

**ANALYSIS OF THE VARIATION OF THE OXYGEN ISOTOPIC
COMPOSITION OF MAMMALIAN BONE PHOSPHATE**

By

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OXYGEN ISOTOPES IN MAMMALIAN BONE PHOSPHATE

ABSTRACT

For the oxygen isotopic composition of bone and other biogenic phosphates to be a useful palaeoclimatic tool it is necessary to process large numbers of samples precisely and rapidly. This study describes the development of robust and rapid analytical methods to achieve this and demonstrates their use in two practical studies.

Silver orthophosphate was chosen as the most suitable compound for oxygen isotopic analysis of phosphates due to its defined stoichiometry and lack of structural or adsorbed water. Biogenic phosphates are processed to silver phosphate by dissolution in acetic acid, followed by removal of the calcium as calcium oxalate and sequestering of the phosphate as lead phosphate. Organic material is then oxidized by heating with concentrated nitric acid and hydrogen peroxide before lead phosphate is reprecipitated and the lead removed as lead sulphate, leaving the phosphate in solution. The phosphate is precipitated as coarse crystals of silver orthophosphate by evaporation of an ammoniacal silver nitrate solution.

Oxygen is liberated from the silver orthophosphate by polymerization and reaction with bromine gas at 550°C in a vacuum line. Bromine is a catalyst and a reactant in the process; electrons are transferred from the oxygen to the bromine to catalyze the reaction and the silver is stabilised as silver bromide to prevent back-reaction. The isotopic composition of the gaseous oxygen is determined only by the temperature of the reaction and the isotopic composition

of the silver orthophosphate. The product polymer consists of three to four phosphate ions, so that the reaction yields rather less than one oxygen atom from each phosphate ion or about 17% of the total oxygen.

Sixteen beaver (*Castor canadensis*) were obtained from October through May in southern Ontario. Enamel was ground from their continuously growing incisors, and a small sample of bone was removed from each of their mandibles. The phosphate was analyzed using the methods described above. The results showed a seasonal variation in the $\delta^{18}\text{O}$ of their incisor enamel of approximately 4‰. The $\delta^{18}\text{O}$ of the beaver's body water decreases gradually through the winter and spring, rising rapidly in the summer. Seasonal fluctuation was not reflected in the $\delta^{18}\text{O}$ of the adult beaver bone which was almost constant over the whole area. A Sangamonian giant beaver (*Castoroides ohioensis*) incisor obtained from Hopwood Farm, Illinois gave a climatic response curve similar to the *Castor* samples but the tooth grew more slowly and recorded 1.3 years of growth, with seasonal fluctuation in $\delta^{18}\text{O}$ of 5‰ and a mean value about 5‰ heavier than in the modern beaver studied, reflecting a warmer climate with significant annual temperature fluctuation.

The city of Teotihuacan, near modern Mexico City, expanded in size rapidly from 100 BC to 200 AD due to substantial immigration from areas including the Gulf and Pacific seaboards where the $\delta^{18}\text{O}$ of rain is substantially different from rain at Teotihuacan. Individuals who died before their bones could completely remodel should preserve this different signature in the $\delta^{18}\text{O}$ of their bones. Samples (mostly ribs) from sixty-four individuals were analyzed, representing the major ethnic barrios in the city, the mass sacrifices associated with the foundation of the Temple of Quetzalcoatl and inhabitants of the Valley of

Oaxaca: the supposed homeland of some of the immigrants. The $\delta^{18}\text{O}$ of the bones had apparently not altered, despite diagenetic effects which were identified by infrared spectra, x-ray diffraction and elemental analyses. Distinct differences in $\delta^{18}\text{O}$ were found between different groups in the city but these were not clearly related to their ethnic origins. The samples from the hotter and lower Valley of Oaxaca were analyzed and found to have lower $\delta^{18}\text{O}$ than the inhabitants of Teotihuacan, the opposite of the expected relationship. This reversal was ascribed to Oaxaca being in the rain shadow of the mountains.

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TABLE OF CONTENTS

ABSTRACT	iii
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	viii
PREFACE	xi
LIST OF FIGURES	xiii
LIST OF TABLES	xv
CHAPTER ONE: INTRODUCTION	1
1.1 Basis of study	1
1.2 The analytical studies	5
1.3 The studies of distributions of $\delta^{18}\text{O}$ in bone	7
CHAPTER TWO: A NEW METHOD FOR PURIFYING BIOGENIC PHOSPHATES FOR OXYGEN ANALYSIS	9
2.1 Abstract	9
2.2 Introduction	9
2.3 Equipment and standards	11
2.4 Method	13
2.5 Testing of the method	20
2.6 Results	22
2.7 Yields and completeness of precipitations	25
2.8 Isotopic properties of silver orthophosphate	28
2.9 Discussion	33

CHAPTER THREE: OXYGEN ISOTOPIC ANALYSIS OF SILVER

ORTHOPHOSPHATE USING A REACTION WITH BROMINE	39
3.1 Abstract	39
3.2 Introduction	39
3.3 Equipment	43
3.4 Method	44
3.5 Results and discussion	48
3.6 Characterization of the reaction and conclusion	51

CHAPTER FOUR: OXYGEN ISOTOPIC DETERMINATION OF CLIMATIC VARIATION USING PHOSPHATE FROM BEAVER BONE, TOOTH

ENAMEL AND DENTINE	54
4.1 Abstract	54
4.2 Introduction	55
4.3 Introduction to beaver	58
4.4 Samples	60
4.5 Analytical procedure	66
4.6 Results	67
4.7 Tooth growth rate estimation	69
4.8 Reliability of data	71
4.9 Influences controlling <i>Castor</i> δ_{pe} and δ_{pb}	72
4.9a Sources of local, hydrologic variability in <i>Castor</i> δ_{pe}	74
4.9b Regional, climatically related variability in <i>Castor</i> δ_{pe}	78

4.9c	Data from <i>Castor</i> δ_{pb} and the relationship with δ_{pe} . . .	84
4.10	The climatic signal in a <i>Castoroides</i> incisor	87
4.11	Conclusions	92
CHAPTER FIVE: THE ISOTOPIC COMPOSITION AND DIAGENESIS OF HUMAN BONE FROM TEOTIHUACAN AND OAXACA, MEXICO . .		94
5.1	Abstract	94
5.2	Introduction	95
5.3	Analytical procedures	98
5.4	Isotopic analyses	105
5.5	Crystallinity, softness, density and organic content	108
5.6	Elemental analyses	113
5.7	Discussion: Climate and regional variability	115
5.8	Discussion: Examination of possible diagenesis and diagenetic models	120
5.9	Summary and conclusions	126
CHAPTER SIX: POSTSCRIPTS AND FUTURE WORK		128
6.1	Postscript	128
6.2	A comment on the archaeology of oxygen isotope data from Teotihuacan	130
6.3	Future work	131
REFERENCES		135

PREFACE

Chapters 2 - 5 of this thesis are modified versions of papers either already published or in the process of being published. All four papers are first-authored by the writer.

Chapter 2:

Title: The development of a new method for preparing silver orthophosphate for oxygen isotopic analysis.

Journal: *Geochimica et Cosmochimica Acta?*

Authorship: Stuart-Williams, H. le Q. and Schwarcz, H.P.

Candidate's contribution: Essentially all concepts and laboratory work, with comment and editorial input by the second author.

Chapter 3:

Title: Oxygen isotopic analysis of silver orthophosphate using a reaction with bromine.

Journal: *Geochimica et Cosmochimica Acta* (1995) **59**(18): 3837-3841

Authorship: Stuart-Williams, H. le Q. and Schwarcz, H.P.

Candidate's contribution: Essentially all concepts and laboratory work, with comment and editorial input by the second author.

Chapter 4:

Title: Oxygen isotopic determination of climatic variation using phosphate from beaver bone, tooth enamel and dentine.

Journal: *Palaeogeography, Palaeoclimatology, Palaeoecology?*

Authorship: Stuart-Williams, H. le Q. and Schwarcz, H.P.

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Chapter 5:

Title: The isotopic composition and diagenesis of human bone from Teotihuacan and Oaxaca, Mexico

Journal: Palaeogeography, Palaeoclimatology, Palaeoecology (in review)

Authorship: Stuart-Williams, H. Le Q., Schwarcz, H.P., White, C.D. and Spence, M.W.

Candidate's contribution: Nearly all laboratory work, writing of the paper and development of isotopic and diagenetic concepts. Original idea of identifying immigrants using isotopes by H.P. Schwarcz and other authors. Comment and editorial input by other three authors. Some laboratory assistance from other workers, as identified in the acknowledgements.

LIST OF FIGURES

Figure	Page
2.1 Flowchart of bone processing for oxygen isotopic analysis	14
2.2 Relationships between yield and $\delta^{18}\text{O}$	27
2.3 Frequency of $\delta^{18}\text{O}$ analyses of a Ag_3PO_4 standard	30
3.1 Vacuum line for reacting phosphates with bromine	42
3.2 Relationship of Br_2 analyses of standards to BrF_3 analyses	49
3.3 Relationship of phosphate $\delta^{18}\text{O}$ analyses of KH_2 standard to temp.	49
3.4 X-ray diffraction spectra and peaks for reaction products	52
4.1 Location map for samples analyzed	61
4.2 Illustrations of <i>Castor</i> and <i>Castoroides</i> teeth used in study	64
4.3 $\delta^{18}\text{O}$ of incisors and bone from beaver samples	68
4.4 Food and water oxygen fluxes in beaver	73
4.5 Oxygen isotopic analysis of precipitation at Chicago	79
4.6 Normalized and rapid growth beaver δ_{pe} profiles	83
4.7 Analyses of giant beaver (<i>Castoroides ohioensis</i>) incisor	88
5.1 Location of sites in Teotihuacan and Oaxaca study	97
5.2 Isotopic distribution of all bone samples	106
5.3 Isotopic composition of groups of bones	107
5.4 Changes in bone characteristics resulting from alteration	110
5.5 Changes in carbonate and uranium content of bone during alteration	112

5.6	Elemental analyses of Teotihuacan bones	116
5.7	Changes in crystallinity of Byzantine & modern bones	123
5.8	Changing $\delta^{18}\text{O}$ profiles of bone during diagenesis	123

LIST OF TABLES

Table	Page
2.1 Reagents for processing of bone phosphate	12
2.2 Reagent quantity and pH limiting factors	15
2.3 Results of processing a liquid phosphate standard	21
2.4 Inter-laboratory comparison of horse tooth enamel $\delta^{18}\text{O}$	23
2.5 Inter-laboratory comparison of Ag_3PO_4 $\delta^{18}\text{O}$ analyses	32
3.1 Identification and $\delta^{18}\text{O}$ of Ag_3PO_4 standards used	46
4.1 Sample information for beaver in this study	62
5.1 Isotope, crystallinity and site data for samples in Teotihuacan and Oaxaca study	99-102
5.2 Physical properties, isotopic composition and uranium content . .	114

CHAPTER ONE

Introduction

1.1 Basis of study

Attempts to predict climate change or place the evolution of *Homo* in a climatic context must be based on palaeoclimate data, but information on the ancient climates of continents is poorly preserved due to a scarcity of sedimentary rocks that might contain such a record. A frequent difficulty with relating proxy climatic records, such as pollen data or oxygen isotopic analyses of ice cores, to archaeological or palaeontological remains is that both the remains and the climatic data must be very precisely dated to permit correlation. In many cases, especially on older sites, such dating is currently impossible. It is substantially more convenient to utilize information that can be gathered from the bone samples themselves or from remains or sediments that are intimately associated with them, even though few long sequences of bones are available. The only climatic information that is known to be integral within the mineral portion of bone (which is probably more stable than the organic portion after burial) is the oxygen isotopic composition of the phosphate or carbonate. In general, phosphate is more resistant than carbonate to isotopic exchange after the death of the organism (Shemesh *et al.*, 1988; Wright and Schwarcz, in press) and is probably more useful for isotopic analysis of older material.

The isotopic composition of meteoric water in an area is controlled largely by the temperature of the air-mass from which the precipitation fell (Dansgaard,

1964). As there is a good correlation between air temperature and ground temperature, the $\delta^{18}\text{O}$ of meteoric water (δ_{mw}) is also strongly correlated with surface temperatures. The $\delta^{18}\text{O}$ of an animal's body fluids (δ_{bw}) is usually dominated by the $\delta^{18}\text{O}$ of the water it drinks (δ_w) and the water in its food (δ_f) (Ayliffe and Chivas, 1990; Bryant and Froelich, 1995; D'Angela and Longinelli, 1990; Longinelli, 1984; Luz and Kolodny, 1985, 1989; Luz *et al.*, 1984). This relationship varies from species to species or genus to genus (Ayliffe *et al.*, 1992; Bryant *et al.*, 1994; D'Angela and Longinelli, 1990; Longinelli, 1984; Luz *et al.*, 1990; Yoshida and Miyazaki, 1991) and must be determined by modelling (Ayliffe and Chivas, 1990; Bryant and Froelich, 1995; Luz and Kolodny, 1989) or examination of modern examples where δ_{mw} is known. In herbivores, water in food and water drunk are usually derived from the same source: precipitation. Biogenic phosphates are deposited in equilibrium with the body fluids at body temperature (Luz and Kolodny, 1989) and preserve the isotopic composition of the body fluids and are therefore a record of ground surface temperatures. Animals which do not regulate their body temperature have a variable offset between δ_{bw} and δ_p (the $\delta^{18}\text{O}$ of their body phosphates) (Kolodny *et al.*, 1983; Longinelli and Nuti, 1973a & b; Luz and Kolodny, 1989). Mammals larger than a kilogram maintain a steady temperature of about 37°C which results in a constant offset of about 17.8‰ between δ_p and δ_{bw} (Bryant and Froelich, 1995; Longinelli and Nuti, 1973a; Luz and Kolodny, 1985). Thus, bones or teeth from mammals can be used to determine their δ_{bw} from which δ_{mw} and temperature can be calculated.

The first step in an oxygen isotopic study of bone phosphate is selecting a site or sites with samples that may yield the required information. The construction of palaeotemperature curves requires that one or more sites in a small area must contain long, relatively continuous sequences of phosphatic remains from species of animals which have a well-understood response to changing climatic conditions. The remains must be in good condition or the oxygen isotopic signal may have been altered unpredictably, and destructive sampling must be permitted. In practice, such sites are extremely difficult to find and no long, continuous palaeotemperature curves have been published based on bone data. Attempting to map different temperatures from a single period in time over a large area requires widely spread samples of one suitable species with very accurate dating for all the samples. This is probably not possible at the present because the frequency of oscillation of climate is higher than can be defined by dating techniques for all but the most recent radiocarbon datable periods. In addition, the isotopic integrity of each sample must be assured: tooth enamel has become the preferred medium as the large size of its crystallites and low organic content (LeGeros, 1981; Lowenstam and Weiner, 1989) make it more resistant to recrystallization and alteration (Ayliffe *et al.*, 1994). For these reasons, coupled with the difficulty of processing and reacting phosphate samples, no major palaeoclimatic studies have been published based on mammalian phosphatic material. Published works have concentrated on evaluating the relationship between δ_{bw} , δ_{mw} , temperature and humidity (Ayliffe *et al.*, 1992; Bryant *et al.*, 1994; D'Angela and Longinelli, 1990; Longinelli, 1984; Luz *et al.*, 1990; Yoshida and Miyazaki, 1991) as well as the identification and correction of diagenetic alteration (Ayliffe *et al.*, 1994; Luz, 1992; Shemesh, 1990).

This study was initiated to use a long, apparently well dated sequence of bones from La Quina, France, to produce a record of climatic change. After some research it was found that the bones were probably unsuitable for the original purpose as dating of the strata was becoming less certain, the stratigraphic relationships were complicated and the original isotopic signal was likely to have been destroyed in the highly altered bones available. It had also become clear that the existing analytical methods were too laborious to permit the extensive sampling necessary to give credibility to such a study. The emphasis of the project consequently shifted to improving the analytical methods and then examining some better controlled samples that would test the analytical techniques and result in useful data.

The four papers that comprise this thesis cover the gamut from processing of the bone to a refined and purified phosphate; reacting the product to release O_2 from the phosphate with a precision suitable for isotopic analysis, and then two studies of data obtained from human and animal bones using the techniques.

1.2 The Analytical Studies

Once samples have been obtained they must be prepared by separating the enamel from the remainder of the tooth and crushing it, or by grinding up bone samples. The samples are then dissolved in acid and processed using one of several methods, discussed in chapter 2. Preparing phosphate for analysis was originally a time consuming process, taking at least five working days (Tudge, 1959, 1960), but has now become more rapid and can be completed in not more than two days (Crowson *et al.*, 1991; Method in Chap. 2; O'Neil *et al.*, 1994). Processing typically includes multiple dissolutions and precipitations to remove undesirable products or purification using ion exchange resins. Two single precipitation methods have also been proposed (O'Neil *et al.*, 1994; Schwarcz *et al.*, 1991), with varying degrees of success. The purified phosphate is then precipitated as a final product which must include no oxygen containing impurities which will interfere with the isotopic analysis. The first material used (BiPO_4) (Tudge, 1959, 1960) typically contained some water which had to be removed by careful dehydration and storage in a desiccator (Karhu and Epstein, 1986; Shemesh *et al.*, 1988). Many workers, including ourselves, now use the anhydrous chemical Ag_3PO_4 (Crowson *et al.*, 1991). The chemical techniques used for producing the final analytical compound are specific to it: the processing method used to produce BiPO_4 cannot be used to make Ag_3PO_4 , and *vice versa*.

The final analyte, Ag_3PO_4 or BiPO_4 , must then be reacted to liberate O_2 , as discussed in chapter 3. The first method relied on active fluorination of BiPO_4 with highly reactive BrF_3 or BrF_5 (Tudge, 1959, 1960). Products of the reaction are Br_2 , BiF_3 , PF_5 , O_2 and mixed bromine/fluorine compounds. The same method is used for reacting Ag_3PO_4 (Crowson *et al.*, 1991), producing AgF ,

AgF_2 , PF_5 , Br_2 , O_2 and mixed bromine/fluorine compounds. Silver phosphate will polymerize rapidly when heated to high temperatures and two new methods of liberating the O_2 have been proposed based on this principle, using Br_2 (Stuart-Williams and Schwarcz, 1995; Chap. 3) or carbon (O'Neil *et al.*, 1994) to stabilize the reaction products. Reaction of Ag_3PO_4 with BrF_3 or BrF_5 takes 12-18 hours (Crowson *et al.*, 1991; Lécuyer *et al.*, 1993) and there are substantial health and safety risks as well as high costs associated with the use of fluorination lines. As Ag_3PO_4 is substantially easier to handle than BiPO_4 it has become the preferred analytical material, while the polymerization techniques appear to be faster, cheaper and safer than fluorination of this compound.

Due to statistical scatter in the δ_p of most animals, as well as uncertainties regarding diagenetic effects on buried bones and teeth, most workers are tending to process large numbers of samples in order to identify data aberrations (Bryant *et al.*, 1994; Luz *et al.*, 1990; Sánchez Chillón *et al.*, 1994; Chaps. 4 & 5) . This trend has also been encouraged by the improvement and shortening of processing and reaction methods; for example well over 1000 phosphate samples were reacted during the development of the techniques described in chapters 2 and 3. This trend toward larger sample databases is symptomatic of the approaching maturity of biogenic phosphate oxygen isotope studies.

1.3 The studies of distributions of $\delta^{18}\text{O}$ in bone

Most studies of δ_p have concentrated on the relationship between δ_p and climatic variables, while a very small minority have looked at other uses of the method, such as identifying immigrant groups in archaeological material on the basis of preserved isotopic signatures (Luz and Kolodny, 1989; Schwarcz *et al.*, 1991). Both aspects were tackled as part of this all-inclusive study: the relationship between the δ_p of beaver and climate is examined in chapter 4 while ethnic groups within the ancient population of Teotihuacan, Mexico, are studied in chapter 5.

The study of the beaver (*Castor canadensis*) resulted from a need to examine the δ_p of the tooth enamel (δ_{pe}) of a living group of animals. It was considered probable that the tooth enamel of most animals would contain an isotopic signal that was heavily biased by the behaviour of the young animal, including perhaps an unusual diet and consumption of milk isotopically fractionated by the mother (Luz and Kolodny, 1989). For this reason an animal with continuously growing teeth was favoured, as enamel forming during the early part of the animal's life would have been lost. Low relative humidity influences the body fluid composition of some animals (Ayliffe and Chivas, 1990; Luz *et al.*, 1990; but also D'Angela and Longinelli, 1990; Yoshida and Miyazaki, 1991). I obtained beaver that were being killed for other reasons so as to avoid ethical difficulties. This sample collection policy worked well, except that communications with trappers were somewhat sporadic and usually failed after a few months. While many trappers were killing beaver as part of pest control programmes, they still preferred to trap in the winter or spring so as to be able to get extra money for the thicker winter pelts. This resulted in a lack of data over

the summer that could only have been rectified by killing beavers myself or paying enough money to encourage a trapper to do it, which would have raised ethical questions and little extra data would have been obtained. No similar study of variation in the δ_p of one species within a reasonably small area, based on so many samples, has been published. A Sangamonian giant beaver (*Castoroides ohioensis*) tooth was obtained from Illinois and analyzed for comparison with modern *Castor* and found to be similar in its response to seasonal changes in δ_{mw} and δ_w , although the tooth grew much more slowly than in *Castor* and recorded a longer period of time. The modern beaver analyses provide a context within which future studies of giant beaver and other aquatic rodents can be placed.

Analysis of the Teotihuacan population resulted from a project already in progress at the University of Western Ontario, led by Dr. Christine White and Dr. Michael Spence. The population of Teotihuacan expanded extremely rapidly from 100 BC to 200 AD, suggesting substantial immigration (Cowgill, 1992). Ethnic barrios can be identified on the basis of cultural differences within Teotihuacan (Spence, 1992) for several hundred years after 200 AD. If the immigrants came from areas with water isotopically distinct from water at Teotihuacan and died before all their bone had been remodelled, then it would be possible to identify their origins on the basis of different δ_p (Schwarcz *et al.*, 1991). This project also provided an excellent opportunity to examine statistical scatter in δ_p and diagenesis within a defined group of people. This study represents the largest sample of δ_p in an ancient population that has been published.

CHAPTER TWO

A new method for purifying biogenic phosphates for oxygen isotopic analysis¹

2.1 Abstract

Bone phosphate can be prepared rapidly for oxygen isotopic analysis using a simple method based on the precipitation of $Pb_3(PO_4)_2$. The precision of analyses (σ) of inorganic phosphates is approximately 0.1‰, including errors arising from the liberation of O_2 from Ag_3PO_4 and mass spectrometry. Analysis of biogenic phosphates yields a precision of approximately 0.15‰ but presents particular difficulties arising from the interaction of organic residues and phosphate which appear to have been underestimated in previous studies. Differences in values obtained for similar materials using various analytical techniques are attributed to variable yields, complexation of phosphates by organic materials and differing extent of hydrolysis of polyphosphates.

2.2 Introduction

The first highly precise technique for phosphate purification for isotopic analysis was that of Tudge (1959,1960) in which the phosphate is initially dissolved in 10 M HNO_3 , precipitated as $(NH_4)_3PO_4 \cdot 12MoO_3 \cdot 2HNO_3 \cdot H_2O$, reprecipitated as $MgNH_4PO_4 \cdot 6H_2O$ and finally precipitated from dilute HNO_3 as α $BiPO_4 \cdot \frac{1}{2}H_2O$. This is carefully dehydrated to $BiPO_4$ while remaining in the

¹A modified version of this chapter, authored by H. Le Q. Stuart-Williams and H.P. Schwarcz, has been submitted for publication in *Geochimica et Cosmochimica Acta*.

original hexagonal form (Karhu and Epstein, 1986; Mooney-Slater, 1962). This compound produces relatively good repeatability ($< 0.1\%$ precision) but has to be handled with great care to prevent rehydration. Karhu and Epstein (1986) proposed heating and conversion to the monoclinic β phase without structural water. Their results indicated that the original technique produced a reduction in the oxygen isotopic value of $> 1\%$ depending on the preparation procedure. While not all workers agreed with their results (Shemesh *et al.*, 1988) it is clear that the use of a compound which nominally includes water, and perhaps even relies on it for structural stability in the hexagonal phase (Mooney-Slater, 1962; Tudge, 1960), is undesirable because the oxygen isotopic composition of any water present may influence the analysis. An alternative (anhydrous) compound was sought and Ag_3PO_4 was selected as a final product for a processing sequence using anion exchange resins (Wright and Hoering, 1989; Crowson *et al.*, 1991; Lécuyer *et al.*, 1993). Silver phosphate has a reliable stoichiometry and does not contain structural water (Anbar *et al.*, 1960; Baxter and Jones, 1910; Firsching, 1961). We have also used Ag_3PO_4 as a final product in our development of a quick, robust method of purifying phosphate using rapid precipitations. In particular we wanted to design a method which is unaffected by the more common impurities in fossil bone, such as Fe^{2+} and Fe^{3+} and which would be suitable for modern biogenic phosphates with a high organic content.

In the method presented here, bone or tooth is dissolved in acetic acid and calcium is then precipitated as calcium oxalate and removed by centrifugation. The addition of lead acetate causes the phosphate to be precipitated from the supernate as lead phosphate which is then dissolved in nitric acid before organic residues are destroyed by treatment with strong oxidants at elevated

temperatures. Lead phosphate is then reprecipitated and centrifuged to separate it from residual oxidants and nitrate. After dissolution of the lead phosphate the lead is removed as lead sulphate, leaving only phosphate and non-interfering ions in solution. The amount of work required to process samples is minimized by not filtering or rinsing precipitates and by using the minimum number of pieces of glassware for each sample. Some phosphate is lost by not washing precipitates but the isotopic composition is not altered. Because the solubility of lead phosphate increases rapidly at a $\text{pH} < 6$ or > 8 , pH is regulated carefully using indicator dyes or a pH probe when forming precipitates.

2.3 Equipment and standards

All samples were prepared in borosilicate glass which was cleaned by brief submersion in 6 M HCl, scrubbed in water with low phosphate detergent and rinsed once under distilled water.

Most of the testing of inorganic phosphates was conducted on a laboratory internal standard of 0.018 M BDH KH_2PO_4 solution. By using a dissolved material all aliquots are guaranteed to be analytically identical. Davis food gelatine was used as the organic portion of artificial bone for testing. An internal standard of archaeological bison bone (BIS II) was used for testing of biogenic phosphates.

Most of the reagents used in the procedure are stored premixed and dispensed by volume, as listed in table 2.1. All reagents are dissolved in deionised water,

Table 2.1: Reagents that can be premixed and stored. In the text only the volume of dissolved reagent to be used is given, assuming a solution molarity as shown here. Indicators should be mixed according to standard instructions.

Chemical Name	Molarity	mL/30 mg sample
Acetic acid	3	5-18
Nitric acid	6	2
Nitric acid	0.5	3
Potassium hydroxide in DIW	8	2
Potassium oxalate	0.55	1
Lead acetate trihydrate in 0.05 M acetic acid	0.5	5
Ammonium sulphate in DIW	0.25	2
Ammonium nitrate	1.25	1.5
Bromocresol	-	-
Bromothymol blue in DIW	-	-
Phenolphthalein in alcohol	-	-

apart from $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ (lead acetate trihydrate) which is dissolved in approximately 0.05 M acetic acid to achieve a pH of 5-6.

Oxygen was released from the prepared Ag_3PO_4 by reacting it with Br_2 at 550°C in an electronically controlled furnace (Stuart-Williams and Schwarcz, 1995). The precision of analysis of duplicate standards was $<0.1\%$. Isotopic ratios were determined using a VG SIRA isotope ratio mass spectrometer.

2.4 Method

A schematic diagram of the processing sequence is presented in figure 2.1. The quantities of reagent and sample used may be varied within defined limits, as shown in table 2.2. Biogenic phosphates which contain a high proportion of organic material, such as recent bone or dentine, require oxidation of the organic material in hot H_2O_2 before being dissolved in acetic or nitric acid. Dissolution in acetic acid is slower but discriminates against solution of diagenetic phosphate and other minerals than bone apatite (dahllite), e.g. fluorapatite (Okazaki *et al.*, 1982). For example, after dissolution of Miocene horse tooth enamel samples (supplied by D. Bryant) in acetic acid, an insoluble residue remained which was identified by x-ray diffraction as more crystalline calcium hydroxylapatite resulting from recrystallization during burial. The same material was completely soluble in 3 M HNO_3 . Organic compounds, especially collagen-like substances, are also less soluble in acetic acid as the remaining collagen "pseudomorphs" are more substantial than those left after dissolution in HNO_3 , although the acidity is sufficient to separate adsorbed phosphate from the collagen

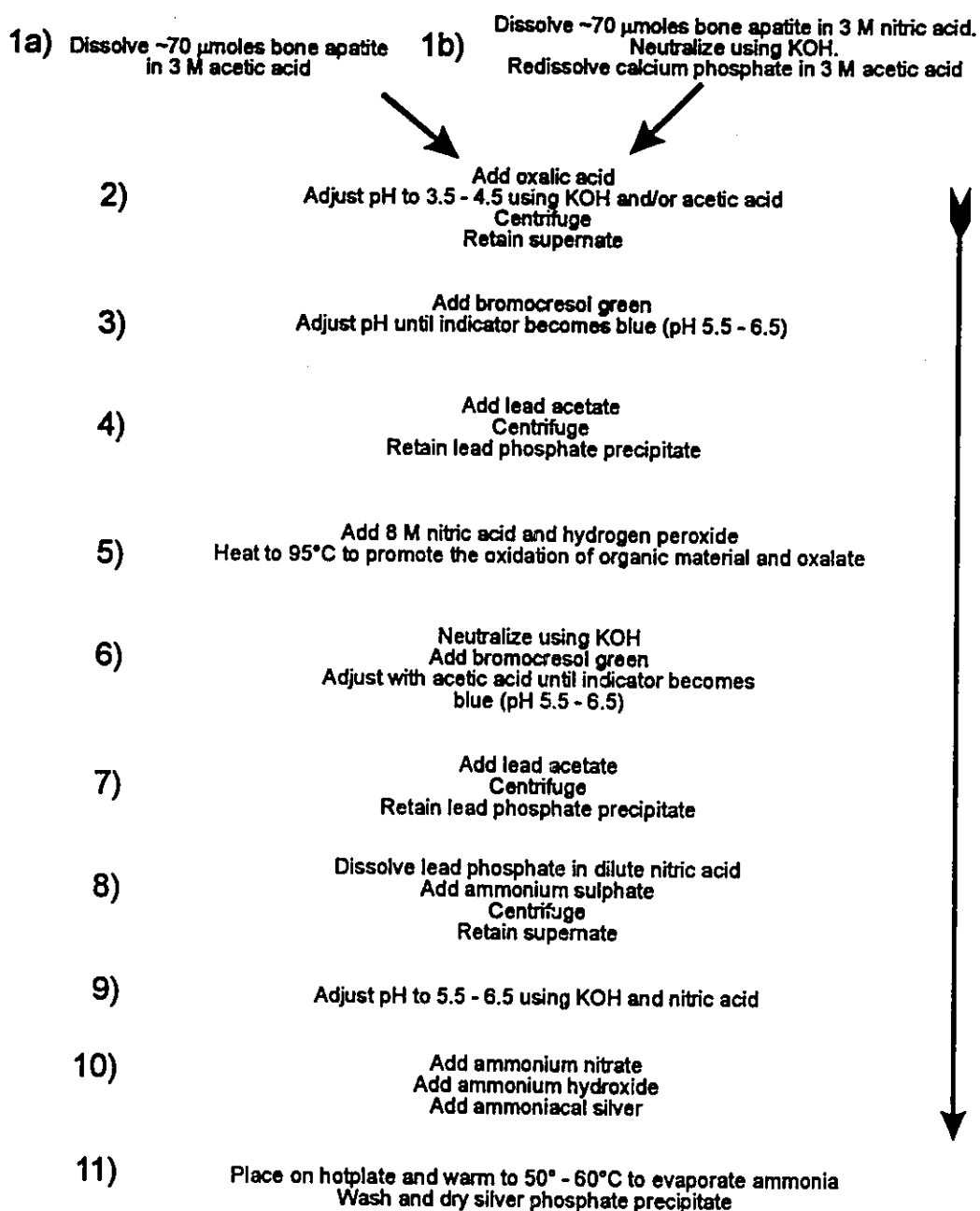


Figure 2.1: Flowchart of bone processing for oxygen isotopic analysis of phosphate.

Table 2.2: Reagent quantity and pH limiting factors.

Step	Controlling limits	Reason
Dissolve bone in acid	Finishing pH < 3. Avoid large excess of nitrate	Phosphate not adsorbed on collagen. Nitrate increases solubility of lead phosphate
Add oxalic acid	Moles oxalic acid \geq (moles apatite x 7)	All calcium must be chelated by oxalate
Precipitate calcium oxalate	pH 3.5 - 4.5	Facilitate precipitation and centrifuging of calcium oxalate
Prepare to precipitate lead phosphate	pH 5.5 - 6.5, minimize fluid and nitrate	Optimize precipitation and centrifugation, reduce solubility losses
Add lead acetate	Moles lead > > ((moles oxalic - (moles apatite x 5)) + (moles apatite x 4.5))	Sufficient lead to precipitate phosphate and remaining oxalate plus excess to reduce solubility of lead phosphate
Oxidation of organic material	Finishing pH < 1. All organic material and oxalate oxidized	Residual oxalate will be precipitated with final product. Organics cause low yield and fine-grained precipitation of silver phosphate
Prepare to precipitate lead phosphate	pH 5.5 - 6.5	Optimize precipitation and centrifugation, reduce solubility losses
Add lead acetate	Total moles lead > (moles apatite x 12), minimize excess nitrate	Reduce solubility of lead phosphate
Dissolve lead phosphate	Minimum amount of nitric acid and water	Maximize removal of lead as lead sulphate
Precipitate lead sulphate	Moles sulphate approximately (moles apatite x 7)	Excess sulphate up to 20 μ mol/20 mL water reduces solubility of lead sulphate
Silver phosphate precipitation	Ending pH \leq 7.5	Maximize precipitation of silver orthophosphate

(Koutsoukos and Nancollas, 1986). All samples should be dissolved using a similar method within a project and the weights should be recorded in order that samples with very low yields can be rejected. We usually process 16 samples in a batch over a period of 1.5 days.

(Step 1a, Fig. 2.1) To dissolve samples in acetic acid, grind 30-35 mg of bone (approximately 150-200 μmoles of PO_4^{3-}) finely and stand in 15 mL of 3 M acid in a 40 mL Pyrex centrifuge tube at room temperature for 24 hours or more, stirring occasionally. Warming to about 35°C will accelerate dissolution.

(Step 1b, Fig. 2.1) If the samples are relatively pure (for example modern bone) or more speed is desirable, samples of approximately 30-35 mg of bone can be dissolved in 2 mL 3 M HNO_3 in Pyrex 40 mL centrifuge tubes. Dissolution of modern bone is complete within several hours, but diagenetically altered or coarsely ground samples may take longer. Add one drop of bromothymol blue indicator solution to each tube and drip in 8 M KOH solution while stirring until neutrality is achieved, as indicated by the precipitation of a white or cream calcium phosphates. Add 1.5 mL of 3 M acetic acid to redissolve phosphate.

(Step 2, Fig. 2.1) Following either method of sample dissolution, add 2 mL of $\text{H}_2\text{C}_2\text{O}_4$ (oxalic acid) solution to precipitate the calcium and adjust the pH to 3.5-4.0 using drops of 8 M KOH solution. Let the sample stand for approximately 5 minutes before centrifuging rapidly and then decant the liquid into a clean 40 mL centrifuge tube and dispose of the precipitate of CaC_2O_4 and other insoluble material, including collagen, from the bone.

(Step 3, Fig. 2.1) Add a drop of bromocresol green indicator and adjust the pH using KOH until the indicator becomes blue at about pH 4.5 , then add 5 mL of $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$ solution, stirring vigorously (Step 4, Fig. 1). Stand for 5 minutes to ensure complete precipitation of all the $\text{Pb}_3(\text{PO}_4)_2$, then centrifuge at high speed and retain precipitate, disposing of the supernate. Ensure that the tubes are labelled using a permanent, waterproof pen. Add 2 mL of 8 M HNO_3 and 3 mL of 30% H_2O_2 (Step 5, Fig. 2.1) to oxidize any organic compounds. Place samples upright in 5 cm of gently boiling water for 2 hours, maintaining the sample fluid level with H_2O_2 when less than 1 mL remains. Continue to heat for 2 more hours keeping sample fluid levels at a minimum of 1 mL with deionised water. When decomposition of the H_2O_2 (indicated by the formation of bubbles) has ceased and the residue is evaporated almost to dryness, remove the tubes from the bath to cool. Wash down the insides of the tubes with the minimum amount of deionised water. Neutralize with KOH (Step 6, Fig. 2.1) until a white precipitate of $\text{Pb}_3(\text{PO}_4)_2$ forms, then add 1 drop of bromothymol green and drops of acetic acid until the indicator changes to a green-blue at about pH 5.5 - 6.0. Note that the precipitate will dissolve due to complexation of the lead if an excess of OH^- is present. A brown coloration of PbO may form if all the H_2O_2 was not evaporated or decomposed but this can be ignored. Add 3 mL of lead acetate solution (Step 7, Fig. 2.1) and adjust the pH to 5-6 using acetic acid or KOH. The addition of Pb^{2+} is necessary at this point to ensure that a substantial excess of Pb^{2+} is present to reduce the solubility of the $\text{Pb}_3(\text{PO}_4)_2$ precipitate and compensate for Pb that may have been lost as PbO . Let stand for 5 minutes, then centrifuge at high speed and retain the precipitate, disposing of the supernate. Dissolve the precipitate in approximately 3 mL of 0.25 M HNO_3

and the minimum number of drops of 3 M HNO_3 (Step 8, Fig. 2.1). Do not use more HNO_3 than is required for dissolution or the solubility of the succeeding precipitation will increase and Pb^{2+} will be found in the final precipitation. A coarse precipitate of lead nitrate may form, which can be ignored. Add 2 mL of $(\text{NH}_4)_2\text{SO}_4$ solution to precipitate the lead as PbSO_4 and stir. We usually halt processing at this point and complete the processing and final precipitation of Ag_3PO_4 the following day. Stand overnight or for at least 5 minutes and stir vigorously to sink floating crystals before centrifuging at high speed. Decant the supernate into a 50 mL borosilicate glass beaker, ensuring that all the precipitate is removed. Recentrifuge if any precipitate remains.

Add 1 drop of bromothymol blue (Step 9, Fig. 2.1) and adjust the pH to slightly < 7 (indicator will change colour from blue to yellow as the pH falls from 7.5 to 6.5) with drops of 8 M KOH and 3 M HNO_3 .

The phosphate can now be precipitated as Ag_3PO_4 by ammonia volatilization (Firsching, 1961). Do not commence the precipitation unless there is sufficient time to complete it and wash the precipitate. (Step 10, Fig. 2.1) To each sample, add 1.5 mL of NH_4NO_3 solution and 1 mL of concentrated NH_4OH . Prepare a quantity of ammoniacal AgNO_3 solution adequate for all the samples in the batch by mixing 600 mg AgNO_3 and approximately 10 mL of deionised water for each sample in a borosilicate glass beaker (for 16 samples a 400 mL beaker is appropriate). Add concentrated NH_4OH (approximately 10 mL) until the solution becomes clear. Divide the ammoniacal silver solution equally into the beakers of purified phosphate solution and wash down the sides of the beakers with a minimum of deionised water. Make up the volume of each sample to about 25-30 mL and place on a hotplate that will bring the solutions to $50^\circ - 60^\circ\text{C}$.

During precipitation maintain the fluid level with deionised water and stir if necessary to break and sink any large masses of coarse floating crystals that form. After 2-3 hours precipitation is usually complete, fine crystals form on the floor of the beaker and $\text{pH} < 7.5$. The hot sample beakers may still have an odour of NH_3 , but this should not be present once they have cooled. After cooling for 10 minutes, pour the supernate and floating crystals onto a medium fritted-glass filter previously cleaned with 3 M HNO_3 (Step 11, Fig. 2.1).

Carefully wipe the Ag_3PO_4 crystals from the sides and bottom of the beaker with a nylon spatula, washing the loosened precipitate into the bottom of the beaker with deionised water from a squirt bottle. Allow the fine crystals to settle for a moment before pouring the supernate into the filter to help prevent the loss of fine material in the fritted glass element. Repeat the rinsing of the precipitate in the beaker twice and then wash the precipitate in the fritted glass filter 3 times with deionised water. Wash the precipitate in the filter back into the beaker and carefully pipette off excess water before air drying at approximately 60°C for several hours. Clean the filter with 3 M HNO_3 after each sample. To avoid an accumulation of extraneous fibres in the final precipitate all equipment, for example spatulas used for stirring, should be rinsed with deionised water rather than wiped with laboratory tissues. Typically the procedure, apart from dissolution, is completed in about 1.5 working days.

2.5 Testing of the method

The liquid standard was prepared in six different ways to examine any effects on the isotopic composition (Table 2.3). 1) Direct precipitation of the KH_2PO_4 liquid standard from aqueous solution by ammonia volatilization was used to produce a most probable value for the material. 2) The liquid standard was processed by the method presented here, steps 1a - 11. 3) The liquid standard was processed through steps 1a - 11, but without the step for the oxidation of organic material (step 5). The $\text{Pb}_3(\text{PO}_4)_2$ was dissolved in nitric acid and reprecipitated but the extended heating and the addition of H_2O_2 were omitted. 4) One possible source of error is the content of higher polymers of phosphate which are present in most orthophosphates, for example the KH_2PO_4 used to make the liquid standard. To test for this, concentrated HNO_3 was added to about 500 mL of the liquid internal standard to make a 1 M HNO_3 solution. This was heated in a 100°C bath for 1 hour. This hydrolysed standard was then precipitated as Ag_3PO_4 . 5) We wanted to test the method on a bone standard but unfortunately no bone was available with a well known isotopic and chemical composition and it is probable that the processing effects produced would have been smaller in magnitude than possible differences in the values reported by different laboratories (O'Neil *et al.*, 1994 and see below and section 2.6). For this reason we prepared a synthetic bone mixture consisting of the liquid standard with an amount of calcium acetate to approximate the stoichiometry of calcium hydroxylapatite. This was processed through steps 1a - 11. 6) A second synthetic bone was prepared by adding powdered gelatine dispersed in water to the previous calcium phosphate solution to approximate a bone with 25% soluble organic material and this was also processed through steps 1a - 11.

Table 2.3: Results of processing a liquid phosphate standard.

Material	Processing	$\delta^{18}\text{O}$ SMOW (‰)	Offset from KH_2PO_4 std. (‰)
KH2 liquid	Precipitation as Ag_3PO_4	12.33 ± 0.06	0.00
"	Standard processing (Steps 1a - 11)	12.50 ± 0.15	+0.17
"	As above, without full H_2O_2 treatment	12.25 ± 0.10	-0.08
"	Hydrolysed in 1 M $\text{HNO}_3 \rightarrow \text{Ag}_3\text{PO}_4$	12.02 ± 0.13	-0.31
"	Synthetic bone with gelatine	11.65 ± 0.28	-0.68
"	Synthetic bone without gelatine	12.20 ± 0.32	-0.13

In addition the method was tested on some internal standard bone and used for processing biogenic phosphates in several research projects, including an interlaboratory comparison. 7) Five aliquots of bison bone (BIS II, internal standard) were processed through steps 1a - 11. 8) About 80 samples of modern and ancient beaver bone, dentine and tooth enamel, and 68 analyses of ancient human bone and other bone standards were processed using this method (Chaps. 4 & 5). 9) Samples of modern and Miocene horse tooth enamel were supplied by D. Bryant for interlaboratory comparison (Table 2.4). These were processed by a number of laboratories using rather different methods, of which three are presented here with the permission of D. Bryant. B. Luz in Jerusalem processed the enamel to produce BiPO_4 which was reacted with BrF_5 (Tudge, 1960). In North Carolina the enamel was processed by D. Bryant using an ion exchange resin to produce Ag_3PO_4 which was reacted with BrF_5 (Crowson *et al.*, 1991). At McMaster the first author processed the material using the lead phosphate method presented here and liberated the oxygen by high temperature polymerization of Ag_3PO_4 with bromine (Stuart-Williams and Schwarcz, 1995).

2.6 Results

All results are given in permil (‰) relative to the VSMOW standard (Table 2.3) using the identification numbers from the previous section. 1) The analytical precision (σ) of the liquid standard precipitated by the Firsching technique (Firsching, 1961) is about $\pm 0.13\text{‰}$ when multiple aliquots of a

Table 2.4: Inter-laboratory comparison of horse tooth enamel $\delta^{18}\text{O}$.(Data from other sites *Pers. Comm.* D. Bryant)

Sample Identification	BiPO ₄ ‰	Ag ₃ PO ₄ ‰	Ag ₃ PO ₄ ‰
	Jerusalem	North Carolina	McMaster
AMNH-M 83453 Modern horse	24.9	25.41	25.90
AMNH 17599A Miocene horse	13.7	14.70	14.75
F:AM 128751 "	20.4	21.85	21.50
F:AM 114059-P4 "	22.2	22.75	22.75
F:AM 108221 "	17.9	18.25	18.20

single precipitate are compared, improving to 0.06‰ for analyses of entire precipitates. This standard has been analyzed numerous times over 3 years and the composition appears to be stable. 2) Processing of the liquid standard through steps 1a - 11 resulted in a small deterioration of the precision and a +0.17‰ offset. 3) Processing of the liquid standard as above, but omitting step 5 except for dissolution in HNO₃, resulted in almost no deterioration of the precision and a -0.08‰ offset. 4) Hydrolysis of the liquid standard shifted the δ¹⁸O by -0.31‰, with a precision of ± 0.13‰. 5) The precision of the analyses deteriorated significantly to ±0.32‰ when the synthetic bone without gelatine was processed through steps 1a - 11 and there was an offset of -0.13‰ relative to the liquid standard. 6) Addition of gelatine to the synthetic bone barely altered the precision but the offset increased to -0.68‰. The quantity of organic material present in solution was substantially greater than in normal processing of bone or enamel, as only a small proportion of the collagen-like compounds in teeth or bone are soluble. 7) 5 aliquots of BIS II bison bone yielded a mean of 10.9±0.13‰. The precision improved to ±0.06‰ if one analysis with a slightly low yield was removed. 8) Precision (σ) of analysis of ancient and modern bones and teeth was found to be 0.15‰, based on duplicate samples of bone and enamel. The total spread of isotopic analyses of 10 samples of a single ethnic group (Tlajinga, Teotihuacan) was less than 1‰ (see Chap. 5). This is a very narrow range of phosphate δ¹⁸O when compared with modern studies of δ¹⁸O variance in a population (Levinson *et al.*, 1987; Longinelli, 1984; Luz *et al.*, 1984) and indicates no large increase in the range due to the analytical method. Detailed isotopic analysis of beaver incisor enamel showed smooth δ¹⁸O trends along the teeth and virtually identical δ¹⁸O profiles for teeth from two

beavers inhabiting the same pond and killed at the same time (see Chap. 4).

9) The analyses of Miocene and modern horse teeth are very similar between laboratories (Table 2.4). The results based on Ag_3PO_4 produced from the enamel are all isotopically heavier than the BiPO_4 analyses and are generally more similar to each other than to the BiPO_4 analyses, with the exception of the analysis of modern horse enamel.

2.7 Yields and completeness of precipitations

Yields for analyses of inorganic phosphate were 70-90% using typical production techniques. This is due to some loss of phosphate when precipitated and centrifuged waste products are disposed of with phosphate adsorbed on them and dissolved in the trapped fluids. Yields for bone could not be calculated as neither the proportion of organic material nor the stoichiometry could be determined.

If a 100% yield could be achieved in processing then an absence of isotopic offset would be assured, but this is seldom possible in practice and it is difficult to identify isotopic offsets produced in this way except by comparison of standards prepared using different methods and resulting in different compounds. This was not readily possible for earlier workers between when research on phosphates commenced, using BiPO_4 (Tudge, 1959, 1960) and the development of an alternative method using Ag_3PO_4 (Crowson *et al.*, 1991).

To achieve a correct analysis, phosphate must not be lost during processing, or material that is lost must be isotopically identical to the remaining portion, as is the case for adsorbed and dissolved phosphate removed with waste precipitates in our processing. To test the effects of losses caused by incomplete precipitations, we added less lead acetate than was required to combine all the available PO_4^{3-} as $\text{Pb}_3(\text{PO}_4)_2$. This resulted in yields being reduced more than was proportional to the reduction in quantity of Pb^{2+} below stoichiometric requirements and produced a maximum offset in $\delta^{18}\text{O}$ of approximately +1‰ (Fig. 2.2a). A similar effect is apparent in analyses of the liquid standard processed through steps 1a - 11 but with insufficient control of the pH during $\text{Pb}_3(\text{PO}_4)_2$ precipitations (Fig. 2.2b). Low yields are apparently associated with high $\delta^{18}\text{O}$ in both cases (Fig. 2.2a&b), as a result of partitioning of the phosphate between a dissolved and a solid phase. To prevent such effects the compositions of the solutions during precipitations should be carefully regulated. For example lead phosphate becomes significantly soluble at $\text{pH} < 5$ or with low ratios of $\text{Pb}^{2+}/\text{NO}_3^-$. During processing the pH is adjusted and high ratios of $\text{Pb}^{2+}/\text{NO}_3^-$ are established before precipitations to avoid these losses and no significant correlation was found between the slightly varying yields and the apparent isotopic value. Phosphate lost in solution or adsorbed onto precipitates is therefore shown to be isotopically identical to the phosphate remaining in solution. This is not the case for phosphate strongly adsorbed onto, or complexed with, collagen-like compounds. There is a sizeable isotopic offset associated with adsorption onto gelatine, also causing higher apparent isotopic values at lower yields (Fig. 2.2b). This effect may also be present in the analysis of unburnt BIS II noted above, although the relationship between lower yield

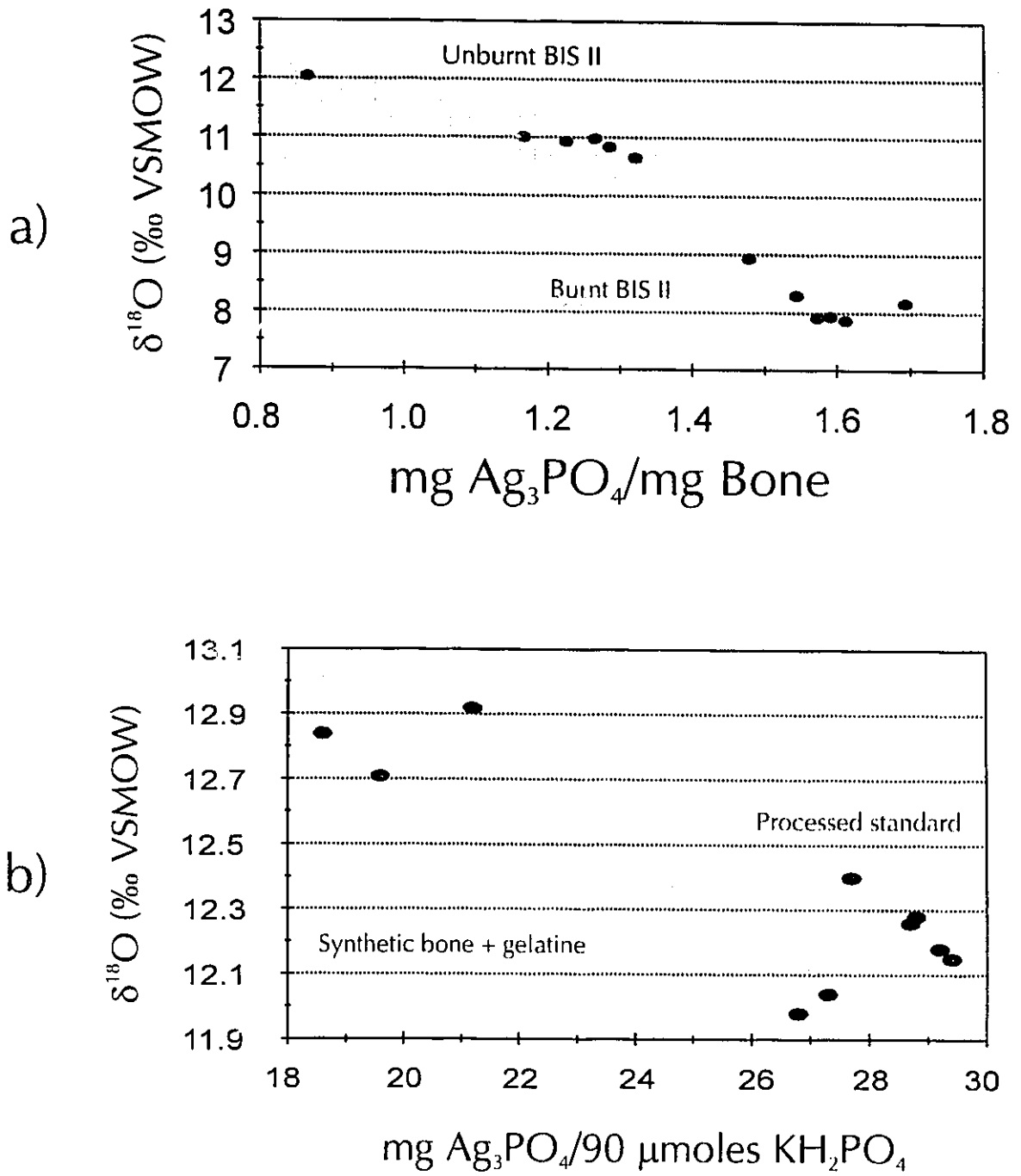


Figure 2.2: Relationship of oxygen isotopic analysis to yield
 a) Low yields caused by restricting the amount of available Pb^{2+} (see text). b) Processing of the liquid standard without correct pH control and processing of synthetic bone + gelatine.

and increasing isotopic value appears to be similar to the burnt BIS II analysis which completely lacked organic material (Fig. 2.2a). Testing of this processing method on bone has not shown reduced yields associated with increased isotopic values and normal quantities of soluble organic materials do not interfere with processing.

Testing for the completeness of precipitations was performed in < 20 mL of fluid in centrifuge tubes, to match conditions during normal processing. As noted above, control of pH during precipitations is essential and loss of phosphate due to solubility of $Pb_3(PO_4)_2$ was shown to be negligible at pH 5.5 - 7; the solubility of $Pb_3(PO_4)_2$ in water is .000014 g/100 mL at 20°C (Weist, 1985). Despite the care taken to minimize losses, the small positive offset on the fully processed standard (test 2) probably results from very slight solubility of the $Pb_3(PO_4)_2$ precipitate following digestion in H_2O_2 and HNO_3 , due to the high concentration of NO_3^- during the precipitation.

2.8 Isotopic properties of silver orthophosphate

A fundamental problem with any method using Ag_3PO_4 precipitated by ammonia volatilization is heterogeneity of the Ag_3PO_4 itself. O'Neil *et al.* (1994) reported several tenths of a permil difference between fine and large crystals in a precipitate and we have found 2-3‰ difference (Stuart-Williams and Schwarcz, 1995) between the bulk composition of crystals formed during the first half of a precipitation and crystals formed during the second half. If a constant offset is maintained between phosphate precipitated and phosphate in solution then the final phosphate precipitated will be highly isotopically fractionated.

This effect is illustrated clearly by 31 separate analyses in our BrF_3 line of 25 mg aliquots of dry Ag_3PO_4 crystals from a bottled standard. The standard comprised a large, single precipitation of dissolved KH_2PO_4 as Ag_3PO_4 with complete recovery of the phosphate. The standard deviation of the 31 analyses was 0.5‰, compared with the usual precision of reproducibility of the line of $< 0.1\text{‰}$. Values ranged from 2.3-4.4‰ in an approximately normal distribution (Fig. 2.3a) without skew, indicating that the dispersion was not due to admixture of atmospheric oxygen. Crystals from the first half of the precipitation have a fairly uniform composition with less than 3‰ variation from the first precipitates to the last, as shown by the offset between two batches quoted above. The precipitation then proceeds as a Rayleigh process. This continues until the last part of the silver phosphate precipitate is substantially depleted in ^{18}O . The precipitate now has a bulk isotopic composition identical to the original dissolved phosphate (Fig. 2.3b) and consists of a majority of grains of very similar isotopic values and a small minority of substantially ^{18}O depleted grains that were precipitated at the end. The composition of the ^{18}O depleted grains is sufficiently offset from the bulk composition that in a 25 mg aliquot of several hundred crystals the number of depleted crystals controls the precision of the analysis (Fig. 2.3c&d). A more typical variance for aliquots of Ag_3PO_4 standards analyzed repeatedly (Table 3.1) is about 0.15‰, probably because in smaller precipitations the isotopic variability is intracrystalline, not intercrystalline. The best precision is obtained when all crystals from a single precipitation are reacted, as is demonstrated by the improvement in precision from 0.15‰ to 0.06‰ when this was done using eight aliquots of Ag_3PO_4 precipitated directly from the KH_2PO_4 liquid standard.

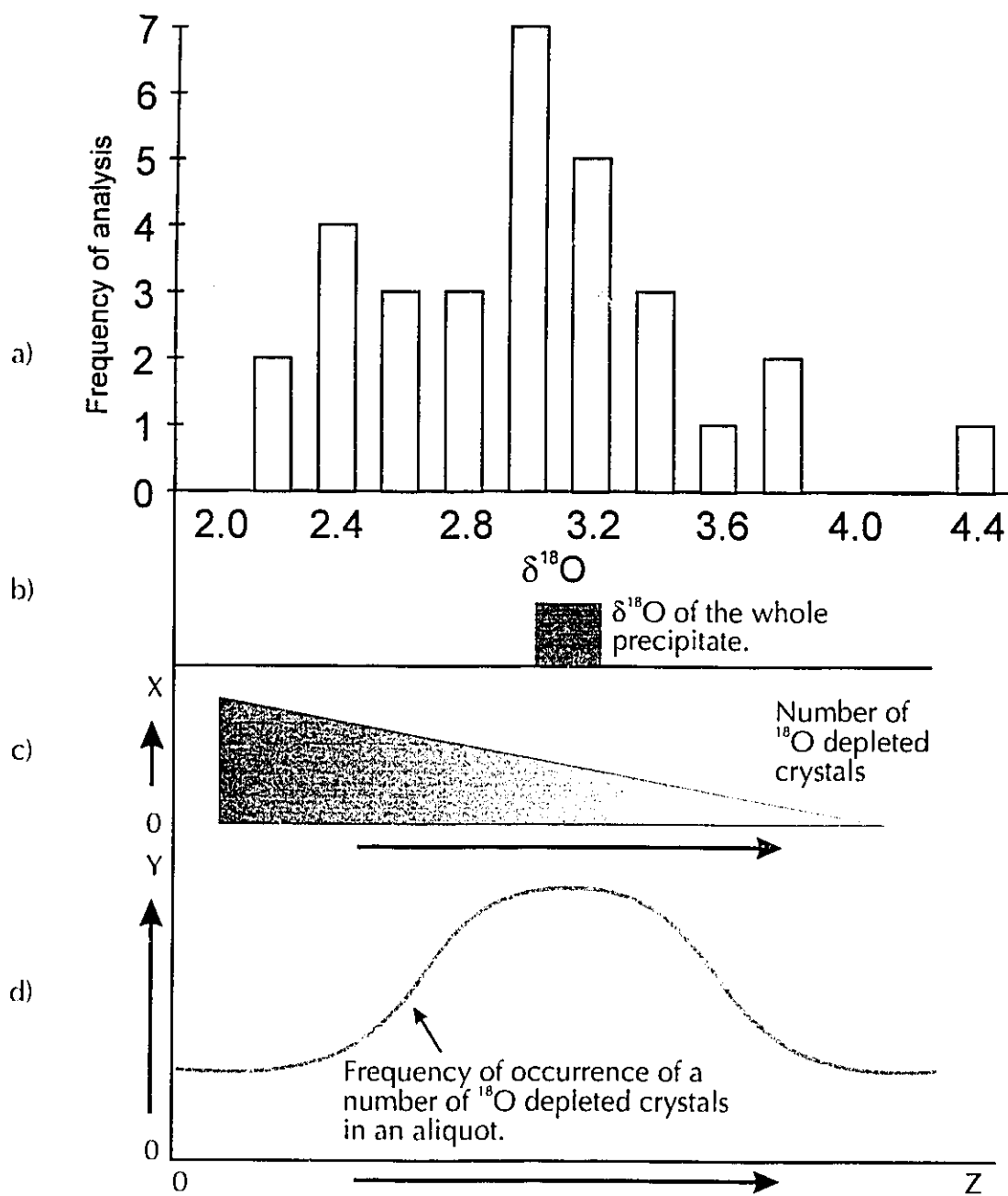


Figure 2.3: Examination of analyses of aliquots from a single precipitation of Ag_3PO_4 used as a standard for a BrF_3 reaction line. a) Frequency of $\delta^{18}\text{O}$ analyses of aliquots. b) Probable bulk composition of the whole precipitate. c) Number of ^{18}O depleted crystals in an aliquot. d) The frequency of occurrence of a number of depleted crystals in aliquots.

This shows that approximately 0.09‰ of a typical deviation in crystalline Ag_3PO_4 results from sample inhomogeneity. Complete recovery of the precipitate is difficult to achieve in practice as the last part of it (with very high $\delta^{18}\text{O}$) occurs mainly as very fine crystals on the bottom of the beaker, unlike the first precipitates which form large crystals on the surface. The finer material tends to be preferentially lost during filtering and washing and by clinging to the walls of storage vials. This effect is likely to result in analyses being isotopically heavier than the true value and can also lead to poor precision.

Silver phosphate was analyzed several times in interlaboratory comparisons to examine errors resulting from mass spectrometry and the liberation of oxygen from the phosphate (Table 2.5). All these samples were reacted with BrF_3 (Crowson *et al.*, 1991). The sizeable errors must result from reaction in the BrF_3 line or from true differences in the isotopic composition of distributed aliquots of the Ag_3PO_4 , as good standards are available for calibration of mass spectrometers analyzing CO_2 . Most of the samples were analyzed at least three times with standard deviations (1σ) on the analyses of 0.15‰ or less. Our analyses are not systematically offset from other laboratories; in the case of comparisons with L. Roe our analyses are offset by +1.4‰ on one standard and -0.8‰ on the other. The cause of these errors is unknown but adsorbed or trapped water in the samples may be a contributing factor.

Table 2.5: Inter-laboratory comparison of Ag_3PO_4 $\delta^{18}\text{O}$ analyses

Sample identification	L. Roe, Michigan	E. Wright Washington	J. O'Neil Michigan	McMaster
E. Wright Ag_3PO_4		19.6‰		20.2‰
L. Roe Ag_3PO_4 , NBS 120C	21.2‰			22.6‰
L. Roe Ag_3PO_4 , CaHPO_4	20.5‰			19.7‰

2.9 Discussion

Alteration of the isotopic value of the analyses results from two particular causes: the inclusion of oxygen in the phosphate from the processing environment or the loss of fractionated phosphate.

While isotopic exchange is improbable during the processing sequence described because concentrated strong acids or bases and high temperatures are avoided (Brodskaa and Sulima, 1953; Tudge, 1960), oxygen may be incorporated into the phosphate from the aqueous medium by hydrolysis of polyphosphates. This was examined by hydrolysing the liquid standard (test 4) which resulted in an offset of +0.31‰ of the $\delta^{18}\text{O}$ relative to the unhydrolysed standard (Table 2.3). The most probable cause for the offset is that the KH_2PO_4 used in the standard contains some polyphosphates, dominantly $\text{P}_2\text{O}_7^{4-}$ or $\text{P}_3\text{O}_{10}^{5-}$ and that oxygen derived from the heated aqueous medium was introduced. This effect may not be significant when processing bone as an analysis in this laboratory of modern white-tailed deer bone using NMR (Gard *et al.*, 1992) showed only orthophosphate to be present within detection limits of approximately 0.2%. The bone was dissolved in the weak acid EDTA at room temperature where hydrolysis would probably have occurred only very slowly. Polyphosphates may not be precipitated effectively by lead as unhydrolysed liquid standard processed using the method described did not show a lighter isotopic value, even though hydrolysis and incorporation of isotopically light oxygen should have occurred during step 5 when the sample was heated with H_2O_2 and HNO_3 .

The loss of any fractionated phosphate will lead to incorrect analyses.

The main causes of phosphate loss resulting in fractionation are:

- a) Slight solubility of the precipitates, with division of the phosphate between phases with different isotopic values.
- b) Improper removal of interfering cations.
- c) The presence of residual organic material which strongly adsorbs phosphate with an accompanying fractionation.

a) Both the solubility of precipitates and the presence of extraneous cations result in phosphate being partitioned between phases of probably different isotopic composition. As examples, both Ag_3PO_4 and BaHPO_4 precipitates have been found in this laboratory to be about 2‰ lighter than phosphate remaining in solution with them. The fractionation of Ag_3PO_4 precipitate relative to the remaining dissolved phosphate was determined by recovering crystals from a typical ammonia volatilization in two parts. Fractionation of BaHPO_4 crystals relative to remaining dissolved phosphate was examined by the evaporation of a solution of $\text{Ba}(\text{NO}_3)_2$ and KH_2PO_4 in 3 M acetic acid, with sampling of the precipitates and solution at three stages during the drying. The $\delta^{18}\text{O}$ of BiPO_4 precipitates has also been shown to be 4.1‰ isotopically lighter than the 0.2‰ of phosphate remaining in solution (Karhu and Epstein, 1986; Tudge, 1960). All three demonstrated offsets almost certainly result from kinetic effects as oxygen isotopic exchange does not occur rapidly in aqueous solutions at low temperatures (Brodskaa and Sulima, 1953), or during crystallization or dissolution (Tudge, 1960).

b) The cations most likely to interfere are calcium and iron. If calcium is not removed completely, calcium hydroxylapatite (which was identified using x-ray diffraction) is formed during the ammonia volatilization procedure. If very fine-grained apatite is present admixed with Ag_3PO_4 , it will not react with bromine to release oxygen in the method used here. If iron is present it will interfere with silver phosphate precipitation by forming stable phosphates or hydroxides during ammonia volatilization which prevent the growth of coarse Ag_3PO_4 crystals. Fine grained silver, calcium and iron phosphates are also likely to be lost during washing while hydroxides will release oxygen when reacted with BrF_5 .

c) The major problem with analyzing biogenic phosphates is interference by organic materials due to the fractionation of phosphate during complexation by collagen-like compounds. Even small amounts of organic material can strongly influence the isotopic result, for example in the study of horse teeth noted above, the greatest offset between the results of N. Carolina and McMaster is in the analysis of the modern tooth, even though probably less than 2% of organic material was present. While developing this method using the BIS II internal standard, we commonly obtained a precision worse than 0.25‰ within batches of samples and a > 1.5‰ range of mean values between batches. At the same time the precision of analyses of inorganic standards was <0.14‰ with repeated analyses identical within the reported precision. Most collagen, insoluble humic material and iron oxide is removed by centrifuging at pH < 4 early in the processing with very little adsorbed phosphate (Gerke, 1993; Koutsoukos and Nancollas, 1986), but soluble collagen-degradation products and humic compounds, which are particularly common in ancient bone, are carried through

the processing complexed with the dissolved phosphate. This strong complexation results in significant fractionation, discoloured precipitates and reduced yields.

Commonly the bone is treated initially with H_2O_2 to oxidize the organic compounds (O'Neil *et al.*, 1994) but we have found that H_2O_2 is not an effective way of digesting collagen as it is incapable of penetrating deeply into coarser particles of bone. Organic material is often present in the final Ag_3PO_4 precipitate even after the bone has been digested in 30% H_2O_2 for several days. Discoloration, low yields and poor precision have also been noted when precipitating $BiPO_4$ from solutions of bone in HNO_3 , after digesting the bone in H_2O_2 for several weeks (Schwarcz *et al.*, 1991). Because bone crystallites are imbedded in collagen and other proteins (Lowenstam and Weiner, 1989 and references therein) some organic matrix is probably present unless the bone is completely separated into its component crystallites. $NaOCl$ will destroy the organic matrix in about one week but results in dispersed fine grains that resist concentration even by centrifuging (R. Chopra in this laboratory).

Organic compounds interfere especially with the preparation of silver phosphate by ammonia volatilization because Ag_3PO_4 precipitates mainly at $pH > 7$. In extreme cases the precipitate is a fluffy, light brown mass but more commonly organic residues cause the precipitation of a reduced amount of fine, dark green or black crystals. The presence of residual organic materials may explain why O'Neil *et al.* (1994) found it necessary to reduce the volume of their final precipitation by evaporation, while the published ammonia volatilization method (Firsching, 1961) achieved precipitation of 1.9 mg P_2O_5 from 175 mL of

solution with an error of less than 0.1 mg. By decreasing the volume of fluid the high ratio of Ag^+ to organic-complexed phosphate shifts the equilibrium toward Ag_3PO_4 formation.

Good precision is not proof of good accuracy or of repeatability. Using a bison bone internal standard (BIS II) we have shown that it is possible to achieve a precision of better than 0.15‰ on 6 analyses with a rather low yield, with a mean value of the analyses that is about 1.0‰ heavier than the value obtained when the bone is correctly processed and high yields obtained. The original method of BiPO_4 preparation (Tudge, 1960) called for dissolution of the bone in 10 M HNO_3 followed by oxidation of the organic compounds with KMnO_4 . A modified form of this method has been used by subsequent workers (Ayliffe *et al.*, 1994; Bryant *et al.*, 1994; Sánchez Chillón *et al.*, 1994). Unfortunately we have not been able to incorporate this step into our processing without interference by residual manganese, but digestion of the dissolved phosphate with H_2O_2 and HNO_3 has proven to be effective. In an effort to remove organic compounds entirely before processing we experimented with heating powdered BIS II bone in air at 550°C for 1 hour, stirring the sample once after 30 minutes. The mean of the isotopic analyses of burnt BIS II was 3‰ lighter than for unburnt BIS II. This offset may result from the breaking of hydrogen bonds in $\text{PO}_4\text{-H-PO}_4$ groups and polymerization of the phosphate (LeGeros and LeGeros, 1984) or incorporation of non-bone phosphate, but it is also possible that the higher $\delta^{18}\text{O}$ of the unburnt bone results from loss of a very small portion of the phosphate which is complexed with organic material.

In conclusion it appears that failure to oxidize organic materials causes low yields, often with higher isotopic values and poor precision . The importance of complete removal of organic compounds cannot be overemphasized and removal of organic compounds can only be avoided by researchers using completely fossilized or inorganic samples (Crowson *et al.*, 1991). Testing of phosphate processing methods for oxygen isotopic analysis using only inorganic standards cannot identify effects arising from peculiarities of bone chemistry. Because of the uncertainties associated with the dissolution and processing of bone, it is important that all samples to be compared within a project should be dissolved and processed using a single method.

All Pb^{2+} must be removed before precipitation of Ag_3PO_4 or fine-grained $Pb_3(PO_4)_2$ will form and alter the $\delta^{18}O$ of the final product. After centrifuging $PbSO_4$, no lead could be detected either as fine white crystals during Ag_3PO_4 precipitation or by adding Na_2S to the solution.

The amount of ammonium nitrate used in the final precipitation was tested for any effects on the product Ag_3PO_4 . Approximately 200 mg of ammonium nitrate was found to give the best results as use of 400 mg or more resulted in less well crystallized silver phosphate and sometimes lower yields, whereas using 25 mg or less results in the silver phosphate having a considerable content of silver carbonate. The silver carbonate is removed during sample preheat in the bromine reaction method (Stuart-Williams and Schwarcz, 1995) as it decomposes at about 218°C (Weist, 1985), releasing CO_2 , and the remaining Ag_2O decomposes further, releasing O_2 .

CHAPTER THREE

Oxygen isotopic analysis of silver orthophosphate using a reaction with bromine¹

3.1 Abstract

A simple and precise method of determining the oxygen isotopic composition of Ag_3PO_4 has been developed in which orthophosphate is condensed to pyrophosphate and higher polymeric forms at elevated temperatures using Br_2 as a reactant in a heated quartz vessel, releasing about 17.5% of the oxygen present as O_2 gas. The O_2 is converted to CO_2 using a heated carbon rod and analyzed using a mass spectrometer. The precision of the $\delta^{18}\text{O}$ determination is better than 0.07‰ when reaction furnace temperature is regulated to within $\pm 0.5^\circ\text{C}$. This method offers advantages of speed, safety and cost over conventional BrF_3 decomposition of silver phosphate.

3.2 Introduction

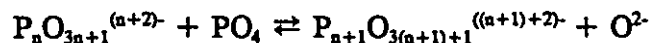
The relative stability of the oxygen isotopic composition of phosphate has led to its use for determining the isotopic composition of the aqueous fluid with which it was equilibrated (Brodskaa and Sulima, 1953; Karhu and Epstein, 1986; McArthur and Herczeg, 1990; Shemesh, 1990). In particular the $^{18}\text{O}/^{16}\text{O}$ ratio of animal tooth and bone phosphate [$\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$ with carbonate substitutions at the phosphate and hydroxyl sites] can be used to infer the isotopic composition of

¹ A modified version of this chapter, authored by H. Le Q. Stuart-Williams and H.P.Schwarz, has been accepted by *Geochimica et Cosmochimica Acta*.

ancient bodily fluids, from which the isotopic composition of ancient meteoric waters can be approximated and mean annual temperature and other climatic data deduced (Kolodny *et al.*, 1983; Longinelli, 1984; Luz *et al.*, 1990). Recent work has concentrated on terrestrial herbivorous mammals but cetaceans and dinosaurs have also been examined (Barrick and Showers, 1994; Yoshida and Miyazaki, 1991). Concerns with the degree to which the oxygen isotopic composition of biological apatites may be altered during diagenesis will probably serve to increase the number of samples which must be processed to give some confidence in the results. Unfortunately conventional methods for oxygen isotopic analysis of phosphates are lengthy and complex. The first practical method (Tudge, 1960) required extensive wet chemical procedures to convert bone phosphate to highly hygroscopic BiPO_4 which must be carefully stored and dried prior to reaction with BrF_3 . New techniques for preparing phosphates as Ag_3PO_4 are somewhat simpler but require increased reaction times with BrF_3 : about 12 -18 hr as compared with 2 hr needed for BiPO_4 (Crowson *et al.*, 1991). The use of BrF_3 is itself expensive and undesirable for health and waste disposal reasons: the compound is highly toxic and explosively reactive with many organic materials. Increasingly restrictive safety regulations make the construction of new lines logistically awkward. Recently a method has been presented for reacting Ag_3PO_4 with carbon at 1000°C with yields of about 25% of the total oxygen with a reproducibility of $\pm 0.2\%$ (O'Neil *et al.*, 1994). An older technique of reacting $\text{Ba}_3(\text{PO}_4)_2$ with carbon released about 72% of the oxygen, but was not developed for high precision work (Cohn, 1957).

The present method is based on our observation that heating of Ag_3PO_4 to 450°C in the BrF_3 reaction line, without addition of BrF_3 , produced O_2 but the

oxygen isotopic ratios were extremely variable. A phosphate condensation reaction of the general form:



was apparently occurring. The presence of O_2 gas indicated some reduction of the silver. In retrospect, this reaction must have resulted from residual bromine compounds as condensation of Ag_3PO_4 does not occur in the absence of bromine at $450^\circ C$. Further experiments using very high temperatures in quartz tubes and electrically-heated platinum ribbons similarly produced O_2 of a variable isotopic ratio together with metallic silver, Ag_2O and unidentified products. The variety of oxidation states of Ag present indicated that it was necessary to prevent undesirable back-reactions and provide an electron donor and acceptor to facilitate the production of O_2 from $2O^{2-}$. Bromine was selected as it is known not to form stable oxides (Spekkens, 1977; Tudge, 1959, 1960) under the conditions of this reaction. Experimentation with trial setups demonstrated that careful temperature control was required to achieve a high precision result using the following reaction:



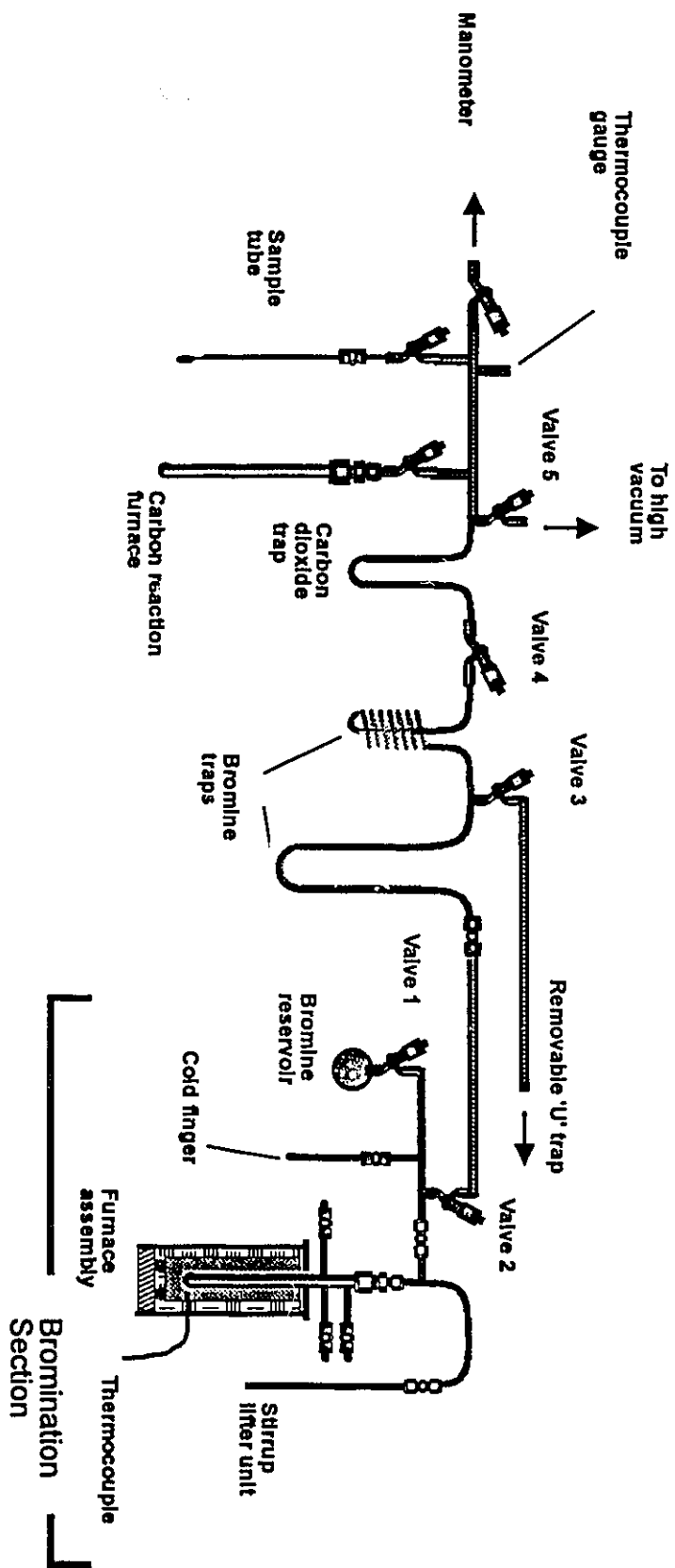


Figure 3. 1: Vacuum line using for reacting phosphates with bromine at high temperature.

3.3 Equipment

The system used for decomposing Ag_3PO_4 is shown in figure 3.1. The reaction line is composed entirely of Pyrex glass, apart from the quartz furnace vessel, and is designed to have the minimum possible volume. A vacuum of ca. 30 millitorr is maintained with a mercury diffusion pump and a liquid N_2 trap. An oil diffusion pump is recommended for future lines as after extended use HgBr_2 was found to coat the inside of the glass line. Samples are loaded in cups prepared by cutting 10 mm from the end of a 6x50 mm Kimble borosilicate glass culture tube. The cups are used once only. 40-50 mg of Ag_3PO_4 is weighed into a cup and then tamped down under a thin layer of borosilicate glass wool. Valves 1 and 2 are closed and groups of 10 or more samples are then inserted into the upper 9 mm O.D. side-arm of the quartz reaction tube, followed by a glass encased magnet, and the end of the tube is sealed with an Ultra-Torr fitting. Brass, nickel or PFA fittings should be used as bromine attacks stainless steel vigorously. The side-arm below the sample inlet contains a glass encased magnet with a glass rod welded to it which is used to push the spent samples into the opposite side-arm. The quartz furnace vessel projects 15 cm into a hole 1 mm larger in diameter, drilled lengthwise into a 2 inch copper bar, nickel plated to reduce oxidation and supported by ceramic bars in a vermiculite filled steel case. A thermocouple is inserted into the furnace adjacent to the bottom of the quartz tube. The core is heated by nichrome wire wound around it, electrically insulated from it by very thin mica sheets. In an improved version of the furnace the core is heated by three nichrome coils arranged along it with the central coil regulated by a Syscon RKC REX-C100 controller. The sample is placed in the furnace by moving it from the sample side-arm to a quartz stirrup which is

lowered and raised by means of a Chromel-A ribbon and another glass encased magnet. The glass vessel containing liquid Br_2 is sealed by a Young glass and plastic valve. Oxygen from the reaction passes through another Young valve and two liquid nitrogen traps, to remove all bromine, and to the CO_2 conversion unit consisting of a group of 10x30 mm lengths of 2.5 mm Union Carbide carbon spectrograph electrode, treated with PtCl_4 , suspended with platinum wire in a quartz tube and heated to red heat (ca. 600°C) by a nichrome coil wound around the outside of the tube. A Veeco thermocouple gauge is attached to the line next to valve 5.

3.4 Method

The furnace core is heated to $550 \pm 0.5^\circ\text{C}$ for the reactions. The bromination portion of the line is isolated by closing valve 2 and Br_2 is frozen from the reservoir into the cold finger using liquid N_2 . A small quantity of liquid Br_2 should be present in the cold finger at all times during reactions to ensure that Br_2 is present in the line at its room temperature vapour pressure. During preheating valve 2 is closed and the line is filled with Br_2 vapour to react any remaining phosphate. Once the furnace has reached 550°C the Br_2 is pumped out to be frozen into a waste trap. A fresh aliquot of Br_2 is then frozen into the cold finger and valve 2 opened to pump away non-condensibles. A sample is placed on the stirrup and lowered until it rests on the bottom of the furnace to preheat for 3 minutes. Valve 2 is then closed and the Br_2 thawed with a hot air blower. The sample is reacted for 7 minutes in the Br_2 atmosphere, after which the Br_2 is frozen back into the cold finger. During this period a 6 mm Pyrex sample tube is evacuated and the remainder of the line pumped with most valves open (except

valve 3) and the cold trap between valves 4 and 5 brought to room temperature to remove any residues. Liquid N_2 traps are then placed under the carbon reaction tube and the 'u' trap between valves 4 and 5. Valve 4 is then closed and valve 2 opened. After a delay of 10 seconds to allow traces of Br_2 to freeze out, valve 5 is closed, valve 4 is opened and the O_2 reacted with the preheated and degassed carbon rod to form CO_2 which condenses into the bottom of the carbon reactor and the 'U' trap next to valve 4. Progress of the reaction is monitored with the thermocouple vacuum gauge to determine when a minimum pressure is reached. Once the reaction is complete, valve 5 is opened to pump out non-condensable gases and the electric heating of the carbon rod is turned off. Valve 4 is closed once a good vacuum has been obtained in the line. The CO_2 gas is then transferred to a mercury manometer and its volume measured before being transferred to the sample tube and cut off with a torch. The CO_2 is opened to high vacuum after each freezing down to remove non-condensable impurities. Once valve 3 has been closed the used sample cup is withdrawn from the furnace and pushed into a side-arm with the magnet opposite; the Br_2 in the cold finger is then thawed and passed into the waste trap between valve 3 and valve 2. The entire process takes about 20-30 minutes, depending on the age of the carbon rods: older rods convert the O_2 to CO_2 more slowly. The isotopic composition of the CO_2 produced was determined using a VG 602 mass spectrometer.

Table 3.1: Identification of silver orthophosphate standards with bromine and bromine pentafluoride analyses.

Standard	Origin	Mean BrF ₅ δ ¹⁸ O	n	Mean Br δ ¹⁸ O	n
2ndFS	KH ₂ PO ₄ (1st split)	2.646 ± 0.42‰	3	-8.401 ± 0.32‰	3
Na-1	NaH ₂ PO ₄ (1st split)	7.811 ± 0.17‰	3	-2.831 ± 0.13‰	4
Na-2	NaH ₂ PO ₄ (2nd split)	10.816 ± 0.29‰	4	-0.180 ± 0.16‰	4
KH2	KH ₂ PO ₄ (Unsplit)	10.851 ± 0.26‰	4	-0.042 ± 0.14‰	11
NH4-1	(NH ₄)H ₂ PO ₄ (1st split)	17.751 ± 0.07‰	4	6.701 ± 0.16‰	4
NH4-2	(NH ₄)H ₂ PO ₄ (2nd split)	20.966 ± 0.25‰	4	9.995 ± 0.26‰	3
Pooled s		0.27‰		0.18‰	

Bromine is removed from the line at the end of each day by closing valve 4, opening valve 3 and freezing the Br_2 into a removable 'U' trap which is then thawed in a fumehood. Typically 10 samples are processed in each run. After no more than 50 samples the silica reaction vessel and the stirrup are cleaned by soaking in 3 molar NaI solution and subsequently washed with phosphate-free detergent.

The Ag_3PO_4 standards used were precipitated from $(\text{NH}_4)\text{H}_2\text{PO}_4$ supplied by Fisher Scientific, and KH_2PO_4 and NaH_2PO_4 from BDH Chemicals using an ammonia volatilization technique (Firsching, 1961) (Table 3.1). In the course of attempting to assess the precision of the results, we discovered a striking lack of homogeneity between aliquots of single preparations of Ag_3PO_4 . This appears to be a result of the NH_3 volatilization (so-called Firsching-) technique used for precipitating the material, in which silver is initially complexed with ammonia but is released gradually to combine with the phosphate as the ammonia volatilizes (Firsching, 1961). More than 40 reactions of the 2ndFS standard with BrF_3 have shown 20 mg samples to have a range of $\delta^{18}\text{O}$ in excess of ± 1 ‰ of the mean. Split standards were produced by separate collection of the first and last parts of a typical NH_3 volatilization precipitation. There is more than 2 ‰ difference between these fractions, with the first material precipitated being isotopically lighter in each case. This inhomogeneity is believed to result from the very slow formation of the crystals as the $\text{Ag}(\text{NH}_3)_2$ complex dissociates, and is only noticeable when large batches are produced to serve as standards; smaller samples are precipitated over a shorter period of time and probably have variation within crystals rather than between crystals. The greater σ_{pooled} of the BrF_3 results (0.27 ‰) than the Br_2 results (0.18 ‰) may be caused partly by the smaller

aliquots used (20 mg vs. ~50 mg), with less averaging between crystals of different composition. While the NH_3 volatilization precipitation method is ideal for gravimetric and BrF_5 analysis where the exclusion of water and impurities is essential, a more rapid method with less isotopic differentiation may be desirable for use with the Br_2 method in which non-phosphatic oxygen, for example from water, is unlikely to be released. Better precision can be obtained by precipitation from a solution containing only the amount of phosphate required for 1 analysis, for example ten separate precipitations of the KH_2 standard solution were totally reacted with BrF_5 , with a precision of 0.1 ‰. With an experimental electronically controlled furnace the precision on seven reacted precipitates improved to 0.057‰.

3.5 Results and discussion

All results are presented here as $\delta^{18}\text{O}$ in permil (‰) relative to the SMOW standard. The results are compared with analyses determined by BrF_5 reaction of aliquots of the same Ag_3PO_4 preparations. Analyses using the Br_2 and BrF_5 methods are shown in table 3.1 and figure 3.2. BrF_5 analyses were made of 20 mg samples. There is an excellent correlation between the two data sets, with values of $\delta^{18}\text{O}$ obtained using Br_2 consistently 10.9‰ lower than those obtained using BrF_5 , over the 2.6-21.0 ‰ (SMOW) range tested with a standard error in the Y estimate of 0.17‰. The pooled population deviations (σ pooled) of the BrF_5 and Br_2 reacted samples are 0.27 and 0.18‰ respectively.

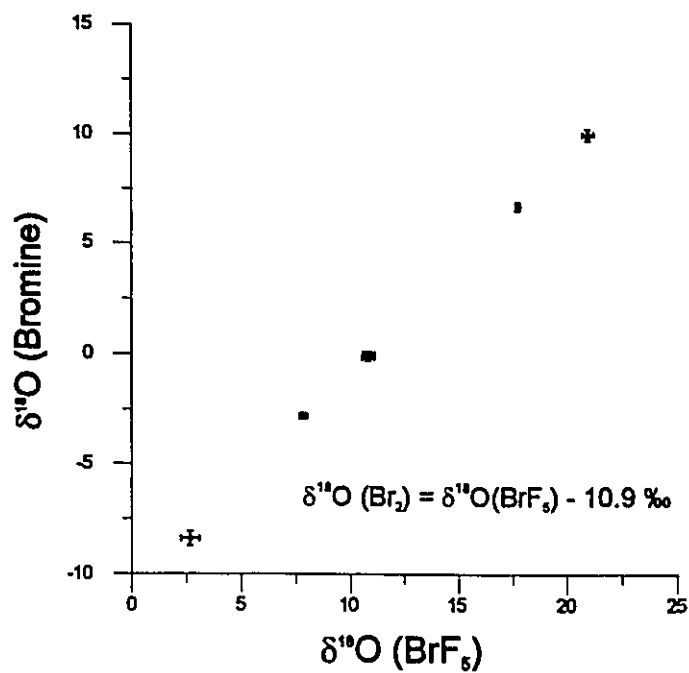


Figure 3.2: Relationship of Br_2 analyses of standards to BrF_5 analyses

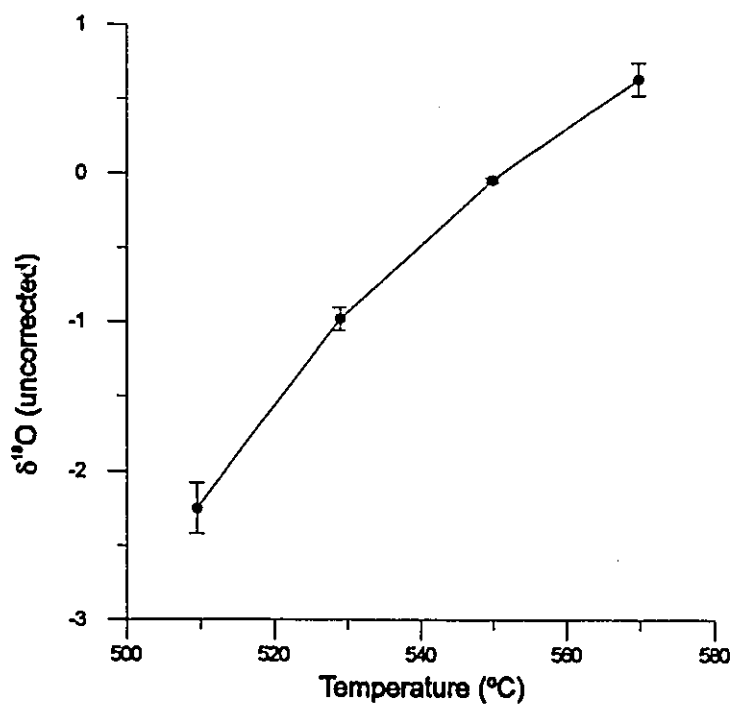


Figure 3.3: Relationship of phosphate $\delta^{18}\text{O}$ analyses of KH2 standard to temperature

Careful temperature control during the reaction is critical in obtaining high precision. The copper cored furnace was designed to achieve very uniform temperatures within the reaction vessel: less than 3°C temperature variation is found at any time along the length of the furnace at 550°C. As it was only possible to regulate the furnace within $\pm 1^\circ\text{C}$ of the chosen temperature, it was necessary to determine whether temperature variation contributed to the data variance. Three KH2 aliquots were reacted at each of the temperatures 510°C, 530°C and 550°C. A mean value for the determinations at 550°C was included and a temperature function generated over that range (Fig.3.3). The multiple samples for the KH2 standard were then corrected by 0.0375‰ / 1°C, assuming that the majority of the polymerization occurred at the initial temperature, 549°C, 550°C or 551°C. No improvement in the variance was produced, indicating that the apparent furnace temperature variation of $\pm 1^\circ\text{C}$ is undetectable in the results, although as noted above an electronically controlled furnace which was stable to within $\pm 0.5^\circ\text{C}$ of 550°C produced a significant improvement in the precision.

Reaction temperature and sample composition are the only significant variables in the analysis. The duration of the reaction is not critical as an aliquot of the KH2 standard reacted for an extra 18 minutes produced a $\delta^{18}\text{O}$ value within 1 standard deviation of the mean for the material. Using twelve values obtained from four runs of the KH2 standard, tests were made for a dependence of $\delta^{18}\text{O}$ on sample mass or yield: no significant correlations resulted within the mass range 45-55 mg or yield variation 67-77%. A sample size of ca. 50 mg was chosen so as to produce ca. 40 μMoles of CO_2 which can be analyzed directly on the VG 602 mass spectrometer without using the cold finger.

There is no memory effect between samples differing in $\delta^{18}\text{O}$ by up to 20‰. An early version of the apparatus in which the sample pellets remained in the hot part of the reaction vessel showed continuing release of O_2 during subsequent reactions but there was no trend toward a changing isotopic value within multiple samples of the same material, demonstrating that the isotopic value of the O_2 released is not related to the degree of polymerization of the phosphate.

3.6 Characterization of the reaction and conclusion

A maximum of 25% of the oxygen atoms present could be liberated if all the phosphate polymerised into a single chain. In practise, the degree of polymerization is rather less than this as we obtained yields of about 17.25%. This indicates that the mean chain length must be between 3 - 4 condensed orthophosphate molecules.

X-ray diffraction data for the reacted contents of the sample pellets reacted for 7 minutes are presented in figure 3.4. Only clearly defined peaks for AgBr are present, no Ag_3PO_4 is visible. The broad peak centred at ca. $2\theta = 32.5$ corresponds with the primary peak for $\text{Ag}_4\text{P}_2\text{O}_7$ while the even broader peak in background scattering around it may be due to the multiple peaks present in the $\text{Ag}_5\text{P}_3\text{O}_{10}$ and the poor crystallinity of the polyphosphate product.

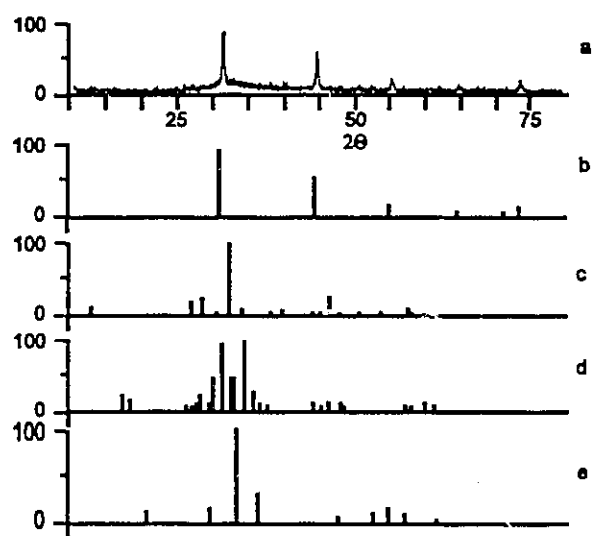


Fig. 3.4: X-ray diffraction spectrum and peaks for reaction products and compounds that may be present. a) Contents of sample cup after reaction. b) AgBr c) $\text{Ag}_4\text{P}_2\text{O}_7$ d) $\text{Ag}_5\text{P}_3\text{O}_{10}$ e) Ag_3PO_4

Polymerization of the Ag_3PO_4 does not proceed at 550°C in the absence of Br_2 ; during the preheat of the sample before reaction no appreciable quantity of O_2 is produced. Polymerization is largely complete after 7 minutes with yields varying by only $\pm 5\%$. After 25 minutes of reaction the yield rises to 19.5% with no detectable shift in $\delta^{18}\text{O}$. Higher yields can be obtained at greater temperatures but the service life of the furnace and reaction vessel would be shortened and the convenience of using borosilicate sample cups would be lost.

In conclusion, these are the advantages of this technique over existing methods:

- Analyses are more rapid than BrF_3 reactions and have a comparable or better precision.
- Health hazards and waste disposal difficulties are considerable reduced.
- The costs of a phosphate analysis line are very much reduced.
- Careful weighing of reactants is not required.
- The analysis is not sensitive to water from the orthophosphate or the atmosphere.

CHAPTER FOUR

Oxygen isotopic determination of climatic variation using phosphate from beaver bone, tooth enamel and dentine¹

4.1 Abstract

We have analyzed 16 incisors and jaws from Canadian beaver (*Castor canadensis*) in southern Ontario to determine whether a climatic signal can be found in the changing $\delta^{18}\text{O}$ of the enamel and bone. The resulting data for the enamel are complex as the beaver respond rapidly to changes in the isotopic composition of their environmental water. In adult beaver the bone oxygen isotopic composition is uniform ($11.9 \pm 0.5\text{‰}$) over the study area. Seasonal variation of 4‰ is present in the enamel, which indicates that a high proportion of the expected 10‰ seasonal variation in the $\delta^{18}\text{O}$ of meteoric water is reflected in isotopic variation of the beaver body water. Attenuation of the δ_{mw} seasonal fluctuation results mainly from the mixing of meteoric water with a large groundwater and surface water reservoir. Other variability in the data appears to result from local hydrological effects, including change in the amount and composition of precipitation and variability of influence of lake water and stream water influxes. A climatic signal is clearly preserved in a Sangamonian giant beaver (*Castoroides ohioensis*) incisor from Hopwood Farm, Illinois, which shows variability of 5‰ in the $\delta^{18}\text{O}$ of the enamel phosphate. This represents an annual seasonal range between mild winters and hot summers. A pattern of a

¹ A modified version of this chapter, authored by H. Le Q. Stuart-Williams and H.P. Schwarcz, has been submitted to *Palaeogeography, Palaeoclimatology, Palaeoecology*.

rapid increase in enamel $\delta^{18}\text{O}$ during the summer and a slow decrease during the winter and spring is seen in *Castor* but is not seen in *Castoroides*.

4.2 Introduction

The isotopic composition of meteoric water is a product of the cooling history of the moist air mass from which the precipitation is derived (Dansgaard 1954, 1964; Yurtsever and Gat, 1981) modified by topographic effects, amount effects and distance travelled across land. By examining the composition of meteoric water, mean annual temperatures and seasonal fluctuations in temperature can be established. The isotopic composition of surface water at a locus (δ_w) is primarily controlled by the composition of precipitation (δ_{mw}), including melting snow, modified primarily by evaporation and mixing with old or non-local water, such as springs and rivers. Despite these limitations, δ_w is strongly correlated with δ_{mw} (Fritz, 1981; Cook, 1970; Siegenthaler, 1970).

Herbivorous animals obtain their body water from their food or as water drunk, the isotopic composition of which is dominantly controlled by precipitation. An exception is animals (such as macropods: Ayliffe and Chivas, 1988, 1990) which depend on plant-derived metabolic water for most of their needs and are greatly influenced by humidity effects. The oxygen incorporated in bone phosphate is isotopically equilibrated with body fluids during metabolic processes such as the synthesis and metabolism of ATP (Kolodny *et al.*, 1983; Longinelli, 1984; Luz *et al.*, 1984) while the animal is alive, but phosphate minerals of bones and teeth are highly resistant to oxygen isotopic exchange after

the death of the organism (Brodskiĭ and Sulima, 1953; Tudge, 1960). The isotopic composition of bone phosphate depends also on the temperature at which it is equilibrated with the body fluids (Longinelli, 1965; Longinelli and Nuti, 1973a) so that in poikilothermic animals both the $\delta^{18}\text{O}$ of the biogenic phosphate (δ_p) and temperature must be known before the isotopic composition of body water (δ_{bw}) can be calculated, which is seldom possible. Research using mammals with a constant body temperature has shown great potential for assessing local δ_w (Longinelli, 1984; Luz and Kolodny, 1985), often with good correlations between δ_p and δ_{mw} modified by relative humidity (Luz *et al.*, 1990; Cormie *et al.*, 1994).

Tooth enamel is preferred to bone or dentine for isotopic analysis of ancient biogenic phosphates because it preserves its signature longer after burial, as the crystallites are substantially larger and more densely packed (Ayliffe *et al.*, 1994; Lowenstam and Weiner, 1989) and the small content of organic matter is much less liable to attack by microbes. Tooth enamel is not remodelled once it emerges above the gum line and thus records the isotopic composition of the body water during the relatively short period when the enamel was forming. The isotopic composition of tooth enamel (δ_{pe}) may not be representative of the average δ_{bw} of the animal throughout the year, especially in young animals born during a particular season when tooth emergence follows after a relatively fixed period. Teeth which grow throughout the animal's life, such as rodent incisors, provide a continuous record of δ_{bw} which can be used to identify season of death, to quantify seasonal variability or to determine mean annual temperatures if

enough samples are present or each tooth grows slowly enough to record δ_{bw} for much of a year.

Within a single species of mammal, δ_{bw} bears a fixed relationship to δ_w which is specified in terms of the slope of δ_w/δ_{bw} (the metabolic slope) and an intercept (Longinelli, 1984; Luz *et al.*, 1984). Large mammals are expected to have a body water composition close to δ_w , and a δ_w/δ_{bw} slope close to unity, according to the most recent model (Bryant and Froelich, 1995). The δ_{bw} of small herbivorous mammals, with a higher metabolic rate, is calculated to have a greater positive offset from δ_w and a slope of less than unity. The oxygen flux model proposed for mammalian δ_{bw} (Luz *et al.*, 1984) precludes slopes of greater than 1 but several studies of δ_p apparently contradict this expectation (D'Angela and Longinelli, 1990; Ayliffe *et al.*, 1992). In addition δ_{bw} of some animals, such as macropods (Ayliffe and Chivas, 1990), rabbits (Huertas *et al.*, 1995) and white-tailed deer (Luz *et al.*, 1990) has been found to be significantly influenced by evaporative fractionation of their vegetable foods.

Because attempts to model δ_w/δ_{bw} (Bryant and Froelich, 1995) have not yielded results precise enough to use for palaeoclimatic determinations, the largest sources of deviation from a predictable δ_w/δ_{bw} relationship must be avoided. Relatively large mammals (> 1 kg) must be used and mammals relying heavily on metabolic water should be avoided. As it is difficult to recognize the effects of low relative humidities in ancient animals (Luz *et al.*, 1990; Cormie *et al.*, 1994) mammals from humid habitats are preferred. Cetacea have a metabolic slope of 0.773 and a positive offset at the zero intercept of δ_w of only

0.5‰ (Yoshida and Miyazaki, 1991), a substantially smaller offset than in most terrestrial mammals. Unfortunately fossil Cetacea can seldom be used to determine continental climate, whereas large aquatic rodents are relatively widespread. The Canadian beaver (*Castor canadensis*) was selected for this study as it lives on a moist diet (Novak, 1987), and spends much of its life in the water or in a high humidity environment. A pilot study of beaver from Erin County, southern Ontario, (Stuart-Williams and Schwarcz, 1993) showed that the $\delta^{18}\text{O}$ of beaver incisor enamel appeared to record seasonally changing δ_{bw} . Seasonal fluctuations of the $\delta^{18}\text{O}$ of carbonate in tooth enamel and tusks have also been detected in Mastodonts, mammoths and black bear (Koch *et al.*, 1989). A large data set of analyses of beaver incisors are presented here with analyses of a single Sangamonian giant beaver (*Castoroides ohioensis*) incisor for comparison.

4.3 Introduction to beaver

Beaver are large rodents of the genus *Castor*, many or all of which are aquatic. The Canadian beaver (*Castor canadensis*) is found in most of North America south of the arctic tree-line, apart from peninsular Florida, parts of the Midwest and the arid southwestern United States (Novak, 1987). The very similar European beaver (*Castor fiber*) is very widely distributed across Eurasia although it is extinct in some restricted areas, such as Britain where it was finally exterminated in the Middle Ages (Coles, 1992). Beaver are the largest rodents in North America apart from the Capybara (*Hydrochoerus sp.*) with an average size in Canada of about 17 kg when full grown, ranging up to nearly 40 kg

(Novak, 1987), and a length of up to 1 m. These are small in comparison with the giant beaver (*Castoroides ohioensis*) which had an overall length of about 2.5 m and weighed up to 200 kg. It became extinct near the end of the last glacial period, about 15 000 years ago (Martin, 1967; Martin and Guilday, 1967). The oldest beaver known in North America is the Oligocene *Anotocastor* from Wyoming (Emry, 1972).

Under most conditions modern beaver (*Castor sp.*) will build a dam to pond water around their lodge and winter food reserves. Evidence of dam building is lacking for ancient beaver. The impact of the beaver on landscapes in temperate zones in the Holocene has probably been significant (Coles, 1992) due to the continual construction, silting and abandonment of dammed ponds. Breeding usually takes place in the winter with kits produced after the spring thaw in northern areas. The kits stay in the home lodge through one more breeding cycle of the parents but are usually ejected before the next brood is produced. The number of beaver having lodges in a pond is quite variable, but when food reserves are limited young beavers will be forced to leave the home area and establish their own colony. Beaver do not hibernate and subsist in winter on the bark of branches stored underwater in the summer and autumn. During the summer the beaver eat more soft vegetable food, including soft water plants (particularly water lilies) and their roots. Trees are gnawed down in a large area around the pond and canal system, with branches, twigs and leaves dragged to the pond to be eaten, added to the food reserve or incorporated into the dam. The fur is waterproof and keeps the beaver dry and warm (Novak, 1987).

4.4 Samples

Beaver lower incisors were obtained free or for a nominal fee from trappers killing them either for pest control or for their pelts in 1993 and 1994. Because the pelts are thin during the summer no specimens were procured from June through September in either year. All samples come from southern Ontario: from around Parry Sound at the east end of Lake Huron and from Georgetown and Erin west of Lake Ontario (Fig. 4.1, Table 4.1). Parry Sound is on the edge of the Laurentian shield which in this area comprises a suite of Precambrian metasediments and igneous rock, stripped and planed off by glaciation. Permeability is poor and water sits in numerous low areas exposed by the retreat of the ice sheets. Some beaver from this area may have lived in inlets connected to Lake Huron. Georgetown and Erin are situated on Palaeozoic sediments, mostly Silurian and Upper Ordovician dolostones and shales. The surface is thickly clad in periglacial deposits with marshes and kettles capturing the surface water. Most swampy areas are isolated from one another and of limited extent. Exact kill sites were not provided by the trappers so it is not possible to correlate δ_p exactly with environmental controls. The Erin beaver were obtained from the Ontario Ministry of Natural Resources and were not originally acquired for this study. Only the month of death was recorded by the trappers. Only heads were given for the study and some of the Parry Sound beavers had the roots of the incisors cut off when the jaw was removed by the trapper. In a few of these cases small amounts of enamel were lost and the missing portion was approximated from comparison with other teeth and jaws. Neither the sex nor the adult body mass of the beavers could be determined.

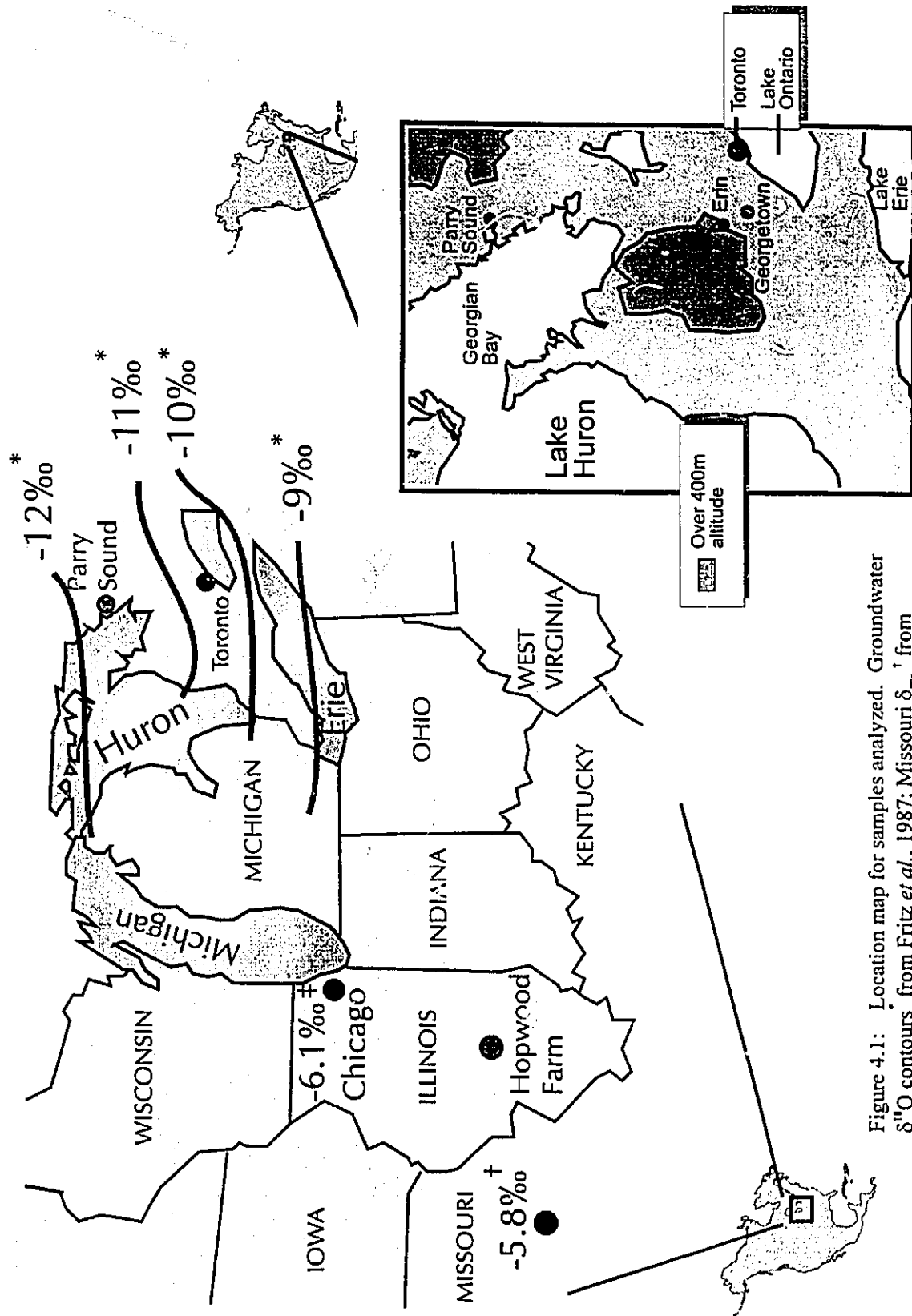


Figure 4.1: Location map for samples analyzed. Groundwater $\delta^{18}\text{O}$ contours from Fritz *et al.*, 1987; Missouri $\delta^{18}\text{O}$ from Luz *et al.*, 1990; Chicago $\delta^{18}\text{O}$ from Yurtsever and Gat, 1981.

Table 4.1: Sample information for beaver in this study.

ID	Sample part	Length x width mm	Month	Yr	Place	no. samples
TL1	Left l. incisor	12.0x6.3	4/5	94	Parry Sound	5
TL2	Right l. incisor	12.5x0.7	4/5	94	Parry Sound	5
TL3	Right l. incisor	12.0x0.75	1	94	Parry Sound	5
TL4	R.l.inc.No root	7.0x0.5	1	94	Parry Sound	5
TL5	L.l.inc.No root	7.7x0.7	12	93	Parry Sound	4
TL6	L.l.inc.No root	8.5x0.7	12	93	Parry Sound	4
TL7	L.l.inc.No root	7.0x0.5	2	94	Parry Sound	3
TL8	L.l.inc.No root	12.0x0.7	2	94	Parry Sound	5
EBA1	Lower incisor	11.0x0.7	5	93	Erin	7
EBA2	Lower incisor	11.0x0.7	5	93	Erin	16
EB2	Left.l. incisor	8.5x0.5	1	93	Erin	3
GT1	Right l. incisor	8.7x0.45	10/11	94	Georgetown	3
GT2	Right l. incisor	8.5x0.45	10/11	94	Georgetown	3
GT3	Right l. incisor	12.5x0.73	10/11	94	Georgetown	5
GT4	Right l.incisor	11.0x0.65	10/11	94	Georgetown	5
GT14	Right l. incisor	12.5x0.8	10/11	94	Georgetown	5

It was found that the beavers could be divided into three groups on the basis of the width of the lower incisors. Adults have lower incisors ≥ 7 mm wide, yearlings have incisors from 5-7 mm wide and young beaver incisors are ≤ 5 mm wide. Almost all ages are represented from kits to fully grown. A total of 95 samples from 16 beavers was used for this survey (Table 4.1).

Left or right lower incisors were sampled every 20 mm from the tip (Figs. 4.2a & 4.2b) apart from an initial study of EBA 1 and EBA 2 which were sampled every 5 mm (Stuart-Williams and Schwarcz, 1993) because at that time we were unaware of the rapidity of incisor growth. Samples of about 15 mg of enamel were ground as a strip across the front of the tooth using a diamond burr. The first sample was taken at the occlusal end and measured toward the root from there. In an adult beaver about two centimetres of the incisor at the root is very thin walled and enamel is absent. Above this for about 1.5 cm toward the tip the enamel is very spongy and contains a great deal of organic material. The portion of the tooth toward the occlusal surface from this is evenly coated with hard enamel but the yellow ferric coating on the enamel is only emplaced shortly before that portion of the enamel appears above the gum line. The pulp cavity is increasingly filled with dentine so that at the occlusal surface it is almost closed. While every effort was made to use only enamel, some irregularity in the results may be due to minor amounts of dentine having been ground off and included. As some seasonal variation is probably also present in the dentine, we used ~50 mg samples of bone from the back of the jaw of each beaver to obtain an average δ_{bw} .

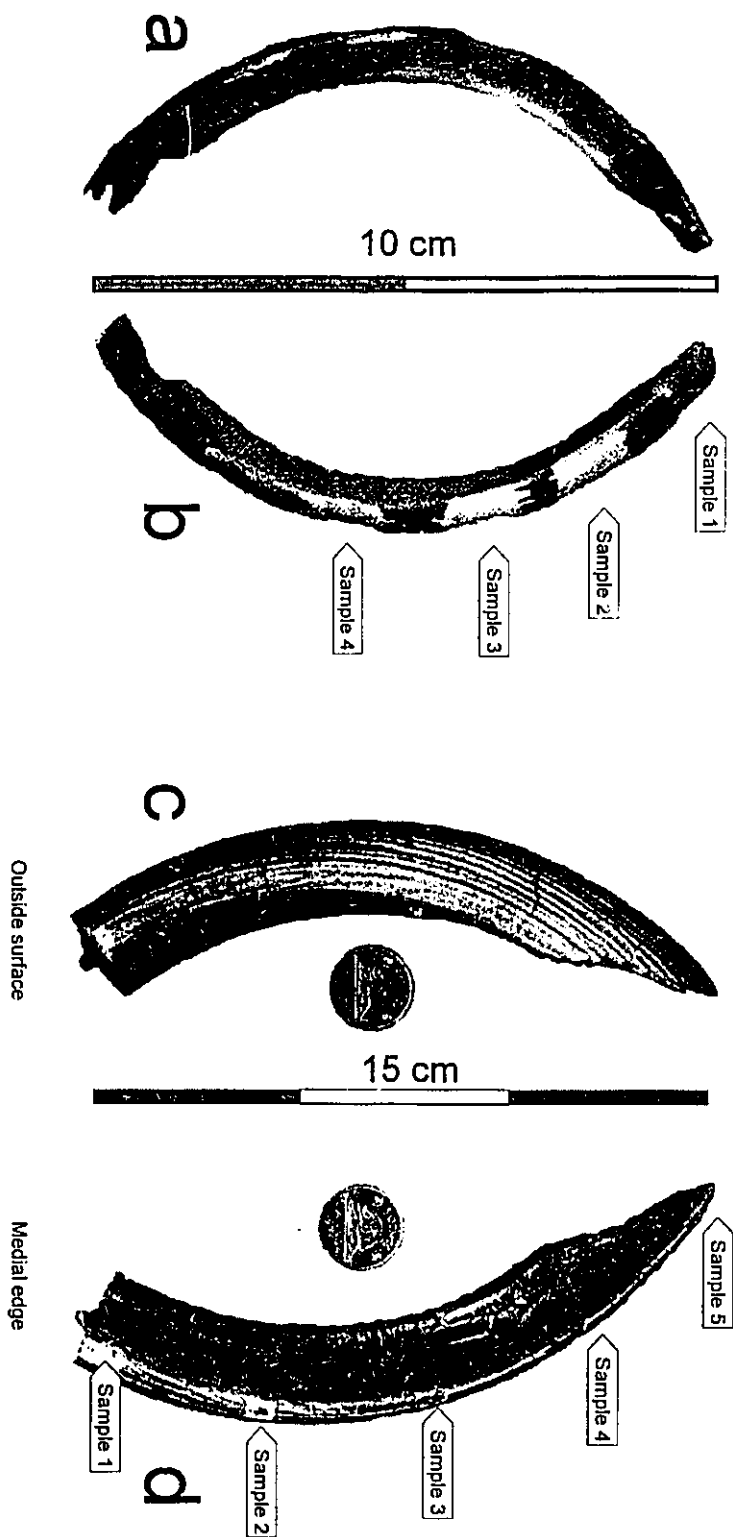


Figure 4.2: a) *Castor* right lower incisor medial surface. Black lines are sampling measurements. Note thin bone at root which breaks up during drying. b) As (a) but outer surface. Light patches are where the iron-rich outer coating has been ground away prior to sampling the enamel. c) *Castoroides* lower left incisor outer surface. The base has been sawn off. Enamelled surface is ridged. d) As (c) but medial surface is shown with sampling locations.

A giant beaver (*Castoroides ohioensis*) lower incisor from the Hopwood Farm locality (Blackwell *et al.*, 1995; King and Saunders, 1986) was kindly provided by the Illinois State Museum (Figs. 4.2c & 4.2d). The tooth comes from a buried kettle depression filled with Sangamonian interglacial deposits (Blackwell *et al.*, 1995). The sediments comprise gyttja, fossiliferous silty clay, alluvium and loess resting on scoured Illinoian till. The beaver incisor sampled rested above a "mastoturbated" horizon at the base of stratum 2 with *Mammut americanum* (mastodont) remains, *Trionyx sp.* (soft-shelled turtle), *Castor sp.* and a variety of other bones (Blackwell *et al.*, 1995). The layer immediately below (stratum 3) includes a fauna of *Lepistosteus platostomus* (shortnosed gar) and *Geochelone crassiscutata* (giant tortoise). Pollen is lacking from the horizon containing the giant beaver remains but was analyzed from stratum 3 below and indicates few *Picea* and *Pinus* trees with increasing amounts of *Quercus*, *Ulmus*, *Carya*, *Poaceae* and *Ambrosia* and greatly decreasing amounts of *Chenopodiaceae*, perhaps suggesting a preponderance of open deciduous woodland in Stratum 2. The tooth was complete apart from a 29 mm length sawn from the base for electron spin resonance dating, yielding dates of 33 - 86 ka depending on the uranium uptake model used (Blackwell *et al.*, 1995). A mastodont molar from the same horizon was ESR dated at 71 - 122 ka. The reason for the disparity between the dates may be due to either dating problems or allochthony of the samples (Blackwell *et al.*, 1995). Five samples of about 20 mg were ground as elongated rectangles parallel with the length of the tooth. This sampling method resulted in some reduction in time resolution but

was less disfiguring to the specimen than grinding the enamel transversely. An 80 mg piece of dentine from the interior of the sawn end was analyzed in order to obtain an averaged value for δ_{DIN} .

4.5 Analytical procedure

15 mg enamel samples from specimens apart from Beavers EBA 1 and EBA 2 were processed using a lead phosphate/lead sulphate/ Ag_3PO_4 method (Stuart-Williams and Schwarcz, in prep (*a*) and Chap. 2). The silver phosphate produced was reacted with bromine at high temperatures (Stuart-Williams and Schwarcz, 1995). Beavers EBA 1 and EBA 2 were processed in a similar manner except that after oxidation of the organic compounds the phosphate was precipitated as $\text{Ba}_3(\text{PO}_4)_2$ which was converted to silver phosphate and reacted with BrF_3 for 18 hours (Crowson *et al.*, 1991; Tudge, 1960). The oxygen liberated from all samples was reacted with a heated carbon rod to convert it to carbon dioxide which was analyzed using a VG 602 or a VG SIRA isotope ratio mass spectrometer. The total standard deviation of analysis of all samples is less than 0.15‰. Only two duplicate samples could be processed and analyzed because of the limited amount of isotopically similar sample available. The differences between the analyses were 0.14‰ and 0.03‰. Analysis of inorganic phosphate using these techniques has a 1σ precision of $\pm 0.11\%$. All results are presented relative to the VSMOW standard and include the necessary corrections for the offset induced by the bromine reaction.

4.6 Results

The results of the δ_{pe} analyses of *Castor* are presented in figure 4.3a. In order to present the data as profiles a date was calculated for the formation of each sample of enamel as $X \text{ (Julian day)} = \text{KillDay} - ((\text{EnamelBase}(\text{mm}) - \text{SamplePoint}(\text{mm})) / 0.75)$. The assumption made that the tooth grew at 0.75 mm day^{-1} is discussed in section 4.7. The point of initial deposition of enamel on the incisor surface is difficult to determine, but in all cases samples are probably a few days to three weeks older than was calculated. The first 120 days of the year are characterized by a trend of falling $\delta^{18}\text{O}$ of tooth enamel phosphate (δ_{pe}) (Fig. 4.3b), with δ_{pe} diminishing by 0.8 - 3.0‰. The densely sampled Erin samples (EBA 1 and EBA 2) are similar in value and decrease in δ_{pe} less than the samples from Parry Sound. There is a gap in the record between day 120 and day 196 for which we have no data. Following day 196 all the δ_{pe} values have increased by several permil. Some of the teeth (GT 4, GT 14, TL 3, TL 6 and TL 8) show increasing values initially followed by a flattening out and then decreasing δ_{pe} (Fig. 4.3a). Other teeth show only decreasing values and none show decreasing spring values starting to increase in the summer. In adult animals the $\delta^{18}\text{O}$ of bone phosphate (δ_{pb}) is greater than δ_{pe} in the spring and less than δ_{pe} in the autumn and early winter (Fig. 4.3b) which is compatible with a model in which the isotopic composition of tooth enamel being deposited is in instantaneous equilibrium with the body fluids whereas bone represents an average over a longer period. The isotopic composition of the bone ranges from 10.8 - 13.7‰.

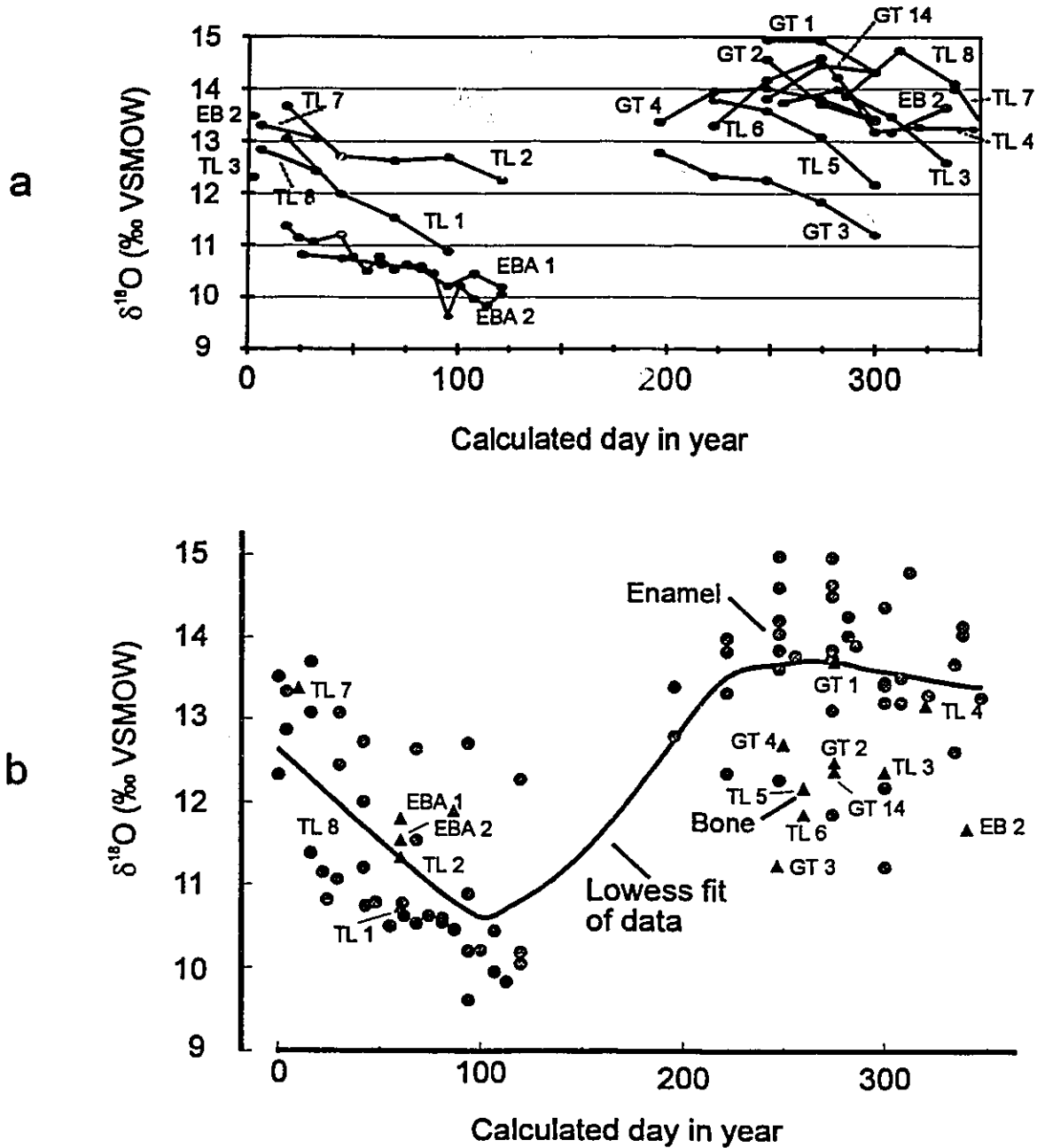


Figure 4.3: a) Profiles from sample *Castor* incisors. Individuals mentioned in the text are identified. Some samples from early in the year wrap around to the previous year. Day zero is January 1st. b) The same data as above but displayed as a scatter. Jaw bone analyses are shown as black triangles with the identity of the beaver. A locally weighted regression (LOWESS) fit is shown, for comparison with a postulated curve for δ_e in figure 4.6c. The X coordinate for both graphs was calculated by assuming a growth rate for the teeth of 0.75 mm day^{-1} .

The δ_{pe} for *Castoroides* ranges from 15.4 - 20.4‰ and $\delta_{dentine}$ is 18.7‰ which is 0.8‰ greater than the median of the δ_{pe} distribution which is 17.9‰, about 5‰ heavier than the mean δ_{pe} of the *Castor* studied.

4.7 Tooth growth rate estimation

It is essential to determine a growth rate for the incisors to match δ_{pe} to time and the changing seasons but no beaver incisor growth rate data were available (M. Novak, *pers. comm.*). The only enamel which can be dated moderately precisely is the portion at the base of the incisor deposited immediately before death. The rate of growth can be constrained in several ways:

- a) matching the isotopic data to seasonal fluctuations;
 - b) using small marks on the sides of the teeth which may be growth lines;
 - c) relating changing skull and tooth sizes in immature beavers.
- a) The two beaver initially analyzed (EBA 1 and EBA 2) both showed monotonically decreasing values for δ_{pe} with time (Figs. 4.3a), apparently representing only a part of the year when δ_{bw} was decreasing from the high summer values. This showed that the entire incisor was cycled in less than approximately 8 months before the beginning of May kill date, representing a minimum tooth growth rate of 0.7 mm day⁻¹. The highest δ_{pe} from the EBA beavers was similar to δ_{pb} from their jaw-bones. In mature beaver δ_{pb} is expected to represent the weighted annual mean value of δ_p , and should therefore also represent a mean value for δ_{pe} . This indicates that the downward trend of δ_{pe}

present in the teeth of the EBA beavers represents only about half of the total range of decreasing values in the annual cycle and that a growth rate of 1 mm day⁻¹ or more is possible.

- b) Fine lines, perhaps daily growth lines, are present on the sides of adult teeth with a spacing of 0.65 - 0.8 mm.
- c) The data indicate that juvenile beaver teeth are about 4.5 mm wide by October of their first year. This has increased to about 5 mm wide by January or February of the following year and to 7 mm by the end of the next summer, when further growth becomes substantially slower. The taper along the length of the juvenile incisors is about 0.5-1.0 mm, and the 85 mm teeth have therefore been completely used up by wear about three times in a year, at a growth rate of about 0.75 mm day⁻¹.

We have therefore used a growth rate of 0.75 mm day⁻¹ when graphing the data. This is more probably an underestimate than an overestimate as analysis of other beavers in the study has shown that adult beaver killed in December may already retain no trace of δ_{pc} increasing through the summer. In the cases of beavers GT 3, GT 4 and TL 5, a growth rate of 0.75 mm day⁻¹ appears to create difficulties with an isotopic interpretation as discussed below and a growth rate of 1.2 mm day⁻¹ was used for some graphs.

No comparable growth lines were visible on the giant beaver tooth, but as they are superficial features in *Castor* they might well have been destroyed during fossilization. More than a complete annual cycle of $\delta^{18}\text{O}$ variation is present in the tooth and the growth rate was about 15 cm year⁻¹, or about 0.4 mm day⁻¹.

4.8 Reliability of data

Three aspects of data reliability require verification:

- a) Are the analyses of δ_{pe} precise and reliable?
- b) Is change in δ_{pe} correlated with change in δ_{bw} ?
- c) Do beaver reliably record changes in δ_w ?

a) The reproducibility of analyses of bone using these methods is typically $\pm 0.15\%$. The smoothness of the δ_{pe} profiles and data reproducibility are compatible with this. The variation in δ_{pe} along an incisor resulting from environmental changes is typically an order of magnitude greater than this.

b) Many of the beaver incisors show 1.5-2.0‰ variation along their length, whereas differences between bones of the same individual are expected to be $\pm 0.3\%$ (Luz and Kolodny, 1985; Luz *et al.*, 1990). Part of the 0.3‰ variation in δ_{pb} from one bone to another may result from differences in body temperature (Barrick and Showers, 1994) and from changing δ_{bw} during the formation of the bones, for example during weaning (Luz *et al.*, 1984; Luz and Kolodny, 1985). Beaver tooth enamel is formed over a short distance near the middle/rear of jaw where spatial and temporal temperature changes are expected to be small. Because bone or tooth enamel is deposited from a phosphate pool equilibrated with body water (Longinelli, 1984; Luz *et al.*, 1984; Luz and Kolodny, 1985) the systematic variation of δ_{pe} observed must be caused by similar changes in δ_{bw} . In post-weaning beaver, changes in δ_{bw} must result from changes in environmentally controlled oxygen fluxes (Luz *et al.*, 1984; Luz and Kolodny, 1985; Bryant and Froelich, 1995). There is a clear relationship between changing δ_{pe} and time, with beaver throughout the study area showing

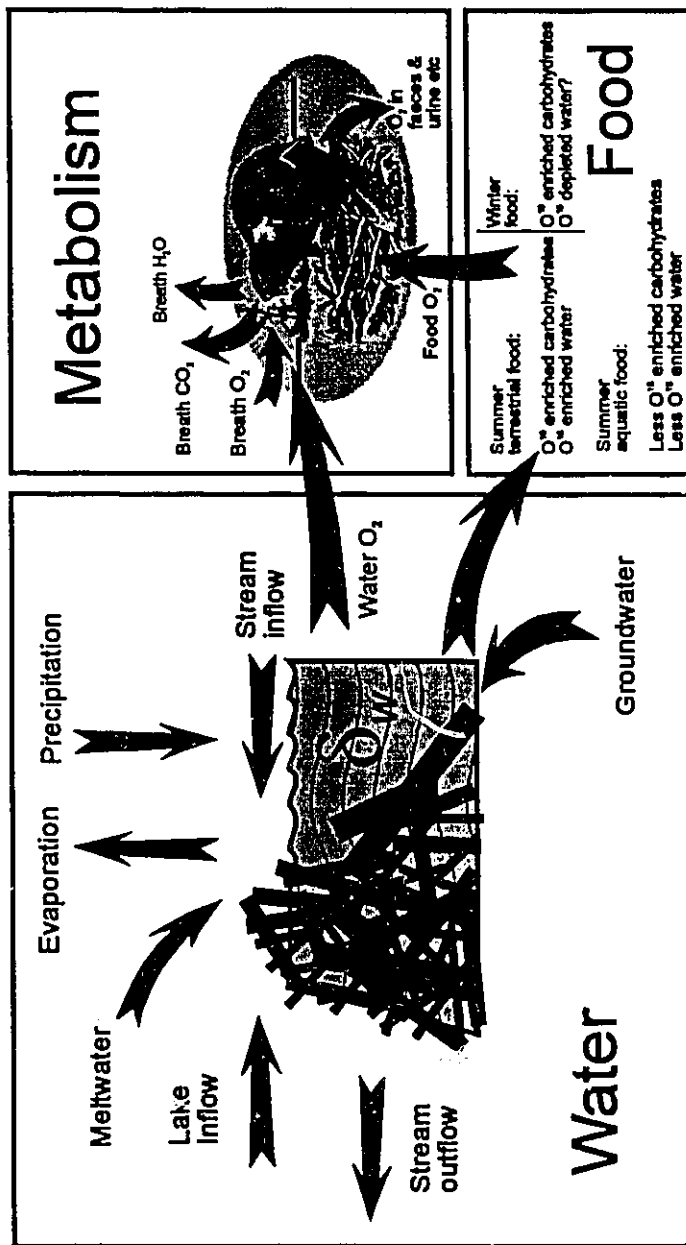
simultaneous and generally similar rates of increase or decrease in δ_{pe} as a result of a widespread change in their oxygen fluxes. As beaver are obligate drinkers, living in a humid environment, the major variability in oxygen flux is seasonal variation δ_w (IAEA 1969,1970; Mook, 1970; Yurtsever and Gat, 1981). Beaver δ_{pe} fluctuation is about 3 months delayed relative to expected seasonal changes in δ_w , with the lowest values in the spring or early summer and the highest values in the late summer.

c) We do not have detailed δ_w data from the beaver ponds because we could not select which beavers were killed. Despite this, the very similar data from EBA 1 and EBA 2, which lived in the same pond and were killed at the same time, show that the δ_{pe} of a beaver is controlled by the δ_w of its environment.

4.9 Influences controlling *castor* δ_{pe} and δ_{pb}

Figure 4.4 summarizes the oxygen fluxes affecting the beavers. These are shown diagrammatically as dimensionless arrows because great variability is expected from one beaver pond to another. The complexity of variation in δ_w experienced by beaver in temperate zones alters the δ_w oxygen flux of previous models (Ayliffe and Chivas, 1990; Bryant and Froelich, 1995; Luz and Kolodny, 1989; Luz *et al.*, 1984) which have regarded the water input as being relatively homogenous. Increase in δ_w due to evaporation is dependent on the isolation of the water body, which in turn depends on the stream outflow, stream inflow, lake inflow and groundwater fluxes. All these fluxes vary depending on the local geologic and geographic setting as well as on such

Fluxes controlling δ_{BW}



Fluxes controlling δ_w

Figure 4.4: The input liquid water oxygen flux in beaver is particularly subject to external controls that influence the beaver rapidly. These are identified in the left-hand box. Dietary controls must also be considered, as in the lower box at the right. The beaver's metabolic fluxes (upper right box) then act within the context of these major inputs.

intangibles as the leakiness of the dam, if there is one. The influence of the winter diet on the beaver is also uncertain due to the unknown isotopic composition of the water-saturated stored bark. Given this diversity of inputs, it is doubtful whether we could construct an accurate model to predict beaver δ_{bw} .

Three types of $\delta^{18}\text{O}$ environmental data are recorded in the beaver teeth and bone. These are considered separately in the following sub-sections:

- a) Small scale variation due to local hydrologic factors, such as recharge variation, evaporation, depth of the beaver pond, the rate of groundwater influx into the pond and the leakiness of the dam.
- b) Regional variation in δ_w resulting from large scale climatically related changes in δ_{mw} .
- c) Information recorded in the $\delta^{18}\text{O}$ of the beaver jaw-bones, representing long term averaging of δ_w variation.

4.9a Sources of local, hydrologic variability in *Castor* δ_{pw}

Variation of δ_{bw} in beaver should be a function of variability in local δ_w (Fig. 4.4) which will be an attenuated, altered and fractionated function of seasonal and regional variation in δ_{mw} . Seasonal fluctuation of δ_w in ponds and streams is significantly less than the variation in δ_{mw} in the same area. δ_{mw} for Hakone Caldera in Japan had a range of 8‰ while variation in groundwater, the lake and a river was only about 1‰ (Matsuo *et al.*, 1979). Three Dutch rivers (Mook, 1970) had a variability in δ_w of about 1.0 - 1.5‰ over a year, whilst variation in δ_{mw} was about 6‰. The amplitude of seasonal change in δ_w is decreased by groundwater inputs (Fig. 4.4), which generally induce a lag in the

response of δ_w to changing δ_{mw} (Fritz, 1981). Most ponds and rivers receive considerable input from groundwater, particularly during periods when the water table is higher than the pond or river surface. This may happen during the summer when evaporation depletes surface water rapidly or when the water table is raised by influx of rain or melting snow (Fritz, 1981). Groundwater δ_w is usually close to weighted mean annual δ_{mw} (Gat, 1981; Mook, 1970; Siegenthaler *et al.*, 1970) but is slightly less than the mean of maximum summer δ_w and minimum winter δ_w due to the larger contribution of isotopically light rain in the spring and autumn. Because of the intermediate δ_w of groundwater relative to seasonal δ_{mw} fluctuation, the greatest δ_w variation will be seen in closed shallow ponds with significant evaporation. The least variation in δ_w is seen in groundwater fed rivers. The amplitude of the beaver δ_{pe} response is perhaps greater than seasonal δ_w fluctuation in the beaver ponds due to the beavers consuming evaporatively ^{18}O enriched food in the summer.

Beavers EBA 1 and EBA 2 (Fig. 4.3a) have very low δ_{pe} in the spring and also appear to have a low amplitude of total δ_{pe} variation over the period of time sampled, when compared with beavers TL 1 and TL 2. EBA 1 and EBA 2 probably lived in a groundwater dominated waterway with substantial flow in the summer, whereas TL 1 and TL 2 lived on the Precambrian shield where groundwater contribution is small and evaporative effects are greater. Without definite tooth growth rates, however, the apparent amplitude variation may also result from faster incisor growth and a consequently shorter time sample for the EBA beavers.

Attenuation of short-term variation in δ_{mw} will result from the mixing of the water from a precipitational episode with the water already present in waterways and groundwater (Fritz *et al.*, 1976). Small amounts of precipitation relative to the volume of the water already present will have little influence on δ_w but considerable amounts of precipitation may have a significant effect on smaller ponds. The downward spike present in δ_{pe} in beavers EBA 1 and EBA 2 (Fig. 4.3a) at about day 90 may represent a sudden influx of that type.

The relative contributions of surface water and groundwater may vary at different times of year. Beaver TL 4 is anomalous in that the δ_{pe} begins to decrease into the autumn and then becomes stable. A similar response is seen in beaver TL 2 in the early spring and is the major factor causing its unusually high δ_{pe} at the beginning of the summer. In both cases δ_{pe} is about 13‰ which indicates a δ_w of about -10‰. Two likely sources of water with a relatively high δ_w in the winter and spring are possible: groundwater or Lake Huron. The crystalline metasediments around Parry Sound are not prolific aquifers and the influence of lake water is more probable. It is unknown whether this represents a change of residence by the beavers or whether they are living in inlets (perhaps fed by streams) that are influenced variably by the lake at different times.

The relationship between the δ_{pe} of the Erin beavers is noteworthy as EBA 1 and EBA 2 lived in the same pond and were stated by the trapper to be the parents of the juvenile EB 2. The close coincidence of the δ_{pe} profiles for the two adults demonstrates the strong control of δ_{pe} by the beavers' environment. The δ_{pe} of the young beaver EB 2 is 2‰ enriched over the parents at about day

zero. This is unlikely to be a milk-feeding effect as the baby was probably about 9 months old when killed and perhaps results from a raised metabolic rate associated with rapid growth causing a slightly larger offset between $\delta_{b,w}$ and δ_w .

Evaporation (Fig. 4.4) results in increasing δ_w particularly during the summer, when $\delta_{m,w}$ is also rising as a function of temperature: the δ_w of some Dutch rivers increases substantially through the summer with no departure in isotopic composition from the meteoric water line (Mook, 1970). The amount of enrichment in lakes may be quite substantial even in cool climates, for example the water of Lake Titicaca at 3800 m in Peru is apparently enriched more than 10‰ over the rivers flowing in (Fontes *et al.*, 1979) although enrichment of temperate rivers is likely to be small (Fritz, 1981). In the humid environments inhabited by beaver, evaporation of closed water bodies can cause δ_w to increase until it is at equilibrium with moisture in the air (Gat, 1981).

The greatest effects of evaporative enrichment are probably found in the vegetable food of the beaver. The winter diet of the beaver consists of stored branches and twigs from caches under the ice. This plant food grew mainly during the summer and the carbohydrates will contain evaporatively fractionated oxygen although the branches are essentially waterlogged and saturated with water of the same δ_w as the pond. The summer diet includes much greater amounts of soft herbaceous material, including roots of soft aquatic plants with δ_w approximately equal to the δ_w of the ponds. Terrestrial plants consumed grow in humid areas and will be substantially less evaporatively fractionated than would be typical of the diet of most other land mammals, such as groundhogs or deer.

4.9b Regional, climatically related variability in *Castor* δ_{pe}

Castor δ_{pe} is derived from local δ_w , which originates from δ_{mw} . Because no record is available of seasonal fluctuation of δ_w in a nearby river, we have used precipitation data in this study. The most suitable set of δ_{mw} data available, on the basis of approximate equivalence of climate, topography, distance into the continent from the sea and proximity to large lakes, is from Chicago through the years 1962-1965 inclusive (IAEA, 1969,1970) (Fig. 4.5). This demonstrates an annual variability in δ_{mw} of about 10‰ from about -13 ‰ in winter to -3‰ in summer. These data represent the total precipitation for each month, so greater variability is expected for the δ_{mw} of any single precipitation event. Increase in δ_{mw} is fast from February through June, followed by more constant values and then a rapid reduction in δ_{mw} from October through January. A weighted mean δ_{mw} is -6.11‰ (Yurtsever and Gat, 1981), which is approximately 5‰ heavier than the $\delta^{18}\text{O}$ of groundwater in the study area (Fritz *et al.*, 1987).

The interpolated annual beaver δ_{pe} profile (Fig. 4.3b) is substantially different from the Chicago δ_{mw} curve. The amplitude of the response is greatly reduced from about 10‰ δ_{mw} variation to 3-4‰ δ_{pe} fluctuation and whereas δ_{mw} begins to increase in February, the δ_{pe} curve continues to fall until at least the end of April (day 120). The continuing decrease of δ_{pe} into the spring is perhaps

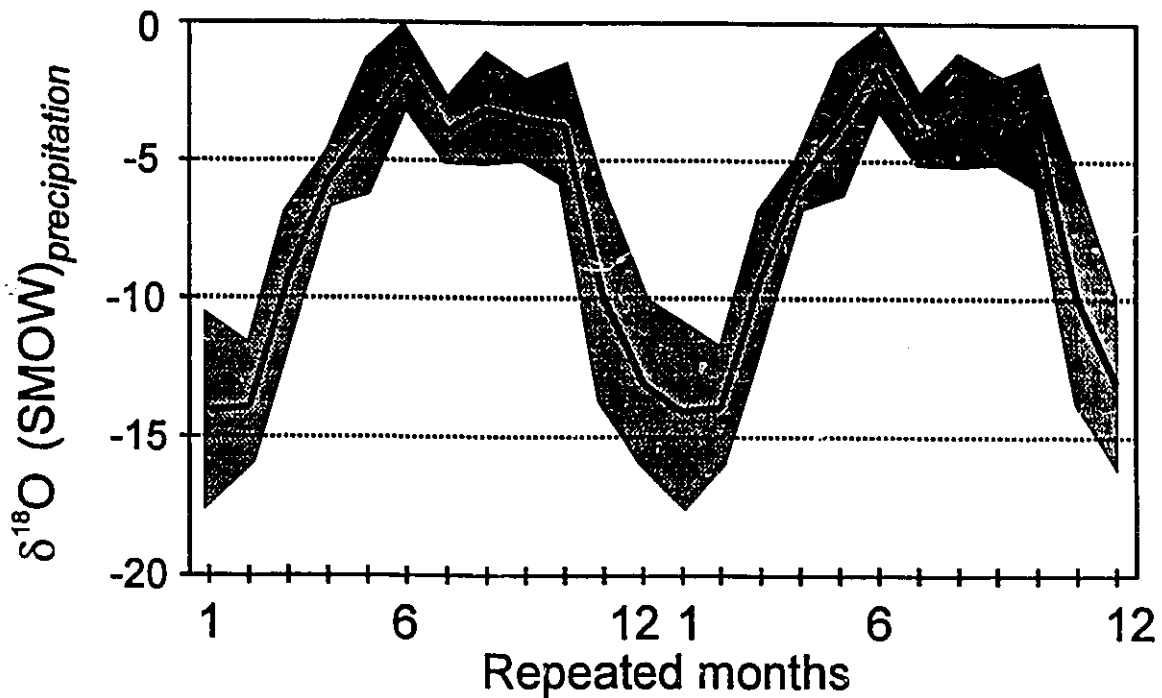


Figure 4.5: Oxygen isotopic analysis of precipitation at Chicago. The data are from four years (1962, 1963, 1964, 1965) repeated twice to emphasize the seasonal variation. The black line is the mean of the four years of data; the grey zone is one standard deviation. (IAEA 1969, 1970)

diagnostic of areas where there is considerable snow cover; melting of snow in the spring causes a characteristic shape of the seasonal δ_w curve, with a gentle decrease in values in the spring followed by a rapid rise in the late summer. This response is present in the annual δ_w curve for the Rhine which is fed by substantial quantities of melting snow in the Swiss Alps (Mook, 1970). The contribution of isotopically light melted snow from the Alps through the spring until mid-summer continues to depress δ_w until the annual melt has reached a maximum. The freezing of winter precipitation results in a substantial time lag between when the precipitation falls and when it becomes apparent in the river. A similar profile is visible in *Castor* δ_{pe} , from the study area, with δ_{pe} continuing to fall until about the end of April. This snow melt response is surprising as snow cover is not substantial over most of the region and is almost entirely melted by rain before late April. Other time lags in the system may enhance this effect, in particular δ_{bw} will not respond immediately to changes in δ_w and the beaver will still be consuming food stored underwater through the winter until new spring growth is available.

There is a gradient of increasing δ_w southwards across the study area: δ_w in locally recharged Parry Sound (TL) water is about -11.5‰ and the δ_w of the Georgetown (GT) and Erin (EB) samples is about -10.5‰ (Fritz *et al.*, 1987). This offset is not apparent in the δ_{pe} profiles (Fig. 4.3) where the Erin beavers show the lowest δ_{pe} . Local hydrologic variation in the beaver ponds and streams, due to evaporation in standing or flowing water, or local recharge variability, perhaps conceals the expected differences.

Considerable seasonal fluctuation was found in δ_{pe} but coeval analyses from all the beavers could not be time corrected and laid over one another exactly. The δ_{pe} profiles presented in figure 4.3a indicate variability among the samples of three different types: a) the amplitudes of changing δ_{pe} over the year appear to vary from beaver to beaver; b) the median value of δ_{pe} oscillation varies and, particularly, c) the responses appear not to be synchronously phased.

a) Difference in the amplitudes of the δ_{pe} responses can only be inferred as there is no complete annual record from a single incisor or from multiple beaver living in one pond. Beavers TL 5 and TL 6 have very similar δ_{pb} , although beaver TL 5 apparently reached a lower maximum δ_{pe} earlier in the year than the maximum δ_{pe} reached by beaver TL 6. A possible hydrologic interpretation for this is that beaver TL 5 lived in a waterway with a higher ratio of groundwater to surface runoff; groundwater influx will reduce the amplitude of the winter and summer δ_w excursions from annual mean δ_w , and reduce the amplitude of seasonal $\Delta_{\delta_{pe} - \delta_{pb}}$. The slope of the δ_{pe} profiles cannot be used to infer the amplitude of the δ_{pe} seasonal fluctuation as the tooth growth rate is not known with any certainty and may be quite variable: teeth growing more rapidly will appear in the figures to have a reduced rate of changing δ_{pe} .

b) The isotopic analyses of the beaver jaw-bones show some scatter of δ_{pb} even in adult beavers (see following section). Beaver δ_{pe} in most cases is close to weighted annual mean δ_{pe} and variation in δ_{pb} between adult beavers from the same area indicates probable offsets between δ_{pe} profiles.

c) The form and phase relationships of the seasonal oscillations in δ_{pe} are best seen between days 200-325 where increasing summer values change to decreasing fall and winter ones. In some beavers (e.g. GT 3, GT 4 and TL 5) δ_{pe} has already begun to decrease by day 250 while other beavers (e.g. TL 3 and TL 6) show increasing values until past day 275. Beaver TL 8 still shows increasing values after day 300. It also appears that where the change to decreasing values occurs later in the year that it is associated with higher δ_{pe} so that in figure 4.3a the profiles appear to be stacking up and to the right. This effect is found both in the Parry Sound beavers and the Georgetown beavers. A possible explanation is that areas not diluted by isotopically light autumn precipitation until later in the year, continue to be isotopically fractionated by evaporation longer and reach heavier δ_w . Groundwater influx could also be a critical factor: where a water body is receiving more groundwater influx, fractionation by evaporation may be reduced rapidly as the period of maximum solar influx passes. This, however, does not explain why beaver GT 3 shows decreasing δ_{pe} by day 200. We feel that the bulk of evidence (lower slopes and unreasonably early autumn inflections on at least GT 3 and TL 5) support very high incisor growth rates in some beavers. This is not general to all large beaver and may be related to dietary or behavioral differences; for example the largest beaver, GT 14, shows increasing δ_{pe} later in the year than any of the other beavers. To demonstrate the effect on the data an arbitrarily accelerated growth rate of 1.2 mm day⁻¹ has been applied to GT 3, GT 4 and TL 5 in figure 4.6b, which is otherwise identical to figure 4.3a. This removes some of the apparent phase differences for these beavers and to some extent makes it appear that their low δ_{pe} may result from a more rapid response to low δ_w later in the year.

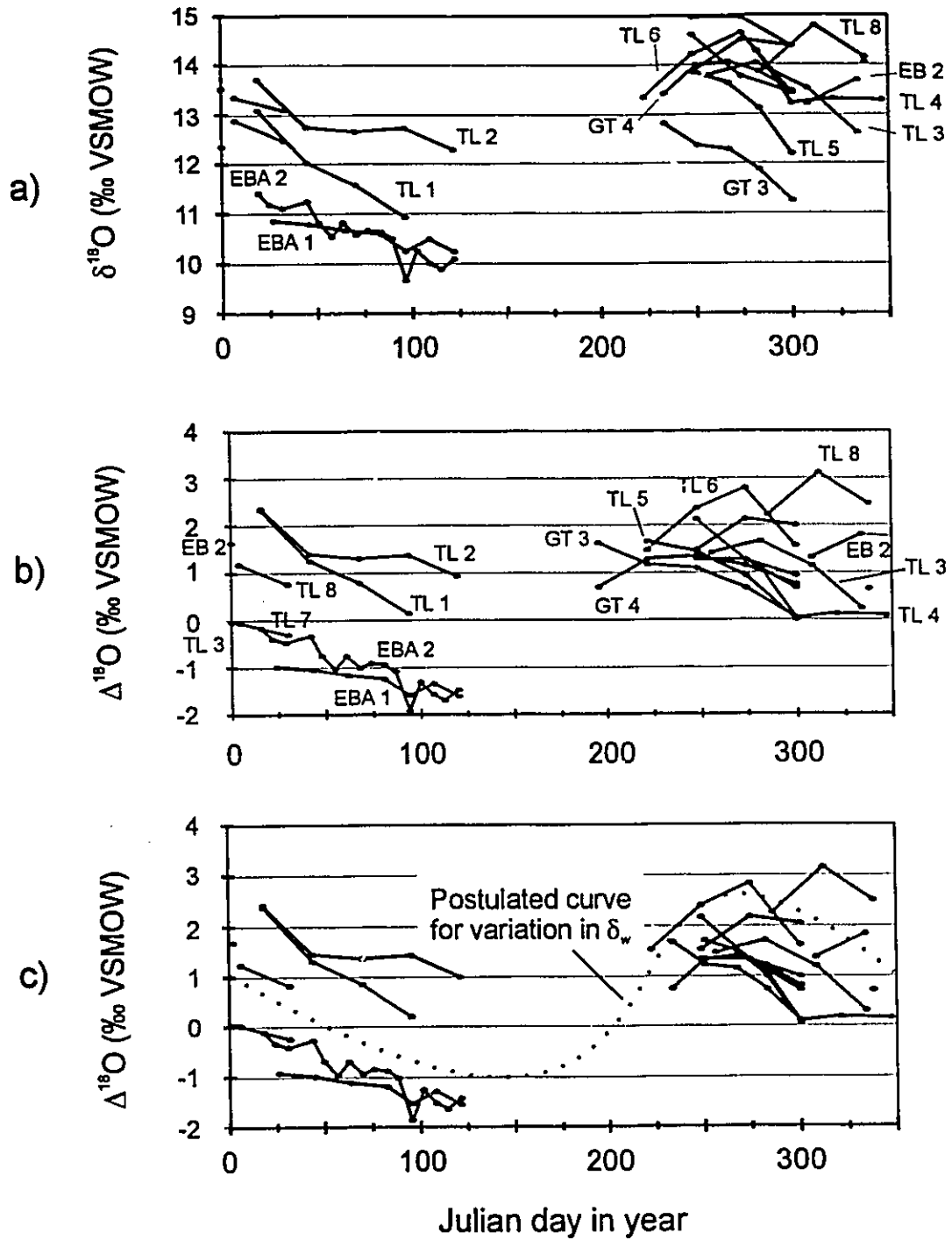


Figure 4.6: Profiles for *Castor* δ_p . a) Data as in figure 4.3a, but with growth rates for GT 3, GT 4 and TL 5 increased to 1.2 mm day^{-1} .
 b) δ_p values converted to offsets from δ_{pb} for the jaw-bone of each beaver.
 c) Data as in a) above, but converted to offsets from δ_{pb} as in b) above.

The reason why these three beavers from two different areas should be different from the other beavers sampled is unknown, but GT 4 may provide a clue: it is a yearling. Young beaver are tolerated in the home pond for varying amounts of time, depending on, among other factors, population pressure on available food supplies. They are then forced by the parents to leave and establish new territory and build their own dams and lodges (Novak, 1987 and references therein). This may happen in their second year or at any point thereafter. This explanation is compatible particularly with the Georgetown samples which were killed as part of a pest control program: new colonies are more likely to be selected for extermination. The three anomalous beavers may have worked their teeth down more rapidly building dams and lodges and accumulating food piles for the winter.

4.9c Data from *Castor* δ_{pb} and the relationship with δ_{pw}

Our data for beaver δ_{pb} range from 10.7 - 13.7‰ which is more scattered than expected (Figs. 4.3a & 4.3b). However the range decreases to 11.2 - 12.5‰ when only the 8 adult beavers (incisor diameter ≥ 7 mm) are included, with a mean of 11.9 ± 0.5 ‰. Despite the wide diversity of environments and hydrological regimes sampled (with different bedrock, groundwater contributions and amounts of evaporation) average δ_{bw} in adult beaver is quite constant over the sample area. A variance in δ_{pb} less than 1‰ is improbable due to variation in mean weighted δ_{mw} from year to year even at a single locus (IAEA, 1969, 1970; Mook, 1970) and variation within a population even under laboratory conditions (Luz and Kolodny, 1985). Juvenile and adolescent beaver δ_{pb} is very heavily influenced by the isotopic composition of δ_{bw} during the previous season. For

example the three highest δ_{pb} are from the juvenile beavers GT 1, TL 4 and TL 7. These were killed in October, January and February, after most of their bone had formed or remodelled during the summer when δ_{bw} was elevated. Similarly the lowest δ_{pb} is from an adolescent beaver (TL 1) which was probably killed in early May and had been growing to maturity through the winter and spring when δ_w was low. Little remodelling of the bone is expected once the beavers reach maturity, with only minor fluctuations in bone composition resulting from seasonally changing δ_w . These data indicate that bones, and perhaps teeth, of non-adult animals of other types may also have non-typical δ_{pb} .

Some of the variation in local hydrology, for example varying quantities of groundwater contribution or differing amounts of evaporative ^{18}O enrichment of deep and shallow ponds, may result in different annual mean δ_w in the beaver ponds. This will result in correspondingly different values for δ_{pb} in adult beaver if they grew to maturity in that habitat. Most beaver are forced to leave their parents' pond at about 2 years old, by which time bone remodelling will have slowed considerably. Some variation will also result from differences in the cumulative weighted mean of δ_{mw} from year to year: the $\delta^{18}\text{O}$ of bone of mature beaver reflects a long term average δ_{mw} whereas δ_{pe} records instantaneous δ_{bw} . Figure 4.6b shows Δ_{pe} profiles constructed by subtracting δ_{pb} for each beaver from the δ_{pe} profiles which should reduce any spread in the data that results from variation in mean weighted δ_{mw} or the δ_w of groundwater. Some reduction of the data spread does result during the autumn period; GT 3 in particular is moved to fit better with the other profiles. The spread of the spring δ_{pe} data is not

improved at all. This non-normalized scatter in δ_{pe} results from one or more of three probable causes: a) local hydrological effects that do not significantly alter the annual mean δ_w of the pond, for example brief periods of evaporative enrichment in the late summer; b) the beavers are fairly recent immigrants to that hydrologic system, or c) short term variation in annual cumulative mean weighted δ_{mw} . Long term (greater than about 4 years) variation in mean annual δ_{mw} as a result of geographic or topographic influences does not appear to be the cause of the scatter in δ_{pe} . Figure 4.6c shows a bone normalized version of figure 4.6a, with accelerated growth rates for GT 3, GT 4 and TL 5.

In mammals $\Delta_{\delta_{bw}-\delta_{pb}}$ is probably constant at about 17.8‰ (Luz and Kolodny, 1985) and using this, mean δ_{bw} can be calculated from δ_{pb} . The beaver studied, where environmental δ_w is approximately -11‰ (Fritz *et al.*, 1987), showed an offset of 5.2‰ between δ_{bw} and δ_w . This compares with an offset of about 7.2‰ for white-tailed deer (*Odocoileus virginianus*) in the same area (Luz *et al.*, 1990). It is not possible to calculate a δ_p/δ_{mw} relationship for beaver as there is insufficient variation of δ_{mw} within the area, but the smaller $\Delta_{\delta_{bw}-\delta_w}$ of the beaver is contrary to body mass-based predictions (Bryant and Froelich, 1995).

To summarize section 4.9, adult beaver δ_{pb} appears to be a good record of mean annual δ_{mw} . We find that juvenile δ_{pb} is greatly influenced by δ_{mw} of the previous season and cannot be used to estimate mean annual δ_{mw} . Beaver δ_{pe} fluctuates significantly throughout the year in response to changing δ_{mw} and melting of frozen winter precipitation as reflected in changing δ_w . While seasonal

fluctuation in δ_{mw} is probably approximately 10‰, annual variation in beaver δ_{pe} is reduced to about 4‰ as a result of attenuation by mixing of groundwater and surface waters. The effect of the beaver's behaviour patterns on the signal amplitude is unclear but the apparent phase of the δ_{pe} seasonal cycle is changed through variations in incisor growth rate which may depend on age or metabolic stress. The true phase of the δ_{pe} response is modified by combinations of evaporation, groundwater mixing, and the size of the water body. In beaver of unknown kill date, a season of death can be determined approximately by comparison of changing δ_{pe} with δ_{pb} along the incisor. Because dentine grows with the rapidly growing tooth, $\delta_{p_dentine}$ is not a reliable guide to mean annual δ_{bw} .

4.10 The climatic signal in a *Castoroides* incisor

Six δ_{pe} analyses of *Castoroides* are presented in figure 4.7. An interpolated smooth sinusoidal curve has been fitted to the 5 enamel analyses representing the total annual variation of δ_{pe} . Sample 1 was duplicated. Total interpolated variation of δ_{pe} in the enamel is from 15.4 - 20.8‰. The variation in the interpolated curve is estimated to be 5.6‰, which is almost 2‰ larger than variation in the grouped analyses of *Castor*, and very much larger than in any single *Castor* incisor. The δ_{pe} signature is compatible with slightly more than one year of climatic record being recorded in the tooth. About 3 cm of tooth were removed for ESR analysis (Blackwell *et al.*, 1995), equivalent to about one extra sample at the base of the tooth in figure 4.2, indicating that the entire

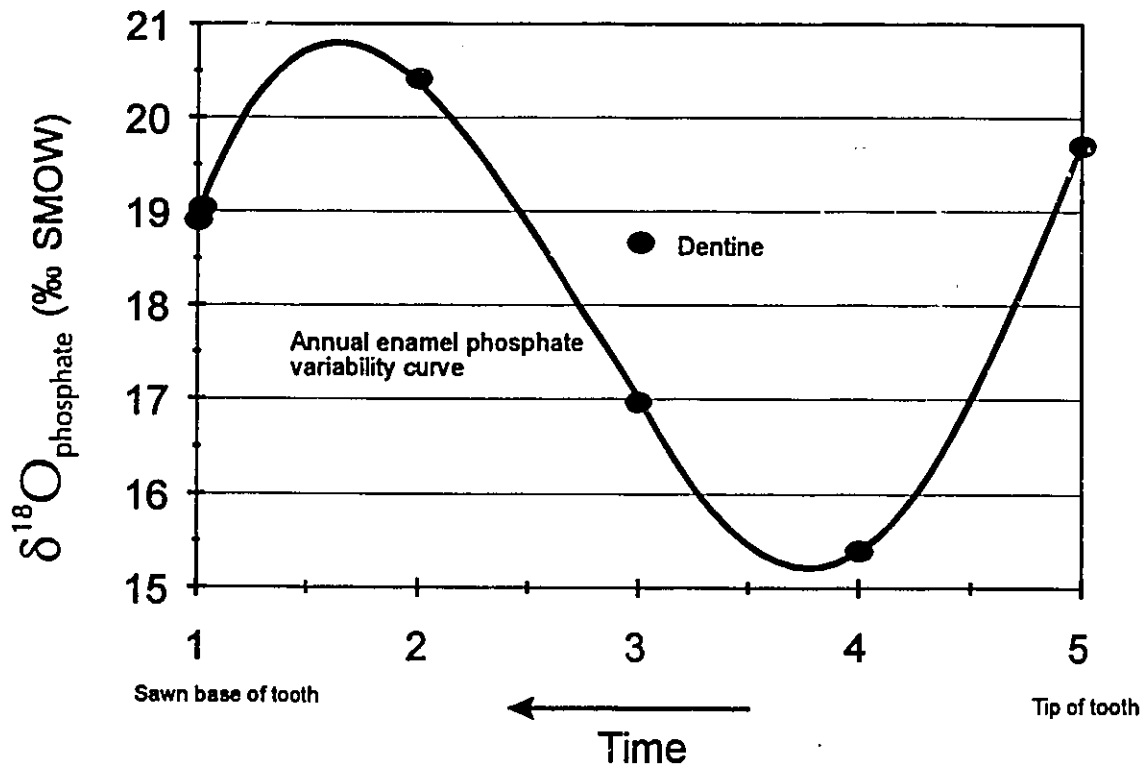


Figure 4.7: Analyses of giant beaver (*Castoroides ohioensis*) tooth from Hopwood Farm, Illinois. Unlike *Castor*, the rate of increase in $\delta^{18}\text{O}$ during the summer is similar to the rate of decrease during the remainder of the year.

enamel record of the tooth is about 1.3 years. $\delta_{p_dentine}$ from a sample at the sawn base of the tooth is close to the mean value of the interpolated variation in δ_{pe} . Dentine grows in concentric, approximately surface parallel, layers by progressive filling of the pulp cavity. A sample taken across these layers samples a much longer period of time than is represented by a small sample of enamel. The similarity in the δ_p of the dentine and the enamel of sample one is therefore probably a result of δ_{pe} for sample 1 being close to mean annual δ_p for the animal, rather than because the samples are close together on the tooth. It is better to estimate δ_{pb} from the middle of the range of δ_{pe} than from dentine because a) the preservation of seasonal variation in δ_{pe} can be used to verify that diagenesis has not completely eradicated the original signal and b) enamel is more resistant to diagenetic change than dentine is (Ayliffe *et al.*, 1994).

The form of the *Castoroides* δ_{pe} profile is similar to the δ_{pe} of *Castor*, showing strong seasonal oscillations. These are more difficult to interpret in *Castoroides* as the season of death is unknown, but longer periods are recorded and a rate of growth estimate is not required. There is little evidence of a "snow melt" effect in *Castoroides*, with the rate of the spring/summer reduction in δ_{pe} being similar to the increase in δ_{pe} at the end of summer. This is to be expected as the presence of giant tortoise (*Geochelone sp.*) fossils in the deposit indicates mild winters, but does suggest that the snow melt effect in *Castor* is probably partly a climatic effect. It is not possible to determine whether the variations in *Castoroides* δ_{pe} are synchronous with the seasonal δ_{mw} fluctuations.

In order to compare the isotopic data from *Castoroides* with *Castor* it is necessary to examine their respective lifestyles. While the habits of *Castor* are relatively well understood, those of *Castoroides* can only be guessed from the occurrence of its bones and its physical characteristics. No evidence is known for tree felling or dam building by *Castoroides* (Kurten and Anderson, 1980) and its convex-faced, rather blunt ended slower growing teeth (0.4 - 0.6 times the rate for *Castor*, based on annual fluctuation in δ_p) are quite unlike the chisel teeth of *Castor* and more similar to the incisors of the groundhog (*Marmota monax*). The constantly growing cheek teeth of *Castoroides* are similar to those of the Capybara (*Hydrochoerus sp.*). Its forelimbs were rather reduced and it probably moved even more awkwardly on land than does *Castor* (Kurten, 1968) making it difficult to regard it as a "browsing element" (Graham and Lundelius, 1984). Some writers have suggested that *Castoroides* failed to survive through unsuccessful competition with *Castor*, as the bones of both types are commonly found together (Kurten and Anderson, 1980) but this may indicate that they were not competing: their ranges apparently overlapped significantly for an appreciable period of time and therefore they presumably occupied rather different ecological niches. *Castoroides* lived during relatively warm periods without severe winters, particularly in the area south of the Great Lakes. It may have preferred a muskrat-like (*Ondatra*) existence in lakes and ponds, surrounded by swamps, (Kurten and Anderson, 1980) although in some cases (as in the Adams fauna of Kansas) its bones are found with those of more typical plains animals such as Scott's horse (*Equus scotti*) (Schultz, 1967) suggesting a river with a relatively

narrow or intermittent palustrine zone. Capybara like a riparian environment but consume substantial quantities of grass on land. The similarity of both the incisors and the molars to known grazing forms (*Marmota monax* and *Hydrochoerus sp.* respectively) may indicate that *Castoroides* also grazed. It probably failed relative to *Castor* in more seasonal northern climates because it could not survive on bark in the winter.

δ_{pe} of the *Castoroides* incisor varied by more than 5‰ over the year, at least 1.5‰ greater than the probable range for δ_{pe} in a single *Castor*. It is possible that *Castor* may increase its lowest winter δ_{pe} , and reduce the total range of δ_{pe} , by consuming summer grown vegetable material during the winter months while *Castoroides* may have grazed on evaporatively ^{18}O enriched grass in summer. Despite this, it is difficult to explain how *Castoroides* can have a range of δ_{pe} , reflecting significant seasonal variation in δ_w and/or a significant contribution of evaporatively enriched food, which exceeds that of *Castor* which lives in a significantly less equable climate (King and Saunders, 1986). In addition, the δ_{pe} of big animals is expected to be largely independent of dietary habits (Bryant and Froelich, 1995). The median δ_{pe} for *Castoroides* is about 18.5‰, approximately 6‰ heavier than in the *Castor* studied. As noted above, $\Delta_{\delta_{pe}-\delta_{bw}}$ is about 17.8‰ in mammals, so *Castoroides* δ_{bw} was about 0.7‰. If $\Delta_{\delta_{bw}-\delta_{mw}}$ was about 5‰ as in *Castor*, then δ_{mw} at that time in the Sangamonian was about -4.3‰, which is approximately 2‰ heavier than δ_{mw} for modern Chicago. If the Sangamonian δ_{mw}/T relationship was the same as the modern weighted mean relationship of $\delta_w = 0.69T - 13.6‰$ (Dansgaard, 1964), then the mean annual

temperature of Sangamonian Illinois was about 3°C warmer than at present.

Castoroides $\Delta_{\delta_{bw}-\delta_{mw}}$ was probably less than *Castor* $\Delta_{\delta_{bw}-\delta_{mw}}$ due to its much greater size (Bryant and Froelich, 1995) which suggests that Sangamonian temperatures were probably more than 3°C warmer than at present. This estimate is highly uncertain, as we do not know the metabolic slope of *Castor* or *Castoroides*, the δ_{mw}/T relationship may have changed and δ_{mw} is sensitive to continental atmospheric circulation as well as temperature (see Chap. 5).

However, both the isotopic and palaeontological data indicate that the Sangamonian climate was warmer and perhaps less humid. Winters were seldom below freezing (King and Saunders, 1986), summers were proportionately hotter and drier, and open woodland may have led to significant evaporative fractionation of the water in the kettle holes during summer.

4.11 Conclusions

The oxygen isotopic composition of *Castor* enamel is an excellent monitor of short term variation in δ_{bw} which probably tracks changes in δ_w well, although the δ_{mw} signal is attenuated, mainly by mixing with groundwater. Because beaver are relatively restricted in the water that they sample, as well as consuming water freely, they are apparently sensitive to even small hydrological effects such as differences in amounts of runoff or evaporation at the end of summer and changes in the balance between lake and stream water. Beaver can record short term variation not found elsewhere because of their rapid tooth growth but a number of teeth must be analyzed to define a full annual cycle. On a longer scale, the bone of adult beaver has a relatively constant value of $11.9 \pm 0.5\%$ within the study

area and is suitable for the interpretation of mean annual temperatures. If δ_{pb} is unreliable due to diagenesis, then it could be determined by examination of multiple δ_{pe} profiles if they were available. The following can additionally be learned from beaver δ_{pe} :

Degree of seasonality (variation in δ_w over a year).

Relative rates of spring increase in δ_w versus winter decrease.

The presence of a meltwater signal as a delayed pulse of low δ_w .

Season of death (for example the *Castoroides* studied probably died in the winter).

Major influences of lake water or ground water.

An approximation of the rate of tooth growth.

The teeth contain a reliable, self-checking, mechanism for detecting diagenesis as no teeth should have a similar δ_{pe} along their length. Bone or dentine δ_p can be checked for validity against the δ_{pe} data.

By combining results from *Castor* with less water dependent mammals, such as deer or marmots (*Marmota*), it may be possible to identify effects resulting from changes in relative humidity in periods when reliable hydrogen isotopic data is not available. An initial examination of the giant beaver (*Castoroides ohioensis*) indicates that its teeth preserve an excellent record of seasonal variability in δ_w and the relative temperatures of interglacial periods can be compared using them. If δ_w can be determined from *Castoroides*, then fish and aquatic reptile bones can be used to determine the temperature of the water.

CHAPTER FIVE

The isotopic composition and diagenesis of human bone from Teotihuacan and Oaxaca, Mexico¹

5.1 Abstract

A sample of archaeological human bone from Teotihuacan and Oaxaca, dating from 0 to 750 AD has been analyzed to examine the possibility of using oxygen isotopes to distinguish ethnic groups within Teotihuacan. Sixty-eight oxygen isotopic analyses of bone phosphate have been made of 64 individuals. Diagenetic and isotopic considerations are examined here while anthropological analysis of these data will be presented elsewhere. In addition to oxygen isotopic analysis, the bones have been examined using FTIR spectra, with some additional DNA and ICP-MS analyses. Little change occurs in the bone apatite until the amount of collagen (as combustible organic material) has been reduced considerably. An assessment of bone softness correlates excellently with phosphate infrared crystallinity. The bones have undergone extensive diagenesis and, probably, solution but the $\delta^{18}\text{O}$ of the phosphate (δ_p) is apparently unaltered. On FTIR plots the relative area of the carbonate peak to the main phosphate peak decreases with diagenetic level. Elemental analysis shows that the bones appear to absorb some metals rapidly after death, for example uranium, which are then leached out as diagenesis of the bone apatite commences. Other metallic elements increase irregularly in concentration as alteration proceeds.

¹ A modified version of this chapter, authored by H. Le Q. Stuart-Williams and H.P. Schwarcz, is under review for *Palaeogeography, Palaeoclimatology, Palaeoecology*.

5.2 Introduction

The isotopic composition of a mammal's body fluids is controlled by its total water intake (Luz *et al.*, 1984; Luz and Kolodny, 1985) (including water generated metabolically from food) and the animal's phosphate reservoir is equilibrated with the fluids at body temperature during energy transfer processes (such as ATP synthesis and destruction) and bone mineral precipitation (Levinson *et al.*, 1987; Longinelli, 1984; Luz *et al.*, 1984). Equilibration proceeds very rapidly, for example teeth from the lower jaws of whales have equilibrated at about 1-1.5°C cooler temperature than vertebrae (Barrick *et al.*, 1992). Once the phosphate is fixed as carbonate hydroxyapatite (dahllite) the isotopic values remain relatively unchanged except for the effects of remodelling. For example tooth enamel does not remodel during the life of the animal, while long bones and ribs are altered relatively rapidly while the animal is growing and more slowly thereafter.

The oxygen isotopic composition of human body fluids is dominantly controlled by the $\delta^{18}\text{O}$ of their drinking water, with smaller contributions from food water and food metabolism. The isotopic composition of local water is strongly influenced by the composition of local precipitation, which in turn reflects the cooling history of the air mass carrying the moisture (Dansgaard, 1954, 1964). The temperature history of the moist air is modified by an amount effect and topographic effects, such as the distance travelled over land and passage over ranges of mountains. It is apparent that once an air mass has been cooled and moisture has precipitated, heating it again cannot restore the precooling isotopic composition. In most areas a good relationship exists

between the mean annual temperature and the $\delta^{18}\text{O}$ of local meteoric water (δ_{mw}) (Dansgaard, 1964). This isotopic signature is passed on to plants and animals that directly or indirectly take up the water (Longinelli, 1984; Luz *et al.*, 1984) although the moisture may be enriched in ^{18}O by evaporation (Ayliffe and Chivas, 1990; D'Angela and Longinelli, 1990; Luz *et al.*, 1990).

In a population of mixed origin such as at Teotihuacan it may be possible to identify immigrant groups on the basis of isotopic variation in their bones, if the isotopic signature of their area of origin has not been destroyed by remodelling. Sixty-four humans from Teotihuacan and Oaxaca were analyzed so as to identify separate groups living in Teotihuacan and provide comparative material.

The ruins of Teotihuacan are situated on a relatively flat plain about 30 km north of Mexico City (Fig. 5.1). At the height of Teotihuacan's development ca. 500 AD it was the largest city in pre-Columbian America. From about 100 BC to 200 AD it expanded very rapidly (Cowgill, 1992), drawing people from areas as distant as the Oaxaca valley, 450 km to the south. Some of the immigrant groups maintained an ethnic identity for a considerable period: the Oaxacan barrio (Tlailotlacan) lasted as a distinct entity from 200-750 AD. In addition other groups were buried in selected areas, for example 200 victims of unknown ethnic affiliations were sacrificed over a very brief period of time and buried, early in the history of Teotihuacan, in the environs of the Pyramid of Quetzalcoatl. Teotihuacan samples for this study came from the Merchant's Barrio, mass burials around the Temple of Quetzalcoatl, a burial from the Tlamimilolpa site just north of Merchant's Barrio, a group of Tlajinga burials and Tlailotlacan. The Temple of Quetzalcoatl samples are from the east and

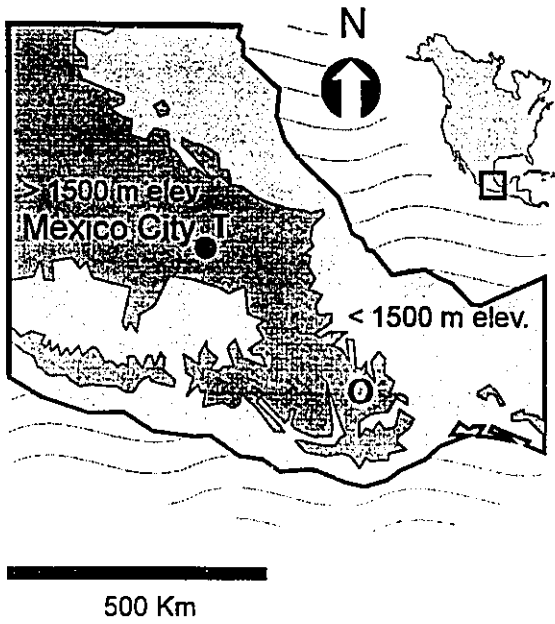


Figure 5.1: Location map of sites studied. T = Teotihuacan, O = Oaxaca.

south sides of the pyramid, where they were covered by concrete floors, and from burials under the structure itself. Samples were also obtained from Monte Alban in the Valley of Oaxaca, as the inhabitants of Tlailotlacan are believed to have come from somewhere in the Valley of Oaxaca. The soils of both areas are similar, being composed of Cenozoic lavas and pyroclastic deposits decayed *in situ*. Too few tooth samples were available to study the $\delta^{18}\text{O}$ of tooth enamel, which is preferred to bone for oxygen isotopic studies as it is less subject to diagenetic alteration (Ayliffe *et al.*, 1994).

5.3 Analytical procedures

Samples of about 100 mg of cortical bone (primarily ribs from Teotihuacan, a variety of bones from Oaxaca) (Table 5.1) were processed by either of two in-house methods (Stuart-Williams and Schwarcz, in prep (*a*)): the first uses lead phosphate and barium phosphate intermediate products to produce silver orthophosphate; the second method uses only a lead phosphate intermediate. The bone is dissolved in 3 molar acetic acid and phosphate is initially extracted as lead orthophosphate. Organic compounds are removed by heating to 95°C in a hot water bath with 6 molar nitric acid and 30% hydrogen peroxide. Previous testing by the first author has shown that the isotopic composition is not altered by this process. The final solution (after potentially interfering cations have been removed) contains only ammonium, some sulphate and phosphate in 0.1 M nitric acid. The solution is neutralised with potassium hydroxide, and silver orthophosphate is precipitated by ammonia volatilization (Firsching, 1961).

Table 5.1: Isotope, crystallinity and site data for samples

Sample No	Burial	Description	Period	$\delta^{18}\text{O}$ (‰)	FTIR crystallinity	CO_2/PO_4 ratio
01	542	Merchants' barrio, adult	EX	13.9	3.2	0.61
02	576	Merchants' barrio, adult	"	14.2	3.7	0.37
04	578	Merchants' barrio, adult	"	14.7	3.5	0.39
05	585	Merchants' barrio, adult male	"	14.4	3.9	0.24
10	153-155	Merchants' barrio, child	LX	16.6	3.1	0.27
23	34	Tlamimilolpa, adult	EX	15.7	3.1	0.58
25	6	Tlajinga, female	ETL	14.4	4.3	0.25
28	61	Tlajinga, male	LX	15.2	3.8	0.52
30	39	Tlajinga, 6 month old	EX	14.8	2.9	0.35
31	21a	Tlajinga, 2yr old	LX	14.6	3.6	0.33
34	11	Tlajinga, male	LTI	15.0	3.9	0.25
36	17b	Tlajinga, Female	LX	14.7	3.5	0.25
39	28a	Tlajinga, 3-4 yr old	LTI	14.6	3.8	0.28
42	42a	Tlajinga, male	LTI	14.3	3.7	0.31
45	5	Tlajinga, perinatal	MET	15.1	3.6	0.47

46	46a	Tlajinga, female	EX	14.9	3.4	0.30
47	56	Tlajinga, male	ETL	14.6	4.1	0.34
49	1	Quetzal', south, male	Ca. 200 AD	15.8	4.3	0.24
50	2-G	Quetzal', interior, female	"	16.3	4.6	0.18
51	2-N	Quetzal', interior, female	"	16.2	4.1	0.19
52	4-A	Quetzal', interior, male	"	15.6	4.6	0.20
53	4-L	Quetzal', interior, male	"	15.5	4.4	0.16
54	5-f	Quetzal', east, male	"	15.4	3.9	0.31
54	5-f	Quetzal', east, male	"	15.4	3.9	0.31
55	5-H	Quetzal', east, male	"	14.2	4.1	0.29
56	6-I	Quetzal', east, male	"	13.4	3.8	0.35
57	6-D	Quetzal', east, male	"	14.1	3.7	0.38
58	10-A	Quetzal', east, female	"	14.3	3.6	0.48
59	10-D	Quetzal', east, female	"	14.1	4.0	0.26
61	11-C	Quetzal', east, female	"	13.6	3.7	0.39
62	12	Quetzal', interior, male	"	15.9	4.5	0.19
63	13	Quetzal', interior	"	16.4	4.7	0.10
64	14-C	Quetzal', interior, male	"	17.7	4.3	0.23
65	14-F	Quetzal', interior, male	"	16.6	4.5	0.23
66	15	Quetzal', interior, male	Pre 200 AD	16.3	3.6	0.26
67	7-P	Quetzal', south, male	Ca. 200 AD	14.6	3.7	0.32

68	7-M	Quetzal', south, male	"	14.2	4.0	0.28
69	F322	Tlailotlacan	4	14.4	4.8	0.35
73	F345N	Tlailotlacan, child	6	16.4	3.5	0.45
74	F223	Tlailotlacan, child	15	15.5	3.4	0.35
75	F133	Tlailotlacan, child	20?	16.5	4.3	0.42
76	F130	Tlailotlacan, child	19	15.3	4.2	0.56
79	F408	Tlailotlacan, infant	4	15.2	3.8	0.44
80	F409	Tlailotlacan	4	15.0	4.7	0.27
83	F381	Tlailotlacan	-	15.6	4.9	0.27
88	F113	Tlailotlacan, young female	20	14.1	3.9	0.30
89	South tomb	Tlailotlacan	20	15.1	4.1	0.48
90	F372	Tlailotlacan	-?	14.2	4.7	0.43
oax1949	Tomb 120	OAX 1949	IV	13.2	4.5	0.21
oax1959	Tomb 113	OAX 1959	II	13.1	3.3	0.53
oax1960	Tomb 111	OAX 1960	I	13.4	3.0	0.82
oax1965	Bur. 4	OAX 1965	II	12.5	3.9	0.40
oax1968	Tomb 2	OAX 1968	I	12.2	3.1	0.40
oax1969	Bur. 17	OAX 1969	IIIa	14.0	3.6	0.43
oax1973	Tomb 63	OAX 1973	V	13.9	4.4	0.17
oax1975	Tomb 121	OAX 1975	IV	13.7	4.3	0.24
oax1999	Bur. VI-7	OAX 1999	IIIa	12.4	3.4	0.49

oax2011	Bur. IV-32	OAX 2011		V	12.5	3.4	0.33
oax2021	Tomb 21	OAX 2021		IIIa	12.5	4.1	0.49
oax2025	Bur. IV-36	OAX 2025		IIIb	12.9	3.3	0.37
oax2034	Tomb 77	OAX 2034		II	12.7	4.4	0.24
oax2045	Bur. V-20	OAX 2045		V	13.2	3.7	0.30
oax2048	Tomb 58	OAX 2048		IV	13.9	4.8	0.31
oax2148	Tomb 153	OAX 2148		IIIb	12.1	5.3	0.27

EX Early Xolapan LX Late Xolapan ETL Early Tlaminiloipa TL Tlaminiloipa LTI Late Tlaminiloipa Met Metepoc

Site or period	Mean $\delta^{18}O$	Standard deviation
Merchant's Barrio	14.76	$\pm 1.1\%$
Tlaminiloipa	15.7	-
Tlajinga	14.75	$\pm 0.3\%$
Quetzalcoatl (interior)	16.3	$\pm 0.7\%$
Quetzalcoatl (east side)	14.2	$\pm 0.6\%$
Quetzalcoatl (south side)	14.4	-
Tlalilolacan	15.2	$\pm 0.8\%$
Monte Alban (Oaxaca)	13.0	$\pm 0.6\%$

These processing methods do not decrease the precision of the phosphate analyses when applied to soluble standards, although improper silver orthophosphate precipitation can produce errors of 2-3‰ (Stuart-Williams and Schwarcz, 1995).

Oxygen was liberated from the silver orthophosphate using a technique of polymerization at high temperature in a bromine atmosphere (Stuart-Williams and Schwarcz, 1995) or by fluorination in bromine pentafluoride (Tudge, 1960). The results are reported as $\delta^{18}\text{O}$ values with respect to the SMOW scale. When the whole of a precipitate is reacted the standard deviation of the analysis is 0.06‰ using the bromine method but when aliquots of a precipitate are used the best precision falls to 0.12‰, as a result of heterogeneity within the silver phosphate precipitate. In addition the precision (σ) is reduced by heterogeneity in the bone which was found to be as great as 0.4‰ in the samples used. Precision using bromine pentafluoride to liberate oxygen from the phosphate is comparable. The oxygen was converted to carbon dioxide with a heated carbon rod and analyzed using a VG SIRA isotope ratio mass spectrometer.

Fourier transform infrared spectrometry was performed using a Bio-Rad FTS 40 infrared photospectrometer. Pellets for analysis consisted of 2 mg of bone with 200 mg of thoroughly dried potassium bromide. The crystallinity index (CI) was calculated using peak splitting of the PO_4^{3-} peaks at 605 and 565 cm^{-1} (Shemesh, 1990). Delayed neutron activation analysis was undertaken using the McMaster Nuclear Reactor. Inductively-coupled plasma mass spectrometric analyses were performed on a SCIEX Elan model 250. The ICP-MS analyses were all normalized to convert the minimum value for each element in the data set to zero and the maximum to 10.

An assessment of the softness of the bones was made by breaking 2 mm fragments of a broken end between the finger nails and rating them from 1 (hardest) to 5 (softest). This method does not promise great repeatability but is reasonably coherent and objective when performed over a short period of time on a small sample set for which other variables are not yet known.

Bone density was determined on combusted fragments from 75 to 300 mg. These were weighed dry and then soaked in water. The excess water was removed so that the fragment would displace its full volume including interstices but not absorb glycerine. The bone fragment was suspended in a beaker of glycerine on a balance so that the mass of the glycerine filled beaker increased proportionately to the volume of glycerine displaced. The volume of the bone fragment was determined as $(\text{increased beaker mass} - \text{initial beaker mass}) / 1.27 \text{ mL}$.

The carbonate and phosphate peak ratios were calculated from the heights of the carbonate peak at 1405 cm^{-1} and the phosphate peak at 1035 cm^{-1} on the infrared spectra. The area of each peak was estimated as the product of the height and the half-height width. The CO_3^{2-} area was divided by the PO_4^{3-} area to produce a dimensionless ratio which removes the majority of analytical variation. This ratio has been found to correlate with the CO_2 wt% yield obtained by manometry in this laboratory (Wright and Schwarcz, submitted):

$$C/P = 0.039 (\% \text{ CO}_2) + 0.025; r = 0.80.$$

5.4 Isotopic analyses

Each archaeologically defined group of burials had a distinct oxygen isotopic composition although some overlap was present between the groups (Table 5.1, Fig. 5.2). The Oaxacan samples in particular formed a distinct population which was isotopically lighter than almost all samples from the Teotihuacan area apart from the lightest from the Pyramid of Quetzalcoatl sacrifices. There was no isotopic overlap between the Tlailotlacan group and the Oaxacan samples. If the samples from the Temple of Quetzalcoatl are regarded as two discrete groups then the most scattered populations are the Tlailotlacan and Merchant's Barrio groups with spreads of about 2.5‰ which largely result in both cases from isotopically heavy results from children's bones; the least scattered are the Tlajinga burials with a scatter of less than 1‰ (Table 5.1, Fig. 5.3). There are few studies of variance of δ_p in bone of modern human populations (Longinelli, 1984; Luz *et al.*, 1984) but analyses of teeth of known origin (Levinson *et al.*, 1987) show minimum scatters of 1.7‰ in 3 populations of 4 or more samples and a maximum of 2.3‰. A total variation of about 1.1‰ exists between different samples from the same bone of individual laboratory rats (Luz and Kolodny, 1985). From this it would appear that the archaeological data set examined here is rather homogeneous but not essentially dissimilar to a modern population. Variance in the δ_p of humans may tend to be higher than in many other animals as a result of the more varied water sources exploited, including variations in dietary water content. For example white-tailed deer collected over much of Oklahoma showed only a 1.7‰ spread in δ_p , and deer from southern Ontario only 0.7‰ (Luz *et al.*, 1990). In the southern half of

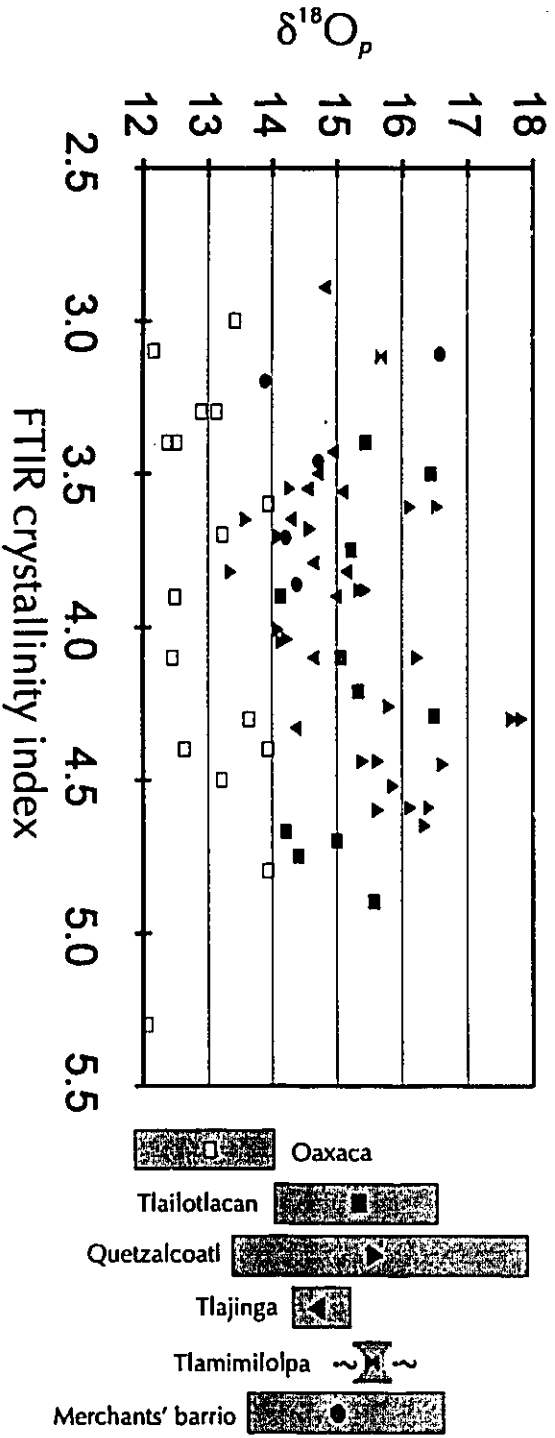


Figure 5.2: Isotopic distribution of all samples. Bars at right show isotopic spread of groups. No errors are shown as populational diversity is uncertain. Analytical error is 0.12 ‰ (see text). Maximum inter- and intra-bone variability noted is 0.4 ‰.

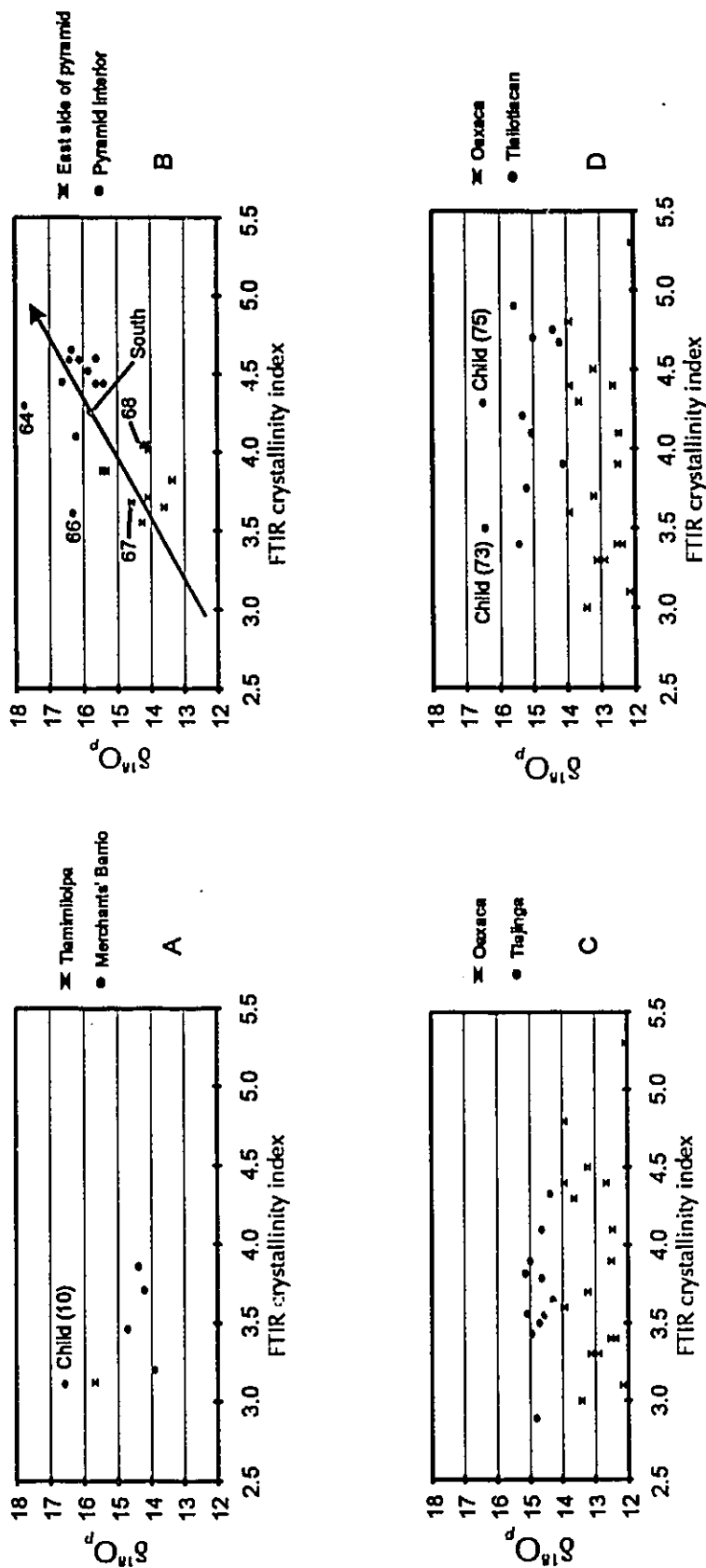


Figure 5.3: Isotopic composition of groups. A) Merchants' barrio with outlying value. B) Temple of Quetzalcoatl. Arrow shows possible diagenetic trend. C) Comparison of indigenous Tlajinga group and Oaxacan samples. D) Comparison of Oaxacan and Tlaloatlacan samples. Key samples are identified.

Alberta, deer showed a larger variance of 4.3‰ which may result from very strong topographic effects and isotopic heterogeneity on the eastern slopes of the Cordillera.

5.5 Crystallinity, softness, density and organic content

Bone is highly prone to isotopic alteration after burial due to its small crystallite size (platelets 250-350 Å wide by 25-50 Å thick) and very high surface area (100-200 m² g⁻¹ (Posner *et al.*, 1984)). Oxygen isotopic change is commonly mirrored by changes in one or more measures of diagenetic alteration, prompting workers to propose that the phosphate is being re-equilibrated with local soil water by biological activity or by unspecified diagenetic processes over geologic time (Luz, 1992; McArthur and Herczeg, 1990). Inorganic exchange of oxygen between phosphate and water is negligible under common burial conditions of archaeological material (Brodskaa and Sulima, 1953).

The crystallinity of the bone was assessed using Fourier transform infrared spectra (Shemesh, 1990). Modern bone typically has an initial CI of about 2.8 to 3.0 while this value tends to increase in ancient bone and most of the archaeological samples examined had values between 3.5 to 4.8 (Fig. 5.2). As the CI increases the probability of isotopic alteration becomes greater but increased crystallinity values need not result in a change in the oxygen isotopic content of the bone phosphate. Apparent crystallinity may increase for a number of reasons, including dissolution of the more disordered outer surfaces of the bone crystallites without recrystallization of the remaining phosphate; or recrystallization in a closed system without alteration of the isotopic value. Despite these reservations, CI is a useful measure of diagenetic change with

which other variables may be compared. It was noted that sub-adult bones (for example samples 10 & 30) with a high collagen content were the least recrystallized, which is compatible with the findings of a number of workers that the combination of collagen and bone mineral strengthens and stabilises the bone synergistically (e.g. Tuross *et al.*, 1989). Some adult bones (for example sample 23) had also survived relatively unscathed, perhaps as a result of particular burial conditions. From Fig. 5.3 we see that there is no significant correlation of isotopic alteration (changing δ_p) with increasing CI in any of the groups of bone samples, with the possible exception of the bones from the Temple of Quetzalcoatl. The correlation between increasing softness/friability and CI is good ($r = 0.65$) (Fig. 5.4a) and the softest bones have a powdery appearance. This finding is compatible with bone weakness and increasing CI being closely related to collagen loss and exposure of the crystallites to chemical attack.

Eight samples were selected from the Temple of Quetzalcoatl group on the basis of diversity of CI and δ_p to obtain a wide spectrum of possible diagenetic alteration. Bones from the east side of the pyramid are different in their CI and δ_p from samples from the south/interior of the pyramid. The organic content of the bone, which ranged from 4.4 to 11.2%, was determined by weighing a dried sample before and after combustion in air at 450°C for 4 hours. This was believed to be adequate to oxidize the organic compounds in the highly permeable bone but not decompose any carbonates. The correlation between mass

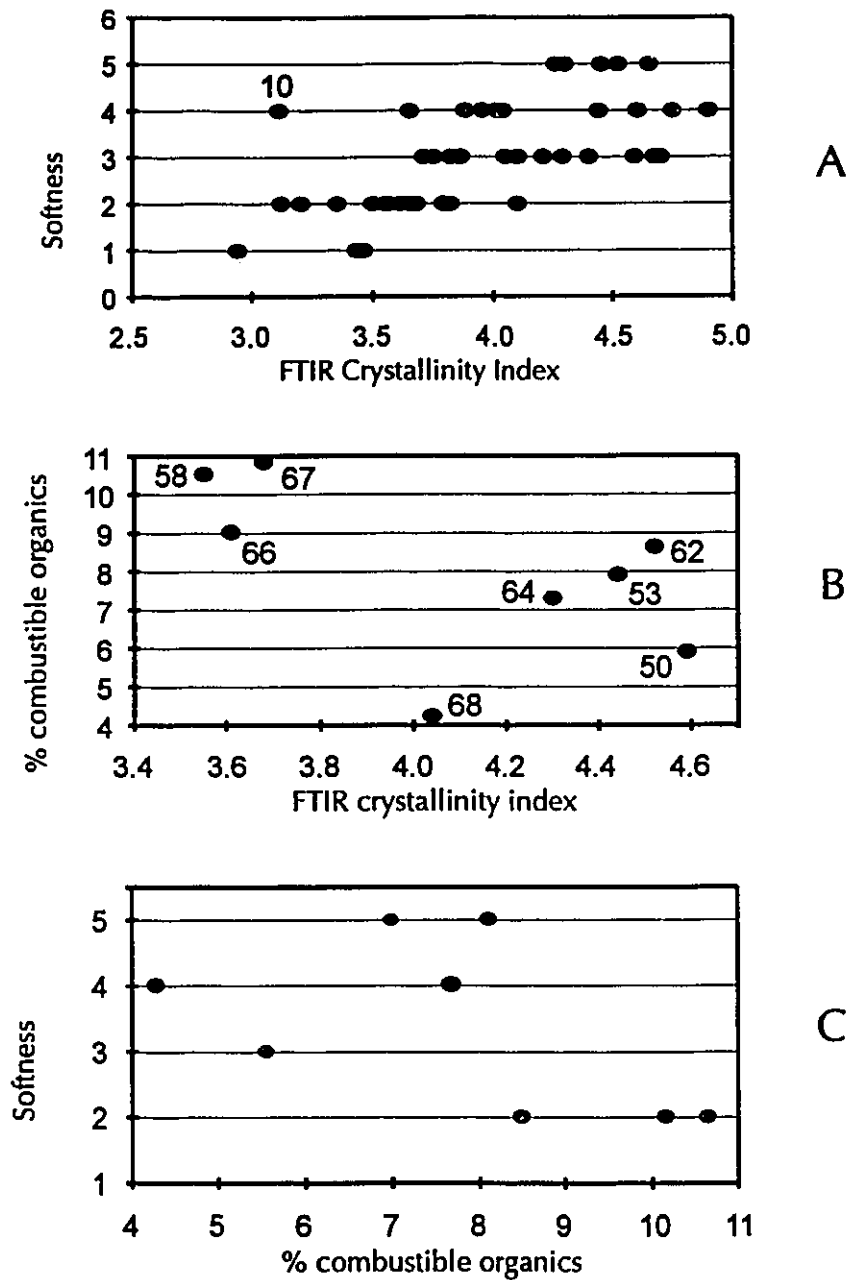


Figure 5.4: Physical alteration of bone. A) Bone crystallinity increase related to loss of strength. $r = 0.59$ with sample 10, increasing to $r = 0.81$ without. B) Crystallinity change as collagen is removed. Note that sample 68 appears to be an outlier. C) Structural weakening as collagen is removed. All samples are from the Temple of Quetzalcoatl, Teotihuacan

lost (indicating the percentage of organic material remaining from the ancient bone) and CI (Fig. 5.b) is good, $r = 0.59$, rising to 0.81 if sample 68 is removed from the data set. In addition there is a positive correlation between the ratio of mass lost during combustion and softness (Fig. 5.4c) of $r = 0.53$. Comparisons using δ_p were not meaningful because the great differences between the CI and δ_p of the south/east side and interior samples, possibly of diagenetic origin, make it difficult to identify more minor effects.

Bone density was determined on the same subset of 8 samples. The results varied from a bulk relative density of 0.55 to 1.14. g mL^{-1} for the cortical bone, indicating very substantial mass loss during burial. No meaningful correlations with other variables were detected in the results, perhaps indicating that initial density variations in the bone were much greater than any effects resulting from diagenesis.

The results (Fig. 5.5a) of the carbonate:phosphate peak ratio analysis made of all 64 samples show that the CO_3^{2-} content decreases as recrystallization of the bone proceeds. Carbonate is flushed from the bones by groundwater as the volcanic ash-like subsoil is carbonate deficient. A similar effect is found in weathering marine phosphorites in low-carbonate settings (Flicoteaux and Lucas, 1984). Bone apatite is initially at least 4% carbonate, of which more than half is on the surface of the crystallites (Posner *et al.*, 1984). This is readily removed during initial solution of the bone crystallites with the most soluble outer layer of apatite where lattice imperfections are most numerous.

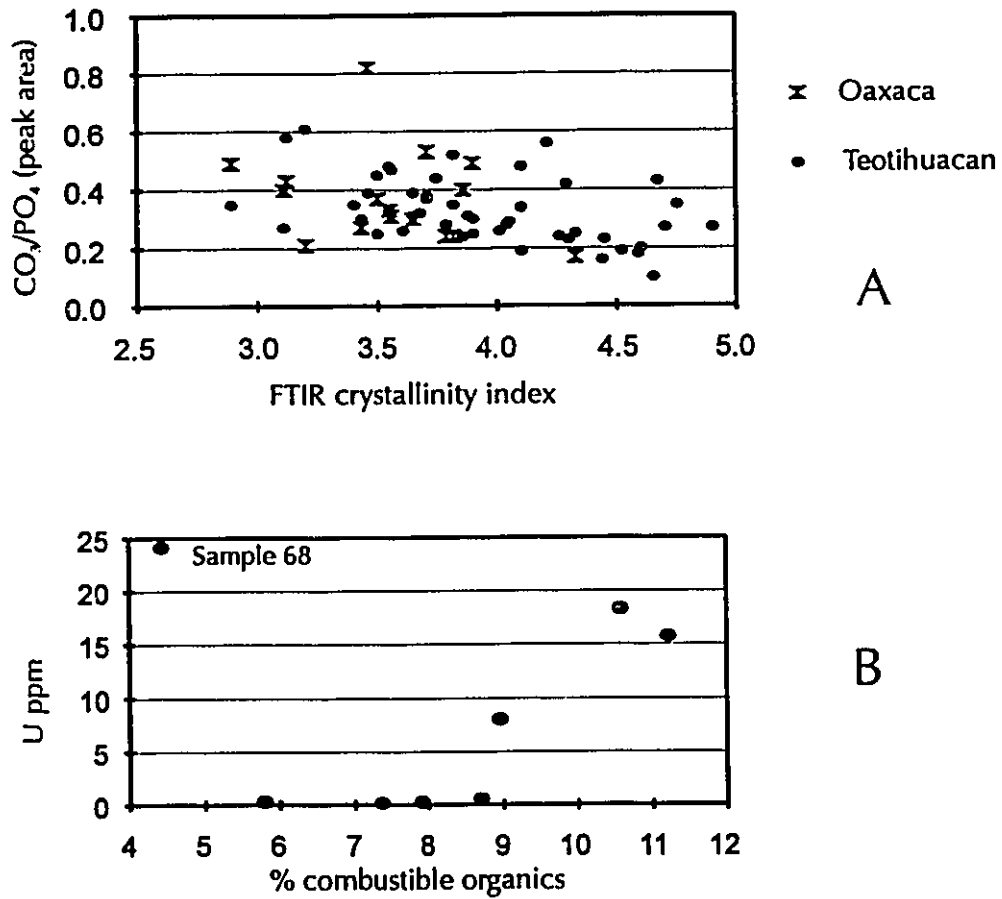


Figure 5.5: A) The amount of carbonate decreases as bone crystallite change progresses. B) Uranium appears to leach out as the organics are removed.

5.6 Elemental analyses

The sample of 8 bones used for density and organic content determinations was also analyzed for its uranium content using delayed neutron activation analysis (DNAA). Uranium was selected in particular as being a mobile element very susceptible to changes in pH and Eh. In addition the analysis could be made rapidly and precisely as the laboratory routinely performs uranium measurements for dating purposes. The U content ranged from 0.21 to 24.2 ppm (Table 5.2). Samples from the south side/interior of the pyramid had lower U contents of 0.21 to 8.03 ppm while values for samples from the east sides ranged from 15.76 to 24.2 ppm. Uranium content is negatively correlated with CI when the group is viewed as a whole: less altered bone contains more uranium. This is probably not an artefact of the groupings as pyramid interior sample 66, which antedates the pyramid, has a CI similar to the east side samples and a high uranium content. In most of the samples uranium content is positively correlated with the organic content (Fig. 5.5b). The exception is sample 68 which has the highest uranium content but a low organic ratio and an intermediate CI. This sample is anomalous in most of the correlations of organic content and may have been altered under unusual conditions, although sample 67 from a similar setting is similar in its properties to other bones in the study.

Four of the eight bones from the subset used for DNAA were also elementally analyzed by ICP-MS in an effort to examine as many measures of diagenetic change as possible. These were samples 50, 53 and 64 from the pyramid interior and sample 68 from the south side. This was also intended as a pilot study from which only tentative conclusions can be drawn.

Table 5.2: Physical properties, isotopic composition and uranium content of selected samples from the Temple of Quetzalcoatl

Sample No.	Burial	$\delta^{18}\text{O}$	FTIR crystallinity index	% combustible organics	Bulk density	Uranium (ppm)	Softness
50	Q2-G	16.13	4.59	5.8	0.96	0.34	3
53	Q4-L	15.40	4.44	7.9	0.67	0.28	4
58	Q10-A	14.28	3.55	10.6	0.91	18.33	2
62	Q12	15.86	4.52	8.7	0.55	0.57	5
64	Q14-C	17.70	4.30	7.4	1.14	0.21	5
66	Q15	16.12	3.61	9.0	0.83	8.03	2
67	Q7-P	14.59	3.68	11.2	1.02	15.76	2
68	Q7-M	14.24	4.04	4.4	0.64	24.20	4

Calcium:phosphorus ratios appear to fall as recrystallization proceeds. Analyses of some of the elements are presented below (Fig. 5.6). Many of the elements show no clear relationship between concentration in the bone and the degree of diagenesis. Sample 68 from the east side of the pyramid has the lowest CI and is depleted in all elements apart from uranium, barium, tin and tellurium. From this limited investigation it was apparent that attempts to correlate degree of bone diagenesis with trace element concentrations would be unsuccessful at this site as no monotonic increase or decrease in element concentrations could be detected.

5.7 Discussion: Climate and regional variability

As humans are obligate drinkers, their δ_p reflects local δ_w ($\delta^{18}\text{O}$ of consumed water) although this may not be equivalent to δ_{mw} ($\delta^{18}\text{O}$ of meteoric water) due to consumption of river and well water, as well as evaporatively enriched water from plant and animal foods. Human δ_p is considerably greater than local δ_w according to equation 1.

$$\delta_p = 0.46\delta_w + 19.4 \quad \text{Levinson } et al., 1987 (1)$$

Two other δ_p/δ_{mw} relationships have also been published based on data sets of human bone and teeth. They are both related to meteoric water values, rather than drinking water samples, and have significantly steeper slopes: 0.64 (Longinelli, 1984) and 0.78 (Luz *et al.*, 1984) with constant offsets of about 22.5 ± 0.2 . For this study the formula of Levinson *et al.* (1987) may be the most appropriate because samples from the present study area are better represented than in the other data sets. Even though local tooth samples were used, the

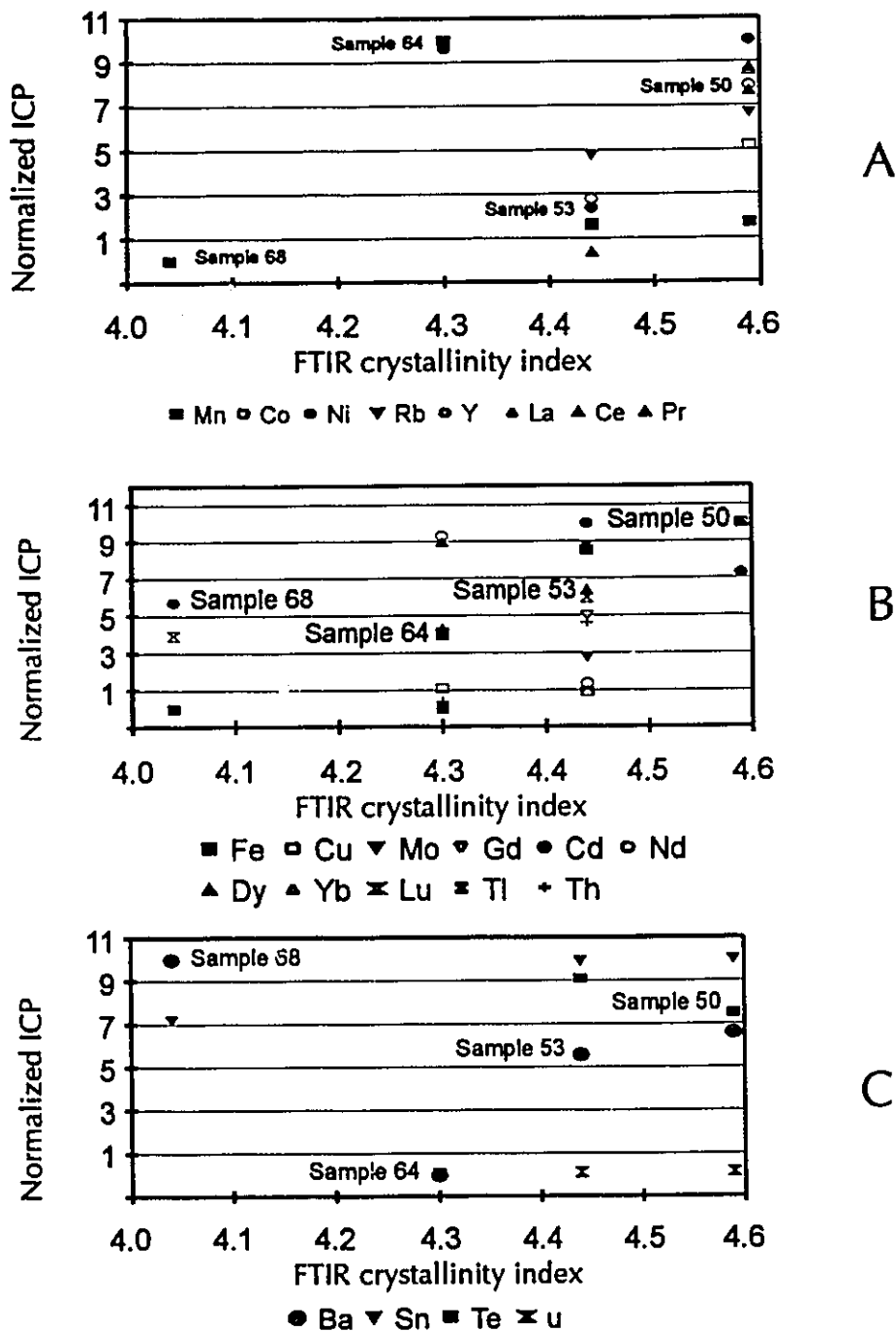


Figure 5.6: Elemental analyses of Teotihuacan bones. Sample 68 is from south of the Temple of Quetzalcoatl and is unlike the other samples from the pyramid interior. It is depleted in the elements from diagram A, has comparable amounts to B and is enriched relative to elements in C, including uranium. Plots are normalized with the maximum for each element equal to 10 and the minimum to zero.

equation may not however be applicable as the Mexico City sample used was from a 15 year old female and may have been influenced by consumption of water with a composition different from local meteoric water, for example deep well water and imported food and drink. However well water and river water sources available at Teotihuacan and Oaxaca are also likely to differ in composition from pure local meteoric water. The majority of the Teotihuacan sample groups have δ_p between 14 and 17‰, which is comparable with a variety of groups ranging from Hamilton, Ontario to Brazil (Levinson *et al.*, 1987). The Oaxacan group is unexpectedly light at 12-14‰. There are potential difficulties when comparing teeth and bone in that teeth are produced during a relatively short period early in the life of the individual in which δ_w may not be representative of the mean consumed by the population. For example human body fluids are isotopically fractionated to 2 to 5‰ above local meteoric water values (Longinelli, 1984) and milk passed from a mother to her child during breast feeding will be further fractionated by the child to a similar extent. The child's body phosphates incorporated into its teeth are therefore also doubly fractionated. This breast feeding enrichment may be present in an isotopically heavier juvenile bone from Merchant's Barrio and the two isotopically heaviest bones from Tlailotlacan but is not present in the juveniles from Tlajinga. Little remodelling of teeth occurs during life (for example D-aspartic acid accumulates during life in dentine (Gillard *et al.*, 1990)) but some structural bone remodels virtually continuously so that bone and tooth values may become different in a migratory adult. Despite these difficulties, published values for δ_p of bone from non-migratory people (Longinelli, 1984) are broadly comparable with dental analyses.

The isotopic data for Teotihuacan and Oaxaca are relatively opposite to initial expectations based on altitude and proximity to the ocean: δ_p for Oaxaca is $< \delta_p$ for higher and cooler Teotihuacan. The average for the Oaxacan bones with a mean annual temperature of 20.5°C at an altitude of about 1900 m is 13‰, whereas Teotihuacan is about 500 m higher with a mean annual temperature of 15.6°C but mean δ_p for the Tlajinga sample is approximately 14.75‰. The climate of Oaxaca is hot and dry with an annual rainfall of about 650 mm with semi-desert vegetation (Tamayo, 1962), whereas Teotihuacan is temperate-humid with a mean annual rainfall of 746 mm. All these factors indicate that Oaxacan δ_p should be substantially greater than Teotihuacan δ_p , due to temperature, amount and altitude effects (Dansgaard, 1954, 1964) but there is every indication that the Oaxacan bone material retains original isotopic values. Analyses of well water from the two areas are in general agreement with this finding, although well water may not be isotopically equivalent to meteoric water. $\delta_w = -9.8‰$ for samples from wells at Teotihuacan; $\delta_w = -8.9$ to $-9.8‰$ for well water samples from the Valley of Oaxaca around Monte Alban, while $\delta_w = -11.5‰$ for the fringes of the valley. The lighter isotopic value from the elevated edges of the valley is perhaps more similar to local δ_{mw} for Monte Alban as the water in the valley bottom is more influenced by isotopically fractionated water from greater distances, including evaporatively enriched river water. Effects of climate change can probably be ruled out as the Oaxacan samples come from periods I-V, which more than span the period of the Teotihuacan samples, with no clear isotopic separation of bones from different periods. The differences between the sites must result from the combination of topography and atmospheric circulation patterns. At both sites the majority of the rain falls

during the middle of the year when the winds are blowing from the east or northeast (Newell *et al.*, 1972). Water vapour is transported from the Gulf of Mexico or the Pacific over the mountains, cooling and condensing as it travels. Oaxaca is therefore in the rain-shadow of the Cordillera and receives only precipitation from clouds already depleted in ^{18}O . This is comparable to the contrast between Holsteinsborg and Sdr. Strømsfjord in Greenland noted by Dansgaard (1964): these sites have similar temperatures but the latter receives precipitation about 10‰ lighter. This emphasizes the importance of atmospheric circulation in the control of oxygen isotopic values: if the dominant weather pattern shifted to westerlies both Teotihuacan and Oaxaca would have very much increased δ_w and their relative isotopic positions would be reversed.

As well as the relative isotopic reversal, both Teotihuacan and Oaxacan populations have unexpectedly light δ_p values. We can estimate an average of -5‰ for water based on modern measurement of other, mostly lower, areas of Mexico (-4.8 (Chihuahua (IAEA, 1969)), -4.6 (Veracruz (Yurtsever and Gat, 1981) and values extrapolated from maps of about -5.6‰ (Taylor, 1974) and -2 to -4‰ (Yurtsever and Gat, 1981)). Using equation (1) we would expect an average δ_p of ~ 17 ‰, about 2‰ heavier than observed for Teotihuacan, and 4‰ heavier than at Oaxaca.

5.8 Discussion: Examination of possible diagenesis and diagenetic models

Although the bones do not appear to have undergone isotopic alteration as shown by the lack of a significant correlation between δ_p and CI, a further check was made by calculating the δ_p that might be expected for diagenetic phosphate. As the bone phosphate is usually expected to move towards equilibrium with the burial conditions, an isotopic value for phosphate at equilibrium with local meteoric water at soil temperature was calculated for comparison. δ_p in equilibrium with local water can be calculated as a two step process. First (as comprehensive water analyses are not yet available) the isotopic composition of local water was derived from the known composition of the bone using equation 1 (Levinson *et al.*, 1987). Then the $\delta^{18}\text{O}$ of phosphate biologically equilibrated with that water at soil temperature was determined using equation 2 (Friedman and O'Neil, 1977; after Longinelli and Nuti, 1973a).

$$t^{\circ}\text{C} = 111.4 - 4.3(\delta_p - \delta_w + 0.5) \quad (2)$$

For the indigenous Tlajinga group (lying close to the centre of the $\delta^{18}\text{O}$ distribution (Figs. 2 & 3)) with mean $\delta_p = 14.75\text{‰}$ a calculated δ_w using equation 1 is -10.2‰ , which is in good agreement with our own measurements of well water from close to Teotihuacan which have a mean value of -9.8‰ . This is similar to measured values for teeth and water respectively from Mexico City of 15‰ and -11.4‰ (Levinson *et al.*, 1987). At a soil temperature of 15°C (the mean annual temperature of Mexico City about 30 km away) the δ_p of equilibrated phosphate in the soil = 12‰ . For the Oaxaca group δ_p of phosphate equilibrated at 20°C (mean annual temperature) in the soil is 7‰ , assuming a calculated δ_w of -14‰ using equation 1. Our measurements of well water from

the area are isotopically heavier and range from -8.9‰ for a sample from the base of Monte Alban, to approximately -9.5‰ on the alluvial plain and -11.5‰ from the flanks of the valley. Using a δ_w of -9.5‰ (approximately the modal value for the Oaxacan valley floor water samples), diagenetic phosphate would have a δ_p of 11.25‰, which is a probable maximum value. If δ_{sw} ($\delta^{18}\text{O}$ of soil water) is similar to δ_{mw} and to δ_w (despite enrichment by evaporation and other effects) then the δ_p of the archaeological bone from Teotihuacan and Oaxaca is close to equilibrium with soil conditions initially: the enrichment of body fluids in ^{18}O is similar to the enrichment of ^{18}O in phosphate equilibrated at a lower temperature in soil. As a result of the proximity of the diagenetic and original δ_p , equilibration effects on the phosphate are expected to be small with a tendency for the δ_p to move toward lower values. This effect is not statistically verifiable in any of the groups and isotopic equilibration is unlikely to be present.

Data from the Temple of Quetzalcoatl when viewed as a whole appear to display a rapid increase in δ_p as the CI increases, with an initial δ_p of about 12‰, determined using the intersection of a regression line through the two groups of samples with the crystallinity index at 2.8 (Fig. 5.3). This interpretation is possible but seems unlikely as no similar effect is visible in the Tlajinga or Tlailotlacan data sets. The material from the east side of the pyramid was buried about 4 metres deeper than the bones from the south side and might have been exposed to less flushing by soil water. The interior samples were even more deeply buried, but the south and east side burials were covered by concrete floors with a substantial carbonate content which must have had an effect on soil water chemistry. It is difficult to devise a model in which groups of bone buried under such disparate conditions would define the same diagenetic path. Further,

for the data to fit an apparently straight line with a steep slope, ultimate re-equilibration with water with an unrealistically high $\delta^{18}\text{O}$ is required. Precedents for this do exist, for example the data from Byzantine and modern human bones from Rehevoth, Israel (Luz, 1992)(Fig. 5.7). The most altered bones from Rehevoth have $\delta_p = 22.5\text{‰}$ with no indication of a lessening in the rate of isotopic alteration versus the crystallinity index, implying that δ_p at equilibrium with burial conditions must be $\gg 23\text{‰}$. δ_w for modern humans in the sample is -4.5‰ but at a soil temperature of 20°C , $\delta_{s,w}$ must be $> 2.25\text{‰}$ which implies an improbably great evaporative enrichment of 6.75‰ (Cerling, 1984; Allison and Hughes, 1983). As inorganic equilibration of the phosphate is very improbable, equilibration must occur by enzymatic activity of soil organisms, particularly bacteria and fungi. Equation 2 is based upon studies of organic phosphates, with all known organisms equilibrating their phosphates with their body fluids according to that relationship. It is unlikely that bacteria or fungi equilibrate phosphate very differently: the mitochondria of higher organisms which are responsible for a considerable portion of the phosphate equilibration and manipulation (Lowenstam and Weiner, 1989) are of bacterial origin. Highly isotopically fractionated body fluids in the soil organisms would result in highly fractionated phosphates but all "water breathing" aquatic organisms examined have body fluid compositions identical to the surrounding medium (e.g. Kolodny *et al.*, 1983; Longinelli and Nuti, 1973a&b) due to the need for efficient

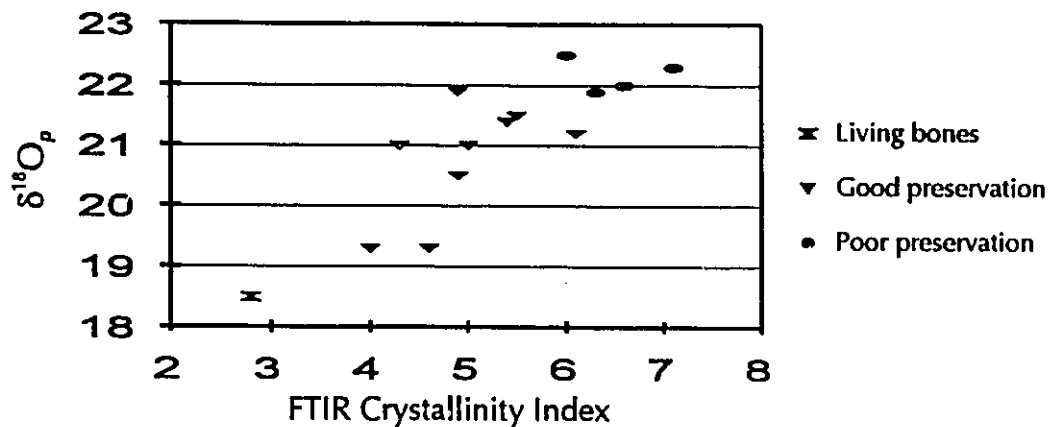


Figure 5.7: Byzantine and modern bones from Rehovoth, Israel. (Luz, 1992)

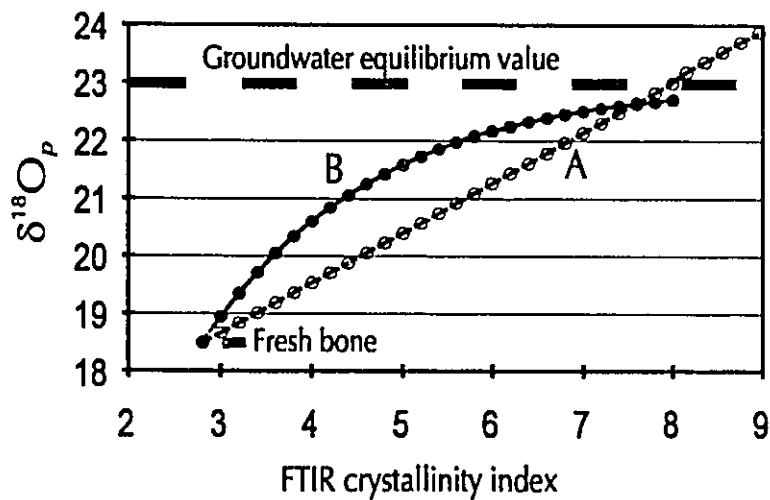


Figure 5.8: Phosphate alteration curves. A) Bone phosphate being equilibrated to 23‰ at 10% exchange for 0.2 change in crystallinity index. B) Phosphate undergoing selective solution/ recrystallization to reach 23‰ at crystallinity index 8.

exchange of gas and nutrients. Other considerations also indicate that a simple re-equilibration with groundwater is not the case. As bone exchanges with soil water it should asymptotically approach some equilibrium value (Fig. 5.8). The data in figure 3 do not show any such decrease in slope with increasing $\delta^{18}\text{O}$, which would require that the region of decreasing slope is encountered at $\delta^{18}\text{O}$ values much greater than the values observed here ($> 20\text{‰}$). This implies that the water with which the phosphates had been exchanging would have been anomalously heavy ($> \sim 23\text{‰}$) when compared with local meteoric waters.

Alteration of the bone phosphate and recrystallization are strongly associated with the final stages of the removal of the organic portions (Fig. 4b). Bone crystallites would apparently provide no energy to an organism that attempted to subsist on them although phosphate is an essential nutrient for all organisms. Conversely collagen is known to adsorb phosphate strongly (Koutsoukos and Nancollas, 1986) and to be associated with the initial formation of bone crystallites (Lowenstam and Weiner, 1989) and the removal of it destabilises the bone matrix as well as rendering the bone more permeable and mobile. Collectively these results indicate that the processes altering the isotopic composition of the bone phosphate are probably inorganic and result from effects associated with the dissolution and recrystallization of the bone mineral, most probably with entire PO_4^{3-} ions being involved rather than O^{2-} ions being exchanged between phosphate ions and the soil water. Oxygen isotopic offsets of 2 to 3‰ have been shown to exist between PO_4^{3-} ions in solution and in a slowly precipitated solid phase (Stuart-Williams and Schwarcz, 1995).

The uranium contents of the bones may be used to indicate burial conditions soon after interment, as the conditions under which uranium is mobilised or fixed are known from geological studies of uranium ores. Uranium is mobile as the uranyl ion, UO_2^{2+} under oxidizing conditions. In neutral to alkaline fluids that contain abundant dissolved CO_2 the uranyl is complexed with carbonate or organic compounds (Gilbert and Park, 1986; Hosteller and Garrets, 1962). In its reduced form as U^{4+} , uranium is highly insoluble in soil waters. This combination of factors may explain the higher concentration of uranium in the samples with less recrystallization and a higher organic content. Initially the body decays and releases organic compounds and CO_2 into the surrounding oxic soil under slightly basic conditions. Uranyl in the soil water is then fixed in proximity to the corpse as U^{4+} by decreasing pH and strongly reducing conditions, in a manner similar to a roll-front uranium deposit (Gilbert and Park, 1986). As the soft tissues decay the uranium tends to become more concentrated around the bones, where reducing conditions and phosphate tend to immobilize it. Once the soft tissues have completely decayed and the bone collagen begins to be lost from the bone, the uranium is oxidised by molecular O_2 in the soil water and dissolved in the groundwater. The anomalous composition of sample 68 suggests that it was perhaps sheltered from flushing by soil water after the soft tissues had decayed or that conditions remained rather more reducing in the neighbourhood of this bone.

In conclusion to this section, it is unlikely that the Temple of Quetzalcoatl samples have suffered isotopic alteration as they were all buried at the same time under very different conditions and could not have followed a similar diagenetic pathway. In addition it is improbable that δ_{U} was sufficiently high for

equilibration to be a possible mechanism but enrichment by fractionation of entire PO_4^{3-} ions is possible. The retention or uptake of uranium in the bone samples does not follow a monotonic pathway and cannot be interpreted to give a concise description of burial conditions.

5.9 Summary and conclusions

The oxygen isotopes of the Oaxacan burials form a distinct and well defined group with little overlap with the Teotihuacano data (Fig. 5.2). While quite considerable recrystallization and/or solution has occurred, this has not apparently affected the $\delta^{18}\text{O}$ of the bone. Both the degree of recrystallization and δ_p appear to be uncorrelated with the relative ages of the samples, with δ_p remaining approximately constant and recrystallization depending on burial conditions. An average δ_p for the Oaxacan population is $13.0 \pm 0.6\text{‰}$. The relatively narrow spread of values may imply consumption of drinking water from a single, not highly variable, source. Climate and atmospheric circulation probably remained relatively constant over the period represented by the samples. High frequency fluctuations are possible, for example interannual variation, but lower frequency fluctuations on a 10 year or greater wavelength are unlikely.

The total spread of values from Teotihuacan is rather greater, perhaps representing a mixture of peoples of different origins but with little indication of isotopic alteration after burial. The Tlajinga group has a low variance, suggesting a regionally discrete population with a very constant weighted mean annual δ_w . The Merchant's Barrio and Tlailotlacan groups have rather greater

isotopic spread while the Temple of Quetzalcoatl samples appear to form two discrete groups. The single Tlamimilolpa sample has a value similar to the mean of the Tlailotlacan group.

It is possible that some of the isotopically heavier groups may have been derived from the Gulf coast, while isotopically lighter samples are from people who are native to the highlands and the rain shadow areas or have been there long enough that substantial remodelling of their bone has occurred.

As collagen removal from the bone accelerated after burial, exposing the bone crystallites to attack by oxidizing, slightly acid fluids, the bone crystallites were progressively dissolved removing the more soluble defect- and carbonate-rich outer layers. Little recrystallization took place and the flushing conditions did not favour kinetic effects that might produce an isotopic fractionation, so the removed phosphate had a composition close to δ_p and the composition of the remaining bone is unchanged.

CHAPTER SIX

Postscripts and future work

6.1 Postscript

It is now routinely possible to obtain analytical precisions of better than 0.06‰ on analyses of silver phosphate, without concerns regarding the use of hydrous compounds, such as $\text{BiPO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$. Guaranteed accuracy, however, remains elusive, especially with regard to analyses of biogenic phosphates. There is no method which guarantees a correct value, and therefore no standard with a value that can be universally accepted. This is emphasized by the lack of a clear explanation for the widely varying analyses of Ag_3PO_4 examined in chapter 2. Even the initial publication of a phosphate fractionation function (Longinelli, 1965) was marred by a 3.5‰ offset of the results relative to those of J.R. O'Neil. A revised version was published (Longinelli and Nuti, 1973*a*) once the author has replaced the silver-soldered joints on his BrF_3 reaction line with flareless fittings but the process which caused problem was not explained. The organic compounds in biogenic phosphates complicate the processing even further, as described in chapter 2, so that no two workers can apparently reproduce the expected $\delta^{18}\text{O}$ analysis of a bone. The processing of phosphate has progressed greatly during the last 30 years but there would appear still to be some subtleties which are evading workers in the field.

The situation is complicated further by the poor preservation of most ancient material. While all workers agree that the $\delta^{18}\text{O}$ of bone changes after

burial, there is little agreement about the mechanisms involved. Inorganic equilibration of phosphate oxygen with diagenetic water is proposed for ancient examples, such as Mesozoic dinosaur bones (Barrick and Showers, 1994) or Pliocene phosphorites (McArthur and Herczeg, 1990). Equilibration with soil water is also proposed for more modern remains, for example Byzantine human bones (Luz, 1992) or Proboscidean remains in hot climates (Ayliffe *et al.*, 1994) and cold climates (Ayliffe *et al.*, 1992). It is not clear from these studies whether the isotopic equilibration is inorganic or biologically mediated. In other studies, for example the Sangamonian giant beaver incisor examined in chapter 4 or the human remains from Teotihuacan examined in chapter 5, there is no clear evidence for diagenetic alteration of the isotopic signature. No rate controlling mechanism has been identified: Byzantine human bone is diagenetically enriched by 4‰ (Luz, 1992) while subtle isotopic variations due to body temperature changes can be measured in Cretaceous *Tyrannosaurus rex* (Barrick and Showers, 1994). In addition many of the isotopic analyses obtained for 'equilibrated' buried bone seem improbable: for example δ_p for the most diagenetically enriched Israeli Byzantine bones is 22.5‰. If all the phosphate in the bone had equilibrated with soil water and soil temperature was 10°C, δ_w must equal 1.6‰ (Longinelli and Nuti, 1973a); but the bone was not 100% recrystallized, local soil temperatures are greater than 10°C and δ_w is probably considerably less. An alternative model based on enrichment by kinetic isotope effects during partial solution of bone (Stuart-Williams and Schwarcz, 1995b) needs to be tested further.

Research opportunities in this field are far from exhausted: there is probably more work on the oxygen isotopes of biogenic phosphates being published now than ever before.

6.2 A comment on the archaeology of oxygen isotope data from Teotihuacan

No archaeological interpretation was made of the isotopic data for human bone from Teotihuacan and Oaxaca in chapter 5, because it was agreed by the four authors (see preface) that the resulting paper must not interfere with publication of the results by the anthropological authors.

There is clear variation between the different groups analyzed (Figs. 5.2 and 5.3, pages 106 and 107). The δ_p of the Oaxacan samples overlaps slightly with the two Teotihuacan analyses, a male and a female from the east side of the Temple of Quetzalcoatl, suggesting that these could be bones of sacrificed Oaxacans. Differences between the δ_p of groups analyzed at Teotihuacan are assumed to result from immigration and the retention of regional isotopic signatures, as it is improbable that there is significant variation in the δ_w of ingested water within the city. The Tlajinga group are archaeologically recognized as being indigenous to Teotihuacan. The δ_p of Tlajinga adults ranges from 14.3 - 15.2‰. This overlaps with the three samples of Tlailotlacan bone definitely from adults (samples 69, 89 and 90) which demonstrate a δ_p range from 14.2 - 15.1‰. If the Tlailotlacan group immigrated to Teotihuacan, then the δ_p of the rapidly growing bones of Tlailotlacan infants and children (which comprise the majority of the data set) should be similar to δ_p for an adult member of the

Tlajinga group, but instead ranges from 15.0 - 16.5‰. There is little evidence for immigration in these results; the adults sampled from Tlailotlacan appear to have been raised there and the substantially greater δ_p of the Tlailotlacan children is very unlikely to be a regional signature and may result from metabolic or dietary differences. The bones from the Temple of Quetzalcoatl were all of adults. Samples from the east side of the pyramid were isotopically similar to the adult Tlajinga and Tlailotlacan examples, suggesting the sacrifice of indigenous people. The samples from the pyramid interior are isotopically more enriched, with δ_p ranging from 15.5 - 16.6‰ with an outlier at 17.7‰. These bones are isotopically distinctly heavier than bones from any adults living in Teotihuacan and must represent the sacrifice of people from a distant area, perhaps the Gulf coast. The inhabitants of Merchant's Barrio were not isotopically distinct from other indigenous groups at Teotihuacan.

6.3 Future work

Most studies of δ_p in mammals have found samples which did not display the expected direct relationship between δ_p and δ_{mw} or δ_w . These include scatter in human tooth and urinary stone δ_p (Levinson *et al.*, 1987); outliers of unexplained origin in archaeological human δ_p and apparent reversals of δ_p/δ_{mw} relationships (Chapter 5; Stuart-Williams and Schwarcz in review); unpredictable hydrological influences in beaver (Stuart-Williams and Schwarcz, 1995); scatter and outliers in horse (*Equus sp.*) δ_p (Bryant *et al.*, 1994); Scatter of δ_p in deer (*Odocoileus sp.*) relative to measured temperature and necessity of correction

using humidity data (Luz *et al.*, 1990); diagenetic effects and scatter of δ_p relative to δ_w in elephants (*Loxodonta sp.*, *Elephas sp.* and *Mammuthus sp.*) (Ayliffe *et al.*, 1992, 1994); scatter in bovine (*Bos taurus*) data from different continents and a red deer (*Cervus elaphus*) δ_p/δ_w slope which is greater than the theoretical maximum (D'Angela and Longinelli, 1990); δ_p controlled almost entirely by relative humidity in Australian macropods (Ayliffe and Chivas, 1990) and very substantial scatter in predicted δ_p relationships with physical characteristics of the animals (Bryant and Froelich, 1995). While it is often possible to identify such anomalous behaviour in modern samples, this is probably not possible with single species studies of ancient material. For example, the Teotihuacan and Oaxacan δ_p relationships cannot be interpreted unless the weather patterns and their relationship with the topography are understood. If a sequence of bones of a genus of deer from a cave site were examined, it would be extremely difficult to determine whether an apparent warm period resulted from actual warming or from a change in the dominant winds that carried most of the moisture or simply changes in relative humidity.

The current trend among workers is to obtain a $\delta_p/\delta_{mw}/T$ relationship for a single species or genus that can be applied to the interpretation of an ancient environment. This is equivalent to early pollen research where characteristic species were identified and used to make environmental interpretations. As the study of oxygen isotopes in mammal bones comes to maturity, workers must be prepared to process very much larger sample databases and to work with assemblages of animals, not a single genus or species. These assemblages should

include the widest possible range of responses to changing δ_{mw} available in an area that has a relatively uniform δ_{mw} , spanning a range from water independent rodents like desert mice to large aquatic mammals. A typical assemblage from southern Ontario would include bats (*Chiroptera*), beaver (*Castor canadensis*), muskrat (*Ondatra sp.*), mouse (*Cricetidae*), groundhog (*Marmota monax*), porcupine (*Erethizon dorsatum*), hare (*Leporidae*), white-tailed deer (*Odocoileus virginianus*), bison (*Bison bison*), racoon (*Procyon lotor*), skunk (*Mephitis mephitis*), wolves and coyotes (*Canidae*), snapping turtle (*Chelydra serpentina*), map turtle (*Graptemys sp.*), some larger fish (deep and shallow water), and a variety of birds ranging from small seed-eaters to large raptors and water-fowl. The choice of animals is made on the basis of obtaining the widest possible selection of habitats, with emphasis on those most likely to appear on sites with human remains. Wherever possible adult teeth should be obtained, as well as bone from extant animals and ones which are now scarce or extinct in the area. Introduced animals, such as rats (*Rattus sp.*) in southern Ontario, are also perfectly acceptable members of an assemblage. Reptiles and fish may have more variable responses due to fluctuating body temperatures, but if environmental temperature can be calculated from other animals then they may provide invaluable information about δ_w of local waterways. In addition they will provide a greater span of δ_p to the assemblage and by refining estimates of δ_w may assist with calculation of relative humidity in the model. Initially the data from assemblages will be difficult to interpret, but as the number of sampled assemblages increases, patterns and relationships between the members will begin

to emerge. The use of assemblages will help to identify inconsistent responses by particular species as their relative position within the δ_p/δ_{mw} space will change. If ecological niches are more constant than the species that fill them (aquatic rodents, seed-eating birds, small carnivores, browsing deer *etc.*) then assemblages will continue to be useful even in past periods when the behaviour of the organisms cannot be exactly ascertained. Similarly, diagenetic effects will become apparent by changes in the internal relationships of the members of the assemblage. Sophisticated modelling of δ_p/δ_{mw} relationships could be combined with assemblage data to improve the precision of the results. If modern assemblage examples include a sufficiently wide variety of representatives of ecological niches, then subsets from archaeological or palaeontological sites can be placed confidently within them.

REFERENCES

- Allison, G.B. and Hughes, M.W. ,1983. The use of natural tracers as indicators of soil-water movement in a temperate semi-arid region. *J. Hydrol.* **60**: 157-173
- Anbar, M., Halmann, M. and Silver, B. (1960) Determination of oxygen-18 in phosphate ion. *Anal. Chem.* **32**: 841-842
- Ayliffe, L.K. and Chivas, A.R. (1988) Australian Macropods (kangaroos and wallabies): Palaeoenvironmental potential of bone phosphate oxygen isotopes. *Chem. Geol.* **70**: 114
- Ayliffe, L.K. and Chivas, A.R. (1990) Oxygen isotope composition of the bone phosphate of Australian kangaroos: Potential as a palaeoenvironmental indicator. *Geochim. Cosmochim. Acta* **54**: 2603-2609
- Ayliffe, L.K., Lister, A.M. and Chivas, A.R. (1992) The preservation of glacial-interglacial climatic signatures in the oxygen isotopes of elephant skeletal phosphate. *Palaeogeog. Palaeoclimat. Palaeoecol.* **99**: 179-191

Ayliffe, L.K., Chivas, A.R. and Leakey, M.G. (1994) The retention of primary oxygen isotope compositions of fossil elephant skeletal phosphate. *Geochim. Cosmochim. Acta* **58**: 5291-5298

Barrick, R.E. and Showers, W.J. (1994) Thermophysiology of *Tyrannosaurus rex*: evidence from oxygen isotopes. *Science* **265**: 222-224

Barrick, R.E., Fischer, A.G., Kolodny, Y., Luz, B. and Bohaska, D. (1992) Cetacean bone oxygen isotopes as proxies for Miocene ocean composition and glaciation. *Palaios* **7**: 521-531

Baxter, G.P. and Jones, G.J. (1910) A revision of the atomic weight of phosphorus. First paper - the analysis of silver phosphate. *J. Am. Chem. Soc.* **32**: 298-318

Blackwell, B., Saunders, J.J., Woodman, N., Curry, B.B. and Schwarcz, H.P. (1995) Dating the Sangamon interglacial near the type locality: electron spin resonance (ESR) dating mammal teeth and ear scales from Hopwood Farm, southern Illinois. *in press*.

Brodskii, A.I. and Sulima, L.V. (1953) Isotope exchange of oxygen in solutions of Phosphoric Acids. *Dokl. Akad. Nauk* **92**: 589-593 (in russian)

Bryant, J.D. and Froelich, P.N. (1995) A model of isotope fractionation in body water of large mammals. *in press*.

Bryant, J.D., Luz, B. and Froelich, P.N. (1994) Oxygen isotopic composition of fossil horse tooth phosphate as a record of continental paleoclimate. *Palaeogeog. Palaeoclim. Palaeoecol.* **107**: 303-316

Cerling, T.E., 1984. The stable isotopic composition of modern soil carbonate and its relationship to climate. *Earth Planet Sci. Lett.* **71**: 229-240

Cohn, M. (1957) O^{18} containing phosphate and related compounds. *Methods in enzymology* **4**: 905-914.

Coles, B. (1992) Further thoughts on the impact of beaver on temperate landscapes *in* *Alluvial archaeology in Britain*. Ncedham, S. and Macklin, M.G. (Eds.) Oxbow books, Oxford. pp 93-99

Cormie, A.B., Luz, B. and Schwarcz, H.P. (1994) Relationship between the hydrogen and oxygen isotopes of deer bone and their use in the estimation of relative humidity. *Geochim. Cosmochim. Acta* **58**: 3439-3449

Cowgill, G.L., 1992. Toward a political history of Teotihuacan. In A. Demarest and G. Conrad (Editors), *Ideology and pre-Columbian civilizations*. School of American research press, Santa Fé, New Mexico

Crowson, R. A., Showers, W.J., Wright, E.K. and Hoering, T.C. (1991) Preparation of phosphate samples for oxygen isotope analysis. *Anal. Chem.* **63**, 2397-2400

D'Angela, D. and Longinelli, A. (1990) Oxygen isotopes in living mammal's bone phosphate: Further results. *Chemical Geology (Isotope Geoscience Section)* **86**: 75-82.

Dansgaard, W. (1954) The O^{18} -abundance in fresh water. *Geochim. Cosmochim. Acta* **6**: 241-260

Dansgaard, W., (1964) Stable isotopes in precipitation. *Tellus* **16**: 436-468

Emry, R.J. (1972) A new species of *Agnotocastor* (Rodentia, Castoridae) from the early Oligocene of Wyoming. *Am. Mus. Novitates* **2485**: 1-7

Firsching, F. (1961) Precipitation of silver phosphate from homogeneous solution. *Anal. Chem.* **33**: 873-887

- Flicoteax, R. and Lucas, J., 1984. Weathering of phosphate minerals. In Nriagu, J.O. and Moore, P.B. (Editors), Phosphate minerals. Springer-Verlag, Heidelberg. pp 292-317
- Fontes, J.-Ch., Boulange, B., Carmmouze, J.P. (1979) Preliminary oxygen-18 and deuterium study of the dynamics of Lake Titicaca *in* Isotopes in lake studies. Panel Proceedings series. IAEA, Vienna. pp 145-150
- Fritz, P. (1981) River waters *in* Stable isotope hydrology: Deuterium and oxygen-18 in the water cycle. Gat, J.R. and Gonfiantini, R. (eds.) IAEA (Vienna) pp 177-201
- Fritz, P., Drimmie, R.J., Frape, S.K. and O'Shea, K. (1987) The isotopic composition of precipitation and groundwater in Canada *in* Isotope techniques in water resources development. Proceedings series. IAEA, Vienna. pp 539-550
- Gard, D.R., Burquin, J.C. and Gard, J.K. (1992) Quantitative analysis of short-chain phosphates by phosphorus-31 nuclear magnetic resonance and interlaboratory comparison with infrared and chromatographic methods. Anal. Chem. 64: 557-561
- Gat, J.R. (1981) Lakes *in* Stable isotope hydrology: Deuterium and oxygen-18 in the water cycle. Gat, J.R. and Gonfiantini, R. (eds.) IAEA (Vienna) pp 203-221

Gerke, J. (1993) Phosphate adsorption by humic/Fe-oxide mixtures aged at pH 4 and 7 and by poorly ordered Fe-oxide. *Geoderma* 59: 279-288

Gillard, R.D., Pollard, A.M., Sutton, P.A. and Whittaker, D.K., 1990. An improved method for age at death determination from the measurement of D-aspartic acid in dental collagen. *Archaeometry* 32(1): 61-70

Graham, R.W. and Lundelius, E.L. Jr. (1984) Coevolutionary disequilibrium and Pleistocene extinction *in* Martin, P.S. and Klein, R.G. (Eds.) *Quaternary extinction: a prehistoric revolution*. Univ. of Arizona Press, Tucson. p 228.

Guilbert, J.M. and Park, C.F. Jr., 1986. *The geology of ore deposits*. W.H. Freeman and Co., New York. pp 910-921

Hostetler, P.B. and Garrels, R.M., 1962. Transportation and precipitation of uranium and vanadium at low temperatures, with special reference to sandstone type uranium deposits. *Econ. Geol.* 57: 226-237

Huertas, A.D., Iacumin, P., Stenni, B., Sánchez Chillón, B. and Longinelli, A. (1995) Oxygen isotope variations of phosphate in mammalian bone and tooth enamel. *Geochim. Cosmochim. Acta* 59: 4299-4305

- IAEA (1969) Environmental isotope data No. 1: World survey of isotope concentration in precipitation (1953 - 1963). Technical reports series no. 96. International atomic energy agency. Vienna
- IAEA (1970) Environmental isotope data No. 2: World survey of isotope concentration in precipitation (1964 - 1965). Technical reports series no. 117. International atomic energy agency. Vienna
- Karhu, J. and Epstein, S. (1986) The implications of the oxygen isotope records in co-existing cherts and phosphates. *Geochim. Cosmochim. Acta* **50**: 1745-1756
- King, J.E. and Saunders, J.J. (1986) *Geochelone* in Illinois and the Illinoian-Sangamonian vegetation of the type region. *Quat. Res.* **25**: 89-99
- Koch, P.L., Fisher, D.C. and Dettman, D. (1989) Oxygen isotope variation in the tusks of extinct proboscideans: a measure of season of death and seasonality. *Geology* **17**: 515-519
- Kolodny, Y., Luz, B. and Navon, O., 1983. Oxygen isotope variations in phosphate of biogenic apatites: 1. Fish bone apatite-rechecking the rules of the game. *Earth Planet Sci. Lett.* **64**: 398-404

- Koutsoukos, P.G. and Nancollas, G.H. (1986) The adsorption of inorganic phosphate by collagen. *Colloids and Surfaces* 17: 81-90
- Kurten, B. (1968) *Pleistocene mammals of Europe*. Weidenfeld and Nicolson, London. p 197
- Kurten, B. and Anderson, E. (1980) *Pleistocene mammals of North America*. Columbia University press, New York. pp 236-238
- Lécuyer, C., Grandjean, P., O'Neil, J.R., Cappetta, H. and Martineau, F. (1993) Thermal excursions in the ocean at the Cretaceous-Tertiary boundary (northern Morocco): $\delta^{18}\text{O}$ record of phosphatic fish debris. *Palaeogeog. Palaeoclim. Palaeoecol.* 105: 235-243
- LeGeros, R.Z. (1981) Apatites in biological systems. *Prog. Crystal Growth* 4: 1-45
- LeGeros, Racquel Z. and LeGeros, John P. (1984) Phosphate minerals in human tissues *in* *Phosphate minerals*. Nriagu, J.O. and Moore, P.B. (Eds.). Springer-Verlag, Heidelberg. pp 351-385
- Levinson, A.A., Luz, B. and Kolodny, Y., 1987. Variations in oxygen isotopic compositions of human teeth and urinary stones. *Applied Geochem.* 2: 367-371

- Longinelli, A. (1965) Oxygen isotopic composition of orthophosphate from shells of living marine organisms. *Nature* **207**: 716-719
- Longinelli, A. (1984) Oxygen isotopes in mammal bone phosphate: A new tool for paleohydrological and paleoclimatological research? *Geochim. Cosmochim. Acta* **48**: 385-390
- Longinelli, A. and Nuti, S., 1973*a*. Revised phosphate-water isotopic temperature scale. *Earth Planet Sci. Lett.* **19**: 373-376
- Longinelli, A. and Nuti, S., 1973*b*. Oxygen isotope measurements of phosphate from fish teeth and bones. *Earth Planet Sci. Lett.* **20**: 337-340
- Lowenstam, H.A. and Weiner, S. (1989) *On biomineralization*. Oxford University Press, New York.
- Luz, B., 1992. Oxygen isotopes in bone apatite. Address at the 52nd meeting of the Society of Vertebrate Paleontology, Royal Ontario Museum, Toronto.
- Luz, B. and Kolodny, Y. (1985) Oxygen isotope variations in bone phosphate of biogenic apatites.IV. Mammal teeth and bones. *Earth Planet Sci. Lett.* **75**: 29-36

Luz, B. and Kolodny, Y. (1989) Oxygen isotope variation in bone phosphate.

Applied Geochemistry 4: 317-323

Luz, B., Kolodny, Y. and Horowitz, M. (1984) Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental drinking water.

Geochim. Cosmochim. Acta 48: 1689-1693

Luz, B., Cormie, A. & Schwarcz, H. (1990) Oxygen isotope variations in phosphate of deer bones. *Geochim. Cosmochim. Acta* 54: 1723-1728

Martin, P.S. (1967) Prehistoric overkill *in* Martin, P.S. and Wright, H.E. (Eds)

Pleistocene extinction: the search for a cause. Vol. 6. Proc. 7th Congr. Int.

Assoc. Quaternary Res. Yale University Press, New Haven. p 80.

Martin, P.S. and Guilday, J.E. (1967) A bestiary for Pleistocene biologists *in*

Martin, P.S. and Wright, H.E. (Eds) Pleistocene extinction: the search for a cause. Vol. 6. Proc. 7th Congr. Int. Assoc. Quaternary Res. Yale University

Press, New Haven. p 25.

Matsuo, S., Kusakabe, M., Niwano, M., Hirano, T. and Oki, Y. (1979) Water budget in the Hakone Caldera using hydrogen and oxygen isotope ratios *in*

Isotopes in lake studies. Panel Proceedings series. IAEA, Vienna. pp 131-144

McArthur, J.M. and Herczeg, A., 1990. Diagenetic stability of the isotopic composition of phosphate-oxygen: palaeoenvironmental implications. In Northolt, A.J.G. and Jarvis, I. (Editors), Phosphorite research and development. Geological Society special publication 52: 23-31

Mook, W.G. (1970) Stable carbon and oxygen isotopes of natural waters in the Netherlands. Isotope hydrology 1970 (Proc. Symp. Vienna, 1970), IAEA, Vienna. pp 163-189

Mooney-Slater, R.C.L. (1962) Polymorphic forms of bismuth phosphate. Zeitschrift für Kristallographie 117: 371-385

Newell, R.E., Kidson, J.W., Vincent, D.G. and Boer, G. J., 1972. The general circulation of the tropical atmosphere and interactions with extratropical latitudes. Vol. 1. The MIT Press. Cambridge, Massachusetts. pp 122-123

Novak, M. (1987) Beaver *in* Wild furbearer management and consevation in North America. Novak, M., Baker, J.A., Obbard, M.E. and Malloch, B. (Eds.), The Ontario Trappers Association with the Ontario Ministry of Natural Resources. pp 283-312

Okazaki, M., Takahashi, J., Kimura, H. and Aoba, T. (1982) Crystallinity, solubility, and dissolution rate behavior of fluoridated CO₃ apatites. *J. Biomed. Mat. Res.* **16**: 851-860

O'Neil, J.R., Roe, L.J., Reinhard, E. and Blake, R.E. (1994) A rapid and precise method of oxygen isotope analysis of biogenic phosphate. *Isr. J. Earth Sci.* **43**: 203-212

Posner, A.S., Blumenthal, N. C. and Betts, F., 1984. Chemistry and structure of precipitated hydroxyapatites. In Nriagu, J.O. and Moore, P.B. (Editors), *Phosphate minerals*. Springer-Verlag, Heidelberg. pp 330-350

Sánchez Chillón, B., Alberdi, M.T., Leone, G., Bonadonna, F.P., Stenni, B. and Longinelli, A. (1994) Oxygen isotopic composition of fossil equid tooth and bone phosphate: an archive of difficult interpretation. *Palaeogeog. Palaeoclim. Palaeoecol.* **107**: 317-328

Schultz, G.E. (1967) Four superimposed Late-Pleistocene vertebrate faunas from southwest Kansas *in* Martin, P.S. and Wright, H.E. (Eds) *Pleistocene extinction: the search for a cause*. Vol. 6. Proc. 7th Congr. Int. Assoc. Quaternary Res. Yale University Press, New Haven.

Schwarcz, H.P., Gibbs, L. and Knyf, M. (1991) Oxygen isotopic analysis as an indicator of place of origin *in* Snake Hill: An investigation of a military cemetery from the war of 1812. Pfeiffer, S. and Williamson, R.F. (*Eds.*). pp 263-268

Shemesh, A. (1990) Crystallinity and diagenesis of sedimentary apatites.

Geochim. Cosmochim. Acta 54, 2433-2438

Shemesh, A., Kolodny, Y. and Luz, B. (1988) Isotope geochemistry of oxygen and carbon in phosphate and carbonate of phosphorite francolite. *Geochim.*

Cosmochim. Acta 52: 2565-2572

Siegenthaler, U., Oeschger, H. and Tongiorgi, E. (1970) Tritium and Oxygen-18 in natural water samples from Switzerland *in* Isotope hydrology 1970 (proc. Symp. Vienna, 1970) IAEA, Vienna, pp 373-384

Spekkens, P. H. (1977) Oxyfluoro compounds of bromine. Ph.D. thesis, McMaster Univ.

Spence, M.W. (1992) Tlailotlacan, a Zapotec enclave in Teotihuacan *in* Berlo, J.C. (Ed.) Art, ideology, and the city of Teotihuacan. Dunbarton Oaks Research Library and Collection, Washington, D.C.

Stuart-Williams, H. Le Q. and Schwarcz, H.P. (1993) Oxygen isotopic environmental information showing possible seasonal variation in large rodent incisors. GSA abstracts, Annual meeting, Boston.

Stuart-Williams, H. Le Q. and Schwarcz, H.P. (1995) Oxygen isotopic analysis of silver orthophosphate using a reaction with bromine. *Geochim. Cosmochim. Acta* 58(18): 3837-3841

Stuart-Williams, H. Le Q. and Schwarcz, H.P. (1995b) Processes controlling the post-burial oxygen isotopic composition of bone and other phosphates. GSA abstracts, Annual meeting, New Orleans.

Stuart-Williams, H. Le Q., Schwarcz, H.P., White, C.D. and Spence, M.W. (in review) The isotopic composition and diagenesis of human bone from Teotihuacan and Oaxaca, Mexico

Stuart-Williams, H. Le Q. and Schwarcz, H.P. (in prep) A new method for purifying biogenic phosphates for oxygen isotopic analysis.

Stuart-Williams, H. Le Q. and Schwarcz, H.P. (in prep) Oxygen isotopic determination of climatic variation using phosphate from beaver bone, tooth enamel and dentine.

Tamayo, J. L., 1962. Atlas geografico general de Mexico. Instituto de investigaciones económicas, Mexico D.F.

Taylor, H.P. Jr., 1974. The application of oxygen and hydrogen isotope studies to problems of hydrothermal alteration and ore deposition. *Econ. Geol.* **69**: 843-883

Tudge, A.P. (1959) A method of analysis for oxygen isotopes in orthophosphate. Ph. D. Thesis, Univ. Chicago

Tudge, A.P. (1960) A method of analysis of oxygen isotopes in orthophosphate - its use in the measurement of paleotemperatures. *Geochim. Cosmochim. Acta* **18**: 81-93

Tuross, N., Behrensmeier, A.K., Eanes, E.D., Fisher, L.W. and Hare, P.E., 1989. Molecular preservation and crystallographic alteration in a weathering sequence of wildebeest bones. *Applied Geochem.* **4**: 261-270

Weist, R.C. (Editor) (1985) CRC handbook of chemistry and physics. 66th Edition. CRC Press Inc. Boca Raton, Florida

Wright, E. and Hoering, T. (1989) Separation and purification of phosphates for oxygen isotope analysis. Annual report of the Carnegie Institution Geophysical Laboratory. pp 137-141.

Wright, L.E. and Schwarcz, H.P., (Submitted). Infrared evidence for diagenesis of bone apatite as Do: Pilas, Guatemala: paleodietary implications.

Yoshida, N. and Miyazaki, N. (1991) Oxygen isotope correlation of cetacean bone phosphate with environmental water. J. Geophys. Res. (Oceans) 96: 815-820

Yurtsever, Y. and Gat, J.R., 1981. Atmospheric waters. In Gat, J.R. and Gonfiantini, R. (Scientific editors), Stable isotope hydrology: Deuterium and oxygen-18 in the water cycle. IAEA (Vienna)