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Variation in $\delta^{13}C$ Values of Post-medieval Europeans

by

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ABSTRACT

This study explores variation in $\delta^{13}C$ values of human bone collagen, using samples selected from seven post-medieval European skeletal collections (N = 138). While European remains have been the subject of previous isotopic investigations, this study is the largest to date, and extends the existing data base significantly beyond the primarily Scandinavian focus it has had to this point in time.

Techniques of collagen extraction and carbon isotope analysis used in this study follow those of DeNiro and Epstein (1981) and Schoeninger and DeNiro (1985), with minor modifications. Estimates of preservation quality are based on collagen yield and elemental analyses (carbon and nitrogen content and atomic C/N ratios).

Mean $\delta^{13}C$ values for the study samples range from -19.7‰ to -17.2‰. Sample variation is low (1σ ranges from 0.2‰ to 0.4‰), reflecting the degree of control over sample composition. Results confirm, however, that $\delta^{13}C$ variation is a complex phenomenon, combining the effects of analytical procedures, preservation quality, and sample attributes (spatial and temporal representation, demographic and social profiles, and element choice).

Archaeological inquiries are supplemented by laboratory

investigations of the effects of NaOH treatment on collagen extract quality, and the impact of nutrition on carbon isotope values of rats. Results show alkali treatment reduces yield, but produces no significant alterations of collagen amino acid profiles or $\delta^{13}C$ values. Data collected from a one-year animal feeding experiment demonstrate nutritional status has a pronounced impact on the incorporation of dietary carbon in bone collagen, and must be given serious consideration in future isotope studies.

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CHAPTER 1

INTRODUCTION

In the latter part of the 1970s, stable isotope analysis was added to the arsenal of techniques employed in paleodiet reconstruction (van der Merwe and Vogel 1978; Vogel and van der Merwe 1977). Subsequent isotopic investigations have provided critical information on the adoption of maize agriculture in the Americas, and the relative importance of marine and terrestrial resources in diets around the globe (see reviews by van der Merwe 1982 and DeNiro 1987). Assessments of more diverse dietary patterns have also added to our knowledge of the past (e.g., Ambrose 1986; Ambrose and DeNiro 1986a; Sealy and van der Merwe 1985, 1986).

Despite the increasing use of stable isotope analysis, the variables affecting isotope abundances in archaeological samples remain poorly understood. Recognizing this deficiency, recent attention has been focused on diagenetic effects on stable isotope values (DeNiro 1985; DeNiro and Hastorf 1985; DeNiro and Weiner 1988; DeNiro et al. 1985; Masters 1987; Nelson et al. 1986; Stafford et al. 1988; Tuross et al. 1988). Less concerted attention has been directed toward "anthropological effects" on stable isotope values. These include: a) physiological factors, such as

sex, age, metabolism, nutrition, and pathology (Bumsted 1984; DeNiro and Schoeninger 1983; Krueger and Sullivan 1984; Lovell et al. 1986a); and b) cultural factors related to subsistence strategies, such as the extent of dietary diversity, seasonal or annual variations in food sources, food preparation techniques, and differential access to food resources (Bumsted 1984; Johansen et al. 1986; Sealy and van der Merwe 1985, 1986). Both diagenetic and anthropological effects require further investigation if applications of stable isotope analysis are to rest on firm ground.

The present study is intended as a contribution to the investigation of isotopic variability, exploring carbon isotope ratios of human bone collagen in a series of samples from post-medieval Europe. While European populations have been the subject of previous isotopic analyses (Johansen et al. 1986; Schoeninger n.d.; Tauber 1981, 1983), this study is the largest to date, incorporating 138 adult individuals from seven sites. Variation in carbon isotope values is assessed in terms of quality of preservation, sample composition, and available dietary information.

HISTORICAL CONCERNS IN ISOTOPIC ANALYSIS

The brief history of using stable isotopes in diet reconstruction records a natural progression from establishing the broad parameters of investigation to

refining specific techniques and applications. The changing nature of stable isotope investigations is illustrated in the following review, which summarizes literature from three periods: the late 1970s, early 1980s, and late 1980s. This chronological division is arbitrary, and imposed only as an aid to delineate shifts in emphasis in isotope research.

Pre-1980 Research

The earliest applications of stable isotope analysis in archaeology were not directed toward diet reconstruction, but rather toward the calibration of radiocarbon dates. In the 1960s and early 1970s, consistently poor radiocarbon dates obtained on maize, versus most other North American plants, were correlated with differences in the utilization of carbon isotopes during photosynthesis (see Chapter 2). Stable isotope assessments were added to radiocarbon procedures as a measure of photosynthetic bias, and as a means of calibrating the carbon isotopic composition of samples to "modern standard carbon," the reference material employed in ^{14}C determinations.

As differences in the stable isotopic composition of dated materials, particularly skeletal remains, became evident, the potential utility of such data for diet reconstruction was recognized (see review by van der Merwe 1982). In 1977-78, Vogel and van der Merwe published two reports in which the carbon isotopic signature of bone

collagen was used to chart the adoption of maize agriculture in New York, Illinois, Ohio, and West Virginia (van der Merwe and Vogel 1978; Vogel and van der Merwe 1977). In an ancillary application, Burleigh and Brothwell (1978) used carbon isotope values of mummified dog hair to identify maize consumption in Peru. Contemporaneous work by DeNiro and Epstein (1978) detailed the relationship between the carbon isotope values of diet and body tissues using laboratory animal data.

1980-1984 Research

In the first half of the 1980s, further studies established the utility of carbon isotopes in assessing the appearance of maize agriculture (Bender et al. 1981; Boutton et al. 1984; van der Merwe et al. 1981). Other research focused on a new application for carbon isotope data -- evaluating the relative importance of marine and terrestrial food sources in past diets. First demonstrated using skeletal material from northern Europe (Tauber 1981), this application soon was extended to material from North America (Chisholm and Nelson 1983; Chisholm et al. 1982, 1983a, 1983b) and Australia (Hobson and Collier 1984).

Also in the early 1980s, DeNiro and Epstein (1981) proposed the use of *nitrogen* isotopes to establish the relative importance of legumes or marine resources in past diets. The legume application was illustrated in an

analysis of archaeological material from Tehuacan (DeNiro and Epstein 1981; these data were later re-evaluated by Farnsworth et al. 1985). Schoeninger et al. (1983) illustrated the marine application, using a variety of samples from the Americas and Europe. Further data relating to the isotopic dichotomy of marine and terrestrial resources were supplied in an extensive study of animal bones by Schoeninger and DeNiro (1984).

While the principal concern of most stable isotope investigations of this time period was the demonstration of *between-sample* dietary differences (maize/no maize, less maize/more maize, less marine/more marine, etc.), the importance of *within-sample* variation was not ignored. DeNiro and Schoeninger (1983) explored age, sex, and element effects and the general nature of isotopic variability in animals fed controlled diets. Bumsted (1984) considered similar issues in her study of human remains from an archaeologically-recorded massacre site. Her work remains the only investigation of isotopic variation in a human *population* (using the term "population" in its biological sense).

Methodological issues were given increasing attention during this period. Sullivan and Krueger (1981) and Schoeninger and DeNiro (1982) debated the validity of isotopic assessments of bone mineral. Chisholm et al. (1983c) explored the relative merits of various collagen

extraction techniques. Krueger and Sullivan (1984) proposed models to explain diet-collagen spacing in herbivores, carnivores, and omnivores. Theirs was the first detailed consideration of macronutrient contributions to collagen carbon content.

1985-present Research

Archaeological applications of stable isotope analysis have become increasingly common in the latter part of the 1980s. A number of studies have appeared which use carbon isotopes, or carbon and nitrogen isotopes, to assess the relative importance of maize as a dietary staple throughout the Americas (e.g., Buikstra et al. 1988; Katzenberg and Schwarcz 1986; Schwarcz et al. 1985; White 1988; Yesner 1988), and an even larger number to assess the relative dietary importance of marine resources in diets of Europe, Australia, and the Americas (e.g., Collier and Hobson 1987; Johansen et al. 1986; Keegan and DeNiro 1988; Lazenby and McCormack 1985; Lovell et al. 1986b; Walker and DeNiro 1986; Yesner 1988). At the same time, several innovative applications have been investigated. Sealy and van der Merwe (1985,1986) have used carbon isotope data to assess seasonal resource exploitation in South Africa (see also, Parkington 1987 and Sealy and van der Merwe 1987). Ambrose and DeNiro (Ambrose 1986; Ambrose and DeNiro 1986a) have used both carbon and nitrogen to distinguish a number of

related subsistence strategies employed in eastern and southern Africa, establishing the power of nitrogen isotopes to quantify reliance on animal products (see also, Schoeninger 1985). Further afield, Hastorf and DeNiro (1985) have extended the use of stable isotope techniques to the analysis of pot residues, and applications of strontium (Aufderheide et al. 1988; Yesner 1988) and sulphur isotopes (Krouse and Herbert 1988) have been investigated.

In recent years, greater attention has been devoted to the exploration of within-sample variability and to the factors affecting isotopic values. Age and sex effects on carbon isotope values of wild animal populations and their implications for paleodiet studies have been discussed by Hobson and Schwarcz (1986); age and sex effects in humans have been explored by Lovell et al. (1986a). Ambrose (1986), Ambrose and DeNiro (1986b, 1987), Heaton et al. (1986), and Sealy et al. (1987) have debated physiological and environmental influences on nitrogen values. Diagenetic effects on both carbon and nitrogen isotope ratios have become a special area of concern, as indicated by the sudden appearance of a number of papers devoted to this subject (DeNiro 1985; DeNiro and Hastorf 1985; DeNiro and Weiner 1988; DeNiro et al. 1985; Masters 1987; Nelson et al. 1986; Stafford et al. 1988; Tuross et al. 1988).

It is clear that, having established the general

utility of stable isotope analysis in diet reconstruction, the emphasis of current research is on refining our understanding of the factors which contribute to isotopic variation. Since variation is a statistical phenomenon, studies of larger samples representative of controlled space and time frameworks are required. The present study was designed with this need in mind.

THIS STUDY

Method

Samples were selected from seven post-medieval European skeletal collections. All collections were associated with sites of established provenience: the Tudor warship *Mary Rose* which sank in Portsmouth Harbour, England (1545); the cemeteries of Saint-Martin, Saint-Pierre-le-Puellier, and Saint-Pierre-du-Chardonnet in Tours, France (selected burials date from the fifteenth to the seventeenth centuries); the Spitalkirche site in Tübingen, Germany (early sixteenth century); a Danish-Norwegian whaling station at Jensenvannet, Svalbard (seventeenth century); and a Basque whaling station at Red Bay, Labrador (sixteenth to early seventeenth century).

Techniques employed in collagen extraction and carbon isotope analysis follow those outlined by DeNiro and Epstein (1981) and Schoeninger and DeNiro (1984), with minor

modifications. Estimates of preservation quality are based on collagen yield and elemental analyses.

Variation in carbon isotope values within and between samples is reviewed in light of preservation quality and relevant information on diet, geographic location (site location, as well as normal place of residence if different from site), demographic profiles (age and sex), and social attributes (status and/or occupation).

Archaeological inquiries are supplemented by laboratory investigations on the effects of NaOH treatment on collagen extract quality, and the effects of nutrition on carbon isotope values (animal feeding experiment).

Results

The results of this analysis confirm variation in carbon isotope values is a complex phenomenon, combining the effects of analytical procedures, preservation quality, and the original nature of the sample. Analytical effects are minimal, whether or not NaOH treatment is employed. Diagenetic effects, on the other hand, have a significant impact on the range of variability expressed. (These can be minimized if careful consideration is given to the interrelation of measures of preservation quality and carbon isotope values.) Residual variation reflects sample attributes which have dietary correlations. These attributes include spatial and temporal representation,

demographic and social profiles, and element choice (physiological effects related to bone remodelling and dietary change).

The variation displayed in the study samples is less than that found in many other isotopic investigations, likely reflecting the degree of control exercised over sample composition. Differences of less than 5% in marine carbon contribution to bone collagen are statistically significant.

Laboratory investigations demonstrate nutritional status has a pronounced impact on the incorporation of dietary carbon in bone collagen, and must be given serious consideration in future studies.

Format

The presentation of information in this study begins with a review of carbon isotopes and food webs in Chapter 2. Much of this discussion is devoted to the relationship between the isotopic signature of bone collagen and diet, but a portion focuses on the impact of nutrition on isotope values, using data collected from a one-year animal feeding experiment. Chapter 3 explores the effects of diagenesis on stable isotope values, and possible methods of eliminating contaminants and detecting diagenetic change. Chapter 4 outlines the specific analytical techniques employed in this study, and includes a description of an experiment conducted

to test the effects of NaOH treatment on collagen composition. Chapter 5 describes the archaeological samples which constitute the primary focus of this investigation. Archaeological and historical data which pertain to the nature of the sites and the skeletal remains sampled, as well as the principal dietary components, are summarized. Specific reference is made to features which may influence patterns of isotopic variability expressed in the samples, both dietary and otherwise. Chapter 6 reports the results of analysis of the archaeological material, and Chapter 7 presents the conclusions and their implications for future research.

CHAPTER 2

CARBON ISOTOPES AND DIET

The application of stable carbon isotope analysis to diet reconstruction is based on several observations: a) the carbon isotopic composition of living organisms varies; b) this variability reflects differences in the carbon sources utilized; and c) the primary carbon source for animal tissue formation is food (van der Merwe 1982). While this foundation is frequently reduced to the adage, "you are what you eat," this reduction belies the underlying complexity of chemical relationships.

This chapter focuses on the dietary relevance of bone collagen "carbon signatures." The discussion is prefaced by a summary of general principles of isotopic analysis and an introduction to carbon isotope variability in terrestrial and marine food webs.

ISOTOPES, ISOTOPE RATIOS, AND FRACTIONATION

The term "isotope" was coined by Sir Frederick Soddy from the Greek terms *iso* (same or equal) and *typos* (place), in reference to the fact that isotopes occupy the same place in the periodic table (Hoefs 1987). Specifically, isotopes are forms of a single element with different mass numbers.

Most elements are represented by two or more isotopes, with one form generally predominating and others present in only trace amounts. Isotopes are referred to as stable if they are not subject to radioactive decay (according to existing methods of decay detection).

Isotopes of an element commonly exhibit differences in physical and chemical properties, which can be attributed to their mass differences. These *isotope effects* almost invariably result in *fractionation*, a change in isotope abundance ratios in specific parts of a system. Fractionation in biological systems is mainly the result of *kinetic isotope effects*, which occur "when the rate of a chemical reaction is sensitive to atomic mass at a particular position in one of the reacting species" (Hoefs 1987:11). Reaction products generally are enriched in the lighter isotope, but isotopic mass balance is always maintained, so that enrichment in one part of the system results in depletion in another.

Isotope abundances are determined by mass spectrometry. "A mass spectrometer separates charged atoms and molecules on the basis of ... their motions in magnetic and/or electrical fields" (Hoefs 1987:19). The accepted unit of isotopic measurement is the "del-value" (δ), which expresses isotope abundances in parts per thousand (per mil or ‰) relative to a standard. This value is defined as

$$\frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

or

$$\left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000$$

where R is the abundance ratio of the heavy to the light isotope.

Fractionation effects are expressed using the capital delta notation, Δ:

$$\Delta_{AB} = \delta_A - \delta_B$$

CARBON ISOTOPES AND FOOD WEBS

The earth's carbon is distributed in a variety of reservoirs: carbon dioxide in air and dissolved in water, carbonates in the earth's crust, carbonate ions in sea water, and terrestrial and marine organic compounds. This carbon exists in three isotopic forms, ^{12}C, ^{13}C, and ^{14}C. The first two of these are stable, while the third is radioactive.

Stable carbon isotope abundances are expressed as $\delta^{13}C$ values, measured relative to the international standard for carbon, Chicago PDB (Peedee Belemnite), a Cretaceous mollusc (Craig 1953, 1957). (The original PDB sample has long been depleted, so current measurements are related to PDB through a series of secondary standards [e.g, NBS-20: Solenhofen

limestone; or NBS-21: spectrographic carbon].) Since PDB, a marine carbonate, has a higher $^{13}C/^{12}C$ ratio than most terrestrial materials, $\delta^{13}C$ values are generally negative. A $\delta^{13}C$ value of -26‰ means the sample contains 26 parts per thousand (2.6%) less ^{13}C than PDB.

Terrestrial Food Webs

Plants. The primary carbon source for terrestrial plants is atmospheric CO_2 which has a $\delta^{13}C$ value of approximately -7‰ (Craig 1953; Keeling 1961). (The two naturally-occurring stable isotopes of carbon, ^{12}C and ^{13}C, comprise approximately 98.9% and 1.1%, respectively, of atmospheric carbon; radioactive ^{14}C comprises some 10^{-10} % [Freyer 1979]). Three different photosynthetic pathways have been defined on the basis of reactions employed in the utilization of CO_2: the C_3 (Calvin or Calvin-Benson), C_4 (Hatch-Slack), and CAM (Crassulacean acid metabolism) pathways (reviewed by O'Leary 1981).

Plants which use the C_3 photosynthetic pathway, convert CO_2 to 3-phosphoglycerate (a phosphoglycerate compound with three carbon atoms) via ribulose biphosphate carboxylase in the first photosynthetic step (Calvin and Benson 1948; Calvin and Bassham 1962). Within this category are included "all trees, most shrubs, and those grasses adapted to temperate or shaded forest conditions" (van der Merwe and Vogel 1983:36). Nuts, most fruits, and root crops are all

C_3 plants (Ambrose 1987).

Plants which use the C_4 photosynthetic pathway convert CO_2 to dicarboxylic acid, a four-carbon compound via phosphoenolpyruvate carboxylase during the first photosynthetic step (Hatch and Slack 1966; Hatch et al. 1967; Kortschak et al. 1965). The C_4 pathway is common in plants from hot and arid environments, and temperate-zone salt marshes (van der Merwe 1982). Maize, sugarcane, sorghum, and some millets, amaranths and chenopods are C_4 plants.

The CAM photosynthetic pathway is employed by a limited number of plants, most of which are succulents and cacti, (Kluge and Ting 1978). These plants are capable of reducing carbon via either the C_3 or C_4 pathway, depending on growth conditions.

During photosynthetic assimilation of CO_2, all plants discriminate against the heavier isotope of carbon, resulting in a lower ^{13}C content in the plants than in the atmosphere (Bender 1971). This discrimination is more pronounced in C_3 plants, however, which consequently have more negative $\delta^{13}C$ values. C_3 plant values range from -20‰ to -35‰, with a mean of -26.5‰ (Sealy and van der Merwe 1987), while C_4 plant values range from -9 to -16‰, with a mean of -12.5‰ (Vogel et al. 1978). Values for CAM plants overlap these two distributions, ranging from -12 to -27‰ (Kluge and Ting 1978), with a mean of around -16.5‰ (van der

Merwe and Vogel 1983). (Prior to the Industrial Revolution, average values for atmospheric CO_2, and hence plants, may have been slightly more positive, though probably not by more than 1‰ [Pang et al., cited in DeNiro 1987].)

Animals. Studies of laboratory and wild animal populations have shown that the "isotopic signatures" of plants are passed along food webs to the animals consuming them, and from these animals to higher trophic levels (Bender et al. 1981; DeNiro and Epstein 1978; Minson et al. 1975; Teeri and Schoeller 1979; Tiezsen et al. 1983; Vogel 1978). Associated with this process, however, are measurable changes in isotopic abundance (DeNiro and Epstein 1978; Vogel 1978), referred to as "diet-consumer spacing." This spacing may reflect isotope effects inherent in the conversion of dietary carbon to tissue carbon (e.g., kinetic isotope effects related to enzymatic reactions), or may reflect the partitioning of dietary carbon for different tissue purposes (a process Bumsted [1985] refers to as "isotopic bias").

Diet-consumer spacing varies for different animals, diets, and tissues, and is generally not a well-understood phenomenon. Experiments reported by DeNiro and Epstein (1978) indicate animals (whole body) are enriched by about 1‰ relative to their diet, while individual tissues show a range of variation in isotopic values (e.g., $\delta^{13}C$ lipid <

flesh < collagen) (DeNiro 1977; DeNiro and Epstein 1978; Tieszen et al. 1983; Vogel 1978). This study is primarily concerned with diet-*collagen* spacing, a subject which is considered in greater detail in the second half of this chapter.

Aquatic Food Webs

Marine and freshwater carbon systems are less well understood than terrestrial systems. Aquatic carbon sources include carbonates, bicarbonates, and dissolved CO_2 (some aquatic plants depend on atmospheric CO_2). Fractionation during the absorption of CO_2, and in the subsequent formation of bicarbonate, results in ^{13}C enrichment relative to the atmosphere of approximately 7‰, yielding bicarbonate with $\delta^{13}C$ values close to zero (Emrich et al. 1970). (Enrichment effects can vary slightly, depending on water temperature and the degree of oceanic upwelling [Fontugne and Duplessy 1981; Parker 1964; Sackett et al. 1965].) As bicarbonate is assimilated by marine plants during photosynthesis, kinetic effects again lead to depletion of the heavy isotope, ^{13}C. Phytoplankton fractionate carbon (on average) to approximately the same extent as terrestrial C_3 plants, maintaining the 7‰ difference between atmospheric and oceanic carbon (Chisholm et al. 1982, 1983b). (There are no marine equivalents of terrestrial photosynthetic pathways.) As in terrestrial

systems, plant values are passed on to higher trophic levels (Craig 1953; Degens 1969; Sackett et al. 1965; Smith and Epstein 1970).

The $\delta^{13}C$ values of fresh water show less (if any) enrichment over atmospheric CO_2 values, varying from -4‰ in hard water to -8‰ in soft (van der Merwe 1982). Thus fresh water plants resemble terrestrial C_3 species in their carbon values, though the resemblance is closer in soft water environments (van der Merwe 1982). Fish in freshwater systems also display isotopic signatures close to those of C_3 plants (Chisholm et al. 1982; Hobson and Collier 1984; van der Merwe and Vogel 1978, 1983).

CARBON ISOTOPES AND HUMAN DIET

Vogel and van der Merwe (1977) were the first to apply stable carbon isotope analysis to the reconstruction of past human diets, with subsequent developments in the field reviewed by van der Merwe (1982) and DeNiro (1987). The principles employed in human studies are the same as for all isotopic assessments of food webs. Attention is focused, however, on the relationship between diet and bone, the most commonly preserved tissue in archeological situations.

Bone Chemistry

Bone has both organic and inorganic components. Stable

carbon isotope assessments of the inorganic fraction have generally been avoided in archaeological reconstructions of diet (cf. Krueger and Sullivan 1984 and Sullivan and Krueger 1981) due to the long-established propensity for carbon from bone mineral to exchange with carbon in ground water and the atmosphere (Berger et al. 1964; Schoeninger and DeNiro 1982). The majority of studies have focused, instead, on the organic component of bone, and specifically the protein collagen. Carbon in bone collagen has been generally assumed to be less susceptible to diagenetic alteration (Armstrong et al. 1983; Berger et al. 1964; DeNiro and Epstein 1978; Hassan and Ortner 1977; Nelson et al. 1986; Olsson et al. 1974; Wyckoff 1972). (Further discussion of collagen preservation is reserved for Chapter 4.)

Estimates of the proportion of organic material in modern (fresh) bone range from 20-25% (Hare 1980) to 35% (McLean and Urist 1968). Collagen comprises about 90% of this organic matrix (Hare 1980; McLean and Urist 1968). The remainder includes various other proteins (glycoproteins, plasma proteins), peptides, lipids, and proteoglycans (Herring 1972).

Bone collagen is composed of large rod-like molecules, consisting of three polypeptide chains (α-chains) linked in a coiled formation (triple helix); these molecules are, in turn, arranged in higher order assemblages of microfibrils and fibrils (Vaughan 1981). The characteristic amino acid

profile of modern mammalian collagen (Table 1) shows a high glycine and relatively high proline content, and the presence of hydroxyproline and hydroxylysine (Taylor 1980).

Diet and Bone

The dietary implications of bone collagen isotope values can be established only if the relationship between diet and collagen is known (Sealy and van der Merwe 1986). In this regard, the following lines of inquiry are of specific relevance: a) differential contributions of macronutrients to collagen synthesis; b) diet-collagen spacing (Δ_{dc}); and c) collagen turnover times.

Macronutrients and Collagen. Food sources contain varying amounts of the primary macronutrient groups, with proteins, carbohydrates, and lipids of concern here. Each of these groups is utilized in different ways by the body, and each differs in its isotopic composition (lipids are isotopically lighter than proteins and carbohydrates [DeNiro 1977; Vogel 1978]). The precise relationship of the various macronutrient groups to collagen formation has not been clearly established in isotope studies. Chisholm (1986) and Krueger and Sullivan (1984) suggest collagen largely reflects protein sources. Schoeninger (n.d.) and Sealy and van der Merwe (1986), on the other hand, clearly state that collagen reflects both the carbohydrate and protein portions

Table 1: Amino Acid Analysis of Modern Mammalian Collagen

Amino Acid	Residues per 1000	
	Modern Human[a]	Modern Bovine[b]
Aspartic acid	44	50
Hydroxyproline	89	100
Threonine	17	19
Serine	36	35
Glutamic acid	74	78
Proline	130	109
Glycine	333	330
Alanine	112	112
Valine	25	21
Methionine	5	4
Isoleucine	9	11
Leucine	23	28
Tyrosine	3	4
Phenylalanine	12	14
Hydroxylysine	5	4
Lysine	27	25
Histidine	5	4
Arginine	50	53
Total	999[c]	1001

[a] Type I collagen data from Bornstein and Piez (1964)

[b] Bone collagen data from Hare (1980)

[c] Deviation from 1000 due to rounding

of the diet.

Collagen synthesis, like that of all proteins, requires the simultaneous availability of the constituent amino acids. Since the body does not store excess amino acids, these must be available from dietary sources on a regular basis, either directly (as preformed amino acids) or indirectly (by providing the compounds the body requires for amino acid synthesis) (Stryder 1988; Zubay 1988). Humans can form 12 of the 20 amino acids they normally require; the remaining eight, referred to as "essential amino acids," must be ingested preformed. These essential amino acids are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. (Histidine is also essential for infants.)

Approximately 12% of the amino acid residues in collagen consists of essential amino acids (calculation based on data presented in Table 1), and hence can be directly attributed to dietary protein (the source of preformed amino acids) (cf. Sealy and van der Merwe 1986 who say 20% essential). The remaining 88% of the amino acid residues are non-essential forms which may derive directly from dietary protein sources or from one of the body's paths of amino acid synthesis. These pathways are diverse (see Table 2), but share an important common feature: carbon skeletons for amino acid synthesis are supplied by glycolysis, the pentose phosphate pathway, or the citric

Table 2: Synthesis of Nonessential Amino Acids of Bone Collagen

Amino Acid	Mode of Synthesis
GLUTAMIC ACID	Transamination of α-ketoglutarate (Citric acid cycle derivative)
Arginine	From glutamic acid
Proline	From glutamic acid
ASPARTIC ACID	Transamination of oxaloacetate (Citric acid cycle derivative)
ALANINE	Transamination of pyruvate (Citric acid cycle derivative)
SERINE	3-phosphoglycerate (Glycolysis intermediate)
Glycine	From serine
TYROSINE	Hydroxylation of phenylalanine

Post-synthetic Modification of Amino Acids

Hydroxylysine	Lysine
Hydroxyproline	Proline

acid cycle (Stryder 1988).

While the pathways for amino acid synthesis are clearly understood, the frequency with which they are utilized has yet to be demonstrated. Smith et al. (1983:602) note that, while the human body is capable of forming nonessential acids, "At the same time, these amino acids are abundant in proteins and their absence in a diet of natural foodstuffs is impossible." Further, they note "In normal circumstances, animals ingesting proteins as sources of the essential amino acids have little demand for directed synthesis of the nonessential ones" (1983:604). These statements should not be taken to suggest nonessential amino acid synthesis is a rare process (cf. Chisholm 1986). In fact, amino acid synthesis and breakdown are integral parts of various metabolic pathways in the body, particularly those pertaining to the flow of nitrogen to body cells (Macko et al. 1982).

The isotopic effects of nitrogen channeling are demonstrated by Gaebler et al. (1966). The authors measured $\delta^{15}N$ values of liver and muscle tissue in two groups of rats fed diets in which the protein components were casein and soybean, respectively (the diets were identical in other respects, consisting of dextrin, sucrose, salt mixture, cellulose, vitamin mixture, and corn oil). Nitrogen isotope values for *nonessential* amino acids, as well as valine and leucine, were found to be significantly heavier in tissue

samples than in the corresponding amino acids of the dietary protein. These amino acids are extensively involved in nitrogen transfer. The clear implication of these data is that, despite the fact the rats were fed a diet which supplied all the amino acids required for tissue synthesis, the nonessential amino acids (as well as some of the essential acids) were not incorporated directly into body tissues, but were involved in a series of chemical reactions.

The precise patterns of enrichment differed for the two groups of rats, with less enrichment seen in those fed the casein protein than those fed soy protein. This suggests animal protein is utilized more directly in tissue formation than plant protein. Differential metabolism of animal and plant protein has been demonstrated in other studies which compare the immediate urinary excretion of protein waste after meals incorporating animal versus plant protein (Phansalkar and Patwardhan 1955, cited in Davidson et al. 1975). Up to this point in time, however, this differential has not been considered a significant concern in isotope studies (Schoeninger n.d.).

Further animal experiments are required to understand the specific contributions of macronutrient sources of carbon to bone collagen. Nevertheless, available evidence suggests both protein and carbohydrate sources figure prominently in collagen synthesis, with perhaps a tendency

for more direct incorporation of amino acids derived from animal protein.

Diet-collagen Spacing. Current estimates of diet-collagen spacing in humans, the difference between the isotopic value of the diet and that of collagen, are approximations based on assessments of skeletal samples. The most frequently employed estimate is +5.1‰, from a study by van der Merwe and Vogel (1978). Their study compares isotope values of North American hunter-gatherers ("pre-maize" groups) and horticulturalists. An average $\delta^{13}C$ value of -21.4 ± 0.78‰ for the "pre-maize" groups is compared to the average value of the food-web, calculated to be -26.5‰, to arrive at a diet-collagen spacing of +5.1‰.

Sealy and van der Merwe (1986) acknowledge one of the possible sources of error in this calculation:

> If, however, the people were consuming an appreciable proportion of animal food isotopically more positive than -26.5‰, the true diet-collagen spacing would probably be somewhat smaller than 5.1‰ (1986:141).

A second source of error may be the composition of the so-called "pre-maize" sample. This sample incorporates Middle and Late Woodland period sites which sometimes include maize remains in limited quantities. Van der Merwe and Vogel (1978) justify inclusion of these sites in the pre-maize category by arguing they display no obvious shift in average $\delta^{13}C$ values from earlier sites. However, values for these

samples (-21.2‰, -20.9‰, -20.9‰) are slightly more positive than those for Archaic samples (-21.7‰, -21.9‰, 21.7‰). If they are excluded from the pre-maize category, the mean is revised to -21.8‰ and the diet-collagen spacing to +4.7‰. Keeping Sealy and van der Merwe's comment in mind, this also represents a maximum value subject to refinement if the people were eating foods which were isotopically heavier than -26.5‰.

The +4.7‰ value is very close to the +4.5 ± 0.5 diet-collagen spacing proposed by Chisholm (1986) on the basis of animal data. Estimates of diet-collagen spacing in various animal species are summarized in Table 3. (Entries are restricted to mammals in keeping with the concerns of this study.) Variation in the animal Δ_{dc} estimates may reflect a number of factors. Differences between laboratory animals may be due to metabolic differences between species or strains, the macronutrient composition of the diets, the length of time the diets were administered, or the age of the animals studied. The disparity between the results of laboratory and field studies is more difficult to assess. Metabolic differences related to animal size may explain the larger values found in wild animal populations. On the other hand, the differences may reflect the fact that field studies lack the degree of control possible in laboratory studies (Chisholm 1986).

An average diet-collagen spacing value of +4.5 ± 0.5‰

Table 3: Estimates of Diet-collagen Spacing

Sample	N	Diet	Δ_{dc} (‰)	Ref.[a]
LABORATORY STUDIES				
Mice	3	JAX911A	+3.7/+4.4[b]	a,b
Mice	3	Wayne Lab Blox F6	+2.8/+3.8[b]	a,b
Mice	4	Purina Rat chow	+3.5	b
Mice	3	Mixed grains	+1.0[c]	c
Mice	3	Corn	-1.0[c]	c
Mice	20	Rodent chow	+4.6	d
Rats	11	Rodent chow	+4.3	d
Rats	8	Wayne Lab Blox	+2.9	e
Cats	16	Cat food and milk	+4.4	d
FIELD STUDIES				
Browsers S. Africa		C_3 plants	+5.3	f
Browsers N. America and Kenya		C_3 plants	+5.0	g
Grazers N. America and Kenya		95% C_4 plants	+6.0	g

[a] References cited:
- a DeNiro and Epstein 1978
- b DeNiro and Epstein 1981
- c Bender et al. 1981
- d Chisholm 1986
- e Kennedy, this study
- f Vogel 1978
- g Sullivan and Krueger 1981

[b] First value for collagen prepared without NaOH, second value for collagen prepared with NaOH

[c] Diet shift -- animals probably not maintained on new diets long enough to reflect accurate Δ_{dc}

is employed in this study. This decision is based on the suggestion, stated above, that van der Merwe and Vogel's (1978) estimate may be exaggerated by two sources of error, and on laboratory studies of a variety of animal species which all produce smaller values than proposed for humans.

Collagen Turnover. Bone remodelling and the associated turnover of collagen are ongoing processes throughout life. The rate of collagen turnover has not been clearly established, but is among the slowest of all body tissues (Boutton et al. 1984; Tieszen et al. 1983). This rate determines the period of dietary history reflected in the isotopic composition of collagen.

A variety of turnover-time estimates have been employed in stable isotope studies. These range from ≤ 10 years (Bumsted 1984; Tauber 1981; van der Merwe and Vogel 1978) to ≥ 30 years (Chisholm et al. 1982, 1983a; Lovell et al. 1986a; van der Merwe 1982; van der Merwe and Vogel 1983). Since estimates of turnover time determine, to a large extent, the interpretations assigned to isotope values, future studies must attempt to refine these estimates, clarifying the extent and nature of bone remodelling and its isotopic implications.

At the present time, it is known that remodelling rates vary according to age and bone type. Klepinger (1984:75) writes

> ...in general, infants and young children have a higher turnover rate than do adults. For normal adults the turnover rate for trabecular bone is estimated to be three to ten times faster than for cortical bone. Although there are variations, the annual turnover rate for trabecular bone averages 10% and that for cortical bone 2.5%.

Thus, from the perspective of stable isotope analysis, the bones of children do not reflect the same diet period as do the bones of adults and, within adult skeletons, ribs, for example, do not reflect the same diet period as tibiae. It follows that if adult individuals change the isotopic composition of their diet, this change will be manifested at different rates throughout the skeleton, up to that point in time when complete turnover has occurred in all bones. The fact that most researchers report no significant differences in isotope values of different skeletal elements from a single individual (DeNiro and Schoeninger 1983; Lynott et al. 1986; Schwarcz et al. 1985) may be explained by the absence of any dietary shift during the critical time period preceding death. In the present study, differences in $\delta^{13}C$ values between elements are identified, and are related to dietary change.

Effects of Nutrition

Clearly, more controlled studies are required to clarify the precise relationship between diet and collagen. To augment the existing data base, results are reported here for an animal feeding experiment conducted by the author in

1983-84. Though originally designed to test the effects of eating manioc on the trace sulphur and stable sulphur isotope composition of bone, carbon isotope determinations were made in the course of the present study as a measure of variation in animals known to have consumed the same diet. The results add a new dimension to the study of diet and bone collagen -- nutritional quality.

Procedure. Twenty-four 4-week-old female albino rats (Sprague Dawley strain) were obtained from a laboratory colony raised for generations on a diet of Wayne Lab Blox. These were divided into two groups of 12 rats each, and housed three animals to a cage. One group was fed *ad libitum* the Wayne Lab Blox diet, while the other was fed a diet composed largely of raw manioc (*Manihot esculenta*) supplemented by a small portion of Lab Blox (10% of the average intake of rats eating only Lab Blox). Because the supplement was small, it was provided on a single day, the same day each week. The absolute amount of supplement (approximately 7 g per rat) remained the same for the duration of the experiment. Since manioc is an extremely rich source of carbohydrates, but contains only 0.8% protein, this was not an optimal diet for rats.

It is difficult to present a quantitative estimate of the composition of the "manioc diet," because measuring the amount of manioc consumed proved to be a difficult task

(manioc has a substantial water content, and significant evaporation occurred each day, complicating determination of average consumption rates). On the basis of the supplement size, however, it is reasonable to assume the diet consisted of at least 90% manioc and 10% Lab Blox by weight. This description can be converted to a percent kilocalories estimate using available statements of the nutritional compositions of the two food sources. (The estimates presented here represent the carbohydrate and protein contents of the diet, but not the lipids, since lipids are not involved in collagen formation). Per 100 g, Lab Blox provides 351 kcal (manufacturer's statement of average nutrient composition) while the same amount of manioc provides 145 kcal (Wu Leung and Flores 1961, cited in Roosevelt 1980). Using these estimates, the manioc diet can be described as 79% manioc and 21% Lab Blox by kilocalories.

Rats were weighed weekly. To measure the rate of change in isotopic composition of body tissues, hair samples were obtained at the beginning of the experiment and at 5 weeks; thereafter this procedure was abandoned due to lack of hair regrowth in the manioc-fed rats. After 20 weeks, 4 rats from each group were sacrificed. The remaining rats were subdivided into four groups of four individuals each. Two groups were maintained on their previous diet (i.e., one group continued on the Lab Blox diet, another continued on the manioc diet), while the other two groups had their diets

reversed (four rats previously fed Lab Blox were put on the manioc diet, while four rats previously fed manioc were put on the Lab Blox diet). All were maintained for a further 32 weeks, and then sacrificed.

The sacrificed animals were skinned, gutted, and defleshed, with the defleshing process completed by dermestid beetles in the Zoology Museum of the University of Calgary. Bones were cleaned ultrasonically, then freeze-dried, and any residual debris was removed with tweezers or by scraping. Lipids were extracted from crushed bones using the methods of Bligh and Dyer (1959). (Chisholm et al. [1983c] extract lipids after demineralizing the bone; the effect of this difference in procedures has not been established.) Distilled water and HPLC grade chemicals were employed in this phase of collagen preparation. After lipid extraction, the bones were cleaned well to remove all traces of chemicals, and freeze-dried. Collagen was extracted as per the methods outlined in Chapter 4, but without the use of NaOH.

Results. The general response to the two diets is demonstrated by the patterns of weight gain and loss recorded for the animals (Table 4). Rats fed Lab Blox showed a consistent pattern of weight increase over the course of the first 20 weeks, with a normal reduction in the size of the growth increment over time. Their average

TABLE 4: Patterns of Weight Gain in Rats Fed Diets of Different Nutritional Quality

Group[a]	Weight (g)		
	0 weeks	20 weeks	52 weeks
I	91.30 ± 6.85	273.68 ± 19.49	303.32 ± 22.16
II	93.02 ± 4.73	120.45 ± 13.18	164.45 ± 27.05
III	87.78 ± 6.67	266.95 ± 9.88	221.60 ± 9.28
IV	87.41 ± 10.27	109.15 ± 12.63	288.23 ± 32.57

[a] Groups are defined on the basis of their diets:

I: 52 weeks @ 100% Wayne Lab Blox

II: 52 weeks @ 90% manioc and 10% Wayne Lab Blox by weight

III: 20 weeks @ 100% Wayne Lab Blox followed by 32 weeks @ 90% manioc and 10% Wayne Lab Blox by weight

IV: 20 weeks @ 90% manioc and 10% Wayne Lab Blox by weight followed 32 weeks @ 100% Wayne Lab Blox

weight gain in 20 weeks was 180.78 ± 16.58 g. Rats fed manioc lost weight for the first month, but then displayed an erratic pattern of minimal gains and losses for the remainder of the first half of the experiment. Their average weight gain in 20 weeks was only 24.58 ± 9.41 g. In the second part of the experiment, those animals continuing to eat Lab Blox gained a further 30.76 ± 7.47 g, demonstrating that growth was almost complete when the second part of the experiment was initiated. Those continuing to eat manioc gained an average 49.64 ± 23.60 g, showing some adjustment to the deficient diet. Those switched from manioc to Lab Blox exhibited a dramatic growth spurt over the next 32 weeks, gaining 181.50 ± 20.40 g, and showing no signs of having ceased growing at the time they were sacrificed. Those switched from Lab Blox to manioc manifested a general tendency toward weight loss. The average loss at the end of the 32-week period was 43.48 ± 7.19 g.

The significant weight differential between the rats eating Lab Blox and those eating manioc was mirrored in the rate of hair regeneration after samples were collected. While the Lab-Blox-fed rats showed complete hair regrowth within five to seven weeks of sampling, most of the manioc-fed animals showed poor to minimal regeneration after 15 weeks. Despite these differences, rats eating manioc were energetic and displayed no susceptibility to illness.

Carbon isotope values for the food sources and animals are presented in Table 5. Rats fed Lab Blox for 20 weeks show a diet-collagen spacing of +2.9‰. After 12 months, the value is slightly larger at +3.0‰, with an average value of +2.9‰ when data for both groups are combined. Rats fed the manioc diet for 20 weeks show carbon isotope values intermediate between what would be expected for rats eating the manioc or Lab Blox diets. If the diet-collagen spacing is assumed to be +2.9‰, a mass balance assessment suggests 36% of the collagen carbon came from manioc and 64% from Lab Blox:

$$\delta^{13}C_{col} = [(x)(\delta^{13}C_{Blox}) + (x-1)(\delta^{13}C_{Manioc})] + \Delta_{dc}$$

$$-18.2‰ = [(0.64)(-19.5‰) + (0.36)(-23.9‰)] + 2.9‰$$

After 52 weeks on the manioc diet, the percentages shift to 41% manioc and 59% Lab Blox. These estimates are significantly less than the 90% manioc and 10% Lab Blox predicted from the weight composition of the diet, and the 79% manioc and 21% Lab Blox predicted from the caloric composition.

Rats whose diet was changed from manioc to Lab Blox display average $\delta^{13}C$ values the same as those who had consumed Lab Blox all their lives (-16.5‰). In contrast, the results for rats whose diet changed from Lab Blox to manioc show carbon isotope values which differ little from

Table 5: $\delta^{13}C$ Values of Rats Fed Diets of Different Nutritional Quality

Sample	N	$\delta^{13}C$ (‰)
DIET		
Lab Blox[a]	3	-19.5 ± 0.4
Manioc[a]	3	-23.9 ± 0.1
RATS		
Lab Blox diet:		
(20 weeks)[b]	3[c]	-16.6 ± 0.1
(52 weeks)	4	-16.5 ± 0.1
Manioc diet:		
(20 weeks)	4	-18.2 ± 0.1
(52 weeks)	4	-18.4 ± 0.2
Mixed diet:		
Manioc diet (20 wk), followed by Lab Blox diet (32 wk)	4	-16.5 ± 0.1
Lab Blox diet (20 wk), followed by manioc diet (32 wk)	4	-16.9 ± 0.08

[a] Samples of both Lab Blox and manioc were freeze-dried and powdered prior to combustion and analysis (see Chapter 4 for details of analytical procedures).

[b] Length of the diet period is measured from commencement of the experiment. All rats were originally from groups maintained on the Lab Blox diet for generations.

[c] Results are reported for only three of the four rats sacrificed at 20 weeks due to loss of one of the skeletons during preparation procedures.

values at 20 weeks (-16.9‰ versus -16.6‰), and do not approach the values of the rats who consumed manioc early in their lives (-16.9‰ versus -18.2‰ and -18.4‰). A mass balance assessment, using +2.9‰ as the diet-collagen spacing, suggests 93% of the collagen carbon is derived from Lab Blox while only 7% is from manioc -- this despite the fact that these animals consumed a diet that was largely manioc for over half their existence.

Discussion. The results for Lab Blox-fed rats obviously present no challenge to current models of isotope behaviour. The same holds true for results from rats whose diet was changed from manioc to Lab Blox. In contrast, results for rats who consumed manioc for the duration of the experiment and for rats whose diet was changed from Lab Blox to manioc raise a number of questions in terms of the basic tenet of isotope research, "you are what you eat."

Several explanations are possible for the apparent under-representation of manioc in the $\delta^{13}C$ values of rat collagen:

a) reduced collagen synthesis (and turnover) due to nutritionally-imposed restrictions on tissue formation;

b) recycling of carbon from previously built collagen to compensate for dietary deficiencies;

c) preferential incorporation of carbon from protein sources; or

d) restriction of collagen synthesis to optimal diet periods; i.e., when the Lab Blox supplement was available.

The hypothesis that collagen synthesis was significantly reduced in the manioc-fed rats derives from the observed patterns of growth and hair regeneration. If it were true, collagen formed prior to the imposition of the manioc diet at 4 weeks or 20 weeks (collagen formed when these rats ate Lab Blox) would be retained for a longer period of time than normal, and this would explain the under-representation of manioc in isotopic signatures.

It is obvious that rats introduced to the manioc diet at 4 weeks were able to synthesize new collagen during their growth (as indicated by the significant, if not predicted, representation of manioc in collagen $\delta^{13}C$ values), though perhaps at a reduced rate. Thus, even if collagen formed prior to age 4 weeks were retained, it should represent less and less of the total isotopic signature with age, and the signatures at 20 and 52 weeks should be significantly different. This is not the case. The $\delta^{13}C$ value of the manioc-fed rats at 20 weeks suggests 64% of the collagen carbon is from Lab Blox, while the signature at 52 weeks suggests 59%, not a notable difference. Therefore, a significant reduction in collagen synthesis (and turnover) would not appear to be the principal explanation for the under-representation of manioc in the isotopic signatures of rats fed the manioc diet from the age of 4 weeks. The same

considerations apply if recycling of collagen carbon is offered as a possible explanation for the values. Hence it, too, is rejected.

For rats who were placed on the manioc diet at 20 weeks, the situation is clearly different. In contrast to the rats who were given manioc at 4 weeks, these rats display almost no manioc signature. No doubt, this reflects the fact that these rats were past the maximum growth period when the manioc diet was imposed. The maintenance of the 20-week value may suggest that bone turnover (with its implied collagen synthesis) is an extremely slow process in adult rats. Alternately, it may suggest that adult rats are able to compensate for nutritional deficiencies by radically reducing the rate of turnover, and/or by recycling previously-formed collagen.

Alternative hypotheses for the unusual isotopic signatures of the manioc-fed rats relate to specific synthesis requirements. The possibility that collagen synthesis involves preferential reliance on protein carbon cannot be dismissed, since the percentage contribution of manioc to bone collagen in rats fed the manioc diet is significantly lower than predicted by the caloric proportions. To test the possibility that protein might be the critical carbon source, the composition of the diet was converted to protein proportions. Per 100 g, manioc contains 0.8 g protein, while Lab Blox contains 24.5 g.

Thus the protein composition of manioc diet is 23% manioc and 77% Lab Blox. The rat collagen values suggest a significantly greater contribution from manioc (41%) than can be accounted for by this estimate, and hence the protein hypothesis is rejected.

The final possibility considered here is that collagen values reflect optimal periods of tissue synthesis. While it is true that the overall diet proportions (caloric) of manioc and Lab Blox in the manioc diet are 79% and 21%, respectively, the diet was not administered in these percentages each day. Rather, the Lab Blox supplement was presented only once a week (there were also two occasions in the first part of the experiment when the weekend feeder gave Lab Blox to all rats). If tissue synthesis were confined to the periods when Lab Blox was provided, then the general dietary proportions estimated are irrelevant. Unfortunately, the dietary proportions for the period when the supplement was provided are difficult to estimate with any precision, since manioc was available *ad libitum*. Nevertheless, it is certain that the Lab Blox contribution would increase significantly from the 21% of the overall estimate, though it would not likely exceed 50%, given the ubiquitous presence of manioc. The approximate 60-40% split seen in the rat bone collagen may reflect the dietary proportions of this period.

The results of this study suggest nutritional

considerations play a major role in determining isotopic signatures. This finding may be of special relevance in the interpretation of stable isotope values of prehistoric peoples, given their often precarious nutritional status.

CHAPTER 3

COLLAGEN PRESERVATION

The term "collagen" is used in this study, as in most archaeological investigations, in a very liberal sense, to refer to that portion of the organic matrix of bone having the same solubility characteristics as collagen (DeNiro and Weiner 1988). Since collagen comprises 90% of the organic matrix, it is not unreasonable to assume that extracts from well-preserved bone will consist largely of collagen, though this does not preclude the preservation of noncollagenous components (e.g., polysaccharides [DeNiro and Weiner 1988] and noncollagenous proteins [Masters 1987]). It is clear, however, that collagen is not always preserved intact in bones (see, for example, DeNiro 1985; DeNiro and Weiner 1988; Nelson et al. 1986; Tuross et al. 1988). Therefore, the residue produced by so-called "collagen extraction techniques," cannot always be assumed to be pure collagen. While it may be unscientific to continue to use this term, given these limitations, any alternate term would likely be so vague as to be meaningless. The term collagen is thus retained in this study to refer to extracts produced from bone samples, but with the understanding that it is an operational definition only.

This chapter explores the issue of collagen

preservation, possible sources of contamination, and methods of removing contaminants and/or identifying their presence. Its purpose is to stress the possible contribution of diagenetic factors to collagen isotopic variability.

COLLAGEN DEGRADATION

It has been established that collagen concentration in buried bone decreases with time (Berger et al. 1964; Ho 1965, 1967; Olsson et al. 1974). Berger et al. (1964) identify the following environmental factors as important in determining the extent of collagen degradation: "(i) invasion by saprophytes, possibly fungi or algae which feed on the organic material ..., (ii) [bacterial] collagenase activity in the bone, and (iii) ground-water erosion" (1964:999-1000). Temperature also influences the rate of bone destruction (von Endt and Ortner 1984). Some bones may be more resistant to the forces of degradation (Nelson et al. 1986), however, and hence have a better chance of survival.

A tendency toward patterned change in amino acid profiles has been associated with collagen degradation: a relative loss of glycine, alanine, and proline, accompanied by an increase in aspartic and glutamic acid (Bada 1985; Hare 1980). Several explanations for these alterations have been suggested: preferential retention of acidic amino acids

during collagen degradation, due to charge reactions between these amino acids and the mineral portion of bone (Bada 1985; Masters 1987); or preferential retention of noncollagenous protein with a more dominant acidic amino acid composition (Hare 1980; Masters 1987). (The possibility that the alterations might also represent exogenous contamination [Bada 1985] is considered below.) Because amino acids vary in their isotopic composition (Abelson and Hoering 1961; Hare and Estep 1982; Macko et al. 1982), changes in amino acid composition will have an effect on isotope ratios. The changes identified above imply isotopic depletion.

Whether bone degradation typically involves other changes with related isotope effects remains to be determined. Studies of the general degradation of organic materials in soil, however, suggest diagenetic processes may lead to ^{13}C enrichment or depletion. Deines (1980:346) lists the following potential sources of fractionation:

> (1) isotope effects during bacterial degradation of organic matter, (2) preferential elimination of compound groups and preferential preservation of others which differ significantly in $\delta^{13}C$ from the average plant material, and (3) decarboxylation reactions, which would remove ^{13}C-enriched groups from the organic material leading to ^{13}C depletion in the residual.

CONTAMINATION

Bone is a porous substance, and thus easily

contaminated by soil organic matter. (Teeth do not show the same degree of contamination because they are less porous.) Soil organics include soil animals, micro-organisms, plant roots, plant and animal residues, and humic substances (Limbrey 1975). While the particular identity of organic contaminants is difficult to establish (DeNiro and Weiner 1988), particular attention has been focused on humic substances, largely due to their predominance in soil organic profiles.

Humates

Eighty-five to ninety percent of soil organic matter consists of humic substances (Limbrey 1975), a heterogeneous group of polymers frequently subdivided into fulvic acids, humic acids, and humin, based on solubility characteristics (Stevenson and Butler 1969). Fulvic acids are acid and alkali soluble; humic acids are alkali soluble (especially if Na ions, and sometimes if Mg ions, are present in the solution), but tend to precipitate in acid; humin is insoluble in alkali and acid. Fulvic acids are typically yellow to red in colour, whereas humic acids are typically dark (brown to black or grey) (Olsson 1972), perhaps reflecting attached proteinaceous and carbohydrate residues (Limbrey 1975). Humic substances generally contain from 15% to 36% amino acids by dry weight, with acidic amino acids dominating the profile (Cunningham and Mitterer 1980).

Thus, the suggestion that exogenous contaminants, like humic acids, may be related to the altered amino acid profiles seen in degraded collagen (Bada 1985) has clear merit. (Amino acid excretions by bacteria [Smith 1972] may also be a factor in changing bone collagen profiles.)

The $\delta^{13}C$ values of humates generally reflect those of the original plant cover. Nissenbaum (1974) found soils covered by C_3-type vegetation showed values of C_3 plants, while those covered by C_4-type vegetation showed values of C_4 plants. Marine humates display a range of $\delta^{13}C$ values, varying from -10 to -30‰, but more than 90% cluster between -20 and -27‰ (Deines 1980).

Isotopic Effects of Contamination

The effects of organic contaminants like humates on bone isotope values depend on the amount and nature of both the contaminant material and the collagen. Bone specimens with little organic matter are more likely to show the effects of extraneous material, but the significance of these effects will be determined by the relative carbon content and relative isotopic composition of the contaminant vis-à-vis the sample (Grant-Taylor 1972). This issue is given closer consideration later in this chapter.

COLLAGEN EXTRACTION AND CONTAMINANTS

Extracting collagen from archaeological samples for carbon isotope analysis is an exercise in balance, aiming to eliminate carbon-bearing contaminants, while recovering the maximum amount of preserved collagen. Various methods of collagen extraction have been employed in isotope studies, with much of the groundwork laid by those interested in radioactive isotopes. All procedures incorporate steps to eliminate contaminants from the collagen extract.

The most frequently used extraction technique in stable isotope analysis is that developed by Longin (1971) for the preparation of bone collagen for radiocarbon dating. As originally proposed, the method involves a short acid pretreatment phase (generally < 20 minutes in 1M HCl) to remove bone mineral, any soluble protein residues, and some organic contaminants (e.g., fulvic acids). This is followed by a gelatinization step, in which collagen is hydrolyzed in slightly acidic (pH = 3) hot water (90°C). Maintaining a pH of 3 promotes hydrolysis, and is intended to avoid dissolution of base-soluble contaminants (humic acids). The end product is filtered to remove insoluble residues.

Opinions vary on the efficiency of the Longin method in preventing inclusion of organic contaminants in the collagen extract (Bumsted 1984; Chisholm 1986; Chisholm et al. 1983c; DeNiro and Epstein 1981). Bumsted (1984) suggests the

relatively short HCl pretreatment of bone samples may not remove all acid soluble contaminants, particularly fulvic acids, whereas Chisholm (1986) argues this pretreatment may not remove all inorganic material. Other researchers reject the reliance placed on pH to avoid humic acid contamination (e.g., Boutton et al. 1984 and DeNiro and Epstein 1981, among others).

Consequently, various modifications of the Longin technique have been proposed, involving adjustments of the acid concentration used and the timing of the pretreatment phase (e.g., Boutton et al. 1984; Bumsted 1984; Chisholm et al. 1983c), as well as the addition of procedures employed in radiocarbon analysis for the removal of humic contaminants: NaOH treatment (e.g., Boutton et al. 1984; DeNiro and Epstein 1981) or use of a resin column (Bumsted 1984). The most controversial, and yet most widely accepted, modification has been the addition of a NaOH treatment.

The controversy surrounding the use of NaOH centres on its possible effects on collagen composition. These include reduced yields (Boutton et al. 1984; Chisholm et al. 1983c; Katzenberg 1989) and changes in isotopic signatures (Chisholm et al. 1983c; DeNiro and Epstein 1981). Changes in $\delta^{13}C$ values of +0.7‰ to +1.0‰ have been reported by DeNiro and Epstein (1981) for modern animal bone collagen extracted with and without NaOH. (This difference is

incorporated into dietary calculations by Lynott et al. 1986.) It is important to note, however, that the opposed extractions were not concurrent, and hence other sources of error may have contributed to the difference. Chisholm et al. (1983c) report a difference in $\delta^{13}C$ of up to 0.6‰ in extractions made with and without NaOH, using the traditional Longin method, and up to 0.8% in extractions made with and without NaOH, using the Longin method with Grootes modification (in which acid pretreatment is revised to consist of repeated extractions in 0.25 M HCl until stable pH is reached). The direction of the shift (enrichment or depletion) is not consistent, and since archaeological bone is employed in the tests, it is impossible to assess whether the changes are due to removal of collagen or elimination of contaminants.

Chisholm et al. (1983c) and Chisholm (1986) suggest the changes may reflect preferential removal of certain amino acids by NaOH. This hypothesis is based largely on effects demonstrated in commercially prepared gelatins (produced mainly from skin or hide collagen), described by Veiss (1964). Paradoxically, alkali treatments are employed in commercial manufacturing processes because they produce a "purer product" (i.e., one with a lower glycoprotein and mucopolysaccharide content), while not significantly reducing yield (Eastoe 1967). Commercial alkali treatments are not comparable to those employed in stable isotope

studies, however, since the typical times involved are on the order of weeks and months; calf skin, for example, may be pretreated for 4-8 months before collagen is extracted, not the 20-24 hours commonly used in isotope studies (Veiss 1964). *Under these circumstances*, some amino acids are affected, notably arginine, tyrosine, serine, and threonine (Meister 1965; Veiss 1964).

Also under these circumstances, nitrogen content decreases, because amide nitrogen is removed by the alkali solution (Veiss 1964). Milder alkaline treatment may produce the same results. Hannig and Nordwig (1967:77) write: "After incubation with 5% NaOH in the cold for several days, [collagen] cross-linkings are broken up and the major part of amide nitrogen is split off; there is, however, no degradation of the chains." Chisholm (1986) argues that atomic C/N ratios obtained by DeNiro (1985) on specimens extracted with NaOH are too high, and may indicate such a loss of amide nitrogen. His argument, however, is based on a misunderstanding of *atomic* C/N ratios, which he equates with *percent weight* C/N ratios (see later discussion of these values). It is clear, however, that NaOH treatment may have a greater impact on nitrogen values (both content and isotopes) than on carbon.

Clearly, additional data are required to assess the effects of NaOH on the isotopic composition of collagen. Experimental investigations conducted in the course of this

study indicate the treatment has no significant impact on collagen quality or $\delta^{13}C$ values (see Chapter 4). Effects on $\delta^{15}N$ values will be a subject of future research.

Before leaving the subject of NaOH treatments, it should be noted that radiocarbon research has suggested that NaOH will not remove all humics if levels of contamination are high. Some ^{14}C labs use sodium pyrophosphate ($Na_4P_2O_7$) to enhance the effectiveness of NaOH extraction (Browman 1981; Goh and Malloy 1972; Goh and Stout 1972), a modification that has not yet been investigated by stable isotope researchers.

MEASURES OF COLLAGEN PURITY

The purity of collagen extracts (both in terms of degradation and contamination) is assessed using various measures. The two most frequently employed are yield and atomic C/N ratios. Less commonly used techniques include amino acid analysis, and infrared spectroscopy.

Collagen yield

The percent yield of collagen from an archaeological sample is the extract return per dry weight of bone powder processed. (This measure is referred to as collagen "concentration" by DeNiro and colleagues [DeNiro and Schoeninger 1983; DeNiro and Weiner 1988; DeNiro et al.

1985].) Normal, dry, fat-free bone is 20-30% collagen. Collagen yields from archaeological bone which exceed this figure indicate significant contamination, either inorganic or organic in nature. Yields below this figure provide a rough approximation of preservation status, or degradation, but do not exclude the possibility of contamination. There is some suggestion that low collagen yields (< 2%) are associated with problematic isotope values (DeNiro and Weiner 1988).

Yield estimates generally are seen as only approximate indicators of quality, since the potential for sample loss at various stages of the procedures is significant. To complicate matters further, the extracted collagen begins to absorb water immediately upon contact with air.

Atomic C/N ratios

In the early 1960s, Oakley (1963) proposed micro-nitrogen determinations could be used as an index of residual collagen for radiocarbon dating, arguing that there was a specific carbon-to-nitrogen (weight) ratio in bone collagen. Estimates of the value for this ratio in radiocarbon and other studies have varied from 2.7 to 3.0 ± 0.5 (Table 6).

Carbon-to-nitrogen ratios have come to hold an increasingly prominent position in the field of stable isotope analysis, largely due to recommendations by DeNiro

TABLE 6: Collagen C/N Ratios

Wgt% Estimate	Atomic C/N Equivalent[a]	Reference
2.75	3.21	Tibbles 1912 [b]
2.80	3.27	Young 1963
2.50	2.92	Oakley 1963
3.0 ± 0.5	3.5 ± 0.6	Berger et al. 1964
2.69	3.14	Chisholm 1986

[a] Atomic C/N values are calculated by multiplying percent weight estimates by 1.167 (at. wgt. nitrogen / at. wgt. carbon).

[b] Cited in Bumsted (1984)

(1985). DeNiro examined the relationship between atomic C/N ratios (the molecular equivalent of percent weight ratios) and isotope values in a series of modern and archaeological bone specimens, with the purpose of identifying diagenetic changes. Archaeological samples with isotope values consistent with their modern equivalents displayed C/N values between 2.9 and 3.6, leading DeNiro to recommend exclusion of samples with values outside this range. Such exclusion, he says, may eliminate some samples with accurate isotope ratios, but will ensure altered ones will not be included. DeNiro provides no theoretical explanation for his range of acceptable C/N values. Its basis, however, can be shown to lie in the elemental composition of collagen, and errors associated with the analytical techniques employed.

There are few precise estimates of the carbon and nitrogen composition of collagen. Indeed, only one was located by the author. Young (1963) states collagen is 50.75 wgt% carbon and 17.90 wgt% nitrogen (unfortunately, the derivation of his collagen sample is not specified). A number of determinations of the nitrogen content only of various collagens (and gelatins) were located, however, many of which were made in the course of amino acid analysis. Eastoe (1967) includes the following nitrogen estimates in his discussion of the amino acid composition of collagen:

18.0 wgt% (cattle collagen)

18.1 wgt% (ox collagen)

18.26 wgt% (ox collagen)

18.45 wgt% (human collagen)

18.10 wgt% (ox gelatin)

18.38 wgt% (cod gelatin)

Based on these values, the elemental composition of collagen may be estimated to lie in the range of 50-51 wgt% carbon and 18 wgt% nitrogen. The average percent weight C/N ratio of bone collagen is thus 2.8. The atomic C/N equivalent of this value is 3.3 (obtained by multiplying the percent weight ratio by 1.167, i.e., the ratio of the atomic weight of nitrogen to the atomic weight of carbon), a value which is at the approximate centre of DeNiro's range of 2.9-3.6.

In a table of C/N values for 103 animal bones provided by DeNiro, the majority fall in the range 3.2-3.4. Their specific distribution is as follows:

< 2.0	2
-- break in values --	
3.2	37
3.3	28
3.4	9
3.5	2
3.6	2
3.7	2
3.8	3
-- break in values --	
≥ 4.3	18

The two values with the highest frequency are 3.2 and 3.3, in agreement with the theoretical value of 3.3 suggested above. Deviations from the mean by uncontaminated collagen

probably reflect the measurement error associated with C/N assessments, which DeNiro reports to be ± 0.1 for his manometric determinations. Techniques which reduce the size of this measurement error may be expected to reduce the size of the acceptable range of C/N values.

Most aberrant C/N ratios in DeNiro's sample err on the large side; i.e., they are greater than 3.6. All are associated with alterations in the carbon or nitrogen isotope values of the samples. DeNiro attributes the alterations in both sets of values to collagen contamination, resistant noncollagenous components, or degradation of certain portions of collagen. Masters (1987) has suggested a bimodal distribution of C/N values, with those slightly beyond the normal range indicating the presence of noncollagenous proteins, and those significantly beyond normal (e.g., values of 10 to 20) indicating admixtures of humate-like substances. While it is, no doubt, true that values in the 10 to 20 range imply the presence of contaminant carbon, the possibility that less deviant values could signify smaller amounts of contaminates cannot be dismissed.

To gain a better understanding of the effects of contaminant on the atomic C/N ratios and isotope values of collagen, a series of computer models were generated as part of the present study. Specific attention was given to the effects of varying: a) the state of collagen preservation

(as reflected in the percent carbon and nitrogen content); b) the amount of contaminant; c) the contaminant C/N ratio; and d) the contaminant $\delta^{13}C$ value. A base atomic C/N value of 3.3 was assumed for collagen, but the carbon and nitrogen content was varied from 10% carbon (3% nitrogen) to 40% carbon (12% nitrogen). C/N values of 6, 10, and 14 were chosen for the contaminant, with the carbon content set at 50%, and the nitrogen contents determined by the designated C/N ratios (e.g., for C/N = 10, C = 50% and N = 5%). These estimates are based on Hare's (1969) assertion that soil and recent sediments have C/N values ranging from approximately 5 to 15, and Schnitzer's (1976) assertion that humic acids are approximately 50-60% carbon and 2-6% nitrogen. (Fulvic acids are approximately 40-50% carbon and less than 1-3% nitrogen.) Contaminants were assigned a $\delta^{13}C$ value of -25.0‰, a reasonable value for humic substances from C_3 environments (Deines 1980). Uncontaminated collagen extracts were assigned a value of -19.0‰ to simulate the average of samples analyzed in this study.

Collagen yield is not a critical factor in the models, since the effects of contaminant do not focus on the amount of collagen, *per se*, but on the percentage carbon and nitrogen. The models generated, however, did require a dummy value for yield, which was set at 10%. The models employed the following mathematical reasoning:

IF

E = Weight of pure collagen extract from bone

C_E = Percent carbon in pure extract (E)

N_E = Percent nitrogen in pure extract (E)

$\delta^{13}C_E$ = $\delta^{13}C$ of pure extract

$X*E$ = Weight of contaminant, expressed as percent (X) of extract (E)

C_{XE} = Percent carbon in contaminant

N_{XE} = Percent nitrogen in contaminant

$\delta^{13}C_{XE}$ = $\delta^{13}C$ of contaminant

THEN

the C/N value of the total yield (E + [X*E]) is expressed as:

$$C/N = \left[\frac{\text{carbon (wgt) in collagen and contaminant}}{\text{nitrogen (wgt) in collagen and contaminant}}\right] * 1.167$$

$$= \left[\frac{(E*C_E) + ([X*E]*C_{XE})}{E + (X*E)}\right] \div \left[\frac{(E*N_E) + ([X*E]*N_{XE})}{E + (X*E)}\right] * 1.167$$

$$= \left[\frac{C_E + C_{XE}X}{N_E + N_{XE}X}\right] * 1.167$$

$$\text{where } 1.167 = \frac{\text{atomic wgt N}}{\text{atomic wgt C}}$$

The $\delta^{13}C$ value of the total yield is expressed as:

$$\delta^{13}C = \left[\frac{C_E}{C_E + C_{XE}} * \delta^{13}C_E\right] + \left[\frac{C_{XE}}{C_E + C_{XE}} * \delta^{13}C_{XE}\right]$$

Each model began with a pure (uncontaminated) collagen extract. Increments of contaminant equal to 0.125% of the collagen content were successively added to the extract. One hundred additions were made, with the total amount of contaminant added thus equal to 12.5% of the collagen content. This would bring the total yield to 11.3% for an extract containing 10.0% collagen, and to 22.5% for an extract containing 20.0% collagen. In the course of routine investigations, these values would not alert the observer to the presence of contaminants.

The simulation was repeated for collagen extracts of different quality (represented by variations in carbon and nitrogen content) and contaminants with different C/N values. The results (Table 7) clearly demonstrate that as the carbon and nitrogen content of the collagen decreases, the effect of contamination on the C/N ratio of the extract increases. Furthermore, the higher the C/N ratio of the contaminant, the greater the effect on the C/N ratio of the extract.

The effect of contaminants on the $\delta^{13}C$ value of the extract is also significantly greater as the carbon and nitrogen content of the collagen decreases. Within a given preservation level, however, equal amounts of contaminants with different C/N values have the same impact on $\delta^{13}C$ values; i.e., the effect of 12.5% contaminant with C/N = 6 is the same as 12.5% contaminant with C/N = 14. This is

Table 7: Effects of Contamination on Collagen Extracts

Collagen Preservation	Contaminant		Total Extract	
	C/N	Amount (%)[a]	C/N	$\delta^{13}C$ (‰)
Poor (wgt% C = 10)[b]	6	0.0	3.3	-19.0
		3.1	3.6	-19.7
		6.3	3.8	-20.2
		9.4	4.0	-20.6
		12.5	4.2	-21.0
	10	0.0	3.3	-19.0
		3.1	3.7	-19.7
		6.3	4.0	-20.2
		9.4	4.3	-20.6
		12.5	4.6	-21.0
	14	0.0	3.3	-19.0
		3.1	3.7	-19.7
		6.3	4.1	-20.2
		9.4	4.5	-20.6
		12.5	4.8	-21.0
Moderate (wgt% C = 25)	6	0.0	3.3	-19.0
		3.1	3.4	-19.2
		6.3	3.5	-19.3
		9.4	3.6	-19.5
		12.5	3.7	-19.6
	10	0.0	3.3	-19.0
		3.1	3.4	-19.2
		6.3	3.6	-19.3
		9.4	3.7	-19.5
		12.5	3.8	-19.6
	14	0.0	3.3	-19.0
		3.1	3.4	-19.2
		6.3	3.6	-19.3
		9.4	3.8	-19.5
		12.5	3.9	-19.6

Continued next page

Table 7 (Continued)

Collagen Preservation	Contaminant		Total Extract	
	C/N	Amount (%)[a]	C/N	$\delta^{13}C$ (‰)
Good (wgt% C = 40)	6	0.0	3.3	-19.0
		3.1	3.4	-19.0
		6.3	3.4	-19.1
		9.4	3.5	-19.1
		12.5	3.5	-19.2
	10	0.0	3.3	-19.0
		3.1	3.4	-19.0
		6.3	3.5	-19.1
		9.4	3.6	-19.1
		12.5	3.6	-19.2
	14	0.0	3.3	-19.0
		3.1	3.4	-19.0
		6.3	3.5	-19.1
		9.4	3.6	-19.1
		12.5	3.7	-19.2

[a] Wgt% N may be determined by dividing wgt% C by C/N.

[b] Amount of contaminant added to the sample is measured as a function of the percentage collagen. Since the standard increments of contaminant were equal to 0.125% of the percentage collagen, the values of 3.1%, 6.3%, 9.4%, and 12.5% represent the addition of 25, 50, 75, and 100 units of contaminant, respectively. See text for details.

because the $\delta^{13}C$ of the contaminant is the same (-25.0‰), no matter what the C/N value. The magnitude of the effect of carbon contaminants on isotopic composition, then, is related to the quality of collagen preservation and the amount of contaminant present. The relative difference in $\delta^{13}C$ values of the collagen and contaminant is also a critical factor, though it is not incorporated in the model shown here.

Other Techniques

Amino acid analysis. As noted in Chapter 2, collagen has a characteristic amino acid profile consisting of approximately 33% glycine and about 20% hydroxyproline and proline. This profile may be altered in archaeological bone specimens due to any of several proposed diagenetic factors. Such alterations may imply isotopic fractionation.

A number of researchers have employed amino acid analysis as a measure of collagen integrity (e.g., Bumsted 1984; Chisholm 1986; DeNiro and Weiner 1988; Lynott et al. 1986), though on a very limited basis, for it is a complicated and relatively expensive addition to analytical procedures. DeNiro and Weiner (1988) have suggested amino acid profiles of low molecular weight products of laboratory reactions of collagen and collagenase may also serve as a measure of preservation quality. They acknowledge, however, that the technical expertise required for such assessments

will severely restrict their use in isotopic studies.

Infrared Spectroscopy. The use of infrared spectroscopy to detect the presence of contaminants has been suggested only recently (DeNiro and Weiner 1988). Contaminated or degraded collagen samples fail to exhibit absorption peaks around specific wavenumbers characteristic of collagen. Since this technique remains largely untested, it is difficult to assess its future contribution to stable isotope studies.

IMPLICATIONS

In summary, the issue of bone preservation remains a contentious one in stable isotope studies. Clearly, more work is needed to identify the nature of diagenetic changes in bone collagen and the associated isotopic effects. For the present, it is imperative that all studies incorporate some measure of preservation quality for every specimen analyzed. It is not sufficient to test a portion of the sample, since the intricacies of preservation are such that conditions within a single site may vary markedly. Only through the continued accumulation of such data will the patterns of isotopic variation due to diagenesis ultimately be clarified.

CHAPTER 4

ANALYTICAL TECHNIQUES

Techniques of sample preparation and analysis are vital aspects of stable isotope research since methodological improprieties can significantly impact results. This chapter details the methods employed in this study. With one exception, the use of a NaOH treatment to eliminate humic contaminants, the procedures are not controversial. Experimental evidence justifying the use of NaOH is provided.

SAMPLE PREPARATION

Collagen Extraction

Fragments of bone weighing 5-10 g were selected for analysis. Ribs were chosen whenever possible to minimize potential variation due to element choice.

Collagen was extracted according to the method outlined by DeNiro and Epstein (1978) and Schoeninger and DeNiro (1984), with minor modifications. These procedures were judged to be the most efficient in terms of conserving sample (by minimizing transfers from one vessel to another), reducing the possibility of laboratory contamination of samples (also by minimizing the number of vessels used), and

reducing labour input. Specific aspects of the extraction procedures are discussed below.

Bone fragments were cleaned manually, and then ultrasonically, using distilled water. Clean specimens were freeze-dried for a minimum 24 hours, then frozen in liquid nitrogen, and ground to a powder using a mortar and pestle. Approximately 1.0 g of bone powder was placed on a bed of Pyrex glass wool in a 50 ml coarse fritted-filter funnel (previously fitted with a stopcock). To prevent passage of small particles of bone powder (and any residual soil particles) through the fritted disc (pore size = 40-60 µm), a glass fibre filter (Whatman GF/A) was placed over the disc. Fifty ml of 1 M HCl were added, and the mixture stirred to break up lumps. After 18 minutes, the liquid was filtered and the residue rinsed to stable pH. Gravity filtration techniques were employed at this and most subsequent stages of the procedure due to the tendency for the funnel apparatus to block when vacuum filtration was attempted. (Acid pretreatment times incorporate the approximately two minutes required for gravity filtration.)

Fifty ml of 0.125 M NaOH solution were added to the bone residue, let stand for 20 hours, and rinsed to stable pH. The decision to incorporate a NaOH treatment in the extraction procedures is based on experimental evidence which demonstrates no significant effect of NaOH on collagen extract qualities (atomic C/N ratios, amino acid

composition, and $\delta^{13}C$ values). Specific details of this experiment are discussed later in this chapter.

To solubilize the collagen, 50 ml of 0.001 M HCl were added, and the mixture placed in a preheated oven (90°C) for 20 hours. Heating denatures and partially hydrolyses intact collagen, rendering a gelatinous product; a pH of 3 promotes this process (Veiss 1967) and avoids dissolution of any acid-insoluble contaminants (Longin 1971). A 20-hour hydrolysis period (a departure from the 10 hours employed by DeNiro and Epstein [1978] and Schoeninger and DeNiro [1984], but within the range reported by Longin [1971]) was used both for the increased yield it implied in trial runs, and the convenience it afforded in terms of the length of a normal working day. The pH was adjusted at 5 hours (further adjustments were generally unnecessary). The solubilized collagen extract was filtered into a clean labelled flask, with a light application of vacuum filtration at the final stage to minimize collagen retention in the glass wool and glass fibre filter. The filtrate was reduced to approximately 10-15 ml in an oven preheated to 75°C, then transferred to a pre-weighed scintillation vial, reduced to 2 ml, and freeze-dried for a minimum 48 hours. The percentage yield of collagen extract was determined as a function of the difference in the weight of the vial to that of the vial plus collagen and the original amount of bone powder processed.

All glassware used in the extraction procedures was Pyrex, to minimize alkali reactions. Glass items were cleaned as per normal laboratory procedures and placed in an acid bath (Chromerge) for 24 hours to remove any adhering protein residues, then rinsed well in distilled water and annealed. (Annealing temperatures for the filter funnels were restricted to 450°C to avoid damage.) All solutions were made with distilled water and reagent grade chemicals.

Three blank runs (trials which incorporated no bone powder) were made to determine if the extraction method, itself, produced any residue. An average yield of 0.026 ± 0.03 g was obtained. This residue may be sodium silicate produced by the reaction of NaOH with the Pyrex glassware. Elemental analysis of two samples, as per the techniques outlined below, indicated a carbon content of 1.14% and 2.48%, and a nitrogen content of 0.00% and 0.03%. These values likely reflect NaOH impurities. (The NaOH used included 0.6% Na_2CO_3, and 0.0005% N compounds. Additional Na_2CO_3 can be expected because NaOH reacts with the atmosphere [Schugar et al. 1981]). No measurable quantities of CO_2 or N_2 gas were produced in three attempts at mass spectrometric analysis of the residue.

Sample Combustion

Samples were combusted using a modified version of the Stump and Fraser technique (Northfelt et al. 1981; Stump and

Fraser 1973). Approximately 3 mg of collagen were placed in a 20 cm length of 6 mm Vycor tubing with 1 g CuO (wire form), 1 g Cu (pellets), and 1 small piece of silver foil. (CuO oxidizes any CO to CO_2 during combustion; Cu removes N oxides; Ag catalyzes N_2 formation and aids in the removal of S. All tubing and chemicals were annealed prior to use [Ag and Cu under vacuum].) The tubes were evacuated and sealed. Prepared samples were then combusted in a preheated furnace (800°C) for 3 hours, and slowly cooled. Sofer (1980) suggests failure to preheat the oven may result in incomplete combustion which may contribute to carbon fractionation, though Chisholm (1986) points out that heating to 900°C should avoid this problem. Slow cooling allows any excess O_2 to recombine with Cu (Chisholm 1986). All isotope measurements were made within 48 hours of combustion, since gases left in tubes for an extended period of time may also be subject to fractionation (Struiver et al. 1984).

Mass Spectrometry

Isotopic measurements were made in the Stable Isotope Laboratory of the Department of Physics, University of Calgary. Tubes were scored with a file, placed in a tube cracker connected to a mass spectrometer calibrated for nitrogen isotope analysis, and broken under vacuum by flexing (DesMarais and Hayes 1976). Gases were passed

through a liquid nitrogen trap to remove water vapour and CO_2. N_2 was expanded into the mass spectrometer for analysis. (Nitrogen results are not reported here, due to the experimental nature of nitrogen determinations at the University of Calgary at the time of analysis, and the consequent high level of variability displayed). CO_2 was released with dry ice/methanol slush and collected for analysis in a sample tube cooled by liquid nitrogen. Carbon isotope measurements were made on a mass spectrometer built around Micromass 903 components.

Carbon and Nitrogen Content

The carbon and nitrogen contents of all archaeological specimens were determined using a Perkin Elmer Model 240B Elemental analyzer in the Department of Chemistry, University of Calgary. Samples of approximately 2-3 mg were used for each analysis. The Perkin Elmer analyzer combusts samples in pure oxygen at a temperature of 950°C (reducing temperature = 700°C), and the combustion products (CO_2, H_2O, and N_2) are analyzed in a series of thermal conductivity cells. The percent weight carbon, nitrogen, and hydrogen in the sample are computed through a series of simple calculations, based on daily calibration values and the sample signals. To calculate atomic C/N ratios, weights of carbon and nitrogen were converted to micromoles and divided.

The primary disadvantage of using the Perkin Elmer analyzer to determine C/N ratios is that a different collagen sample is assessed than is combusted for isotopic analysis. Provided the extract is homogeneous, however, this should not pose a significant problem.

Effects of NaOH

As indicated in the previous chapter, incorporation of a NaOH treatment in collagen extraction procedures is a somewhat controversial procedure, though no controlled test of the effects of NaOH has been reported. Since NaOH is known to reduce the possibility of humic contamination of collagen extracts, clearly this procedure should not be dismissed out of hand. Hence the motivation for the following experiment.

Procedures. A sample of fresh animal bone (moose) was obtained for testing. As was the case with the rat bones reported in Chapter 3, defleshing was completed with assistance from dermestid beetles. Lipids were extracted from crushed bone using the methods of Bligh and Dyer (1959). After careful rinsing to remove chemicals used in the lipid extraction procedures, the bone was freeze-dried (24 hours), then frozen in liquid nitrogen, and crushed to a fine powder. Ten aliquots of bone powder (1 g each) were weighed out. Five were extracted as per the techniques

discussed above, with NaOH, while five were extracted without NaOH.

Percentage yield, atomic C/N ratios (estimated for 5 samples only), and $\delta^{13}C$ values were determined as described above. The amino acid compositions of six samples (three from each group) were determined using a LKB4155 Alpha Plus amino acid analyzer in the Protein Chemistry Laboratory of the Department of Biological Sciences, University of Calgary. Samples destined for amino acid analysis were dried to a constant weight. Less than 0.5 µg of collagen were hydrolyzed in 0.5 ml of 6 N HCL (ultrapure grade) in evacuated sealed tubes for 24 hours at 110°C. Tubes were scored with a file and opened, and the hydrolysate dried under vacuum for a minimum of 15 minutes, then refrigerated. Approximately 20 µl of the hydrolysate were dissolved in 200 µl of lithium buffer, and the amino acid composition determined on a 100 µl subsample of this solution.

Results. Results are summarized in Tables 8 and 9. Yield was reduced by 2.8% in the samples treated with NaOH. The percent weight carbon and nitrogen in the extracts, in contrast, were greater when NaOH was employed, with a 5% increase in carbon and 6% increase in nitrogen. Average C/N ratios are the same for both groups. Carbon isotope values are very consistent within the two groups, with the NaOH samples averaging 0.1‰ lighter than the untreated bones.

TABLE 8: Effects of NaOH Treatment on Extract Quality
I. Yield, %C, %N, Atomic C/N Ratios, and $\delta^{13}C$

Variable	Without NaOH	(N)	With NaOH	(N)
Yield (%)	22.6 ± 1.4	(5)	19.8 ± 1.0	(5)
Carbon (wgt%)	38.79 ± 0.31	(3)	40.54	(2)
Nitrogen (wgt%)	13.86 ± 0.02	(3)	14.68	(2)
Atomic C/N	3.3 ± 0.06	(3)	3.3	(2)
$\delta^{13}C$ (‰)	-23.1 ± 0.04	(5)	-23.2 ± 0.05	(5)

TABLE 9: Effects of NaOH Treatment on Extract Quality
II. Amino Acid Composition [a]

Amino Acid	Residues per 1000	
	Without NaOH	With NaOH
Aspartic acid	36 ± 1	38 ± 1
Hydroxyproline	84 ± 3	80 ± 4
Threonine	14 ± 0	15 ± 0
Serine	26 ± 1	28 ± 2
Glutamic acid	59 ± 2	60 ± 1
Proline	110 ± 2	104 ± 5
Glycine	406 ± 54	396 ± 31
Alanine	143 ± 43	152 ± 35
Valine	16 ± 0	17 ± 1
Methionine	4 ± 0	4 ± 0
Isoleucine	7 ± 0	8 ± 0
Leucine	19 ± 0	21 ± 1
Tyrosine	3 ± 0	3 ± 0
Phenylalanine	10 ± 0	10 ± 0
Hydroxylysine	3 ± 0	3 ± 0
Lysine	19 ± 1	20 ± 0
Histidine	4 ± 1	4 ± 0
Arginine	37 ± 1	38 ± 1
Total	1000	1000

[a] Values are skewed by use of large samples. In the interpolation of concentration peaks, glycine was over-represented, and other amino acids were correspondingly under-represented.

There are no significant differences in the amino acid profiles of the two groups. Both profiles show more glycine than expected for bone collagen, but this can be attributed to instrument interpolation problems linked to the use of samples which, in hindsight, were too large.

Discussion. The reduction in yield with the use of NaOH is not surprising given previous tests (Boutton et al. 1984; Chisholm et al. 1983c; Katzenberg 1989). The higher percentage nitrogen in the NaOH-treated samples does not support the idea that this reflects collagen degradation and loss, since existing evidence suggests nitrogenous groups are more likely to be affected if degradation occurs (Hannig and Nordwig 1967). Neither is collagen degradation indicated in the amino acid profiles of the two groups which, contrary to statements by Chisholm and colleagues (Chisholm 1986; Chisholm et al. 1983c), do not show any significant differences. While the distortion caused by the use of large samples hampers comparisons with modern bone profiles, the fact that the two test groups show no significant difference demonstrates the lack of any effect of the 20-hour 0.125 M NaOH treatment on amino acid composition. Any collagen loss during NaOH treatment would seem then to involve whole molecules.

The differences in carbon and nitrogen content more likely reflect preferential loss of non-collagenous organic

substances (e.g., carbohydrates) when NaOH is employed. The lighter isotopic values of the NaOH-processed group are not consistent with loss of residual lipids, since this would imply enrichment of the final extract.

The possibility that differences between the two groups are tied to contaminants in the NaOH must also be considered. Once again, however, this explanation would not account for the higher nitrogen content found in the NaOH-treated group, since carbon contaminants are more common than nitrogen in NaOH.

It is clear that the use of NaOH does not alter the quality of collagen extracts to an appreciable extent. The consistent difference in $\delta^{13}C$ values (0.1‰), the primary concern in dietary studies, appears due to the preferential removal of noncollagenous organic molecules when NaOH is employed. Given the results of this experiment, the use of NaOH in collagen extraction procedures for $\delta^{13}C$ analysis is clearly justified. It should be noted, however, that the effects of NaOH on nitrogen isotope values must also be critically evaluated, and cannot be presumed insignificant on the basis of the carbon isotope results presented here.

MEASUREMENT PRECISION

The accuracy of isotopic determinations is dependent on

extract quality and measurement precision. The estimates of extract quality used in this study have been presented earlier in this chapter. It remains to discuss estimates of measurement error for the various phases of the analysis. These have been determined largely through examination of the results of repeat analyses (on single extracts, as well as different extracts of the same bone specimen).

Yield

The reproducibility of the collagen extraction procedures, in terms of yield, are clearly indicated in the results of the NaOH tests. The standard deviation (1σ) for 5 aliquots prepared with NaOH was 1.0%.

Atomic C/N Ratios

Repeat analyses for archaeological and modern bone specimens showed an average difference in C/N ratios between same extract pairs of 0.1 ± 0.1 (1σ, N = 12 pairs). The average difference between different extract pairs was also 0.1 ± 0.1 (N = 7 pairs).

$\delta^{13}C$ Values

Repeat $\delta^{13}C$ analyses on same gas samples from archaeological and modern bone collagen specimens showed an average difference of 0.09 ± 0.09‰ (N = 129 pairs). Repeat analyses of gas samples from different extractions (NaOH

tests) gave a standard deviation of 0.05‰ (with NaOH) and 0.04‰ (without NaOH).

All analytical work reported in this study was conducted by the author, with the exception of carbon isotope analysis in which the author was assisted by two technicians in the Stable Isotope Laboratory, University of Calgary: Nenita Lozano and Luda Yakobi. Personal differences in technique, therefore, contribute little to the reported variability.

CHAPTER 5

THE SAMPLES

Sample selection is a critical element in the study of isotopic variability. To assess the sources of variation influencing isotope values, a clear understanding of temporal, spatial, biological, and cultural parameters of the populations is required. In archaeological material, such information is often limited, or too vague to permit valid discussion of the results of stable isotope analysis. Sample control tends to improve when historical collections are utilized, because documentary sources frequently provide valuable information.

This study focuses on isotopic variability in post-medieval Europeans. Seven samples are assessed, all of which are restricted temporally to the fifteenth to seventeenth centuries; dating is based on historical records and/or the presence of diagnostic artifacts. All samples represent individuals native to northern and western Europe, although two samples come from burials on foreign soil (Figure 1). The specific nature of the sites represented is known (to varying extent) from documentary evidence.

In this chapter, archaeological, physical anthropological, and historical information on each sample is presented, accompanied by pertinent dietary information.

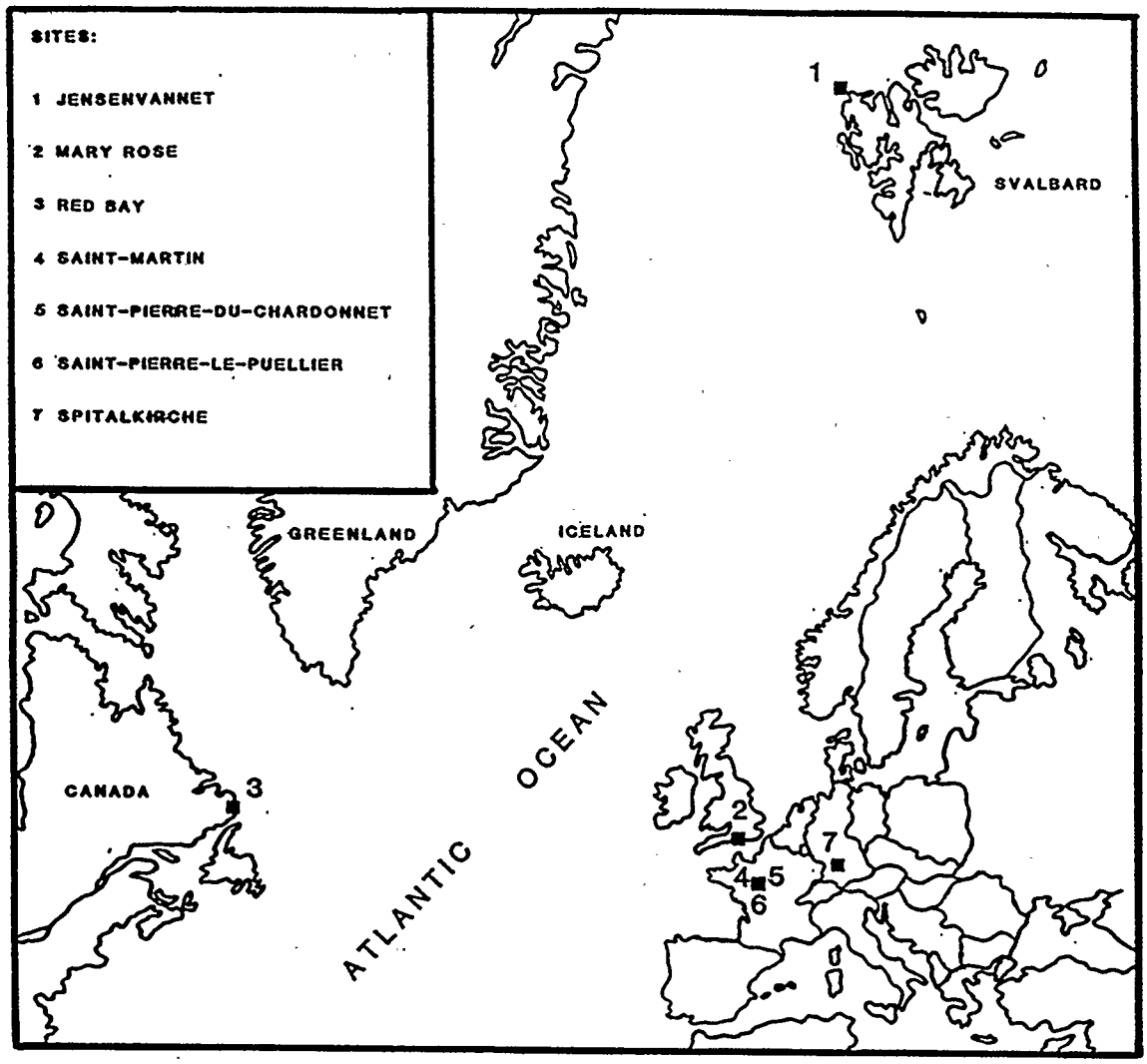

Figure 1: Site Locations

Isotopic implications are summarized at the end of the discussion.

THE *MARY ROSE*

The Site (see Rule 1982, 1983)

King Henry VIII's flagship, *Mary Rose*, sank in Portsmouth Harbour, England, on July 19, 1545. Emerging from her home port to engage the French who were moored off the Isle of Wight, she was caught by a gust of wind as she turned to starboard, and capsized. The ship is reported to have carried over 700 men (415 regular crewmen and 300 extra soldiers - skilled archers). Only 35 survived, since the decks had been covered with anti-boarding netting which prevented the men from evacuating the sinking ship.

From the late 1960s to the early 1980s, the *Mary Rose* was the subject of archaeological investigations led by Margaret Rule. These culminated in the much-heralded raising of this ship which is now on display in the town of Portsmouth.

Skeletal remains of the ship's crew were located during the course of excavations (Stirland 1984, n.d.). Bone, like most organic material associated with the *Mary Rose*, was extremely well-preserved, due to the rapid silting and sealing of the wreck in the years subsequent to 1545. The articulation of skeletons was frequently disturbed, however,

and there was considerable commingling of bones both within and between the surviving sections of the four decks. At least 179 individuals have been identified on the basis of cranium and mandible counts (A. Stirland, personal communication 1986). Individuals isolated from the sometimes mixed assemblage range in age from 14 to 44 years, with the majority falling between 18 and 25 years. All appear to have been in reasonably good health at the time of death, though Stirland (n.d.) suggests they were subject to a relatively high frequency of childhood dietary deficiencies based on the following osteological evidence: cribra orbitalia, enamel hypoplasia, porotic hyperostosis, and tibial bowing.

The Sample

A sample of 12 rib fragments was obtained from the *Mary Rose* collection. Due to the commingling problem, specimens were selected from separate areas of each of the four decks, limiting the possibility that any two specimens were from the same individual. All individuals are presumed male. All are adult, though the specific age at death cannot be established given the mixed nature of the assemblage. The age range is not expected to exceed the upper limit recognized for the individuals noted above, i.e., 44 years. All bones used in this study were in an excellent state of preservation, though some displayed a charcoal-grey caste.

When these specimens were crushed for collagen extraction, the interior bone matrix was entirely grey, the apparent result of mineral replacement.

The *Mary Rose* collection represents a single moment in time, a very positive feature in a study of isotopic variability. The sample is also confined to a single sex and relatively restricted age group, young to middle adult (as defined for this period by Webster [1979]). Sample control ends here, however, as these individuals represent a variety of English communities and possibly a variety of social strata, factors likely to increase the isotopic variability displayed by the sample. Records of place of residence of the individual crewmen of the *Mary Rose* are not available. It is known, however, that when England joined Spain in the fight against the French, seamen were recruited from as far away as Bristol and Norfolk, and in the English campaign against the Scottish, which culminated in 1544 with the capture of Edinburgh, men had been recruited from fishing fleets of the east and south coasts of England (Rule 1982).

Faunal remains were recovered from the *Mary Rose*, but it was not possible to obtain samples for comparative isotopic assessments.

Dietary Information

While childhood dietary deficiencies have been posited

for the men from the *Mary Rose*, their shipboard diet appears to have been more than adequate. Preserved food items include fish, meat (venison, beef, chicken, mutton, and pork), vegetables (peas), fruits (wild cherries and plums), and nuts (hazelnuts). Fishing equipment recovered from the wreck would have provided fresh supplements to the diet. For the regular crewmen and soldiers, there was beer to drink, while wine was available for officers.

Historical sources provide further insights into the diets of sixteenth-century English seamen. In general, sailors seem to have eaten well, as reflected in the provisioning lists for sea voyages. These lists include ship's biscuit, flour (heavily salted), cheese, onions, garlic, dried peas, beans, and chick peas. Salt meat and salt fish (sardines and anchovies in the south; herring in the north) were always stocked, with catches of fresh fish expected to supplement supplies. There was generally dried fruit for the officers.

The supply list for Martin Frobisher's second voyage (1577) gives a more precise idea of the rations for this time period (though it should be noted that Frobisher intended his men to eat heartily). For a crew of 120 men the supplies were as follows:

1 lb biscuit	per man per diem
1 gal beer	per man per diem
1/4 lb butter	per man per diem
1/2 lb cheese	per man per diem
1 lb salt beef or pork	per man per diem (for flesh days)

1 dried cod	per 4 men per diem (fast days)[a]
Oatmeal and rice to use if fish stores gave out	
Honey for sweetening	
1 hogshead oil	
1 pipe vinegar	
Fresh cod and salmon, wild game, and fowl as available when they reached their destination	

[a] The list of provisions for Frobisher's next voyage in 1578 indicates an average of 14 fast days a month.

The diets of most Englishmen of this time period were not as complete as those of seamen. Between 1500 and 1660, one English harvest in six was a serious failure, and London experienced almost continual food shortage (Youings 1984). For the less advantaged, barley, oats, peas, and beans were standard fare (Youings 1984). Salt beef was important as a winter dish, but significant meat consumption was reserved for wealthier individuals. The importance of fish seems to have varied in rural and urban diets. Youings (1984) describes fishing as a seasonal occupation, ancillary to agriculture in rural areas, and notes that in the larger seaports there were those who concentrated on fishing. Yet urban fish consumption does not appear significant. In 1538, Henry VIII relieved Lenten fast restrictions for some of his richer subjects, permitting them "because of *the scantiness and high price of fish*, to consume milk, butter, eggs, cheese and other white meats" (Youings 1984:188, emphasis not in original).

SAINT-MARTIN

The Site (see Galinié and Randoin 1979, 1981, 1982)

The city of Tours is located in the heart of France, nestled between two rivers, the Loire and the Cher. In the sixteenth and seventeenth centuries, the city encompassed an area of some 120 hectares, sub-divided into a number of parishes (Bisson and Galinié 1986). The present study incorporates samples drawn from three parish cemeteries used during this time period: Saint-Martin, Saint-Pierre-du-Chardonnet, and Saint-Pierre-le-Puellier. Excavation of these cemeteries was the responsibility of the Laboratoire d'Archéologie Urbaine, Tours, directed by Henri Galinié.

Archaeological excavations were conducted at the site of the Cloitre Saint-Martin between 1979 and 1982. A large number of burials were encountered during the course of excavations with the earliest dating to perhaps the third century A.D. A collection of 36 post-medieval burials from zone 3 of the site was sampled for this study. These burials have been classed as sixteenth-century on the basis of burial practices, funerary vases, and a 1592 coin.

All burials were aligned east-west, with heads to the west in 33 cases. Twenty-nine burials were in wooden coffins (only the nails are preserved), 5 were in plain ground or possibly in burial shrouds, while two were in monolithic sarcophagi. With the exception of four

individuals, the deceased were placed on their backs with hands crossed on the abdomen. No personal objects were recovered from the burials. Fourteen of the coffin burials, however, were accompanied by ceramics which lay broken on the lid or in the surrounding soil. These ceramics are of the same type as found at the Saint-Pierre-le-Puellier cemetery discussed below. It seems the ceramics were utilized as part of the burial ceremony.

The burials represent non-ordained members (canons) of the Saint-Martin monastery (C. Theureau, personal communication). Names and personal histories of some of these men are known. Canons came from the city of Tours and would have entered monastery life as early as nine years of age. (More senior positions in the monastery hierarchy were occupied by individuals from outside the city who were buried apart from the canons.) These men worked and prayed together, but lived in the surrounding parts of the city.

The Sample

Rib fragments were obtained from 23 burials. All individuals are male, but age at death has not been determined. Galinié (personal communication 1987) describes this collection as probably the most homogeneous of those from Tours. Given the common lifestyle of the men, a minimum range of isotopic variability is expected.

None of the cemetery collections from Tours are

associated with direct archaeological evidence of diet (e.g., faunal remains). However, a contemporary animal bone sample from another site in the city was provided by the Laboratoire d'Archéologie Urbaine. This sample includes pig, sheep, cattle, chicken, and fish.

Dietary Information

Historical sources supply ample information on food habits in post-medieval France, though much of it focuses on peasant rather than urban lifestyles. Since one of the cemeteries from Tours (Saint-Pierre-du-Chardonnet) is marginally rural, such information is relevant to this study. However, the daily fare of other urban dwellers is expected to differ somewhat, simply by virtue of their higher economic status and access to more varied markets.

General dietary conditions in post-medieval France have been described as worse than in England (Braudel 1973; Mennell 1985). Famine was widespread (Braudel 1973; Le Roy Ladurie 1987), and peasant diets were meagre (Goubert 1986). Wheat and bread were central items in the diet. Peasants consumed at least 3 pounds of bread a day, the cheapest source of calories available to them. Other grains (barley, oats, millet, buckwheat) were used to make porridge and pancakes. Soup was a standard part of the diet, made with herbs and root vegetables (radishes, carrots, turnips, and leeks). Other vegetables were available in season,

including cabbages, peas, and beans. In rare areas where pigs were kept, a piece of salt pork would be added to soup; in areas situated near the sea or large rivers, fish was added. There was a widespread absence of dairy products, fruit, and especially meat in the peasant diet (Goubert 1986); eggs, poultry, butter, and cheese, as well as the best fruits and vegetables, were sold at markets.

SAINT-PIERRE-DU-CHARDONNET

The Site (see Randoin 1983)

In 1983 salvage excavations (preceding construction activities) were carried out at the site of an important medieval cemetery known to be located at 20-24 Rue de Clocheville, Tours. City plans dating to the sixteenth and seventeenth centuries indicated the presence of the cemetery anterior to the church of Saint-Pierre-du-Chardonnet. Though this church was situated on the perimeter of the city of Tours, straddling city and countryside, it served the city until 1777, shortly after which it was demolished.

The cemetery seems to have been established in the eighth or ninth century (on the basis of a small number of recovered ceramic fragments), with the most recent burials dating to the fifteenth and sixteenth centuries. Two hundred twenty-seven burials have been excavated from the densely packed site. The deceased are oriented east-west,

with heads to the west, lying on their backs. Site stratigraphy indicates three phases of burials, separated by temporary interruptions in the use of the cemetery. The first and second phases incorporate 170 to 180 burials and predate the period of concern here. The third phase of burials accounts for the remainder of the graves. Limited published information is available on the nature of the burial practices other than to note that the remains of wooden coffins are evident. Galinié (personal communication 1986) suggests the people interred in the cemetery of Saint-Pierre-du-Chardonnet were likely members of the lower class.

The Sample

Ribs fragments were obtained from 35 adult individuals clearly associated with the third burial phase. Both sexes are represented in the sample: 17 males and 18 females (sex determinations by Christian Theureau, Laboratoire d'Archéologie Urbaine, Tours). All individuals are adult, but specific estimates of age at death are not available.

Population mobility is a consideration in sampling parish cemeteries like this one. In the sixteenth and seventeenth centuries, men in search of employment commonly migrated to nearby towns and cities. Such migration might increase the range of $\delta^{13}C$ variability in interred remains, if there were differences in rural and urban diets.

Dietary Information

See Saint-Martin discussion.

SAINT-PIERRE-LE-PUELLIER

The Site (see Galinié 1976; Theureau 1985)

The parish of Saint-Pierre-le-Puellier was created in the tenth century, and became part of the city of Tours in the fourteenth century. It subsequently became one of the most heavily populated parishes in the city. When the parish was dissolved in 1782, three cemeteries surrounded its church. One of these was the subject of archaeological investigations between 1969 and 1974.

Four hundred six graves were excavated, dating from the eleventh to the seventeenth centuries. Three periods of use were identified on the basis of the persistance and abandonment of funerary practices:

Period I (or A)	11-12th centuries
Period II (or B)	13-14th centuries
Period III (or C)	15-17th centuries

The Saint-Pierre-le-Puellier burials demonstrate a number of constant features throughout the period of use of the cemetery. These include: a) position of the corpse on the back, feet together, arms crossed on the chest or hands joined at the pelvis; b) use of a burial shroud to wrap the dead; c) burials in plain ground (i.e., with no coffins or

other burial enclosures); d) E-W orientation with head to the west; e) shallow depth of the burial pit; f) absence of grave markers; and g) few cases of truncated earlier graves.

Specific to Period III is the use of wooden coffins (poorly preserved at this site), though coffin burials are not universal. Sometimes a simple stretcher was used instead, and a large number of individuals were simply placed in the ground. The use of linen burial shrouds is evidenced only by pins which served to fasten the cloth. No clothing accompanied the burials, but several pieces of clothing seem to have been removed at the site. Funerary vases were placed at the head or feet of the deceased in some cases. Only two surface markers were identified.

Individuals of both sexes and varied ages were interred in the cemetery. According to Galinié (personal communication 1987), the skeletal remains represent people from various social classes.

The Sample

Rib fragments were obtained from 16 adult individuals from Period III burials. Both sexes (8 males and 8 females) are represented in the sample. Estimates of age at death range from 17-19 years to more than 60 years. Both age and sex determinations are by Christian Theureau, physical anthropologist at the Laboratoire d'Archéologie Urbaine, Tours.

Dietary Information

See Saint-Martin discussion.

SPITALKIRCHE

The Site (see Deschner 1948)

The city of Tübingen is located in southern Germany, on the Neckar River. During World War II (1943), destruction of a local bomb shelter revealed human burials, and personnel from the University of Tübingen were called to investigate. Subsequent excavations revealed a series of single and multiple interments, incorporating the remains of both mature and immature individuals. For the most part, bodies were aligned east-west with heads to the west, and placed in rows (with the exception of five infant skeletons found in the lower levels, which were interred with no particular orientation).

The burials have been associated with the St. Jacob's chapel cemetery (the site name "Spitalkirche" refers to the broader activities associated with the chapel; Spitalkirche means "hospital church"). St Jacob's chapel was used from 1326-1508, but its cemetery was used until at least 1541. In 1500, the cemetery was expanded, a process involving removal of the surrounding houses, but by 1541 it ceased to be used except on rare occasions.

Archaeological investigations at the site demonstrate

that the remains recovered in 1943 come from the post-expansion phase of the cemetery. The skeletons were situated above a series of infilled cellars and a lime floor which contained late fourteenth- to early fifteenth-century ceramics; more recent ceramics occurred in the grave fill. Since the cemetery was rarely used after 1541, the graves are presumed to date from the early part of the sixteenth century.

The precise identity of the individuals buried at this site is not clear, though presumably the people were from Tübingen or the immediately surrounding area. Deschner (1948) suggests the possibly hurried nature of the infant burials may indicate they were plague victims. Alternatively, he postulates these deaths may have resulted from malnutrition, though the general level of health in Tübingen at this time is unknown.

His most intriguing hypothesis as to the identity of the deceased is that they represent cadavers used for anatomy classes at St. Jacob's. St. Jacob's chapel served as the anatomy department of the local high school from the fifteenth century onward. In 1483, the medieval faculty at the church were given permission from Pope Sixtus IV to dismember cadavers of criminals. While it is not clear whether dismemberment began at this time (or if it may have begun earlier), it is known that in 1538, Professor L. Fuchs at the University of Tübingen changed faculty statutes to

read that twice a year dismemberments could take place, rather than every three to four years. Dismembered cadavers were given church burials.

The Sample

Ten tibial shaft fragments were obtained from adult individuals in the Spitalkirche collection. Ribs could not be selected for analysis since the recovered remains include few of these elements (likely due to bias at the time of excavation; skulls and long bones are the most common elements.) Tibiae were substituted because a) they were prevalent, and b) virtually all specimens had been subject to previous damage. Shaft fragments adjacent to the damaged areas were removed for this study. Eight of the ten samples come from a single grave (46). Since the bones from this multiple interment were not sorted, only left tibiae were used so as to avoid sampling the same individual twice. Catalogue information indicated the presence of six individuals in this grave but, in selecting specimens for sampling, the remains of at least 12 individuals were identified. Only three of the eight tibiae selected for analysis had been labelled (a, b, d); the designations c, e, f, g, and h were randomly assigned to the remaining five for the purpose of this study only. All individuals are adult based on fusion of the tibial epiphyses, but more specific estimates are obviously not possible. Sex estimations are

impossible due to the mixed nature of the remains. Information on the age and sex of the other two individuals included in the sample is omitted, since it is of no significance in the absence of other data.

Faunal remains were not associated with the Spitalkirche site, but a sample of animal bone from a comparable site (Marbach) was provided by Hans-Peter Uerpmann of the Institut für Urgeschichte, University of Tübingen. The sample includes cattle, chicken, sheep, pig, and fish, as well as human bone.

Dietary Information

According to Wurm (1984), the animal protein content of German diets increased in the Late Middle Ages and early post-medieval period. This shift is linked to a decrease in population and the restructuring of agriculture to include cattle breeding. The population decrease reflected both outward migration, as well as the effects of plague deaths which released large areas of land for raising animals. More specific information on diet composition is provided by a German ordinance of 1482, which declared that craftsmen must receive four-course meals at midday and evening: on meat days, this would include soup, two meat, and one vegetable course; on Fridays or meatless days, soup, fresh or salted fish, and two vegetables (Braudel 1973). During periods of extended fast, five courses were ordered, soup,

two fish, two vegetables, plus bread and beer.

Beginning in the early sixteenth century, meat consumption began to decrease, and by the nineteenth century much of the diet was vegetarian in nature.

RED BAY

The Site (see Tuck 1982, 1985, 1987; Tuck and Grenier 1981)

Basques have been identified as a distinct ethnic group in western Europe for the entire period of recorded history. These people, renowned for their unique language (as well as certain distinctive elements of their blood chemistry) now occupy a small area (approximately a hundred miles across) where the western Pyrenees meet the Bay of Biscay, an area straddling the modern French-Spanish border (Douglass and Bilbao 1975).

In the sixteenth century, a long established Basque whaling industry shifted its focus to the New World, specifically the rich resources of the Strait of Belle Isle, that narrow passage of water between the northern peninsula of Newfoundland and southern Labrador (Barkham 1978). Documentary evidence suggests the principal period of the Basque whaling operations in Labrador was from the 1540s to the 1580s or 1590s, with the industry abandoned by the 1620s (Barkham 1978; Belanger 1971). At its peak period the industry may have employed as many as 2000 men. Those ships

going to the early hunt would leave their home ports in the Bay of Biscay in April or early May and return in December or early January before ice closed the Labrador ports (Barkham 1977). Later sailings were more common, however (Tuck and Grenier 1981), with ships leaving by the end of June or early July.

An archaeological survey in the late 1970s revealed the Basque presence at a number of sites on the Labrador coast. Subsequent investigations (both on land and under water) focused on Red Bay, a site thought to be identifiable with "Butus," one of the two most important ports in the Basque operations. At the height of the whaling industry, nine or ten galleons would arrive in Red Bay each summer carrying as many as 600-900 men (Barkham 1977, 1978, 1980). Land excavations at the site, directed by James Tuck of Memorial University, began in 1977 and continue to the present day; underwater excavations, directed by Robert Grenier of Environment Canada Parks, began in 1978 and ended in 1985.

Of particular interest in the present study is the cemetery located in 1982, at the eastern end of Saddle Island in Red Bay harbour. Excavations of this site from 1982-1984 revealed a series of 57 burial features, including both single and multiple interments (these containing from 2 to 11 individuals), as well as a group of unburied dead (Kennedy 1985; Tuck 1985, 1987). (A limited number of burials located outside the limits of this

cemetery (Tuck 1987; Tuck, personal communications 1984-1987) are not included in these estimates.)

The following characteristics describe the majority of Saddle Island graves: a) shallow burial; b) position of the corpse extended on the back with feet together and arms crossed on the pelvis or chest; c) orientation of grave east-west, with heads to the west; d) interment in plain ground, generally without the use of a coffin ; and e) general absence of grave inclusions, with the exception of occasional remnants of clothing (textile fragments, a few clothing clasps, and a series of lead discs thought to be clothing weights).

The following contrasts in burial patterns are notable: a) the presence of both single and multiple interments; b) the use of grave markers (a row of large rocks placed along the long axis of the grave) in only the eastern portion of the cemetery; and c) occasional use of coffins (identified by fragments of wood and nails).

The skeletal remains of at least 125 individuals are represented in the Red Bay cemetery. Acidic soils, however, have severely degraded many skeletons, in some cases leaving only dark silhouettes in the soil to represent the interred individuals. A small group of skeletons (less than 25% of the total individuals interred in the cemetery) were removed from the ground for further analysis. (Most of these were interred in a section of the cemetery marked by the presence

of a shell lens which enhanced bone preservation.) The poorly preserved remains were reinterred in situ.

The available osteological sample is that of a group of generally healthy men killed in early to middle adulthood (several boys are also represented in the cemetery, as judged by stature estimations and preserved dentitions). Deaths may be attributed largely to accidents while hunting or processing whales, though some, no doubt, relate to disease and those years when ice closed the harbour at an earlier date than had been anticipated, forcing crews to overwinter in the area (recorded in 1574-75, 1576-77, and 1604-05, according to Barkham 1978, 1982).

The Sample

Bone specimens were obtained from 32 individuals interred in the Red Bay cemetery. Wherever possible, ribs were selected; where these were unavailable due to poor preservation, other elements were chosen. The effects of element variability will be considered in discussing the results of the analysis. All individuals are adult males. Specific age estimations are not possible in most cases, due to limited preservation of diagnostic sites such as the pubic symphysis. Histological age estimates for a sample of 10 individuals by Susan Pfeiffer, University of Guelph, were also problematic (tending to favour a much older group of individuals than expected), and again may reflect poor

preservation of skeletal material at the site.

Like the *Mary Rose* collection, the Basque whalers from Red Bay represent a group of men who were linked by their common occupation, rather than their place of residence. In the Red Bay case, however, these men are known to have resided together (on the coast of Labrador) for a significant part of each year. Moreover, the whalers' home ports are known to some extent. Barkham (1982) reports that in the 1570s, at least, most came from the area between Capbreton and Santander, with the majority from the short stretch of coast between the Cubo de Machichaco and Fuenterrabia. Unlike the *Mary Rose*, the Saddle Island cemetery represents deaths which took place over an extended period of time (1530-1630, at maximum); this may or may not have an effect on the variability evident.

A sample of animal bone specimens from the Red Bay site was provided for this study by Stephen Cumbaa, National Museum of Civilization, Ottawa. Included in the sample are seal, whale, cod, bear, and dog.

Dietary Information

Some information is available on the whalers diet in Labrador in the form of provisioning lists. One document reported in the original Spanish by Barkham (1982) details the following food supplies for a voyage to "Terranoba":

750 hanegas of wheat
8 quintals of salt pork

14	hanegas of broadbeans
14	hanegas of peas
8	quintals of olive oil
2	hanegas of mustard seed
6	quintals of cod
4000	sardines
8	botas sherry
120	botas cider
	hardtack, i.e., ship's biscuit
	garlic
	flour for the coopers

Measures may be converted to imperial and metric units as follows:

1 hanega = 1.6 bu = 0.058 m^3
1 quintal = 100 lb = 45.4 kg
1 bota = 125 gal = 568 l

The last item on the list suggests there were status differences in the diet of the men in Labrador. The higher social position of coopers is demonstrated, also, in personal possessions like glassware and pottery, and hence comes as no surprise.

Physical remains of dietary items have also been recovered from the Red Bay site. Land excavations have produced the bones of a number of animal species (terrestrial and marine, domestic and wild), with an especially large selection of native bird species. Underwater excavations have produced shells of walnuts and unidentified nuts. The amount of animal protein available in southern Labrador in the form of fish, birds, and mammals meant the whalers were generally well-fed. Whether this diet was better than they received at home is unclear. Historical evidence indicates sheep and cattle were the mainstay of the traditional Basque agricultural economy

(Gómez-Ibañez 1975). The principal grain crops were wheat and millet or barley, with wheat the most important of the three; oats and rye are also mentioned in historical sources. According to Gómez-Ibañez (1975), maize was probably first introduced in the Basque Country around 1570-75 (in Guipuzcoa) but did not become established as a significant dietary element until the seventeenth century. (He says references to earlier maize in Bayonne (1523) are based on an incorrect translation.) Vetch, peas, turnips, and beets were common items in the diet in the post-medieval period. No reference to dietary hardships in the Basque Country are encountered in the available sources. Whereas this may be a case of omission rather than fact, it is possible the Basques were relatively better off in terms of diet than much of Europe.

JENSENVANNET

The Site (see Albrethson 1985, 1986)

The Basque monopoly on whaling was broken when the Dutch and English opened the Svalbard whale fishery in the second decade of the seventeenth century (Spence 1980). Svalbard was discovered in 1596 by Wilhelm Barents in his search for a northeast passage to Asia. The economic potential of this group of Arctic islands was soon recognized, and in 1611 the British and Dutch sent whaling

ships to the area. (Skeletal remains from the Dutch operations have been described in a series of publications by Maat (e.g., 1981, 1984, 1987). In 1615, the Danish-Norwegian king (at this time Denmark and Norway were a single country), Christian IV, claimed Svalbard as part of Greenland, and Danish-Norwegian ships were sent to the area to collect tolls and evict those without permission to be there. The expedition was a fiasco, with the Dutch and English largely ignoring the Danes. At the same time, however, several Danish-Norwegian companies received permission to hunt whales in Svalbard. Dutch whalers cooperated with the Danish-Norwegians during the early years of their enterprise, but the amicable relations ended in 1623 when the Dutch destroyed some of the Danish-Norwegian holdings on the east coast of Amsterdameiland (Amsterdam Island). The Danish-Norwegians re-established themselves on Danskoya (Danes' Island), and in the regular course of affairs buried their dead there.

In 1984, a Danish-Norwegian archaeological expedition was organized and sent to Danskoya to assess the archaeological remains of the seventeenth-century whaling efforts. A well-preserved oven-complex was located on a small beach terrace on Kobbe fiord on the west coast of the island. These are believed to relate to the Copenhagen Bay whaling station set up by the Danes when they first moved to Danskoya. A second oven complex with three associated

burial areas was located at Jensenvannet on the north coast of the island. Jensenvannet is not mentioned in written records pertaining to the Svalbard whaling operations, and hence may be presumed Dutch, British, Basque, or Danish-Norwegian. According to Albrethsen (1985), if the station were Dutch, it would be highly unusual that it was not recorded since Dutch historical sources relating to whaling are very thorough. The fact that the site is only 4 km southwest of the large Dutch station at Smeerenberg supports the notion that it is Danish-Norwegian, since this was the only group of whalers known to have had reasonable relations with the Dutch. Oven construction is very similar to the ovens constructed at Kobbe fiord (and different from the identified Dutch construction). Moreover, textiles found in association with the burials differ from those found at Dutch stations and seem to be Scandinavian (based on the type of wool used as well as the dyes). Taken together, this evidence suggests the Jensenvannet station was Danish-Norwegian.

Archaeological excavations at Jensenvannet concentrated on the three burial locations at the site since some of the burials were washing out of the ground. Fifty-three graves were recorded in total. The method of interment was similar in all cases. The body was placed on a bed of sawdust in a wooden coffin (in many cases, a pillow stuffed with sawdust or down was placed under the head), and covered by more

sawdust. The coffins were buried as deep as permafrost permitted, and stones were piled in an oval or rectangular pile on the top (a single pile sometimes covered up to three coffins). The majority of graves were aligned in the typical Christian pattern of head to the west, though there were considerable exceptions. A wooden cross inscribed with the name of the deceased was placed at the head (these are not well-preserved). Textiles were found in many graves.

Skeletal material is well-preserved due to the Arctic nature of the environment. Deaths are presumed due largely to work-related accidents, though scurvy is also a possibility. All graves are believed to date to the seventeenth century (Albrethsen, personal communication 1987).

The Sample

The sample employed in this study was selected from the group of 19 skeletons excavated in 1984 at the Gravplads 2 location, Jensenvannet. Rib fragments were selected from 10 individuals. All are adult males. Specific age estimates by J. B. Jorgensen (unpublished data) indicate the men varied in age between 20 and 60 years.

Since Danish/Norwegian ships sometimes had very mixed crews, it is possible that Basque or Dutch men are interred with Danish-Norwegians at Jensenvannet. This potential source of variation will be considered in assessing the

results of this study.

No animal bones were recovered from the site; hence no sample could be incorporated in this analysis.

Dietary Information

Published information on the diet of the Svalbard whalers relates exclusively to Dutch stations. Van Wijngaarden-Bakker and Pals (1981) review Dutch archaeological and historical evidence which indicates that "rations were plentiful but of low quality, and the exploitation of local resources was indispensible for survival, especially for the overwinterers" (van Wijngaarden-Bakker and Pals (1981:149). The following foods are indicated:

Meat	salt beef, salt and smoked pork, live chickens, reindeer, arctic fox, seal (limited), many bird species
Fish	cod (dried or salted)
Grains	rye (flour used for bread), barley, buckwheat, oats and wheat (perhaps combined in breakfast porridge) (broomcorn millet is represented by a few chaffs, but was probably used as a packing material)
Vegetables	beans and peas, scurvy-grass (*Cochlearia officinalis*)
Dairy products	cheese, butter
Fruit	plums, raisins, figs
Beverages	brandy, beer
Other	salt, mustard seed, linseed, hops, cleavers (*Galium aparine*)

While the Dutch whalers were clearly distinct from the Danish-Norwegians, the limited variety of preservable foods available to provision European crews for extended sea voyages and the finite resources of Svalbard suggest the diets of the two groups would have been similar.

ISOTOPIC IMPLICATIONS

The information presented above is intended to guide the interpretation of isotopic variation observed in the samples. The following observations are central to this investigation:

I. Whereas sugar, millet, and maize are mentioned in post-medieval historic records, C_4 plants do not constitute a significant part of the diet of any group in this study. Hence C_4 plant signatures are unlikely to have an impact on $\delta^{13}C$ values of the populations discussed.

II. Freshwater fish constitute an important resource in inland areas, but are unlikely to have an impact on $\delta^{13}C$ values, since they tend to resemble C_3 plants in their isotopic composition.

III. Marine resources constitute the primary source of enriched carbon in diets of the study area. Reliance on

marine resources is expected to vary with distance from the sea, though the importance of inland trade cannot be dismissed.

IV. Heterogeneity of group composition, in terms of age, sex, social class, and place of origin, will be reflected in isotopic values, insofar as the dietary histories of individuals differ.

V. Significant dietary shifts implied in seasonal occupations, like whaling, will be reflected commensurate with employment histories.

VI. Differences in preservation quality may have an impact on the range of variability displayed in the samples.

CHAPTER 6

RESULTS

The samples included in the present study are restricted to a specific time and space framework, post-medieval Europe. At the same time, however, they exemplify a number of factors which may influence isotopic variability. Samples incorporate inland and coastal dwellers, males and females, individuals of high and low social status, natural populations, and occupational aggregations. One sample is confined to men who died at a single moment in time, while others reflect periods of up to several hundred years. Burial environments range from temperate Europe to the Arctic, and the waters of the English Channel. These factors are given special consideration in the following analysis of $\delta^{13}C$ variation.

PRESERVATION

Results

The preservation status of each specimen included in this study was assessed in terms of four variables: yield, carbon and nitrogen content, and atomic C/N ratio (Table 10). The composition of modern bone (see Chapters 2 and 3) was used as a standard for this analysis.

TABLE 10: Sample Preservation Status

Sample (N)	Yield (%)	Carbon (wgt%)	Nitrogen (wgt%)	Atomic C/N
(Expected	20 - 30	50 - 51	18	3.3)
JV (10) [a]	17.4 - 25.7 [b] 22.3 ± 2.5 11.2	36.12 - 40.00 37.90 ± 1.19 3.1	12.83 - 13.65 13.20 ± 0.30 2.3	3.3 - 3.4 3.4 ± 0.05
MR (12)	14.4 - 22.7 19.0 ± 2.0 10.5	37.06 - 41.38 39.43 ± 1.50 3.8	12.78 - 14.88 13.74 ± 0.62 4.5	3.2 - 3.4 3.4 ± 0.07
RB (32)	4.9 - 19.4 9.3 ± 3.2 34.4	18.03 - 38.52 29.08 ± 5.28 18.2	4.26 - 13.63 9.83 ± 2.36 24.0	3.2 - 5.0 3.5 ± 0.4
SM (23)	7.5 - 18.8 12.2 ± 3.3 27.0	8.57 - 37.71 31.90 ± 6.18 19.4	2.84 - 13.86 11.40 ± 2.30 20.2	3.1 - 3.5 3.3 ± 0.08
SPC (35)	6.5 - 16.1 10.0 ± 2.4 24.0	12.27 - 36.84 21.99 ± 6.03 27.4	4.35 - 13.15 7.37 ± 2.25 30.5	3.07 - 3.90 3.51 ± 0.19
SPP (16)	7.7 - 17.3 11.7 ± 2.8 23.9	19.81 - 35.92 28.47 ± 4.99 17.5	7.04 - 12.75 10.25 ± 1.81 17.7	3.1 - 3.4 3.2 ± 0.09
SK (10)	5.2 - 11.5 7.6 ± 1.9 25.0	13.45 - 31.20 2.97 ± 6.62 28.2	4.25 - 10.80 8.00 ± 2.52 31.5	3.2 - 3.7 3.4 ± 0.1

[a] Sample names are abbreviated as follows:

JV	Jensenvannet	SPC	Saint-Pierre-du-Chardonnet
MR	*Mary Rose*	SPP	Saint-Pierre-le-Puellier
RB	Red Bay	SK	Spitalkirche
SM	Saint-Martin		

[b] Entries for each sample include the range, followed by the mean and standard deviation (1σ), and the coefficient of variation ($v = 100s/x$), where applicable (ratio level data only [Lark et al. 1968]).

Samples exhibit a considerable range of variability in preservation quality. The most favourably preserved specimens are from Jensenvannet (an Arctic environment) and the *Mary Rose* (a marine environment). Both samples show high collagen yields, and consistent carbon and nitrogen contents and atomic C/N ratios. The most variable preservation is seen in the Red Bay sample (an acidic soil environment).

With one exception (Saint-Martin), yield and carbon and nitrogen content data are normally distributed for each sample (Lilliefors test, $p > .05$ [Lilliefors 1967]). With one exception (Saint-Pierre-du-Chardonnet), atomic C/N ratios are *not* normally distributed ($p < .05$). Carbon isotope values are normally distributed for four samples (Jensenvannet, *Mary Rose*, Red Bay, and Saint-Pierre-le-Puellier). At least some of the deviation from normality in C/N and $\delta^{13}C$ data appears due to rounding procedures. Tests of the same data expressed to two decimal places, rather than one, indicates only two deviations from normality in C/N ratios (Red Bay and Saint-Martin) and one exception in $\delta^{13}C$ (Saint-Martin). The isotope results are in closer agreement if a .01 confidence level is assumed. In this case, only one deviation from normality is seen in the data expressed to two decimal places (Saint-Martin), and one in the data rounded to one place (Saint-Pierre-du-Chardonnet).

The interrelation of preservation variables and $\delta^{13}C$

values was estimated using the Pearson product-moment correlation coefficient (for normally distributed data) and Spearman's rank correlation coefficient (for non-normal data). Results are summarized in Table 11. The following significant correlations were identified among preservation variables: carbon content with nitrogen content in all samples; carbon content with yield in five samples; carbon content with C/N in three samples; nitrogen content with yield in four samples; nitrogen content with C/N in three samples; and yield with C/N in two samples.

Significant correlations between the state of preservation and carbon isotopic composition were identified in four samples. In all cases, the correlations are multiple; i.e., more than one variable is correlated with $\delta^{13}C$. In the *Mary Rose* sample, $\delta^{13}C$ is negatively correlated with yield and nitrogen content. In the other three (Red Bay, Saint-Martin, and Spitalkirche), the opposite pattern is evident: $\delta^{13}C$ is positively correlated with yield and carbon and nitrogen content, and negatively correlated with C/N (the last of these correlations is significant only in the Red Bay sample).

Many of these correlations are due to a small number of specimens with relatively high C/N values. The majority of C/N ratios in the study samples are restricted to the 2.9 to 3.6 range outlined by DeNiro (1985), but some values from Red Bay, Saint-Pierre-du-Chardonnet, and Spitalkirche are

TABLE 11: Significant Correlations Among Preservation Variables and $\delta^{13}C$ [a]

	Yield	Wgt% C	Wgt% N	C/N
Wgt% C	MR+, RB+,[b] SM+, SPC+, SK+			
Wgt% N	RB+, SM+, SPC+, SK+	JV+, MR+, RB+, SM+, SPC+, SPP+ SK+		
C/N	RB-, SM-	RB-, SM-, SPC-	RB-, SM-, SPC-	
$\delta^{13}C$	MR-, RB+ SK+	RB+, SM+ SK+	MR-, RB+ SM+, SK+	RB-

[a] Pearson product-moment correlation coefficients were calculated for normally distributed data, and Spearman's rank correlation coefficients for non-normal data ($p < .05$). See text for details of data distributions.

[b] Site names are abbreviated as per Table 10. The direction of the correlation is indicated by the sign (+ or -) appended to each abbreviation.

greater than 3.6.

Discussion

Collagen yield and atomic C/N ratios have been employed as indicators of preservation quality in a number of isotope studies (see Chapter 3), but little attention has been given to absolute abundances of carbon and nitrogen in collagen extracts. Both the carbon and nitrogen content of collagen, and its atomic C/N ratio, have fixed values. Thus, whether a bone specimen yields 5% or 20% collagen, this collagen should be approximately 50-51 wgt% carbon and 18 wgt% nitrogen, with an atomic C/N ratio of 3.3. It is apparent in Table 10, however, that there is considerable deviation from these values in the archaeological samples analyzed in this study, with carbon and nitrogen contents varying more than C/N ratios.

This deviation indicates the presence of inorganic inclusions in the extract. In Chapter 4, it was noted that the collagen extraction procedures employed in this study produce a residue when blanks are processed, implying a residue is also present in extractions of bone specimens. This residue clearly represents a greater part of yield when the collagen content of bone is low than when it is high, and will cause the carbon and nitrogen content of the collagen to be under-represented in the total extract.

To evaluate the effects of residue on the elemental

composition of collagen, the yield for each specimen in this study was adjusted by the average 26 mg of residue produced in the blank runs. (Adjusted yield = [collagen - residue] ÷ amount of bone powder processed.) The elemental composition of the total extract was then corrected to the adjusted yield. The results are presented in Table 12.

While yield remains a variable quantity, carbon and nitrogen contents are much more consistent from one sample to the next after the residue adjustment. The results clearly illustrate that an inorganic residue will explain the shift in carbon and nitrogen content with decreasing yield. The relatively low adjusted values for the Saint-Pierre-du-Chardonnet, Saint-Pierre-le-Puellier,and Spitalkirche samples may suggest additional inorganic inclusions (possibly clay particles).

The "blank residue" may also explain a tendency for higher C/N values to be associated with low yields, and their implied low carbon and nitrogen contents. (The relation between C/N values and nitrogen content is illustrated in Figure 2.) As noted in Chapter 4, elemental analysis of blank residues obtained in this study indicated the presence of a very small proportion of carbon (1.1-2.5%) and nitrogen (0.0-0.3%), contaminants presumed to originate in NaOH. The effect of this carbon and nitrogen on the elemental composition of high-yield specimens is minimal, since their collagen content overwhelms the residue content,

TABLE 12: Adjusted Preservation Estimates

Site	N	Yield [a] (%)	Elemental Composition [b] Carbon (wgt%)	Nitrogen (wgt%)
(Expected		20 - 30	50 - 51	18)
JV	10	19.8 ± 2.5	42.68 ± 1.36	14.87 ± 0.31
MR	12	16.6 ± 1.9	45.08 ± 1.33	15.70 ± 0.58
RB	32	6.9 ± 3.2	40.82 ± 5.73	13.70 ± 2.63
SM	23	9.6 ± 3.3	40.88 ± 6.64	14.60 ± 2.46
SPC	35	7.7 ± 2.4	29.05 ± 6.05	9.71 ± 2.31
SPP	16	9.3 ± 2.8	36.56 ± 6.18	13.16 ± 2.21
SK	10	5.1 ± 1.9	34.62 ± 6.70	12.02 ± 2.71

[a] Yield estimates are adjusted to take into account the average 26 mg residue produced by the extraction procedures when no bone is included (see Chapter 4):
Adjusted yield = (collagen - residue) ÷ amount of bone powder

[b] Elemental composition is modified to reflect adjusted yield:
Adjusted carbon = (Wgt% C)(Adjusted yield / Yield)
Adjusted nitrogen = (Wgt% N)(Adjusted yield / Yield)

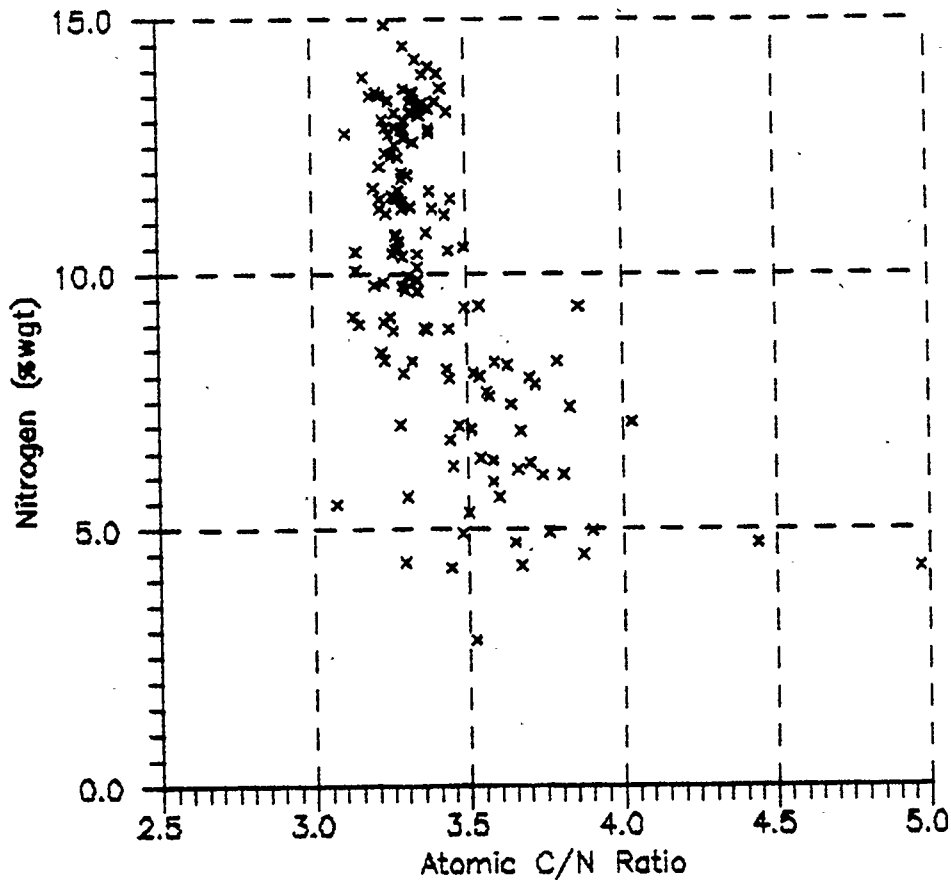

FIGURE 2: Scatter diagram of nitrogen content and atomic C/N ratio data for the study samples.

but may become significant in low-yield specimens. If the total yield is only 5%, and presuming that 1 g of bone powder is used, a residue of 26 mg would constitute 50% of the total. Under these circumstances, the carbon content of the residue, though slight, could bias the elemental composition of the extract and, therefore, its C/N ratio.

The Saint-Pierre-du-Chardonnet sample may be a case in point. On average, specimens from this sample contain a much smaller percentage of carbon and nitrogen than other samples. While no significant correlations exist between $\delta^{13}C$ values and preservation indicators, the average C/N value for this sample is significantly larger than in other samples. Perhaps residue composition is a significant factor here.

This is not to suggest extraction residues are entirely responsible for variability in the carbon and nitrogen content of collagen extracts. Machine error may be implied; i.e., as the amount of nitrogen in the specimen decreases, the magnitude of measurement errors may increase. At the time the Saint-Pierre-du-Chardonnet specimens were analyzed, the supply of helium required for elemental analysis was low, and this may have contributed to variation in values for this sample.

Extracts of archaeological bone which deviate from the normal elemental composition of collagen also may reflect diagenetic change. This effect, however, should be evident

in supplementary correlations with $\delta^{13}C$ values. In this study, *all* correlations between preservation variables and $\delta^{13}C$ are presumed indicative of diagenetic effects on isotope values, unless proven otherwise. In the absence of such correlations (as in the Saint-Pierre-du-Chardonnet sample), no diagenetic effects are suspected.

In three of the four samples displaying correlations between $\delta^{13}C$ and preservation state (Red Bay, Saint-Martin, and Spitalkirche), lighter isotope values are associated with a pattern of decreasing yield, lower carbon and nitrogen contents, and higher C/N values. This pattern is consistent with the presence of humic contaminants, which generally have lighter $\delta^{13}C$ values and larger C/N ratios than human bone. Moreover, as noted in Chapter 3, humics are more likely to have a significant impact on low yield specimens than high. The possibility that the pattern of association is tied to bone degradation (with an implied preferential loss of nitrogen and ^{13}C), rather than contamination, is not as favourable an explanation, since correlations are not consistently demonstrated in low yield specimens.

The *Mary Rose* sample displays the opposite pattern of correlations to that noted above. In this case, heavier isotope values are associated with decreasing yields, lower carbon and nitrogen contents, and higher C/N values. Burial in a marine environment must account for this difference.

Since marine humic substances typically have values in the -20 to -22‰ range, they cannot account for the shift. It is possible, however, that deposits left by marine bone-boring organisms are isotopically enriched relative to collagen.

In all samples showing evidence of a correlation between preservation status and $\delta^{13}C$, outlying specimens which appear responsible for the relationship can be identified. Significant correlations disappear when these specimens are removed. Specific revisions made in the composition of study samples are detailed below.

Sample Refinement

The Mary Rose. The three heaviest isotope values from the *Mary Rose* sample are associated with specimens displaying low yield (Figure 3), and low carbon and nitrogen contents. When these are removed from the sample, significant correlations with $\delta^{13}C$ disappear. The statistical effects of removing these specimens are illustrated in Table 13.

Removal of outlying values has a greater impact on the variability evident in the sample than on mean values, which change by less than 5% in all cases. Variability, by comparison, is reduced by up to 50%.

Red Bay. The Red Bay sample demonstrates the most significant correlations between isotope values and

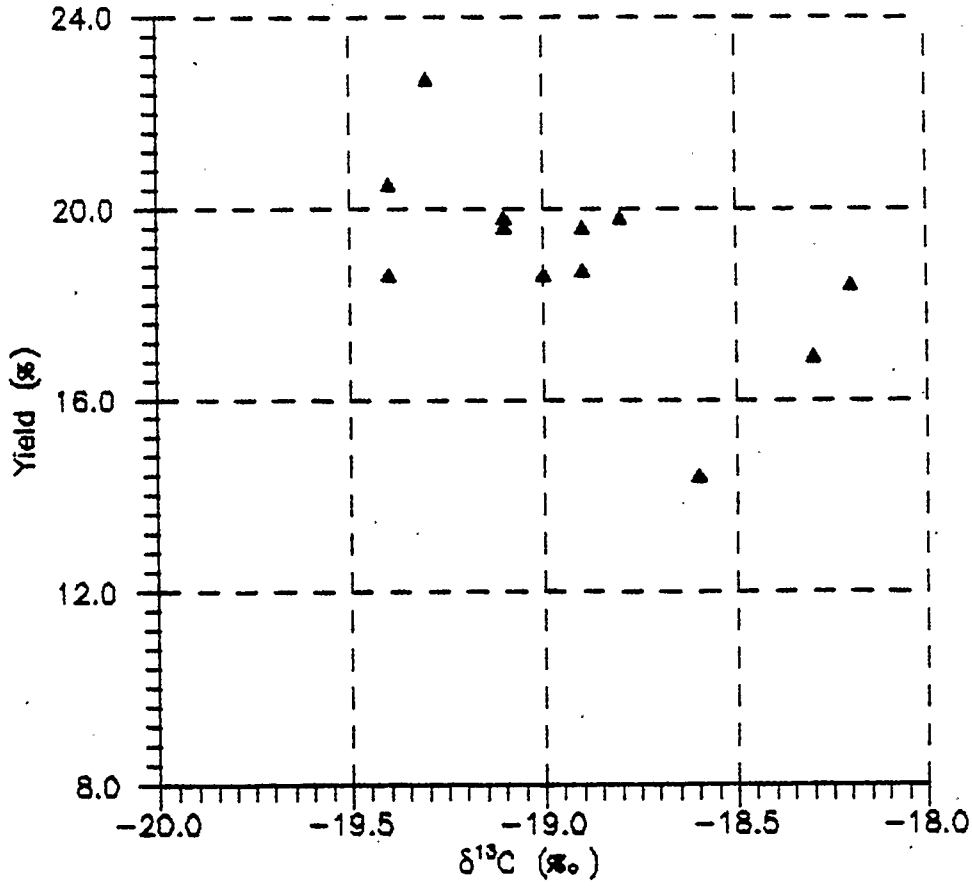

FIGURE 3: Scatter diagram of yield and $\delta^{13}C$ data from the *Mary Rose* sample.

TABLE 13: Comparison of Data from the Original and Revised *Mary Rose* Samples

Variable		Original Sample	Revised Sample	Change (%)[a]
Yield (%)	$\bar{x}$	19.0	19.8	4
	s	2.0	1.3	-35
	v	10.5	6.6	-37
C (wgt%)	$\bar{x}$	39.43	39.87	1
	s	1.50	1.41	-6
	v	3.8	3.5	-8
N (wgt%)	$\bar{x}$	13.74	13.92	1
	s	0.62	0.57	-8
	v	4.5	4.1	-9
At. C/N	$\bar{x}$	3.4	3.3	-3
	s	0.07	0.07	0
$\delta^{13}C$ (‰)	$\bar{x}$	-18.9	-19.1	1
	s	0.4	0.2	-50

[a] $$\text{Change} = \left[\frac{\text{Value for Revised Sample}}{\text{Value for Original Sample}} - 1\right] \times 100$$

preservation indicators of any of the study samples. This is not surprising since, as stated in Chapter 5, bone preservation was generally very poor in the Red Bay cemetery, with less than 25% of the individuals interred at the site removed from the ground for further osteological analysis. Even in the latter cases, many bones were degraded, and root-invasion was a serious problem.

The distribution of C/N values in the Red Bay sample peaks at the predicted value of 3.3, but is then positively skewed to a high of 5.0. Specimens with C/N values above 3.6 are, in general, isotopically lighter than those with C/N values below 3.6 (Figure 4). The latter values were removed from the sample in a step-wise process until the correlations were no longer significant, a point reached when all specimens having C/N values > 3.6 were removed, followed by a single specimen displaying low values for $\delta^{13}C$ and carbon and nitrogen content.

The overall effects of the Red Bay sample revision are illustrated in Table 14. It is clear that removal of outlying C/N values had a significant impact on the range of variability in elemental composition (a 47% reduction in the coefficient of variation for wgt% N, and a 36% reduction in the coefficient of variation for wgt% C), C/N ratios (a 75% reduction in the standard deviation), and $\delta^{13}C$ values (a 43% reduction in the standard deviation). Variation in yield is not as strongly affected (a 7% reduction in the

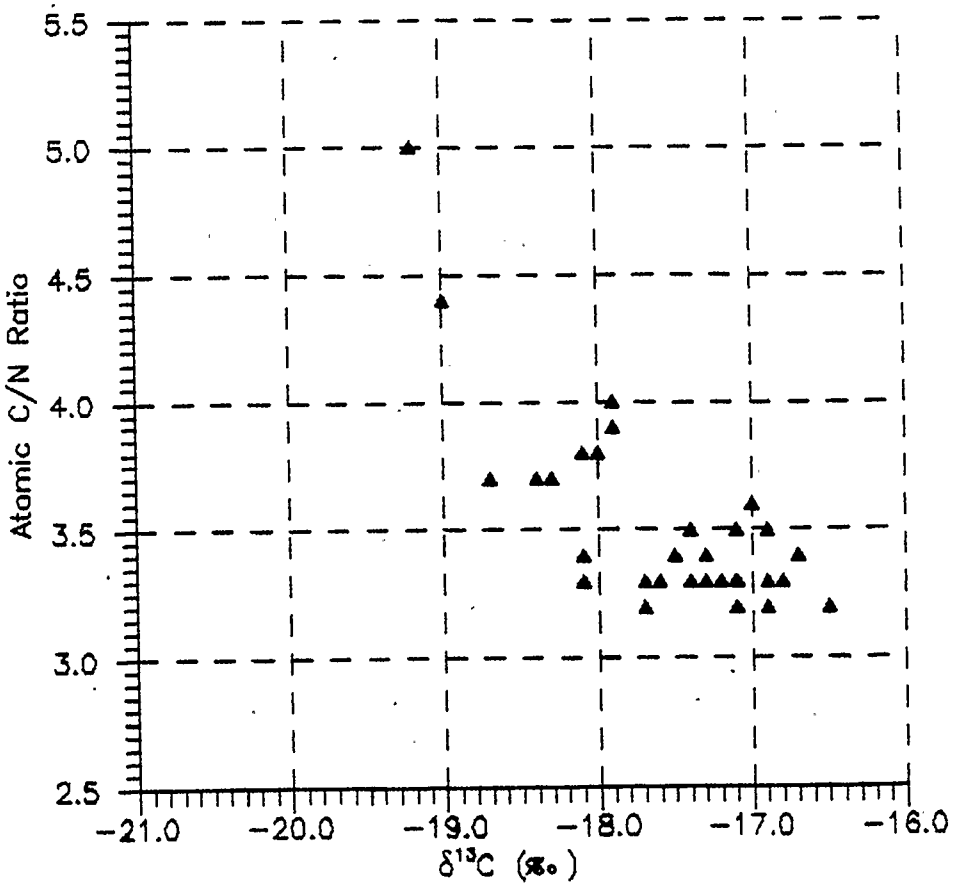

FIGURE 4: Scatter diagram of atomic C/N ratio and $\delta^{13}C$ data from the Red Bay sample.

TABLE 14: Comparison of Data from the Original and Revised Red Bay Samples

Variable		Original Sample	Revised Sample	Change (%)[a]
Yield (%)	$\bar{x}$	9.3	10.3	11
	s	3.2	3.3	3
	v	34.4	32.0	-7
C (wgt%)	$\bar{x}$	29.08	31.56	9
	s	5.28	3.67	-30
	v	18.2	11.6	-36
N (wgt%)	$\bar{x}$	9.83	11.07	13
	s	2.36	1.41	-40
	v	24.0	12.7	-47
At. C/N	$\bar{x}$	3.5	3.3	-6
	s	0.4	0.1	-75
$\delta^{13}C$ (‰)	$\bar{x}$	-17.6	-17.2	-2
	s	0.7	0.4	-43

[a] $$\text{Change} = \left[\frac{\text{Value for Revised Sample}}{\text{Value for Original Sample}} - 1\right] \times 100$$

coefficient of variation). Change in mean values is ≤ 13%.

Saint-Martin. Significant correlations between preservation variables and $\delta^{13}C$ disappear in the Saint-Martin sample if a single specimen with aberrant values for these variables (relative to the rest of the sample) is removed (Figure 5). It is notable that the C/N ratio for this specimen (3.5) does not exceed the limits set by DeNiro (1985), but is larger than other sample values, which range from 3.1 to 3.4.

The overall effect of this sample revision is indicated in Table 15. Once again, there is minimal change in mean values (< 3%), but a significant reduction in variation in wgt% C (44%) and wgt% N (43%). Yield, C/N ratios, and $\delta^{13}C$ values are less affected (3%, 13%, and 8%, respectively).

Spitalkirche. Correlations indicative of diagenesis are eliminated when a single specimen which displays the lightest carbon value, and a C/N ratio of 3.7, is removed (Figure 6). The effects on sample statistics are illustrated in Table 16. Again, the net impact is a reduction in variation, ranging up to 25%. Changes in mean values are ≤ 5%.

The sample revisions made in this study may not remove all specimens affected by diagenetic change. It is assumed,

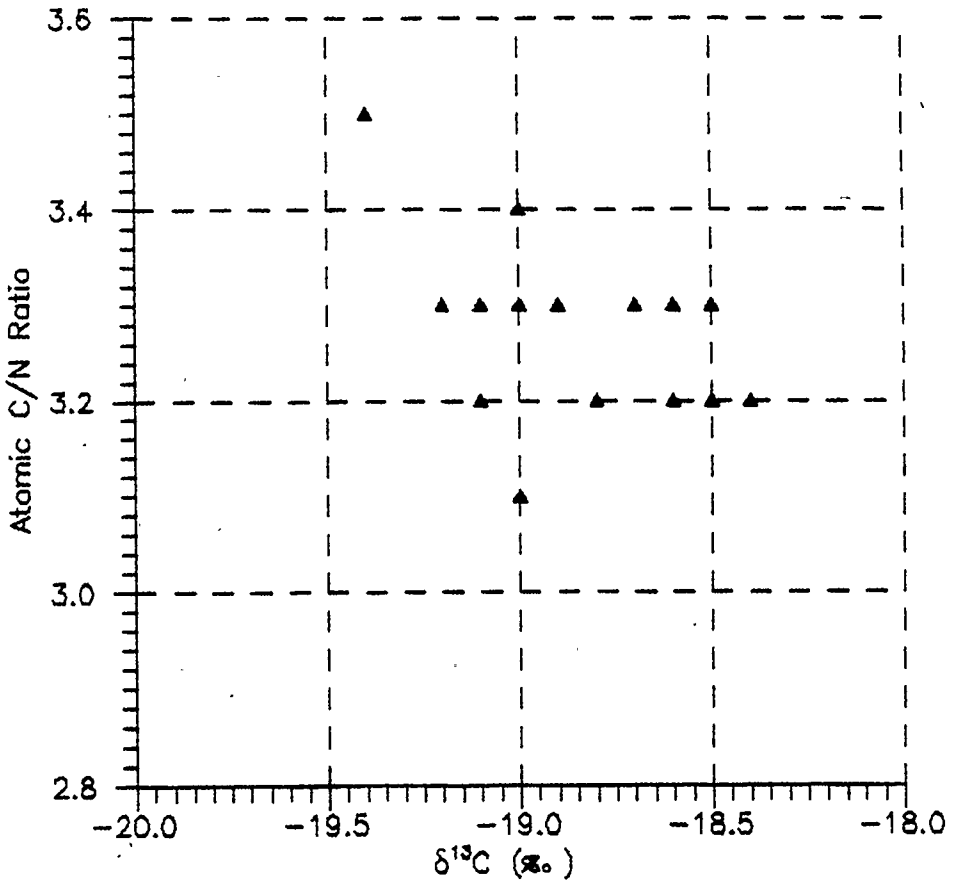

FIGURE 5: Scatter diagram of atomic C/N ratio and $\delta^{13}C$ data from the Saint-Martin sample.

TABLE 15: Comparison of Data from the Original and Revised Saint-Martin Samples

Variable		Original Sample	Revised Sample	Change (%)[a]
Yield (%)	$\bar{x}$	12.2	12.4	2
	s	3.3	3.2	-3
	v	27.0	26.2	-3
C (wgt%)	$\bar{x}$	31.90	32.96	3
	s	6.18	3.59	-42
	v	19.4	10.9	-44
N (wgt%)	$\bar{x}$	11.40	11.79	3
	s	2.30	1.37	-40
	v	20.2	11.6	-43
At. C/N	$\bar{x}$	3.3	3.3	0
	s	0.08	0.07	-13
$\delta^{13}C$ (‰)	$\bar{x}$	-18.8	-18.8	0
	s	0.3	0.2	-33

[a] $$\text{Change} = \left[\frac{\text{Value for Revised Sample}}{\text{Value for Original Sample}} - 1\right] \times 100$$

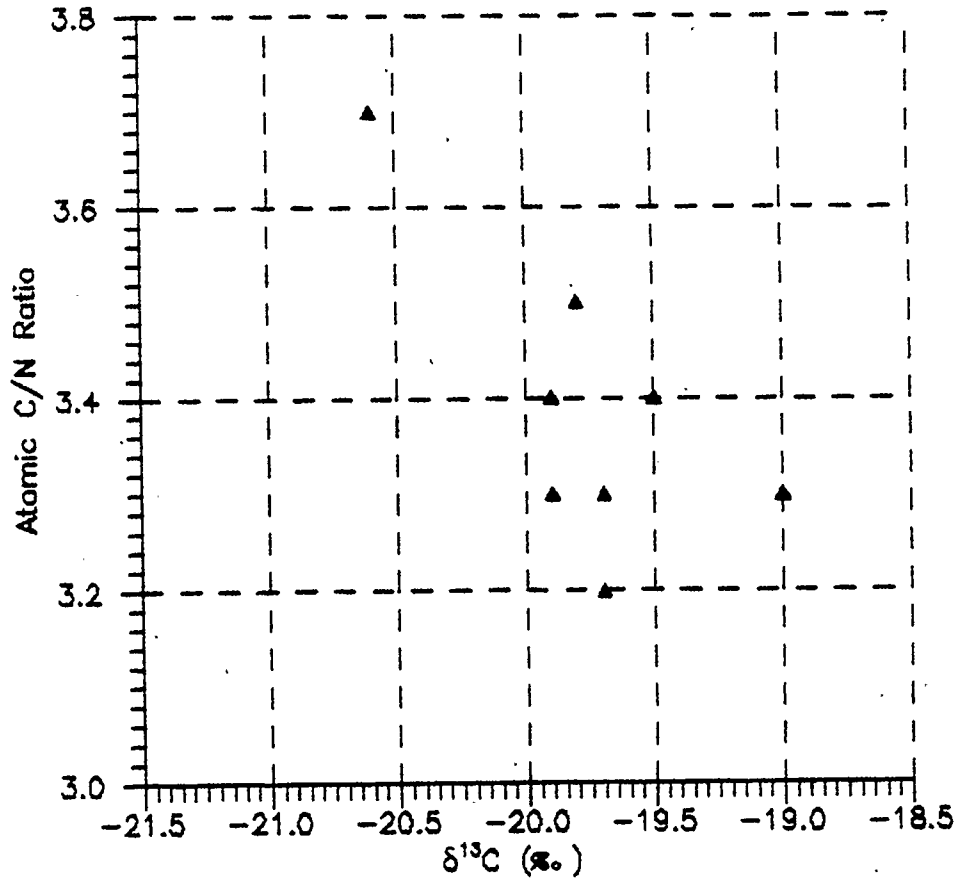

FIGURE 6: Scatter diagram of atomic C/N ratio and $\delta^{13}C$ data from the Spitalkirche sample.

TABLE 16: Comparison of Data from the Original and Revised Spitalkirche Samples

Variable		Original Sample	Revised Sample	Change (%)[a]
Yield (%)	$\bar{x}$	7.6	7.8	3
	s	1.9	1.9	0
	v	25.0	24.4	-2
C (wgt%)	$\bar{x}$	22.97	24.02	5
	s	6.62	6.07	-8
	v	28.8	25.3	-12
N (wgt%)	$\bar{x}$	8.00	8.41	5
	s	2.52	2.29	-9
	v	1.5	27.2	-14
At. C/N	$\bar{x}$	3.4	3.3	-3
	s	0.1	0.09	-10
^{13}C (‰)	$\bar{x}$	-19.8	-19.7	0
	s	0.4	0.3	-27

[a] $$\text{Change} = \left[\frac{\text{Value for Revised Sample}}{\text{Value for Original Sample}} - 1\right] \times 100$$

however, that variation in the retained specimens (N = 123) is closer to the "true" sample variation than would have been otherwise the case. The four samples which were revised to eliminate diagenetically-altered specimens show no significant correlations between $\delta^{13}C$ and preservation variables. Nevertheless, it is notable that the pre-revision pattern of relationships is retained; i.e., a tendency toward the association of lighter isotope values, lower yields, lower carbon and nitrogen contents, and higher C/N ratios. Until precise methods of identifying collagen contamination and/or alteration are devised, however, to reduce these samples further would risk the introduction of observer bias.

VARIATION IN CARBON ISOTOPE VALUES

Statistical Analysis

Variation in $\delta^{13}C$ values in the revised study samples is summarized in Table 17. All sample means fall within a range of 2.5‰. Five of the seven samples have average values close to -19.0‰. Included in this category are the men from the *Mary Rose* ($\bar{x}$ = -19.1‰) and Jensenvannet ($\bar{x}$ = -19.1‰), as well as individuals from the three sites in Tours: Saint-Pierre-du-Chardonnet ($\bar{x}$ = -19.0‰), Saint-Martin ($\bar{x}$ = 18.8‰), and Pierre-le-Puellier ($\bar{x}$ = -18.7‰). The most negative values are seen in the Spitalkirche sample

TABLE 17: $\delta^{13}C$ Values of Revised Samples

	$\delta^{13}C$ (‰)					
Sample	N	Min	Max	Range	Mean	SD
JV	10	-19.6	-18.1	1.5	-19.1	0.5
MR	9	-19.4	-18.0	0.6	-19.1	0.2
RB	23	-18.1	-16.5	1.6	-17.2	0.4
SM	22	-19.2	-18.4	0.8	-18.8	0.2
SPC	35	-19.9	-18.9	1.5	-19.0	0.3
SPP	16	-19.6	-18.3	1.3	-18.7	0.3
SK	10	-19.9	-19.0	0.9	-19.7	0.3

($\bar{x}$ = -19.7‰), and the most positive in the Red Bay sample ($\bar{x}$ = -17.2‰).

The equivalency of sample means was tested by analysis of variance. ANOVA assumptions of normality and homogeneity of variance were evaluated using Lilliefors and Bartlett's tests, respectively. With two exceptions, (Saint-Martin and Saint-Pierre-du-Chardonnet), the $\delta^{13}C$ values of the revised samples were normally distributed ($p > .01$). A test of values expressed to two decimal places showed all samples to be normally distributed ($p > .05$). Since sample variances are equivalent, the decision was made to proceed. ANOVA results indicated at least one mean to be significantly different from the others ($p < .05$). To establish more precise relationships, samples were paired and contrasted. The results of these paired analyses, presented in Table 18, show the Spitalkirche and Red Bay sample means to be significantly different from all others. The Jensenvannet, *Mary Rose*, and Saint-Pierre-du-Chardonnet means are not significantly different from one another. The Saint-Martin and Saint-Pierre-le-Puellier means are also equivalent.

Within-sample variation in $\delta^{13}C$ values is greatest for samples from Jensenvannet (range = 1.5‰, 1σ = 0.5‰) and Red Bay (range = 1.6‰, 1σ = 0.4‰). The least variation is seen in the samples from the *Mary Rose* (range = 0.6‰, 1σ = 0.2‰) and Saint-Martin (range = 0.8‰, 1σ = 0.2‰). Other samples have intermediate values.

TABLE 18: $\delta^{13}C$ Variation Between Sample Pairs [a]

	MR	RB	SM	SPC	SPP	SK
JV	-	+	+	-	+	+
MR		+	+	-	+	+
RB			+	+	+	+
SM				+	-	+
SPC					+	+
SPP						+

[a] This table presents results of analysis of variance, contrasting the effects of different samples on the overall pattern of significant difference between sample means ($p < .05$). A "+" sign indicates a significant difference between paired samples. A "-" sign indicates no significant difference. (Sample names are abbreviated as per Table 10).

The Saint-Pierre-du-Chardonnet and Saint-Pierre-le-Puellier samples incorporate both males and females. There is no significant difference in $\delta^{13}C$ values between the sexes (t-test, $p > .05$) (Table 19). Neither is there any suggestion that one sex displays more variation in $\delta^{13}C$ values than the other.

In the samples for which age estimates were available (Jensenvannet and Saint-Pierre-le-Puellier), variation among age categories was also assessed. Men from Jensenvannet were assigned to one of two age groups: Group 1 ≤ 40 years or Group 2 > 40 years (these categories reflect the precision of the age estimates supplied). Figure 7 illustrates a trend toward lighter and more variable $\delta^{13}C$ values in the older men. The mean value for the younger group (-19.1 ± 0.6‰, N = 5), however, is not significantly different than the mean for the older group (-19.0 ± 0.4‰, N = 5) (t-test, $p > .05$).

Individuals from Saint-Pierre-le-Puellier were assigned to 10-year age categories, based on the estimates supplied. Statistical assessment of these data is limited by the number of individuals in the 50-59 and 60+ categories. Nevertheless, no difference between these older individuals and the more widely distributed younger values can be identified (Figure 8).

In the Red Bay sample, it was possible to assess the effects of element choice on $\delta^{13}C$ values. Isotope values

TABLE 19: Sex Differences in $\delta^{13}C$ Values

Sample[a]	Sex	N	Mean ± 1σ (‰)	t-test Probability
SPC	M	17	-19.0 ± 0.4	
	F	18	-19.1 ± 0.3	0.57
SPP	M	8	-18.7 ± 0.3	
	F	8	-18.8 ± 0.4	0.28

[a] Sample names are abbreviated as per Table 10.

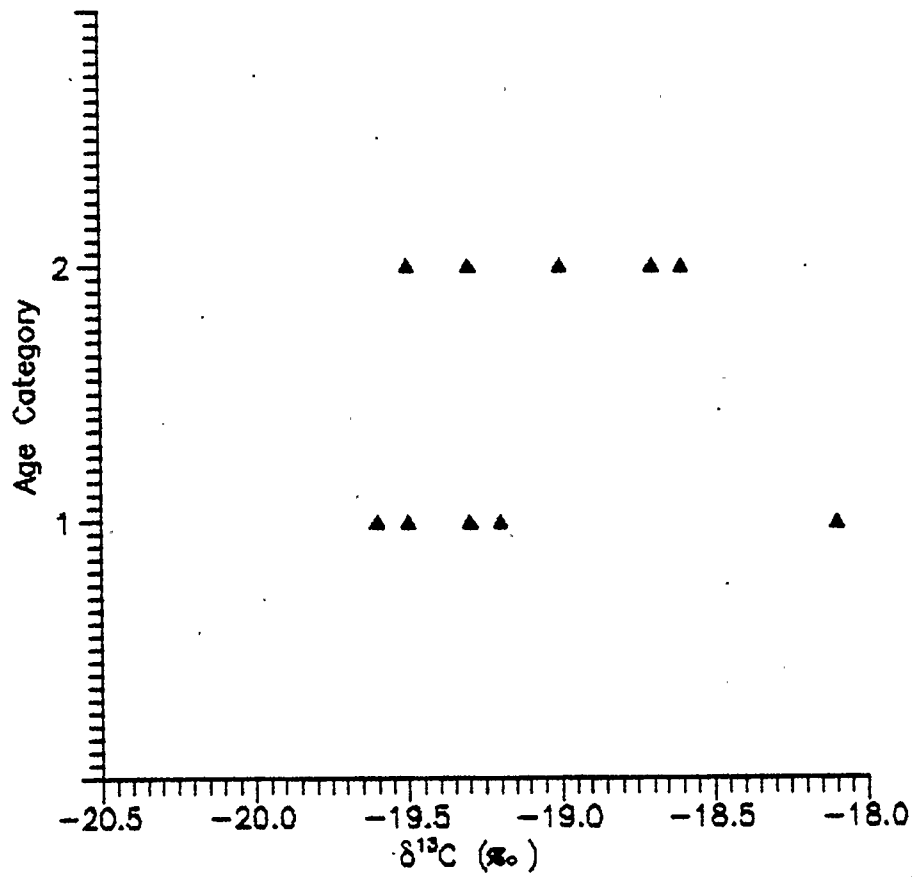

FIGURE 7: $\delta^{13}C$ variation by age category‡ in the Jensenvannet sample.

‡ Category 1: < 40 years; Category 2: ≥ 40 years

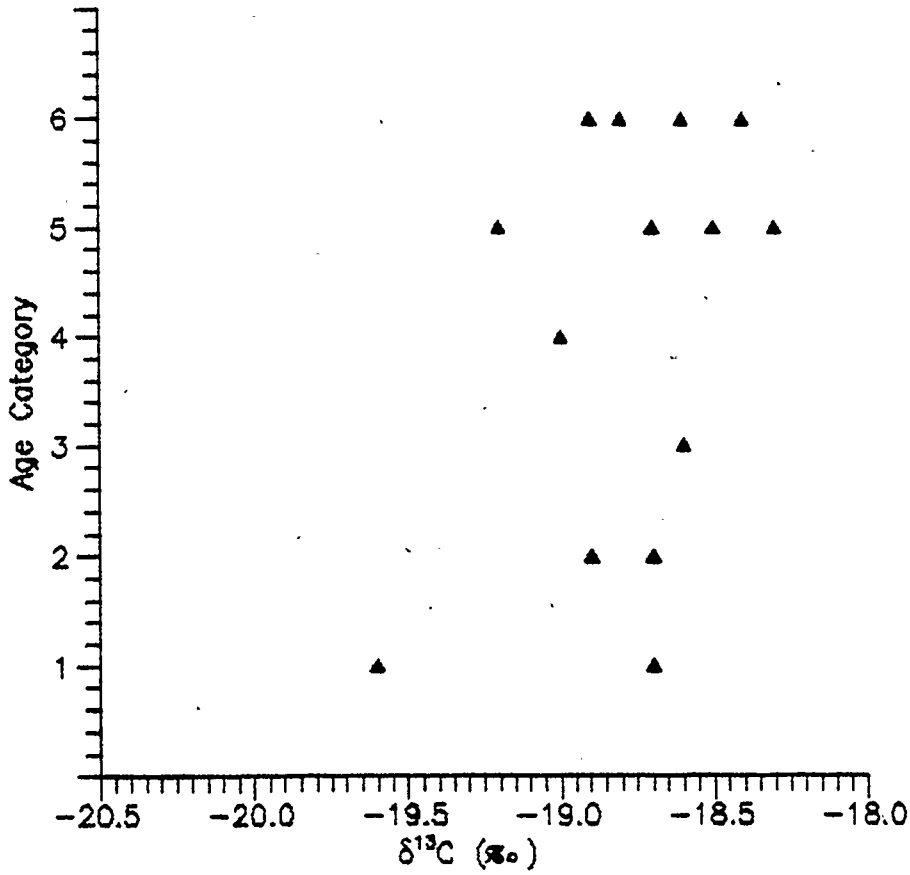

FIGURE 8: $\delta^{13}C$ variation by age category* in the Saint-Pierre-le-Puellier sample.

* Category 1: 17-19 years; Category 2: 20-20 years; Category 3: 30-30 years; Category 4: 40-49 years; Category 5: 50-59 years; Category 6: ≥ 60 years

for ribs, as a group, were contrasted with those of non-rib elements as a group. The mean value for ribs (-17.0 ± 0.3‰, N = 12) is significantly different from the mean for non-ribs (-17.5 ±0.3‰, N = 10) (t-test, $p < .05$), though both groups display the same variability. The difference between rib and non-rib elements is obvious in Table 20 which ranks $\delta^{13}C$ values from lightest to heaviest.

Also obvious is the homogeneity of values from multiple burials. Individuals from multiple burials generally follow one another in the $\delta^{13}C$ ranking (Table 20). The burials which break this pattern include at least one value from a non-rib specimen. Even breaking this pattern, however, the values do not approach what would be expected in a random sample from the site.

Dietary Interpretation

The contributions of marine and terrestrial carbon sources to the carbon isotopic composition of bone collagen can be assessed using mass balance equations. To do so, however, requires the establishment of average $\delta^{13}C$ values for the marine and terrestrial carbon reservoirs available to consumers. End-points used in this study were established from animal bone samples from the same temporal and geographic range as the human samples.

A total of 17 specimens were obtained for analysis, two of which proved to contain no collagen when extracted. The

TABLE 20: Variation Among Specimens from the Red Bay Sample

$\delta^{13}C$ (Rank)	$\delta^{13}C$ (‰)	Burial	Element
1	-18.1	36I	Non-rib
2	-17.7	38B	Non-rib
3	-17.7	36H	Non-rib
4	-17.6	36C	Non-rib
5	-17.5	8A	Rib
6	-17.4	9B	Non-rib
7	-17.4	9C	Non-rib
8	-17.3	11B	Non-rib
9	-17 3	36G	Non-rib
10	-17.3	36A	Non-rib
11	-17.2	35	Non-rib
12	-17.2	11A	Rib
13	-17.1	6E	Rib
14	-17.1	6C	Rib
15	-17.1	6F	Rib
16	-17.0	6B	Rib
17	-16.9	3	Rib
18	-16.9	4C	Rib
19	-16.9	4D	Rib
20	-16.8	4A	RIb
21	-16.7	4C	Rib
22	-16.5	7	Rib

results of analysis of these specimens are reported in Table 21. The overall value for terrestrial animals (including the freshwater fish sample) from Marbach and Tours is -20.9 ± 1.1‰, while the value for marine specimens from Red Bay is -14.0 ± 0.6‰. (Bear and dog values are not included in this estimate.) The difference between the two data sets, 6.9‰, is very close to the 7‰ difference in marine and terrestrial carbon reservoirs noted in Chapter 2. The marine end-point is reasonably close to the -13.7‰ value determined by Tauber (1983) for 16 marine mammal specimens from Denmark and Greenland. The terrestrial value is lighter than Tauber's average (-20.1‰), but this reflects the fact that 50% of Tauber's sample of 40 animals is composed of elk, muskox, and reindeer, with average values more positive than -20‰.

Assuming a diet-collagen spacing of +4.5‰ (see Chapter 2), the values for terrestrial and marine animals translate into values of -25.4% for the terrestrial food-web base and -18.5‰ for the marine food-web base. Using these estimates, the proportion of terrestrial and marine carbon in bone collagen can be estimated by the following mass balance equation:

$$\delta^{13} C_C = \delta^{13} C_T \ (x) + \delta^{13} C_M (1-x) + \Delta_{dc}$$

where,

$\delta^{13}C_C$ = carbon isotope value in collagen;

TABLE 21: Assessment of Animal Bone $\delta^{13}C$

Site	Animal	$\delta^{13}C$ (‰)
Marbach, Germany	Cattle	-21.2
	Chicken	-19.5
	Pig	-20.1
	Sheep	-20.6
Tours, France	Cattle	-21.3
	Chicken	-21.3
	Pig	-21.9
	Sheep	-19.4
	Fish	-22.8
Red Bay, Labrador	Cod	-14.3
	Seal	-13.3
	Whale	-14.5
	Bear	-16.5
	Dog	-14.3

$\delta^{13}C_T$ = average carbon isotope value of terrestrial plants (-25.4‰);

$\delta^{13}C_M$ = average carbon isotope value of marine plants (-18.5‰); and

Δ_{dc} = average diet-collagen spacing (+4.5‰).

This model provides only an approximate estimate of terrestrial and marine input, given the averaging process implied in determining end-point values and diet-collagen spacing. It is also important to note that, while the equation models the contribution of dietary carbon to collagen carbon, this cannot be translated directly into dietary proportions (van der Merwe 1982), since to do so requires a more complete understanding of the relationship between macronutrient sources, nutrition, and collagen than currently exists. Nevertheless, it is assumed the results give an approximation of the relative importance of marine and terrestrial foods in human diets.

The results of assessments of the study samples are presented in Table 22. The contribution of marine carbon to bone collagen carbon content ranges from 54% in the Red Bay sample to 18% in the Spitalkirche sample. Significant differences in $\delta^{13}C$ indicated by the ANOVA analysis translate into differences in marine carbon contribution as small as 2%.

TABLE 22: Mass Balance Assessment of Study Samples

Sample[a]	Carbon Contribution (%)	
	Terrestrial Sources	Marine Sources
JV	74	26
MR	74	26
RB	46	54
SM	70	30
SPC	72	28
SPP	68	32
SK	83	17

[a] Sample names are abbreviated as per Table 10.

Discussion

Variation Among Samples. The presence of significant differences in the $\delta^{13}C$ values of samples included in this study comes as no surprise, given the geographic separation of many of the sites. It was predictable that the Spitalkirche sample, from the most inland site included in this study, would display the least input of marine-based carbon. It was also predictable that the Red Bay sample would display one of the heaviest inputs, given the maritime occupation of these whalers. It was not anticipated, however, that two samples from the inland site of Tours (Saint-Martin and Saint-Pierre-le-Puellier) would have heavier $\delta^{13}C$ values than the soldiers and sailors of the *Mary Rose*, and the whalers of Jensenvannet. The third Tours sample (Saint-Pierre-du-Chardonnet) shows an average value close to the English and Danish-Norwegian samples. The difference in marine carbon contribution between the Saint-Pierre-du-Chardonnet and the other French samples is less than 5%, but is statistically significant.

Differences in values for the three Tours samples may be linked to social status. The people buried at Saint-Pierre-du-Chardonnet were of a lower social class than those interred in the other cemeteries. Given that fish was not always a cheap commodity in post-medieval Europe, it is possible that these people could not afford to purchase the same amount of marine resources or did not have the same

market access. The slightly lighter values of the *Mary Rose* and Jensenvannet values versus two of the Tours sites is more difficult to explain, since it is assumed these sailors and whalers would have had more liberal access to cod and other marine fish than the citizens of Tours. These values may relate to religious prescriptions regarding fish consumption in Catholic France versus Protestant England and Denmark, with fewer fast days in Protestant countries.

The average values for the samples included in this study fall in the mid-range of results reported for other European samples to date, approaching neither the established upper or lower limits (Table 23). Previous studies have focused on Scandinavian samples, with some of the data derived from radiocarbon determinations of $\delta^{13}C$ values. (In Table 23, radiocarbon data are limited to published summaries from restricted temporal and/or spatial ranges.) Results from Jensenvannet, the only Scandinavian sample included in this study, echo the -18 to -20‰ range most common in Neolithic specimens from Denmark, reported by Tauber (1983; see also Schoeninger et al. 1983). They also overlap the values from seventeenth-century Oslo, reported by Johansen et al. (1986). The mean value is significantly heavier than that for Dutch whalers from Svalbard reported by Schoeninger (n.d.), supporting Albrethsen's (1985) assertion that the Jensenvannet whaling station was not Dutch. (The possibility that the station was English cannot

TABLE 23: $\delta^{13}C$ Variation in Europeans: Previous Studies

Provenience	N	Mean ± 1σ (‰)	Ref.[a]
RADIOCARBON DETERMINATIONS			
Norway			
West and North, Stone Age	4	-16.3 ± 0.6	a
South, Stone/Bronze Age	3	-20.3 ± 1.7	a
East, Early Iron Age	3	-21.8 ± 1.0	a
North, Medieval and Post-medieval	3	-15.2 ± 2.5	a
North Central, Medieval and Post-medieval	4	-18.4 ± 1.2	a
Denmark			
Coastal, Mesolithic	8	(-11 to -16)[b]	b,c
Coastal, Neolithic	31	(-17 to -27)[b]	b,c
Iron Age	40	-19.4 ± 0.7	d
DIET DETERMINATIONS			
Norway			
Coastal, Stone Age	2	-13.4	a
Coastal, Late Iron Age	5	-17.0 ± 1.0	a
Coastal, Early Medieval	11	-16.9 ± 1.2	a
Inland, Medieval	10	-20.6 ± 0.3	a
Oslo, 17th Century	2	-18.6	a
Svalbard			
Dutch, 17-18th Centuries	6	-19.9 ± 0.5	e
Spain			
Basque, 13th Century	6	-16.6 ± 0.9	f

[a] References cited:
- a Johansen et al. 1986
- b Tauber 1983
- c Schoeninger et al. 1983
- d Sellevold et al. 1984
- e Schoeninger n.d.
- f B. V. Kennedy, unpublished data

[b] Range extrapolated from Tauber (1983): Figure 3

be ruled out on the basis of isotopic evidence, however, since average values for the men of Jensenvannet and the men of the *Mary Rose* are identical.) Basque values from Red Bay ($\bar{x}$ = -17.2‰) are not as positive as those from the thirteenth-century Basque site of Mendraka ($\bar{x}$ = -16.6‰). The more positive values at the latter site, however, are likely due to millet rather than marine resource consumption, since millet was an important crop in the Basque Country in medieval times (Gómez-Ibañez 1975). Nitrogen isotope data would clarify this difference.

Variation Within Samples. Within-sample variation in the present study is generally lower than in other European studies, with the exception of a Medieval Norwegian sample from Heidal (Johansen et al. 1986) and Dutch whalers from Svalbard (Schoeninger n.d.). This likely reflects the more restricted nature of the samples employed in this study. The samples showing the greatest variation in the present study, Jensenvannet and Red Bay, represent collections of men united in common economic pursuits, and living a large part of the year in an environment far removed from their normal place of residence. These men were drawn from a number of different communities, spent varying numbers of seasons engaged in the whaling enterprise, and died over a period of up to a hundred years. The Jensenvannet sample is slightly more variable than that from Red Bay, perhaps

because, as stated in Chapter 5, the Danish-Norwegian whaling crews were often mixed groups, including Basques, Dutchmen, and Englishmen at varying times. While the range of variation expressed in the Jensenvannet does not extend to the Basque range, it does overlap that of the English from the *Mary Rose* and the Dutch from Svalbard (Schoeninger n.d). The Basque crews, on the other hand, were Basque only, and then from a small portion of the Bay of Biscay coast.

One of the least variable samples in this study also represents an occupational aggregate, rather than a residential group -- the *Mary Rose* sample. Reduced variability, in this case, may reflect the fact that these soldiers and sailors all died at the same moment in time. Moreover, unlike the whalers discussed above, their occupations did not imply regular seasonal residence in a different part of the world. The Saint-Martin sample is equally homogeneous, despite the fact it represents a longer time period. This homogeneity no doubt reflects the common place of residence and common lifestyle of the canons comprising this sample.

In the Red Bay sample, men who were buried in a single grave displayed very consistent $\delta^{13}C$ values. This similarity may reflect a tendency for men who were related or from the same community to work closely together, sometimes dying together as a result of industry-related

accidents.

The lack of significant sex differences in the study samples is not surprising, in light of past research on this subject. Studies of both laboratory and wild animals have failed to demonstrate any sex difference (DeNiro and Schoeninger 1985; Hobson and Schwarcz 1986). Similarly, a number of studies of past human groups show no difference in male and female $\delta^{13}C$ values (Bender et al. 1981; Chisholm 1986; Hobson and Collier 1984; Lovell et al. 1986a). Taken together, these data establish the fact that sex does not imply differences in isotope metabolism. Thus differences which have been noted in human samples (e.g., Bumsted 1984; van der Merwe and Vogel 1978) appear to be due to cultural differences in access to food resources.

The lack of significant variation in $\delta^{13}C$ values with age is also not surprising, since the samples are composed of adult individuals, and previous evidence has suggested such correlations are limited to comparisons of children and adults (Chisholm 1986; Chisholm et al. 1983b; Johansen et al. 1986). Metabolic or dietary factors may be implied in these differences. The data from Jensenvannet are intriguing, however, in that there is a tendency for older individuals to display more positive carbon isotope values. This suggests that the whalers' diet contained a larger proportion of marine resources than the regular home fare, and that with increasing number of seasons in the industry,

this dietary shift was becoming manifest in the bones.

This same trend would seem to be in evidence in the Red Bay sample, in which values for ribs suggest a greater reliance on marine protein than those for non-rib elements, such as long bones, vertebrae, and the skull vault. If, as noted in Chapter 2, the rate of collagen turnover is greater for cancellous bone, ribs may be expected to reflect a more recent part of the dietary history than long bones. For the Basque whalers of Labrador their more recent dietary history included more marine resources, a change that may have been typical of all Basques, but more likely was directly related to their occupation and the available fresh food sources in Labrador (fish, mammals, and birds).

CHAPTER 7

SUMMARY AND CONCLUSIONS

This study is the single most extensive analysis of $\delta^{13}C$ variation in European skeletal remains to date. One hundred thirty-eight specimens from seven historically-documented collections of burials dating to the fifteenth to seventeenth centuries are assessed. The results of this analysis confirm that $\delta^{13}C$ variation is a complex phenomenon, which combines the effects of analytical procedures, preservation quality, and the original nature of the sample.

ANALYTICAL EFFECTS

Techniques employed in collagen extraction and carbon isotope analysis follow those outlined by DeNiro and Epstein (1981) and Schoeninger and DeNiro (1985) with minor modifications. These procedures are an adaptation of the Longin (1971) method, with the addition of a NaOH treatment to ensure removal of humic contaminants. For the purpose of this study, the effects of NaOH on extract quality and $\delta^{13}C$ values were evaluated experimentally, using fresh animal bone. Despite a 3% reduction in yield, no significant alterations of collagen amino acid profiles were detected in

alkali-treated specimens. A consistent depletion in $\delta^{13}C$ values of 0.1‰ with NaOH appears due to preferential removal of noncollagenous organic molecules. (The effects of NaOH on nitrogen isotope values remain to be evaluated.) The analytical techniques employed appear to have had minimal effect on isotopic variation. This is clearly indicated by analyses of replicate extractions of a single bone sample for the NaOH experiments (N = 5); these have a standard deviation of only 0.05‰.

DIAGENETIC EFFECTS

Diagenetic effects, on the other hand, had a significant impact on variation in four samples in this study (*Mary Rose*, Red Bay, Saint-Martin, and Spitalkirche). These effects were identified by statistical analysis of the interrelation of measures of sample preservation (yield, carbon and nitrogen content, and atomic C/N ratios) and $\delta^{13}C$. With one exception (*Mary Rose*, a unique burial environment), these samples showed a consistent pattern of association of isotope values and preservation variables. Lighter $\delta^{13}C$ values were correlated with decreased yield, carbon content, and nitrogen content, and increased C/N ratios. This pattern is consistent with the presence of humic contaminants, as illustrated in computer models presented in Chapter 3. If this is the case, the analytical

procedures employed in this study are not always effective in eliminating such contaminants from the collagen extracts and must be improved. It is notable that the changes in isotopic composition evident in diagenetically-altered specimens did not exceed the general range of $\delta^{13}C$ variation exhibited by samples in this study. In the absence of preservation and correlation data, these changes may not have been evident.

With two exceptions (one from each of the Red Bay and Saint-Martin samples), C/N ratios of altered specimens exceeded the range of acceptable values proposed by DeNiro (1985), i.e., 2.9 - 3.6. (The two exceptional values were in the upper part of this range.) On the other hand, the Saint-Pierre-du-Puellier sample showed relatively high C/N ratios (some of which exceeded DeNiro's range) with no apparent effects on $\delta^{13}C$ values. These values may be indicative of collagen degradation, extraction residues (see Chapters 4 and 6), or machine error. Since no isotopic effect was implied, no specimens were eliminated. These data suggest C/N values should not be the sole criterion used to identify diagenetic change in bone collagen, but should be considered in relation to other lines of evidence.

The procedures employed in this study to identify diagenetic change focus on outlier values. Minimal effects on mid-range values are much more difficult to distinguish by existing methods, and cannot be presumed to be

eliminated. Nevertheless, the procedures permit a closer approximation of "true" sample variation than would otherwise have been the case.

It is clear that the problem of diagenetic change must remain a serious concern of stable isotope studies. Further work is required to establish extraction methods which eliminate the effects of contaminants. More critical assessments and comparisons of existing analytical procedures are required. Evaluations of extract quality must become an established feature of all isotopic analyses.

SAMPLE EFFECTS

Residual variation in the study samples, i.e., variation not due to analytical or diagenetic effects was related to sample attributes having dietary correlations. These attributes include spatial and temporal representations, demographic and social profiles, and element choice (physiological effects related to bone remodelling and dietary change).

Variation is less than found in many other isotopic investigations (1σ varies from 0.2‰ to 0.5‰), likely reflecting the degree of control exercised over sample composition. Differences of less than 5% in marine carbon contributions to bone collagen are statistically significant.

Such approximations of terrestrial and marine carbon contributions to collagen isotopic composition cannot be translated into dietary estimates, however, until we have a better understanding of the complex relationship between diet and bone collagen. This understanding must include an appreciation of: a) the contributions of various macronutrient carbon sources to collagen formation, b) the differential metabolism of plant and animal protein, c) isotope effects associated with the use of diet carbon for collagen synthesis, d) the implications of rates and processes of bone growth and remodelling, and e) the impact of overall nutritional status. Results of a one-year animal feeding experiment reported in this study (Chapter 2) indicate the complexity of these relationships. Further animal studies employing isotopically and nutritionally distinct food groups are needed to supply information which is critical to the interpretation of isotope values.

In 1986, John Parkington commented, "An obvious limitation of carbon isotope readings is that they represent a distillation of several distinct constituents, an average of a set of averages, an answer for which there are numerous questions" (1986:145-146). In exploring the nature of isotopic variation, we hope to gain a better understanding

of the diverse factors which influence isotopic abundances. With continued research in this area, we *will* find the right questions to ask of our data.

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