable which is explained by the regression model. In the divariate case, $R^2=r^2$.

rh — Short form for the words "relative humidity".

RH — Percent growing season average of daily (24h) rh derived for each sampling location using data obtained from NOAA and Environment Canada weather stations. The growing season is defined as the months of the year in which Tm>0°C

RH_2 — For Canadian weather stations, percent growing season average of rh for the same two observation periods in each time zone as was used by NOAA.

RH_{day} — For Canadian weather stations, percent growing season average of daytime (daylight) rh calculated using the 7am and 1pm LST time periods from Environment Canada (1984).

RH_m — Percent average monthly relative humidity for a particular observation period during the day for each NOAA and Environment Canada weather station.

RHy — Same as RH except annual (12 mo) average.

RHy_2 — Same as RH_2 except annual (12 mo) average.

RHy_{day} — Same as RH_{day} except annual (12 mo) average.

SEAL	A bone standard: powdered tibia of a ringed seal from the same site as MUSK on Banks Is. NWT (App. B).
SMOW	Standard Mean Ocean Water (Vienna). The ocean water normalization standard used for all δD and $\delta^{18}O$ determinations.
T	Growing season average of Tm for each sampling location from NOAA and Environment Canada weather stations. The growing season is defined as the months of the year in which Tm>0°C.
T_6	Average temperature over the winter months (October through March).
T'	Condensation temperature for a vapor cloud.
Tc	Sample combustion temperature.
T_{EQ}	Temperature of equilibration experiments in which H_{EX} of a sample are equilibrated with H_2O-vapor of a known isotopic composition. T_{EQ} for the experiments here was ≈24°C [T_{EQ}(room)]. Also represents the temperature of equilibration of any sample before it is outgassed.
Tm	Average monthly temperature for each NOAA and Environment Canada weather station.
Tm'	Average monthly temperature for each IAEA location.
Tp	Sample pre-heating (or outgassing) temperature.
Ty	Same as T except annual (12 mo) average.
w.b.	Powdered whole bone samples.
X/Y	General term for relative percent yield as given by eq. 4.2. X/Y_g is of gel and X/Y_b is of w.b. preparations.
X/g	General term for absolute percent yield as given by eq. 4.3. X/g_g is of gel and X/g_b is of w.b. preparations.
Z'/Z	General expression for the ratio of the heavy to light isotope

A.2 Additional Formulae Used for Stable Isotope Work:

1. The δ-notation (in permil or ‰) for all isotopic determinations compares ratio of the heavy (Z') to light (Z) isotopes in a sample gas to this same ratio in a standard gas:

(eq. A.1)
$$\delta Z' = \left[\frac{(Z'/Z)_{SPL}}{(Z'/Z)_{STD}} - 1\right] \cdot 1000$$

2. In order to convert the Z' of a sample compared to a working reference ($\delta Z'_{SPL/REF}$) to standard notation ($\delta Z'_{STD}$ or $\delta Z'_{SPL/STD}$):

(eq. A.2)
$$\delta Z'_{STD} = \delta Z'_{SPL/REF} + \delta Z'_{REF/STD} + \delta Z'_{SPL/REF} \cdot \delta Z'_{REF/STD} \cdot 10^{-3}$$

where: $\delta Z'_{REF/STD}$ is the δZ' value of working reference normalized to the standard (STD).

3. Any comparison on the mass spectrometer such as a sample to a reference gas ($\delta Z'_{A/B}$) can be recalculated to reverse the order of the comparison, i.e.:

(eq. A.3)
$$\delta Z'_{B/A} = -\left[\frac{10^3 \cdot \delta Z'_{A/B}}{(10^3 + \delta Z'_{A/B})}\right]$$

4. At the University of Waterloo all $\delta D_{SPL/REF}$ values were converted to standard notation using the SMOW/SLAP linear calibration method which corrects for various machine errors including H_3^+ contribution (Gonfiantini pers. comm. 1980, 1981; Hoering 1974):

(eq. A.4)
$$\delta D_{SMOW/SLAP} = \left[\frac{(\delta D_{SPL/REF} - \delta D_{SMOW/REF}) \cdot \delta D_{SLAP/SMOW}}{\delta D_{SLAP/REF} - \delta D_{SMOW/REF}}\right]$$

where: $\delta D_{SMOW/REF}$ was the V.SMOW compared to the working reference (REF), $\delta D_{SLAP/REF}$ was SLAP compared to REF and $\delta D_{SLAP/SMOW}$ was the δD of SLAP normalized to SMOW.

The variance ($\sigma^2_{SPL/SLAP/SMOW}$) was calculated as:

$$\text{(eq. A.5)}\quad \sigma^2_{SMOW/SLAP} = (\delta_{SLAP/SMOW})^2 \cdot \left[\frac{\sigma^2_{SPL/REF}}{\delta D_{SLAP/REF} - \delta D_{SMOW/REF})}\right] + \ldots$$

$$\ldots + A/(\delta D_{SLAP/REF} - \delta D_{SMOW/REF})^4$$

where: $A = [(\delta D_{SPL/REF} - \delta D_{SLAP/REF})^2 \cdot \sigma^2_{SMOW/REF} + \ldots$
$\ldots + (\delta D_{SPL/REF} - \delta D_{SMOW/REF})^2 \cdot \sigma^2_{SLAP/REF}]$

and: $\sigma^2_{SMOW/REF}$, $\sigma^2_{SLAP/REF}$, $\sigma^2_{SPL/REF}$ were the variances on $\delta D_{SMOW/REF}$, $\delta D_{SLAP/REF}$ and $\delta D_{SPL/REF}$ respectively.

5. The isotope fractionation factor ($\alpha_{A/B}$) will be defined as:

$$\text{(eq. A.6)}\quad \alpha_{A/B} = \left[\frac{(Z'/Z)_A}{(Z'/Z)_B}\right] = \left[\frac{(\delta Z'_A + 10^3)}{(Z'_B + 10^3)}\right]$$

where: $\delta Z'_A$ and $\delta Z'_B$ were normalized to STD and $\alpha_{A/B} = 1/\alpha_{B/A}$.

6. The isotope separation factor (ϵ_{A-B}) will be defined as:

$$\text{(eq. A.7)}\quad \epsilon_{A-B} = (\alpha_{A/B} - 1) \cdot 10^3 \approx \delta Z'_A - \delta Z'_B$$

7. Isotope fractionation will be defined as:

$$\text{(eq. A.8)}\quad \Delta_{A-B} = (\ln\alpha_{A/B}) \cdot 10^3 \approx \delta Z'_A - \delta Z'_B$$

8. Equilibrium (e) isotopic fractionation between liquid (L) and vapour (V) and between ice (I) and vapour phases of water:

$$\text{(eq. A.9)}\quad {}^2\epsilon e_{L-V} \approx {}^2\Delta e_{L-V} = 24.844 \cdot 10^6 T^{-2} - 76.248 \cdot 10^3 T^{-1} + 52.612$$

(Majoube 1971):

(eq. A.10) $^{2}\epsilon e_{I-V} \approx {}^{2}\Delta e_{I-V} = 16.289 \cdot 10^{6} T^{2} - 94.5$

(Merlivat and Nief 1967 as in Fritz and Fonts 1980):

(eq. A.11) $^{18}\epsilon e_{L-V} \approx {}^{18}\Delta_{L-V} = 5800/T(^{\circ}K) - 10.5$

(Baertschi and Thurkauf 1969):

APPENDIX B
THE SAMPLES AND SAMPLE INFORMATION

Modern deer samples:

Notes: Unless indicated otherwise, all samples are tibia of white-tailed deer (*Odocoileus virginiana*). Map locations for each specimen are identified by their $\delta^{15}N$, $\delta^{13}C$ or δD result. Most specimens were road-killed in 1984. Gelatins only were analyzed from ND-1 and LA-8.

AB-1 One year old pregnant female (one fetus) from Edmonton, Alberta. Killed May, 1984.

AB-2 Two year old male from Edmonton, Alberta. Killed May, 1984.

AB-3 One year old male from Edmonton, Alberta. Killed May, 1984

AB-4 Left tibia of female from Lethbridge, Alberta.

AB-5 Right tibia of same specimen as AB-4.

AB-6 Male of white- or black- tailed deer from 3 miles east of Foremost, Alberta. Killed January, 1977.

AB-7 Female (140 lbs fresh weight) from Drumheller, Alberta. Killed January, 1974.

AL-1 Tarsal of 1½ year old male (105 lbs) from Hale County, Alabama. Killed November, 1984.

AL-8 Two year old female from Big Lake Wildlife Management Area, Louisiana. Killed August, 1984.

AZ-1 Male, 5-6 years old, from Bumbelbee Washington Tonto National Forest, ≈10 miles from Roosevelt in Gila County, Arizona. Killed November, 1984.

BC-2 Right tibia of yearling male mule deer from 5 km. south of Little Fort, British Columbia. Feed included green up-land grasses in hay-alfalfa fields. Killed May, 1984.

BC-3 Left tibia of same specimen as BC-2.

BC-4 Mature Sitka Black-tailed male from Rennell Sound on Graham Island, Queen Charlotte Islands, British Columbia.

FL-1 Metatarsal of 1½ year old male, (dressed weight of 45 lbs.) from St. Vincents National Wildlife Refuge, St., Vincent Island, Apalachicola, Florida. Killed December, 1984.

KS-1 Metatarsal from Flint Hills National Wildlife Refuge near Hartford, Kansas.

LA-2 Five year old female, 140 lbs, from Lake Providence, Louisiana. Killed November, 1984.

MI-1 From Section 4, Lapeer County, Michigan. Feeding included small oak and maple woodlots, small marshes, corn, beans and sugar beets. Killed May, 1984.

MI-1b Metatarsal from same leg as tibia of specimen MI-1.

MI-2 One year old female from Section 22 Benzie County, Michigan. Feeding included conifer swamps, oak, aspen and fruit orchards (mainly cherry). Autopsied May, 1984.

MI-3 Two year old female from Section 9, Delta County, Michigan. Feeding included aspen, conifer and cedar swamps. Autopsied May 1984.

MI-4 One year old Male from Section 3, Tuscola County, Michigan. Feeding included small oak, maple woodlots, small marshes, corn, beans, and sugar beets. Killed June 1984.

MI-5 One year old male from Ingham County, Michigan. Feeding included Small oak and maple woodlots, corn, beans and winter wheat. Autopsied May 1985.

MI-6 From Section 31, Oscoda County, Michigan. Feeding included mixed conifer and hardwood conifer swamps and jackpine stands. Killed May 1984.

MI-8 Three year old female from Section 10, Leelanau County, Michigan. Feeding included mixed conifer and hardwood and fruit orchards (mainly cherry). Autopsied May 1984.

MI-9 Three year old female from Section 22 Charlevoix County, Michigan. Feeding included mixed conifer and hardwood and fruit orchards (mainly cherry). Killed May 1984.

MI-10 Five year old female from Section 18, Wexford County, Michigan. Feeding included oak, beech, aspen, maple and some large cedar swamps. Killed May, 1984.

MI-11 One year old female (small for age) from Section 19, Ingham County, Michigan. Feeding included small oak, maple woodlots, corn, beans, and winter wheat. Killed May 1984.

MI-12 Three year old male (in poor health) from Olkand County, Michigan. Feeding included small oak, maple woodlots, small marshes, corn, and beans. Killed May 1984.

MI-13 One year old male from Oakland County, Michigan. Feeding included small oak, maple woodlots, small marshes, corn and beans. Killed May 1984.

MI-14 Two year old female from Section 25, Grand Traverse County, Michigan. Feeding included oak, aspen, conifer swamps and fruit orchards (mainly cherry). Autopsied May 1984.

MO-4 Yearling male from Howard County, Missouri. Killed November, 1984.

MS-1 Female, 2½ years, from Chickasaw Wildlife Management Area, Chickasaw County, Near Houston, Mississippi.

MT-1 Female, 4 to 6 years old, from prairie herd of Medicine Lake National Wildlife Refuge, Sheridan County, Montana. Feeding included buck brush, prairie rose, choke-cherry, some agricultural grain and alfalfa. Killed September, 1984.

NB-1 Two year old female from 10 miles east of Fredericton, New Brunswick. Killed May, 1984.

ND-1 Two year old male from Section 14, McLean County, 6 miles north of Washburn, North Dakota. Habitat includes cultivated land and native mixed grass prairie. Killed June, 1984.

NE-2 Male, 2½ year old, from Crescent Lake National Wildlife Refuge, Garden County, Nebraska. Killed November, 1984.

NS-1 Three year old male (poor condition with 10% marrow fat) from Hwy. 101, about 1 mile from Yarmouth, Nova Scotia.

NS-2 Female from Guysborough County, Nova Scotia. Feeding in blueberry field when shot in April, 1984.

OH-1 Male, 14 month old, from Rome Township, Athens County, Ohio. Killed August 1984.

OH-2 Female, 14 month old, from Waterloo Township, Athens County, Ohio. Raised in captivity on sweet mix feed containing cracked corn, steamed oats, soy beans, alfalfa pellets, salt, minerals and molasses. Killed August, 1984.

OH-3 Female, 14 month old, from Dover Township, Athens County, Ohio. Killed August, 1984.

OK-1 Male, 1½ year old, (110 lbs) from McCurtain County Oklahoma. Killed September, 1984.

OK-2 Female, 1½ year old, (79 lbs) from McCurtain County, Oklahoma. Killed September, 1984.

OK-5 Female, 4½ years old, (116 lbs) from Osage County, Oklahoma. Killed August 1984.

OK-9 Metatarsal of 3 year old female (121 lbs) from Cherokee, Oklahoma. Killed July 1984.

OK-10 Metatarsal of 1 year old female (70 lbs) from Cherokee, Oklahoma. Killed July 1984.

ON-1 From Sidney Township, Ontario (district 64, Lot 3, Concession 4). Habitat includes rolling fields and deciduous forests. Killed April, 1984.

ON-3 Female fawn (74 lbs and poor condition) from Glamorgan (Lot 15, Concession 7) near Minden, Ontario. Killed April 1984.

ON-6. Four year old from Mowat Twonship, Parry Sound District, Ontario. Killed March 1984.

ON-8 Four year old male from Kenora, Ontario. Killed May, 1984.

OR-1 Male mule deer, 1½ year old, from Section 26, Harney County, Oregon. Killed October 1984.

SA-1 Metatarsal from Hwy. 11 about 15 miles south of Saskatoon. Killed August 22, 1984.

QC-1 From Saintes-Veronique, Quebec. Killed June 1984.

QC-2 Male, 26 months old, (excellent conditon) from International Airport of Mirabel, near Montreal, Quebec. Killed June, 1984.

QC-3 Adult specimen from Route 299, about 40 km south east of Sainte-Anne-des Monts in Gaspesie region, Quebec. My notes indicate that the fragment which arrived was too thick to be a tibia. The bone was in poor shape, porous and stained brown.

QC-8 Female from Gatineau Valley, Quebec. Killed summer, 1984.

TX-1 From Tom Green County, about 3 miles north of Christoval (intersection of Farm Road 2335 and South Concho River). Killed April, 1984.

TX-2 Metatarsal of male, 2½ to 3½ years, from Aransas National Wildlife Refuge, about 50 miles north of Corpus Christi, Texas. Animal is of mcilhennyi sub-species of w.t. deer found along Texas and Louisiana Gulf Coasts. Killed October, 1984.

TX-3 Mcilheynnyi sub-species from Aransas National Wildlife Refuge, 50 miles north of Corpus Christi, Texas. Killed October, 1984.

TX-5 Metatarsal from same leg and same specimen as TX-3.

TX-6 Female from Palo Pinto County, Texas (Hwy. 4 bridge, Brazos River at Dark Valley Creek).

WI-1 One and half years old (dressed weight of 134 lbs) from Section 7, Oneida County, Wisconsin. Habitat includes aspen and red pine, near potato fields. Killed October, 1984.

WV-1 Female, 3½ years old, good physical condition, (95 lbs) from Hardy County, West Virginia (Sloan Parsons' farm). Killed September, 1984.

WV-2 Male, 1½ year old, good physical condition from Hardy County, West Virginia (Sloan Parsons' farm). Killed September, 1984.

WV-3 Female, 4½ years old, good physical condition from Hardy County, West Virginia (Sloan Parsons Farm). Killed September, 1984.

WY-2 Six year old female, poor condition, from Crook City (Section 17), near Sundance, Wyoming. Riparian habitat includes ponderosa pine, scrub, oak forests; diet could include alfalfa, winter wheat and other domestic crops.

Muskox standard:

MUSK This standard was prepared using bone fragments from a number of muskox (*Ovibos moschatus*) individuals from a 100 year old archaeological site (PjRa 18) on the northern part of Banks Island, North West Territories. The site had been occupied for about 35-40 years (cf. Will 1982). A large amount of standard was prepared by placing the bone fragments in a tungsten carbide mill. The cancellous and stained bone was gound off and saved using several episodes of 10 minutes grinding. The powder from the spongy bone was saved for gelatin extraction. Fragments of dense compact bone which had remained after the initial grinding were hand picked for cleanliness and ground to a fine powder. The powder fraction made up of compact bone is the standard. This standard was prepared early before degreasing techniques were established but it is assumed that any lipids entering the bone from the marrow were naturally and mechanically removed.

Seal standard:

SEAL This standard was prepared from a right tibia of ringed seal (*Phoca hispida*) from the same site as the muskox standard on Banks island (PjRa 18). The sample was not degreased but the outer surface and stains were ground off using the Dremel tool. The sample had been air-dried for 3 days at 70°C prior to grinding to a fine powder in a tungsten carbide mill.

Seal-1 This is a second fragment of tibia of the same specimen as Seal-0. This sample had been prepared by the standardized methods with the exception that the lipid removal step was not included.

Gelatin Standard:

GEL A gel standard made from a large amount of thoroughly mixed commercially bought gelatin.

Tooth Enamel Standard:

ENAM Enamel from a modern bison (Bison bison) tooth from Bow Island near Medicine Hat, Alberta. Enamel was carefully cleaned of dentine using a dental drill with a diamond drill bit. It was then pulverized in a tungsten carbide ball mill for 10 min. as described for whole bone.

Fossil seal:

Seal-2 Right tibia of ringed seal (≈2500 y.B.P.) from the Lagoon Site (OjRl-3) on the southern part of Banks Island, North West Territories. The bone was hard and dense, had a dark brown stain on the outer rim and was permeated with brown staining. It also had a label (black ink and clear nail polish) which was ground off with the Dremel tool.

Seal-3 Left tibia from a harbour seal (*Phoca vitulina*) (≈4500 y.B.P.) from Namu (ElSx-1) in British Columbia. The sample appeared very weathered. It was light weight and porous. The bone was fragile and had been broken open. It appeared to be glued at a seam and this portion of the bone was avoided during sampling.

Seal-4 Right tibia of harbour seal (≈3400 y.B.P.) from Namu (ElSx 1) British Columbia. The sample was very weathered, light weight and porous and buff stained. The shaft had been split open and the bone was very friable. The bone was labeled with black ink which was scraped off.

Seal-5 Left tibia of ringed seal (500 to 950 y.B.P.) from the Washout Site on Hersheel Island, North West Territories. The bone appeared well preserved and was dense with a light brown stain on the outside and buff stain on inside.

Seal-6 Left humerus of Harbour seal (100-600 y.B.P.) from the Waterlogged Site (FaSu-1) near Kwatna British Columbia. Bone appeared to be relatively unweathered.

Seal-7 Left femur of Harbor Seal (200-1200 y.B.P.) from Kwatna (FaSu 10) British Columbia. Sample was very porous and weathered with light buff stain.

Fossil bison:

BIS Bison tibia (Bison sp.; ≈1500 y.B.P) from the Muhlbach site (FbPf 100) near Drumheller, Alberta (cf. Gruhn 1971). Tibia was lightweight and stained buff to brown.

OLD-C Juvenile Bison rib (Bison sp.; ≈12,000 y.B.P.) from a gulley of Porcupine River near Old Crow in the Yukon Territory, Canada.

Animal Flesh:

Pyrolysis experiment:

Marine:

1) Herring (*Culpea harengus*) from Georgia Straights, B.C.

2) Shrimp from Georgia Straights, B.C.

3) Greasy herring sample from Georgia Straights, B.C.

Terrestrial:

1) Beaver (*Castor canadensis*) from Telegraph Creek, B.C.

2) Moose (*Alces americana*) from Telegraph Creek, B.C.

3) Muledeer (*Odocoileus hemionus*) from Sushwap, B.C.

Combustions in Pyrex (P) and Quartz (Q) Tubing:

M047 Shrimp from Georgia Straits, B.C.

M047 Herring from Georgia Straits, B.C.

I010 Ptarmigan from Telegraph Creek, B.C.

I014 Showshoe Hare from Telegraph Creek, B.C.

Human Bone:

NOTE: All samples except Annabon were gelatins donated by Dr. Brian Chisolm and extracted as per Chisolm et al. (1983a). Information on marine or terrestrial diet and $\delta^{13}C$ was also provided by Dr. Chisolm and has been discussed in Chisolm et al. (1982, 1983b) and in Lovell et al. (1986). Annbon was whole bone powder donated by Dr. Ann Katzenberg and was analyzed both as a whole bone powder and as a gelatin extract.

M028P Subsisted mainly on marine foods. From Crescent Beach, B.C. (DgRr 1; ≈2300-3000 y.B.P.).

M032P Subsisted mainly on marine foods. From Crescent Beach, B.C. (≈2300-3000 y.B.P.).

M048P Subsisted mainly on marine foods. From Namu, B.C. (ElSx 1; ≈1500-3000 y.B.P.).

M033Q Subsisted mainly on marine foods. From Namu, B.C. (≈2880-3800 y.B.P.).

M040Q Subsisted mainly on marine foods.

I066P Subsisted mainly on interior foods. From near Lilloet, B.C. (≈100 y.B.P.).

I068P Subsisted mainly on interior foods. From Alexis Creek, near Williams Lake B.C. (≈200 y.B.P.).

I039P Subsisted mainly on interior foods. Early historic site from near Lilloet, B.C.

I067Q Subsisted mainly on interior foods. From Alexis Creek, near Williams Lake (≈200 y.B.P.).

I058Q Subsisted mainly on interior foods.

I060Q Subsisted mainly on interior foods.

I056Q Subsisted mainly on interior foods.

Annbon Ground bone from Huron Ossuary Ossossane' (A.D. 1636) near Midland, Ontario. Various bone fragments from nine individuals were ground in a tungsten carbide mill. No other pre-treatment was carried out.

APPENDIX C

STABLE ISOTOPE ANALYSES -PREPARATION AND EXTRACTION OF GASES FOR MASS SPECTROMETRY

C.1 Cryogenic Separation of H_2O, N_2 and CO_2 Combustion Products:

Details of the use of the specially designed vacuum extraction line shown in Fig. 4.1 for the extraction of the H_2O, CO_2 and N_2 are described here. The whole bone or gelatin sample has been previously combusted in the 9mm breakseals. The sample was then attached to the line at (B) using a tube cracker (Desmarais and Hayes 1976) designed to fit the 9mm o.d. size of the breakseal tubing. Capillary tubing (6 mm o.d.) with one end sealed using a torch, is attached to the line at positions (C,H,I) and will eventually be used to receive the cryogenically separated combustion products (H_2O, N_2, CO_2). The tube at (C) will receive the H_2O. The Breakseal at (I) will receive the CO_2 following its manometric determination at (E) and (G). Previously outgassed activated charcoal at N_2(l) temperature is used first to collect the N_2 into the manometer for yield measurement and then into the Pyrex breakseals at (H) which all contain previously outgassed activated charcoal. The activated charcoal in the manometer (F) is outgassed between samples using heating tapes. The activated charcoal in each sample breakseal (H) has been outgassed overnight and is continuously outgassed until ready to receive the N_2. All sample tubing (both 9mm and 6mm) have

been previously pre-cleaned through heating at 550°C for ≈1-2 h. The 500 ml bulb at (A) will initially receive both the CO_2 and H_2O combustion products while the U-tube at (D) ensures that the combustion product to be transferred into the manometer (either N_2 or CO_2) is completely purified and that no H_2O (or CO_2) escapes out of the bottom line. To ensure that the bottom line remains clean, spare bulb and U-tube are available and the entire line may be dismantled and cleaned if necessary.

The extraction procedure is as follows: Following attachment of the sample and tube cracker and all other aparatus as shown in Fig. 4.1, the line is evacuated. The bottom portion of the line is isolated from the rest of the line by closing stop cock 8 (SC8). Since explosive decompression may result when the sample tube is broken open under vacuum, the sample gasses are first frozen down with N_2(l) before the breakseal is cracked. Liquid N_2 is then placed on the 500 ml bulb and the sample gradually heated with an air gun to transfer all CO_2 and H_2O into the bulb. To ensure complete outgassing of the bone mineral, a small furnace is placed over the sample at the bottom of the tube (B) which is then heated at 450°C for 15 minutes to allow all H_2O to transfer into the bulb. Heating continues throughout the extraction procedure to ensure that no water or gases re-adsorb onto the bone mineral.

Liquid N_2 is then placed on the U-tube (D) and on the N_2 manometer trap (F), and the N_2 gas is cryogenically transferred through the U-tube and into the N_2 manometer trap (F) where it is temporarily stored by closing SC6. As in all other transfers the appropriate SC's must be adjusted accordingly. For instance during the N_2 transfer SC's 1,3,5,7 will be closed and SC's 2,8,4,6 will be open.

Liquid N_2 is then placed on the CO_2 manometer trap (E), SC5 is opened, and the N_2(l) baths on the bulb and U-tube are changed to isopropyl and dry ice thus allowing the CO_2 to evaporate from the bulb trap while the H_2O remains frozen. The bath is changed first under the U-tube and then under the bulb trap and the evaporating CO_2 allowed to transfer through the U-tube water trap (D) and into the CO_2 manometer trap (E).

The bottom line containing the H_2O is then isolated from the rest of the system by closing SC8 and the H_2O breakseal (C) is cooled with N_2(l). The isopropyl-dry ice baths are removed from the U-tube and the bulb trap. Both traps (A,D) are heated using an air gun and heating tapes for 10 minutes to complete the transfer of the H_2O into the breakseal (C). This breakseal is then sealed off with a torch.

After the CO_2 gas yield is measured manometrically, the CO_2 is cryogenically transferred into the CO_2 breakseal (I) which is then sealed off with a torch. Similarly, the N_2 gas yield is measured and the N_2 cryogenically transferred into its designated breakseal (H) using the charcoal sponge at N_2(l) temperature. The activated charcoal in the manometer trap (F) remains at room temperature during both the yield measurement and the transfer of N_2 into (H).

C.2 <u>Calibration of Extraction Line for Yield Determination:</u>

A bulb of known volume was attached to the vacuum line and evacuated. Tank CO_2 was allowed to fill the vacuum line and bulb and the gas pressure was measured manometrically. From the known pressures of CO_2 and the volume of the bulb, the exact number of moles of CO_2 in the bulb could be calculated. Calibrated quantities of CO_2 gas

were then created and transferred into the CO_2 manometer for measurement at room temperature. A regression of the data allowed determination of moles of CO_2 gas per centimeter manometric reading and the uncertainty about the regression curve gave the error of this measurement.

Once the line was calibrated for CO_2, calibration for N_2 and accuracy of this yield was determined. Pure tank nitrogen was allowed to fill the CO_2 manometer and the number of moles of N_2 calculated on the basis of the CO_2 calibration. The N_2 was then frozen onto the charcoal manometer trap and the empty CO_2 manometer trap was closed. The N_2 manometer readings were thus determined at room temperature for various known quantities of N_2 gas. Regression of the data was used to determine the number of moles of N_2 gas per cm of manometer reading and the uncertainty of the measurement.

C.3 <u>$\delta^{13}C$ Mass Spectrometry:</u>

The CO_2 produced by combustion of collagen was cryogenically isolated and stored in sealed 6mm o.d. Pyrex breakseals for later isotopic analyses. Using a tube-cracker, the CO_2 is then introduced into the on-line vacuum line attached to the mass spectrometer. Since all H_2O and N_2 have been previously removed, the CO_2 may be introduced directly into the mass spectrometer. However, using precautions standardized by the McMaster Lab, the CO_2 was first passed through an isopropyl/dry ice bath (which ensures complete removal of H_2O) and frozen onto a cold finger. The line is then re-evacuated to remove any residual N_2, prior to introducing the CO_2 into the mass spectrometer.

The in-house reference is a large amount of CO_2 ($\delta^{13}C \approx 0‰$) released, using phosphoric acid, from a calcite standard and stored for a period of time in a Pyrex vessel on-line to the mass spectrometer. For each the reference and sample gas, both the mass 45 ($^{13}C^{16}O^{16}O$ + $^{12}C^{17}O^{16}O$) and 46 ($^{12}C^{18}O^{16}O$) peaks were measured. An average of six determinations for each of the sample and reference gas for each peak were determined for $\delta(45)_{REF}$ and $\delta(46)_{REF}$ calculations (see eq. A.2). The component in the mass 45 peak due to a small amount of ^{17}O was then subtracted through knowledge of natural $^{17}O/^{16}O$ abundances. The analytical uncertainties were: ±0.05‰ for $\delta(45)_{REF}$ or $\delta(46)_{REF}$ and ±0.1‰ for $\delta^{13}C_{PDB}$. Equation A.2 was used to calculate conventionally normalized ($\delta^{13}C_{PDB}$) values.

C.4 $\delta^{15}N$ Mass Spectrometry:

The N_2 gas was stored in Pyrex breakseals containing activated charcoal (Ch. 3). The N_2 was then introduced directly into the on-line vacuum line and mass spectrometer using a tube-cracker and glass adapter containing glass wool to prevent any charcoal from entering the line. It was found that machine variability was improved slightly (without altering the $\delta^{15}N_{REF}$ values) if, after the N_2 was introduced into the vacuum line, $N_2(l)$ was placed on an adjacent cold finger prior to introducing the N_2 into the mass spectrometer. This ensured that any residual CO_2 or H_2O in the adapter or line remained frozen and did not enter the mass spectrometer.

Tank N_2 ($\delta^{15}N$ = -6.9‰) was the in-house working reference and was placed into a sample container for use as a reference gas each day of analysis. Samples of atmospheric N_2 (ATM) were also prepared and

cross-referenced to tank N_2. Here, 6mm o.d. Pyrex breakseals containing copper turnings and a small amount of CuO was thinned at the open end. The tubes were allowed to cool and air was allowed to freely move in and out of the tubes before the thinned end was quickly sealed off with a torch. These tubes containing air were then cooked at 550°C to convert any small amounts of naturally occurring N-oxides to N_2. The atmospheric N_2 was then purified and collected into Pyrex breakseals containing charcoal in a manner previously described for sample N_2.

An average of six determinations of each of the sample and the reference gas was included in the $\delta^{15}N_{REF}$ calculation. The analytical uncertainties are ±0.05‰ for $\delta^{15}N_{REF}$, ±0.1‰ for $\delta^{15}N_{ATM}$ and the standard deviation is ±0.15‰ for replicate preparations and analysis of atmospheric N_2 ($\delta^{15}N$(ATM/ATM), n=20.

In the McMaster Lab N_2 from combusted samples is introduced directly into the mass spectrometer. Previously outgassed NaOH and glass wool in a glass adapter is used as a pre-filter to remove H_2O and CO_2. The $\delta^{15}N$ results collected on charcoal of this work were compared to those of the same samples filtered through NaOH and introduced directly. It was discovered that the $\delta^{15}N$ results from samples collected on charcoal were offset by ≈-0.45‰ relative to traditional preparations. Using the in-house reference value of: ($\delta^{15}N_{REF/ATM}$ + 0.45‰) in eq. A.2 corrected for this small bias for all the $\delta^{15}N$ results of this work.

Previously outgassed molecular sieve at N_2(l) temperature was also tried for collection of N_2 gas but it was found that the uncertainty in the $\delta^{15}N$ results was three times larger (±0.6‰) and the results were biased by -2.0‰ (see Table D.1).

C.5 δD Mass Spectrometry: Conversion of H_2O to H_2, H_2 Yield Measurements, Controls and Precautions:

The uranium furnace and vacuum line (H_2-line) used at the University of Alberta for reducing H_2O to H_2 gas over hot uranium metal (Begeleisen et al. 1952) at ≈800°C is shown in Fig. C.1. The possibility of memory effects from adsorption of H_2 or H_2O onto the uranium (i.e. Friedman and Hardcastle 1970) was reduced by evacuating the line and preheating and outgassing the uranium for at least one hour prior to use. Results were monitored for memory effects (below) and the uranium replaced with fresh uranium turnings if significant effects began to occur. Memory effects were also reduced by preparing samples and standards in groups of similar isotopic composition and by preparing large amounts of water (≈15 μl) in order to reduce the significance that a small memory effect would have on the final δD results.

The water sample, which had been previously purified and stored in 6mm o.d. Pyrex breakseals was introduced into the vacuum-line at (C) (Fig. C.1) using a tube-cracker. Once the breakseal had been broken under vacuum (with SCs 11 and 13 closed), the H_2O was frozen onto the cold finger (B) using $N_2(l)$, and the line was re-evacuated. The H_2O was then cryogenically transferred to the U-tube (E) and the line re-evacuated. Then, with SCs 11, 10 and 7 closed, the $N_2(l)$ was removed from the water trap at U-tube (E) and placed on the U-tube (G). This allowed the H_2O to slowly transfer through the uranium furnace. The H_2 gas which was formed was pumped into the manometer

Figure C.1. Hydrogen Line for Reducing H_2O to H_2.

Hydrogen Line at the University of Alberta for Reducing H_2O to H_2 Over Hot Uranium.

Pirani gauges (A,H)

Cold finger and two U-tubes (B,E,G)

Uranium furnace (F,D)

Automatic Toepler pump (J) with electronic box (I) for switching from air to vacuum to control mercury level

Manometer (L)

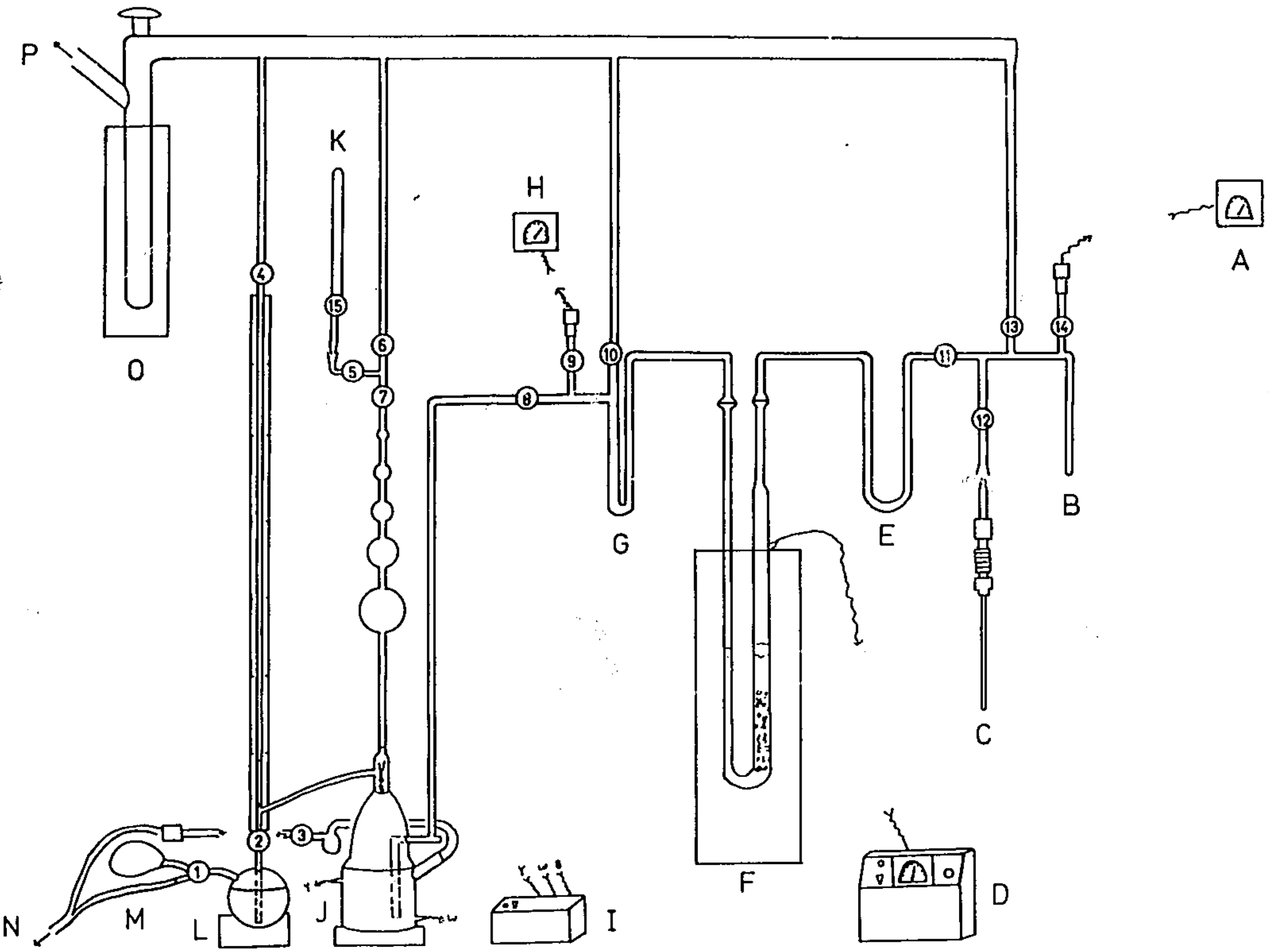
Sample vessel (K)

Hand pump (M) for forcing mercury up Toepler pump to compress H_2 gas into sample vessel.

Vacuum to Toepler pump (N)

Cold trap at $N_2(l)$ temperature (O)

To vacuum (P) (through mercury diffusion pump) for hydrogen line and uranium furnace.


A
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(J, L) using the automated Toepler pump (J, I). Waiting for 1/2hr allowed all the H_2 to form and be collected. This also reduced possible memory effects from the uranium furnace. If any H_2O transferred through the uranium furnace (to G) without being converted to H_2 gas, the uranium in the furnace was replaced.

Once the H_2-yield was determined at (L), the H_2-gas was manually pumped into a previously evacuated glass sample container fitted with a stop-cock using viton O-ring seals (K and SC-15) using the hand pump (M and L) and with SC-6 closed, SCs-7,5 and 15 open. Closing SC-15 sealed the H_2 gas into the pyrex container. The H_2 gas was thus stored until it was introduced into the mass spectrometer.

Loss of H_2 from the H_2-gas-containers was prevented by use of Viton O-rings, by storing the H_2-gas samples at liquid nitrogen temperature and by analyzing each gas sample on the same day it was prepared. Each H_2 sample took ≈1hr to prepare and, typically, ten samples and one or more reference gasses were prepared on a single day. All were analyzed on the mass spectrometer during a short period towards the end of the day. By knowing the H_2 yields for each sample and monitoring the gas pressure gage on the mass spectrometer any serious leak in a sample containers was easily detected.

H_2-gas from a H_2O standard (ABC-TAP, -55‰) was prepared towards the end of each day for use as the in-house working reference gas on the mass spectrometer. In addition, a series of secondary H_2O standards were prepared and analyzed at the beginning of the visit to the Alberta lab and at various intervals during the visit as cross-references in order to monitor and correct errors due to H_3^+ contribution to the DH peak in the mass spectrometer (cf. Friedman 1953,

Horibe and Kabayakawa 1960, Gonfiantini 1981); memory effects, loss of H_2 gas from the sample containers over a days storage and loss of H_2 reference gas in the mass-spectrometer during the time of analysis. The secondary calibration standards were: V.SMOW (δD = 0‰), SLAP (δD = −428‰), NBS-1A (δD = −182‰), NBS-1 (δD = −47‰) (Gonfiantini 1978); GISP (δD = −186‰; Gonfiantini lett. 1980, Miller 1984) and, at the University of Waterloo, SWAT (δD = −76‰; Miller 1984). A Hamilton syringe was used to transfer ≈20 μl of each H_2O standard from its bottle, fitted with a septum, into the H_2-line at (C) which was, itself, fitted with a glass adapter and Ultra-torr with septum.

The ABC-TAP working reference had a similar δD value to most deer of this study. This reduced the memory effects from the H_2-line, as well as the uncertainty of and bias in the δD_{REF} measurement due to machine operating errors including over or under correction for H_3^+. An average of four determinations of DH/HH for each of the reference and sample gasses was included in the δD_{REF} calculation and analytical uncertainty was typically ≤ ±0.4‰.

Each time a new group of samples was to be prepared on the H_2-line which had considerably different isotopic compositions from the deer samples, the working reference gas was also changed so that it was of similar composition to that of the samples. Memory effects from the H_2-line were evaluated by preparing two very different standards, then preparing a duplicate of the second standard (i.e. ABC-TAP, GISP1, GISP2). If the first replicate of the second standard (GISP1) was identical in composition to its second replicate (GISP2), then there were no memory effects. If the first replicate was biased in the direction of

the first standard (i.e. ABC-TAP) then a coefficient representing the size of the memory effect could be evaluated. i.e.:

$$\text{(eq. C.1)} \qquad A = \left[\frac{\delta D_{GISP1} - \delta D_{GISP2}}{\delta D_{GISP2} - \delta D_{ABC-TAP}} \right]$$

and a linear correction for memory effects performed:

$$\text{(eq. C.2)} \qquad \delta D'' = A \cdot (\delta D''' - \delta D''_{(n-1)}) + \delta D'''$$

where: $\delta D''$ was the value corrected for memory effects, $\delta D'''$ was the uncorrected value, $\delta D''_{(n-1)}$ was the δD value of the previous sample prepared on the H_2-line. Using precautions described above, memory effects were always found to be small with shifts of ≈1‰ per 100‰ difference in δD between two samples prepared in sequence on the H_2-line.

In order to check for shifts in δD due loss of H_2 gas from the sample containers over a days storage, one sample of ABC-TAP was prepared at the beginning of the day (ABC-TAP1) and compared to the ABC-TAP working-reference (ABC-TAP2) prepared at the end of the day. A positive bias in ABC-TAP1 indicated shifts in δD due to a loss of H_2 gas from the sample container during a days storage. A coefficient representing this bias was calculated:

$$\text{(eq. C.3)} \qquad B = \left[\frac{\delta D_{ABCTAP2} - \delta D_{ABCTAP1}}{n'} \right]$$

where: n' was the order of preparation of ABC-TAP2 during the day (i.e. if n'=10 and ABC-TAP2 was the tenth sample prepared on the H_2-line

then ABC-TAP1 was stored for ≈10h). If necessary, a correction for bias due to loss of H_2 could be performed:

(eq. C.4) $\delta D' = \delta D'' + n(B/n)$

where: $\delta D''$ was of the sample corrected for memory effects and n was the order during the day in which that sample was prepared. However, such effects were always found to be small, never exceeding 0.4‰ for every 10 hours of storage.

In order to check for bias in results due to losses of H_2 from the reference gas on the mass-spectrometer during the analysis time, two ABC-TAP's could be prepared at the end of the day. One (ABC-TAP1) was used as the reference gas and the other (ABC-TAP2) was cross checked against it after all other samples had been analyzed. If ABC-TAP2 appeared to be too light isotopically, this would indicate loss of reference gas during the analysis time. Under the analytical conditions employed here, no significant shifts in δD value due to shifts in the composition of the reference gas on the mass spectrometer were ever noticed.

While the H_3^+ contribution on the MICROMASS 602 can be automatically "dialed away", residual errors due to H_3^+ corrections and other machine factors produced a bias in results which was especially noticeable for samples having isotopic compositions considerably different from that of the reference gas.

Linear calibration was, therefore, performed (Hoering 1974) using the series of secondary standards listed earlier. A standard (REF2), significantly different in composition from ABC-TAP or another

working reference (REF1), was used to evaluate bias for samples having δD values lying between REF1 and REF2:

$$(\text{eq. C.5}) \qquad C = \left[\frac{(\delta D'_{REF2} - \delta D_{REF2})}{(\delta D_{REF2} - \delta D_{REF1})}\right]$$

where: δD_{REF2} and δD_{REF1} were the true δ-values of the references (normalized to SMOW) and $\delta D'_{REF2}$ was the apparent δ-value or that containing bias due to machine errors. Corrections could then be performed using:

$$(\text{eq. C.6}) \qquad \delta D = C \cdot (\delta D' - \delta D_{REF1}) + \delta D'$$

where: $\delta D'$ was that of a sample (normalized to V.SMOW) which has been already corrected for other effects (memory and H_2 loss). Typically, the δD results were biased by ≈3‰ for every 100‰ spread in δD between sample and working reference. On one or two occasions, larger biases (6–11‰) were noted and resulted in re-calibration of the MICROMASS for H_3^+ correction. Bias was evaluated at the beginning of each visit to the Alberta lab and was re-evaluated by re-analyzing the secondary standards whenever any MICROMASS operating conditions (such as H_3^+ corrections) were altered.

The uncertainties of the δD measurement using various H_2O standards were (1) analytical: $\delta D_{REF} \pm 0.4$‰, $\delta D_{SMOW} \pm 0.7$‰ (n=221), (2) total variability (pooled standard deviations, σ_P; Johnston 1976:372) from replicate H_2-preparations, analysis with correction for bias: $\delta D_{SMOW} \pm 1.7$‰ (n=48), (3) replicate preparation and analysis without correction for bias:

δD_{SMOW}±1.7‰ (n=57), and (4) replicate preparation and analysis of samples having isotopic compositions close to that of the working reference used: δD_{SMOW}±1.3‰.

A small number of samples were analyzed at the university of Waterloo. H_2O was converted to H_2 over hot uranium at 800°C using a line similar to that shown in Miller (1984, side B, pp. 48) and described by Aravena (1982). Sample water was introduced using a glass adapter and tube cracker into the vacuum line and allowed to pass slowly over hot uranium. A magnetic circulation pump homogenized the H_2 gas in the line and forced any remaining H_2O over the uranium. After ≈5 minutes, liquid N_2 was placed on a sample vessel to help concentrate the H_2 gas into the vessel, which was then sealed and transferred to the mass spectrometer. A rough estimate of H_2 yields was provided by calibrating the vacuum pressure readings on the mass spectrometer to the known amounts of water standards introduced into the uranium furnace using a Hamilton syringe and glass adapter with septum.

In the Waterloo lab, analyses were against an internal working reference (REF, δD≈-250‰) and eq. A.4 and A.5 were used to convert δD_{REF} to δD_{SMOW} with calibration to the SMOW/SLAP line. Uncertainties obtained during this study were: (1) analytical: δD_{REF}±0.2‰ (n=65) for ≈10µl sized samples; δD_{REF}±0.45‰ (n=13) for ≈2µl samples, (2) total between run variability (σ_P) on replicate H_2-preparation, analysis, normalization and SMOW/SLAP calibration for all water standards: $\delta D_{SMOW/SLAP}$±2.5‰ (n=34) and (3) within run (or single day) variability on replicates: $\delta D_{SMOW/SLAP}$±2‰ (n=36).

APPENDIX D

OUTGASSING AND COMBUSTION CONDITIONS - RAW DATA

D.1 Table Notes:

[A] Outgassing conditions: (see Ch. 5 for additional explanations).

1) Samples were placed on a vacuum line and preheated with heating tapes for 1 to 3 days.
2) Samples were placed in a vacuum oven for 3 days then dried on a vacuum line for 1 day using heating tapes.
3) Samples were placed in a vacuum oven for 3 days then dried on a vacuum line for 1 day using voltage controlled furnaces.
4) Samples were placed in the vacuum oven for 1 day and then placed on a vacuum line for varying lengths of time and at varying temperatures using voltage controlled furnaces.
5) Samples were placed directly on vacuum line and outgassed using thermistor controlled furnaces.

Unless indicated otherwise, all gelatins were preheated at ≈100°C and all whole bone at ≈150°C. Combustion tube types are indicated by capital letters as Pyrex (P), Quartz (Q), or Vycor (V). The number (900) indicates that the set of samples were combusted at 900°C. Unless indicated otherwise, all gelatins were combusted at 550°C and all whole bone samples at 450°C.

[B] Analytical uncertainties are: $\delta D \pm 1.5‰$, $\delta^{13}C \pm 0.1‰$, and $\delta^{15}N \pm 0.1‰$.

[C] All yields in percent were calculated as described in Ch. 4.

[D] The new vacuum line was cleaner and held a better static vacuum ($\approx 10^{-3}$ torr) than did the old line. Gas pressures during transfer were reduced in two ways: (a) a 500ml bulb trap for water collection was employed and (b) as the CO_2 evaporated out of the bulb trap it was immediately transferred through a U-tube and frozen down rather than remaining for any length of time as gas in the line volume containing the H_2O. Very high gas pressures occurred when the H_2O samples were collected on the University of Alberta Uranium-line using a cold finger and small line volumes without immediate removal of CO_2. Under such conditions the CO_2 yields may appear high because a fraction of the H_2O is transferred and collected with the CO_2.

[E] Analyzed at University of Alberta unless indicated otherwise.

[F] Samples placed on vacuum line and outgassed together.

[G] Nitrogens collected onto charcoal unless indicated otherwise.

[H] Yields of samples without CO_2 collection were normalized to 0.92, the average C/g yields of 15 samples ($\sigma = \pm 0.02$).

[I] δD analyzed at Waterloo University. H_2 yields estimated using the major ion beam of the MICROMASS 602.

[J] Nitrogens collected on molecular sieve

[K] Parenthesis contain the number of samples included in the average.

[L] Uncertain about time in oven, probably 1 day with 3 days on the vacuum line using voltage controlled furnaces.

[M] N_2 analyzed by conventional methods (i.e. not frozen onto charcoal)

[X] Not included in average.

Appendix D. RAW RESULTS OF SAMPLE PREPARATION

Table D.1 Pre-Heating Conditions and Extraction Methods of Gelatins Combusted in Pyrex at 550°C.

Outgassing Conditions[A]	Isotopes[B]				Yields[C]						Extraction notes[D]
	δD[E]	same time[F]	δ13C	δ15N[G]	C/N	H/N	H/C[H]	N/g	C/g	H/g	
1P	-47		-	-	-	-	85	-	89	76	old
	-44		-	-	-	-	90	-	93	84	calcite
(n=10)	-60		-	-	-	-	86	-	-	79	line
	-54		-	-	-	-	90	-	-	83	variable-
	-49		-	-	-	-	77	-	-	71	low gas
	-54		-	-	-	-	90	-	-	83	pressure
	-49		-	-	-	-	100	-	-	92	"
	-54		-	-	-	-	92	-	89	82	"
	-49		-	-	-	-	77	-	92	70	"
	-44		-	-	-	-	99	-	93	91	"
	-51±6						89±8		91±2	81±7	
2P	-69		-	-	-	-	81	-	90	73	high gas
	-60		-	-	-	-	84	-	90	76	pressure
(n=10)	-55		-	-	-	-	67	-	92	66	"
	-56[I]		-	-	-	-	57	-	-	52	"
	-62[I]		-	-	-	-	59	-	-	54	"
	-58[I]		-	-	-	-	73	-	93	67	"
	-57		-12.5	7.2	-	-	94	-	86	81	low gas
	-69		-12.9	7.5	-	-	92	-	80	73	pressure
	-68		-12.3	6.3[J]	-	-	93	-	84	78	"
	-71		-12.9	7.6	-	-	96	-	69	67	"
	-63±6		-12.7±0.3	7.4±0.2			80±15		86±8	69±10	
	66±6(4)[K]						94±2(4)		80±8(4)	75±6(4)	

Table D.1 (cont)

Outgassing Conditions	Isotopes				Yields						Extraction notes
	δD	same time	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	
3P	-68		-12.3	5.4[J]	-	-	95	-	90	85	low gas
	-68		-	7.5	-	-	95	-	90	85	pressure
	-66	-67±1	-12.7	5.7[J]	-	-	95	-	90	85	"
(n=16)	-67		-	7.3	-	-	95	-	96	85	"
	-68		-12.9	4.7[J]	-	-	95	-	89	85	"
	-65		-12.2	5.3[J]	-	-	94	-	88	83	"
	-69		-12.8	7.4	-	-	80	-	109	87	"
	-68	-67±2	-12.9	6.0[J]	-	-	93	-	89	83	"
	-66		-13.1	5.0[J]	-	-	78	-	90	71	"
	-67		-	6.4	-	-	97	-	90	87	"
	-67		-12.5	8.0	95	88	93	96	91	84	new line
	-68		-12.6	7.7	96	88	91	96	93	85	"
	-67	-68±1	-11.9	7.3	96	88	89	95	94	83	"
	-68		-12.9	7.5	97	86	89	95	92	81	"
	-70		-13.1	7.6	98	88	90	95	93	84	"
	-70		-12.7	7.6	96	88	92	96	92	84	"
	-68±1.4		-12.7±0.4	7.5±0.2(9) 5.5±0.6(7)[J]	97±1	88±1	91±5	96±1	92±5	84±4	

Table D.1 (cont)

Outgassing Conditions	Days	°C	Isotopes δD	same time	δ13C	δ15N	Yields C/N	H/N	H/C	N/g	C/g	H/g	Extraction notes
4P	4	152	-58		-12.9	7.9	98	87	89	-	-	-	new line
	3	135	-56	-57±1	-12.9	7.6	97	88	91	-	-	-	"
(n=8)	1	143	-56		-12.5	7.5	99	92	93	-	-	-	"
	2	149	-53		-12.9	7.7	97	86	88	-	-	-	"
	5	153	-60		-	7.6	99	86	87	96	95	83	"
	5	94	-59	-59±1	-13.2	7.5	100	89	88	92	92	81	"
	3	100	-59		-11.8	7.7	97	90	93	95	92	85	"
	1	100	-59		-12.1	7.7	99	92	93	93	92	85	"
			-58±2		-12.6±0.5	7.7±0.1	98±1	89±3	90±3	94±2	93±2	84±2	
5P (n=1)			-49		-11.8	7.2	102	91	89	89	91	81	"
6P (n=1)			-44		-13.0	7.1	98	87	89	93	91	81	"

Table D.2 Standard Gelatin Samples in Vycor or Quartz Tubes Combusted at 550°C.

Outgassing Conditions			Isotopes				Yields						Extraction notes
	Days	°C	δD	same time	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	
2Q	1	130	-65[I]		-	-	-	-	85	-	91	77	old line-
(n=9)	1	130	-64[I]		-13.0	-	-	-	87	-	93	81	low gas
	1	130	-66[I]		-	-	-	-	86	-	92	79	pressure
	1	130	-68[I]		-	-	-	-	87	-	94	82	"
	1	130	-65[I]		-13.0	-	-	-	86	-	91	78	"
	1	130	-72[I]		-	-	-	-	80	-	93	74	"
			-67±3		-13.0±0				85±3		92±1	79±3	
2Q			-61		-	-	-	-	-	-	-	75	"
(n=3)			-57		-12.4	7.3	-	-	98	-	77	75	"
			-57		-12.8	7.4	-	-	100	-	-	-	"
			-58±2		-12.6±0.3	7.4±0.1			99±1		77±0	75±0	

Table D.3 Standard Gelatin Samples in Vycor or Quartz Tubes Combusted at 900°C

	tube	δD	same time	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	Extraction notes
1QV900	Q	-55		-	-	-	-	72	-	-	66	cold finger
(n=9)	Q	-36		-	-	-	-	35	-	-	32	U.of A
	Q	-51		-	-	-	-	12	-	-	11	very high
	Q	-46		-	-	-	-	71	-	-	65	gas pressures
	V	-49		-	-	-	-	4	-	-	4	"
	V	-48		-	-	-	-	4	-	-	4	"
	V	-46		-	-	-	-	85	-	-	78	low
	V	-47		-	-	-	-	81	-	96	77	gas
	V	-55		-	-	-	-	86	-	-	79	pressure
		-48±6						50±34		96±0	46±33	

Table D.4 Pre-Heating of Muskox Whole Bone Combusted in Pyrex at 450°C

Outgassing Conditions			Isotopes				Yields						Extraction notes
	Days	°C	δD	same time	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	
2P			-169[1]		-	-	-	-	77	-	84	65	old line
(n=2)			-172		-	-	-	-	86	-	63	62	low pressures
			-172±2						82±6		79±8	64±2	
4P			-158[x]		-	5.7[x]	94[x]	89[x]	95[x]	84[x]	79[x]	75[x]	new line
(n=7)	6	95	-148		-19.7	5.9	91	98	107	85	78	83	low gas
	6	150	-151		-	6.4	92	93	101	82	75	76	pressure
	5	144	-157		-20.9	5.7	96	95	99	64	61	60	-problem
	4	156	-149	-151±3	-20.5	6.2	89	94	106	83	74	78	with O_2
	3	160	-151		-20.3	5.9	92	94	102	83	76	78	"
	2	147	-150		-20.0	6.0	90	94	106	83	75	79	"
	5	146	-154		-20.2	6.4	92	93	101	83	77	77	"
			-151±3		-20.3±0.4	6.1±0.3	92±2	94±2	103±3	80±7	74±6	76±7	
5P			-139		-20.7	4.5	100	89	89	83	83	73	new line
(n=6)			-142	-143±1	-20.4	4.8	99	90	91	91	90	82	low gas
			-143		-21.3	4.9	98	88	90	90	88	80	pressure
			-140		-20.7	4.6	100	95	95	84	79	79	"
			-145	-140±4	-20.4	4.7	95	92	97	86	81	79	"
			-137		-20.3	4.7	95	94	99	87	82	82	"
			-141±3		-20.6±0.4	4.7±0.1	98±2	91±3	94±4	87±3	84±4	79±3	

Table D.5 Pre-Heating of Muskox Whole Bone Combusted in Quartz at 450°C.

Sample	δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	Extraction notes
2Q	-174	-20.0	-	-	-	79	-	82	64	old line-
(n=5)	-169	-20.9	-	-	-	87	-	84	73	low gas
	-162	-20.7	-	-	-	74	-	79	59	pressure
	-161	-20.8	-	-	-	95	-	78	74	"
	-166	-21.1	-	-	-	61	-	77	47	"
	-166±5	-20.9±0.2				79±13		80±3	63±11	

Table D.6 Pre-Heating of Muskox Gelatins Combusted in Pyrex at 550°C.

Outgassing Conditions	Isotopes				Yields						Extraction notes
	δD	same time	δ¹³C	δ¹⁵N	C/N	H/N	H/C	N/g	C/g	H/g	
2P	-161		-21.8	3.9*	-	-	77	-	89	68	new line
	-177		-21.7	3.6*	-	-	76	-	90	68	low gas
	-160		-21.9	3.9*	-	-	88	-	80	71	pressure
	-158		-23.8x	3.7*	-	-	94x	-	-	-	"
	-164±9		-22.3±1	3.8±0.2			84±9		86±6	69±2	
			-21.8±0.1(3)				80±7(3)				
5P (n=5)	-145		-21.3	3.8	102	90	88	91	93	82	new line-
	-144		-22.0	3.4	104	95	92	89	92	85	low gas
	-148	-145±3	-20.8	3.6	103	92	89	92	95	85	pressure
	-144		-	3.5	103	90	87	91	94	81	"
	-143		-21.4	3.5	106	97	92	84	89	82	"
	-145±2		-21.4±0.5	3.6±0.2	104±2	93±3	90±2	89±3	93±2	83±2	

Table D.7 Pre-Heating of Muskox Gelatin Combusted in Quartz at 550°C.

Outgassing Conditions	δD	same time	δ¹³C	δ¹⁵N	C/N	H/N	H/C	N/g	C/g	H/g	Extraction notes
2Q	-182		-21.[illegible]	4.7*	-	-	65	-	81	53	old line low gas pressure

Table D.8 Pre-Heating of Seal Whole Bone Combusted at 450°C.

Outgassing Conditions	Isotopes				Yields						Extraction notes
	δD	same time	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	
2P (n=1)	-41		-15.9	-	-	-	79	-	68	53	old line high pressure
5P	59		-15.5	18.7	112	98	87	64	72	63	new line
(n=6)	59		-15.8	18.4	107	99	92	69	74	68	low gas pressure
	61		-16.0	18.8	102	98	96	74	76	72	
	57		-15.2	18.8	104	94	90	74	77	69	
	64		-14.1x	18.9	99x	98	99x	72	71x	70	
	63		-15.7	18.9	103	99	97	74	76	73	
	61±3		-15.4±0.7	18.8±0.2	105±5	98±2	94±5	71±4	74±2	69±4	
			-15.6±0.3(5)		106±5(4)		92±4(5)				

Table D.9 Pre-Heating of Seal Whole Bone Combusted in Quartz at 450°C.

Outgassing Conditions	δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	Extraction notes
2Q (n=1)	5	-15.9	-	-	-	78	-	67	52	old line high pressure

Table D.10 Pre-Heating of SEAL Gelatin Combusted in Pyrex at 550°C.

Outgassing Conditions	δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	Extraction notes
5P (n=2)	92	-16	18.4	102	94	92	81	82	76	new line
	86	-	-	103	94	91	84	86	79	low pressure
	89±4			103±0.7	94±0	92±0.7	83±2	84±3	78±2	

Table D.11 Pre-Heating of SEAL Gelatin Combusted in Quartz at 900°C.

Outgassing Conditions	δD	$\delta^{13}C$	$\delta^{15}N$	C/N	H/N	H/C	N/g	C/g	H/g	Extraction notes
5Q900 (n=1)	93	-16.1	18.2	104	97	93	66	69	64	new line low pressure

D.2 Calibration of $\delta^{15}N$ of Six Samples for Difference in Sample Preparation:

Both AB-3 and MUSK had undergone both the oven and standard (5P) treatments (AB-3 by method 3P in the oven and MUSK by method 4P in the oven). The KS-1 w.b. had undergone the 3P treatment whereas the gel had undergone the standard treatment. The results on the two treatments appear in Table D.12. For KS-1 it was assumed that the gel and col $\delta^{13}C$ and $\delta^{15}N$ values provide a good estimate for KS-1 w.b. undergoing the standard treatment (see Ch. 5).

An examination of the data (Table D.12) shows no consistent difference in $\delta^{13}C$ between the two treatments but $\delta^{15}N$ increases with the oven treatment and this increase is largest for the higher $\delta^{15}N$ values. A simple linear calibration equation using this data from Table D.12 was used to correct the oven-treated data for this bias ($n=3$, $r=0.994$).

(eq. D.1) $\delta^{15}N(\text{corrected}) = 0.1465\delta^{15}N(\text{oven}) + 3.921$

The corrected $\delta^{15}N$ results appear in Table D.12.1 and D.12.2.

D.3 Calibration of δDb Values for Difference in Sample Preparation:

AB-3 had undergone preparation 5P and 3P and had been outgassed at the same time as the other w.t. deer undergoing preparation 3P. For AB-3, $\delta D_{3P} - \delta D_{5P} = -23.5‰$. If it could be assumed that only δDv and ϵe_{H-v} were affected by preparation 3P to produce changes in δD_{EX}, and that $\delta Dx_c'$ or p_{EX} were not affected, then eq. 5.2

Table D.12 Comparison of Deer $\delta^{13}C$ and $\delta^{15}N$ for Different Outgassing Conditions and Correction of $\delta^{15}N$ for Effects of Different Sample Preparation.

Table D.12.1 Results for Calibration Method

sample	5P[a] $\delta^{13}C$	5P[a] n	3P[b] $\delta^{13}C$	3P[b] n	5P[a] $\delta^{15}N$	5P[a] n	3P[b] $\delta^{15}N_o$	3P[b] n	3P[b] $\delta^{15}N(cal)$
MUSK	-20.5±3	17	-20.3±0.4	8	4.8±0.3	17	6.1±0.3	10	4.8
AB-3	-21.3	1	-22.3	1	5.0	1	7.2	1	5.0
KS-1	-22.3±0.1[c]	2	-21.3	1	5.2±0[c]	2	8.8	1	5.2

Table D.12.2 Calibrated Results[c]

sample	$\delta^{15}N_o$	$\delta^{15}N(cal)$[d]
KS-1	8.8	5.2
TX-1	8.7	5.2
NS-1	4.0	4.5
NB-1	5.7	4.8
ON-3	5.0	4.6
ON-6	3.2	4.4

a Normal outgassing conditions
b outgassed in the vacuum oven over 3 days then on the vacuum line for 1 day except for MUSK, oven 1 day and on-line 3 days.
c gelatin.
d $\delta^{15}N(cal) = 0.1465\delta^{15}N_o + 3.921$, n=3, r=0.994.
where $\delta^{15}N_o$ is the original uncalibrated result.

can be used to give $\delta Db_{3P}-\delta Db_{5P}\approx-23.5‰$ for all w.t. deer samples from the 3P preparation.

Evidence in Ch.5, nonetheless, indicates that a change in collagen composition might also occur in the 3P method so that some change in $\delta Dx_c'$ and p_{EX} might occur. Not only were there changes in the $\delta^{15}N$ values (above) but also a continuous decrease in δD value with length of time in the oven. However, additional evidence (Ch. 6) suggests that the value of p_{EX} does not greatly change with outgassing conditions, at the least, with changes in T_P (Fig. 6.1). If p_{EX} remains constant then eq. 5.1 (Ch. 5) can be simplified to:

(eq. D.2) $$\delta Dx_i-\delta Dx_j=p_{EX}(\delta Dv_i+\epsilon e_{H-Vi}-\delta Dv_j-\epsilon e_{H-Vi})+(1-p_{EX})(\delta Dx_c'_i-\delta Dx_c'_j)$$

where the first term, $p_{EX}(\delta Dv_i+\epsilon e_{H-Vi}-\delta Dv_j-\epsilon e_{H-Vi})$, is a constant offset in δD_{EX} for all 3P. It does not seem unreasonable that there may also be a constant offset in the non-exchangeable hydrogens or $(\delta Dx_c'_i-\delta Dx_c'_j)$ for all 3P samples. For instance, using methods for which there was good on-line control of T_P (3P,4P,5P), $\delta Dg_{3P}-\delta Dg_{5P}=-19‰$ for GEL which is not dissimilar to the above (-23.5‰) value. In addition, $\delta D_{4P}-\delta D_{5P}$ for GEL is -9‰ and similar to that for MUSK (-10‰). Thus, all 3P samples may be offset by a constant value from the 5P samples. Using this assumption, +23.5‰ was added to all 3P results in order to calibrate δD for difference in sample preparation.

APPENDIX E

MULTIPLE LINEAR REGRESSION - METHODS AND PRECAUTIONS

The stepwise multiple linear regression procedure used here (STATPRO, Wadsworth Professional Software, Inc. 1984, Boston) first selects the predictor variable showing the highest correlation coefficient with the dependent variable. It then enters independent variables into the regression equation one at a time, according to decreasing values of their F-statistics. Only those variables having F-values with a probability level of 0.10 (two tailed test) are included (for details see Draper and Smith 1981, Bevington 1969, Efroymson 1962, Johnson 1976). The multiple regression equation is expressed as:

(eq. D.1) $$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_i X_i$$

where X_i is the chosen predictor variable, i is the order in which the variable is chosen, β_i the regression coefficient of the ith variable, and β_0 the intercept. For each regression step, The STATPRO program generates partial F-scores, beta-coeffients, and the multiple correlation coefficient (R) which aids in the analysis of the appropriateness of including various predictor variables in the equation. A main reason for excluding variables is multicollinearity between some of the predictor variables. This can produce instability in the beta-coefficients including

possible changes in their sign following the stepwise addition of a particular predictor variable. An additional indication of regression coeffient instability due to poor choice of variables, is the presence of beta-coefficients which have the wrong sign, that is, their signs do not make biological or physical sense or their signs are opposite to those of the divariate correlation coefficient (r) between the dependent variable and each predictor variable (cf. Mullet 1976). Additional problems will occur if the relationship between the dependent variable and a predictor variable is non linear or if outlier results are included in the analysis.

Several tests were performed in order to protect against multicollinearity among the predictor variables and to ensure that the best regression model had been obtained:

1) The dependent variable was plotted against all possible predictor variables in order to visually check for extraneous points or for non-linear relationships. In some instances curvilinear fits to the data would reveal that a transformation of either predictor or dependent variable would allow a linear model to be more appropriately fit to the data. Generally, simple transformations [i.e. $\ln(Xi)$ or Xi^2] were preferred for most work. While quadratic equations [$(Xi)^2 + Xi$] can also be fit using multilinear regression methods (Draper and Smith 1981), these are more complex because some of the predictor variable terms can be naturally highly correlated (i.e. Xi may be highly correlated with Xi^2) which can make it difficult to use some of the tests for multicollinearity that are described below. Quadratic fits also require use of additional terms such as cross products if there is more than one quadratic predictor variable.

2) Multiple stepwise regressions were repeated to include conversions of data for possible non-linearity while other repeats would exclude possible outliers.

3) Stepwise regressions were also repeated while excluding selected variables used in previous models or by using different sets of variables in order to ensure that the best regression model had been chosen.

4) each divariate correlation coefficient comparing the predictor variables to each other were examined to ensure that no two highly correlated predictor variables were included in the same regression model.

5) Examinations of changes in the partial F-scores and ß-coefficients during each step of the stepwise regression also helped identify multicollinearity between preditor variables. When a step in the regression added a variable that was highly correlated with a previous predictor variable, the partial F-score of the previous variable, to which it is correlated, usually decreased sharply and its beta-coefficient would also change considerably. Small beta-coefficients would sometimes change sign. Regressions were recalculated minus the variables causing the instability in the ß-coefficients of any previously chosen variable. Regressions were also repeated to replace the previous variable with the problem variable and so forth.

6) Variance inflation factors (VIF) were used to ensure no major source of multicollinearity existed within a regression model (cf. Mullet 1976). In this method, multiple regressions were used to correlate each predictor variable against all the remaining predictor

variables in a particular model. The VIF of all regressions were then calculated from R as: $(1-Ri^2)^{-1}$, where Ri is the multiple linear correlation coefficient of the ith variable and i is as defined above. As a rule of thumb, all VIF's must be less than 4. In my work all VIF's were less than 3 and most were between 1 and 2 indicating few problems of multicollinearity in the chosen regression models. Therefore, all β-coefficients determined from this work can be assumed to be stable.