THE UNIVERSITY OF CALGARY

Stable Isotope Analyses

of

Contemporary Human Hair

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Stable Isotope Analyses of Contemporary Human Hair" submitted by Lingjian Guo in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Stable isotope analysis is an emerging analytical tool in diet reconstruction. In addition to carbon and nitrogen which have been favoured in previous investigations, this thesis examines the potential use of other elements: sulphur, hydrogen, and oxygen. Various preparation techniques have been evaluated and data obtained for contemporary human hair samples from Calgary, Canada and three regions (Harbin, Beijing and Chengdu) in China.

As with carbon and nitrogen, it is concluded that sulphur, hydrogen, and oxygen isotope data for human hair can be related to diet and the place of residence. Data for hydrogen, and oxygen isotopes revealed general geographical trends (e.g. latitudinal dependence which relates to global processes e.g. photosynthesis, meteorology); in contrast, sulphur isotope composition is more dependent upon localized phenomena (e.g. rock weathering, atmospheric pollution). At a given location the isotope data may reflect some cultural influence e.g. choice of diet. The current study shows that S, H, and O-isotope data for hair can reinforce conclusions derived from C and N-isotope data and also provide additional information which should prove useful in paleodietary reconstruction as well as in applications such as environmental studies, paleoclimate, and forensic science.

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To my parents

for their enduring guidance and moral support

throughout my life

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CHAPTER 1 INTRODUCTION

Although most applications in stable isotope research have been developed in geochemistry, stable isotope analysis is becoming a routine component of anthropological research. In particular, stable isotope analyses have proved invaluable in paleodiet reconstruction (e.g., Ambrose, 1993; Katzenberg et al., 1993; Schoeller et al., 1986). In a review article for the general reader, van der Merwe (1982) traced the early history of carbon isotope analyses from its origins in physics, to discoveries in plant physiology and diet reconstruction in archaeology.

Stable isotope compositions are expressed in δ-values (see 2.1.1). The use of isotopic analyses for dietary reconstruction require constant and reproducible relationships between the isotope abundances of elements in food (and water) and those retained in body tissues and minerals. Such relationships are constrained by both the isotope compositions of dietary compounds and the feeding habit of the animal (e.g., Minagawa et al., 1986; Minagawa, 1992). Previous studies on many kinds of animals have revealed that the isotope fractionation of carbon and nitrogen during feeding and metabolism falls in a limited range. Nitrogen in the tissues of many animals is normally enriched in ^{15}N by about 4‰ over that of dietary proteins (Minagawa and Wada, 1984; Minagawa et al., 1986; Schoeller et al., 1986). $\delta^{13}C$ values for body tissues of animals are usually about 2‰ higher than those of diet (Nakamura et al., 1982; Rounick and Winterbourn, 1986; Minagawa, 1992). Human tissues were found to have sulphur isotope compositions near

the mean value for their diets (Krouse and Levinson, 1984; Krouse et al., 1987). It has been observed that on average, human tissue is enriched in deuterium by about 12‰ relative to diet (Schoeller et al., 1986). Hence the isotope fractionation during human metabolism appears to be small and nearly constant for most food sources. In a food web, the overall isotope fractionation can be significant in that it is the summation of the Δδ-values for each trophic level.

Hair samples have certain unique advantages, such as ready availability, high concentrations of many elements of interest (C, N, S, H, O) and long term preservation under some conditions. The isotope compositions of hair should reflect the food digested during the previous few months. Changing diet and/or change of residence are recorded isotopically along the length of a given hair (Nakamura et al., 1982; Jankowska, 1994). In the latter study, the subject's alternation in residence between Calgary and Poland was clearly seen in the $\delta^{13}C$ data.

The isotope composition of a given element in hair relates to diverse physical and chemical phenomena. In the case of hydrogen and oxygen, the different vapour pressures of isotopic species of water is of fundamental importance. The world is a complex distillation system for isotopic species of water so that the δD and $\delta^{18}O$ values of environmental water vary with location. During photosynthesis, carbon, hydrogen and oxygen isotopes are fractionated. Different photosynthetic processes (Calvin-Benson C_3;

Hatch-Slack C_4 or CAM: Crassulacean Acid Metabolism) are accompanied by different carbon isotope fractionation between atmospheric CO_2 and the generated biomass. In contrast, the sulphur isotope compositions of plants and animals depend upon geochemical processes such as the weathering of the lithosphere and atmospheric pollution. Nitrogen isotope data provide a measure of the relative amounts of marine and continental food in a diet.

Consequently, the isotopic composition of human hair relates not only to diet (e.g., different $\delta^{13}C$ values for C_3 beet sugar and C_4 cane sugar) but also to the place of residence. Thus applications of stable isotope analyses of hair extend beyond dietary reconstruction with possible use in forensic science. Contemporary human hair samples from Canada (e.g., Krouse et al., 1987), USA (Schoeller et al., 1986), Japan (Minagawa, 1992) and South America (Iyer et al., 1995) have been isotopically analyzed during the past few years. Analyses have also been carried on mummy hair in South America (Aufderheide et al., 1994). These studies have revealed latitudinal trends and effects of living different distances from marine resources.

In this thesis, carbon, sulphur, nitrogen, hydrogen and oxygen isotope compositions of contemporary human hair samples from Calgary and three regions in China were analyzed to discern possible geographical differences and associated dietary, cultural and environmental influences.

CHAPTER 2 THEORETICAL AND EXPERIMENTAL BACKGROUND

2.1 Fundamentals of Stable Isotope Techniques

2.1.1 Stable Isotopes

Isotopes can be divided into stable and unstable (radioactive) species. The term "stable" is relative, depending on the detection limits of radioactive decay. Most elements have two or more stable isotopes, although one isotope is usually present in far greater abundance. Natural isotope abundance variation or fractionation depends on thermodynamic equilibrium and kinetic processes. Departures from unity for the equilibrium constants for isotope exchange processes and the ratios of isotopic rate constants are usually small (on the order of a few percent).

The absolute abundances of isotopes are difficult to determine. Hence, isotope ratios are usually expressed using del (delta, δ) notation in parts per thousand (per mil: ‰) relative to a standard:

$$\delta X\ (‰) = [\ (R_{sample} / R_{standard}) - 1\ ] \times 1000 \qquad (2.1)$$

where X is usually the heavier of two chosen isotopes, e.g., D, ^{13}C, etc., and R_{sample} and $R_{standard}$ are the isotope abundance ratios (e.g., D/H, $^{13}C/^{12}C$, etc.) of the sample and standard, respectively.

Table 2-1 lists the average natural abundances of the stable isotopes of the five light elements of the current study, as well as their isotope abundance ratio expressions and standards.

Table 2-1 Average Natural Abundances (Holden, 1995), Isotope Ratio Expressions and Standards of H, C, N, O, and S

Element	Isotope	Abundance (%)	Isotope Abundance Ratio	δ-Value	Reference Standards
Hydrogen	^{1}H (H) ^{2}H (D)	99.985 0.015	D/H	δD	SMOW (Standard Mean Ocean Water), V-SMOW
Carbon	^{12}C ^{13}C	98.89 1.11	$^{13}C/^{12}C$	$\delta^{13}C$	PDB (Peedee Belemnite), V-PDB
Nitrogen	^{14}N ^{15}N	99.634 0.366	$^{15}N/^{14}N$	$\delta^{15}N$	Atmospheric N_2 (Air)
Oxygen	^{16}O ^{17}O ^{18}O	99.762 0.038 0.200	$^{18}O/^{16}O$	$\delta^{18}O$	SMOW (Standard Mean Ocean Water), V-SMOW
Sulphur	^{32}S ^{33}S ^{34}S ^{36}S	95.02 0.75 4.21 0.02	$^{34}S/^{32}S$	$\delta^{34}S$	CDT (Troilite from Canyon Diablo iron meteorite), V-CDT

Many of the standards were based on natural specimens for which original supplies have been exhausted (e.g., SMOW, PDB, CDT). Some of these reference materials were

not isotopically homogeneous (e.g., CDT). The International Atomic Energy Agency (IAEA) has distributed a number of isotopic intercomparison materials. These materials are available to investigators for use in calibrating the working standards within laboratories. The IAEA in Vienna has mixed various waters together to produce V-SMOW (V stands for Vienna, in analogy with V-PDB and V-CDT), which has an isotopic composition nearly identical to that of the original SMOW. The isotope composition of NBS-19 (calcite, calibration material for carbon and oxygen) versus a hypothetical V-PDB (supposed identical to PDB) has been fixed to $\delta^{13}C = 1.95‰$ and $\delta^{18}O = -2.20‰$, whereas the value of $\delta^{34}S = -0.30‰$ versus a hypothetical V-CDT (supposed identical to CDT) was proposed for IAEA-S-1 (Gonfiantini et al., 1995). These steps were necessary to keep the exhausted PDB, CDT, etc. as primary references because most of the results published to date are expressed relative to them.

In practice, several "working standards" or "standard reference materials" are used in a laboratory since the primary standards are too valuable for routine usage. The δ-values of these working standards are related to the universal standards listed in Table 2-1. To convert δ-values from one standard to another, the following equation may be used

$$\delta_{X-A} = [(\delta_{B-A} /1000 + 1) \times (\delta_{X-B} /1000 + 1) - 1] \times 1000 \qquad (2.2)$$

where X represents the sample, A and B represent different standards (Hoefs, 1987).

2.1.2 Stable Isotope Mass Spectrometry

To date, mass spectrometric methods are favoured as the most effective means of measuring isotope abundances. Stable isotope abundance ratios are usually determined with a gas source isotope ratio mass spectrometer (IRMS) based on the design by Nier (1947), with modifications by McKinney et al. (1950). In principle, a mass spectrometer may be divided into four essential components:

(1) The inlet system, symmetrically arranged for the introduction of either sample or standard gases

(2) The ion source, to ionize the gaseous molecules, and accelerate them into the flight tube

(3) The mass analyzer, usually a magnetic field to separate the ions according to their mass-to-charge ratios

(4) The ion detector, a set of Faraday cups to trap the ions at the end of the flight tube

Most isotope ratio mass spectrometers are capable of measuring only low-molecular-weight compounds (some less than mass 64). Elements that do not readily form gases are measured with solid source mass spectrometers. For the isotope abundance analysis of solid samples, a salt of the element is deposited on a filament which is then mounted in the source. The filament (Ta, Re, or W) is heated electrically to a temperature sufficient to volatilize and ionize the element of interest.

Continuous flow isotope ratio mass spectrometry (CF-IRMS) is a new and exciting technique that permits rapid automatic analysis of concentrations and isotope compositions of element(s) in samples. In 1983, Preston and Owens connected an automated nitrogen analyzer to an IRMS system. Their manually collected data showed the method to be sufficiently precise. The technique was soon automated by Barrie and Workman in 1984.

Principles of traditional IRMS and CF-IRMS will be discussed in 3.5.

2.1.3 Sample Preparation

Differences in isotope compositions among samples are often extremely small. Therefore, care has to be taken to avoid any isotope fractionation during chemical or physical treatment of the sample. Any preparation procedure with a yield of less than 100% may yield a reaction product that is isotopically different from the original specimen because the different isotopic species have different reaction rates. A quantitative yield of a pure gas is usually necessary for the mass spectrometric measurement in order to prevent not only isotope fractionation during sample preparation, but also interference in the mass spectrometer. Contamination with gases having the same molecular masses and having similar physical properties may be a serious problem. Contamination may result from incomplete evacuation of the vacuum system, degassing of a sample, and incomplete cryogenic distillation transfer of gases.

The procedure of transforming the raw samples into a form in which isotope abundance ratios can be determined involves: isolation and purification of an uncontaminated fraction of tissues such as hair), quantitative conversion to gases without isotopic fractionation, distillation and collection of gases for isotope abundance ratio analysis of different elements, and finally, isotope ratio mass spectrometry. Gases most commonly used for mass spectrometric analyses of the various elements H, C, N, O, and S are H_2, CO_2, N_2, CO_2, and SO_2, respectively. How these gases are prepared, distilled, transferred, or otherwise processed in vacuum lines will be briefly discussed in Chapter 3.

Tissue, mineral, or fluid that contains carbon, nitrogen, or sulphur etc. can be processed for isotope analysis. These include breath CO_2, body fluids, hair, skin, fingernails, muscle, fat, urine, bone or tooth carbonate, collagen, etc.(Ambrose, 1993). Among these tissues, hair of contemporary living things have certain unique advantages, such as ready availability, high concentrations of elements of interest (C, N, S, H, O), non-destructive or -injurious sampling, and constant isotope discrimination factor between diet and human hair (Nakamura et al., 1982). Hair samples are also relatively easy to clean before analysis. It should be mentioned that the $\delta^{13}C$ value of fats (lipids) is generally 5 to 8‰ more negative than the average for the whole organism, whether plant or animal. Therefore lipids on the hair samples should be removed with appropriate solvents (Chisholm, 1989).

About 50 hair samples from mainland China were collected, including 27 samples from Beijing (capital city, northeast of China), 11 from Harbin (Heilongjiang Prov., far northeast of China) and 11 from Chengdu (Sichuan Prov., southwest of China). For comparison, 11 hair samples were also collected from Calgary, Alberta, Canada. Hair samples from China were collected between March and July, 1995. Those from Calgary were collected in October, 1995. The hair samples are collected from healthy volunteers of both sexes and different ages. Most of the sampled populations are urban dwellers except those from Chengdu, China.

2.2 Natural Variation in Foodweb Stable Isotope Abundance Ratios

Recent research has revealed significant variation in the isotopic composition of foodwebs in different habitats and climates (e.g., van der Merwe, 1982; Stevenson, 1986). Variations can be traced from atmospheric and soil sources through the food chain to secondary consumers. When various environmental effects are understood and their magnitudes determined for specific foodwebs, stable isotopes can serve as reliable natural tracers in foodwebs within natural ecosystems (Ambrose, 1993).

2.2.1 Carbon Isotope Variation in Terrestrial and Marine Foodwebs

The natural ranges of variation in stable carbon and nitrogen isotopes in marine and terrestrial foodwebs are illustrated in Figure 2-1-1.

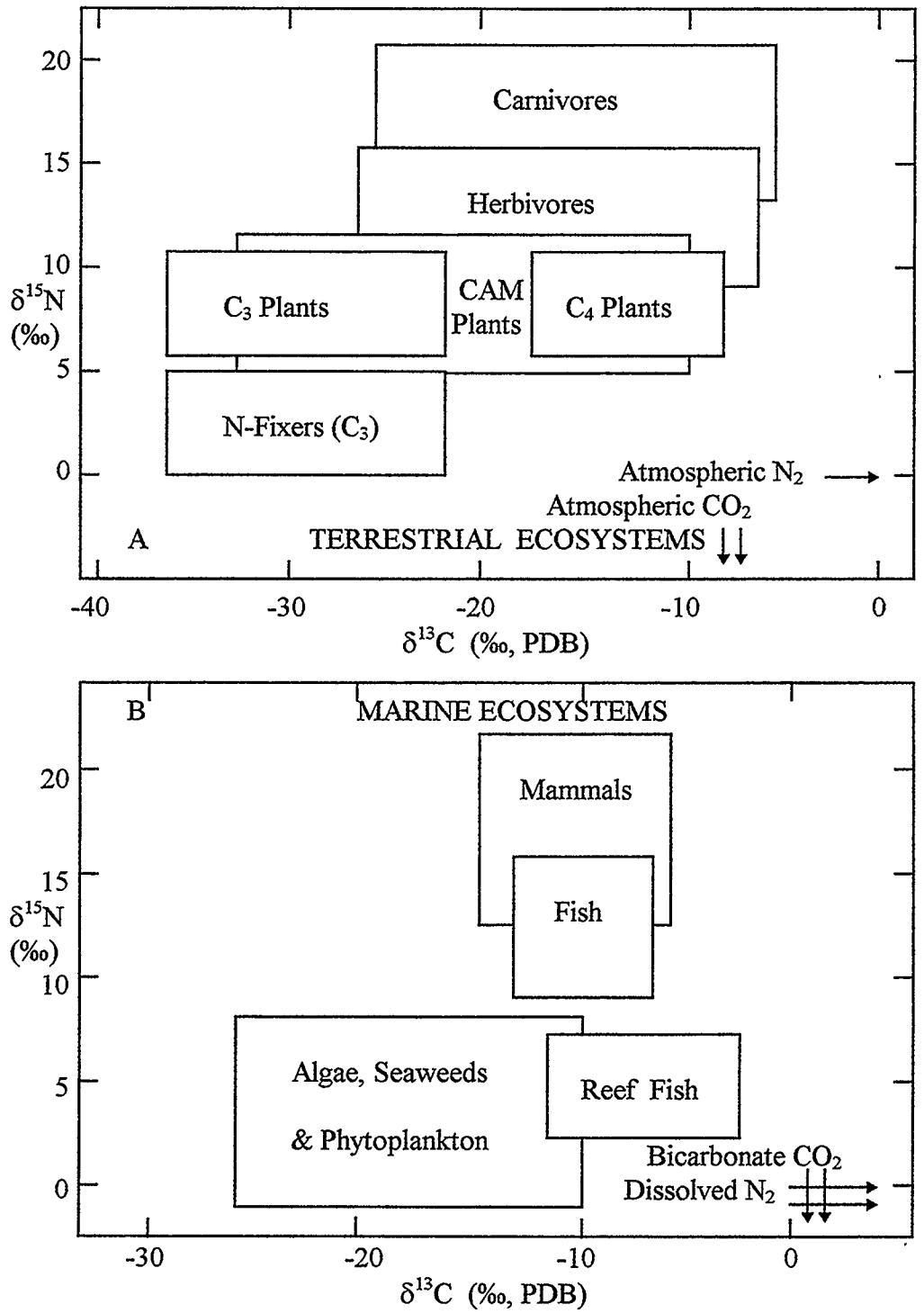

Figure 2-1-1 The Distribution of Stable Carbon and Nitrogen Isotopes in Terrestrial and Marine Foodwebs (after Ambrose, 1993)

The carbon isotope composition of a plant is determined by its mechanism of photosynthesis (C_3 or Calvin-Benson, C_4 or Hatch-Slack, and CAM or Crassulacean Acid Metabolism) and the attending isotope fractionation with respect to the reasonably uniform atmospheric CO_2 reservoir ($\delta^{13}C \approx -8‰$).

In terrestrial environments, carbon isotopes can often be used to separate C_3, C_4 and CAM plants. C_3 and C_4 refer to the number of carbon atoms in a molecule formed during the first stage of photosynthesis. Nutritionally important C_4 plants are sorghum, millets, corn (maize), sugarcane, some chenopods, and tropical pasture grasses. Examples of C_3 plants and food products are wheat and rice, forest, sugarbeets, montane and wetland grasses, all root crops, legumes, nuts, honey, most vegetables and most fruits. CAM plants include cacti, succulents, euphorbias, agaves and bromeliads (pineapples). C_4 plants grow best in hot, sunny and dry microhabitats with high temperatures and strong sunlight during the growing season. They are replaced by C_3 plants in shaded, winter rainfall, high latitude and high altitude environments. There are thus clines in the distribution of C_3 and C_4 plants between tropical and temperate regions, mid-latitude summer and winter rainfall zones, and low and high altitudes on tropical and subtropical mountains. C_3 and C_4 plants fix carbon (by the process of photosynthesis from atmospheric CO_2) with average $\delta^{13}C$ values of -26.5‰ and -12.5‰, respectively (Smith, 1972). There is no overlap in their $\delta^{13}C$ values, with those of C_3 plants ranging from -35‰

to -19‰, and those of C_4 from -15‰ to -9‰. Thus the C_3/C_4 dichotomy between foodweb end members has been widely used in diet reconstruction.

Freshwater aquatic foodwebs have not been intensively studied. Non-tropical foodwebs appear to have C_3-like carbon isotopic compositions (Katzenberg, 1989).

In marine environments, carbon is ultimately derived from dissolved bicarbonate (HCO_3), which has a $\delta^{13}C$ value of about 0‰. Marine foodwebs, based mainly on plants with the C_3 pathway, thus have $\delta^{13}C$ values averaging -19‰ (Smith, 1972). Therefore, where C_3 and C_4 plants both contribute to terrestrial diets, the precise estimation of marine resource consumption requires the use of the stable isotope abundance ratios of additional elements, such as N and S.

2.2.2 Nitrogen Isotope Variation in Terrestrial and Marine Foodwebs

Nitrogen isotopes can be used to distinguish marine from terrestrial plants, and plants with atmospheric nitrogen-fixing symbioses from those that rely on other sources of nitrogen, such as dissolved nitrate, ammonia and ammonium (Figure 2-1-1). The $\delta^{15}N$ values of marine plants are about 4‰ higher than those of terrestrial vegetation. But there are some exceptions in tropical marine reef and mangrove ecosystems (Capone and Carpenter, 1982).

There are significant variations in soil, plant and animal nitrogen isotope abundance ratios within and among terrestrial environments. Foodweb nitrogen is derived from soil nitrates, ammonia and ammonium ion, animal urea, and plants that have symbioses with N-fixing bacteria. The effects of climate on soil $\delta^{15}N$ values can be summarized as follows: cool, moist forest soils have higher N-fixation and mineralization rates, and low $\delta^{15}N$ values; hot, dry savanna and desert soils, or those with significant animal inputs have high $\delta^{15}N$ values. Plant $\delta^{15}N$ values are lower in closed habitats at higher altitudes (Ambrose, 1993).

2.2.3 Sulphur Isotope Variation in Terrestrial and Marine Foodwebs

The initial report of Thode et al. (1949) revealed large variations in the isotopic composition of sulphur in nature. The earth's sulphur can be considered in terms of two main reservoirs that are isotopically uniform: deep seated or igneous sulphur and oceanic sulphate with $\delta^{34}S$ values near 0‰ and 21‰, respectively. In addition, there are numerous smaller reservoirs which vary widely in sulphur isotope composition which reflects more local information of the ecosystem (Krouse and Herbert, 1988).

A major cause of natural $\delta^{34}S$ variations is the large kinetic isotope effects associated with bacterial sulphate reduction. However, sulphur isotope fractionation during oxidation of reduced sulphur compounds is small. Assimilated sulphur in plants may come from many sources. In addition to uptake of SO_4^{2-} from soil solutions through

the root system, land plants can incorporate sulphur oxide from the atmosphere which may be of industrial emission (Krouse, 1977). Higher members of foodwebs must ingest essential organo-sulphur compounds since they lack the ability to assimilate sulphate. Biochemical pathways are available for interconversions of sulphur amino acids. Since these pathways involve large molecules, the accompanying isotopic selectivity is expected to be small (Krouse, 1989). Sulphur isotope compositions for various tissue, fluids and minerals in humans have been determined (Krouse and Levinson, 1984; Krouse et al., 1987). The overall variation in $\delta^{34}S$ of these components in a given human is about 2‰ and appears to approximate the mean sulphur isotope composition of the diet.

Where carbon and nitrogen isotopes fail to discriminate marine and terrestrial resource use sulphur isotopes can provide clear separation (Krouse, 1980). Sulphur is a minor component of collagen (about 0.15%) by weight, exclusively in the amino acid methionine. Since hair has substantial amounts of methionine, cystine, and cysteine, etc., it could be used for sulphur isotope analysis.

2.2.4 Hydrogen and Oxygen Isotope Variation in Foodwebs

Hydrogen isotopes are particularly interesting because of their large relative mass differences and the largest variations in isotope abundances in nature. The hydrogen isotope abundance ratios of both plants and animals are very much dependent on the isotope composition of the water in their growth environment (Yapp and Epstein, 1982). Since the isotopic composition of precipitation depends on latitude, altitude, temperature

and distance inland, this means that independent of any biological processes, we would expect the hydrogen (and oxygen) isotopic composition in organisms to differ among locations.

Mean annual isotope data for hydrogen and oxygen in water in different regions show a tight linear relationship between δD and $\delta^{18}O$ with a slope of 8:1 (Craig, 1961). This pattern is referred to as the Meteoric Water Line (MWL). Atmospheric precipitation generally follows a Rayleigh process under liquid-vapour equilibrium conditions. This process explains why fresh water is isotopically lighter at higher latitude, altitude and increased distance inland (the so-called "continental effect"). There is also an "amount effect" that in areas of high rainfall, for each 100 mm increase in rainfall the value of $\delta^{18}O$ decreases by about 1.5‰. A relationship between the annual averages of $\delta^{18}O$ values of precipitation and local surface air temperature was also found (Dansgaard, 1964). Because evaporation and isotope exchange with water vapour after condensation also affects the isotopic composition, the Rayleigh distillation model can only describe the isotopic composition of rain water in qualitative terms. It is known that precipitation D/H and $^{18}O/^{16}O$ ratios show a strong seasonal cycle, with the highest values in the summer and the lowest in the winter (Dansgaard, 1964). In temperate and humid climates, the isotopic composition of groundwater is similar to that of the precipitation in the area of recharge. In semiarid or arid regions evaporation losses before and during recharge shift the isotopic

composition of groundwater towards heavier values. The δD and $\delta^{18}O$ values of ocean water are near 0‰.

Water taken up by a plant from the soil undergoes little change until evapotranspiration results in leaf water becoming isotopically heavier. Hydrogen isotopes undergo little fractionation by passage through the food chain (although plant parasites tend to be enriched in deuterium as compared to their hosts). The splitting of water in photosynthesis results in the lighter hydrogen isotope being incorporated into organic matter. Additional fractionation is associated with lipid synthesis (Smith and Epstein, 1970) and other processes involving the pyruvate dehydrogenase complex. Differences in metabolic pathway between CAM, C_4, and C_3 species can be detected by D/H ratios (Smith and Ziegler, 1990).

Although oxygen isotope fractionation in precipitation and evapotranspiration is similar to that for hydrogen, different isotope fractionations for oxygen and hydrogen were found during the process of photosynthesis. Studies of several different plants (including plants having different photosynthetic modes) have shown that $\delta^{18}O$ value of cellulose is always 27 ± 3‰ higher than that of the water at the site of synthesis (Epstein et al., 1977; Sternberg et al., 1984). This is possibly due to the fact that the oxygen during photosynthetic process can come from H_2O, CO_2, but hydrogen can only come from H_2O.

Therefore, δD values of vegetation are usually close to those of local precipitation whereas $\delta^{18}O$ values of vegetation are higher than those of precipitation.

2.2.5 The Significance of Multiple Element Isotope Tracer Studies

Sometimes isotopic analysis of one element in foodweb studies cannot provide definitive answers, and stable isotope data for other elements may prove complementary. It is important that the isotope compositions of different biologically significant elements are altered by distinctly different processes. Otherwise, little additional information could be gained from analyzing additional elements. For example, in the hydrological cycle, isotopic variations of hydrogen and oxygen provide essentially the same information. In contrast, carbon isotope abundances in foodwebs are determined to a large extent by photosynthetic pathways, whereas sulphur isotope compositions are determined by many biogeochemical processes. The $\delta^{13}C$ value of a particular plant will be reasonably consistent wherever it occurs, but its $\delta^{34}S$ value can vary widely because sulphur is derived from soil solutions and atmospheric gases.

2.3 Isotope Analyses and Diet Reconstruction from Isotope Data

Diet reconstruction with stable isotope data requires accurate knowledge of: (1) the relationship between the isotopic composition of the diet and tissues (bone, hair, fat, muscle, etc.) or biochemical fractions (protein, carbohydrates, and fats) analyzed, (2) the

isotope composition of classes of dietary resources that may have been consumed, and (3) physiological, nutritional, cultural (e.g., diet selection) or environmental sources of variation in diet-tissue relationships. Given the "menu" of potential dietary resources consumed by a human population, one can construct hypothetical "meals". In other words, if the available resources are known, their proportions can be estimated to yield an average isotope composition for ingested food. That average should embrace possible selectivity of dietary items. For example, the ratio of consumed C_3 to C_4 grasses by a cow may not correspond to the actual ratio by weight or coverage in the pasture area. Beyond isotopic selectivity during intake, there may be varied retention of isotopically different classes of chemical compounds and isotopic fractionation during chemical inter-conversions. Thus the isotope systematics are quite complex and overall isotopic fractionations tend to be estimated between dietary resources (isotopic end members) and tissues. These fractionation factors are incorporated in calculations which attempt to relate the isotopic composition of tissues to the proportions of the "isotopic end members" ingested.

The percentages of marine versus terrestrial resources in a diet has been estimated from the $\delta^{13}C$, $\delta^{15}N$ and/or $\delta^{34}S$ values of bone collagen by White and Schwarcz (1989), Aufderheide et al. (1994). However, uncertainties in our knowledge of diet-tissue relationships correspond to errors in estimates of individual's diet composition of as much as 10%. Improved accuracy in dietary interpretation of collagen carbon and nitrogen isotope abundance ratios can be achieved by consideration of the weight contribution of

these elements (in terms of specific resources) to the tissues analyzed (Spielmann et al., 1990).

Minagawa (1992) has generated a model for dietary reconstruction which assumes that a person has a constant diet of components with known isotope abundance ratios. In the case of hair, the major component is protein, and it is assumed that dietary protein is directly transferred to human hair and other macromolecules do not participate. Although the isotope composition of tissues differ in various human organs, the isotopic trends among organs appear to be similar. Therefore, the isotope abundance ratios of hair may be used as representative of the human body for a human feeding model.

The isotope abundance ratio of the body tissues should be controlled by the isotope abundance ratios of the food, and by the isotope fractionation between food and human tissue. In most animals, including humans, the isotopic fractionation of C and N appears to be nearly constant. If we know the isotope discrimination values ($\Delta_{human\text{-}diet}$) for a certain animal-diet system, the isotope composition of the diet can be estimated by

$$\delta_m = \delta_{human} - \Delta_{human\text{-}diet} \qquad (2.3)$$

where δ_m and δ_{human} are δ-values of the diet and human tissue, and $\Delta_{human\text{-}diet}$ is an offset due to isotope fractionation between diet and human tissue. In the case of a mixed diet

composed of two different food sources, the proportion of each source can be calculated from the mass balance equation:

$$\delta_m = f_1 \times \delta_1 + f_2 \times \delta_2 \qquad (2.4)$$

Substituting $f_2 = 1 - f_1$ gives

$$f_1 = (\delta_m - \delta_2) / (\delta_1 - \delta_2) \qquad (2.5)$$

where f_1 and f_2 are proportions of source 1 and source 2, respectively, and δ_1, δ_2 and δ_m indicate δ-values for source 1, source 2 and a mixed diet, respectively.

In the case of three sources, simultaneous measurements of two tracer elements (such as C and N) are needed to estimate the contribution of each source. This analysis can be extended to those cases with more than three food sources. The isotope abundance ratios of body tissues should be controlled by the isotope abundance ratios of each diet alternative and their mixing proportions, as given by the following mass balance equation:

$$\delta_{human} = \Delta_{human\text{-}diet} + f_1 \times \delta_1 + f_2 \times \delta_2 + \ldots + f_n \times \delta_n \qquad (2.6)$$

where $f_1, f_2, \ldots f_n$ and $\delta_1, \delta_2, \ldots \delta_n$ are mass fractions of the diet alternatives and each isotope abundance ratio (δ-value), respectively. The dietary mixing proportions cannot be calculated exactly, as done in the analytical model. However they can be estimated using a stochastic approach (Monte Carlo Simulation) where dietary

proportions can be randomly generated with a computer. When the calculated (using equation 2.6) isotope abundance ratio was close to the measured data, the dietary proportions yielding that result were recorded as a possible dietary mixture. After many trials (millions of calculations but only those yielding δ-values close to those observed were retained), the possible ranges of proportions for each food source were determined.

CHAPTER 3 ANALYTICAL

This chapter will describe the procedures of transforming the raw samples into a form in which isotope abundance ratios can be determined. These procedures include: sample cleaning (3.1), gas preparation, and isotope abundance ratio mass spectrometry. Gases most commonly used for mass spectrometric analyses of H, C, N, O, and S are H_2, CO_2, N_2, CO_2, and SO_2, respectively. Distillation and collection of these gases (without isotopic fractionation) will be briefly discussed in 3.2 to 3.4. Two kinds of mass spectrometric techniques (traditional Nier-type and on-line continuous flow) will be discussed finally in 3.5.

3.1 Cleaning of Hair

Hair samples were first immersed in the 95% ethanol solution for about half an hour (using 99.5% acetone solvent for a couple of minutes is an alternative way) to dissolve the surface impurity (lipid, shampoo, etc.) as much as possible, although exclusion of this step did not make much difference in the measured isotope abundance ratio (Nakamura, 1982; and trials in our labs). Samples were then washed with distilled water to clean any impurity in the solvent, and dried at 70°C for at least 2 hours before use.

3.2 Preparation of CO_2, N_2, and H_2 from Hair for Carbon, Nitrogen and Hydrogen Isotope Abundance Analyses

3.2.1 Closed Tube Combustion Followed by Cryogenic Separations of H_2O, CO_2, and N_2

One traditional method for conversion of organic matter to H_2O, CO_2 and N_2 is closed tube combustion (sometimes called static combustion), where the sample and an oxidant are heated in an evacuated, sealed tube.

About 10 mg of cleaned hair sample was placed in a clean Pyrex tube (9 mm O.D., 20 cm long, one end closed) with 1 gram cupric oxide (CuO) and a small piece of silver foil (or 10 mg silver powder). The tube was then evacuated vertically to a pressure of 2×10^{-2} torr and collapsed near its upper end and sealed with a natural gas-O_2 torch. The sealed tube was then heated to 550°C for 15 hours. An optional combustion technique used a sealed quartz tube heated to 850°C for 2 to 3 hours and slowly cooled to room temperature overnight. During heating, organic carbon combined with oxygen from the cupric oxide, forming CO_2. Hydrogen combined with oxygen to form water. Some cupric oxide was reduced to copper by organic matter, and the copper reacted with produced nitrogen oxides to form N_2. Silver and copper also reacted with product sulphur oxides (SO_2, SO_3, sulphate), preventing their accumulation. No other gases were detected. Combustion tubes and unreacted reagents were not recycled to prevent memory effects.

N_2, CO_2 and H_2O freeze at different temperatures, and can be separated by cryogenic distillation. H_2O can be frozen with a dry ice-alcohol slush ($T \approx -78°C$) and CO_2 sublimed with liquid nitrogen ($T \approx -196°C$). N_2 is relatively difficult to liquify in vacuum systems. It can be frozen with liquid H_2 or liquid helium which is very expensive, or condensed onto activated charcoal, silica gel or molecular sieve in a liquid N_2 trap. N_2 was not collected during this series of sample preparations. Distillation was performed by cracking the combustion tube in an evacuated distillation line. CO_2 and H_2O were trapped in a liquid N_2 trap. Then N_2 was pumped away. The liquid N_2 trap was replaced with dry ice-alcohol and product CO_2 was distilled into another liquid N_2 trap. The frozen CO_2 was pumped upon to remove any non-condensable gases which may have been trapped in the initial solid CO_2. The liquid nitrogen was removed and upon warming, the CO_2 was transferred to a vertical Pyrex sample tube partly immersed in liquid N_2. An upper part of the tube was collapsed, sealed, and the tube removed using a natural gas-O_2 torch.

3.2.2 Conversion of H_2O to H_2

H_2O was reduced to H_2 using the zinc reduction method described by Coleman et al. (1982). With reference to the static combustion method described in 3.2.1, the dry ice-alcohol trap was removed and water transferred to a Pyrex sample tube partially immersed in liquid N_2 and containing about 100 mg zinc. The sample tube was sealed, removed, and then heated in an oven at 450°C to 500°C for at least half an hour so that the H_2O reacted with Zn to form the ZnO and H_2. The amount of zinc used, which depends on the amount

of water, is important for the complete reaction to ensure that no isotope fractionation occurred during the formation of H_2.

3.2.3 On-line Combustion with Carlo-Erba Elemental Analyzer and Gas Chromatographic Separation of N_2 and CO_2

The automated N/C analysis of biological materials with an on-line elemental analyzer (in our case, a Carlo Erba NA 1500) and continuous flow mass spectrometer has revolutionized stable isotope analysis. Comparing with the traditional IRMS, this technique has many advantages: rapid analysis and small sample size (up to a hundred extra samples per day and up to a hundred times smaller samples can be analyzed). Samples are loaded into a carousel of the autosampler and are automatically combusted. The combustion gases are then chromatographically separated and transferred with the He carrier gas to a continuous flow mass spectrometer. These operations are controlled by a computer, including customized data evaluation and presentation. The overall system is presented in Figure 3-2-1.

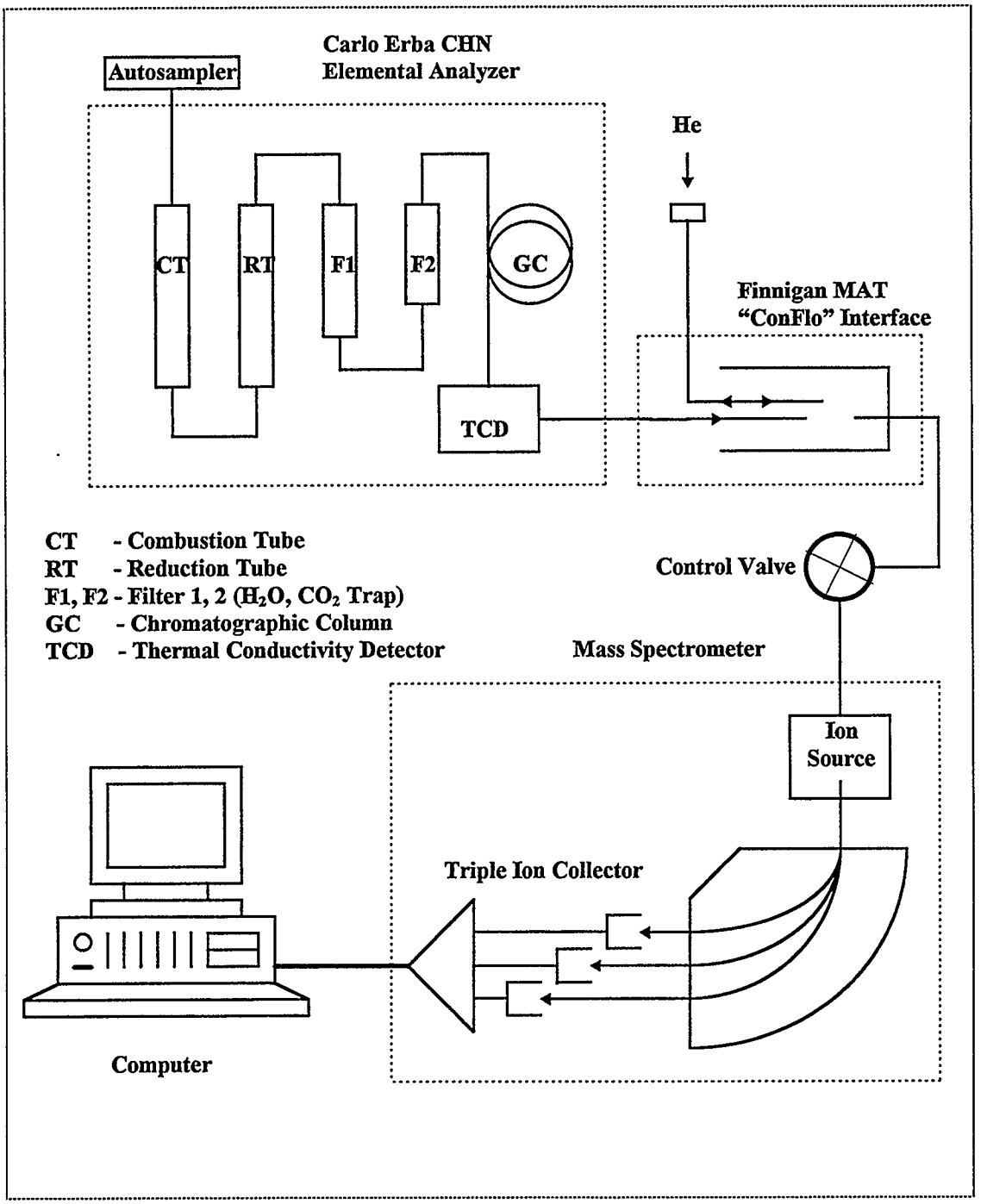

Fig. 3-2-1 Schematic Diagram of Continuous Flow Mass Spectrometer System (TracerMAT)

The sample is weighed in a tin capsule and loaded into the autosampler with up to 49 others. When the autosampler drum is triggered to an open position, a sample is introduced in the Combustion Tube (CT) which is maintained at about 1020°C. The sample and capsule melt and the tin promotes a violent reaction (flash combustion) in a temporary enriched atmosphere of oxygen. Under these conditions (estimated temperature of 1600°C), even thermally resistant substances are completely oxidized. The combustion products carried by a constant flow of helium carrier gas pass through an oxidation catalyst of chromium trioxide (Cr_2O_3) inside the reaction combustion tube. The Cr_2O_3 inhibits the formation of nitrogen oxides and doesn't react with the quartz combustion tube. To ensure complete oxidation, a 5 cm layer of silver coated cobalt oxide is placed at the bottom part of the combustion tube to retain interfering substances produced during the combustion of halogenated compounds. Then the mixture of combustion products (CO_2, N_2, NO_x and H_2O) passes through a second tube known as the Reduction Tube (RT) which contains metallic copper kept at 650°C. The excess of oxygen is removed and nitrogen oxides are reduced to elemental nitrogen which together with CO_2 and H_2O pass through two absorbent filters. The first filter (F1) consists of magnesium perchlorate (or molecular sieve) which absorb H_2O, while a second filter (F2) of ascarite and molecular sieve can be used to retain CO_2 if only nitrogen is being analyzed. The N_2 and CO_2 gases are swept into the chromatographic column (GC) by the helium carrier gas. The gases are separated in the column and detected by the Thermal Conductivity Detector (TCD) which gives output signals corresponding to the concentrations of the individual components of

the mixture. The separated gases are then transferred to an open split inlet system ("ConFlo" interface) and a continuous flow mass spectrometer (discussed in 3.5.2).

3.3 Preparation of CO_2 and H_2 Using a Porous Nickel Tube Reactor for Oxygen and Hydrogen Isotope Abundance Analyses

To determine the oxygen isotopic composition of an organic compound, oxygen must be quantitatively extracted and converted to CO_2. Generally, there have been two effective ways to determine the isotopic composition of oxygen in organic compounds. One is conversion to CO_2 by $HgCl_2$ (or Cu_2Cl_2 or Cl_2) at 360 to 530°C (Rittenberg and Ponticorvo, 1956). The other approach is the nickel-tube pyrolysis method (Thompson and Gray, 1977). In the latter, the sample is pyrolysed in an evacuated nickel reaction vessel at about 1125°C. The products from organic compounds are C, CO_2, CO and H_2. This method can also be used for water sample analysis using extra carbon in the vessel. There are two advantages to the use of nickel-tube: the possibility of isotope exchange with the vessel is eliminated, and the diffusion of H_2 through the porous walls of the reaction vessel drives the reaction to completion. The pyrolysis reaction can be expressed as the following unbalanced equations:

$$(CH_2O)_n \rightarrow CO + CO_2 + C + H_2$$

$$H_2O + C \rightarrow CO + CO_2 + C + H_2$$

The apparatus of Thompson and Grey (1977) proves expensive if a large number of samples are prepared simultaneously. A spark discharge chamber is also needed for the conversion of CO to CO_2. A revised method used in the current study is based on resealable nickel pyrolysis bombs and a simplified operating procedure (Edwards et al., 1994; Motz et al., 1995). The resealable nickel bombs in routine use at the University of Waterloo permit greatly increased service life of individual tubes (>50 cycles), and eliminating the use of a spark discharge chamber to recover oxygen from CO. That is based on the fact that isotopic equilibrium between the CO and CO_2 at high temperature (1050°C for 50 minutes) is retained as the bombs cool to room temperature. A nickel pyrolysis bomb and puncturing device are illustrated in Figure 3-3-1. Pyrolysis bombs were machined from nickel rod in the Faculty of Sciences Technical Services Machine Shop at the University of Calgary. The resealable fitting was fabricated using a 0.5 mm-thick silver-plated nickel disc (Cajon, NI-4-VCR-2-BL) squeezed between raised circular rims. The discs are punctured by a steel sewing needle to release the pyrolysis products. The plated silver on the nickel disc serves to chemically trap sulphur oxides (similar to the use of Ag in the static combustion method described in 3.2.1). The pyrolysis bomb containing the sample (15 to 20 mg for hair) was placed in an inert (argon) environment and sealed. It was then inserted into a quartz tube and the encapsulated bomb was heated at 1050°C for about 50 minutes, during which hydrogen diffusing through the nickel lattice is retained in the quartz tube, and can be used for hydrogen isotope analyses. Satisfactory services for manufacturing the quartz tubes were not available during the

course of this thesis. Two tubes prepared by the glassblower did not withstand the thermal stress of the first heating. Tubes which should be satisfactory were only recently obtained from the University of Alberta.

The distillation line connected to the puncturing device for the collection of CO_2 is shown in Figure 3-3-2. The extraction line oven was preheated at least a hour in advance to keep the section of glass at a temperature of 350°C. The entire line (all valves open) was pumped for several minutes after a cooled bomb was placed inside the puncturing device. The valve #1 was closed and then the bomb was punctured. Contained gases were allowed into the heated portion of the glass line and remained for 3 to 4 minutes. Any traces of $Ni(CO)_4$ that might be produced in the bomb were decomposed immediately upon exposure to the hot glass, precipitating nickel and liberating CO. Valve #3 was closed and #1 opened to allow CO_2 (and possibly some H_2O which indicates uncomplete reaction) to condense in the liquid N_2 trap. Non-condensible gases (mainly Ar and CO) were pumped off slowly and liquid N_2 was replaced with dry ice-alcohol after valve #1 and #3 were closed. The CO_2 was transferred into a vertical Pyrex sample breakseal (partly immersed in liquid N_2) which was then flamed off using a natural gas-O_2 torch.

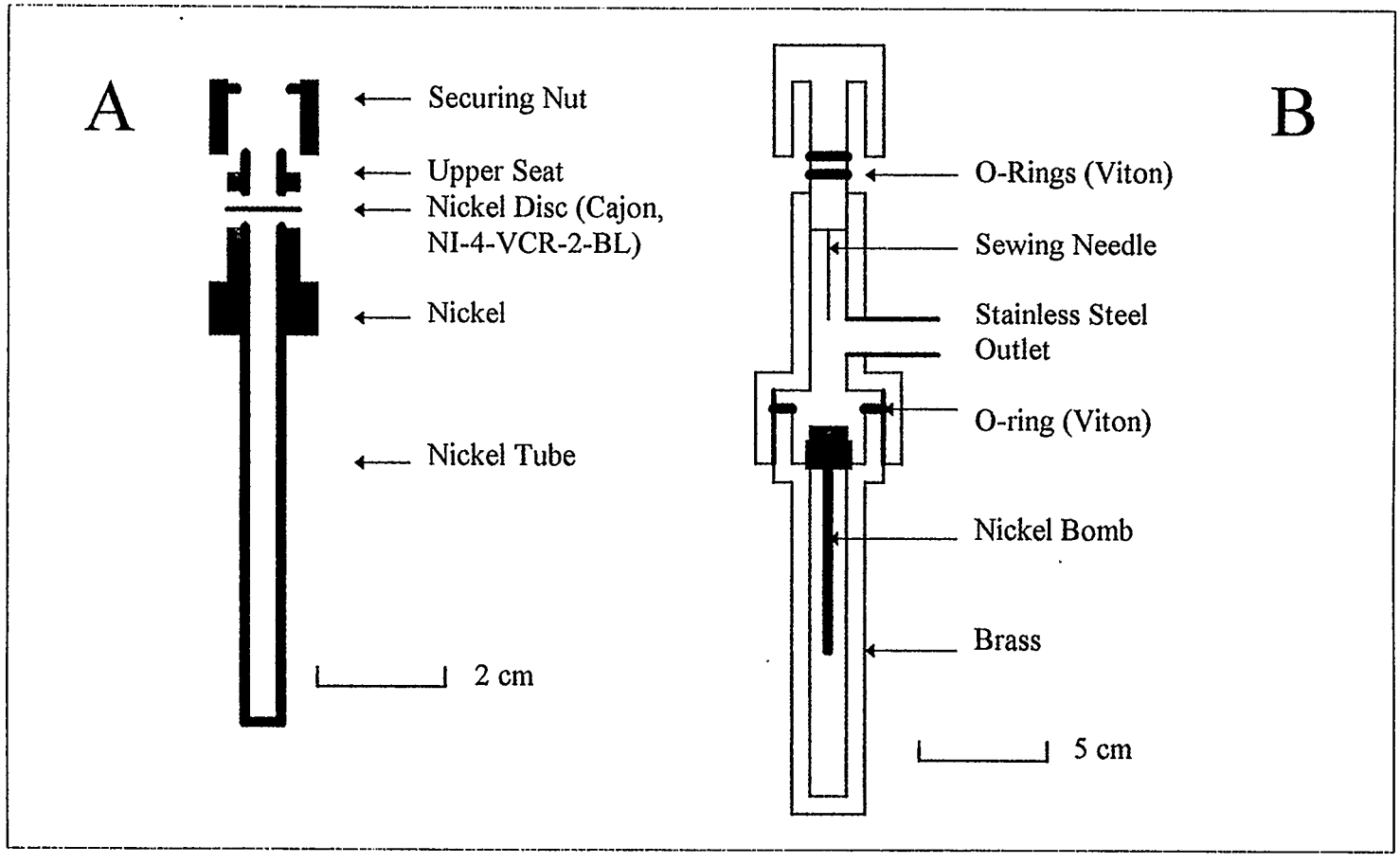

Figure 3-3-1 Sketch of nickel pyrolysis bomb (A) and puncturing device (B)

(After Edwards et al., 1994)

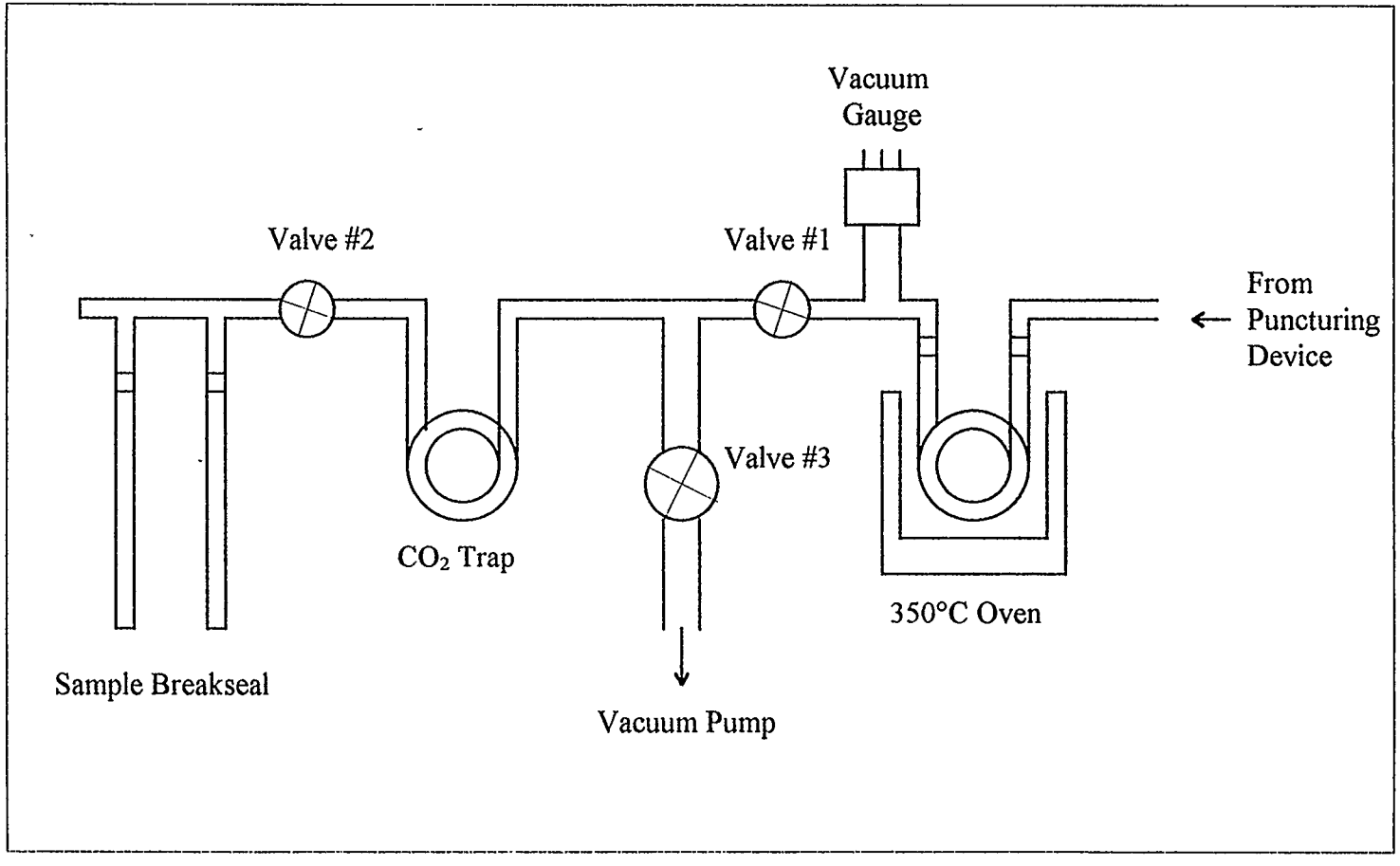

Figure 3-3-2 The distillation line for the collection of CO_2 in the nickel-tube pyrolysis method

3.4 Preparation of SO_2 for Sulphur Isotope Abundance Analysis

Several methods were used for sulphur isotope analysis. The organic sulphur in the hair was changed to either sulphide (usually Ag_2S) or sulphate (usually $BaSO_4$) for subsequent conversion to SO_2 using the method described by Ueda and Krouse (1986). A number of metal sulphides are suitable for combustion to SO_2, but for reproducibility of oxygen isotope composition, the combustion of one sulphide within a laboratory is preferred. The compound Ag_2S has been favored because of its low solubility, reproducible combustion, and the high mass of Ag for gravimetric determinations of S in the sample and monitoring yields of SO_2.

Conversion of sulphide or sulphate to SO_2 for isotope analyses was described by Ueda and Krouse (1986) and Yanagisawa and Sakai (1983). Sulphide and sulphate minerals (approximately 20 μmole or 0.7 mg S) were ground with a mixture of V_2O_5 (50 mg) and SiO_2 (50 mg), placed at the bottom of a quartz tube (9 mm O.D.), and covered with quartz wool and some metallic copper turnings. The quartz tube and reaction mixture were heated up to about 900°C to 1000°C on the combustion line and kept at this temperature for 10 to 15 minutes. The evolved SO_2 was continuously condensed into a U-shaped trap immersed in liquid nitrogen. Any evolved SO_3 was converted to SO_2 upon passing through the hot copper turnings. Non-condensable gases were pumped out. After the reaction, the SO_2 gas was purified to remove CO_2 using vacuum distillation with a liquid nitrogen-pentane bath ($T \approx -131°C$). A dry ice-alcohol slush was used to remove

H_2O. The purified SO_2 was then transferred to a cold finger for admission to the Micromass 602 mass spectrometer situated on-line.

3.4.1 SO_2 Prepared from the Remains of Static Combustion

As described in 3.2.1, the silver foil or powder in the static combustion tube chemically traps SO_2 and other sulphur oxides. There may be some silver sulphide and sulphate created, as well as other solid sulphide and/or sulphate. Thus it should be possible to use the S containing solids and the method described above to prepare SO_2 for isotope analysis. Since the amount of hair sample used in each static combustion tube was about 10 mg, with a 4% weight percentage of sulphur, about 0.4 mg (or 12 μmole) S is available. According to this estimate, about 50 mg of (V_2O_5 + SiO_2) was needed for each sample.

3.4.2 Direct Analysis of SO_2 from Carlo-Erba Analyzer On-Line Combustion

In addition to the total nitrogen and carbon analyses that was described in 3.2.3, determination of sulphur (concentration and isotope abundance) in the same sample is also possible. The principle of operation is the same as that discussed in 3.2.3, except that the SO_2 from combustion is not chemically trapped but comes at a later time off the chromatographic column. This technique was not explored in this thesis although this capability should be available in the laboratory within a few months.

3.4.3 Parr Bomb Combustion Followed by Conversion of $BaSO_4$ to SO_2

The total S in hair may be oxidized to SO_4^{2-} using the Parr combustion bomb according to the method of Siegfriedt et al. (1951). The bomb is a closed heavy wall stainless container where the sample is combusted at an O_2 pressure of 2.0 to 2.5 MPa. The sample resting in a metal sample cup is ignited by passing a current through a thin Ni alloy wire placed just above the cup. Gaseous products are absorbed in 1 ml of de-ionized water added to the bomb prior to closing. Oxygen gas is admitted slowly to the bomb to minimize turbulence. As a safety precaution and a check for leaks, the pressurized bomb is placed under water prior to ignition. To assure complete combustion, at least 15 minutes should pass to allow the product sulphur oxide gases to dissolve in the water, before the unexpended O_2 and the product CO_2 are released by a bleeder valve and the bomb opened. More complete SO_4^{2-} recovery is realized by using de-ionized water to wash interior parts of the bomb and adding the washings to the originally added water (and water of combustion). The water is filtered to remove solid residues (e.g., metal oxides from fusing the filament) and SO_4^{2-} in the filtrate converted to $BaSO_4$ by the addition of 0.1N $BaCl_2$ solution. The sample was also acidified to inhibit the formation of $BaCO_3$ which may have precipitated. The $BaSO_4$ was filtered, dried, weighed and packed with the V_2O_5 + SiO_2 (1:1) mixture for preparation of SO_2 as described at the beginning of this chapter.

3.4.4 Kiba Method of Generating H_2S from Hair and Conversion to SO_2

Tin (II) – Strong Phosphoric Acid (also called "Kiba reagent") was first described by Kiba et al. (1955) as a powerful reducing reagent taking sulphate to hydrogen sulphide.

It has been successfully applied to the rapid quantitative analysis of various forms of both organic and inorganic sulphur. Ueda and Sakai (1983) developed an *in vacuo* Kiba reduction procedure to extract small quantities of sulphide and sulphate separately from silicate rocks. Kiba reagent techniques have been extended to many other materials such as meteorites, marine carbonates and biological specimens (Krouse and Ueda, 1987). The procedure of sulphur extraction and preparation of Kiba reagent was described in detail by Sasaki et al. (1979), as is the method used for the study of this thesis.

Extra pure grade (d = 1.7) orthophosphoric acid (300 ml) is dehydrated in a 500 ml Pyrex flask by heating to 250°C for an hour with a mantle heater. After cooling to 150°C, 30 to 40 grams of extra pure grade dehydrated tin (II) – chloride are added to this "strong" phosphoric acid. The mixture is heated to 280°C for an hour under nitrogen gas flow. Hydrogen chloride evolves from the reaction mixture and any sulphur-bearing impurities in the chemicals are removed as hydrogen sulphide. Nitrogen gas flow through the apparatus is maintained until the reagent cools down below 150°C.

Processing of samples may be carried out with a similar apparatus. After placing the sample and Kiba reagent in the reaction vessel and connecting it to an absorption vessel containing cadmium acetate ($Cd(CH_3COO)_2$) solution. Nitrogen gas is allowed to flow through the system for a few minutes at a rate of some 200 bubbles per minute. The reaction mixture is then heated up to 280°C and maintained there for about 40 minutes

under the same rate of nitrogen flow. Evolved hydrogen sulphide is fixed as cadmium sulphide (CdS) in the absorption vessel. The reaction flask is allowed to cool and N_2 flow stopped. The cadmium sulphide precipitate is converted to silver sulphide by adding silver nitrate solution. The mixture is then boiled for about one minute, so that the silver sulphide coagulates coarsely enough to be easily filtered onto glass wool. The filtered Ag_2S is dried, weighed and packed with V_2O_5 + SiO_2 (1:1) mixture for sulphur isotope analysis.

3.5 Stable Isotope Mass Spectrometer

Two stable isotope mass spectrometer techniques were used, as discussed below. Both techniques used the same mass spectrometer optics but differed in the method of sample introduction and ion current measurement.

3.5.1 Traditional Isotope Ratio Mass Spectrometry (IRMS)

In a traditional Nier-type isotope ratio mass spectrometer, a sample or standard reference gas (usually H_2, CO_2, N_2 or SO_2) is introduced through its viscous leak (a capillary about 1 meter long) to the mass spectrometer source. In the source, an electron beam generates positively charged ions. An ion beam is formed, focused and accelerated out of the ion source down a flight tube. The ions then follow circular paths upon entering an magnetic field. The radius of the path of the lighter ions is smaller than for heavier ones. Thus the beam is split into a mass spectrum of beams of ions of different mass to

charge ratios. The beams (ion currents ranging from 10^{-11} to 10^{-8}A) strike two or more carefully positioned Faraday cup detectors at the end of the flight tube. The ions striking the collectors are neutralized by an electron current which passes through high impedance inputs of amplifiers. The output of the amplifiers undergo voltage-to-frequency conversion followed by counters whose outputs are fed into a computer. The counts are proportional to the intensity of the beams, and thus the abundance of the isotopes.

Mathematically, an ion of mass m and charge q accelerated through a potential difference of V acquires kinetic energy $qV = (1/2)\ mv^2$. In the magnetic field, B, the ion is subjected to a force $\bar{F} = q\ \bar{v} \times \bar{B}$. With $\bar{v}$ normal to $\bar{B}$, the magnitude of the force qvB is equaled to the mass of the ion (m) × centripetal acceleration (v^2/R): $qvB = mv^2/R$, where R is the radius of its path in the magnetic field. These relationships can be solved to equate the mass to charge ratio of the ion to the radius of its path in the magnetic field:

$$m / q = B^2R^2 / (2V)$$

The gas inlets into the mass spectrometer source are symmetrically arranged. Gases are temporarily stored in metal bellows and then are passed through a set of capillaries (one for each side) to ensure viscous flow of the gases. In theory, this ensures that there will be no fractionation of the gases prior to introduction into the mass spectrometer. In any case, any fractionation is the same for the standard and unknown gases. A changeover valve is used to switch between the standard and sample gases.

Accuracy and sensitivity of the system can be enhanced by switching between measurement of reference gas and the sample several times during the course of an isotope ratio determination. Isotope abundance ratios, presented as δ-values (see 2.1.1) are calculated from the ratios of the isotopic ion beams (actually counts as described above) of the sample and the standard. Precision of determining δ-values for pure samples is typically better than ±0.1‰ which corresponds to determining relative isotope abundance ratios to better than 1 part in 10^4. The overall reproducibility for natural samples is typically 2 to 3 times worse than that of the mass spectrometry due to isotope fractionation during chemical processing and/or heterogeneity of the raw material. Precision decreases with small sample sizes, when impure gases are analyzed, when the vacuum system leaks, and when the sample is radically different in isotope composition from the standard.

Two mass spectrometers built with V.G. Micromass 602 components were used for the hydrogen and sulphur isotope determination respectively. The H_2 was corrected for H_3^+ production by regression to zero gas pressure (so-called "slope correction"). A mass spectrometer built with V.G. Micromass 903 components was used for determining the carbon and/or oxygen isotope composition of the CO_2. If the CO_2 isotopically equilibrated with sea water is used as the working standard for oxygen isotope analysis, the following calibration formula is used to calculate the $\delta^{18}O$ value (y) of the organic oxygen (in V-SMOW):

$$y = 0.004\,x^2 + 1.382\,x + 46.173$$

where x is the measurement "raw" $\delta^{18}O$ value. Precisions for the isotope analyses of H, C, O, and S in pure gases are about ±1‰, ±0.1‰, ±0.2‰, and ±0.2‰ respectively.

3.5.2 On-line Continuous Flow Mass Spectrometer

Continuous flow isotope ratio mass spectrometry (CF-IRMS) is a methodology for which the sample preparation is integrated with the mass spectrometer to give "raw-samples in, results out" operation. In the other words, the IRMS-based analyzer is coupled to an automated sample preparation unit, in this case, a Carlo-Erba NA 1500 elemental analyzer (see Figure 3-2-1). This new technique permits rapid automatic analysis of content and the isotope abundances of elements, such as nitrogen, carbon, and more recently sulphur (Norman, 1991; Geisemann et al., 1994). The δD and $\delta^{18}O$ values of water have also been analyzed by a continuous flow method. Samples are normally solids or liquids but gaseous samples (human breath) have also been analyzed successfully (Preston and McMillan, 1988).

In CF-IRMS, the combustion products from the sample are swept through a gas chromatograph by a He carrier. Separated gases are swept through the mass spectrometer as desired. Ionization and ion optics are the same as in traditional IRMS. The difference is that ion currents change in time as the sample passes through the ion source. The isotopic

ion currents are integrated over time so that the abundance ratios are based on total charge reaching the individual Faraday cups.

In the newly-developed TracerMAT continuous flow mass spectrometer (Finnigan MAT, model 1993, for C and N analysis, see Figure 3-2-1) in our lab, a new patented sniffing device, the ConFlo open split interface, is installed. It permanently samples the output carrier stream of the elemental analyzer and can also sample reference gases through capillaries which can be inserted into the device by computer control. One of the capillaries introduces helium make-up-gas to dilute the CO_2 gas eluting from the elemental analyzer. In this way $\delta^{13}C$ values for CO_2 and $\delta^{15}N$ values for lower concentration N_2 gas can be measured using the same amplifier scale settings. Standard working gases can be introduced through the ConFlo and/or solid standards inserted periodically in the Carlo Erba carousel.

In traditional IRMS, less than 1 percent of the sample gas is consumed as viscous flow through the leaks requires a gas pressure of the order of 100 mmHg. In CF-IRMS, the total prepared gas goes through the mass spectrometer source and raw sample size can be reduced to μg quantities (as compared to mg quantities for traditional IRMS). Linearity of the source (ion beam current $\propto$ amount of gas admitted to source) is more critical for CF-IRMS since the yields of gases from the Carlo Erba unit vary with the type of sample. For a single analysis, the precision for a δ-value determination is slightly worse than for

the traditional twin leak IRMS but still within the uncertainties encountered in chemical processing of samples.

CHAPTER 4 RESULTS AND DISCUSSIONS

4.1 Comparison of Hydrogen, Carbon, and Sulphur Isotope Data Obtained with Different Analytical Methods

Appendices A-1 to A-3 give the hydrogen, carbon and sulphur isotope data obtained with different analytical methods.

From A-1, it is seen that most of the δD values obtained using quartz tubes are slightly lower or more negative than those using Pyrex tubes. However the cryogenic distillation process to separate the H_2O and CO_2 seems to be the main factor in the fractionation of hydrogen isotopes, resulting in the observed differences of δD values measured for replicates of the same sample. The standard deviations for δD values are the largest because D has twice the mass as H and isotope fractionation is an order of magnitude higher than for other biologically significant elements.

For the carbon isotopic compositions (A-2), slightly higher $\delta^{13}C$ values are obtained on average using quartz than when using Pyrex tubes. However this difference is within the standard deviation for one sample processed by either method.

Determined sulphur isotopic compositions for different analytical methods are given in A-3. The standard deviations (1~2‰) for different methods are larger than for C.

The two oxidation methods gave more comparable data whereas the $\delta^{34}S$ values for the Kiba technique were found to be lower. The latter is a reduction technique and the lower $\delta^{34}S$ values probably reflect a kinetic isotope effect and incomplete reaction (30~50%). However the standard deviation for the Kiba results were as good or better than for the other preparations. The second method (oxidation from remains of static combustion) offers a quick, economic way of processing. Although consistent results were obtained with the Kiba method, it is not preferred because of the large sample consumption (more than 50 mg), relatively low yield of H_2S, relatively expensive reagents, and longer time spent for each sample.

4.2 Geographical Influence on Stable Isotope Composition; Comparison to Other Locations

The isotope data for human hair in different regions are listed in Appendices A-4 to A-7. The δ-value variations in part are probably due to sample heterogeneity. Carbon has the least isotopic variability, because photosynthesis uses atmospheric CO_2 which is markedly constant in isotopic composition. Water used in photosynthesis may display seasonally dependent δD and $\delta^{18}O$ variations in precipitation. The sulphur isotope composition of hair relates to uptake of atmospheric gases and soil water sulphate by plants. The isotopic composition of these sources can vary markedly over short distances and temporally (wind direction, use of fertilizer, etc.).

Sets of histograms of the isotope data from different regions are plotted in Figures 4-2-1 to 4-2-5. Some multivariate relationships ($\delta^{15}N$ versus $\delta^{13}C$, $\delta^{34}S$ versus $\delta^{13}C$, and $\delta^{34}S$ versus δD values) for hair samples from different regions are shown in Figures 4-2-6 to 4-2-8. Some geographical and geological features of the regions are listed in Table 4-1, while causes of isotope abundance variations for different elements and comparisons of the isotope data from different regions are listed in Table 4-2. The isotope data obtained from other locations in the world are listed in Table 4-3.

Samples from different regions can be distinguished using multivariate analyses because isotope compositions of different biological significant elements are altered by distinctly different processes. Cross-plots of sulphur, hydrogen, and carbon isotope data separates some groups (Figures 4-2-7, 4-2-8), whereas a cross-plot of nitrogen and carbon isotopes has significant overlaps of all groups (Figure 4-2-6).

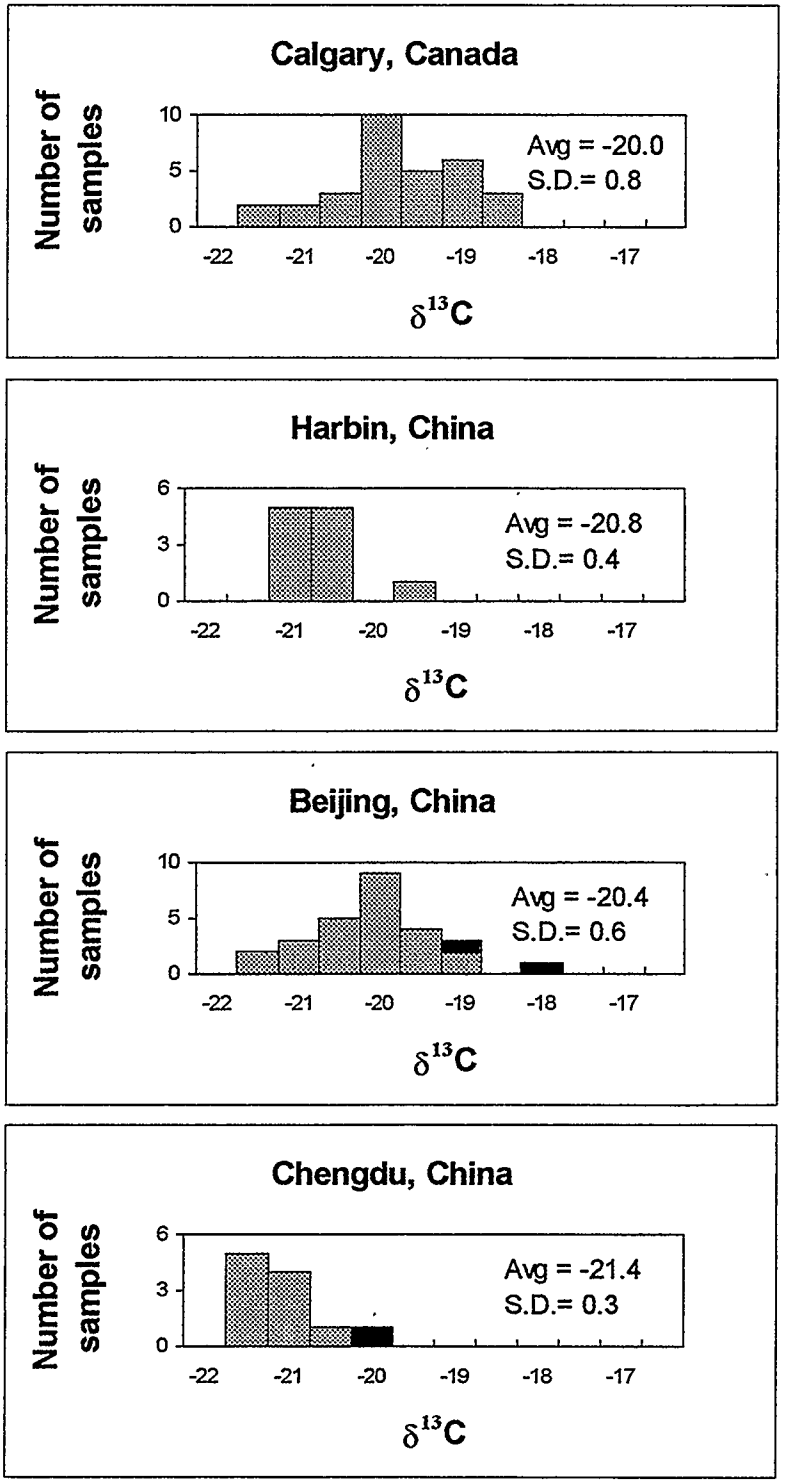

Figure 4-2-1 Histograms of $\delta^{13}C$ for Hair from Studied Locations (Black boxes indicate individuals under 5 years of age)

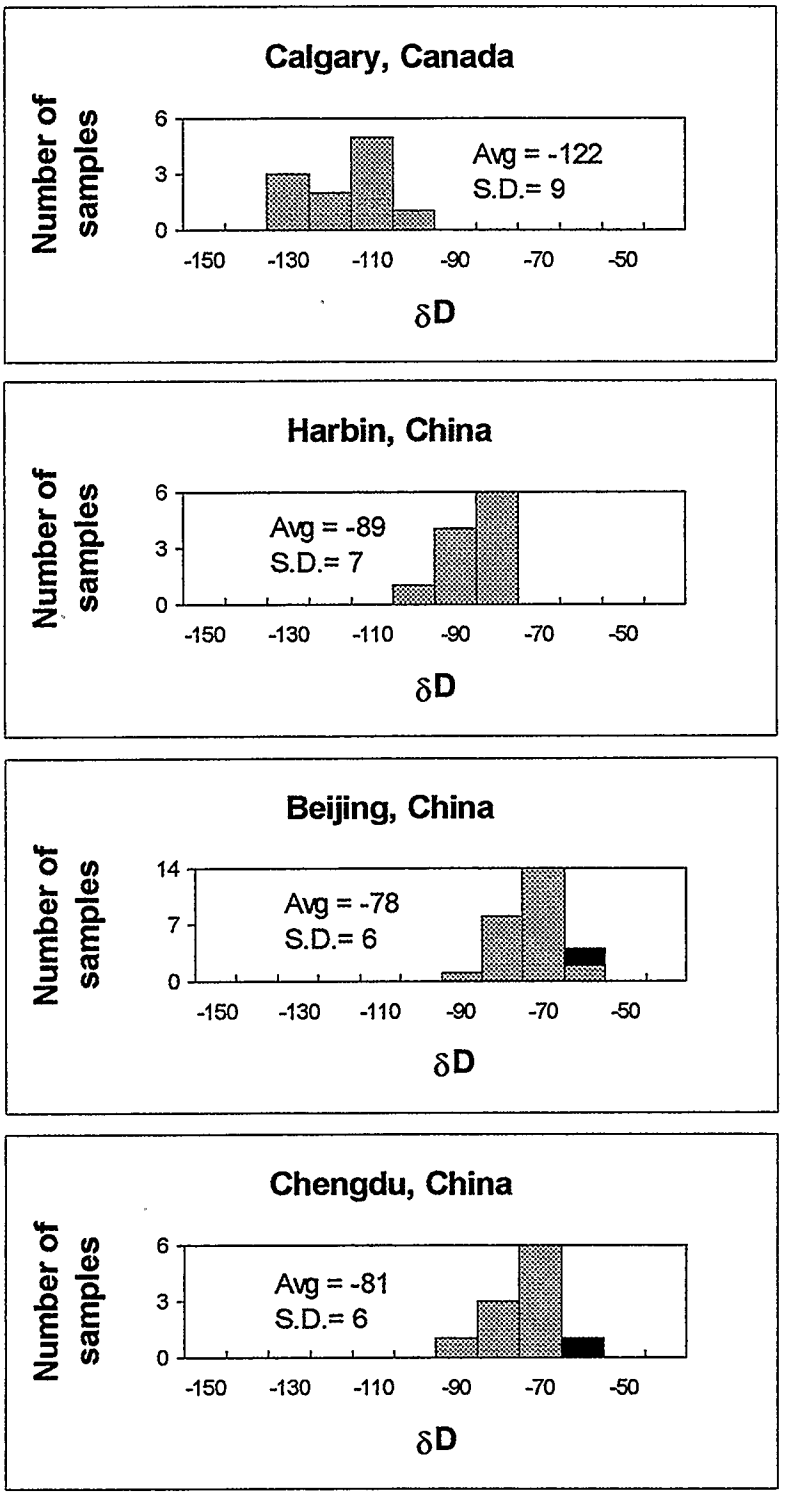

Figure 4-2-2 Histograms of δD for Hair from Studied Locations (Black boxes indicate individuals under 5 years of age)

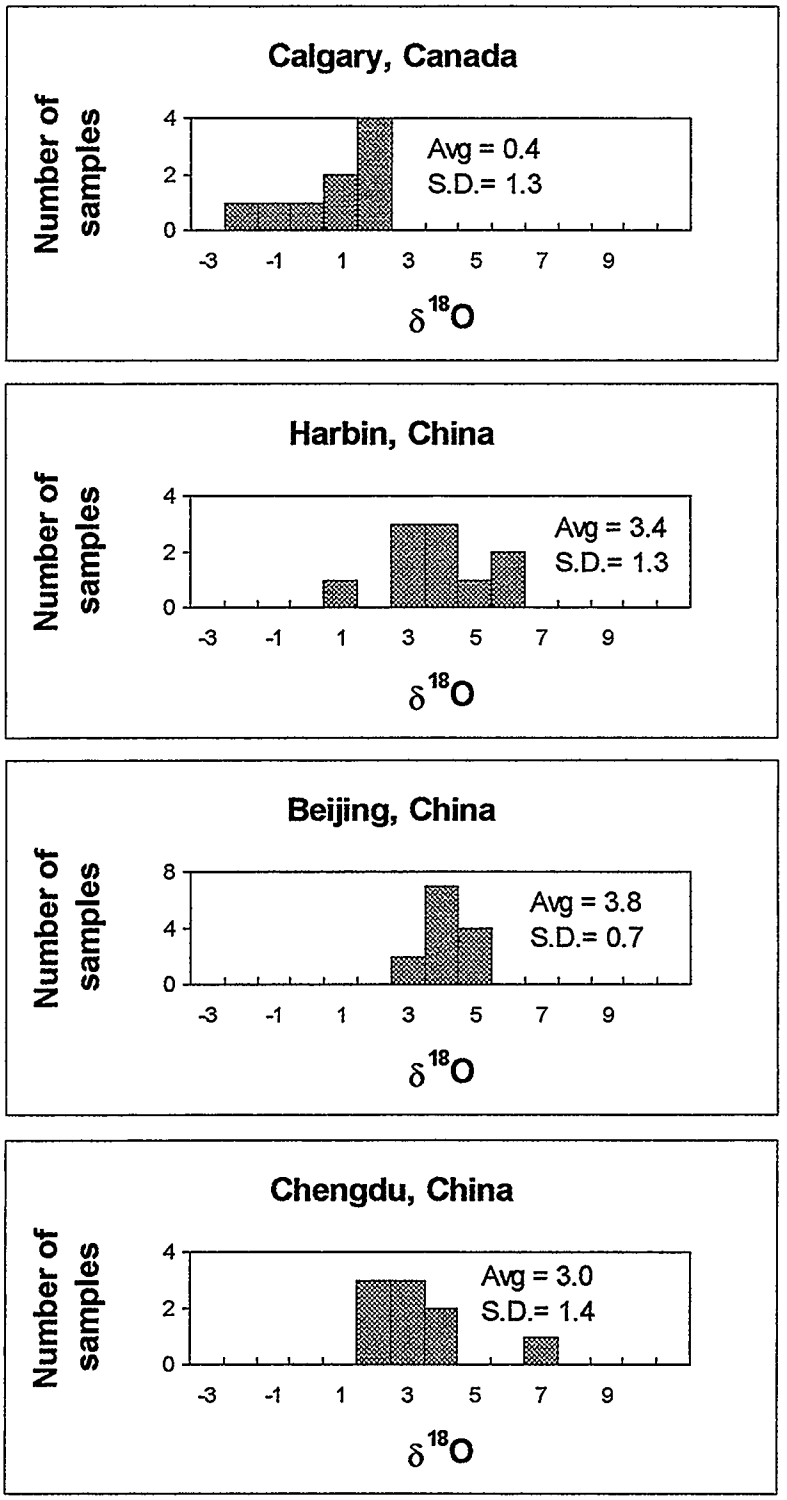

Figure 4-2-3 Histograms of $\delta^{18}O$ for Hair from Studied Locations

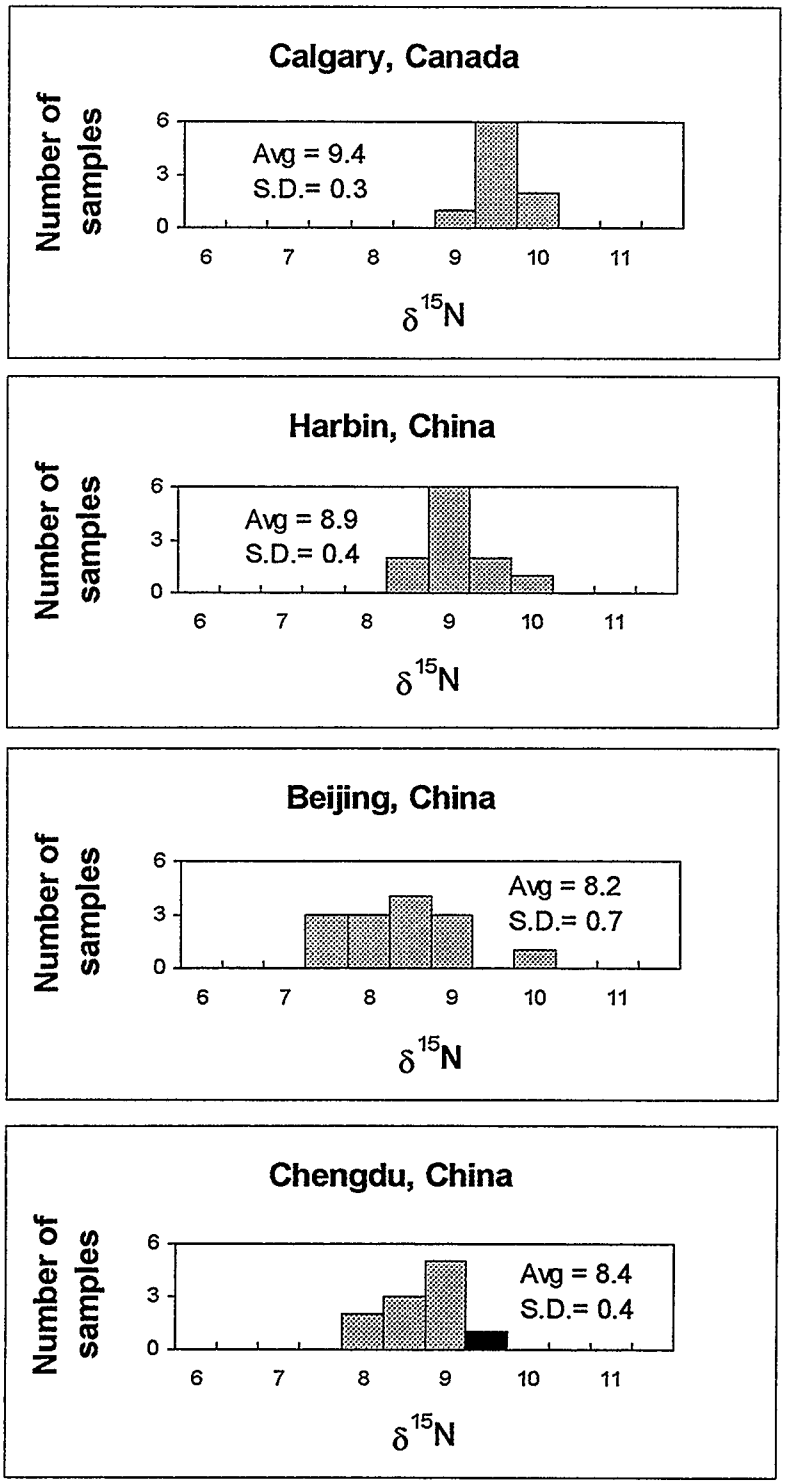

Figure 4-2-4 Histograms of $\delta^{15}N$ for Hair from Studied Locations (Black box indicates an individual under 5 years of age)

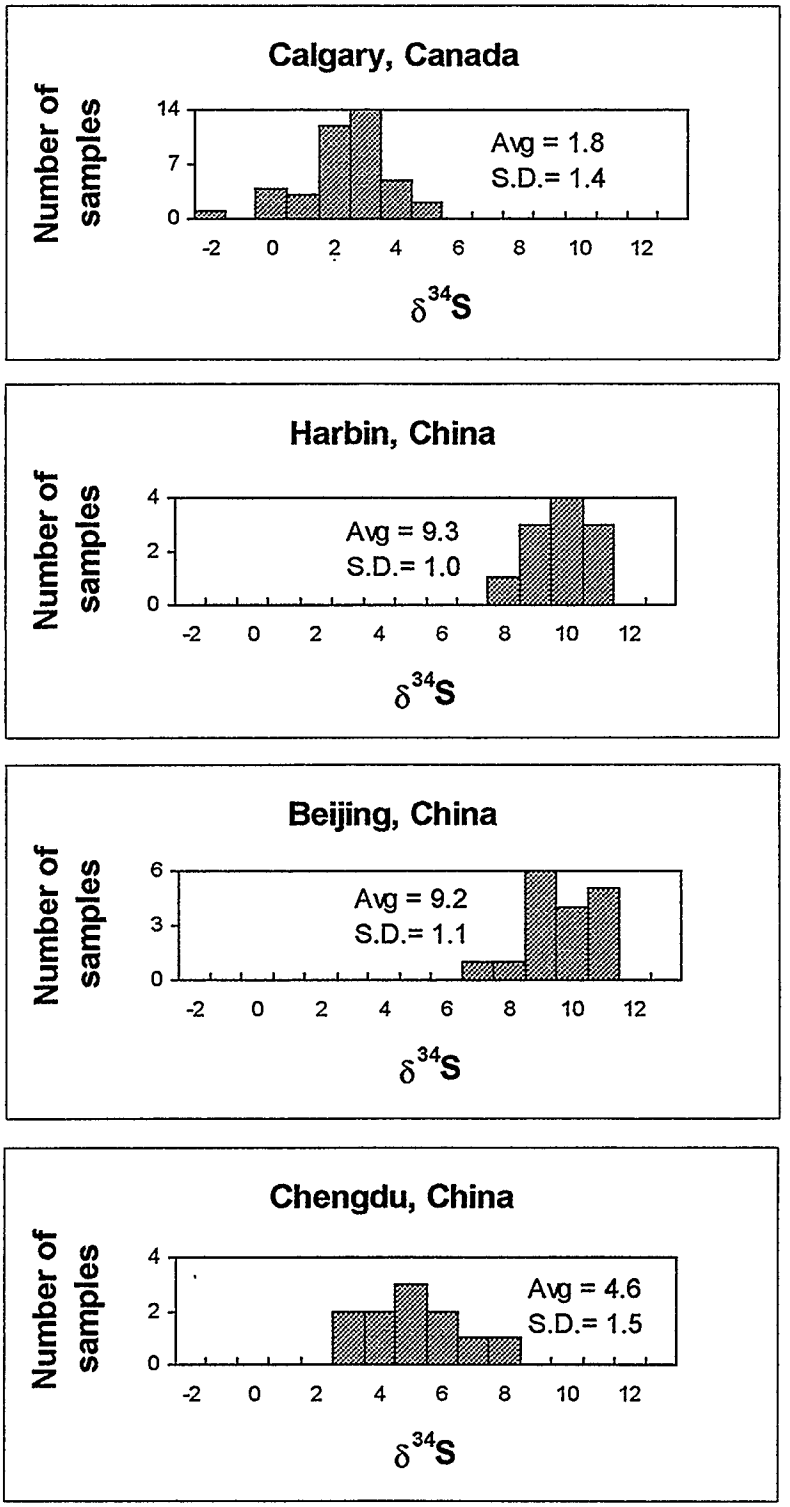

Figure 4-2-5 Histograms of $\delta^{34}S$ for Hair from Studied Locations

Table 4-1 Some Geographical and Geological Features of the Regions under Study (References: Atlas of Alberta, 1984; Hydrological Atlas of Canada, 1978; National Economic Atlas of China, 1994)

	Calgary	Harbin	Beijing	Chengdu
Population (1988)	0.7 million	2.7 million	6.7 million	3.0 million
Latitude	51.0°N	45.7°N	40.0°N	30.7°N
Longitude	114.1°W	126.6°E	116.4°E	104.0°E
Altitude	1048 m	145 m	51 m	506 m
Distance from the Coast	700 km	500 km	200 km	1200 km
Major Industries	Petroleum, Minerals, Food, Beverages, Leather	Heavy Machinery, Electrical Equipment	Machinery, Electronics, Iron & Steel, Chemicals, Textiles	Instrument & Cutting Tools, Light Industry
Where Most Hair Samples Collected	Urban	Urban	Urban	Suburban
Mean Annual Temperature	3°C	3°C	11°C	17°C
Annual Range of Temperature	47°C	43°C	31°C	21°C
Annual Precipitation	500-600 mm	550 mm	650 mm	1000 mm

Table 4-2 Causes of Isotope Abundance Variations for Different Elements and Comparison of Average Isotope Data from Different Regions

Fundamental Isotope Fractionation Mechanism	Element	δ-Value	Comparison of mean δ-Values for different regions (here ≥ means slightly greater than)			
Photosynthesis	C	$\delta^{13}C$	Calgary ≥ Beijing ≥ Harbin > Chengdu			
			-20.0 ±0.8	-20.4 ±0.6	-20.8 ±0.4	-21.4 ±0.3
Meteorology, Evapo-transpiration, Photosynthesis	H	δD	Beijing ≅ Chengdu ≥ Harbin > Calgary			
			-77.9 ±5.6	-80.7 ±5.5	-88.6 ±7.1	-121.8 ±9.1
Meteorology, Evapo-transpiration, Photosynthesis	O	$\delta^{18}O$	Beijing ≥ Harbin ≥ Chengdu > Calgary			
			3.8 ±0.7	3.4 ±1.3	3.0 ±1.4	0.4 ±1.3
Weathering, Bacterial Nitrate Reduction, Fertilizers, Industrial and Biogenic Emissions	N	$\delta^{15}N$	Calgary ≥ Harbin ≥ Chengdu ≥ Beijing			
			9.4 ±0.3	8.9 ±0.4	8.4 ±0.4	8.2 ±0.7
Weathering, Bacterial sulphate Reduction, Fertilizers, Industrial and Biogenic Emissions	S	$\delta^{34}S$	Harbin ≅ Beijing > Chengdu > Calgary			
			9.3 ±1.0	9.2 ±1.1	4.6 ±1.5	1.8 ±1.4

Table 4-3 Isotope Data for Hair Samples Studied at Other Locations in the World†

Location [References]	$\delta^{13}C$ ± S.D.	δD ± S.D.	$\delta^{18}O$ ± S.D.	$\delta^{15}N$ ± S.D.	$\delta^{34}S$ ± S.D.
Calgary, Canada [1]	-20.0±0.8	-122 ± 9	0.4±1.3	9.4±0.3	1.8±1.4
Calgary, Canada [2]	-20.3±1.0				
Chicago, USA [3]	-16.6±0.6	-62 ± 4		9.6±0.5	
Chicago, USA [4]	-16.4±0.9				
Hawaii, USA [2]	-17.7±1.6				
Brisbane, Australia [5]	-17.2				13.8
Canberra, Australia [5]	-19.0				12.6
New Zealand [8]	-21.3			9.9	5.1
Munich, Germany [4]	-20.4±0.5				
Salvador, Brazil [6]	-14.5±0.6	-65 ± 2		10.0±2.3	11.7±0.7
San Pauro, Brazil [6]	-15.3±1.6	-82 ± 15		10.3±1.5	11.4±0.6
Asuncion, Paraguay [6]	-16.9±0.6	-83 ± 3		10.9±0.7	8.8±0.7
Rosario, Argentina [6]	-17.8±0.5	-75 ± 5		10.5±0.5	5.8±0.9
Punta Arenas, Chile [6]	-19.1±0.5	-95 ± 3		8.8±0.8	8.8±1.4
Japan [4]	-18.0±0.8				
Japan [7]	-18.2±0.4			10.3±0.4	
Korea [8]				10.0±0.5	
India [8]				9.0±0.5	
Beijing, China [1]	-20.4±0.6	-78 ± 6	3.8±0.7	8.2±0.7	9.2±1.1
Harbin, China [1]	-20.8±0.4	-89 ± 7	3.4±1.3	8.9±0.4	9.3±1.0
Chengdu, China [1]	-21.4±0.3	-81 ± 6	3.0±1.4	8.4±0.4	4.6±1.5

† All values are in ‰, with respect to international standards in Table 2-1.

[1] Data from current study
[2] Krouse et al., 1987
[3] Schoeller et al., 1986
[4] Nakamura et al., 1982
[5] Krouse and Herbert, 1988
[6] Iyer et al., 1995
[7] Minagawa, 1992; collected from Tokyo, Okinawa and Akita
[8] Unpublished data, Stable Isotope Laboratory, the University of Calgary

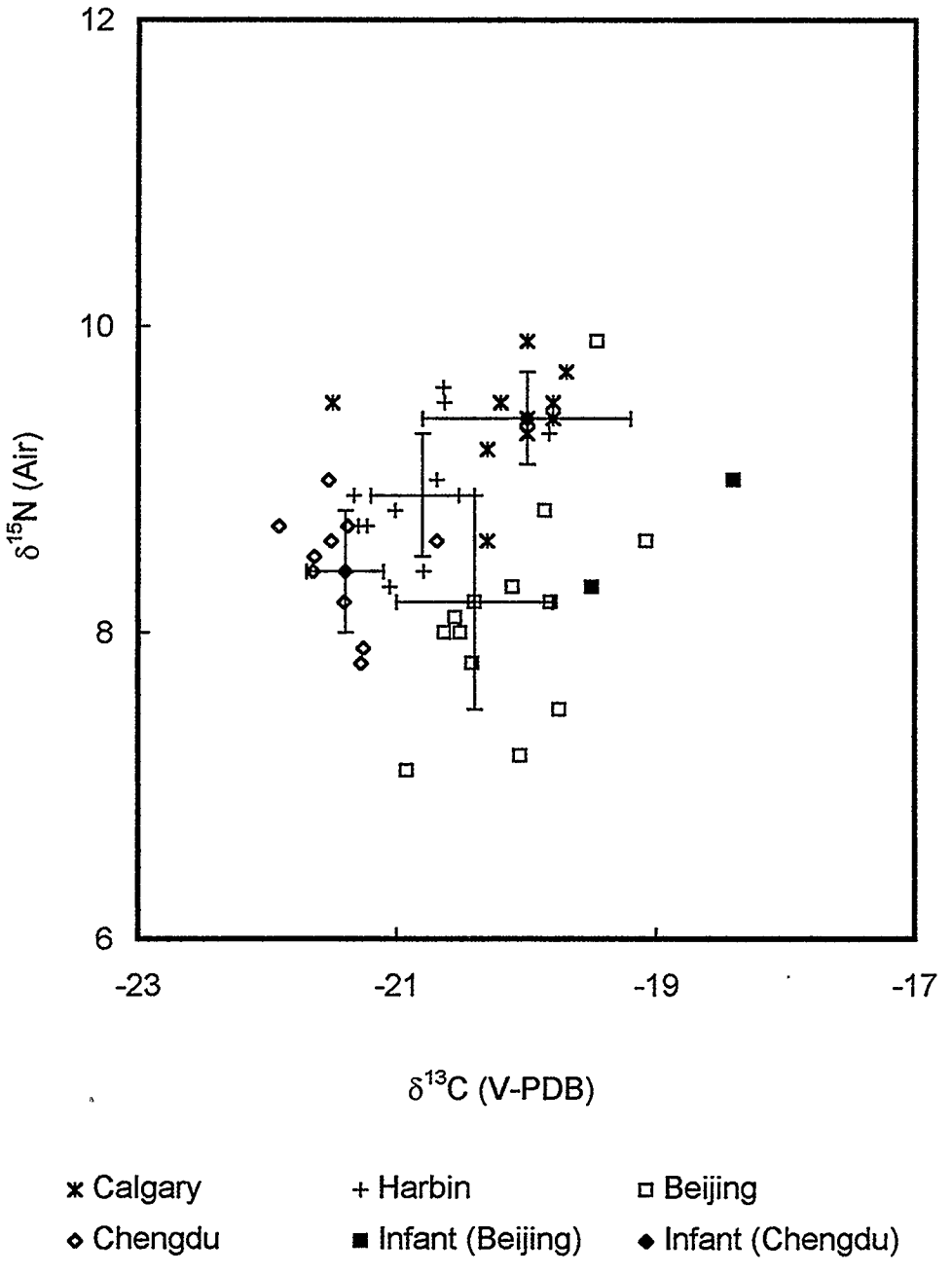

Figure 4-2-6 $\delta^{15}N$ versus $\delta^{13}C$ for Hair from Studied Locations

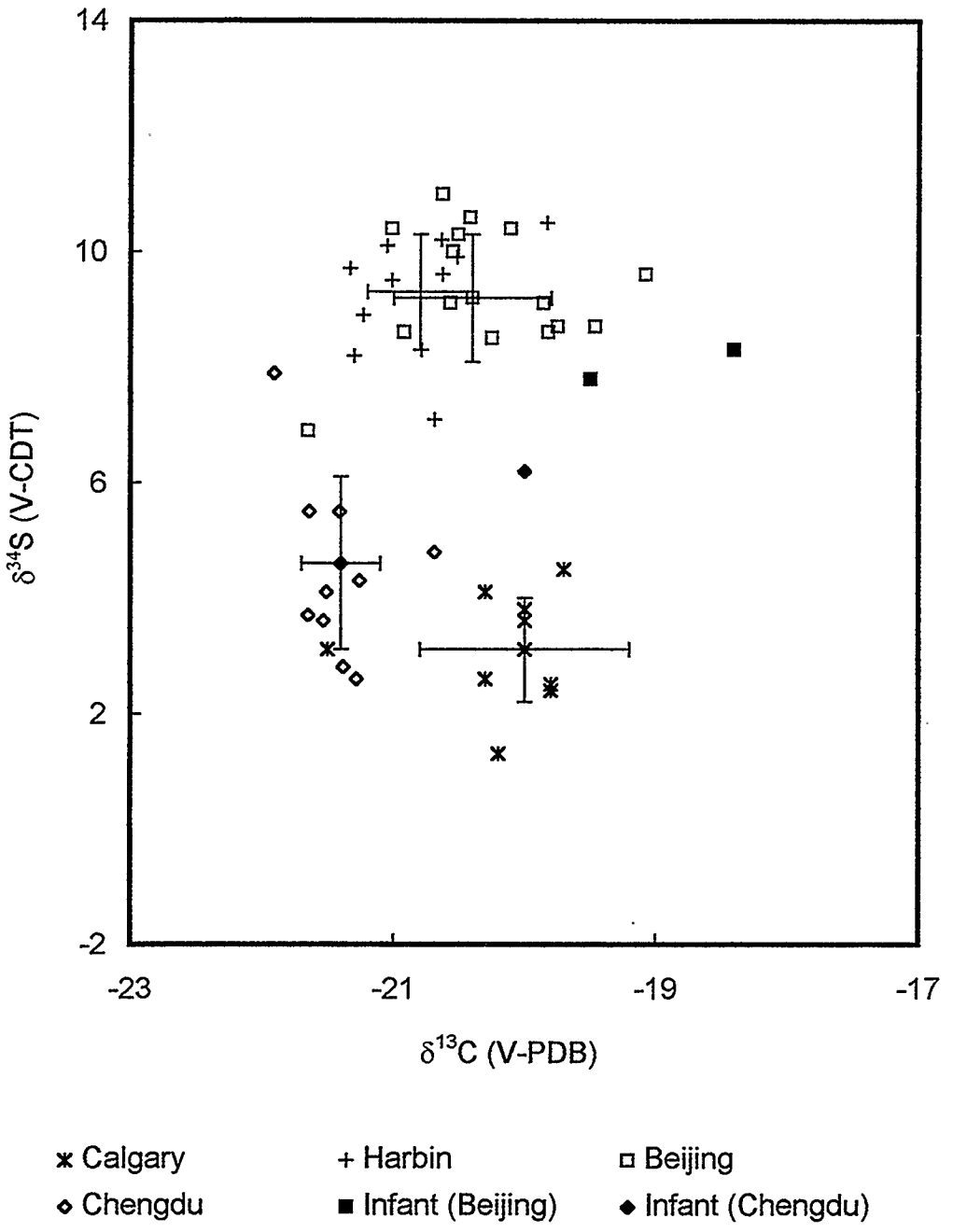

Figure 4-2-7 $\delta^{34}S$ versus $\delta^{13}C$ for Hair from Studied Locations

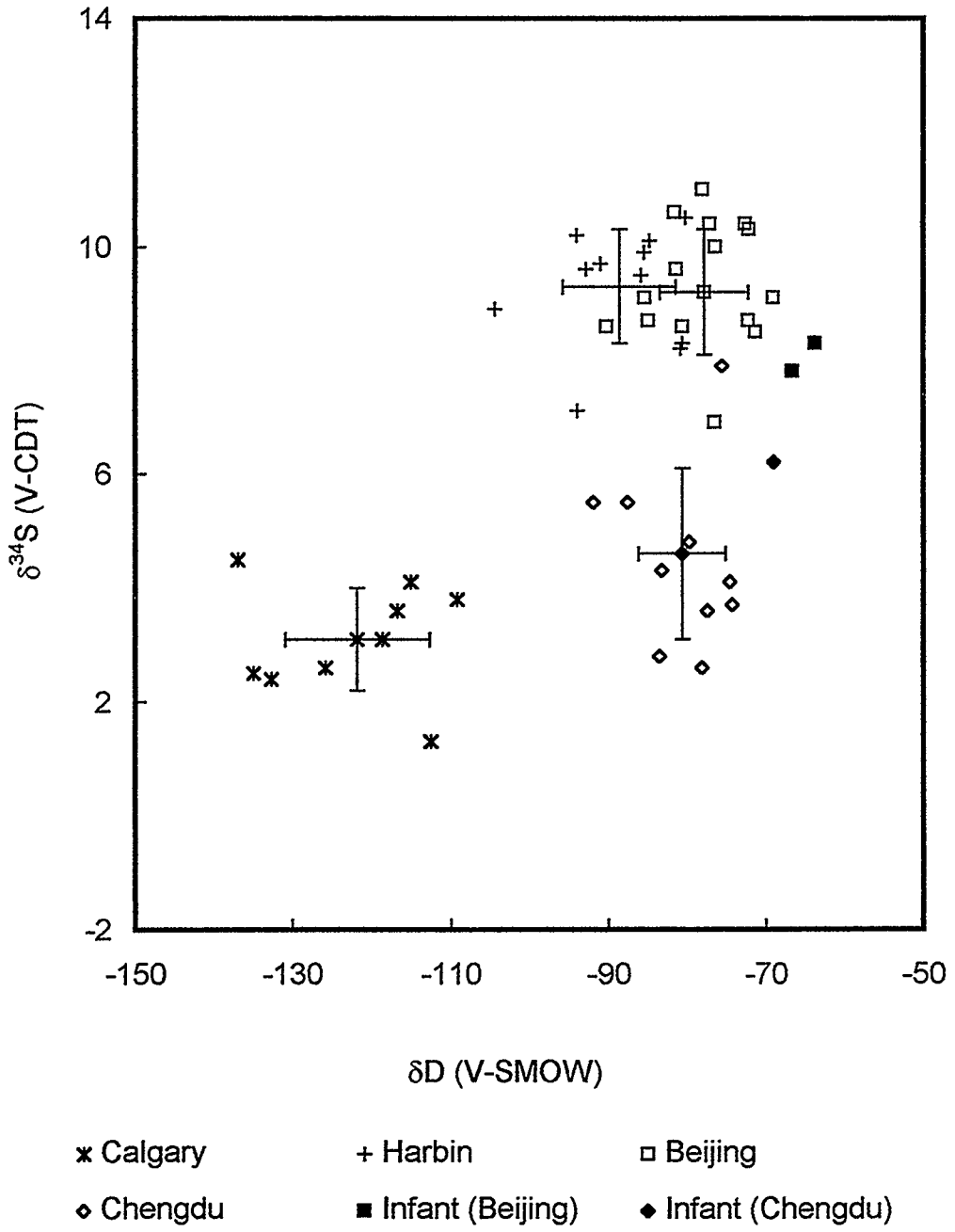

Figure 4-2-8 $\delta^{34}S$ versus δD for Hair from Studied Locations

Not much difference was found for the mean $\delta^{13}C$ values of hair samples from different regions under study, which vary between -20.0‰ to -21.4‰. The carbon isotopic compositions can provide a rough estimate of the quantities of different food sources, especially C_3 and C_4 plants (see discussion in 4.4.1). $\delta^{13}C$ values for samples from Calgary (-20.0‰) are comparable to those from Harbin and Beijing e.g., two-sample unequal variance two-tailed t-test for Beijing and Calgary: $P = 0.033$. The mean $\delta^{13}C$ value for samples from Chengdu is slightly more negative than at other locations. The standard deviation just "touches" or slightly overlaps those of the other locations e.g., t-test for Chengdu and Harbin: $P = 0.002$. A possible interpretation is a greater proportion of C_3 plants (rice, vegetables, fruit) in the diet at Chengdu which is interestingly a suburban rather than urban setting. The carbon isotope data do not show a clear latitudinal trend as observed by others (Minson et al., 1975; Nakamura et al., 1982; Krouse and Herbert, 1988; Iyer et al., 1995). This suggests that C_3/C_4 plant coverage is comparable at all sites and/or other factors compensate for any differences, e.g., most sugar consumed by Calgarians comes from sugar cane (C_4).

The δD and $\delta^{18}O$ values of hair samples from Calgary are much lower than those from China, and those from Beijing are higher than those from the other two regions in China (Figures 4-2-2, 4-2-3, 4-2-9). The hydrogen and oxygen isotopic compositions should reflect the isotopic compositions of the local precipitation. Except for Chengdu, the hydrogen and oxygen isotope data show a clear latitudinal trend. The lower δD and $\delta^{18}O$

values of samples from Chengdu may be attributable to altitude, continent, and amount effects as discussed in 2.2.4.

Figure 4-2-10 gives the weighted mean δD and $\delta^{18}O$ values of local precipitation versus the latitude in some regions of China (data are listed in A-8). The isotope data of precipitation in Beijing, Harbin and Chengdu were estimated from the data of other neighbouring regions. It can be found that the δD value of hair is close to that of local precipitation, whereas $\delta^{18}O$ values are about 15‰ higher (Table 4-4). Thus the H and O-isotope compositions of hair are consistent with those of vegetation, as is expected from the relationship between local precipitation and vegetation (see 2.2.4). It should be noticed that the δD and $\delta^{18}O$ values of tap water may not be the weighted mean values of local precipitation because of evaporation in storage reservoirs and control of precipitation reaching them.

Table 4-4 Comparison of δD and $\delta^{18}O$ Values of Hair Samples and Estimated (from A-8) Weighted Mean Values of Precipitation in Regions Studied

Region	δD (V-SMOW)		$\delta^{18}O$ (V-SMOW)	
	Hair	Precipitation	Hair	Precipitation
Calgary	-121.8±9.1	-130 *	0.4±1.3	-16.5 *
Harbin	-88.6±7.1	-80	3.4±1.3	-10.8
Beijing	-77.9±5.6	-55	3.8±0.7	-7.5
Chengdu	-80.7±5.5	-72	3.0±1.4	-10.7

* Isotope data of tap water in Calgary: δD = -145‰, $\delta^{18}O$ = -19.5‰.

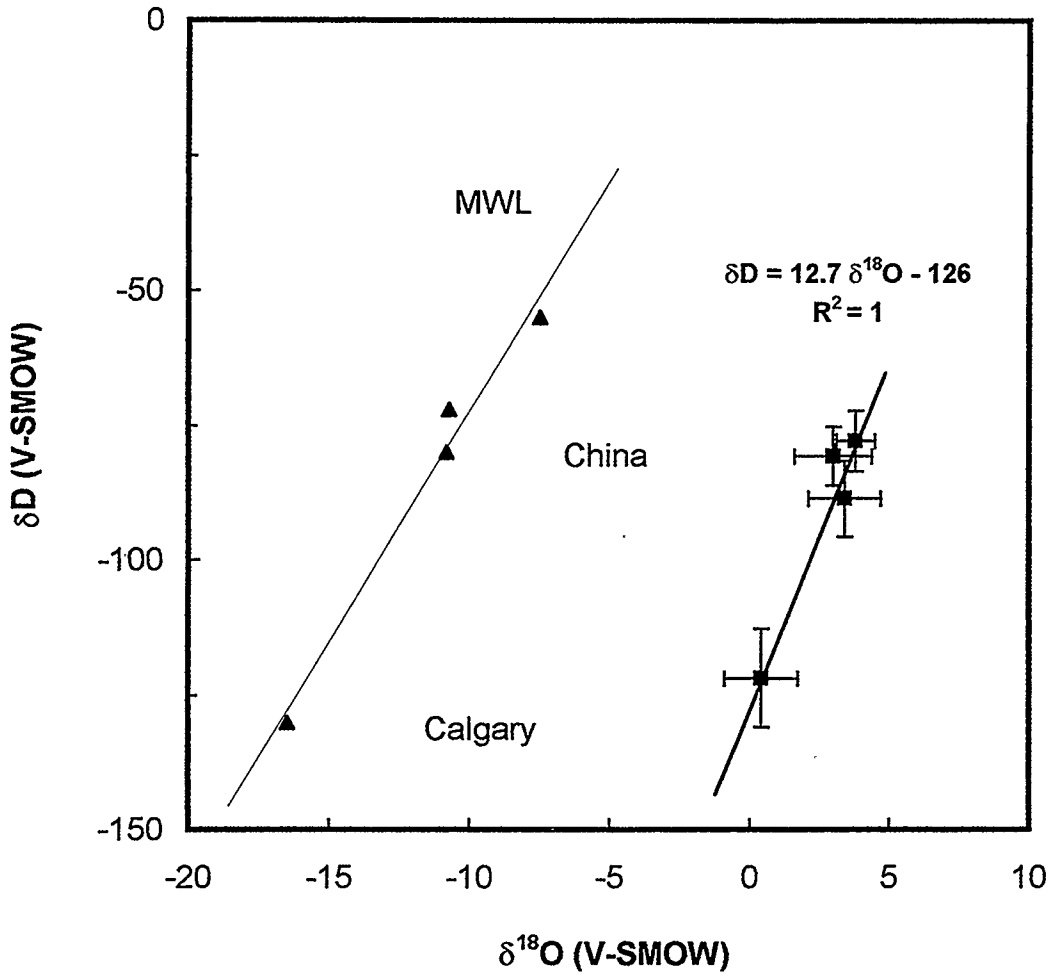

Figure 4-2-9 Relationship of Hydrogen to Oxygen Isotope Compositions of Hair Samples and Precipitation from Different Regions Studied

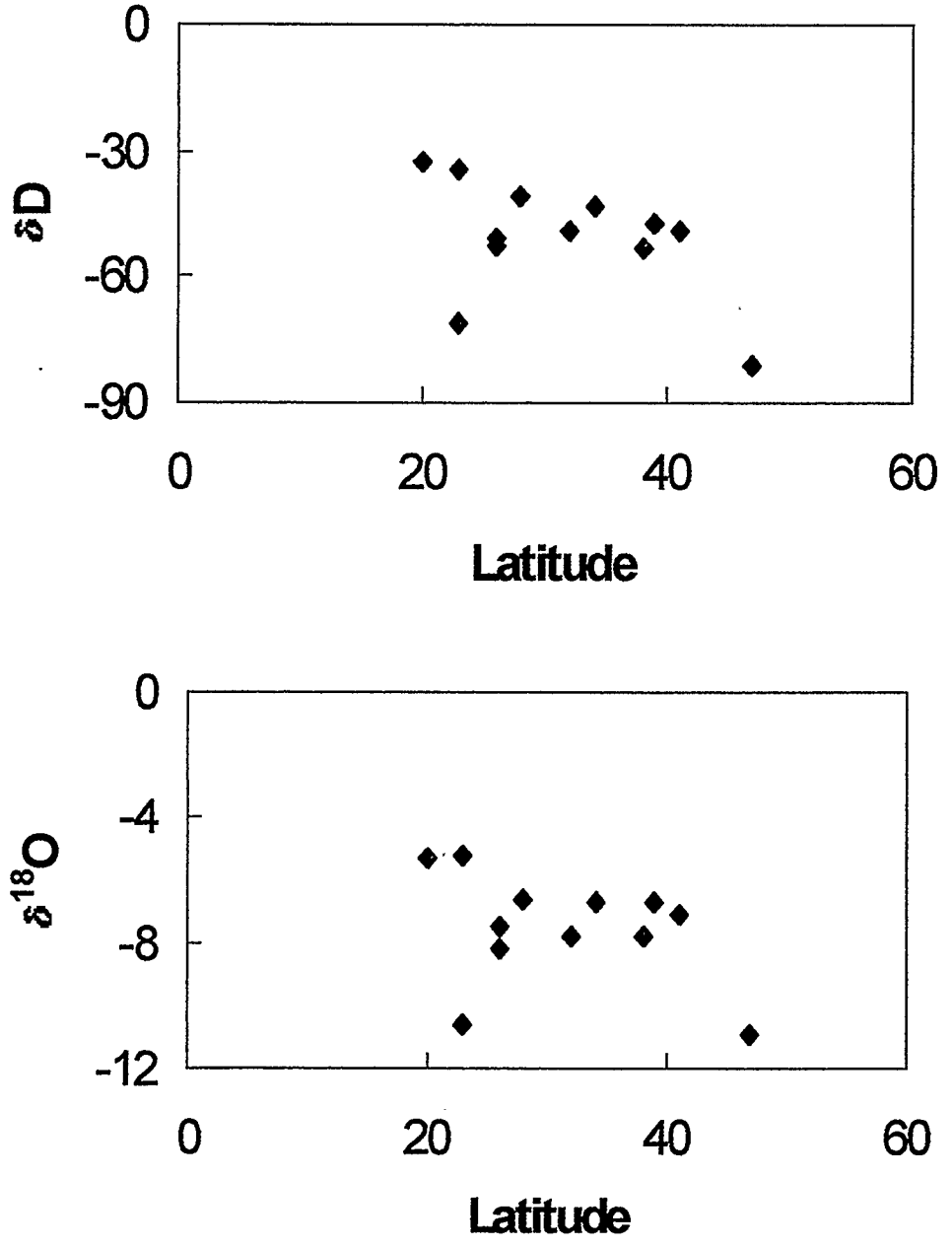

Figure 4-2-10 Weighted Mean δD and $\delta^{18}O$ Values of Precipitation versus Latitude in Some Regions of China (data in A-8)

The mean $\delta^{15}N$ value of hair from Calgary (+9.3‰) was found to be slightly higher than those from China (+8.2 to +8.9‰) e.g., two-sample unequal variance t-test for Calgary and all samples from China: P = 1.2E-05. It has been found that dietary protein nitrogen is enriched in ^{15}N by about 5 to 6‰ relative to atmospheric nitrogen and the enrichment of ^{15}N in hair relative to diet is attributed to *in vivo* isotope fractionation (e.g., Schoeller et al., 1986; Minagawa, 1992). Much higher $\delta^{15}N$ values arise if there is a high marine component in the diet (e.g., Aufderheide et al., 1994). The data imply that at all the locations the diet was dominantly continental.

Sulphur isotope data do not display the same trends as those of other elements, nor are they expected to do so. There are dependences upon weathering, pollution, etc. not associated with the other elements. The $\delta^{34}S$ values for samples from Beijing and Harbin (two-sample unequal variance t-test for the two regions: P = 0.884) are significantly higher than those from Chengdu. The mean $\delta^{34}S$ value for samples from Calgary is near 0‰, which is lower than those from the three regions in China e.g., t-test for Calgary and Beijing: P = 1.7E-22. It has been found that the diet for Calgarians varies in $\delta^{34}S$ values from -8 to +19‰, the latter corresponding to imported seafood (Krouse et al., 1987). The $\delta^{34}S$ value of soil in the vicinity of Calgary is near 0‰, and $\delta^{34}S$ values for grain, poultry, eggs, dairy products, beef and pork in southern Alberta ranged from 0 to +5‰ (Krouse and Herbert, 1988). Therefore human hair of Calgarians has a sulphur isotope composition near the mean $\delta^{34}S$ value for their diets.

4.3 The Influence of Age and Other Human Characteristics

For all samples collected, no difference in the isotopic compositions in the same region could be attributed to the sex of the subject.

Differences in isotopic compositions among the different adult age groups were not discernible, whereas some infant individuals in China have noticeably large δ-value deviations from the average value of other samples in that region. Sample #304 (male, age of 3) had the highest $\delta^{13}C$, δD and $\delta^{15}N$ values among the samples from Chengdu. Sample #218 (female, age of 2), #204 (male, age of 4), and #226 (male, age of 6) from Beijing have almost the same feature. This reflects a difference in the dietary intake of infants e.g., consumption of milk with high protein content. Protein at a higher trophic level has higher $\delta^{13}C$ and $\delta^{15}N$ values than those of rice and vegetables. The differences of $\delta^{13}C$ and $\delta^{15}N$ values for infants and adults have also been found in prehistoric human bone collagen (Katzenberg, 1993; Katzenberg et al., 1993).

In order that the data sets at all locations should be comparable (i.e. pertain to adults), $\delta^{13}C$ and δD values for samples #204, #218, and #304, and the $\delta^{15}N$ value for sample #304 were not included in the calculation of means and standard deviations of these δ-values.

4.4 Diet Reconstruction

4.4.1 Estimated Relative Amounts of C_3 and C_4 Components in the Diet

If a diet is dominately continental, carbon isotope data can be used to estimate relative amounts of C_3 and C_4 components. For example, one can use the $\delta^{13}C$ values of -28.1‰ and -13.5 ‰ quoted by Schoeller et al. (1986) as the means for dietary C_3 and C_4 carbon, as described in 2.3, it is necessary to know the hair-diet carbon isotope discrimination which unfortunately has been reported to range widely from +1.0 to +4.8‰ (Yoshinaga et al., 1996). For the estimates given in Table 4-5, the value of +2.3‰ was used in accordance with the report of Schoeller et al. (1986). It should be noted that the C_4 (or C_3) components in the diet consist not only of the direct input of C_4 (or C_3) plants but also higher trophic level consumer products (dairy, eggs, meat, etc.).

Table 4-5 Estimated Amounts of C_3 and C_4 Components in the Diet

Regional Average, or Individual Sample Number ($\delta^{13}C$ values in brackets)	C_3 Contribution	C_4 Contribution
Calgary (mean -20.0‰)	60%	40%
Harbin (mean -20.8‰)	66%	34%
Beijing (mean -20.4‰)	63%	37%
#218 in Beijing (-18.4‰)	49%	51%
Chengdu (mean -21.4‰)	70%	30%
#304 in Chengdu (-20.0‰)	60%	40%

The calculation gives C_3 and C_4 contributions which seem too low and too high respectively. One can examine the extent to which the results of Table 4-5 are changed by using different values for the mean C_3 and C_4 $\delta^{13}C$ values and the hair-diet discrimination. If +1‰ is used for the latter, the calculated C_3 contributions decrease by about 9 percent relative to the values given in Table 4-5. If +3‰ is used for the discrimination factor, the percent C_3 contributions increase by about 5. The extreme value of +4.8‰ reported by Yoshinaga et al. (1996) increases the C_3 contributions by about 17 percent above those in Table 4-5. The often quoted average isotopic abundances of C_3 and C_4 carbon may be different from those in the local foodwebs. For example, on the basis of purchased food, Schoeller et al. (1986) considered the mean C_3 and C_4 dietary $\delta^{13}C$ values for Chicago to be -25.1 and -10.8‰ respectively. If these values are used with a hair-diet discrimination factor of +2.3‰, the C_3 contributions in Table 4-5 increase by about 20 percent. In the case of Calgary, the higher value seems more realistic. In any case, better estimation of the amounts of C_3 and C_4 components requires isotopic analyses of the dietary resources at the location under study.

Sometimes it is more appropriate to consider the isotopic compositions of the dietary macronutrients (protein, carbohydrate, lipid) rather than total diet, because the major component of hair is protein. It has been found that the carbon isotopic compositions of the individual macronutrients in the body compare well with those of the dietary macronutrients rather than total dietary carbon (Schoeller et al., 1986). On the other hand, hair may be too "pure" and therefore not representative of all proteins. For

example, hair contains 10-14% cystine and therefore will be influenced by the isotopic abundance of the cystine to a larger degree than other proteins (Nakamura et al., 1982). The lack of food samples for analyses does not allow such further refinement to interpretation of our data.

4.4.2 Estimated Marine versus Terrestrial Content of Diet

The sulphur and/or nitrogen isotopic composition of human hair can be used to discriminate the marine and terrestrial food source. Estimate of the marine versus terrestrial content of diet is difficult because many unknown factors still exist, such as isotopic compositions of local terrestrial foodweb (including the environmental influence), unknown fractionation factors in the nitrogen metabolism, etc. Without these data, estimates of marine versus terrestrial content of diet have only been possible when the diet was near one extreme or the other (e.g., Aufderheide et al., 1994).

It was found that the mean $\delta^{15}N$ value for hair from Calgary was slightly higher than those from China (two-sample unequal variance t-test for Calgary and China: P = 1.2E-05). Carbon isotope data (t-test: P = 8.2E-05) had a similar trend. A possible explanation is that there is a slightly larger proportion of marine food (and meat) intake for Calgarians than for the Chinese studied.

Using only the sulphur isotope data, the low $\delta^{34}S$ values of samples from Chengdu may be explained as a smaller proportion of marine source in the diet. However the $\delta^{15}N$ data do not show Chengdu to have a significantly lower marine component than Beijing. Further for Calgary, $\delta^{34}S$ and $\delta^{15}N$ data argue for the lowest and highest marine proportions respectively. The problem is that high $\delta^{34}S$ values are not always consistent with a high proportion of marine food in the diet.

4.5 Other Environmental Influences

If we consider compare the nitrogen and sulphur isotopic compositions of hair samples, it is found that the local environmental influence is not negligible. Whereas the regional differences for nitrogen isotope data are small, there are correspondingly significant differences in sulphur isotope abundances.

Most samples from Chengdu were collected from residents in a small city 50 km away from the metropolitan Chengdu area and most of their food is grown locally. Therefore the isotopic compositions of samples from Chengdu should be more representative of the local ecosystem. For samples collected from Beijing and Harbin (all samples come from the urban area), other food sources from areas far from these cities must be considered. This complicates the interpretation.

CHAPTER 5 CONCLUSIONS

The δ-value variations measured in individual samples of hair are probably due in part to sample heterogeneity. Carbon was found to have the least isotopic variability possibly because photosynthesis uses atmospheric CO_2 which is markedly constant in isotopic composition over time. In contrast, water used in photosynthesis may reflect some of the seasonally dependent δD and $\delta^{18}O$ variations in precipitation. The sulphur isotope composition of hair relates to uptake of atmospheric gases and soil water sulphate by plants. The isotopic composition of these sources can vary markedly over short distances and temporally (such as wind direction, use of fertilizer, etc.).

The range of mean $\delta^{13}C$ values (-20.0‰ to -21.4‰) of hair samples from different regions under study was found to be small. The lower $\delta^{13}C$ values of samples from China may be attributed to a larger dietary intake of C_3 carbon, such as rice, vegetables and fruit. The carbon isotope data do not show a strong latitudinal trend possibly reflecting cultural or nutrient choice overriding any geographical differences. The fact that the $\delta^{13}C$ values were higher for hair from very young subjects is consistent with this statement. Although the carbon isotopic compositions were used to predict the proportions of food derived ultimately from C_3 and C_4 plants, more accurate estimation requires analyses of C_3 and C_4 components in dietary resources in the local foodweb. A particular problem with $\delta^{13}C$ data is that a marine value cannot be distinguished from appropriate mixtures of C_3 and C_4

continental resources. This may not be a significant problem since nitrogen isotope data suggest that the diets were dominately continental at all locations.

The δD and $\delta^{18}O$ values of hair samples reflect the geographical trends found for precipitation in the regions studied. In addition to the latitudinal trend, the continental and amount effect are also found. Consequently, the combination of δD and $\delta^{18}O$ data should provide climatic as well as foodweb data. Higher δ-values attest to greater evapotranspiration, because evapotranspiration enriches foliage water in heavier isotopes (^{18}O and D). In contrast, photosynthesis favours the lighter H-isotope, but the heavier O-isotope. Therefore the H and O-isotope compositions of vegetation have different relationships with respect to the local precipitation: H-isotope composition of vegetations is close to that of precipitation, whereas O-isotope composition of vegetations is significantly higher than that of precipitation. The H and O-isotope compositions of hair are consistent with those of local vegetation.

Human hair of Calgarians was previously shown to have sulphur isotope compositions near the mean value for their diets. Sulphur and/or nitrogen isotopic composition of human hair can be used to discriminate marine and terrestrial food sources, and the isotope data of this study seem reflective of local continental environments.

It was found that the isotopic compositions for some Chinese infants were noticeably different from the average values for adults in that region, indicating a different food intake than for adults. From all samples collected, sexually dependent differences in isotopic compositions in the same region were not found.

Due to the expansion of food trade in the modern world, for contemporary humans in developed countries or metropolitan areas, foodwebs are more complex than those of prehistoric humans who depended solely on local food. That is one reason why geographical influences on isotope composition may not be as large as expected.

Stable isotope analyses of hair can offer a potential quantitative method of diet reconstruction. The isotopic composition of human hair relates not only to choice of diet but also to the place of residence. Prior to this thesis, $\delta^{13}C$ and $\delta^{15}N$ data had been used in a number of foodweb constructions. The Stable Isotope Laboratory at Calgary was among the first to carry out studies with $\delta^{34}S$ values. Besides $\delta^{13}C$ and $\delta^{15}N$, the use of $\delta^{34}S$ has been further advanced. Further, for the first time (to our knowledge), δD and $\delta^{18}O$ data for hair have been shown to have potential for foodweb and climatic constructions. Since the isotopic compositions of diet reflect place of residence, multi-element isotopic data can assist in other applications such as forensic science (e.g., animal poaching investigation) and environmental studies.

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APPENDICES

A-1 Comparison of Hydrogen Isotope Data from Different Closed Tube Combustion Methods: Using Pyrex Tubes (550°C, 15 hours) and Quartz Tubes (850°C, 3 hours)

Sample #	δD (in ‰, V-SMOW)*	
	Pyrex	Quartz
#91	-118.6 (-122.4, -114.9)	-121.7 (-122.3, -121.0)
#102	-104.4	-105.6
#103	-85.5 (-83.7, -84.6, -88.1)	-88.5
#108	-80.9 (-83.9, -80.2, -78.8)	-78.8 (-81.0, -76.5)
#218	-63.7 (-63.6, -63.8)	-67.0
#304	-69.0 (-63.0, -69.4, -74.7)	-73.8

* Values underlined are averages of the values in brackets.

A-2 Comparison of Carbon Isotope Data from Different Closed Tube Combustion Methods: Using Pyrex Tubes (550°C, 15 hours) and Quartz Tubes (850°C, 3 hours)

Sample #	$\delta^{13}C$ (in ‰, V-PDB)*	
	Pyrex	Quartz
#91	<u>-21.5</u> (-21.6, -21.5, -21.6, -21.4, -21.5, -21.4, -21.5, -21.5)	<u>-21.3</u> (-21.4, -21.2, -21.3, -21.3, -21.2, -21.8, -21.3, -21.2)
#102	<u>-21.2</u> (-20.3, -20.1)	-20.9
#103	<u>-20.5</u> (-20.7, -20.5, -20.4)	<u>-20.2</u> (-20.3, -20.2)
#108	<u>-21.3</u> (-21.6, -21.6 -20.8)	-21.0
#218	<u>-18.4</u> (-18.44, -18.32)	<u>-18.4</u> (-18.40, -18.30)
#304	<u>-20.0</u> (-20.2, -20.1, -19.7)	-19.8

* Values underlined are averages of the values in brackets.

A-3 Comparison of Sulphur Isotope Data from Different Analytical Methods: Parr Bomb Combustion (Parr), Oxidation from Remains of Closed Tube Combustion (Closed Tube) and Kiba Method (Kiba)

Sample #	$\delta^{34}S$ (in ‰, V-CDT)*		
	Parr	Closed Tube	Kiba
#91	<u>3.1</u> (2.8, 3.0, 3.1, 3.6)	<u>3.4</u> (1.9, 3.0, 3.0, 5.5)	<u>2.7</u> (2.3, 2.8, 3.1)
#102	8.9	7.9	
#103	<u>9.9</u> (9.9, 10.0)	<u>10.2</u> (8.2, 8.8, 10.6, 13.0)	
#108	8.2	<u>9.8</u> (8.3, 10.1, 10.2, 10.6)	
#109	<u>10.1</u> (9.9, 10.3)	<u>11.7</u> (10.8, 11.6, 12.7)	8.4
#218	<u>8.3</u> (8.0, 8.6)	<u>9.8</u> (9.3, 9.8, 10.2)	7.5
#304	<u>6.2</u> (5.8, 6.7)	<u>6.9</u> (6.3, 7.6)	5.4

* Values underlined are averages of the values in brackets.

A-4a Carbon, Hydrogen and Oxygen Isotope Data for Hair Samples from Calgary, Alberta, Canada (number of subjects in brackets)

Sample Name	Sample #	Sex	Age	$\delta^{13}C$ (V-PDB)	δD (V-SMOW)	$\delta^{18}O$ (V-SMOW)
LJG-ycli	#89	M	33		-115.4	
LJG-jp	#90	F	38	-19.1	-121.3	
LJG-ljg	#91	M	30	-21.5	-118.6	+1.4
LJG-bw2	#92	F	57	-20.3	-125.8	+1.0
LJG-bw3	#93	M	46	-19.8	-132.7	+1.6
LJG-bw4	#94	F	58	-19.7	-137.0	+1.3
LJG-bw5	#95	M	60	-20.2	-112.6	+1.0
LJG-bw6	#96	F	10	-19.8	-135.0	-1.5
LJG-bw7	#97	F	16	-20.0	-116.7	-2.3
LJG-bw8	#98	M	33	-20.3	-115.0	+1.3
LJG-bw9	#99	M	25	-20.0	-109.3	-0.2
Average				-20.1(10)	-121.8(11)	+0.4 (9)
Standard Deviation				±0.6	±9.1	±1.3
Maximum Value				-19.1	-109.3	+1.6
Minimum Value				-21.5	-137.0	-2.3

A-4b Nitrogen and Sulphur Concentrations and Isotope Data for Hair Samples from Calgary, Alberta, Canada (number of subject in brackets; S% based on Parr bomb method)

Sample Name	Sample #	Sex	Age	$\delta^{15}N$ (Air)	N %	$\delta^{34}S$ (V-CDT)	S %
LJG-ycli	#89	M	33				
LJG-jp	#90	F	38				
LJG-ljg	#91	M	30	+9.5	14.1	+3.1	3.98
LJG-bw2	#92	F	57	+8.6	13.9	+2.6	4.19
LJG-bw3	#93	M	46	+9.5		+2.4	4.90
LJG-bw4	#94	F	58	+9.7		+4.5	4.63
LJG-bw5	#95	M	60	+9.5	14.2	+1.3	4.38
LJG-bw6	#96	F	10	+9.4	14.1	+2.5	3.83
LJG-bw7	#97	F	16	+9.3	14.5	+3.6	4.10
LJG-bw8	#98	M	33	+9.2	14.4	+4.1	4.74
LJG-bw9	#99	M	25	+9.9	14.5	+3.8	4.83
Average				+9.4 (9)		+3.1 (9)	
Standard Deviation				±0.3		±0.9	
Maximum Value				+9.9		+4.5	
Minimum Value				+8.6		+1.3	

A-4c Other Carbon and Sulphur Isotope Data for Hair Samples from Calgary, Alberta, Canada (Krouse et al., 1987 and unpublished data of the Stable Isotope Laboratory, the University of Calgary)

	$\delta^{13}C$ (V-PDB)		$\delta^{34}S$ (V-CDT)		
	-19.1	-19.7	+2.0	+3.5	+1.1
	-18.7	-20.2	+1.5	+2.4	+1.2
	-20.1	-20.3	+3.3	-0.5	+0.0
	-19.3	-18.6	+2.0	-2.0	+1.8
	-20.7	-19.1	+1.7	+0.7	+2.1
	-21.2	-18.9	+2.4	+2.8	+1.5
	-21.4	-19.3	+2.9	+2.0	+1.6
	-19.6	-19.1	+2.1	-0.6	+1.2
	-21.7	-20.0	+2.1	+0.1	-0.9
	-20.1	-20.7	+2.0	+0.6	+2.4
	-20.9		+1.6	+1.2	
Total number of subject (this table only)	21		32		
Average	-19.9		+1.4		
Standard Deviation	±0.9		±1.2		
Maximum Value	-18.6		+3.5		
Minimum Value	-21.7		-2.0		
Total number of subject (with A-4a, b)	31		41		
Average	-20.0		+1.8		
Standard Deviation	±0.8		±1.4		
Maximum Value	-18.6		+4.5		
Minimum Value	-21.7		-2.0		

A-5a Carbon, Hydrogen and Oxygen Isotope Data for Hair Samples from Harbin City, Far Northeast of P.R. China (number of subjects in brackets)

Sample Name	Sample #	Sex	Age	$\delta^{13}C$ (V-PDB)	δD (V-SMOW)	$\delta^{18}O$ (V-SMOW)
DMW-x1	#101	F	27	-20.6	-94.0	+2.6
DMW-x2	#102	F	40	-21.2	-104.4	+0.7
DMW-x3	#103	M	24	-20.5	-85.5	+2.4
DMW-x4	#104	F	58	-19.8	-80.3	+4.6
DMW-x5	#105	F	42	-20.6	-92.8	
HDQ-y1	#106	F	57	-21.0	-85.9	+2.5
HDQ-y2	#107	M	36	-20.7	-93.9	+3.4
HDQ-y3	#108	M	33	-21.3	-80.9	+3.2
HDQ-y4	#109	F	57	-21.1	-84.8	+5.3
ZQW-z1	#110	F	28	-21.3	-91.0	+3.6
ZQW-z2	#111	F	29	-20.8	-80.7	+5.2
Average				-20.8(11)	-88.6(11)	+3.4(10)
Standard Deviation				±0.4	±7.1	±1.3
Maximum Value				-19.8	-80.3	+5.3
Minimum Value				-21.3	-104.4	+0.7

A-5b Nitrogen and Sulphur Concentrations and Isotope Data for Hair Samples from Harbin City, Far Northeast of P.R. China (number of subjects in brackets; S% based on Parr bomb method)

Sample Name	Sample #	Sex	Age	$\delta^{15}N$ (Air)	N %	$\delta^{34}S$ (V-CDT)	S %
DMW-x1	#101	F	27	+9.6	13.2	+10.2	
DMW-x2	#102	F	40	+8.7	13.1	+8.9	3.65
DMW-x3	#103	M	24	+8.9	14.4	+9.9	
DMW-x4	#104	F	58	+9.3	14.1	+10.5	
DMW-x5	#105	F	42	+9.5	13.7	+9.6	4.04
HDQ-y1	#106	F	57	+8.8		+9.5	
HDQ-y2	#107	M	36	+9.0		+7.1	3.30
HDQ-y3	#108	M	33	+8.7	14.4	+8.2	4.23
HDQ-y4	#109	F	57	+8.3	13.8	+10.1	
ZQW-z1	#110	F	28	+8.9		+9.7	
ZQW-z2	#111	F	29	+8.4	14.1	+8.3	
Average				+8.9 (11)		+9.3 (11)	
Standard Deviation				±0.4		±1.0	
Maximum Value				+9.6		+10.5	
Minimum Value				+8.3		+7.1	

A-6a Carbon, Hydrogen and Oxygen Isotope Data for Hair Samples from Beijing, Northeast (Capital City) of P.R. China (number of subjects in brackets)

Sample Name	Sample #	Sex	Age	$\delta^{13}C$ (V-PDB)	δD (V-SMOW)	$\delta^{18}O$ (V-SMOW)
ZXG-zxgao	#201	F	58	-20.4	-81.7	+3.4
ZXG-xsguo	#202	M	61	-20.1	-79.5	+2.8
ZXG-li1	#203	M	6	-19.8	-85.0	+3.4
ZXG-tycui	#204	M	4	-19.5	-66.6	+3.7
ZXG-xu	#205	M	29	-20.3	-84.1	
ZXG-song	#206	M	23	-20.1	-72.7	
ZXG-diao	#207	M	18	-19.9	-69.1	
ZXG-lxguo	#208	F	32	-19.8	-80.7	+4.7
ZXG-shcui	#209	M	32	-19.1	-81.4	+4.0
ZXG-yang	#210	F	35	-20.6	-76.5	+4.7
ZXG-zhao	#211	F	29	-20.5	-72.2	+2.5
ZXG-xie	#212	M	50	-20.6	-85.4	
ZXG-zhang1	#213	M	58	-20.3	-71.4	
YJZ-x	#214	F	28	-21.4	-82.3	
YJZ-yqzhu	#215	F	21	-20.0	-77.7	
ZXG-zhang2	#216	F	45	-20.4	-84.1	
ZXG-wang	#217	M	12	-19.9	-78.3	
ZXG-liao	#218	F	2	-18.4	-63.7	+3.9
ZXG-li2	#219	F	26	-20.4	-74.3	
YZH-yzhe	#220	F	27	-20.1	-72.7	+3.1
LPS-wang	#221	M	23	-21.2	-78.2	
LPS-zhao	#222	M	21	-21.8	-66.5	

LPS-sun	#223	M	23	-21.0	-77.2	
LPS-li	#224	M	21	-21.7	-76.6	
ZXG-yu	#225	M	23	-20.9	-90.2	+4.9
ZXG-lian	#226	M	6	-19.5	-72.3	+4.5
ZXG-li3	#227	M	26	-20.6	-78.1	+3.5

Average	-20.4(25)	-77.9(25)	+3.8(13)
Standard Deviation	±0.6	±5.6	±0.7
Maximum Value	-19.1	-66.5	+4.9
Minimum Value	-21.8	-90.2	+2.5

A-6b Nitrogen and Sulphur Concentrations and Isotope Data for Hair Samples from Beijing, Northeast (Capital City) of P.R. China (number of subjects in brackets; S% based on Parr bomb method)

Sample Name	Sample #	Sex	Age	$\delta^{15}N$ (Air)	N %	$\delta^{34}S$ (V-CDT)	S %
ZXG-zxgao	#201	F	58	+7.8	14.4	+10.6	
ZXG-xsguo	#202	M	61	+7.2	14.3		
ZXG-li1	#203	M	6	+7.5	14.4	+8.7	4.65
ZXG-tycui	#204	M	4	+8.3	14.6	+7.8	4.81
ZXG-xu	#205	M	29				
ZXG-song	#206	M	23				
ZXG-diao	#207	M	18	+8.8	14.0	+9.1	
ZXG-lxguo	#208	F	32	+8.2	13.9	+8.6	4.00
ZXG-shcui	#209	M	32	+8.6	14.3	+9.6	4.25
ZXG-yang	#210	F	35	+8.1		+10.0	

ZXG-zhao	#211	F	29	+8.0	13.1	+10.3	
ZXG-xie	#212	M	50			+9.1	4.62
ZXG-zhang1	#213	M	58			+8.5	4.01
YJZ-x	#214	F	28				
YJZ-yqzhu	#215	F	21				
ZXG-zhang2	#216	F	45				
ZXG-wang	#217	M	12				
ZXG-liao	#218	F	2	+9.0	14.1	+8.3	
ZXG-li2	#219	F	26				
YZH-yzhe	#220	F	27	+8.3		+10.4	
LPS-wang	#221	M	23				
LPS-zhao	#222	M	21				
LPS-sun	#223	M	23			+10.4	
LPS-li	#224	M	21			+6.9	
ZXG-yu	#225	M	23	+7.1		+8.6	
ZXG-lian	#226	M	6	+9.9		+8.7	
ZXG-li3	#227	M	26	+8.0		+11.0	

Average	+8.2 (14)	+9.2 (17)
Standard Deviation	±0.7	±1.1
Maximum Value	+9.9	+11.0
Minimum Value	+7.1	+6.9

A-7a Carbon, Hydrogen and Oxygen Isotope Data for Hair Samples from Chengdu City, Southwest of P.R. China (number of subjects in brackets)

Sample Name	Sample #	Sex	Age	$\delta^{13}C$ (V-PDB)	δD (V-SMOW)	$\delta^{18}O$ (V-SMOW)
DFH-qh	#301	F	28	-21.6	-91.9	+1.3
DFH-yw	#302	F	16	-21.4	-87.6	+3.0
DFH-zph	#303	M	57	-21.3	-83.3	+4.0
DFH-slz	#304	M	3	-20.0	-69.0	+1.8
DFH-zyn	#305	M	32	-21.7	-74.4	+2.7
DFH-hchi	#306	M	16	-21.5	-77.5	+2.6
DFH-xmh	#307	F	5	-21.5	-74.7	+6.1
DFH-wliu	#308	M	9	-21.3	-78.2	
DFH-llei	#309	F	6	-20.7	-79.8	
DFH-kw	#310	M	17	-21.4	-83.6	+1.8
DML-x	#311	F	24	-21.9	-75.7	+3.8
Average				-21.4(10)	-80.7(10)	+3.0(9)
Standard Deviation				±0.3	±5.5	±1.4
Maximum Value				-20.7	-74.4	+6.1
Minimum Value				-21.9	-91.9	+1.3

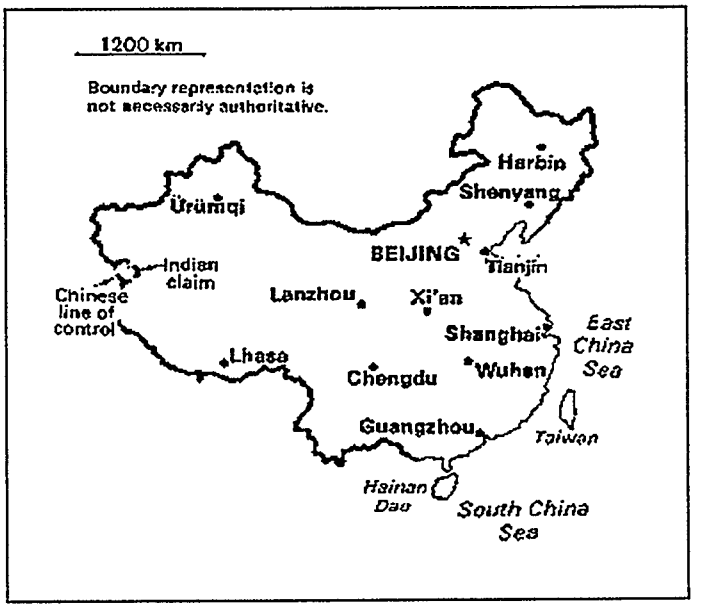
A-7b Nitrogen and Sulphur Concentrations and Isotope Data for Hair Samples from Chengdu City, Southwest of P.R. China (number of subjects in brackets; S% based on Parr bomb method)

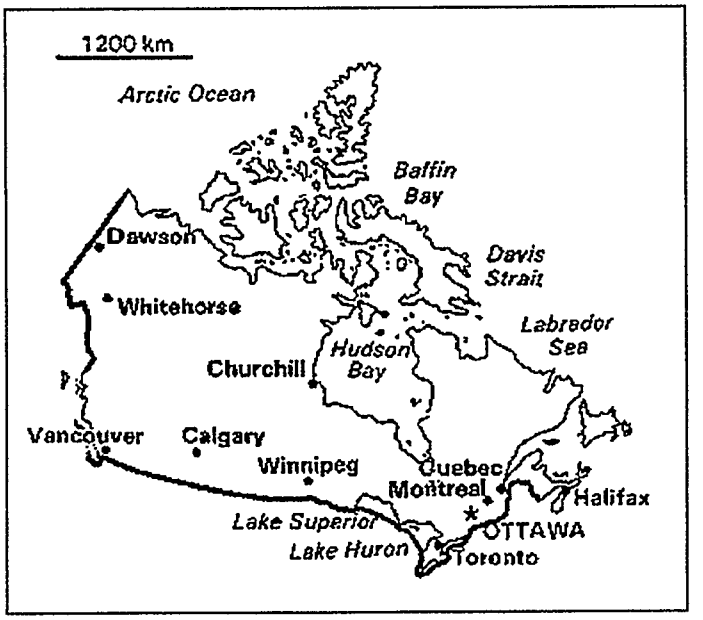
Sample Name	Sample #	Sex	Age	$\delta^{15}N$ (Air)	N %	$\delta^{34}S$ (V-CDT)	S %
DFH-qh	#301	F	28	+8.5	13.2	+5.5	
DFH-yw	#302	F	16	+8.2		+5.5	
DFH-zph	#303	M	57	+7.9	13.9	+4.3	3.98
DFH-slz	#304	M	3	+9.4	14.4	+6.2	
DFH-zyn	#305	M	32	+8.4	14.4	+3.7	4.48
DFH-hchi	#306	M	16	+9.0	14.4	+3.6	3.15
DFH-xmh	#307	F	5	+8.6		+4.1	
DFH-wliu	#308	M	9	+7.8	14.7	+2.6	3.87
DFH-llei	#309	F	6	+8.6	14.2	+4.8	
DFH-kw	#310	M	17	+8.7		+2.8	4.01
DML-x	#311	F	24	+8.7	14.1	+7.9	
Average				+8.4 (10)		+4.6 (11)	
Standard Deviation				±0.4		±1.5	
Maximum Value				+9.0		+7.9	
Minimum Value				+7.8		+2.6	

A-8 Weighted Mean δD and $\delta^{18}O$ Values of Precipitation in Some Regions of China†

Region	Latitude, Longitude, Altitude	Distance from the Coast	Annual Precipitation (year)	δD (V-SMOW)	$\delta^{18}O$ (V-SMOW)
Qiqihar	47N, 124E, 147 m	900 km	638 mm (88, 91)	-81.3	-10.9
Baotou	41N, 110E, 1067 m	700 km	387 mm (90)	-49.4	-7.1
Tianjin	39N, 117E, 3 m	50 km	556 mm (90, 91)	-47.2	-6.7
Shijiazhuang	38N, 114E, 80 m	300 km	472 mm	-53.3	-7.8
Xian	34N, 109E, 397 m	1000 km	530 mm	-43.0	-6.7
Nanjing	32N, 118E, 26 m	200 km	1065 mm (90)	-48.9	-7.8
Changsha	28N, 113E, 37 m	700 km	1294 mm (90)	-40.6	-6.6
Guiyang	26N, 106E, 1071 m	600 km	991 mm (89-92)	-51.1	-8.2
Fuzhou	26N, 119E, 16 m	20 km	1847 mm (90)	-52.7	-7.5
Kunming	23N, 103E, 1841 m	700 km	1115 mm	-71.0	-10.6
Guangzhou	23N, 113E, 7 m	30 km	1748 mm	-34.2	-5.2
Haikou	20N, 110E, 15 m	0 km	2033 mm (89)	-32.8	-5.3

† References: Rozanski et al., 1993; National Economic Atlas of China, 1994; Global Network of Isotopes in Precipitation (by IAEA: International Atomic Energy Agency, and WMO: World Meteorological Organization), at URL: http://www.iaea.or.at/programes/ri/gnip/gnipmain.htm.





A-9 A Map of China and Canada

(from CIA World Factbook 1995)