STABLE CARBON ISOTOPES IN DEEP TIME

The diets of fossil fauna and hominids

Julia Anne Lee-Thorp

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ABSTRACT

This thesis describes the development, testing and application of a technique for extending carbon isotopic dietary tracing millions of years in time, using the mineral phase of calcified tissues (apatite) as alternative sample material to collagen. The results reported here provide empirical evidence for the validity of the technique.

Investigation of the isotopic relationships between diet, collagen and apatite, using a large sample of modern fauna with known diets, confirms that the isotopic relationship between collagen and apatite changes with trophic level. Thus, both collagen and apatite reflect dietary $^{13}C/^{12}C$ ratios, but the results support the hypothesis that different aspects of diet - protein and energy, respectively - are reflected by these tissues. Isotopic analyses of apatite in archaeological dietary studies have been controversial due to argument about the effects of diagenesis on the $^{13}C/^{12}C$ ratios. A comparison of apatite $^{13}C/^{12}C$ ratios from a chronological series of fossil browsers with those of modern browsers reveals a difference between the two which may largely be attributed to diagenesis. This difference increases predictably with time and cannot be eliminated by an acid pretreatment which removes calcite and a more soluble mineral phase. Despite the alteration, browsers and grazers are clearly distinguishable using enamel $^{13}C/^{12}C$ ratios, even after 3 ma.

The technique is applied to three studies of the diets of extinct taxa. Firstly, the extinct springbok, *Antidorcas bondi*, is shown to have been a grazer, in contrast with the intermediate diet of its living relative, *A. marsupialis*. Secondly, a comparison of two fossil baboons with browsers and grazers from Swartkrans Member 1, dating to approximately 1.8 ma, shows that the C_3-based diet of *Papio robinsoni* is clearly distinct from the graminivorous (C_4) diet of *Theropithecus darti*. Thirdly, results for *Australopithecus robustus* enamel specimens from Swartkrans indicate an intermediate diet, mainly C_3-based, but with a significant C_4 contribution, derived either directly from C_4 grasses or indirectly via animal foods. Thus a comparative approach using $^{13}C/^{12}C$ ratio analysis of enamel offers a significant new tool for obtaining unique information about human and faunal diets of great age.

PREFACE

The subject of this thesis is the exploration of a technique for extending carbon isotopic dietary tracing very much further back in time than has been possible using existing techniques and bone collagen as sample material. It represents a significant new development in dietary studies and is capable of addressing previously intractable problems about the diets of early hominids and humans. Not all the aspects and problems of such a vast subject are addressed in this study, but the results provide empirical support for the method. The work reported here and interpretations of the results are my own, although some of the applied studies were of necessity co-operative projects, involving workers from other disciplines. The problem of *Antidorcas bondi* was the first of the co-operative projects and was suggested by Mr James Brink of the National Museum, Bloemfontein. He provided the fossil specimens from Florisbad, and the broader interpretations which follow from the results are largely his ideas. Dr C. K. Brain, Director of the Transvaal Museum, suggested the comparison of two extinct baboon species from Swartkrans Member 1, *Papio robinsoni* and *Theropithecus darti*, and provided the specimens for this study as well as for the *Australopithecus robustus* study. Some of the material in this thesis has been published or is in press, with appropriate co-authors. The publications are listed below.

Lee-Thorp, J.A. and van der Merwe, N.J 1987. Stable carbon isotope analysis of fossil bone apatite. *S. Afr. J. Sci.* **83**: 712-715.

Lee-Thorp, J.A., van der Merwe, N.J. and Brain, C.K. (1989) Isotopic evidence for dietary differences between two extinct baboon species from Swartkrans. *J. hum. Evol.* (in press).

Lee-Thorp, J.A., Sealy, J.C. and van der Merwe, N.J. (n.d.) Stable carbon isotope differences between bone collagen and bone apatite, and their relationship to diet. *J. archaeol. Sci.* (in press).

In the last paper, the section dealing with isotopic analyses of modern fauna was my own work, and is included in this thesis, while the study of archaeological humans was the work of Ms Judith Sealy.

This project was inspired by a seminal paper by Dr C. H. Sullivan and Mr H. W. Krueger, published in 1981, in which collagen $^{13}C/^{12}C$ ratios were compared with those from apatite for a series of prehistoric fauna and humans. Their conclusions were disputed by Dr Margaret Schoeninger and Dr Michael DeNiro who produced conflicting data. The issue has remained unresolved since this exchange in *Nature*. A more convincing test was necessary. Professor Nikolaas van der Merwe persuaded me to undertake the project, pointing out that if it was successful, the method could be applied to dietary problems as remote in time as the australopithecines. This possibility has remained an objective of the research. Professor van der Merwe supervised the thesis, and I am deeply grateful to him for his unfailing support and advice throughout the project.

In addressing the problem of determining the extent and pattern of isotopic alteration of fossil apatite, I have adopted a new approach. The strategy is essentially a comparison of $^{13}C/^{12}C$ ratios for fossil browsers in a chronological series against a mean range determined for modern browsers, following the principle that alteration should cause the browser-apatite ratios to equilibrate towards those of the matrix. The first step, therefore, was to establish a reliable isotopic data-base for modern browsers. At that stage, little was known about carbon isotopes in apatite and their relationship to diet, and it soon became necessary to expand the data-base to include a range of modern fauna, with animals from different trophic

levels. Much of this first sample set was obtained from museums and national parks and I thank all who contributed material. Dr Stanley Ambrose donated specimens from his Rift Valley study in Kenya, as well as providing encouragement and advice. Mrs Elizabeth Voigt and Dr Ina Plug of the Transvaal Museum, Mr P. Swanepoel of the Kaffraria Museum, Dr H. Opperman of the University of Fort Hare, the Natal Parks Board, and Mr Peter Beaumont of the McGregor Museum contributed to the modern browser collection. In particular I thank Dr Richard Bell, formerly Senior Research Officer at Kasungu National Park, Malawi, who provided a large and varied sample of modern fauna from Kasungu and elsewhere in Malawi. A large set of specimens was obtained from the comparative collection at the Bernard Price Institute for Palaeontology at the University of the Witwatersrand, and I thank Professor James Kitching, Acting Director of the Institute, as well as Dr Judith Maguire for their assistance. Ms Sharma Saitowitz of the University of Cape Town helped me collect the material from the Institute. Dr Brett Hendey, Head of the Department of Palaeontology at the South African Museum and Dr Peter Norton of the Cape Department of Nature Conservation kindly donated carnivore specimens. Dr Ed Wilmsen of the University of Boston sent material from Botswana, and Mr Leon Jacobsen of the Windhoek Museum sent some samples from Namibia. Ms Judith Sealy allowed me to analyse many of the specimens she had collected from the southwestern Cape.

The next step was the crucial test: the analysis of a chronological series of browsers. Since no single site provided an uninterrupted series, a "sequence" had to be constructed from several sites. Mr Timothy Robey and Dr Timothy Maggs, Natal Museum, permitted the use of material from Mpambanyoni, an early Iron Age site in Natal. Dr Margaret Avery of the South African Museum provided faunal samples from two coastal sites in the Cape Province, namely Die Kelders and Klasies River Mouth. Later, Professor H. J. Deacon, Head of the Department of Archaeology at

the University of Stellenbosch kindly allowed me to collect deposit samples from the latter site. Promising results were obtained and Mr Peter Beaumont subsequently permitted analysis of faunal specimens from Border Cave, an inland site of roughly the same age. Mr Graham Avery of the South African Museum kindly identified this material. Older, identified samples were obtained from Makapansgat Member 3 (about 3 ma) and Sterkfontein (Dump samples of indeterminate age) with the help of Dr Judith Maguire and Professor James Kitching from the Bernard Price Institute of Palaeontology. Dr Elizabeth Vrba, formerly Deputy Director of the Transvaal Museum, suggested other extinct browsers from Makapansgat.

For the applied studies, I enjoyed close cooperation with Mr James Brink and Dr C.K. Brain. I thank James Brink for his suggestion of the problem of *Antidorcas bondi* diet, for supplying the fossil and some modern material for analyses, and for sharing his ideas about grassland structures and extinctions with me. Preliminary results for this project were presented jointly at a meeting of the Palaeontological Society of Southern Africa, held in Cape Town in 1986. I am particularly grateful to Dr C.K. (Bob) Brain for his prominent role in the applied studies. He supplied all the fossil faunal material from Swartkrans, including the baboons, *Antidorcas bondi* specimens from Member 5, and in addition he permitted the analysis of some *Australopithecus robustus* tooth fragments.

Various people assisted me in the experimental section of the study. I am grateful to Mr Harold Krueger of Geochron Laboratories and Dr Stanley Ambrose of the University of Illinois, for their encouragement and advice on chemical procedures. Dr Michael DeNiro kindly allowed me to work in his laboratory for a short period. Professor Dave Thornton of the Department of Chemistry at the University of Cape Town (who provided much of my earlier education in Chemistry) advised me on infrared spectroscopy and permitted the use the infrared spectrophotometer in the Department of Chemistry. Mr Philip Hall kindly showed me how to use it. I am

grateful for the encouragement and advice on the subject of apatites and infrared spectroscopy from Professor Raquel LeGeros, Director of the Laboratory for Calcium Phosphate Research, College of Dentistry, University of New York.

I would like to thank my teachers, colleagues and fellow students in the Archaeology Department at the University of Cape Town who have helped me along the way. In particular I thank Mr John Lanham, Dr Andrew Sillen, Ms Judith Sealy and Dr Francis Thackeray for their ideas and comments. Dr Sillen and Dr Thackeray commented on earlier drafts of this thesis, and Dr Thackeray kindly helped me with statistical tests. John Lanham provided a great deal of technical and computer assistance. Finally, I thank my husband, Patrick Lee-Thorp, without whose support I could not have completed this thesis.

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CHAPTER 1

INTRODUCTION

This thesis reports the development of a method for studying the diets of humans and animals of great antiquity by means of isotopic analysis of fossil bone and teeth. This has been accomplished using stable carbon isotope analysis of the mineral phase of calcified tissues (apatite), in contrast to the usual sample material, bone collagen. Progress in this direction has been hampered by controversy about the effects of diagenesis on the biogenic $^{13}C/^{12}C$ ratios in fossil apatite. In this study, however, it is shown that these problems may be circumvented to such an extent that differing contributions of C_3 and C_4 foods to the diet may be recognised, even for fossils millions of years of age.

APPROACHES TO DIET

Diet has always been an important area of study in archaeology, because it is so closely related to subsistence patterns and lifestyle in all human populations, past and present. What people eat, and how they exploit the foods available to them, sets effective limits on how they can live. The nutritional adequacy of the diet further affects the health and stature of individuals and populations. Diet and dietary differences amongst the earliest members of the human family have been recognised as crucial elements in hominid evolution, phylogeny and the relationships between contemporaneous species, and have been the subject of numerous investigations and lively debate for many years. The possibility of developing stable carbon isotopic methods as a more direct means of addressing the all-important question of hominid diet has provided the impetus for the study reported in this thesis.

There have been many approaches to the reconstruction of the dietary patterns of our ancestors. The further back in time we delve, the more tenuous the clues become, so that for our earliest forbears, essentially the only direct evidence lies in the fossils themselves. The various approaches for addressing questions about diet fall within the general ambits of traditional archaeology, physical anthropology and chemical or physical analysis of skeletal material. Each approach suffers from a particular set of problems related to assumptions and interpretation. In the traditional archaeological approach, taphonomic problems are an essential element to be considered in the interpretation of food remains recovered from sites. Such debris may be misleading for many reasons. These include the problem of agency, since various animals may be responsible for the accumulation of food debris. Birds may bring in seeds and fruits, and carnivores (e.g. hyenas and leopards) as well as porcupines accumulate bones (Brain 1975, 1980, 1981). This problem is compounded in older sites where faunal bones predominate over less durable material, particularly plants. Finally, the debris represents what was discarded, rather than what was eaten.

The second approach relates sets of dental and masticatory features to broad dietary categories (e.g. frugivory, carnivory) by analogy with modern species (e.g., Kay 1985). Considerable difficulties arise in the choice of morphological features for analysis (i.e., which are diagnostic) and the selection of suitable modern analogues, hence the results are often conflicting or ambiguous and difficult to interpret. Examination of toothwear, either at the macroscopic or microscopic level, may provide some indication of features such as grittiness or toughness of food objects (Wallace 1975). Scanning electron microscopic analysis of occlusal tooth surfaces has provided useful indications about hominid diets (Grine 1981, 1986; Grine & Kay 1988; Walker 1981). This technique is, however, limited to inferences about the consistency of foods from observations of the effects of chewing such substances on

enamel surfaces. For instance grass-eating produces characteristic fine scratches (Walker *et al* 1978), while very hard items cause pitting (Grine 1986). In sum, physical anthropological studies offer some insights into prehistoric human and early hominid diets (reviewed in Walker 1981), but humans (and indeed many other primates) are generalists and omnivores, and therefore dietary diagnosis from physical characteristics is very difficult.

DIETARY RECONSTRUCTION BASED ON STABLE CARBON ISOTOPES

The application to prehistoric dietary reconstruction of various kinds of chemical analyses of actual skeletal material may provide a means of filling some of the gaps. The main advantage is that dietary information recorded in the bones and teeth may be obtained by direct analysis of skeletal material and quantitative, repeatable values are obtained. Constraints common to these techniques include diagenesis or chemical alteration of the original material after deposition, and problems of interpreting the measurements in dietary terms. The most commonly applied techniques in this field are undoubtedly stable isotope ratio (carbon and nitrogen) and trace element (mainly strontium) analysis. Stable carbon isotope analysis is the best understood of these systems, and has been widely applied (for a review see van der Merwe 1982). Until recently, however, applications have been limited to more recent (subfossil) material because of the limited longevity of bone collagen, the tissue usually preferred for $^{13}C/^{12}C$ ratio measurements. The main focus of this thesis concerns an investigation into the use of bone or tooth mineral as an alternative sample material, thus allowing the extension of such studies much further back in time than has been hitherto possible using conventional collagen-based techniques.

Briefly, the principles of dietary reconstruction based on stable carbon isotope measurements are as follows. Two major groups of plants follow different photosynthetic pathways, known as C_3 (described by Calvin & Benson 1948) and C_4 (Kortschak *et al* 1965; Hatch & Slack 1970) respectively, in which the heavier isotope (^{13}C) is discriminated against (Park & Epstein 1960, 1961). This fractionation is greater for plants following the C_3 pathway, which are thus isotopically lighter than those following the C_4 pathway, and therefore the two groups are isotopically distinct (Bender 1968, 1971; Smith & Epstein 1971). These values are passed along the food chain to the various animal and human consumers, with some further fractionation at each step (Vogel 1978; DeNiro & Epstein 1978a). Trees, most shrubs and temperate grasses are C_3, while savanna grasses are C_4. Thus, in most African savanna environments, measurement of the tissues of the consumers will indicate whether the diet was C_3-based (browsers) or C_4-based (grazers), or a mixture of the two (Vogel 1978; DeNiro & Epstein 1978b). In the reconstruction of prehistoric human diets, these principles have been widely applied to demonstrate, for instance, the introduction of a C_4 domesticate into a temperate zone (Vogel & van der Merwe 1977; van der Merwe & Vogel 1978), or dietary differences amongst agriculturalists, pastoralists and hunter-gatherers in the same area (Ambrose & DeNiro 1986).

In most studies to date, the preferred tissue has been collagen, the main component of the organic phase of bone. The advantage is that isotopic alteration of pure intact collagen is unknown (Hassan 1975; Hassan & Hare 1978; DeNiro 1985), but the disadvantage is that collagen hydrolyses and eventually disappears. Heat and water accelerate this process (Hare 1980), so that in warm environments, collagen rarely survives in usable quantities for more than 10 ka. In tropical climates, collagen may not even survive a few thousand years. Thus important transition periods in human evolution remain completely out of reach using present collagen-based methods.

These include the first appearance of anatomically modern humans at about 100 ka, the emergence of *Homo* at about 1.5-2 ma, and even further back in time, australopithecine divergence at 2-5 ma.

The problem is, therefore, whether one can develop reliable methods for obtaining stable carbon isotope measurements from much older samples. There are two possible avenues to the solution of this problem. One is the development of micro-techniques for analysis of traces of biogenic bone protein residues. The difficulties of isolating such residues in material of the order of millions of years of age (if they do survive at all) and measuring their isotope ratios are enormous. This avenue is at present technologically remote and is not discussed further here. The other possibility - the avenue explored in this thesis - is the use of the bone or tooth mineral as sample material. The carbonate content of biogenic mineral reflects carbon isotope values in the diet (DeNiro & Epstein 1978a, 1978b; Land *et al* 1980; Sullivan & Krueger 1981; Lee-Thorp 1983; Krueger & Sullivan 1984), and the mineral phase has the advantage of lasting much longer than collagen (under normal burial conditions). The main problem with the use of mineral as sample material is diagenesis, since chemical alteration may alter the biogenic isotopic ratios to such an extent that they are no longer useful as dietary indicators.

Diagenesis is the reason why isotopic analysis of the mineral phase has been largely ignored. It is known that apatite undergoes some structural changes during fossilisation: one obvious characteristic is an increase in crystallinity, as shown by X-ray diffraction and infra-red studies (Perinet 1957; Hassan 1975; Hassan *et al* 1977; Sillen 1989). Another is the incorporation of ions, such as fluoride, carbonate, strontium and uranium from the deposit (Oakley 1964; Hassan 1975; Parker & Toots 1970; McConnell 1973; Sillen 1989). Early radiocarbon studies showed that dates on whole bone or apatite were unreliable, compared with those on extracted

collagen, a problem presumed to be the result of exchange with soil (bi)carbonates or CO_2 (Berger *et al* 1964; Tamers & Pearson 1965; Sellstedt *et al* 1966). This has led to the assumption that diagenesis also renders stable carbon isotope measurements from bone and tooth mineral so unreliable as to be useless (DeNiro & Epstein 1978a, 1978b; Schoeninger & DeNiro 1982, 1983), a view somewhat akin to "guilty until proven innocent".

Although the radiocarbon data demonstrate that some exchange of diagenetic for biogenic carbonate occurs, it should be noted that radiocarbon dating and stable carbon isotope measurements are not entirely comparable. The former is much more sensitive to sample contamination than the latter because ^{14}C is so much rarer than ^{13}C (^{12}C:^{13}C:^{14}C is $100:1:10^{-10}$), and thus a tiny amount of foreign carbon material could cause a large error in the date. Furthermore, it has been shown that radiocarbon dates on mineral may be improved after suitable chemical pretreatment to eliminate amorphous carbonates such as calcite (Hassan 1975; Hassan *et al* 1977). Hassan's study opened up possibilities for using stable carbon isotope analysis of the mineral phase.

Following a suggestion made by Land *et al* (1980), Sullivan & Krueger (1981) devised a test to check the reliability of stable isotope measurements of suitably pretreated bone mineral from older material, by comparing them with collagen values from the same individual. A constant isotopic difference observed between the two tissues for a series of modern and prehistoric faunal and human specimens led them to conclude that apatite isotopic ratios were indeed reliable, providing that the material was properly pretreated. This finding was disputed by Schoeninger & DeNiro (1982), who showed considerable variation between collagen and apatite $\delta^{13}C$ values in their specimens. These two studies were not entirely comparable because the pretreatment procedures were different, and furthermore, it was later

established that the isotopic difference between the two tissues varied with trophic level (Krueger & Sullivan 1984). Although this relationship is valuable because it suggests that apatite $\delta^{13}C$ values may provide complementary evidence about diet to those from collagen, it does mean that this approach is not satisfactory as a test for diagenesis. More importantly, this test may only be applied as far back as collagen survives, which defeats the object of the exercise.

A different approach is used in this thesis: the reliability of $^{13}C/^{12}C$ ratios from mineral carbonate is tested by analysing animals of known diet, in this case browsers, in a chronological series. Measurements on browsers (all C_3 plant consumers, by definition) from various time periods are compared against a mean value and range established from large numbers of modern browsing bovids. All specimens are pretreated in the same way, in order to eliminate the more labile carbonate ions and to extract the 'structural' carbonate ions substituted in the apatite crystals. Any difference that there might be between values for the fossils and those for the modern browsers is assumed to represent largely a diagenetic alteration which cannot be removed by these chemical procedures.

The approach just outlined forms the main focus of the thesis. If the method can be shown to work, it offers many opportunities for assessing the diets of extinct fauna. Before moving on to applications to unknown diets, however, it is also necessary to gain a more comprehensive understanding of the isotopic relationships between diet, collagen and apatite. Although both collagen and apatite are clearly both isotopically related to diet, it has been suggested that apatite reflects dietary fuel, while collagen is related rather to dietary protein (Krueger & Sullivan 1984). The approach used here to test this model has been to measure the stable carbon isotope ratios of both apatite and collagen from a range of modern animals with different diets (trophic levels), and drawn from various environments. Thus an extensive

modern data-base has been built up. Associated studies have been directed at investigating the effects of chemical pretreatment procedures on apatite structure and carbonate ion content, using a combination of isotopic and infra-red techniques. Results for bone apatite were compared with those for tooth enamel, using the same techniques.

APPLICATION TO FOSSILS

With the information gained in the preceding sections, the methodology described in this study has been applied to an investigation of the diets of several extinct species, using isotopic analysis of tooth enamel. The faunal species included *Antidorcas bondi*, a springbok which became extinct about 7 ka ago (Brown & Verhagen 1985). There have been different interpretations of the diet of this species on palaeontological grounds: one proposal suggests an intermediate, tough diet (Vrba 1973), and the other that *A. bondi* was a specialized grazer (Brink 1987). This problem is amenable to solution using stable carbon isotope analysis. Although there is no dispute about the diets of the extinct baboons, *Papio robinsoni* and *Theropithecus darti*, since it is generally assumed that they followed similar dietary patterns to their modern counterparts, *P. ursinus* and *T. gelada* respectively, they are nevertheless useful for demonstrating whether or not stable carbon isotope analysis of tooth enamel can distinguish between the diets of extinct primates occupying distinct niches. If the method can be shown to work for non-hominid extinct primates, there is good reason to apply the same technique to hominids. A culminating part of this thesis concerns the isotopic investigation of the diet of *Australopithecus robustus* from Swartkrans. The apparent dietary specialisations of this hominid have been the subject of study and controversy for many years (for a review, see Grine 1981) and this study is the first application of stable carbon isotope analysis to the problem.

SYNOPSIS

A brief synopsis of the thesis is as follows. Previous research into stable carbon isotope analyses of mineral carbonate and the controversies surrounding this work is reviewed in Chapter 2. In Chapter 3, some aspects of the chemistry of biogenic mineral apatites are discussed, followed by a discussion about diagenesis, particularly as it relates to alteration of the biogenic carbonate isotopic signal. The research strategy for establishing the reliability of isotopic measurements of bone and tooth apatite in Chapter 4, followed by applications using the methodology, is outlined. The isotopic relationships between diet, collagen and apatite carbonate in modern animals are investigated in Chapter 5, and the methods for sample preparation and analysis of the modern specimens are described. The methods, sampling and results for the series of browsers of increasing age are reported in Chapter 6. In Chapter 7, the procedures to eliminate diagenetic material are evaluated for their effects on both fossil and modern specimens. Fossil bone, dentine and enamel apatites are also compared in this chapter. The background, methods and results of the applied studies, which are isotopic investigations of the diets of extinct animals (*Antidorcas bondi, Papio robinsoni* and *Theropithecus darti*), and the hominid species *Australopithecus robustus* are outlined in Chapter 8. Chapter 9 concludes the thesis with a synthesis of the study, and a discussion of its significance for future archaeological and palaeoanthropological research.

CHAPTER 2

THE USE OF MINERAL IN ISOTOPIC DIETARY STUDIES

Carbon isotopes occur in nature in three forms: ^{12}C,^{13}C and ^{14}C with approximate relative abundances of 10^2:1:10^{-10} (Craig 1953). ^{14}C is by far the least abundant and the only radioactive carbon isotope. Since the fractionation for ^{14}C may be shown to be about twice that for ^{13}C (Libby 1955: 8), this led to the use of stable carbon isotope ratios as correction factors for radiocarbon dates on various materials. Stable carbon isotope studies of archaeological materials developed into a separate field, but a tendency to follow on or borrow from developments in the older, more experienced radiocarbon field, remains. This has certainly been the case for isotopic analyses of bone mineral.

The use of the carbonate ions substituted in biological mineral (apatite) as an alternative sample material to collagen has been largely ignored, mainly because unreliable dates were obtained from whole bone or the mineral. This has been translated, not unreasonably, into the stable carbon isotope field. ^{13}C is far more abundant than ^{14}C, however, thus the presence of a small amount of diagenetic carbon will effect the result much less than is the case for ^{14}C, where even minor exchange could produce large errors. To date, however, the tests devised to check the reliability of apatite for isotopic analyses have not been convincing enough to counter the legacy inherited from radiocarbon dating. Both radiocarbon and stable carbon isotope chemists have continued to treat applications using bone mineral as sample material with a great deal of circumspection. In this chapter, the history of the use of mineral in isotopic studies and the controversies surrounding it, are described in detail.

RADIOCARBON AND MINERALOGICAL STUDIES

Very soon after radiocarbon chemists began to date bone directly, in the 1960's, they found that radiocarbon dates on whole bone were unreliable - either too young or too old (Berger *et al* 1964; Tamers & Pearson 1965; Sellstedt *et al* 1966). Dates from purified organic collagen, on the other hand, provided much more reliable dates. The errors were ascribed to exchange of biogenic carbonate within the mineral with atmospheric CO_2 (Tamers & Pearson 1965) or groundwater carbonates (Berger *et al* 1964). One preliminary report appeared to show some promising results for dates from the inorganic phase of bone (Haynes 1968), but a final report did not emerge. Thereafter bone mineral was not used as sample material for some years. During this period most attention was directed at obtaining more reliable dates from bone collagen by improved extraction and purification procedures (e.g., Longin 1971).

There was an important exception. One study demonstrated that radiocarbon dates from both bone collagen and mineral could be much improved if contaminants were carefully removed from the samples (Hassan 1975; Hassan & Ortner 1977; Hassan *et al* 1977). Some contaminants were visible with the naked eye or with the aid of a microscope, such as rootlets and various crystalline substances deposited into the interstices of bone. The main carbonaceous contaminants affecting the mineral phase were carbonates such as calcite. Various physical (removal of crystals, density fractions) and chemical procedures (triammonium citrate and acetic acid) were evaluated for their effects in removing contaminants. A combination of radiocarbon, stable carbon isotopes, X-ray diffraction and infrared techniques were used to characterise the material before and after treatment. It was shown that such methods improved the radiocarbon dates obtained from both the collagenous and mineral phases, although they were not successful for all specimens.

The mineralogical section of this study, using X-ray diffraction and infrared techniques, suggested that mineralogical changes had occurred in the mineral phase

after burial (Hassan *et al* 1977). These varied between specimens, with burial conditions and length of time in the deposit, but in general, the fossil apatites were more crystalline (as shown in the XRD and infrared spectra) and contained more carbonate than modern bone apatite. Increased fluoride content accompanied these changes, leading frequently to recrystallisation into francolite (a high-carbonate, high-fluoride apatite). Hassan suggested that postmortem changes involved removal of biogenic carbonate ions substituted in the crystal surface and hydroxyl positions, and deposition of diagenetic carbonate ions into the phosphate positions, and that chemical pretreatments might weaken the bonds of the diagenetically introduced carbonates.

Other mineralogical studies using X-ray diffraction have also shown that fossil apatite is more crystalline than modern apatite (Perinet 1957; Sillen 1986; Schoeninger 1982) and that various ions may easily be incorporated into the apatite structure (Oakley 1964; Parker & Toots 1970; McConnell 1973; review in Sillen 1989). Some aspects of Hassan's study have not been supported, however. For instance, calcite is not always detectable as a major contaminant, nor does an marked increase in fluoride necessarily accompany fossilisation (Sillen 1989). Hassan's suggestion about removal of carbonate ions from the hydroxyl position and deposition of diagenetic carbonate in the phosphate position during fossilisation, is inappropriate, as indicated by the discussion of the position of carbonate in biological apatites in the following chapter. The findings from mineralogical studies have reinforced the experiences of radiocarbon chemists with bone mineral, and lent support to the idea that diagenesis renders radiocarbon and stable isotopic analyses of apatite meaningless. Although it had been shown that dates on bone apatite could often be improved by proper pretreatment, the degree to which each individual sample had been affected by diagenetic carbonates remained uncertain. This is unacceptable for radiocarbon age determinations, but stable isotopic analysis

does not require quite the same degree of purity, and the implications of Hassan's study for stable carbon isotope analysis of apatite has been somewhat neglected.

STABLE CARBON ISOTOPE STUDIES

Applications of stable carbon isotope analysis to dietary reconstruction in prehistoric human populations were first reported in 1977 (Vogel & van der Merwe 1977). All of the applied studies have used collagen as sample material, although the first laboratory study using animals on controlled diets showed that both collagen and apatite reflect dietary carbon isotope values (DeNiro & Epstein 1978a). DeNiro & Epstein reported a diet-collagen fractionation of between +2.8 and +3.7‰, and a diet-apatite fractionation of +9.5 and 9.8‰ for mice on two different diets[1]. In another study they reported both collagen and apatite isotopic values for two hyrax species in a natural habitat, but cautioned against using apatite for dietary determinations of fossil fauna, citing the radiocarbon studies as evidence for diagenesis (DeNiro & Epstein 1978b).

Land *et al* (1980) reported the results of a study designed to gain information for use in radiocarbon studies. Their isotopic survey of modern North American white-tailed and mule deer showed that both the mineral and apatite phases reflect dietary carbon isotope values, and that the difference between the two is fairly constant, about 7-9 ‰ (Land *et al* 1980). The concentration of CO_2 in the mineral was calculated at about 3.4 wt% CO_2. One modern specimen was analysed before and after acetic acid pretreatment, and the isotopic results seemed to be similar, although the CO_2 yield was reduced. In addition, oxygen isotope values from the carbonate ions in apatite correlated with latitude, or water temperature combined with evaporation rates. These relationships did not hold for the few (n=6) fossil

[1] $^{13}C/^{12}C$ ratios are reported in the δ notation, relative to the P.D.B. standard, as $\delta^{13}C$ (‰) = $(R_s/R_{ref}-1)x1000$, where $R = {}^{13}C/{}^{12}C$.

deer analysed, although XRD data showed that all calcite had been removed by leaching with dilute acetic acid, and wt% CO_2 concentrations were similar (with one exception where it remained high). The authors concluded that exchange with groundwater bicarbonate had taken place, and that isotopic values of apatite, and in some cases collagen too, had altered, rendering them unsuitable for both radiocarbon and palaeoecological analyses.

There are, however, a few problems with some of the data presented by Land *et al* (1980). Many of the specimens have $\delta^{13}C$ collagen values which are unusually negative (i.e. depleted in ^{13}C), compared to values usually obtained for similar animals from temperate regions (i.e. less than -23‰ compared to about -21.5‰). There are two possible explanations for the depleted values. One is that the animals lived in deep forests, where all the $\delta^{13}C$ values in the food-web (including bone collagen of forest animals) are more negative, a phenomenon often described as the "forest canopy effect" (Vogel 1978a; Medina & Minchin 1980; van der Merwe & Medina 1989). This seems unlikely, however, as far as can be ascertained from the sample site locations given (Land *et al* 1980: Table 1). Another more likely explanation is that lipids, which are isotopically depleted in ^{13}C relative to proteins from the same organism, were not removed from the specimens. The unusually depleted fossil collagen values are more difficult to explain, since lipids usually disappear rapidly from exposed or buried bone specimens, and apparently humates (another frequent cause of anomalously depleted collagen values) were removed with sodium hydroxide solution. Of the 6 fossil specimens, 5 are from Texas, and only one (from Venezuela) might have come from a forested environment, and their ages are only given as Holocene or Pleistocene. The inexplicably depleted collagen values leave the impression that there might have been some methodological problems.

Land *et al* (1980) suggested that the relatively constant isotopic spacing between the two phases seen in the modern specimens may be used as a check for establishing the reliability of apatite isotopic values. This idea was further explored by Sullivan and Krueger (1981) to test the reliability of carbon isotope values from apatite. They compared the isotopic values of collagen and apatite for a number of modern and prehistoric specimens, selected to represent a wide variety of environmental and burial conditions. Their prehistoric samples are categorised as archaeological bone (<2000 years old), archaeological bone (>2000 years) and fossil bone (>10 000 years), but individual dates are not indicated. The specimens were all herbivorous animal bones, with the exception of two archaeological human bones. They were pretreated in dilute acetic acid, under vacuum, to remove what the authors called 'normal' carbonates. It is implied that the latter phase includes diagenetic carbonate material in fossils. They found a consistent diet-apatite enrichment of about +13‰, and a diet-collagen enrichment of about 5‰. A consistent isotopic difference of 8‰ between the two phases (the 'isotopic spacing') was found to exist for both the modern and prehistoric specimens, expressed by the relationship

$$\delta^{13}C_{apatite} = \delta^{13}C_{collagen} + 8‰ \qquad (1)$$

Sullivan & Krueger concluded that apatite carbon isotopic values reflected diet as accurately as those from collagen, and that this continued to be the case for older fossil samples. Thus apatite could confidently be used instead of collagen, and isotopic dietary reconstruction could be extended much further back in time, since apatite generally lasts much longer than collagen.

At about the same time, a preliminary study of much older faunal material was reported (Ericson *et al* 1981). Two faunal tooth specimens (a giraffid and an equid) from the Omo Valley, about 2 ma. in age, were analysed for $^{13}C/^{12}C$ ratios. The results were consistent with the inferred diets for each animal: the giraffid tooth yielded a $\delta^{13}C$ value of -11.8‰, and the equid tooth -3.8‰. Sample pretreatment

was reported to be the same as that used by Sullivan and Krueger (1981). The authors translated these values into percentages of C_3 and C_4 plants in the diet, which was perhaps a little ambitious. It is not clear whether 'tooth apatite' referred to enamel or a mixture of enamel and dentine apatite. Unfortunately, no further results were reported, and the results for the two specimens tended to be dismissed as fortuitous. As it turned out, however, the data anticipated later developments.

Schoeninger & DeNiro (1982) disputed the conclusions drawn by Sullivan & Krueger (1981). Consistent isotopic spacings between collagen and apatite were not observed in their archaeological animal and human specimens from six sites in Mexico and Peru, ranging in age from modern to about 20 000 years BP (Schoeninger & DeNiro, 1982: Table 1). The modern specimens, all *Lama pacos* from La Raya, Peru, had collagen and apatite values consistent with dietary predictions. The data points fell close to equation (1), and the difference between the two phases averaged about +7‰. For the archaeological specimens, however, Schoeninger & DeNiro found that the differences between the observed apatite values and those predicted from collagen according to equation (1), ranged between +4.6 and -12.1‰ (Schoeninger & DeNiro 1982). In addition, they suggested that the higher variability of apatite $\delta^{13}C$ values compared to the consistency of collagen values for archaeological human specimens from Tehuacan was further evidence of diagenetic shifts in apatite. The authors concluded from these results that diagenetic exchange processes had shifted the $\delta^{13}C$ values by as much as 12‰, and therefore, carbon isotope analysis of apatite could not reliably be used for dietary reconstruction.

On re-examination of the data, the picture does not look quite so bleak as the authors believed. Schoeninger & DeNiro have adopted a rather circuitous argument by comparing observed apatite $\delta^{13}C$ values with apatite values predicted from collagen $\delta^{13}C$ values, and in doing so, have exaggerated apparent isotopic shifts.

The actual differences between values for the two phases (Δ) vary between the two extremes of -4.1 and +10.2‰. For the fauna, most of the differences were between 7 and 9‰, as expected, although there was some variability (including +5.1‰ for a *Canis diris* and +12.4‰ for a *L. glama*). Of the eleven fossil faunal specimens, Δ seems too large for four (including one specimen where this may be due to an anomalously depleted collagen value). Most of the animal specimens are from the sites of Pachamachay and Wari in Peru (n=1 *Lama glama* each), dated between ≈1 and 4 ka, and San Josecito, Mexico (n=9, various animals), ≈20 ka. For most of the human specimens, Δ was approximately 3-4‰. Two exceptions with unusual negative differences are undoubtedly altered.

In their response, Sullivan & Krueger (1983) suggested a modified model which provided a metabolic explanation for Schoeninger & DeNiro's results (or at least most of their data). Omnivores and carnivores required different models; as later elaborated, the apatite-collagen difference was reduced in higher trophic levels (Krueger & Sullivan 1984). The data for the human specimens from Tehuacan Valley (Schoeninger & DeNiro 1982) make more sense when trophic effects are considered, since the smaller differences between the two phases of bone might indicate high trophic level diets. (Trophic level effects on the differences between $\delta^{13}C$ values for collagen and apatite are investigated in Chapter 5.)

Sullivan & Krueger (1983) doubted that the slightly different pretreatment procedure (Schoeninger & DeNiro used 50:50 v/v acetic acid, much stronger than 1M acetic acid used by the other group) was efficient at removal of 'normal' carbonates. Although it was stated (Schoeninger & DeNiro 1982) that XRD revealed no trace of calcite after treatment (there is no indication that it existed before treatment either), the detection limit is only of the order of 1-5%. Any calcite remaining in the bone would undoubtedly have an effect on the results,

The dispute remained unresolved, with Schoeninger & DeNiro (1983) expressing their disagreement with Sullivan & Krueger's claims that only minimal exchange of the carbonate subsituted in bone apatite was likely. The matter rested after this exchange in *Nature* and nothing much was heard about stable carbon isotope analysis of fossil bone mineral for some time. One exception was a brief treatment by Nelson *et al* (1986) in which the possible effects of diagenesis were investigated using a variety of chemical and isotopic analyses, including $\delta^{13}C$ and $\delta^{18}O$ from bone apatite. The results for $\delta^{13}C$ were inconclusive.

It is clear from the foregoing discussion that so far, tests to determine the reliability (or unreliability, depending on one's point of view) of fossil apatite $\delta^{13}C$ values have been inadequate and inconclusive. Many problems remain unresolved. The nature of the isotopic relationship between diet and apatite, and between apatite and collagen must be established to avoid both misinterpretations of the data and the development of inappropriate tests. Arguments about the most appropriate pretreatment procedures must also be resolved. After reviewing what is known about the chemistry of biological apatites and the patterns of change during fossilisation in the following chapter, a new approach is developed for testing the reliability of $^{13}C/^{12}C$ ratios from biological minerals.

CHAPTER 3

THE CHEMISTRY OF BIOLOGICAL APATITES AND DIAGENESIS

The calcified tissues - bone, tooth enamel and dentine - are products of metabolic processes, so that aspects of diet and disease are reflected in their chemistry and structure. At present isotopic and trace-elemental analytical techniques are amongst the most promising avenues for obtaining palaeodietary and palaeoecological information directly from calcified tissues that occur in archaeological and palaeontological sites. The biological signals which permit the recovery of such information may, however, be obscured or even erased by processes of diagenesis - the complex chemical changes which occur in tissues after death and burial. Indeed, calcified tissues are most often not preserved at all; only certain circumstances of burial permit their preservation as fossils. The chemistry and structure of these tissues, as well as the depositional conditions and length of time in the deposit, will determine the extent and pathways of diagenesis in fossil material. Thus it is important to understand both the chemistry of bone and tooth mineral, in particular where the dietary information is recorded, and the nature of the post-depositional processes. Only then can we evaluate the consequences of diagenesis on the biogenic signals, and develop methods for circumventing these problems.

Calcified tissues consist of two phases. In adult bone, the inorganic (or mineral) phase constitutes about 70% by weight; the organic phase, consisting mainly of a complex fibrous protein called collagen, makes up the rest. Bone, enamel and dentine differ in the proportions of mineral and organic phases (these proportions also vary with the type of bone, i.e., cortical or cancellous), in crystallinity and aspects of the mineral chemistry, and in the type of protein matrix (LeGeros 1983).

In the discussion which follows, I will refer mainly to bone mineral and tooth enamel. The formation and chemistry of the mineral phase, known mainly from *in vitro* and *in vivo* crystallographic and biochemical studies, is briefly described. Particular emphasis is placed on the carbonate ions present in mineral, since it provides the link to dietary $\delta^{13}C$ values. The carbonate ion content also has some important consequences for the crystalline properties of the mineral. Some differences between tooth enamel and bone and dentine apatite are described. Finally, our present knowledge of diagenetic processes is discussed, again with particular emphasis on the affects on the carbonate ion.

CHEMISTRY OF BIOLOGICAL MINERALS

The form and composition of bone mineral has been a subject of intense research and debate for many years, because of its importance in the medical and dental fields. Although many aspects of the chemistry are still being investigated, there is general agreement that bone mineral is a poorly crystalline analogue of hydroxyapatite, characterized by non-stoichiometry and a high degree of isomorphic substitution (Pellegrino & Biltz 1968; Betts *et al* 1981; LeGeros 1981). The general formula for hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$, but biological minerals are non-stoichiometric because the analytical determination of Ca/P ratios do not conform to theoretical values expected from this chemical formula. It is probably more appropriate to refer to biological apatite (or apatite), rather than hydroxyapatite, since the structure differs due to substitution and adsorption of a whole range of ions (Betts *et al* 1981; Boskey & Posner 1981; LeGeros 1981).

Formation

Bone mineral forms in the extracellular organic matrix from minerals secreted by osteocyte bone cells. The immediate precursor is the subject of an ongoing debate, and may be octacalcium phosphate (OCP) (Brown *et al* 1962; Chickerur *et al* 1980),

or amorphous tricalcium phosphate (ACP) (Termine & Posner 1966; Blumenthal & Posner 1973; Blumenthal *et al* 1975; Posner & Betts 1975). Biological calcification is an extremely complex process and the role of various constituents in the organic matrix is not well understood (Boskey 1981). Extra cellular macromolecules (proteoglycans) and certain cells (osteoblasts, odontoblasts and chondrocytes), are believed to control calcification by increasing local calcium and phosphate concentration, providing sites for crystal growth and deposition, and inhibiting mineralization where it is not required (Blumenthal *et al* 1975; Betts *et al* 1981; Boskey & Posner 1981). Magnesium ions and adenosine triphosphate also have an inhibitory effect (Blumenthal *et al* 1977), as do diphosphonates (Boskey *et al* 1979). This is an essential biological mechanism which prevents uncontrolled mineralization where it is harmful, a point which will be returned to in the discussion about diagenesis. Collagen regulates the size and orientation of the mineral, since deposition is related to the periodicity of the fibrils, along which the crystallites' c-axis is generally aligned (Boskey 1981). The structural components of the immediate precursors and metabolic formation of biological apatites accounts for the highly substituted and imperfect structure. Bone mineral is poorly crystalline, but studies have shown that crystallinity increases somewhat with age of the mineral and age of the individual (Ascenzi *et al* 1977; Bonar *et al* 1983).

Crystal Structure

Apatites belong to a hexagonal system of crystals, characterised by a six-fold axis of symmetry (the longitudinal, c-axis) and three equivalent a-axes at 120° to each other (Figure 1)(Simpson 1972; Jenkins 1978; LeGeros 1981). Calcium ions (Ca^{2+})are arranged in two groups: the columnar group forms a series of hexagons, inside which lies the hexagonal calcium group, arranged in a series of parallel triangles with adjacent triangles rotated through 60°, known as the screw axis. The phosphate ions (PO_4^{3-}) lie in tetrahedra between pairs of Ca^{2+} ions in the outer hexagon, such that 1

phosphorous and 3 oxygen atoms are above the plane, while the fourth oxygen lies below the plane. The next phosphate molecule is reversed. In hydroxyapatite, hydroxyl ions (OH^-) are placed within the Ca^{2+} triangles along the c-axis. Since OH^- is asymmetrical it can be placed as OH^- or ^-OH, resulting in a disordered column with OH^- in both directions (Jenkins 1978, Simpson 1972). The voids in the column may be filled by fluoride ions (F^-), which may also replace some OH^- ions.

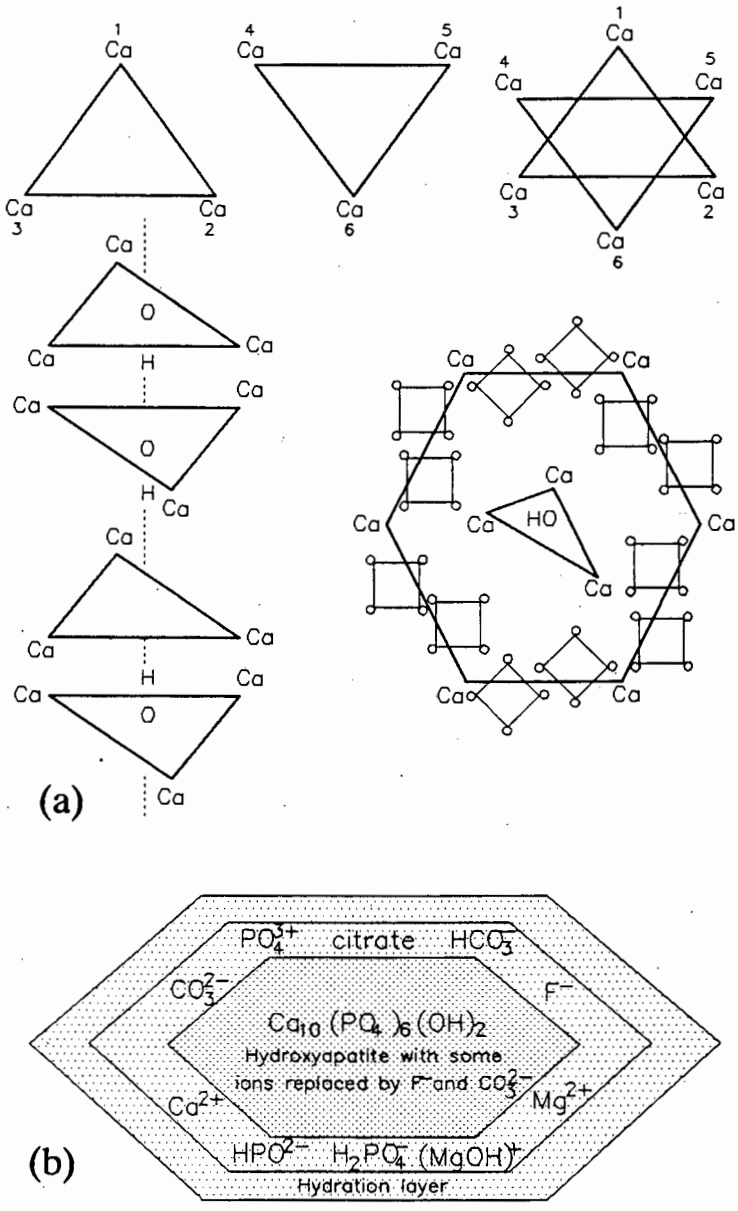

Figure 1 Diagrams of (a) the crystal structure of hydroxyapatite and (b) an apatite crystal showing the hydration layer and adsorbed ions (after Jenkins 1978: 71 & 74).

Biological apatites differ from pure, synthetic apatites in a number of ways, including poor crystallinity, variability in composition and non-stoichiometry (Betts *et al* 1981). Crystallinity, a term implying both large crystal size and lack of structural defects or crystal strain varies within and between tissues. Crystallinity may be determined by measurement of peak width at half-height of the 002 x-ray diffraction (XRD) reflection or the infra-red "splitting function" (SF) from the PO_4^{3-} anti-symmetric bending mode (Termine & Posner 1966; LeGeros 1967; Blumenthal *et al* 1975; Betts *et al* 1981). Bone apatite is poorly crystalline and has been described as submicrocrystalline or paracrystalline (i.e. having no long range order) (Wheeler & Lewis 1977; Nelson *et al* 1982). Bone apatite crystallites are very small, about 32 nm along the c-axis, and plate-like in form (Jackson *et al* 1978).

Two important properties of biological apatite, surface adsorption and ionic substitution, explain these features. Crystal surfaces have a nett electrical charge, attracting ions of equal and opposite charge. Each crystal is surrounded by a hydration layer and ions such as citrate, magnesium and carbonate (or bicarbonate) may be bound to this layer (Simpson 1972; Jenkins 1978)(Figure 1). The extremely small size of bone apatite crystallites increases the importance of surface effects. Ions in the crystal lattice may be replaced or substituted by other ions of similar molecular size and charge (Elliott 1973; Jenkins 1978; LeGeros 1981). Ca^{2+} ions may be replaced by sodium, silicon, manganese, lead, strontium and hydronium ions. Hydroxyl ions may be replaced by fluoride, chloride, possibly carbonate and other anions (Simpson 1972; Elliott 1973). Fluoride is smaller than the hydroxyl ions and fits neatly into the face of the unit cell. The presence of fluoride has a marked effect on apatites: increasing crystallinity and decreasing solubility (Baud *et al* 1977; LeGeros 1981; LeGeros *et al* 1982; LeGeros & Tung 1983). CO_3^{2-} may substitute for PO_4^{3-}, and Na^+ for Ca^{2+} in a simultaneous "coupled substitution" (Simpson 1965, 1972; LeGeros 1967; Driessens *et al* 1978; Eanes 1979; Chickerur *et al* 1980).

Carbonate substitution has most important consequences for the properties of biological, which are discussed below.

Carbonate in biological apatites

Bone apatite and tooth enamel contains about 2-5% by weight of CO_3^{2-} (Chickerur *et al* 1980); the carbonate content of bone is at the upper end of this scale, and that of enamel is at the lower end (LeGeros 1981). There has been some controversy over the position of CO_3^{2-} in biological apatites (Simpson 1965, 1972; LeGeros 1967; McConnell 1973), since synthetic apatites may have CO_3^{2-} substituted either in the OH^- position, or the PO_4^{3-} position. The former, however, only occurs in apatites synthesised at high temperatures, and is no longer considered applicable to biological apatites, and therefore the main ionic substitution of CO_3^{2-} within the crystal structure is in the PO_4^{3-} position (LeGeros 1967; Simpson 1972; Driessens *et al* 1978; Chickerur *et al* 1980). This is referred to here as "structural" carbonate. Some CO_3^{2-} is adsorbed onto the hydration layer or crystal surface, and is referred to as "adsorbed" carbonate. There is some doubt about the relative amounts of CO_3^{2-} in each "compartment", but about 50/50 has been suggested (Neuman & Mulryan 1967; Poyart *et al* 1975a, 1975b). It has been shown that about half of the adsorbed carbonate is thermally labile, and therefore must be in the form of bicarbonate (HCO_3^-)(Neuman & Mulryan 1967; Poyart *et al* 1975a, 1975b). Poyart *et al* (1975a, 1975b) suggested that the labile carbonate acts as a reservoir for release of bicarbonate into the bloodstream should it be required.

Carbonate substitution has a marked effect on the properties of apatites, both synthetic and biological (Blumenthal *et al* 1975; Betts *et al* 1981; LeGeros 1981; Posner 1985). Reduction of the a-axis and crystal size occurs, and solubility increases as a result of increased crystal disorder. Calcium deficiency, which may be explained by missing Ca^{2+} ions or by substitution, has a similar but less marked effect. The nett effect of coupled Na^+ and CO_3^{2-} substitution is a decrease in crystal

size and concomitant increase in the influence of surface effects according to the Gibbs-Kelvin relationship (Blumenthal *et al* 1975; Betts *et al* 1981). The relatively high reactivity of bone apatite is dependent on large specific areas which provide interfaces for chemical exchange with body fluids (Wheeler & Lewis 1977). This enables bone to fulfil its role as a reservoir of Ca^{2+} and PO_4^{3-} to the body.

The poor crystallinity of bone apatite was interpreted earlier as evidence for two co-existing mineral phases - a crystalline apatite and an amorphous calcium phosphate (ACP), the proportion of apatite increasing with age (Termine & Posner 1966, 1967). Comparison of mature and immature osteons by electron spin resonance (ESR) techniques indicate an increase in crystallinity with age (Ascenzi *et al* 1977), and X-ray diffraction studies have shown an increase in crystallinity with the age of the mineral and age of the individual (Bonar *et al* 1983). It is now believed, however, that poor XRD resolution is due rather to small crystal size and internal disorder caused primarily by CO_3^{2-} substitution (Blumenthal *et al* 1975; Boskey & Posner 1981; Betts *et al* 1981). Biological apatites thus vary in form and constituents, depending on conditions during formation and subsequent treatment.

Turnover

Bone is in ionic equilibrium with blood, so that resorption and remineralisation takes place continuously during life. At the histological level of osteons, pockets or compartments of bone tissue are remodelled in turn (Frost 1983), a process which varies for type and function of bone and in response to trauma or disease. Presumably this process should result in the complete turnover of both the organic and the mineral phases. At the crystalline level, the processes of resorption and remineralisation are complex, and there is some evidence that only part of the apatite "pool" (the smaller, more reactive fraction), is available for resorption. Posner (1969) proposed that about 27% of bone Ca^{2+} is part of an exchangeable pool. In osteoporotic Eskimo bones, X-ray diffraction and infra-red studies showed

that the smaller, less crystalline fraction was preferentially resorbed leaving behind bone with larger crystals (Thompson *et al* 1983). Since bone is in equilibrium with blood serum, one would expect that bone carbonates and serum bicarbonates are freely exchangeable. *In vitro* studies have indicated, however, that only part of the carbonate pool in apatite, believed to be the adsorbed carbonate, is freely exchangeable (Neuman & Mulryan 1967; Poyart *et al* 1975a; Biltz & Pellegrino 1977). The results were confirmed by *in vivo* studies (Neuman & Mulryan 1967; Poyart *et al* 1975b), and suggest that structural carbonate is not exchangeable.

Carbonate in the structural position is therefore derived from serum (bi)carbonates at the time of formation and during subsequent remodelling episodes. Adsorbed carbonate, on the other hand, since it is so much more easily exchangeable, is more closely related to prevailing serum bicarbonates. Serum bicarbonates are derived originally from oxidation of foods for energy requirements, and should therefore reflect $^{13}C/^{12}C$ ratios of dietary fuel. Since carbonate ions in apatite are derived from serum bicarbonates, apatite $^{13}C/^{12}C$ ratios are related in this way to diet. There may be some differences between structural carbonate $^{13}C/^{12}C$ ratios and adsorbed carbonate $^{13}C/^{12}C$ ratios because the latter should reflect current serum bicarbonate ratios, rather than those at formation. A laboratory feeding study has confirmed that apatite is isotopically related to diet (DeNiro & Epstein 1978a) as have studies using animals in their natural habitats (DeNiro & Epstein 1978b; Land *et al* 1980; Sullivan & Krueger 1981; Lee-Thorp 1983). The nature of the isotopic relationships between diet and the calcified tissues are discussed and investigated further in Chapter 5.

Tooth enamel

Although enamel is also a highly substituted hydroxyapatite, it differs from bone apatite in several important aspects. Enamel is more crystalline, denser and less porous than bone apatite. There is much less organic matrix, only about 2%, which

in this case consists primarily of phosphoproteins and amelogenins (Boskey 1981; LeGeros 1983) instead of collagen as in bone. The organic matrix decreases in quantity and changes in composition with enamel maturation. There is no sign of any amorphous ACP during or after formation, and therefore it has been suggested that OCP is the sole precursor (Brown *et al* 1962; Eanes 1979; Chickerur *et al* 1980). Enamel apatite crystals are much larger than those of bone and dentine apatite, a difference which has been explained in terms of the combined effects of the nature of the organic matrices and the concentration of "impurities" (LeGeros 1983).

Initially formed crystals appear as long thin "ribbons" having specific orientation with respect to the enamel prisms or rods, and with respect to each other. The pseudo-crystalline rods or prisms are the anatomical units of enamel, extending through its full thickness. The organic matrix is highly structured in the form of tubules, which provide the space and volume necessary for nucleation and allow unique growth and orientation of enamel crystallites (LeGeros 1983). The concentrations of CO_3^{2-}, Mg^{2+} and $P_2O_7^{4-}$ (pyrophosphate) ions are lower in enamel than in bone, but the concentration of F^- is higher. Since the first three ions promote low crystallinity while F^- has the opposite effect, these differences account in some measure for the increased crystallinity of enamel relative to bone and dentine. The other factor is the highly regular orientation of the organic matrix, which is believed to promote crystal growth in particular directions (LeGeros 1983). As a result of larger crystal size, enamel has less surface area per unit weight and is therefore much less reactive.

Enamel is not remodelled and does not maintain an equilibrium with blood serum, as does bone mineral. Substitution of carbonate and fluoride ions occurs during formation; thereafter, under physiological conditions, exchange is limited to crystal surfaces and dislocations (Brown *et al* 1975; Driessens *et al* 1978; Eanes 1979). $^{13}C/^{12}C$ ratios of enamel carbonate will therefore reflect dietary values at the time of

enamel formation, rather than a composite of formation and turnover, as is the case with bone. Unless tooth formation is continuous, as it is for certain teeth in some animals, (e.g., porcupines and elephants), the $^{13}C/^{12}C$ ratios will reflect dietary values during the growth stage in which the permanent dentition formed. Permanent dentition formation and eruption usually occurs over a few years, so the period sampled will vary with the particular tooth used for analysis. For example, in humans, the molars will reflect a more mature stage than the incisors. These factors must be considered, although there is at present no evidence to show that $^{13}C/^{12}C$ dietary fractionations differ with age and sex in humans (Lovel & Nelson 1986).

DIAGENESIS

Diagenesis is a term commonly used to denote the complex chemical changes which occur in calcified tissues after death and deposition of the organism. These changes include loss, addition or exchange of the original material, and may therefore obscure biological tracers such as isotopic ratios. The kind and rate of diagenesis of calcified tissues is affected by the nature of the original structure, the post-mortem environment, and time. Usually, unless they are preserved by burial or other special circumstances, calcified tissues do not survive at all. Exposure in the open results in rapid oxidation and loss of organic material, followed by loss of mineral (Hill 1980; Tuross *et al* n.d.). The rate of change is much slower if burial takes place soon after death of the animal, and is further slowed by optimal soil conditions. Bone and teeth survive longest in dry deposits of neutral or slightly alkaline pH (Salamon & Haas 1967). In certain circumstances, usually where soils are warm and moist, fungi and bacteria may tunnel into bone and digest the collagen (Ascenzi 1969; Piepenbrink 1986). The organic acids they excrete in turn dissolve the mineral, and new apatite may be reprecipitated in the spaces, but the histological structure and biogenic trace element or isotopic signals are lost. Another process, described as

"weathering" of apatite, may also be initiated by organic acids which are formed when groundwater protons displace calcium ions in the apatite structure (White & Hannus 1983).

Preservation of collagen

In optimal depositional circumstances, where attack by micro-organisms is minimal, diagenesis of bone collagen usually takes the form of a process of gradual denaturation and degradation of collagenous proteins into the constituent amino acids, which are subsequently easily washed away and lost (Hare 1980; Tuross *et al* n.d.). The rate of the process is dependent on temperature and hydration (Hare 1980). This form of diagenesis, while it results ultimately in the loss of collagen, does not affect the $^{13}C/^{12}C$ ratios of the remaining collagen material, as long as the original proportions of amino acids remain (Tuross *et al* 1988). Thus measurement of biogenic $^{13}C/^{12}C$ ratios are possible as long as sufficient collagen remains for isotopic measurement. Some non-collagenous proteins apparently survive for long periods (Tuross *et al* 1988), but to date have not been used in isotopic dietary studies. Whether or not protein residues are collagen may be determined by measurement of the C/N ratio or an amino acid profile. The former method, originally proposed by Hassan & Hare (1978), and later by DeNiro (1985), is the simplest, following the principle that the C/N ratio falls within a narrow range for pure collagen. Contaminants such as humates may be removed by suitable preparation procedures. In warm climates, collagen generally does not survive in amounts suitable for isotopic measurement beyond a few thousand years. This period may, however, be considerably longer in some circumstances, for instance consistently very dry or very wet (but anoxic) conditions, and low temperatures.

Preservation of mineral

The mineral in bone and teeth survives long after collagen has disappeared, but in contrast to collagen, the kinds of diagenetic changes which occur may alter or obscure the biogenic signals from which dietary information is derived. It has been suggested that while collagen survives, bone apatite is protected to some extent from exchange with groundwater (Sillen 1989). This suggestion is based on a tentative correlation between the periods in which collagen survives intact (as judged by published C/N ratios) and the period in which there is no evidence from X-ray diffraction patterns for increased crystallinity of apatite. On the other hand, based on data from short-term weathering of wildebeest bones, Tuross *et al* (n.d.) have suggested the converse - that the mineral protects the collagen. Their data indicate an increase in crystallinity of the apatite while collagen still survives. Alteration of the biogenic carbon isotope signal in bone or tooth apatite may occur via several mechanisms, which are discussed below.

One kind of diagenesis is physical inclusion of carbonate-containing substances. As discussed in Chapter 2, Hassan showed that calcite and other crystalline material such as pyrites was frequently deposited in the interstices and Haversian canals of bone (Hassan 1975; Hassan & Ortner 1977; Hassan *et al* 1977). In a number of subsequent studies, the presence of calcite has been confirmed, usually from X-ray diffraction patterns (Land *et al* 1980; Sullivan & Krueger 1981), or hinted at (Schoeninger & DeNiro 1982). Frequently, however, XRD patterns indicate only an apatitic phase; this may imply no calcite, or small amounts below the detection limit for XRD (1-5%). In at least some cases, for instance the fossils from the Omo Shungura formation, no calcite is seen in the XRD patterns, but minor inclusions of calcite may be inferred from high Ca/P ratios and high calcium and carbonate contents (Sillen 1986, 1989) Such minor calcite inclusions would certainly affect the $^{13}C/^{12}C$ ratios. On the available evidence it seems that varying amounts of

diagenetic CaCO3 may be present, depending on the depositional circumstances. Calcite, however, can be effectively removed by treatment in dilute acetic acid solutions as described by various workers (Land *et al* 1980; Sullivan & Krueger 1981); others have used stronger solutions for this purpose (Schoeninger & DeNiro 1982; Nelson *et al* 1986).

Increased crystallinity with age has often been demonstrated from X-ray diffraction studies (Hassan 1975; Hassan *et al* 1977; Schoeninger 1982; Sillen 1981a, 1981b. 1986). The infrared splitting factor (SF) also provides a measure of crystallinity, but has been used less often in fossil studies. The SF is based on the observation that the splitting of the P-O antisymmetric bending mode at 550-600cm^{-1} increases with increasing crystallinity (Blumenthal *et al* 1972, 1975). An increase in crystallinity implies an increase in crystal size and/or increased order, which in turn implies a certain amount of crystal growth and/or recrystallisation. The propensities of apatite for crystal growth are well-known: Ca^{2+} and PO_4^{3+} ions have an extreme affinity and will "scavenge" each other in any environment (Simpson 1972). As noted above, the tendency towards apatite growth is inhibited by a variety of mechanisms, probably acting in concert, while the individual is alive. These mechanisms cease to exist after death, and thus apatite should continue to grow, initially using adjacent constituents from hydration layers or bone fluids, and later those from percolating groundwater.

A sizeable increase in crystallinity, as apparent from X-ray diffraction studies, seems to be a slow process (Sillen 1989). During this process of recrystallisation and crystal growth, extraneous carbonates could enter the crystal structure or exchange with biogenic carbonates. Hassan (1975) suggested that bone apatite underwent a slow transition during fossilisation from a dahllite type of apatite (low CO_3^{2-}, low F^-) to francolite (a high CO_3^{2-}, high F^- apatite) during fossilisation. It seems that the

amounts of F^-, CO_3^{2-} and other ions incorporated during fossilisation are very variable, however, depending on the depositional circumstances.

The effect of recrystallisation on the dietary $^{13}C/^{12}C$ signal is difficult to estimate. It will be minimal if recrystallisation merely involves a rearrangement of the biogenic constituents into larger crystals. Thus an increase in crystallinity will not automatically lead to the erasure of biogenic carbonate $^{13}C/^{12}C$ signals. On the other hand, incorporation of diagenetic carbonates is most likely to occur when abundant (bi)carbonates are present in groundwater, leading to the formation of calcite and high-carbonate apatites. Since apatites with higher carbonate contents are more soluble (Blumenthal *et al* 1975; Betts *et al* 1981; LeGeros & Tung 1983), these diagenetic phases should also preferentially dissolve in dilute acetic acid treatments. If F^- is also incorporated, the effect will be different, since the over-riding effect of F^- is to increase crystallinity and reduce solubility (Baud *et al* 1977; LeGeros *et al* 1982, LeGeros & Tung 1983).

Ionic and isotopic exchange are other mechanisms in which biogenic $^{13}C/^{12}C$ ratios may be altered. Exchange of ions may take place via several diffusion mechanisms. The most likely pathway for exchange of carbonates is via the more reactive hydration layers. Thus groundwater bicarbonates or soil CO_2 could be adsorbed to these layers. Exchange with phosphate and carbonate ions within the crystal structure by solid-state diffusion is a much slower process by several orders of magnitude, as shown by studies of crystal calcite systems (Haul & Stein 1955; Anderson 1969). Diffusion tends to occur mainly along crystal dislocations. Isotopic exchange, in which diagenetic carbon and oxygen atoms exchange with biogenic atoms in carbonate ions, may also occur via similar mechanisms. Isotopic exchange is well-known for $CO_2(g)/HCO_3^-(l)/CO_3^{2-}(s)$ systems, and the fractionations between the phases have been determined experimentally (Emrich *et al* 1970) and theoretically (Bottinga 1968). Oxygen is more exchangeable than carbon, because of

the ease of exchange with water oxygen atoms (McCrea 1950) and the way in which carbonate is bound within the crystal. The presence of water enhances the probability of both ionic and isotopic exchange, resulting in isotopic ratios approaching those of the groundwater. There is probably no way in which these effects can be counteracted, although they may be quantified.

Preservation of tooth enamel

Although enamel is also a substituted apatite, it differs from bone apatite in a number of ways which consequently affect the process of diagenesis. Enamel is denser, less porous and has less organic matrix support, thus there are fewer pathways for entry of groundwater and dissolved ions. This will reduce deposition of calcite and minimise the effects of ionic and isotopic exchange. Its greater crystallinity implies that there is less tendency towards crystal growth, as occurs in bone apatite. At the same time, because the crystals are more stable, there is less tendency towards dissolution and subsequent recrystallisation. This does not mean that there are no pathways for the processes of incorporation of diagenetic CO_3^{2-} and ionic and isotopic exchange, but that these are reduced.

SUMMARY

It is apparent from the foregoing discussion that diagenesis of biological apatites and alteration of biogenic $^{13}C/^{12}C$ ratios are complex processes. The trajectories depend on a number of factors : the nature of the original material, the nature of the depositional circumstances, and time. For bone apatite, much of diagenesis can be attributed to imperfect bone apatite reverting to a more ordered crystal structure (Sillen 1989). This observation does not apply in the same degree to tooth enamel, where the structure is originally more crystalline. The reactivity of bone crystallites and the presence of many adsorbed ions on the hydration layers, should ensure that crystal growth and recrystallisation occurs almost immediately after the *in vivo* inhibitory effects are removed. Incorporation of diagenetic ions probably occurs

mostly during these processes. The most important factor promoting such changes in apatite is probably moisture, which may be provided initially by body fluids and later by groundwater. Whether or not change commences immediately, or after the disappearance of the organic phase, the most important contaminating medium is groundwater. Biogenic $^{13}C/^{12}C$ ratios should equilibrate to some extent with those of the groundwater with the passage of time. However, given the propensity of apatite to grow and stabilise over time, the rate of change might decrease after this occurs, since the crystals are more stable. The extent to which $^{13}C/^{12}C$ ratios equilibrate with groundwater values with the passage of time is addressed in this thesis.

CHAPTER 4

RESEARCH STRATEGY

Research strategies and techniques used in previous studies which have addressed the problem of reliability of stable carbon isotope measurements of the mineral phase have been outlined in Chapter 2. Although their preparative techniques differed, both Sullivan & Krueger (1981) and Schoeninger & DeNiro (1982) employed a similar approach, i.e. a comparison of the $^{13}C/^{12}C$ ratios for collagen and apatite. Shortcomings of this approach include the complexity of the isotopic relationship between collagen and apatite, which was not understood at the time, and restrictions placed on the time scale for the test by the relatively short life of collagen.

To avoid these limitations, a different approach for testing the stability of the biogenic carbon isotope signal in apatite is adopted here. The first step requires an investigation of the isotopic relationships between diet, collagen and apatite, to enable valid interpretations of the apatite ^{13}C values in terms of diet. To this end, apatite and collagen $\delta^{13}C$ values are measured for various species of modern animals at different trophic levels, and from different environments. The second step involves an assessment of the stability of the stable carbon isotope signal of fossil apatites over time. An evaluation of the effects of the usual pretreatment procedures on bone and tooth enamel is also required, to determine firstly whether the treatment preferentially removes diagenetic carbonates, and secondly whether there are any other unexpected effects. Finally, the diets of extinct species are examined using enamel $\delta^{13}C$ measurements.

RESEARCH DESIGN

Isotopic relationship between diet, collagen and apatite

An important part of this study involves the distribution of stable carbon isotope in food webs : these modern studies include two components. The first is the establishment of well-defined ranges $\delta^{13}C$ values for apatite and collagen of modern browsers and grazers, in order to provide a sound comparison for the fossil herbivores. The second component is an investigation of the isotopic relationships between diet, collagen and apatite for different trophic levels and biomes, since an understanding of these relationships is essential when dietary assessments are to be made on the basis of apatite alone.

Some evidence has been presented (Krueger & Sullivan 1984) that these relationships change with trophic level. As discussed in the previous chapter, the $^{13}C/^{12}C$ ratios of carbonate ions in apatite are related to serum bicarbonate, which in turn is derived from oxidation of fuel for energy requirements. Thus apatite may reflect a different aspect of the diet to that obtained from measurement of collagen $^{13}C/^{12}C$ ratios, since the latter must be synthesized from dietary proteins and carbohydrates. Krueger and Sullivan (1984) have proposed models linking these isotopic relationships to the differential consumption of macronutrients in different trophic level diets. These models are tested by determining the relationship between collagen and apatite for herbivores, carnivores and omnivores from a variety of natural environments in southern Africa. The browsing or grazing habits of various species, believed to be either browsers or grazers respectively, are checked using isotopic measurements of collagen and apatite, and the range of values for reliable browsers and grazers is defined. A review of previous work on this subject, together with the methods and results obtained in this study, is given in Chapter 5.

Reliability of older apatite $\delta^{13}C$ values

The rationale for the approach adopted here to test the stability and reliability of apatite carbon isotope values through time, and the efficacy of chemical methods for separating biogenic from diagenetic CO_3^{2-}, is as follows. The $\delta^{13}C$ values for groundwater and deposit carbonates in limestone or dolomite derived sites cluster around 0‰ (Veizer & Hoefs 1976), whereas apatite $\delta^{13}C$ values from animals eating pure C_3 diets, i.e. browsing herbivores, cluster around -14‰ (Sullivan & Krueger 1981, Lee-Thorp & van der Merwe 1987). Thus, in these situations, diagenetic alteration by groundwater carbonates should change the biogenic isotopic signal towards more positive values. Therefore, the difference between apatite $\delta^{13}C$ values for modern and those for fossil browsers (suitably treated to eliminate carbonates not intrinsically part of the apatitic structure) represents the amount of alteration due to diagenesis. Measurements from specimens of differing age should enable an estimation of the extent and mode of alteration of biogenic carbonate $\delta^{13}C$ values, with time.

There are a number of assumptions and conditions to be met if the premise that alteration in browser apatite $\delta^{13}C$ values represents the degree of diagenetic exchange is to hold. The first concerns the consistency of the C_3 diet. Browsing bovids were chosen for the test, since their C_3 foliage diets should yield consistent $\delta^{13}C$ values. A number of criteria had to be satisfied in the choice of species. Firstly, the species must be consistent browsers under all environmental conditions, as demonstrated from isotopic measurements of modern specimens, and the mean and range of values established. This was outlined in the previous section. Further, the species must be fairly common in archaeological sites, as well as be available from modern collections. On preliminary examination, several species seemed to fit the bill:- *Giraffa camelopardalis* (giraffe), *Tragelaphus scriptus* (bushbuck), *Tragelaphus strepsiceros* (kudu), *Raphicerus melanotis* (grysbuck), *Cephalophus*

monticola (blue duiker), and *Sylvicapra grimmia* (duiker). Since no single species was common to all southern African modern environments or archaeological sites, all of these species are included in the tests.

Another condition of the model is that matrix carbonates and groundwater carbonates have $\delta^{13}C$ values very different from those of browser apatite. Most of the sites chosen for this study could be assumed to have matrix values near 0‰, since their carbonates are derived from marine limestone or dolomite. The sites, from which fossil fauna were obtained, and which span some 3 million years in time are: Die Kelders in the southern Cape (1.5 to about 60 ka of age), Klasies River Mouth in the south-eastern Cape (2.5 to about 120 ka of age), Mpambanyoni on the Natal coast (950 BP), Border Cave on the borders between Natal and Swaziland (recent to about 120 ka), and Makapansgat (3 ma) and Sterkfontein (150 ka to about 2.5 ma) in the Transvaal (Figure 2). Groundwater in Die Kelders Cave may be expected to be enriched in ^{13}C (i.e. near 0‰) as a result of the Bredasdorp marine limestone cave roof (Tankard & Schweitzer 1974, 1976). At Klasies River Mouth Cave 1 (KRM 1), the carbonate-rich groundwater is generally attributed to percolation of water through marine shell in aeolian sands on the cliffs above the caves (Butzer 1978, Deacon & Geleijnse 1988). The matrix carbonate isotopic values at KRM 1 can be measured directly by analysis of deposit samples. Matrix samples are not available for Mpambanyoni, an early Iron Age midden, and Border Cave; thus these matrix carbonate values remain unknown. The older sites of Makapansgat and Sterkfontein, both cave-fills within dolomitic formations (Brain 1975, 1981), may be expected to have matrix carbonates enriched in ^{13}C, but this assumption may be checked by isotopic analysis of the frequent calcite inclusions as well as samples of breccia.

If differences should occur between fossil and modern values, are there alternative explanations or is diagenesis the most reasonable or only possibility? One possible

alternative explanation is that $\delta^{13}C$ values were different originally, either because the prehistoric animals ate different foods or because $\delta^{13}C$ values of prehistoric atmospheric CO_2 differed from present-day values, resulting in different average foliage values. The former can be discounted, since by definition, browsers eat C_3 foliage and forbs. The diets of the modern species are well-known from dietary studies based on analysis of stomach contents and feces (Hofman & Stewart 1972; Smithers 1983). As an added precaution, the species chosen for this study have been studied isotopically. The exceptions are extinct varieties. The second scenario remains a possibility. The concentration of CO_2 in the atmosphere has increased over the last 100 years or so, due to the burning of fossil fuels, and a change has been detected in atmospheric CO_2 $\delta^{13}C$ values over the last 20 years (the change is less than $1^{o}/_{oo}$, towards more negative values) (Keeling *et al* 1979). Thus all $\delta^{13}C$ values obtained from present-day foodwebs may be shifted slightly towards more negative values (more depleted in ^{13}C). It has also been shown that the concentration of atmospheric CO_2 has varied in the past; it was about $^{2}/_{3}$ lower during glacial periods, compared to interglacials (Berner *et al* 1978, 1980; Broecker *et al* 1985; Broecker 1987; Barnola *et al* 1987; Jouzel *et al* 1987). At present, it is uncertain whether or not this affected atmospheric CO_2 $\delta^{13}C$ values, due to lack of data, and the two principal theories for the biosphere-ocean-atmosphere CO_2 cycle predict opposite effects (Broecker 1987). The predicted effects, however, are very small.

Applied studies

The results of the study of modern browsers are reported in Chapter 5, and those for fossil browsers in Chapter 6. They show there is some alteration in the biogenic isotopic signal in older apatite, which cannot be chemically removed. Nevertheless, a clear difference between grazers and browsers remains even at the oldest site, Makapansgat. On the basis of these results, I concluded that the method may be

used to solve palaeontological problems concerning diet. Accordingly, three problems were chosen for study. The approach adopted for these applied studies of extinct animals is comparative, i.e. $\delta^{13}C$ apatite values of the species with unknown diet is compared with values of known browsers and grazers from the same site. This procedure may be referred to as the comparative isotopic approach.

The first extinct species chosen for dietary assessments on the basis of enamel $\delta^{13}C$ values, is *Antidorcas bondi* (Bond's springbok). The extinct species is related to the modern springbok, *Antidorcas marsupialis*, but it was smaller and possessed markedly hypsodont teeth. The small size of the animal, the fact that it was a ruminant and the possession of a remarkably hypsodont dentition imply adaptation to a specialised dietary niche. Vrba (1973) suggested a diet similar to *A. marsupialis*, i.e. intermediate, but much coarser. Brink (1987), however, considered that the dental hypsodonty suggested rather a grazing diet. The problem is amenable to carbon isotopic analysis, since most grasses in Southern Africa are C_4, and thus the amount of grass versus browse in the diet may be estimated. Isotopic analysis using collagen is precluded by the age of the fossil specimens, and thus isotopic analysis of apatite provides the only means of estimating diet. Two sets of *A. bondi* specimens are involved : they are from Florisbad and Swartkrans Member 5 (Figure 2), two sites of different age. Comparative faunal specimens i.e. known browsers and grazers from the same stratigraphic context are not available from Swartkrans. The comparative material from Florisbad includes only *A. marsupialis* and *Taurotragus oryx*, a rather doubtful browser, but the only browsing species available. A more comprehensive background for this study, and the methods, results and conclusions are given in Chapter 8.

The second application involves two extinct baboon species from Swartkrans Member 1 formation, *Theropithecus darti* and *Papio robinsoni*. At an estimated age of 1.8 ma, they provide an opportunity for testing the method on very old specimens.

Each species shares a similar tooth morphology with their modern relatives, *T. gelada* and *P. ursinus* respectively (Freedman 1957). The modern Gelada is a graminivore (Dunbar & Dunbar 1974), and the modern *Papio* is predominantly dependant on C_3-based foods (Smithers 1983); it seems likely that the extinct varieties also followed these patterns. At the time of Member 1 formation, about 1.7-1.9 ma ago (Vrba 1982; Brain *et al* 1988), the grasses in the Transvaal would have been C_4, as they are now, and therefore isotopic values for the two species should be distinct. Known browsers and grazers from Member 1 are used as internal references. Modern specimens of *Papio ursinus* from the northern Transvaal also provide a comparison. The results of this study are given in Chapter 8.

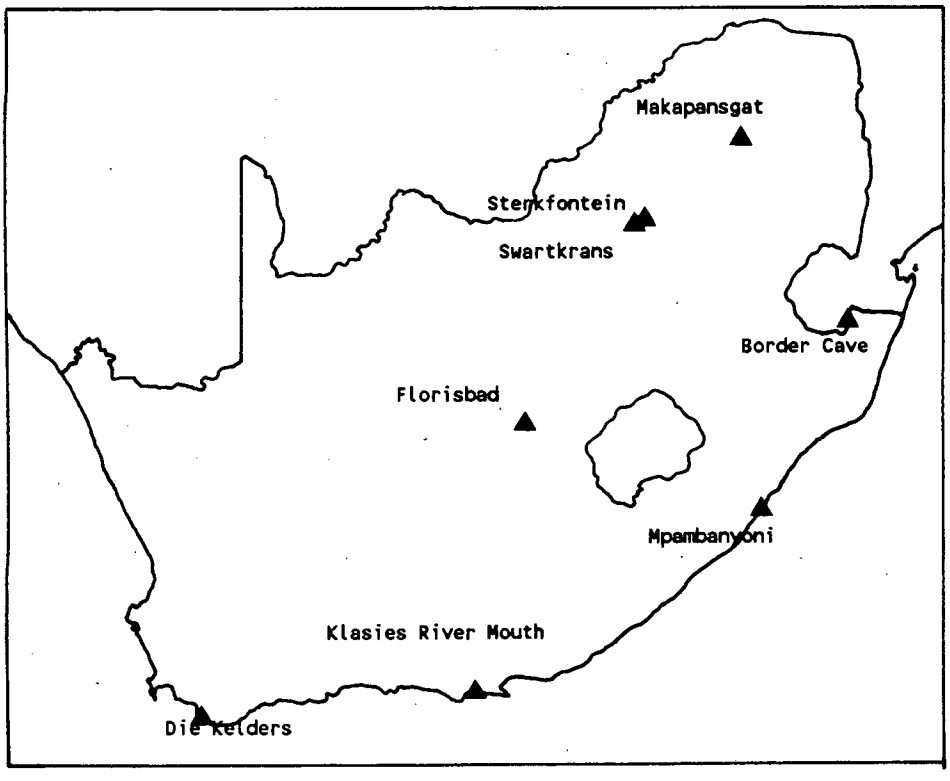

Figure 2 Map of South Africa showing the location of archaeological sites.

The most significant application using the comparative isotopic approach involves the analysis of tooth enamel of an early hominid, *Australopithecus robustus*. Remains of this hominid are found in Member 1 at Swartkrans, along with the baboons. Various attributes strongly suggest that *A. robustus* was adapted to a diet of very tough or hard small objects. These characters include the dentition, which had enormous molars with large grinding surfaces and comparatively small anterior teeth, very robust jawbones, and prominent bony attachments (crests) for the jaw muscles. The question of what they were eating has been the subject of much study and controversy and various dietary hypotheses have been proposed. These include the "seed-eating" dietary stage hypothesis (Jolly 1970), and its refinements (Dunbar 1976). Another, more current hypothesis may be called the "hard-fruit/nuts" hypothesis. These hypotheses may be tested using carbon isotope analysis of tooth enamel, since grasses (including the seeds) in the Transvaal are C_4 foods, whereas fruits and nuts must necessarily be C_3. The fauna from Swartkrans Member 1 from the baboon study provides comparisons for C_3 and C_4 diets. The results are considered in relation to the various conflicting dietary hypotheses (Chapter 8).

SUMMARY

The rationale for developing a fresh approach for testing and applying isotopic measurements of fossil apatite is described in this chapter. The approach involves, in the first place, an isotopic characterisation of modern apatites for different kinds of animals from natural habitats. The approach for testing the stability of older stable carbon isotope measurements is the measurement of fossil fauna of known diet, in a chronological series. Any differences between these values and those for modern browsers is assumed to represent alteration due to contamination and exchange with matrix carbonates. The problem of the efficacy of the method used for eliminating amorphous carbonates and diagenetic apatites in fossil specimens is

addressed using both modern and fossil material. Thereafter, the methods so developed may be applied to the three studies of the diets of extinct species.

CHAPTER 5

STABLE CARBON ISOTOPES IN FOOD WEBS

THE RELATIONSHIP BETWEEN DIET, COLLAGEN AND MINERAL

Laboratory feeding experiments and studies of natural systems have shown that stable carbon isotope ratios in animal tissues reflect dietary ratios (DeNiro & Epstein 1978a, 1978b; Vogel 1978b; Land *et al* 1980, Tiezsen *et al* 1983), although values for individual tissues differ. The most important fractionation in foodwebs occurs during photosynthesis, but significant secondary fractionations also occur in metabolic tissue-formation processes, and these are at present not very well understood. The organic and mineral phases of calcified tissues (collagen and apatite) have markedly different $^{13}C/^{12}C$ ratios, mainly because the formation of apatite fractionates the isotopes much more strongly than the formation of collagen. The isotopic difference between the two phases has also been observed to vary with trophic level (Krueger & Sullivan 1984). Krueger & Sullivan (1984) have proposed a model linking the variations to differential use of macronutrients (proteins, carbohydrates and fats) amongst animals at different trophic levels.

In this chapter the pathways of stable carbon isotopes in modern foodwebs are outlined. The range of $\delta^{13}C$ values for apatite and collagen of various species of fauna are determined. Krueger & Sullivan's foodweb models relating to macronutrients and trophic levels are described and refined on the basis of the faunal data. The results reinforce the idea that apatite reflects fuel $\delta^{13}C$ values and collagen reflects protein values. Thus apatite is not merely an alternative sample

material to collagen: in combination, the two provide information about trophic level. Some implications for isotopic assessments of human diets are discussed.

CARBON ISOTOPES IN THE FOOD WEB

Photosynthesis

The strongest natural fractionation of stable carbon isotopes occurs during photosynthesis. Three photosynthetic pathways exist among plants, the C_3, C_4 and CAM pathways, and these have distinct fractionation patterns. C_3 plants follow the Calvin cycle (Calvin & Benson 1948) in which the first product is a 3-carbon molecule, phosphoglyceric acid (hence the term C_3). Most trees and shrubs, and grasses in temperate environments and those growing in shade and at high altitudes are C_3. C_4 plants follow the Hatch-Slack pathway (Kortschak *et al* 1965; Hatch & Slack 1970) in which the first product is a 4-carbon molecule, oxaloacetate (hence the term C_4). The initial product is then reduced to malate or aspartate and after decarboxylation in the bundle sheath cells enters the Calvin cycle. Kranz anatomy, the arrangement of thick-walled bundle sheath cells around vascular bundles, is the physiological feature which distinguishes C_4 plants (Smith & Brown 1973; Smith & Robbins 1974)). This pathway fixes CO_2 more efficiently, uses less water (Zelitch 1979), and discriminates less against ^{13}C than C_3 plants (Park & Epstein 1960, 1961; Bender 1968, 1971; Smith & Epstein 1971). The main controlling factor seems to be high radiation in the growing season i.e. summer rainfall. C_4 plants are mainly tropical and subtropical (savanna) grasses. A third group of plants follow Crassulacean Acid Metabolism (CAM), in which the plant may switch from C_3 to C_4, depending on conditions (Osmond 1978). C_4 photosynthesis occurs only in the dark and the plants lack Kranz anatomy. CAM plants are usually succulents.

All of these pathways (C_3, C_4 and CAM) preferentially fix ^{12}C and discriminate against ^{13}C, but to different extents. C_3 plants are isotopically lighter than C_4 plants (mean $\delta^{13}C$ values are -26.5‰ and -12.5‰ respectively), but CAM values have a trimodal distribution (i.e. within the C_3 and C_4 ranges or in between)(Mooney *et al* 1977). This is not usually a problem for dietary tracing, however, since the distribution of CAM plants is limited, and with a few exceptions, they are seldom eaten by herbivores. There is one exception amongst the environments covered in this thesis - Addo in the eastern Cape Province (van der Merwe *et al* 1988). The mean values for C_3 and C_4 plants are useful guidelines, but it should be noted that a range of values is implicit. Furthermore, the plant constituents i.e. carbohydrates, proteins and lipids differ isotopically. Thus the carbohydrate and protein components are slightly heavier (about +2 and +1‰ respectively) and the lipid component is uniformly lighter (about -4 to -5‰) than the composite $\delta^{13}C$ value (Jacobson *et al* 1970; Smith & Jacobson 1976; Winkler & Schmidt 1980).

Animals

The distinct C_3 or C_4 isotopic patterns are passed on to the tissues of the consumers, so animals eating C_3 plants can clearly be distinguished from those eating C_4 plants, or a mixture of the two. In most African savanna environments where the grasses are C_4 and foliage and fruits from trees and shrubs are C_3, carbon isotope analysis clearly distinguishes browsers from grazers. The exceptions occur in the limited winter-rainfall areas, such as the south-western Cape Province, shaded forests and high altitude Afro-alpine environments, where the grasses, too, are C_3 (Livingstone & Clayton 1980; van der Merwe & Vogel 1983). Animals at higher trophic levels, e.g. carnivores, also reflect whether their diet is C_3-based or C_4-based, or a mixture, depending on whether the predominant prey species were browsers or grazers.

Living organisms are in isotopic balance, so that the total amount of ^{12}C and ^{13}C entering the organism in food, drink and air, equals the total amount stored in body

tissues and leaving the organism by excretion and respiration (DeNiro & Epstein 1978a). Although all tissues in the body reflect diet, isotopic values of various tissues in the same individual differ in predictable ways (DeNiro & Epstein 1978a, 1978b; Vogel 1978; Tiezsen *et al* 1983) due to factors such as differences in tissue composition, synthesis from different constituents of the diet, tissue turnover time, and metabolic fractionation effects. Some secondary fractionation, observed but not very well understood, takes place in the biochemical conversion of plant material to various animal tissues.

Some details of the isotopic relationships between the diet, collagen and bone apatite are not clear, particularly for higher trophic level animals. It has been established that $\delta^{13}C$ value of bone collagen is about 3-6‰ more positive than that of the animals diet: differences of 2.8-3.9‰ have been reported for laboratory mice (DeNiro and Epstein 1978a), and about 5‰ for free-ranging herbivores in their natural habitats (Vogel 1978b; Sullivan & Krueger 1981; Lee-Thorp & van der Merwe 1987). The reason for the observed difference between laboratory animals and animals in their natural habitats is not clear. Estimates for humans include 5.1‰ (van der Merwe and Vogel 1978) and 6.1 ‰ (Chisholm *et al* 1982). There has been some debate about the extent to which collagen carbon is derived directly from ingested proteins, or manufactured in the body from carbohydrates (Krueger & Sullivan 1984; Klepinger 1984; Klepinger & Mintel 1986; Parkington 1986; Sillen *et al* 1988). Different mixes of foods in the diet may well affect the isotopic values for collagen and apatite.

The carbonate ions substituted in apatite are isotopically heavier than bone collagen: diet-apatite fractionation has been reported as +9.6‰ for mice in a laboratory feeding study (DeNiro & Epstein 1978a), and +12-13‰ for free-ranging herbivores (Sullivan & Krueger 1981; Krueger & Sullivan 1984; Lee Thorp & van der Merwe 1987). It is generally understood that carbonate ions in apatite are

incorporated from serum bicarbonate. As noted in Chapter 3, "structural carbonate" should be related to serum bicarbonates at the time of apatite formation. On the other hand, "adsorbed carbonate", part of which is thermally labile and therefore in the form of bicarbonate (Neuman & Mulryan 1967; Poyart *et al* 1975a, 1975b) is constantly exposed to and directly exchangeable with serum bicarbonates at any time. Enriched apatite $\delta^{13}C$ values may be largely explained in terms of the well-known fractionation between phases in the $CO_2/HCO_3^-/CO_3^{2-}$ system (Emrich *et al* 1970). Using the same fractionations between the phases, serum HCO_3^- should be enriched in ^{13}C by about 8-9‰ with respect to the CO_2 from which it is formed, and further enrichment occurs during the formation of CO_3^{2-} from HCO_3^- (about 2‰). These fractionations add up to about 10‰, close to the observed 9.6 to 12‰, although other fractionations are undoubtedly involved e.g. in the kidneys (Burbea *et al* 1983) and the lungs. Measurements of respired CO_2 $\delta^{13}C$ values have been shown to similar to metabolite (lipid or glycogen) values in laboratory rats (Jacobson *et al* 1972), and humans (Schoeller *et al* 1984).

Foodweb models

The enrichment effect described above, however, does not explain the fact that the differences between the $\delta^{13}C$ values of apatite and collagen may vary considerably. A model has been proposed which relates bone collagen and apatite $\delta^{13}C$ values specifically to the principal macronutrients (protein, carbohydrates and lipids) in the diet (Krueger & Sullivan 1984). The model is supported in part by medical studies of breath CO_2 which relates isotopic changes in respired CO_2 of human subjects to changes in the amounts of fats or carbohydrates (sugars) metabolised (Schoeller *et al* 1984). Similar results also were obtained for laboratory rats (Jacobson *et al* 1972).

The difference between the $\delta^{13}C$ values of collagen and apatite is often called the apatite-collagen spacing, but here Δ is used for convenience. According to the Krueger & Sullivan model, Δ changes with trophic level because different

macronutrients (proteins, carbohydrates and lipids), assume greater or lesser importance in different diets. Thus herbivores derive protein from plant proteins and transamination of keto-acids (derived from carbohydrates), and energy from carbohydrates. Carnivores derive protein directly from prey protein (mainly meat), and energy from prey lipids and proteins. In both cases collagen reflects the growth aspect and apatite the energy aspect of the diet. Since lipids are isotopically lighter than the proteins and carbohydrates of the same plant or organism (Jacobson *et al* 1972; Smith & Jacobson 1976; DeNiro & Epstein 1977; Winkler & Schmidt 1980), an increase in their utilisation for energy results in lighter apatite $\delta^{13}C$ values. These are consequently closer to collagen $\delta^{13}C$ values, and result in a smaller Δ value. In Krueger & Sullivan's report (1984), secondary fractionations associated with a simplified food web were shown, and the relationship between collagen and apatite isotope values was expressed as:

$\delta^{13}C$ apatite = $\delta^{13}C$ collagen + 7 ‰ for herbivores (2)

$\delta^{13}C$ apatite = $\delta^{13}C$ collagen + 3 ‰ for carnivores (3)

Equation (2) is slightly different to equation (1) (Chapter 2) published earlier (Sullivan & Krueger 1981). $\delta^{13}C$ measurements of a sample of modern herbivore and carnivore bones were fitted to these equations, and a range of possible omnivorous human diets was interpolated between the two extremes of herbivory and carnivory. Results for archaeological humans were compared with this theoretical model. The implication is that different kinds of diet produce characteristic differences between bone collagen and apatite isotope values. Thus apatite is not merely an alternative sample material to collagen. In combination, isotopic analysis of both phases may provide unique information on trophic level.

One approach to testing this model is the measurement of $\delta^{13}C$ values from collagen and apatite of faunal species of known diet and trophic level. An advantage of such a method is that it is possible to sample animals from environments with the full

range of carbon isotope values, from C_4 grasslands (enriched in ^{13}C) to C_3 forest (depleted in ^{13}C). This approach is adopted here, and is described below. There are two modern studies involved: the determination of the ranges for browser and grazer $\delta^{13}C$ values, and test of Krueger & Sullivan's (1984) foodweb models, but since they are so closely linked, and the methods are the same, they are described together.

METHODS

Sampling program

Faunal bone or tooth specimens from all over southern Africa and a part of central Africa (Malawi) were obtained from nature reserves and the comparative collections of museums. This range encompasses: an isotopically "light" C_3 forest biome in the southern Cape, a C_3 "Mediterranean" biome in the south western Cape, arid scrubland (Karoo), C_4 savannah/grasslands further north, and the Kasungu *Brachystegia* woodland in Malawi (Figure 3). Browsing herbivores include the following species:- *Tragelaphus scriptus* (bushbuck), *Tragelaphus strepsiceros* (kudu), *Sylvicapra grimmia* (duiker), *Raphicerus melanotis* (grysbok), *Giraffa camelopardalis* (giraffe) and *Cephalophus monticola* (blue duiker). The grazers used to characterise C_4 grazing isotopic ratios include *Alcelaphus lichtensteini* (hartebeest), *Equus burchelli* (Burchell's zebra), *E. zebra* (zebra), *Connochaetes taurinus* (blue wildebeest) and *Connochaetes gnou* (black wildebeest), drawn from areas with C_4 grasses. The entire herbivore sample set includes grazers, mixed feeders and browsers. The carnivore set includes animals which are nearly pure carnivores (felids), animals which are also partly omnivorous (e.g. jackals) and insectivores (bat-eared foxes). The smaller set of omnivores are mostly baboons. A smaller set of browsing herbivores was obtained from an altitudinal transect of the Central Rift Valley, Kenya. The species and locations of all the modern fauna are given in the Appendix.

Laboratory procedures

The condition of the specimens varied because of the wide range of sampling circumstances. The faunal bone and tooth specimens obtained from the museum collections were already defleshed and clean and required no further cleaning. The same applied to most of the specimens from nature reserves, which had all been sun-dried. The sandy specimens were washed in running water, sonicated, and air-dried. The few really fresh specimens were defleshed with a scalpel, soaked in water, the

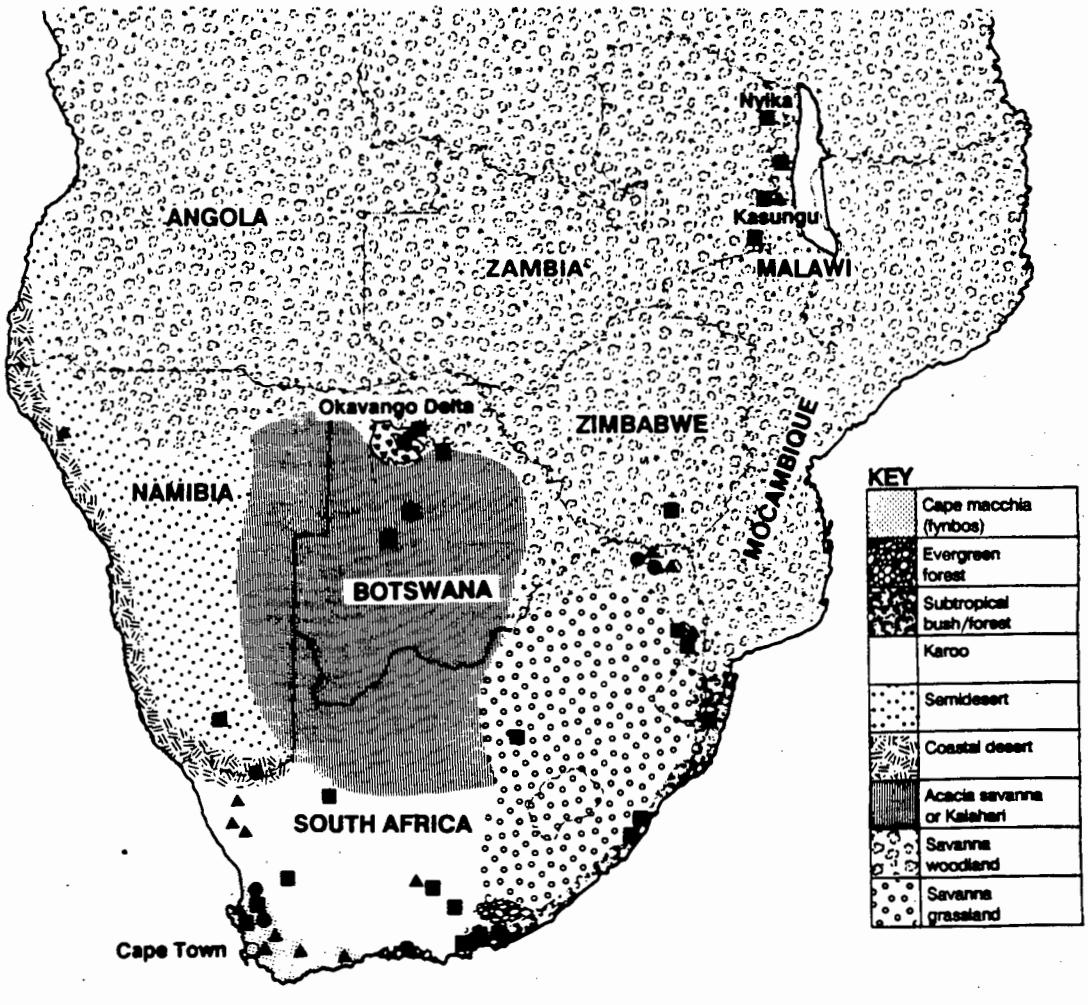

FIGURE 3 Map of southern Africa showing vegetation zones and the approximate location of faunal samples.

remaining adhering tissues removed and air-dried. Where both collagen and apatite were to be analysed, as is the case for many of the modern faunal specimens, specimens were subdivided and the two phases prepared separately. Where possible, collagen and apatite from both tooth and bone of the same individual were prepared to compare the results. In order to standardize the procedure as far as possible, the modern and fossil specimens were pretreated according to the same procedures, and therefore modern mineral specimens were also reacted in acetic acid. This treatment has the effect of dissolving the more soluble fractions of the mineral. Experiments to characterise the effects of acetic acid on modern minerals are described in the following chapter. All samples were cut into pea-sized pieces. The procedures used for collagen and mineral preparation are described below.

Collagen preparation

Collagen preparation followed the "whole bone" method normally used in the UCT Archaeometry Laboratory. The method is an adaptation of the procedure used for many years by radiocarbon chemists, and has been described elsewhere (Sealy 1986; Sealy *et al* 1987). More complex procedures exist (e.g. the gelatine method), but they introduce unnecessary complications for modern samples. The advantage of the "whole bone" method is that it is labour-saving and batches of samples can be processed at a time. Pieces of clean bone, or the whole root of a tooth, were decalcified in a solution of 0,2M HCL until reaction ceased (about a week). This leaves a decalcified isomorph, which after rinsing several times with distilled water, was placed in a mixture of methanol:chloroform:water (2:1:0.8 v/v) overnight to remove lipids (Bligh & Dyer 1959). The treatment was repeated for fatty bones.

After rinsing and freeze-drying, about 10 mg of dried collagen, accurately weighed, was introduced into a 9mm Vycor tube together with approximately 1g of wire-form CuO, 1g of wire-form Cu and a small piece (about 1x2mm^2) of Ag foil, following the procedure outlined by Sofer (1980). The CuO and Cu had been previously sieved

and baked at 500°C to oxidise organic material, and the Cu subsequently reduced under H_2 gas. The tube was evacuated, sealed and baked at 800°C for 6 hours. The tubes were allowed to cool slowly to room temperature over 24 hours to prevent the formation of nitrogen oxides (Sofer 1980).

The sample gas was introduced into a stainless steel vacuum line using an adaptation of a DesMarais-Hayes tube-cracker (DesMarais & Hayes 1976). Clean, dry CO_2 was collected by cryogenic separation, using liquid nitrogen and a slurry of ethanol and dry ice. Gas yields were measured manometrically. The $^{13}C/^{12}C$ ratios were measured in a VG Micromass 602E dual-inlet ratio mass spectrometer against a secondary reference gas calibrated against NBS standards 16,17 18, 19, and 20. $\delta^{13}C$ values were calculated relative to the PDB standard, according to the relationship:

$$\delta^{13}C_{sample}\ (‰) = (R_{sample}/R_{ref} - 1) \times 1000$$

Normal corrections for ^{17}O and machine factors were applied to the calculations.

Mineral Preparation

For tooth samples, enamel and dentine was separated by hand with the aid of jeweller's side-cutters and a scalpel. For bone, clean, cortical bone was cut into pea-sized fragments. The fragments were ground in a Spex Model 7600 freezer mill under liquid nitrogen. In order to remove organic material, 40ml of 2% (v/v) sodium hypochlorite ($NaHCLO_3$) solution was added to 1g of powdered sample in a 50ml centrifuge tube, stirred and allowed to react. The reaction was allowed to continue until effervescence ceased, during which time the mixture was frequently stirred and the $NaHClO_3$ solution twice renewed. After centrifuging, the supernatent was discarded, and the powder alternatively washed with distilled water and centrifuged, until the supernatent was neutral. 40ml of 1M acetic acid (CH_3COOH) was added to the anorganic powder, stirred, and allowed to react

overnight. The resultant material, called apatite here for convenience, was again washed and centrifuged until neutral as before, and freeze-dried.

Between 0.125 and 0.150g of apatite was weighed and introduced into one arm of a reaction vessel (Figure 4). Following the method of McCrea (1950), 3-4ml of 100% phosphoric acid (H_3PO_4) was introduced into the other arm, under vacuum, and the vessel was then removed and evacuated again. With the stopcock closed, the vessel was allowed to equilibrate for 30 minutes at 25°C in a constant temperature waterbath; the acid was then tipped over and allowed to react with the apatite powder. The reaction took several days to complete, when effervescence finally ceased, but the larger fragments frequently did not dissolve in the acid. Clean, dry CO_2 was collected by cryogenic separation in a stainless steel vacuum line (Figure 4), and the $^{13}C/^{12}C$ ratios measured as described above. A schematic representation of apatite preparation is shown in Figure 5.

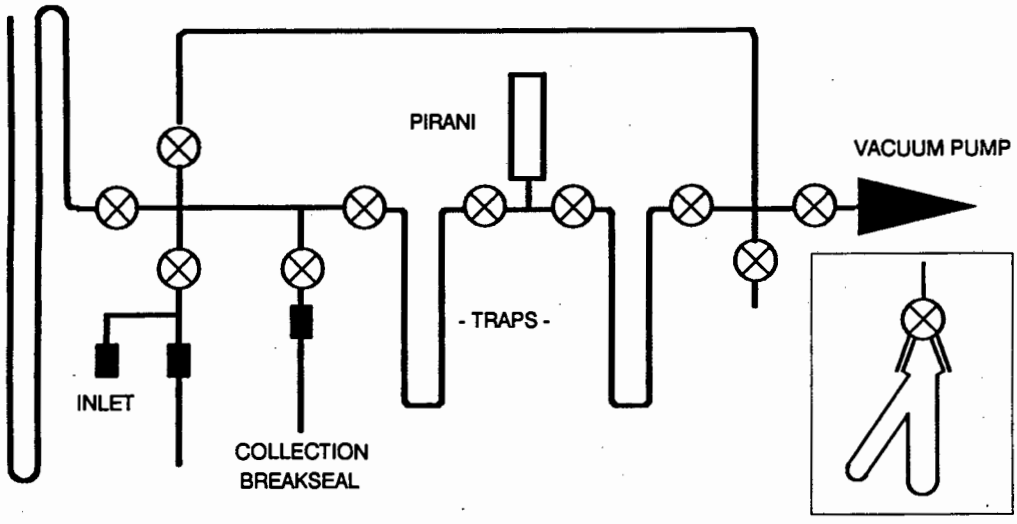

FIGURE 4 The vacuum line (inset reaction vessel).

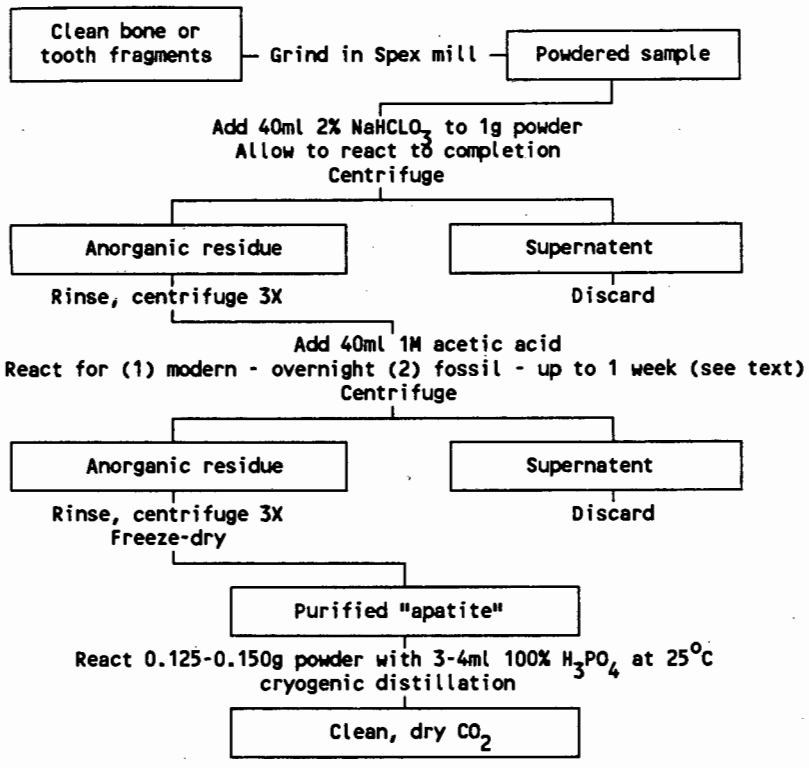

FIGURE 5 Scheme of apatite preparation.

RESULTS

Browsers and grazers

The results for collagen and apatite of the modern browsers and grazers are reported in Tables 1 and 2 respectively. Values obtained for collagen and apatite of some of the kudu specimens (Appendix) indicate that this animal is not always a pure C_3 browser. In more extreme environments (e.g. Keetmanshoop, Namibia) it seems to eat some grass, and in the eastern Cape Province the enriched values may be related to the abundance of an edible CAM shrub, *Portulucaria afra*. Elephants in this region also eat these shrubs (van der Merwe *et al* 1988), but the bushbuck, duiker and grysbok apparently do not (Table 1). Accordingly the kudu has been excluded for the purposes of the calculation of a mean for reliable C_3 browsers. The mean value for browser $\delta^{13}C_{apatite}$ values is calculated as -14.5 ± 1.6‰ (n=31),

and for $\delta^{13}C_{collagen}$ as -21.4 ± 1.1‰ (n=32)(Table 1). Similarly the mean $\delta^{13}C_{apatite}$ value for C_4 grazers is calculated as -0.5 ± 1.3‰ (n=16) and for $\delta^{13}C_{collagen}$ as -8.0 ± 1.1‰ (n=24) (Table 2).

Paired collagen and apatite $\delta^{13}C$ values, all animals

The results are shown in Figure 6 as plots of $\delta^{13}C_{apatite}$ vs $\delta^{13}C_{collagen}$ for herbivores (a), carnivores (b) and omnivores (c) respectively. The $\delta^{13}C$ values are reported in Appendix. A linear regression was calculated for each group of fauna, as follows.

(a) Herbivores: $\delta^{13}C_{apatite} = 7.8 + 1.06 \times \delta^{13}C_{collagen}$

n = 67, R^2 = 0.95, std error of y est. = 1.32

(b) Carnivores: $\delta^{13}C_{apatite} = 3.4 + 0.94 \times \delta^{13}C_{collagen}$

n = 49, R^2 = 0.93, std error of y est. = 0.99

(c) Omnivores: $\delta^{13}C_{apatite} = 6.4 + 1.07 \times \delta^{13}C_{collagen}$

n = 17, R^2 = 0.93, std error of y est. = 0.85

Comparison of Figure 6a and b illustrates the difference between the trophic levels: the relationship between collagen and apatite $\delta^{13}C$ values is clearly different for carnivores and herbivores. The omnivores fall between the herbivores and carnivores, as expected. Mean differences between $\delta^{13}C_{apatite}$ and $\delta^{13}C_{collagen}$ (Δ) are: 6.8 ± 1.35‰ for herbivores, 5.2 ± 0.8‰ for omnivores and 4.3 ± 1.0‰ for carnivores. The results are similar but not identical to those obtained by Krueger and Sullivan (1984), who suggested that the slope of the relationship between $\delta^{13}C_{apatite}$ and $\delta^{13}C_{collagen}$ was unity. In this study, the slope of equation (a) is 1.06 and that of equation (b) is 0.94. While neither of these slopes differs much from 1, they are significantly different from each other, as indicated by a standard statistical test for comparing slopes (t = 8.81; p = 0.05; d.f. = 112) (Moore *et al* 1972:59-61).

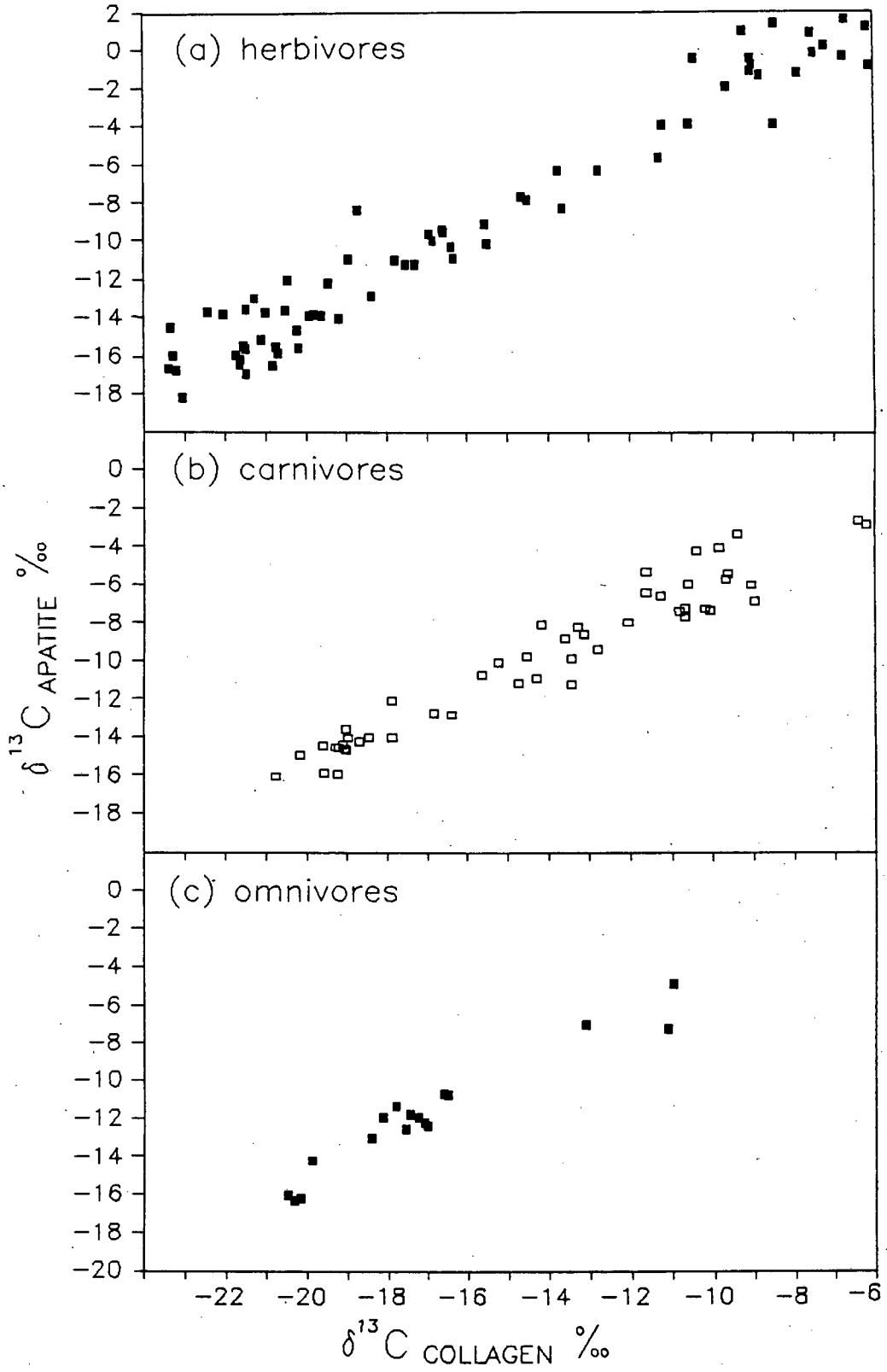

FIGURE 6 $\delta^{13}C_{collagen}$ versus $\delta^{13}C_{apatite}$ for (a) herbivores, (b) carnivores and (c) omnivores.

As a result of the slight difference in the slopes, the regression lines tend to converge at the C_3 end of the range and diverge towards the C_4 end. There is some overlap in the distribution of values for herbivores and carnivores, notably in the lower range of the C_3 spectrum, but this overlap diminishes with increasing $\delta^{13}C$ values. Combined with the fact that the slopes of equations (a) and (b) are significantly different from each other, these observations suggest that Δ is not constant for a given trophic level and varies slightly with environment. For the calculation of apatite $\delta^{13}C$ values from collagen $\delta^{13}C$ values, or *vice versa*, the appropriate equation should be used rather than simple addition (or subtraction) of Δ.

$\delta^{13}C_{collagen}$ and $\delta^{13}C_{apatite}$ values for tooth and bone of the same individual animal are compared in Table 3. There are small isotopic differences between the two tissues for individuals, but these do not show any consistent pattern. Thus, for the purposes of this study, bone and tooth $\delta^{13}C$ values may considered to be interchangeable.

Discussion

Although Krueger and Sullivan (1984) reported a slope for their equations of 1, Schoeninger & DeNiro (1982) pointed out that the slope obtained from the data reported in Sullivan & Krueger (1981) was in fact 1.07. In fact, the differences are probably due to the fact that Krueger & Sullivan (1984) fitted their data to an equation based on a foodweb flow model (Figure 7a), instead of deriving the equations from the data, as was done here. The foodweb models (Figure 7a) used in the derivation of their equations contain some simplifications. For instance, carnivore dietary protein $\delta^{13}C$ values were equated with herbivore collagen values. Meat, the major source of carnivore dietary protein, however, is depleted by 2-3‰ with respect to collagen from the same animal (DeNiro and Epstein 1978a; Vogel 1978b; Sealy 1986). Herbivore lipids are probably slightly more depleted than the assumed (X-1)‰; about (X-3) is more likely (see Vogel 1978b). They assumed that there are no secondary fractionation steps between herbivore protein and carnivore

collagen. Data for fauna from the southwestern Cape, summarised in Figure 8, indicate that the conversion of herbivore tissues into carnivore collagen does involve some additional fractionation. The southwestern Cape flora is predominantly C_3 so that the mean herbivore dietary base is about -26‰ (Sealy 1986). The herbivores have mean $\delta^{13}C$ values of -21‰ for collagen and -23.5‰ for meat, while the mean for carnivore collagen is -19.0‰. Since meat is the main source of protein for carnivores, this represents a secondary fractionation for protein of about +4-5‰. Both herbivores and carnivores have similar apatite values, about -14‰. In terms of Krueger & Sullivan's model (Figure 7a) this represents (X+12)‰ in contrast to the (X+8)‰ they suggested.

Krueger & Sullivan's flow chart appears in revised form in Figure 7(b) to take account of these changes. (To be even more precise, the plant food base should also be separated into proteins, carbohydrates and lipids, but the result is very unwieldy). Using the revised diagram, similar Δ values are derived for herbivores and carnivores as before, but the fractionation for protein synthesis is about +4 to 5‰ and diet-energy fractionation is +12‰, for both trophic levels. This implies that the isotopic values of dietary protein and dietary fuel from which the bone is derived can be calculated separately from the collagen and apatite values, whatever the trophic level.

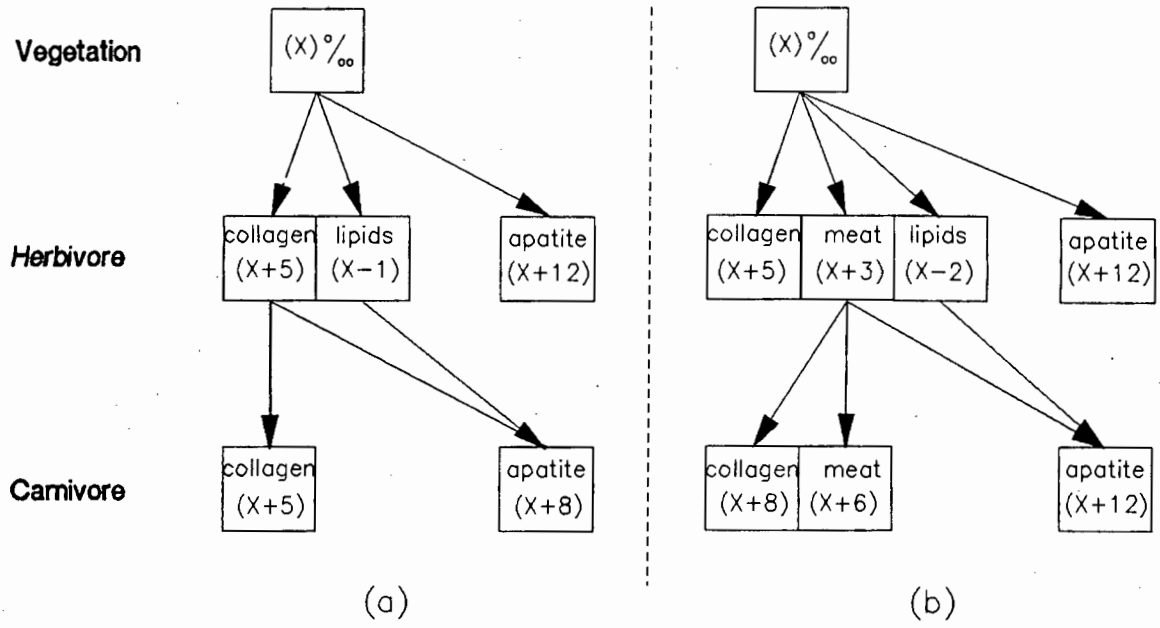

FIGURE 7 Stable carbon isotopes in foodwebs, showing (a) Krueger and Sullivan's foodweb model [adapted from Krueger and Sullivan (1984)] and (b) revised model.

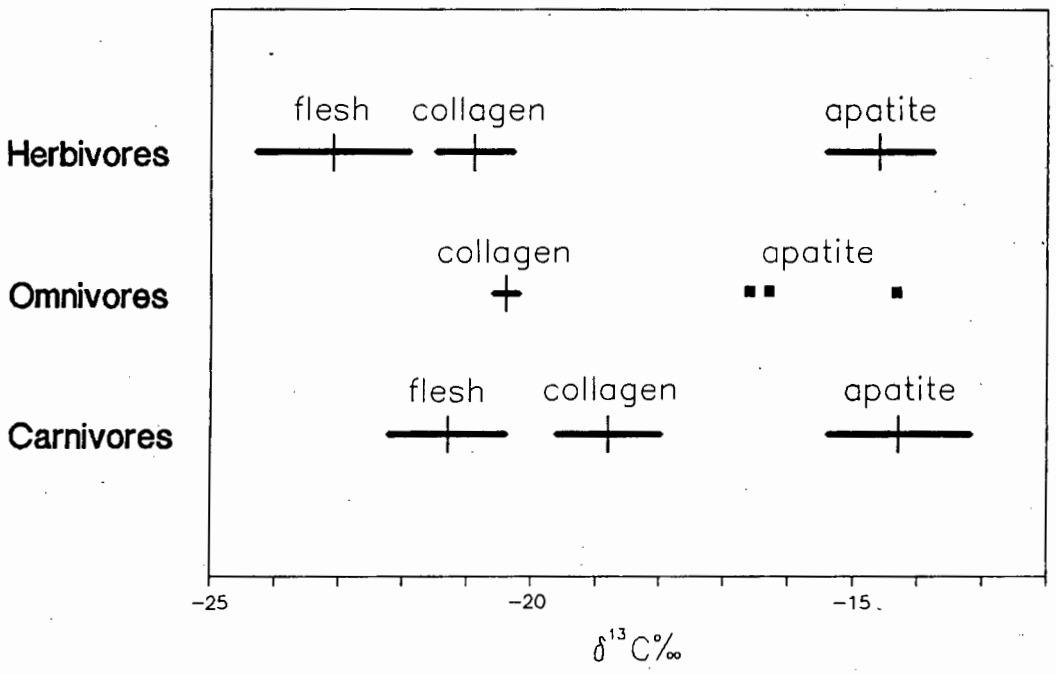

FIGURE 8 Diagram of the ranges of $\delta^{13}C$ values for fauna at different trophic levels from the southwestern Cape Province, South Africa.

IMPLICATIONS AND CONCLUSIONS

The results obtained in this study confirm that the difference between $\delta^{13}C_{apatite}$ and $\delta^{13}C_{collagen}$ is smaller for carnivores than for herbivores, and thus provide support the Krueger & Sullivan's model, which proposes that apatite and protein are related to different aspects of diet. There is a range of Δ values for both groups, however, and some overlap between trophic groups. At least some of the values observed by Schoeninger and DeNiro (1982) are the result of natural phenomena and not necessarily evidence of diagenesis.

The difference between $\delta^{13}C_{apatite}$ and $\delta^{13}C_{collagen}$ values is potentially useful as an index of the importance of meat in prehistoric human diets (ie the relative degree of "herbivory" or "carnivory"). In carnivorous diets the increased use of isotopically depleted lipids as body fuel produces depleted plasma bicarbonate and consequently depleted bone carbonate, hence reducing Δ. Human diets are much more complex and varied, however, and deriving a relative herbivory/carnivory index may not be simple. For instance, a dietary combination of isotopically enriched (e.g. marine) proteins and depleted lipids and/or terrestrial carbohydrates, would also produce reduced Δ values.

The model presented here involves a number of simplifications and assumptions about foodweb fractionations and pathways. These require further investigation if a combination of collagen and apatite $\delta^{13}C$ values is to provide really useful information about prehistoric human diets.

CHAPTER 6

ALTERATION OF APATITE ISOTOPIC VALUES WITH TIME

In this chapter the study for evaluating the stability of stable carbon isotope values from fossil apatites is described. The approach is to measure apatite $^{13}C/^{12}C$ ratios for animals of known diet (browsers) in a chronological sequence, to determine whether or not a difference exists between these animals and their modern counterparts. Any differences which there might be can reasonably be ascribed to exchange with matrix carbonates, although other factors might contribute in a minor way. A brief description of the archaeological sites and the fossil fauna obtained from these sites, is given below. The methods used for the pretreatment and preparation of CO_2 gas samples from the fossil specimens are described. The results indicate that some degree of exchange of biogenic apatite carbonates with those of the matrix occurs, but that this alteration is predictable and not so extensive as previously believed.

INTRODUCTION

The approach for measuring the degree of isotopic alteration over time, using carbon isotopic values from apatite of animals with known diet in a chronological series, is outlined and the results are reported below. The test concentrated on browsers, since browser apatite and matrix $\delta^{13}C$ values are generally distinct by at least 10‰, if the matrix carbonates are derived from marine carbonates such as dolomites or limestone, with $\delta^{13}C$ values close to 0‰ (Veizer & Hoefs 1976). Grazers do not provide such a distinction, since their apatite values are generally close to 0‰ in C_4 grass areas, and thus are similar to matrix values Where grazers

are abundant in the faunal assemblages (as they are in the inland sites), they are included in the analyses, since they provide a distinct internal comparison for the browsers at each site. A description of the sampling program and laboratory procedures follows.

METHODS

Sampling Program

The archaeological faunal specimens included in this study were drawn from sites differing in age and locality (Figure 2). Brief descriptions of these archaeological sites are given below.

Mpambanyoni is an early Iron Age midden on the Natal coast, dating to approximately 950 BP (Robey 1980). Bone samples of *Tragelaphus scriptus* (n=2) and *Cephalophus monticola* (n=3) were obtained from this site.

Klasies River Mouth (KRM) is a complex of cave sites on the south-eastern Cape Province coast (Singer & Wymer 1982; Deacon *et al* 1986; Deacon & Geleijnse 1988). KRM has attracted attention on account of the fragmentary remains of anatomically modern *Homo sapiens* found in an last interglacial context, and also provides early evidence for human exploitation of marine foods (Voigt 1973; Klein 1976; Singer & Wymer 1982; Deacon et al 1986; Thackeray 1988). The caves were wave-cut in Table Mountain quartzite (Butzer 1978), but there are numerous speleotherms and flowstones present in the cave system. The calcium carbonate is believed to percolate from older shell-rich aeolian deposits on the cliffs above the caves (Butzer 1978; Deacon & Geleijnse 1988). The earliest occupation layers are associated with a substage of the Middle Stone Age (MSA) stone tool assemblages designated as MSA I by Singer & Wymer (1982). KRM has a complex stratigraphy and the focus of occupation apparently shifted from KRM 1 to adjacent KRM 1A

and 1B at some stage and back again. MSA I and II substages are present in KRM 1; and the MSA II, Howieson's Poort (HP) and MSA III substages in KRM 1A.

Dating has been a problem at KRM, since the MSA deposits fall beyond the range of radiocarbon dating. Age estimations have been based on various lines of evidence, including oxygen isotope ratios from measurements of marine molluscs (Shackleton, in Singer & Wymer 1982). More recently a uranium series date of 110 ka has been obtained from the base of a speleotherm in Cave 1 (Deacon & Geleinjse 1988). Evidence currently available suggests that the MSA deposits include part or all of oxygen isotope stage 5, with evidence of human occupation some time after 125 ka BP, lasting (not necessarily continuously) until about 60 ka BP (Butzer 1978, Singer & Wymer 1982). After a long hiatus, human occupation resumed in the mid-Holocene with the accumulation of a large shell midden and associated Later Stone Age (LSA) industries, for which radiocarbon dates of about 4500 and 2500 years BP were obtained (Singer & Wymer 1982).

A series of fragmentary bone and tooth specimens of *Tragelaphus scriptus* and *Raphicerus* sp. was obtained from KRM 1. These fauna had been previously identified by Klein (1976). While most of the *Raphicerus* specimens are *R. melanotis*, a reliable browser, a few may have belonged to the related *R. campestris* species, which also includes some grass in its diet. Only two of the specimens are from the LSA midden, the rest are from layers associated with the MSA occupation period (Table 3). In addition, a series of deposit and flowstone samples were taken from various levels at KRM 1 (n=13), in order to determine the range of $\delta^{13}C$ values for the deposit.

At *Die Kelders* cave, on the south coast of the Cape Province, MSA human occupation apparently began somewhat later during the early part of the last Glacial period. The cave was formed along an unconformity between Table Mountain sandstone and the Bredasdorp marine limestone formation (Tankard & Schweitzer

1974, 1976). The MSA occupation continued intermittently until about 45 or 35 ka ago (Tankard & Schweitzer 1974, 1976; Butzer 1979), and the stone tool industry has been assigned as MSA 3 by Volman (1984). In the absence of any radiocarbon dates for these deposits, dating is tentatively based on the geomorphology. After a long hiatus, human occupation recommenced with the appearance of pottery and Later Stone Age stone tool assemblages at about 2 ka BP. The earliest stratified occurrence of domesticated sheep in the Cape Province appears in these upper levels (Schweitzer & Scott 1973). Specimens of *T. scriptus* (n=3) and *R. melanotis* (n=11) were obtained for analysis; 12 are from MSA levels, and 2 from the LSA deposits (Table 3).

The period of occupation at *Border Cave*, an inland site on the western scarp of the Lebombo mountains between Swaziland and Natal (Figure 2), is broadly similar to that of KRM. Border Cave has also yielded potentially very early specimens of anatomically modern *Homo sapiens* (Beaumont *et al* 1978), but their context is doubtful because the fossils were not found *in situ*. Occupation began early in the last Interglacial, and continued well into the last Glacial period (Beaumont *et al* 1978). Again, dating is a problem. Some radiocarbon dates were obtained for the terminal MSA 3 of between about 33 and 48 ka BP (Beaumont *et al* 1978). Beyond this, dating is based on geomorphological estimates (Butzer *et al* 1978). The stone tool assemblages have been assigned to MSA 2a & 2b, HP and MSA 3 by Volman (1984). The MSA is followed by an early LSA assemblage, then a hiatus and finally some Iron Age material (Beaumont 1973; Beaumont *et al* 1978). Faunal analysis of the macromammals (Klein 1977) and micromammals (Avery 1982) has provided some indications of changes in climate and vegetation. Few browsers occur in the faunal assemblage. These consist of *T. scriptus* and *Oreotragus oreotragus*, both reliable browsers, and *Tragelaphus strepsiceros* and *Taurotragus oryx*, which are not consistent browsers. Specimens of the grazers, which dominated the assemblage,

were obtained for comparison with the browsers. They were *Equus burchelli*, *Syncerus caffer*, *Connochaetes taurinus*, *Hippotragus* sp. and *Redunca fulvorufula*. Details of the periods sampled, ranging in age from about 33 to 100 ka BP, are shown in Table 5.

Specimens were also obtained from the older Transvaal sites of Makapansgat and Sterkfontein. These sites are calcified cave-fillings associated with karst formation in the Pre-Cambrian dolomitic limestone (Butzer 1971; Brain 1975, 1981; Partridge 1978, 1979), and have yielded remains of early hominids and considerable quantities of early and later Pleistocene fauna. The sites have passed through the same general stages of formation, but the form and depositional history of the individual sites is complex. Unlike the east African hominid sites, the Transvaal sites cannot be dated by radiometric methods. The taphonomic history and age of the hominid and faunal remains has therefore presented considerable problems.

The *Makapansgat Limeworks* is considered to be the oldest of these sites (Vrba 1976, 1982; Cooke 1978; Delson 1984). Member 3, a richly fossiliferous grey breccia, contains an extremely dense concentration of fauna, which includes *Australopithecus africanus*. The density and particular set of body parts led Dart to propose an "Osteodontokeratic culture" for australopithecines (Dart 1957). Since then, taphonomic studies have shown that agents other than hominids are likely to have contributed to the accumulations (Brain 1975, 1980, 1981). Maguire *et al* (1980) suggested that the bone accumulation in Member 3 originally derived from hyena dens and was subsequently concentrated by water action. Palaeomagnetic dating brackets Member 3 between 2,9 and 3,2 ma (McFadden *et al* 1979), dates which are supported by biostratigraphic studies based on primates (Delson 1984). Similar studies based on the Bovidae initially suggested a younger age of about 2.6-2.8 ma (Vrba 1975, 1976), but this estimate has since been revised to "close on" 3 ma (Vrba 1982). The faunal specimens used in this study were from Member 3 only (Table 6).

Browsers included *Sivatherium* sp. (giraffoids), *Giraffa camelopardalis*, *Ancylotherium hennigi* (a chalicothere), and *Cephalophus pricei*. Two specimens of grazers, *Hipparion* sp. and an equid, were obtained for comparison. Since many of the faunal species at Makapansgat are extinct, dietary assignments are based on palaeontological assessments obtained in discussions with Elizabeth Vrba (pers. comm.). Many of the fossils from Member 3 have well developed calcite crystals in bone and tooth cavities (Maguire *et al* 1980), and the specimens obtained for this study are no exception (Figure 9). The phenomenon is ascribed to the presence of supersaturated solutions of calcium carbonate at the time of formation (Wells 1971). These crystals were extracted for separate isotopic analysis.

Figure 9 *Cephalophus pricei* (UCT# 1878, M 124)) from Makapansgat Member 3, showing well-formed crystals of calcite within the mandible.

Sterkfontein is believed to be slightly younger than Makapansgat. The oldest (hominid-bearing) stratum, Member 4, is about 2.5 ma old, based on biostratigraphic studies of the Bovidae (Vrba 1974, 1975, 1982). It contains *Australopithecus africanus* remains, and the younger Member 5 some specimens tentatively assigned to *Homo*. It would be logical to find *A. robustus* in Member 5, since it occurs at roughly the same time as Swartkrans Member 1 which contains many *A. robustus* specimens, but so far no specimens have been identified (Vrba 1982; Clarke 1988). Provenance of the small number of tooth specimens obtained for this study is uncertain, since they are all "Dump" samples from the spoil heaps of earlier excavations. The sample includes *Tragelaphus scriptus* and *Connochaetes taurinus* from Dump 16 (D16, probably Member 6), which is considered to be about 150 ka old (Vrba 1982), *T. strepsiceros* from D8 (cf. Member 4, about 2.5 ma), *Taurotragus oryx* from D13 (cf. Member 4),and *Connochaetes taurinus* from dump H2 (cf Member 5, about 2 ma) (Table 6). One fragment of breccia from D13 (cf. Member 4) was obtained for comparison with the fauna.

Laboratory procedures

Collagen preparation

Collagen was only extracted from two of the most recent archaeological specimens, from Mpambanyoni. The more recent specimens from KRM (UCT# 1024) and Die Kelders (UCT#'s 695 and 696) may have contained collagen, but the samples were too small to allow the preparation of both apatite and collagen. The rest of the specimens were far too old to contain any collagen. Preparation methods were the same as for modern collagen, outlined in Chapter 5, except that the lipid removal step was dispensed with (fossils are not fatty). Instead, a step was inserted for removal of humates by immersion of the decalcified isomorph in 0.1M NaOH until the solution remained colourless, followed by several rinses with distilled water.

Combustion of the material, and collection and measurement of the CO_2 followed the methods outlined in Chapter 5.

Apatite preparation

Fossil apatite was recovered from bone, tooth enamel and in a few cases, dentine. Where possible, these were prepared from the same individual, in order to compare the results for the different tissues. All specimens were initially cleaned by removing adhering breccia or matrix with a scalpel. Enamel was separated from dentine as described for modern specimens. Any remaining deposit was removed with a scalpel, and calcite crystals found in the interstices of teeth and bones were removed for separate analysis. Discoloured outer layers were scraped off and discarded, as were those parts of the bone samples which were heavily impregnated with breccia. The fragments were treated in acetone for several hours to remove glyptol, and dried before they were ground in a Spex mill under liquid nitrogen. The procedure followed was similar to that used for the modern sample, with the following differences. Less sample (about 0.5g) was used: this was usually dictated by the small size of the fossil specimens available. Such small amounts provide sufficient purified apatite for isotopic analysis, because they include no organic material. Fossil specimens were allowed to react overnight in $NaHCLO_3$ solution, in order to remove traces of bacterial proteins and humates. The acetic acid treatment was more rigorous: samples were left to react for a longer period (up to one week), and if effervescence continued, the reaction was encouraged by sonication until effervescence ceased. Reaction with H_3PO_4 and collection and measurement of the CO_2 followed the procedure outlined in Chapter 5.

Deposit, breccia and calcite

These samples were ground in a mortar and pestle, and allowed to react with $NaHCLO_3$ solution overnight or until reaction ceased, as described for apatite

samples. After rinsing well in distilled water and freeze-drying, CO_2 was produced by reaction with phosphoric acid, collected and measured as described above.

RESULTS

The $\delta^{13}C$ values obtained for various species of fossil browsers and grazers, are reported in Tables 4-8, for Klasies River Mouth, Die Kelders, Border Cave, Makapansgat and Sterkfontein,and Mpambanyoni respectively. Unfortunately, some of the *Raphicerus* specimens from KRM may not be the browsing species *R. melanotis*, but its close relative *R. campestris*, a species which includes some grass in its diet. This would result in somewhat more positive values, a shift caused by diet and not by diagenesis. The two collagen determinations from Mpambanyoni are also reported in Table 8; these values are consistent with browser diets. Means have not been calculated for $\delta^{13}C_{apatite}$ values of the browsers and grazers, since they are drawn from different time periods, and it the relationship with time which is of interest here. In Figure 10 the $\delta^{13}C$ values for the fossil browsers from all the sites are shown arranged in chronological order. Only the reliable browsers are included in Figure 10; thus *T strepsiceros* and *T. oryx* from Border Cave are excluded. $\delta^{13}C$ and $\delta^{18}O$ values from the various matrix specimens from all the sites for which specimens were obtained are reported in Table 9.

DISCUSSION

Contamination of browser apatites with carbonates dissolved in or precipitated from groundwater should cause a shift in their $\delta^{13}C$ values towards that of the matrix, that is, in a positive direction. A shift towards more positive values is readily apparent both from the results in Tables 4-8 and in Figure 10. The shift away from the modern mean apparently increases with age, although the relationship does not appear to be linear. (In order to fit the entire time-scale into Figure 10, there is a

break between 150 ka and 1 ma and the scale changes. This may give a misleading impression that the relationship between the browser apatite $\delta^{13}C$ values and time is linear.) Even recent browser apatite $\delta^{13}C$ values have shifted somewhat towards more positive values, although they fall within one standard deviation of the modern browser mean. For the specimens from MSA strata, dated between about 50-120 ka, most of the values fall just outside this range, and on average, differ from the modern mean by about +2-3‰. Change after this period seems somewhat slower, since specimens millions of years older (Sterkfontein and Makapansgat) have only shifted by another +1-2‰.

Not surprisingly, the oldest (3 ma) specimens from Makapansgat have the most positive $\delta^{13}C$ values. It is possible, however, that the size of the shift might also be related to depositional conditions in Member 3. Many of the specimens from the grey Member 3 breccia contain well-developed calcite crystals (Maguire *et al* 1980,

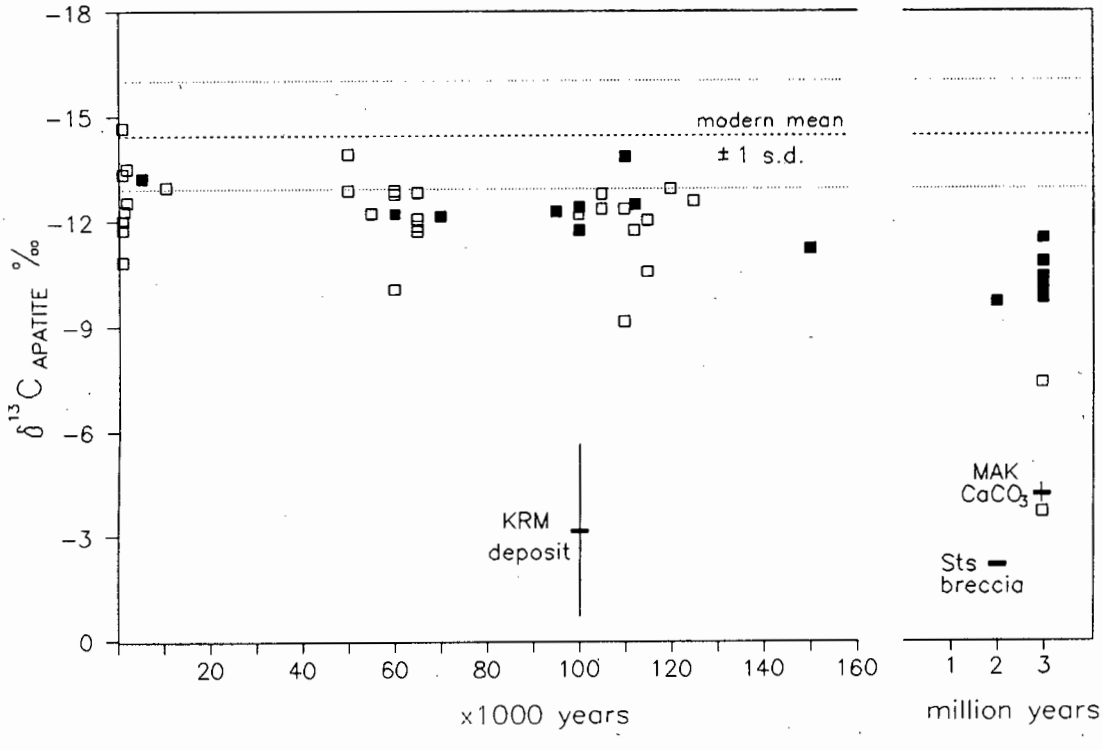

FIGURE 10 $\delta^{13}C$ values of browsers in a chronological sequence. Open symbols represent bone apatite, closed symbols tooth enamel apatite.

this thesis: Figure 9). It has been proposed that the breccia formed from a super-saturated solution of calcium carbonate (Wells 1971; Maguire *et al* 1980). Such conditions provide a "worst-case" scenario for alteration of apatite. One approach to resolving this problem would be a comparison of results from similar fauna from another site of similar age but different depositional circumstances.

There appears to be little difference between modern grazers (Table 2) and fossil grazers from the inland sites. If matrix carbonates are the main cause for alteration of biogenic apatite carbonate values, this is to be expected, since biogenic $\delta^{13}C$ apatite values for grazers are close to matrix values. For the fauna from Border Cave in particular, the fossil grazers have, on average, slightly more positive carbon isotope values. This positive shift, although slight, is unexpected, since one would have assumed a shift towards the slightly more negative matrix values. (Although matrix values for Border Cave could not be determined, it is extremely unlikely that values for this site are more positive than for the other sites reported in Table 9). Possibly the fossil grazer $\delta^{13}C$ values reflect slightly more positive dietary values, as might occur if the C_4 grass cover was more absolute than today (e.g. 100% instead of 95%); or if the ancient C_4 grasses had slightly more positive $\delta^{13}C$ values than present-day values either as a result of different environmental conditions (Smith & Boutton 1981; Tieszen 1988); or because the present-day values are negatively skewed as a result of the burning of fossil fuels. This last possibility was discussed earlier in Chapter 4. Another possibility is that a shift towards more positive values has occurred during diagenetic processes more complex than simple direct contamination with matrix carbonates, for instance, fractionation during crystal growth and recrystallisation.

At this stage, alternative explanations (referred to in Chapter 4) for the enriched values for fossil browser apatite must be considered. The enriched browser apatite $\delta^{13}C$ values might be due to atmospheric CO_2 more enriched in ^{13}C compared to

that of today. This could occur in two ways. Modern browser apatite $\delta^{13}C$ values may be unusually depleted as the result of depleted CO_2 in the present-day atmosphere, caused by the burning of fossil fuels. Such an effect could not be very large, however. Furthermore, collagen should also be affected, but there seems to be no evidence that present-day browser collagen values are different from those a few hundred years ago. Another explanation could be sought in the fluctuation of atmospheric CO_2 concentrations during glacial and interglacial periods, as shown by ice-core studies (Berner *et al* 1978, 1980; Broecker *et al* 1985; Barnola *et al* 1987; Jouzel *et al* 1987). These periods might have CO_2 with different carbon isotope ratios. The two current models for the carbon cycle predict different effects for the carbon isotope ratios, but there are no data available at present to test either one of them. If there is an isotopic change as a result of changing concentrations of atmospheric CO_2, it follows that there should be a glacial-interglacial periodicity in such isotopic fluctuations: interglacials should be similar and glacials similar. The data most pertinent to this prediction are from KRM, where the specimens span parts of the last Interglacial, grading into the last Glacial, and the present Interglacial periods. The results for the two interglacial periods are clearly different, and there is no noticeable difference between specimens from the last interglacial and early last glacial periods (Table 4 and Figure 10). Thus, the data indicate that post-depositional isotopic exchange with matrix carbonates is the dominant effect, and any atmospheric CO_2 isotopic fluctuations that there might be are too small to be apparent in this data set.

SUMMARY

Most importantly, however, regardless of the cause of enrichment or diagenesis, a large carbon isotopic difference continues to exist between browsers and grazers even for the oldest specimens from Makapansgat. This observation only holds for enamel, however, since 3 ma old bone specimens from Makapansgat no longer

reflect dietary values (Table 6). This suggests that enamel is the more reliable mineral tissue for isotopic analyses of older fossil material, and this subject is further investigated in the following chapter. The efficacy of the acetic acid pretreatment procedure for removing diagenetic carbonate fractions is also evaluated in the following chapter. The change (enrichment) is consistent within a given time range, and is not a random process. The isotopic difference between modern and fossil browsers increases with time, but the change is not linear. Rather there seems to be an initial rapid change soon after deposition (i.e within a few ka), followed by slower change within in the next ma or so. This implies that after the initial change, alteration is slowed by some mechanism(s), the possibilities of which are discussed in the concluding chapter.

CHAPTER 7

EXPERIMENTAL STUDIES

Some experimental studies for testing the efficacy of the methods used for eliminating diagenetic material and comparing the reliability of fossil apatite isotopic measurements from enamel, bone and dentine, are presented in this chapter. Although pretreatment with acetic acid has been the procedure most commonly used for eliminating diagenetic carbonates, a characterisation of the effects of this treatment on modern and fossil apatite has been neglected in previous studies. Hassan's study was an exception (Hassan 1975). Most workers have been content with showing that no calcite remains in fossil apatites after pretreatment. Therefore, the first section of this chapter is concerned with an evaluation of the isotopic and mineralogical effects of the standard acetic acid treatment on modern and fossil bone and enamel apatite. Methods are based on an examination of the isotopic results themselves and CO_2 gas yields, together with characterisation of the apatites by infrared spectroscopy. Finally, using the same sets of specimens and methods, the integrity of isotopic results from fossil enamel, bone and dentine are compared. The implications of the results for isotopic studies of fossil apatites are discussed in the concluding remarks.

AN EVALUATION OF THE EFFECTS OF ACETIC ACID PRETREATMENT

Modern mineral

The effects of the acetic acid pretreatment on modern bone and tooth enamel specimens have not been previously evaluated. This would appear to be an essential first step before moving on to evaluate the effects on fossil apatites. A study for

characterising some aspects of modern bone and tooth mineral before and after acetic acid treatment is described below.

Sampling and methods

The effects of acetic acid on modern bone and enamel specimens were evaluated by characterisation of some modern specimens of bone and tooth mineral before and after treatment with 1M acetic acid. The parameters measured included the $\delta^{13}C$ values, CO_2 gas yields and infrared absorption spectra on specimens before and after treatment with acetic acid. One bone specimen was monitored at intervals for two weeks in acetic acid. The specimens used for these tests were drawn from the sample of modern faunal material described in Chapter 5. Details of the species are given in Table 10.

The procedure was as follows. The bone and tooth specimens were prepared as described in Chapter 5 (Figure 5), up to the removal of the organic phase in $NaHCLO_3$ solution, followed by rinsing in distilled water. Some of the resultant anorganic wet powder was removed from the centrifuge tube and freeze-dried. This anorganic portion was analysed separately. The remaining material was reacted in the usual way with acetic acid as described in Chapter 5, and the treated apatite recovered for analysis. For one bone specimen (UCT# 684, *Diceros bicornis*) the reaction in acetic acid was allowed to proceed for two weeks, during which time portions and finally the residue, were recovered, rinsed and freeze-dried after 2 days, 7 days and 14 days respectively, for separate analysis.

CO_2 gas was produced by reaction with 100% H_3PO_4 acid, collected by cryogenic separation, and the $^{13}C/^{12}C$ ratios measured as described in Chapter 5. CO_2 gas yields were measured manometrically during the collection of the gas on the vacuum line, and are calculated as μmol CO_2 per mg dry material (μmol/mg). These calculated gas yields, although accurate for the amount of CO_2 gas introduced into the vacuum line, are not necessarily entirely accurate reflections of the CO_3^{2-}

content of the mineral, because larger fragments remained impervious to acid attack (probably because some collagen remained), and therefore not all of the CO_3^{2-} was released. This calculated yield is, however, suitable for providing relative measurements, as is done here.

Infrared spectroscopy is used to characterise the mineral before and after treatment, for several reasons. Firstly, apatites have a characteristic and unmistakable infrared spectrum, and this applies to biological apatites. Enamel infrared absorption spectra are similar but readily discernible from bone or dentine apatite spectra (LeGeros 1977). The presence of other minerals may be easily detected. Secondly, the sharpness of the spectrum and individual absorption bands indicates the crystallinity (crystal size and perfection) of the material. (A more accurate measure of crystallinity may be obtained by calculation of the splitting factor, or SF, from the splitting of the $\upsilon_4PO_4^{3-}$ band at 600 cm^{-1}, but this procedure was not considered necessary here.) Finally, the CO_3/PO_4 ratio indicates changes in the amount of CO_3^{2-} present in the apatite structure; any excess above the normal range for bone or enamel apatite indicates the presence either of a high-carbonate apatite or another carbonate compound. The CO_2 gas yield and the CO_3/PO_4 ratio are related and should yield similar results. The CO_3/PO_4 ratio may be calculated from the υ_3CO_3 absorption band at 1410cm^{-1} and the υ_4PO_4 band at 600 cm^{-1} (after LeGeros *et al* 1982)(Figure 11). All spectra were recorded on a Perkin Elmer 983 infrared spectrophotometer, as potassium bromide (KBr) pellets.

Results and discussion

The results, summarised in Table 10, show that differences exist between the untreated (anorganic) and the acid-treated mineral, for all the parameters measured. These are discussed below.

The isotopic changes are surprising in that $\delta^{13}C$ values after acetic acid treatment for 1 day are depleted by varying amounts. The difference before and after acid

treatment varies from -0.5 to -2.8‰. The pattern appears to be the same for bone and enamel of the same individual. For example, in UCT# 2424 the differences are small for both tissues, and in UCT# 2547 the differences are similar for bone and enamel (-2.6 and -2.7‰). The results for specimen UCT# 684, which was monitored at intervals during the continued reaction in acetic acid for two weeks, show consistent isotopic values after the first major change. After two weeks in acid the residue is more enriched in ^{13}C (Table 10).

There are accompanying changes in the CO_2 yields and the CO_3/PO_4 ratios between the anorganic and acid-treated mineral samples. Gas yields are substantially reduced after 1 day in acetic acid, and a similar pattern is seen for the CO_3/PO_4 ratios (Table 10) These changes are not unexpected, since a proportion of the total carbonate in apatite (the adsorbed carbonate) is more labile and reactive, and is not bound within the crystal structure, as discussed in Chapter 3. Furthermore, the

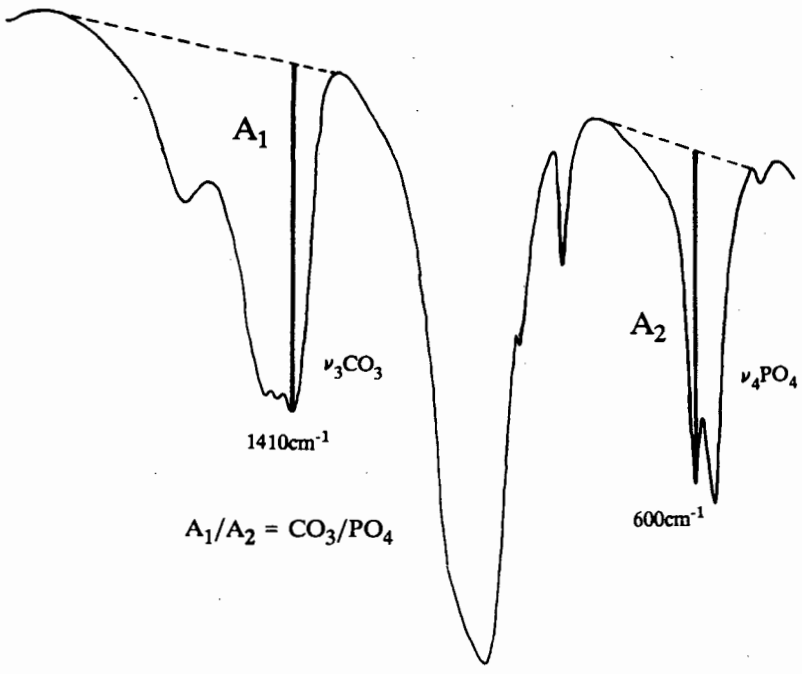

FIGURE 11 The derivation of CO_3/PO_4 ratios from the infrared absorption spectra.

smallest apatite crystallites are more soluble in acid solution. Since the latter also have higher carbonate contents, the apatite remaining after acetic acid treatment should consist mainly of the larger crystals, with carbonate confined to the "structural" (PO_4) position. The CO_2 gas yields and CO_3/PO_4 ratios for UCT# 684 are very small after only a few days in acetic acid, and negligible after two weeks. The reason for this becomes clear on examination of the infrared spectra. The CO_2 yield for anorganic bone is higher than for anorganic enamel, and the reduction after acid treatment is greater for bone than for enamel. These results reflect the lower carbonate content of enamel compared to bone, in agreement with other published work (LeGeros 1983).

Infra red absorption spectra for the specimens before and after acid treatment for 1 day are all typically apatitic, except that the carbonate υ_3 stretch at 1410cm^{-1} is reduced after acid treatment (Figures 12 and 13), resulting in the lower CO_3/PO_4 ratios. This is consistent with the removal of "adsorbed" rather than "structural carbonate. Spectra for enamel are sharper than those for bone, and show splitting of the carbonate out-of-plane bending mode (υ_2) at 870cm^{-1} (Figure 12). These are indications of the greater crystallinity of enamel (LeGeros 1983). A dramatic change is observed in the spectra of the bone specimen allowed to remain in acetic acid (Figure 13). After 2 days in acid, the spectrum indicates the presence of some brushite (DCPD, or dicalcium phosphate dihydrate), a related calcium phosphate mineral, as well as apatite (this spectrum is not illustrated for lack of space). After 7 days in acid, almost only brushite is visible, and the carbonate absorption bands almost disappear. The same applies for the residue after 14 days in acid. These spectra demonstrate recrystallisation to brushite. This phenomenon (recrystallisation to brushite in acid solutions) is well-known from apatite crystal chemistry studies (LeGeros 1983), and explains the dramatic reduction in CO_2 yields and CO_3/PO_4 ratios after a few days treatment in acetic acid.

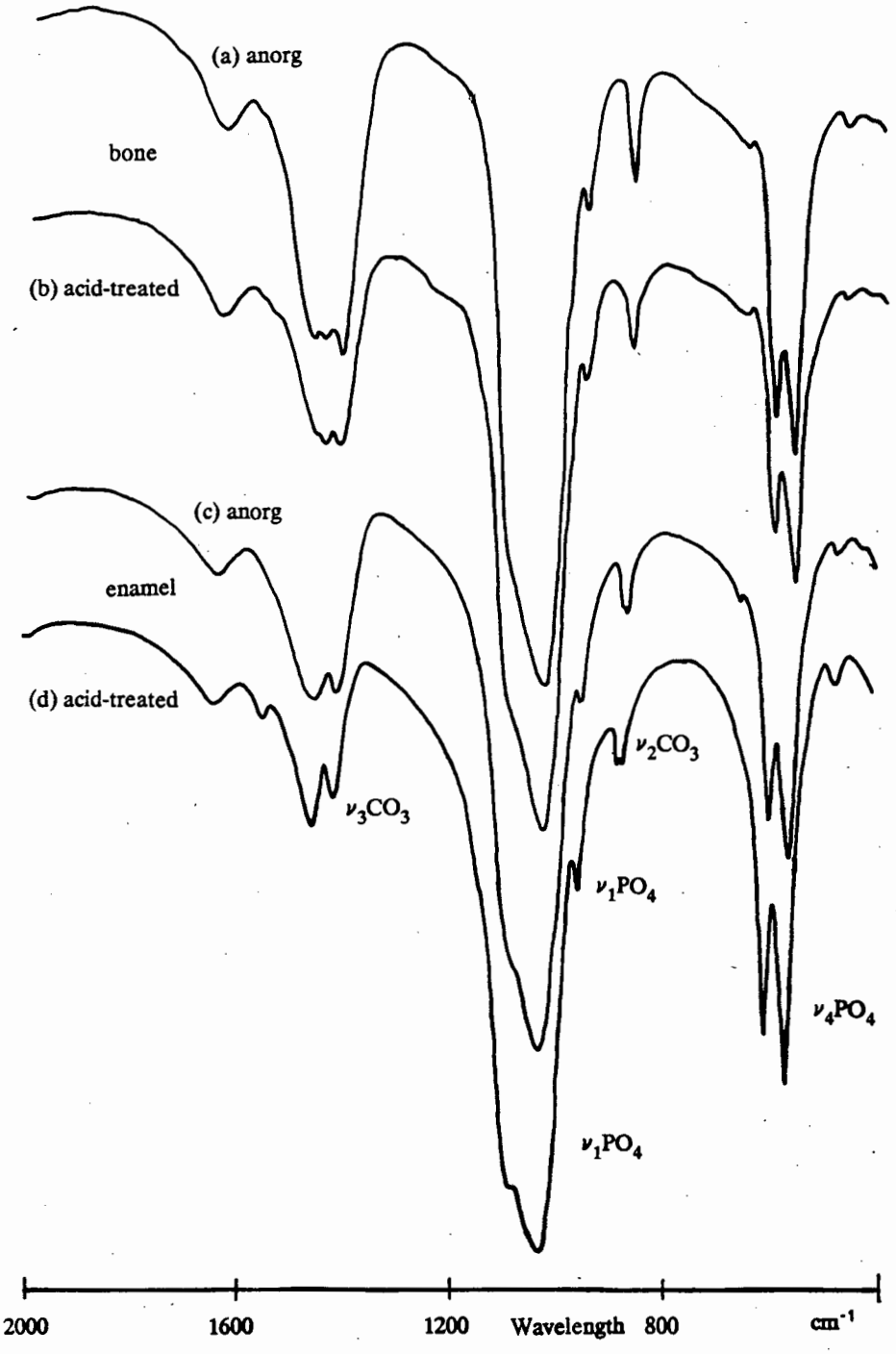

FIGURE 12 Infrared spectra of bone and enamel from a modern baboon specimen UCT# 2547) before and after overnight reaction in 1M acetic acid: (a) and (b) bone, (c) and (d) enamel.

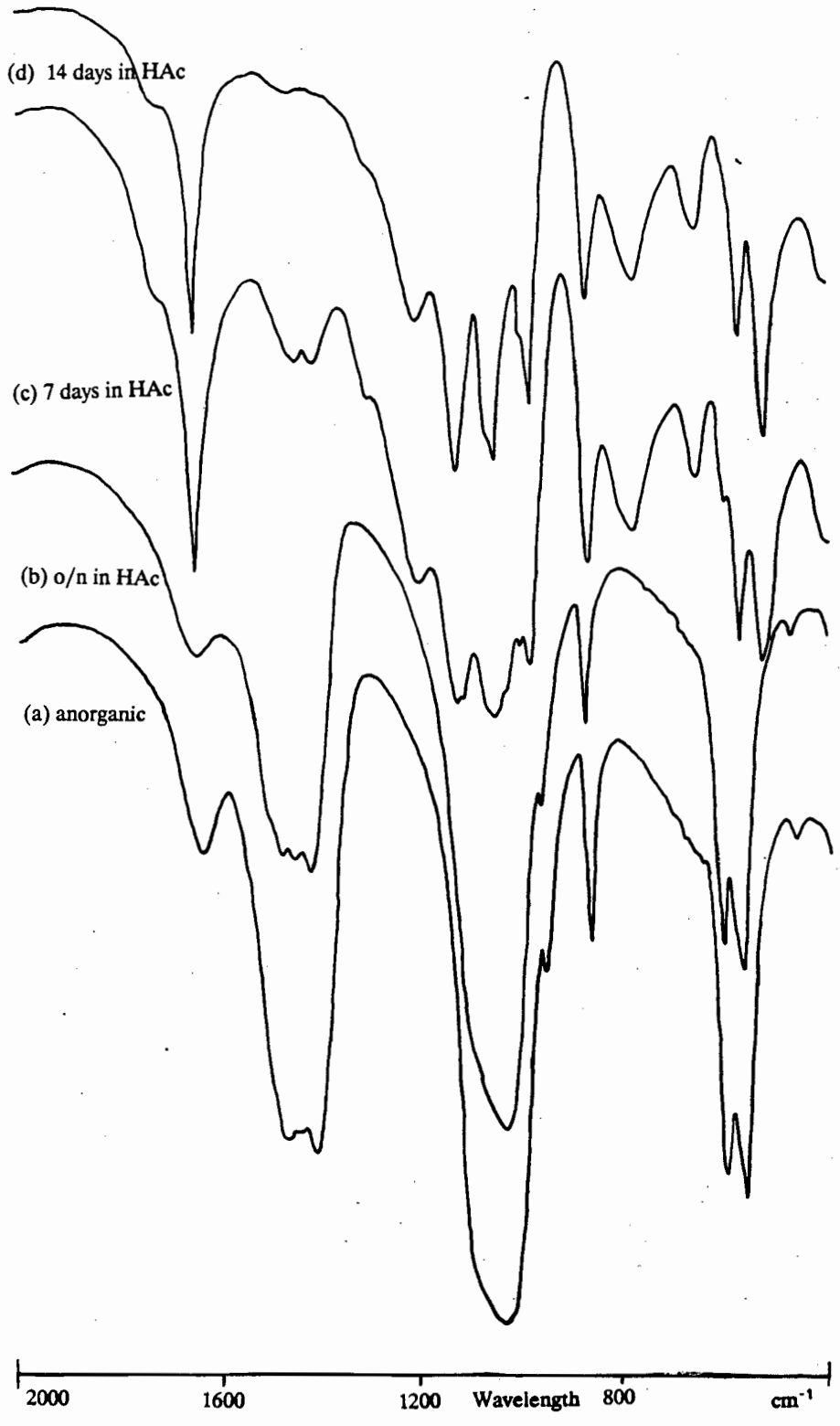

FIGURE 13 Infrared spectra of UCT# 684 for (a) anorganic bone, (b) bone allowed to react in 1M acetic acid overnight, (c) for 7 days and (d) the residue after 14 days in acetic acid. After 7 days the spectrum shows almost only brushite.

Thus the changes in CO_2 yields and CO_3/PO_4 ratios may be readily explained. The reason for the isotopic differences is not so clear, however, and there are at least two explanations. It is possible that fractionation of apatite carbonate occurs during the acetic acid treatment, in which the more enriched carbonates are preferentially leached, although it is difficult to imagine a mechanism for this. This possibility could be tested by subjecting isotopically homogeneous synthetic apatites to the same procedure. Another explanation is that two carbonate-containing phases exist in apatite, which differ isotopically. In this scenario, the more reactive, enriched phase corresponds to "adsorbed" carbonates and possibly the more poorly crystalline fraction of the mineral. The less reactive, more stable phase remaining after acetic acid treatment corresponds to larger apatite crystals containing only "structural" carbonate, and theoretically this fraction should have a lower CO_2 content. This is indeed the case, as seen in the reduced CO_2 gas yields and CO_3/PO_4 ratios for apatite after acid treatment.

Thus the existence of two phases is consistent with the chemistry of biological apatites and the data reported here, but it is not clear why the two "phases" should be isotopically different. As noted in Chapter 3, "adsorbed" carbonate reflects current serum bicarbonates to a greater degree than does "structural" carbonate. This difference might partly account for the observed isotopic heterogeneity in bone apatite, but not in enamel. Furthermore, the differences are patterned in individuals for both tissues. Whether due to fractionation during acetic acid dissolution, or to isotopic heterogeneity of the mineral, the observed differences require further investigation. These observations also suggest that not all $\delta^{13}C$ values previously reported for biological minerals may be comparable, if acetic acid treatment was not used. Land *et al* (1980) concluded that acetic acid treatment made little difference to isotopic values, on the basis of only one 'pair' of values. In other studies where modern material was analysed, it is not clear whether acetic acid was used or not.

Fossil mineral

Sampling and methods

The effects of acetic acid treatment on fossil specimens before and after acetic acid treatment were similarly evaluated as for the modern specimens in the previous section. An additional test was the comparison of the acetic acid treatment described for this thesis (Chapter 6), with the more rigorous treatment using evacuation in addition to acetic acid, described by Sullivan & Krueger (1981).

For the first part of the study, fossil specimens were chosen so that bone, dentine and enamel from the same individual could be compared, where possible. The specimens for this experiment were chosen from other studies in this thesis. The *C. pricei* specimen (UCT# 1878, Figure 9) is drawn from the fossil browser sample (Chapter 6), the *A. bondi* specimen is from Florisbad and the others (UCT#'s 2450, 2452, 2453 and 2463) are from part of the study of fossil baboons (both described in Chapter 8). As described in the preceding section, the approach is to characterise the minerals before (anorganic) and after acetic acid treatment, using in this case the methods outlined in Chapter 6. Anorganic samples were retrieved after the reaction in $NaHCLO_3$ solution, and reaction in acetic acid was allowed to continue until effervescence ceased (up to 7 days). As before, the methods for characterising the products were $\delta^{13}C$ values, CO_2 gas yields and infrared spectroscopy.

For the second part, the comparison of the two acetic acid methods, the specimens were drawn from Border Cave (Chapter 6) and Florisbad (Chapter 8). The same procedure was used, but in addition, after the reaction in acetic acid as described above, the reaction was encouraged by placing a mild vacuum on the centrifuge tube and its contents, using an aspirator. In this case, only the isotopic ratios ($^{13}C/^{12}C$ and $^{18}O/^{16}O$), and the CO_2 gas yields are used for comparison of the treatments.

Results and Discussion

The results for both these experiments are shown in Table 11 and 12, and the infrared spectra for UCT# 1878 and UCT# 2450 in Figure 14 and 15 respectively. The results indicate that after acid treatment there are changes in all parameters measured. For the first experiment, the following changes are noted. Isotopic changes are in general small. For the fossil browser, *T. strepsiceros*, $\delta^{13}C$ values for dentine and enamel are slightly depleted compared to the anorganic material, but slightly enriched for the bone. For the fossil grazer, *Connochaetes* sp., bone and enamel are slightly more enriched after acetic acid treatment. Since these alterations are in the direction of $\delta^{13}C$ values expected for these two diets (i.e. -14‰ in the case of the browser and -0.5‰ for the grazer), the results are compatible with what one would expect if acetic acid preferentially dissolves contaminating carbonates derived from the matrix. The latter are more enriched in ^{13}C than biogenic browser apatite values, but slightly more depleted than biogenic grazer apatite values. Matrix values for Swartkrans Member 1 cluster around -1.3‰ (Table 9). On the other hand, the small changes seen for the *A. bondi* (a grazer) bone and enamel samples are both in the direction of the matrix $\delta^{13}C$ values at Florisbad (about -7.6‰). In general, the change in $\delta^{13}C$ values after acetic acid treatment are very small, about 0.5‰. The largest alteration is 2.2‰ for *Connochaetes* sp. bone. This suggests that usually only small amounts of chemically removable diagenetic carbonate are present.

CO_2 gas yields were reduced after acid treatment, and CO_3/PO_4 ratios followed the same pattern. Gas yields and CO_3/PO_4 ratios for all acid-treated fossil minerals are similar to those for acid-treated modern material, for the same tissue, i.e. enamel or bone. A large reduction after acid-treatment for both these parameters is seen for two specimens (UCT #'s 2450 and 2463, Table 11). These specimens had unusually high CO_2 gas yields and CO_3/PO_4 ratios before acid treatment. The reason for this

becomes clear on examination of the infrared spectra (Figure 15). (Only the spectra for UCT# 2450 is illustrated, but similar results were obtained for UCT# 2463.) An additional infrared band is seen in the spectrum of these samples prior to acid treatment. The absorption band occurs at $700cm^{-1}$, and indicates the presence of calcite. After acid treatment, $\delta^{13}C$ the gas yields and CO_3/PO_4 ratios resemble those of the other acid-treated specimens (Table 11), and indeed those of the acid-treated modern specimens (Table 10). The infrared spectra of all the acid-treated fossil minerals indicate the presence of apatite only.

For the second experiment, similar changes in the isotopic ratios and the CO_2 gas yields are noted between anorganic and acid-treated minerals, as above. The isotopic changes are minimal, with one exception, and so are the CO_2 gas yields. The exception is UCT# 1480 *(E. burchelli)*, which shows a large change in both $\delta^{13}C$ and $\delta^{18}O$ values. The gas yield is altered, although not dramatically, suggesting that a slightly higher carbonate-containing apatite phase has been preferentially leached. There seems to be very little further change in isotopic values or CO_2 gas yields after the more rigorous treatment (Table 12). This observation may not apply universally, however, since only tooth (mainly enamel) specimens were analysed in this experiment, and the results might be a little different for bone specimens.

The data show that contamination is due to the presence of calcite in only two cases. The observed reduction in the gas yields and CO_3/PO_4 ratios, and slight alteration in the isotopic ratios, therefore suggests the preferential removal of a high carbonate apatite phase by the acetic acid. The results indicate that 1M acetic acid pretreatment is effective in preferentially removing calcite and high-carbonate apatites in fossil minerals. It appears that more rigorous treatments are unnecessary, at least for the specimens tested here.

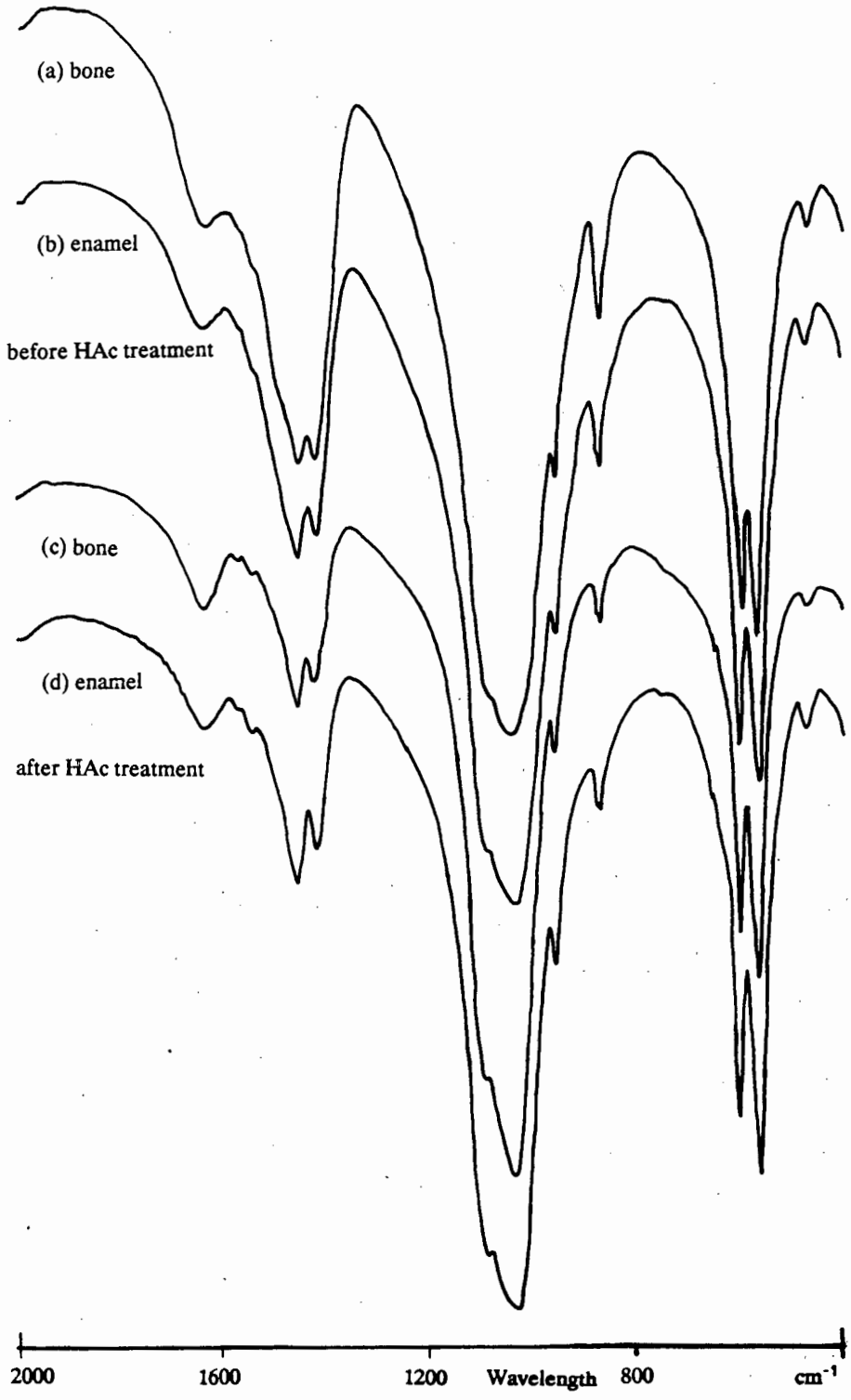

Figure 14 Infrared spectra from a *Cephalophus pricei* specimen from Makapansgat (UCT# 1878, M 124), before acetic acid treatment: (a) bone and (b) enamel, and after acid treatment: (c) bone and (d) enamel.

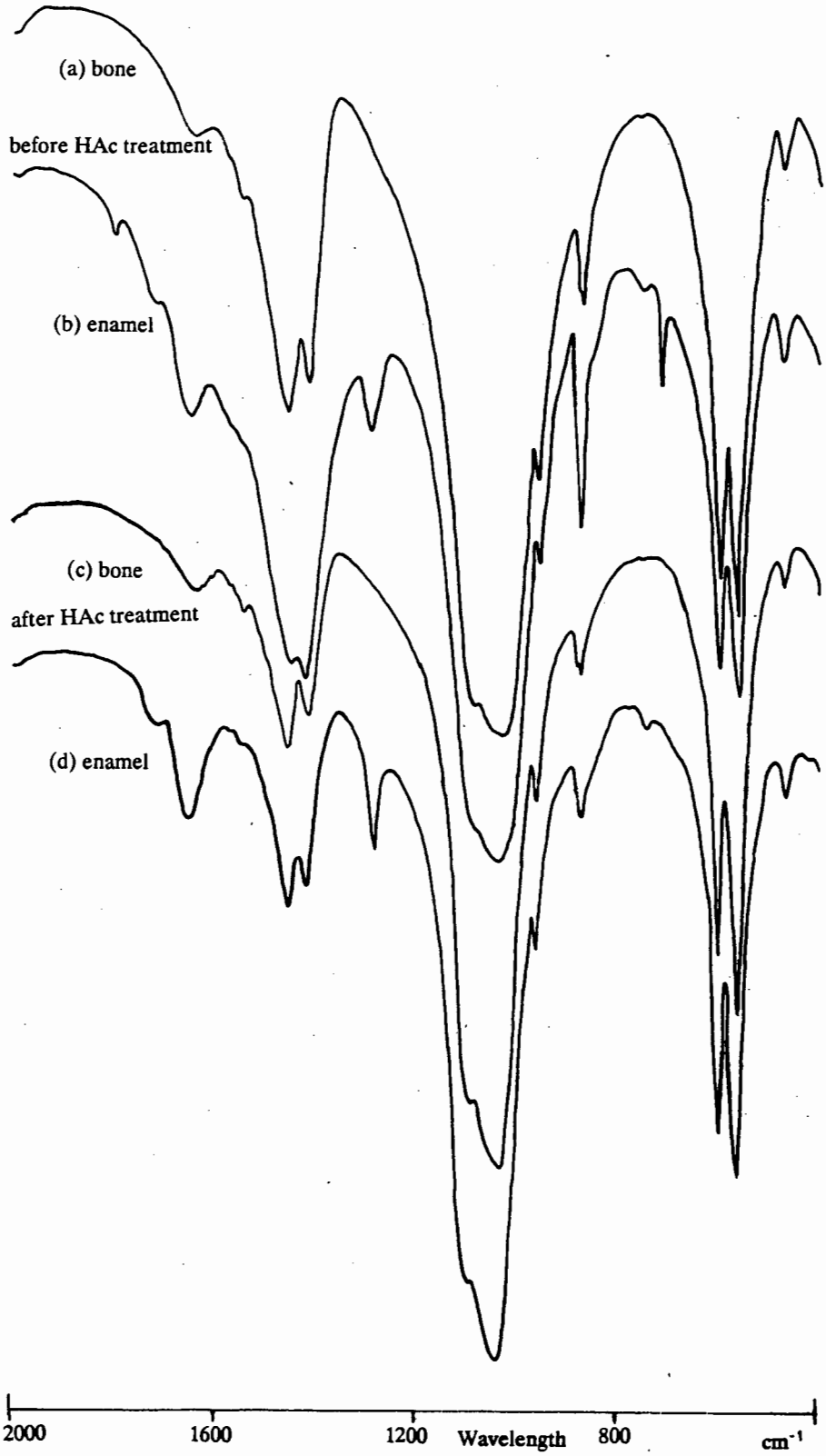

Figure 15 Infrared spectra of bone and enamel from a *Tragelaphus strepsiceros* specimen from Swartkrans Member 1 (UCT# 2450, Sk 2651): (a) bone, showing bands due to calcite and (b) enamel before acid treatment, and (c) bone, no longer showing the calcite bands, and (c) enamel after acid treatment.

An important observation is that the fossil specimens are much more resistant to acid attack than are modern specimens. Fossil specimens do not react as vigorously in acetic acid, or recrystallise after a few days in acid, or even after the more rigorous evacuation technique. This suggests greater stability of fossil apatite, and is consistent with other data showing increased crystallinity in fossil apatites (Sillen 1989). Most of the specimens used in this study were very old, some aged millions of years. The stability and resistance to acid attack may not apply to recent specimens which structurally closely resemble modern mineral, and may therefore be liable to recrystallise during prolonged acid treatment. Unfortunately, recent specimens were not a primary focus of study in this thesis, and therefore it is impossible to be more specific about the age. It would seem, however, that for specimens younger than about 1000 years, care should be exercised in the pretreatment procedures in order to avoid recrystallisation and possible isotopic changes.

A COMPARISON OF FOSSIL BONE, DENTINE AND ENAMEL APATITES

The reliability of the three calcified tissues - bone, dentine and enamel - for preserving biogenic isotopic ratios are compared using by comparing the $\delta^{13}C$ and $\delta^{18}O$ values for these tissues with those of matrix carbonates from the same deposits.

Sampling and methods

The specimens used for this comparison are drawn from the samples used elsewhere in this thesis, where both bone and tooth was available from the same individual. They comprise:- two *Raphicerus* specimens from KRM (UCT#'s 1025 and 1029), an *O. oreotragus* from Border Cave (UCT# 1470), two *C. pricei* specimens from Makapansgat (UCT#'s 1877 and 1878)(Chapter 6), and the rest are from Swartkrans Member 1: a *T. cf strepsiceros* (UCT# 2450), two *Connochaetes* (UCT#'s 2452 and 2453), a *T. darti* (UCT# 2454) and 5 *P. robinsoni* specimens (UCT#'s

2459-2463) (Chapter 8). Matrix isotopic values could be obtained as a comparison for all of these specimens except the one from Border Cave. Samples of bone, dentine and enamel could be compared for 3 of the specimens (UCT#'s 2450, 2452, 2453). All samples were pretreated and the $\delta^{13}C$ and $\delta^{18}O$ values obtained according to the methods set out in Chapter 6.

Results and discussion

The results for the faunal specimens are reported in Table 13. Results for matrix samples are shown in Table 9. For the three younger specimens from Border Cave and KRM (about 100 ka), there appears to be little difference between bone and enamel $\delta^{13}C$ values. For the older specimens from Makapansgat and Swartkrans, which are millions of years old, enamel $\delta^{13}C$ values conform more closely to the values expected for the diet of the particular animal. $\delta^{18}O$ values, too, are distinct from those of the matrix for these older enamel samples, but this relationship is not quite so distinct for bone and dentine.

In Figure 16, $\delta^{13}C$ values for the largest subset of fauna, from Swartkrans Member 1, are plotted against ^{18}O values. The isotopic values for the matrix specimens, which cluster very closely (the $\delta^{18}O$ values remarkably so) are shown on the same diagram. For enamel, $\delta^{13}C$ values correspond in all cases with those expected from the diets (although consistently offset by a factor of about $+3‰$), and $\delta^{18}O$ values are all in the same range (clustering around $0‰$). For bone, most but not all of the $\delta^{13}C$ values conform to the expected pattern, but $\delta^{18}O$ values are more scattered. This pattern is intensified for the 3 dentine specimens, which indicate a scatter for both isotopes, although the sample is small.

Since physical inclusions of calcite and diagenetic high-carbonate apatite phases have been chemically removed from these specimens by treatment in 1M acetic acid solution, the results indicate that isotopic values from structural enamel carbonate are more reliable than those from bone. Dentine is clearly unreliable.

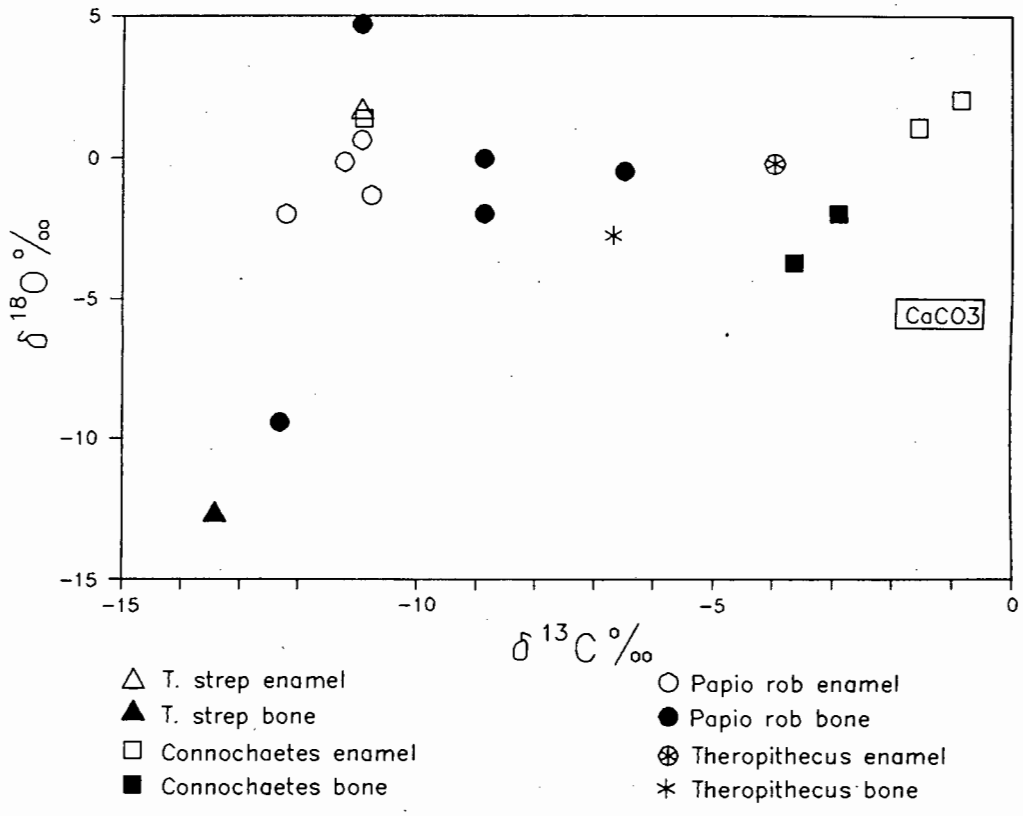

FIGURE 16 $\delta^{13}C$ and $\delta^{18}O$ values for bone, dentine and enamel from various species, Swartkrans Member 1.

SUMMARY AND CONCLUSIONS

The results for the two experiments in this chapter show that 1M acetic acid pretreatment effectively removes calcite from fossil specimens, and also suggest that the treatment preferentially leaches a high-carbonate apatite phase. The most reliable results are obtained from enamel apatite. This is predictable on theoretical grounds, since enamel apatite is more crystalline, more stable and less soluble than bone apatite. It is also much denser, and has very little organic matrix, and therefore fewer pathways and interstices are available for the passage of extraneous ions after the decay of organic material. There is also less opportunity for

recrystallisation and crystal growth, which are also mechanisms by which isotopic and ionic alteration may occur. The observed isotopic differences between fossil specimens and their modern counterparts (Chapter 6), apparently cannot be reduced even by the more rigorous acetic acid treatment (Table 12). Since the acid preferentially attacks the more soluble fractions of the mineral, the data suggest that carbonate in the structural position within the crystallites has been isotopically altered to some extent.

CHAPTER 8

APPLIED STUDIES : DIETS OF FOSSIL FAUNA AND HOMINIDS

In this chapter, three applied stable carbon isotope studies for dietary reconstruction are described, using only tooth enamel as sample material. In all cases, the age of the fossil specimens precluded collagen analysis and isotopic analysis of apatite represented the only way in which isotopic dietary reconstructions could be attempted. Given the results presented in Chapter 7, enamel was chosen as sample material. As far as possible, a comparative method was used, in which the species with unknown diets were isotopically compared with animals of known diet, i.e. browsers and grazers, from the same deposit, in order to provide internal controls.

Of the three applied studies, two are palaeontological, and concern the diets of an extinct springbok, *Antidorcas bondi*, and two extinct baboons, *Papio robinsoni* and *Theropithecus darti*. The third and most significant application is palaeoanthropological: it concerns the diet of the early hominid, *Australopithecus robustus*, whose dietary habits have been the subject of considerable controversy and study for many years. In each section the background to the problem is first described, followed by the results and conclusions.

ANTIDORCAS BONDI

Background

The extinct springbok, *Antidorcas bondi*, was a small antilopine related to the extant springbok *A. marsupialis*. It occurred in great numbers in Southern Africa during the Pleistocene, but declined rapidly and became extinct at the beginning of the Holocene. The last known occurrences date to approximately 7000 years ago at Kruger Cave in the Transvaal (Brown & Verhagen 1985), and at Equus Cave

(Beaumont pers. comm.), a site very near Taung in the Northern Cape. The species still existed in large numbers in the Transvaal about 11000 years ago, since the faunal assemblage of the Member 5 Formation at Swartkrans consists of very little else, and is thus known as the "*Antidorcas bondi* Channel" (Brain 1987). Although the two species of springbok are closely related and similar in size, there are marked differences in dentition between the two species. In contrast to *A. marsupialis*, *A. bondi* had hypsodont teeth.

To account for the difference in dental features, Vrba proposed that *A. bondi* had an intermediate diet consisting mainly of browse and some grass, like the modern springbok, but it consisted of much tougher material (Vrba 1973). Brink considered the dental features to be more characteristic of a grass-eater (Brink 1987). The small size of the animal and the fact that it is a ruminant imposes particular constraints on a grazing diet, however. The nutritional and energy requirements of a small grazing ruminant may only be supplied by a concentration on new grass shoots, the most readily digestible and nutritious part of grass. According to the grazing succession model outlined by Bell (1971), a small-bodied grazer utilises the new grass shoots which emerge after mowing of the tougher, bulk material by the large and medium-bodied grazers. The two dietary hypotheses may be readily distinguished using stable carbon isotope analysis, because a grazing diet implies a C_4 isotopic signature (i.e near 0‰), while an intermediate diet implies a more positive isotopic signature. Vrba's hypothesis predicts that the diets of the two springbok should be similar isotopically, while Brink's predicts that they should be different: *A. marsupialis* should be isotopically depleted in ^{13}C, while *A. bondi* should be more enriched in ^{13}C (i.e. near 0‰).

Methods

Sampling

Specimens of *A. bondi* were obtained from two sites, representing an earlier occurrence from the late Pleistocene, about 125-150 ka (Florisbad), and a later one (Swartkrans Member 5), at about 11 ka (Figure 2). These two sites are briefly described below. (The description for Swartkrans only concerns the relevant member at this point. A fuller description of the site is given in the next section.)

The fossil site of Florisbad in the Orange Free State (Figure 2) is well-known for the archaic *Homo sapiens* skull found in the 1930's (Dreyer 1938). The site is a spring mound, and the faunal assemblage now called the "Old Collection" was excavated from sand bodies in the mound, believed to be extinct spring "eyes". The "Old Collection" represents a faunal assemblage accumulated by various natural agencies, and not by humans (Brink 1987). More recently, a Middle Stone Age occupation site has been excavated in another part of the mound (Brink 1987). The "Old Collection" assemblage is believed to be of Late or late Middle Pleistocene age, although the accumulations in the various spring eyes were only broadly contemporaneous. Grazers dominate the assemblage, which includes several giant extinct forms such as *Equus capensis* and *Megalotragus priscus*, and large numbers of the small extinct gazelle, *A. bondi* (Brink 1987). The sample analysed in this study was obtained from the "Old Collection" assemblage. These fossils are well-preserved and have a characteristic "pearly-white" appearance. Only tooth specimens were chosen, some of which have well-formed calcite inclusions in the pulp cavities, and these were removed for separate analysis. Comparative material was a problem at Florisbad, because the herbivore component of the faunal assemblage consisted mainly of grazers (Brink 1987). *A. bondi* and *A. marsupialis* could be compared, however, and a few specimens of *Taurotragus oryx* (eland), although not a very reliable browser, were obtained (Table 14). In addition to the

fossil specimens, a number of modern specimens of *A. marsupialis* were obtained to serve as a comparison for the fossil specimens. Some were from the nearby Soetdoring Nature Reserve (a C_4 grassland), and others were from different habitats in the Cape Province (Table 15).

In Swartkrans Member 5, remains of *A. bondi* are so abundant in the aptly-named *"Antidorcas bondi* Channel" that there are almost no other fauna present. A radiocarbon date of about 11 ka BP has been obtained from *A. bondi* bone apatite (Brain 1987). This has led Brain to suggest that the channel filling is the result of a rapid depositional episode at the beginning of the present interglacial (Brain 1987). A number of tooth and bone specimens were chosen for analysis. Unfortunately, no comparative material is available for Member 5; it simply does not exist, but some deposit samples were obtained for analysis. It is possible, however, to compare the earlier (Florisbad) and later occurrences of *A. bondi* (Swartkrans). Thus the lack of comparative faunal material did not seriously affect the validity of the results.

Laboratory procedure

The specimens from Florisbad were soaked in acetone to remove glyptol (a preservative), but this step was unnecessary for the Swartkrans specimens, since they had not been coated in it. Both bone and enamel samples were obtained from two Swartkrans specimens (UCT#'s 2217 and 2219). Preparative methods for the tooth and bone specimens followed the procedures for fossil specimens outlined in Chapter 6. Calcite crystals were recovered from the pulp cavities of two teeth from Florisbad, and these were analysed separately according to the methods outlined in Chapter 6. Only one of the deposit samples from Swartkrans Member 5 yielded enough CO_2 gas for measurement of the $^{13}C/^{12}C$ ratios.

Results

The results are reported in Tables 14-16. $\delta^{13}C_{apatite}$ values from enamel are given for all the fossil fauna from Florisbad in Table 14. $\delta^{13}C_{apatite}$ and $\delta^{13}C_{collagen}$ values for modern *A. marsupialis* specimens are reported in Table 15, and $\delta^{13}C_{apatite}$ values for *A. bondi* enamel and bone specimens from Swartkrans Member 5 in Table 16. These results are shown as histograms in Figures 18 and 19.

For Florisbad, the data clearly show a distinction between the two species of springbok from the site. Values for fossil *A. marsupialis* apatite specimens cluster around a mean value of -9.8±1.2‰, slightly more depleted than average values for modern specimens from the area as it is today. $\delta^{13}C_{apatite}$ values for *A. bondi* are quite distinct with a mean of -1.4±2.5‰. Results for two *T. oryx* (-11.2 and -9.9‰) are consistent with a predominantly browsing diet, but the third specimen has a more enriched value (-4.8‰). It is not possible to determine whether this value is due to diagenetic alteration, or simply a reflection of a mixed diet, since *T. oryx* may incorporate a considerable amount of grass under certain conditions (Smithers 1983). The $\delta^{13}C$ values for two calcite crystals extracted from teeth are -7.4 and -7.8‰ respectively (Table 9). These results show that, of the sites examined in this study, Florisbad is the only site where groundwater isotopic values are not near 0‰.

The results for the Swartkrans *A. bondi* specimens are similar, with a mean $\delta^{13}C_{apatite}$ value of -2.5±1.6‰. The distinct outlier at -12‰ has been excluded from the calculation of the mean value. The deposit samples contained very little carbonate, and only one $\delta^{13}C$ reading (-5.1‰) could be obtained (Table 9).

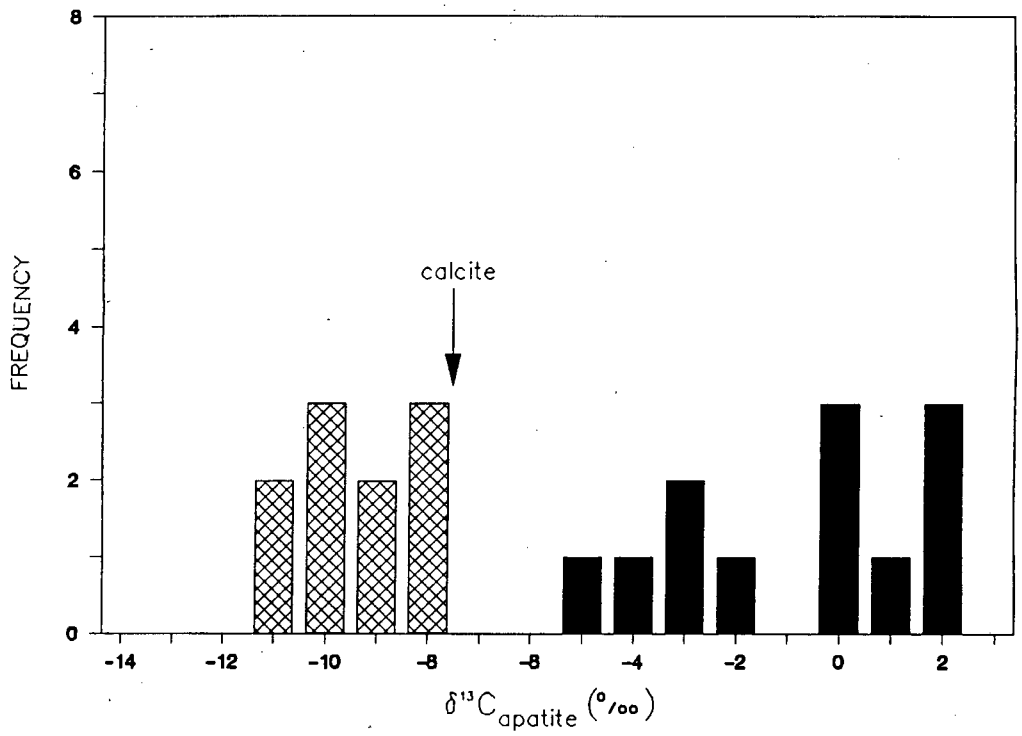

Figure 17 Frequency diagram showing $\delta^{13}C$ values for *Antidorcas* sp. from Florisbad. Matrix values are indicated by an arrow.

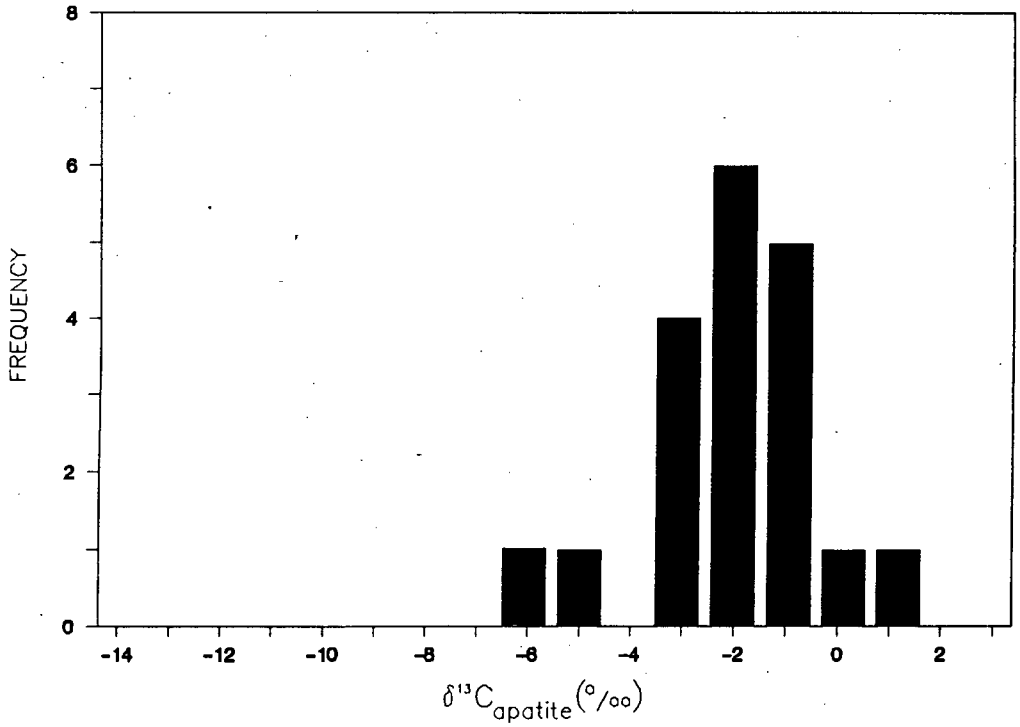

Figure 18 Frequency diagram for $\delta^{13}C$ values for *Antidorcas bondi* from Swartkrans Member 5.

Discussion

The $\delta^{13}C$ results for *A. bondi* are in agreement with the prediction that this species was predominantly a grazer, unlike *A. marsupialis*, which is an intermediate feeder with a preference for browse. The distribution of $\delta^{13}C$ values for *A. bondi* at Florisbad appears to be bimodal, while the distribution at Swartkrans is normal. The reason for the apparent bimodality is not clear. It is possible that the specimens are drawn from depositional episodes differing chronologically, and this might account for the bimodality of the distribution, either because there was a small shift in diet as a result of changing environmental conditions, or because matrix values and therefore diagenetic effects varied slightly between "spring eyes". As discussed above, the "Old Collection" fossils were collected from vertical sand bodies described as "extinct spring eyes" (Dreyer 1938; Brink 1987), suggesting that the assemblage is derived from different depositional episodes. This seems to offer the most likely explanation for the distribution of *A. bondi* values, although it is possible that the bimodal distribution might disappear if more samples are analysed.

There is a small difference of about 1‰ in the $\delta^{13}C$ means for the two occurrences of *A. bondi* at Florisbad and Swartkrans, but the bimodality for the values for the species at Florisbad makes it difficult to interpret such small differences. The results indicate clearly, however, that *A. bondi* was predominantly a grazer, and not an intermediate mixed feeder.

This finding has some implications concerning the eventual extinction of this specialised small grazer, and possibly the other giant grazers which were present in southern Africa until the end of the Pleistocene. The faunal assemblage of Florisbad contains numerous large-bodied grazers, such as *Equus capensis* (giant zebra), *E. burchelli/quagga*, and the giant ruminants *Megalotragus priscus* and *Pelorovis antiquus*, and medium-bodied grazers, such as *Damaliscus niro* and *Connochaetes gnou* (Brink 1987). As demonstrated in this study, it also included *A.*

bondi, a small specialised grazer. The large number of grazers in the faunal assemblage, especially the number utilizing the upper grass-stem level (the large-bodied grazers), suggests a highly productive grassland ecosystem which functioned in a similar way to the Serengeti (Bell 1971). The nett result of the extinctions at the end of the Pleistocene was that the most specialized forms in the large, medium and small grazing categories became extinct. One explanation for this event is that change occurred in the structure of the grassland vegetation - a reduction in primary production - leading to increased competition and the eventual extinction of the specialised forms, including *A. bondi*. In this scenario, human Stone Age predation was not a key factor in the extinctions as has been proposed by Klein (1975), since it is unlikely that humans selectively hunted only the most specialised forms at all three levels of the grazing succession to extinction.

PAPIO ROBINSONI AND THEROPITHECUS DARTI

Background

The comparative approach using fauna of known diet (browsers and grazers) as internal controls provides an appropriate method for studying the diets of two extinct baboon species from the Lower Pleistocene, *Theropithecus darti* and *Papio robinsoni*. When they were first described (Freedman 1957), it was suggested that *P. robinsoni* was a potential ancestor of the modern chacma baboon, *P. ursinus*. More recently it has been proposed that *T. darti* (formerly known as *Simopithecus darti danieli*) was an earlier member of the Theropithecine genus, represented today only by the modern gelada baboon, *T. gelada* (Jolly 1972). On the basis of tooth morphology, it may be inferred that *P. robinsoni* had a diet similar to that of *P. ursinus*, while the diet of *T. darti* was similar to that of *T. gelada*.

P. ursinus is omnivorous, including a wide range of vegetable matter, insects and flesh in its diet (Smithers 1983); *T. gelada* concentrates on grass blades, seeds and rhizomes (Dunbar & Dunbar 1974; Dunbar 1976). Therefore, *T. darti* should have an enriched C_4 isotopic signature (near 0‰), related to its graminivorous diet. That of *P. robinsoni* should be predominantly C_3-based (relatively depleted in ^{13}C), because the C_4 contribution, which can only be derived from grasses and/or grass-eating animals and insects, would be minor. This hypothesis may be tested directly using carbon isotope analysis of tooth enamel.

Methods

Sampling and laboratory procedure

The fossil specimens used for this comparative study are from the Swartkrans Member 1 faunal assemblage, which is believed to be between 1.7 and 1.9 ma old on the basis of comparative biostratigraphy (Vrba 1982; Delson 1984). Swartkrans is a karstic cave-filling in pre-Cambrian dolomite in the Transvaal (Figure 2). The site has a complex stratigraphy, which has been described by Brain (1981, 1982, 1987), and is outlined very briefly here. Five depositional members are currently recognised, although it is likely that others existed and have been removed by miners. Brain has suggested that each of the members were laid down and subsequently partly eroded in a series of depositional and erosional episodes (Brain 1981, 1982). The oldest member is Member 1, which is believed to be between 1.9 and 1.7 ma old on the basis of biostratigraphy (Vrba 1982). Members 2 and 3 are younger, about 1.5-1.6 and 1.1 ma respectively (Vrba 1982). The nature of the hominid remains found in each of these members is described in the next section.

The sample included six specimens each of the two baboon species, and three specimens of a browser, *Tragelaphus strepsiceros* (kudu), and three specimens of a grazer, *Connochaetes* sp (wildebeest). Some of these are illustrated in Figures 19 and 20. The kudu is not entirely satisfactory as a browsing "standard", as pointed out

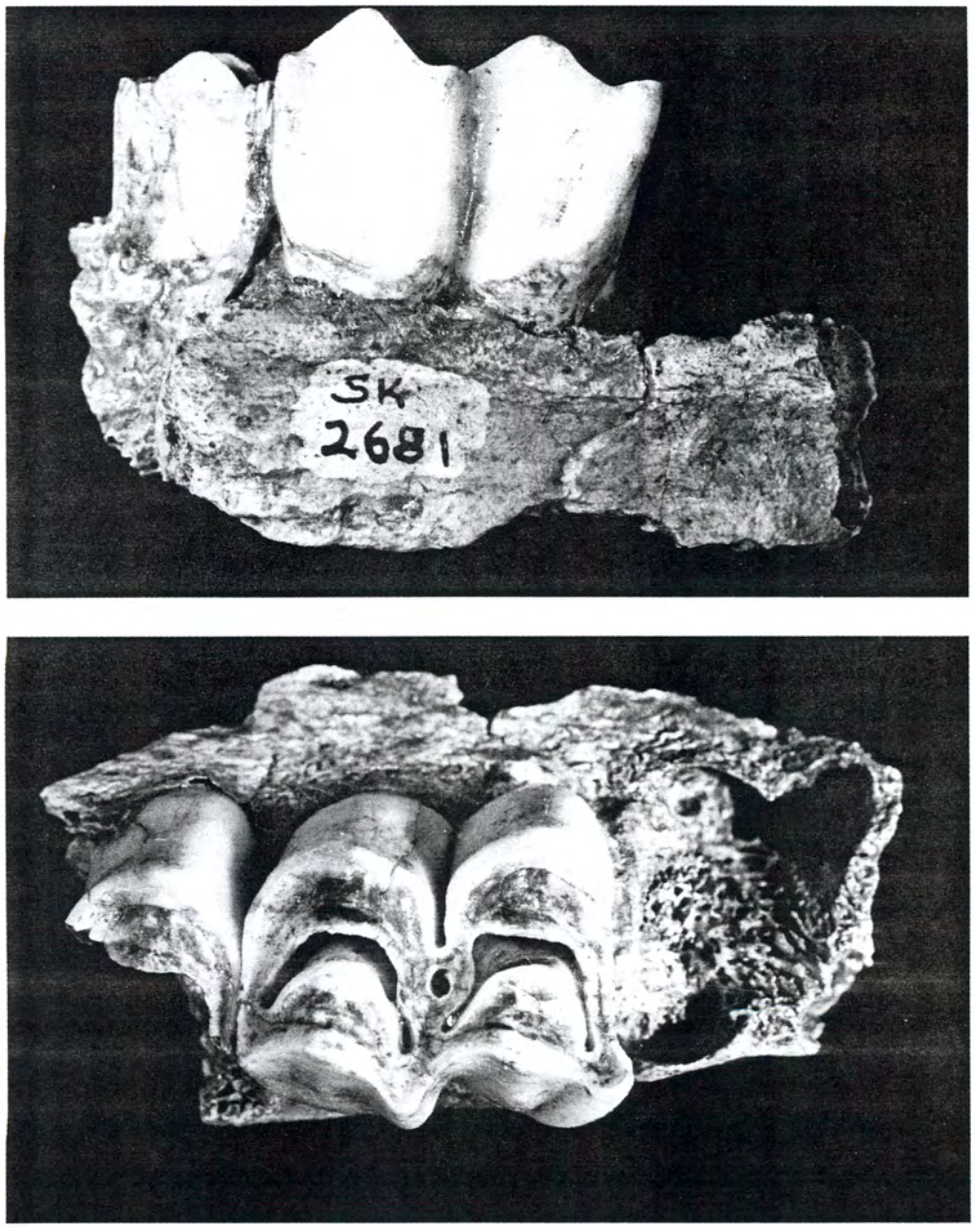

Figure 19 Two views of a *Tragelaphus strepsiceros* maxillary fragment including M^2 and M^3 (UCT# 2350, Sk 2681)) from Swartkrans Member 1.

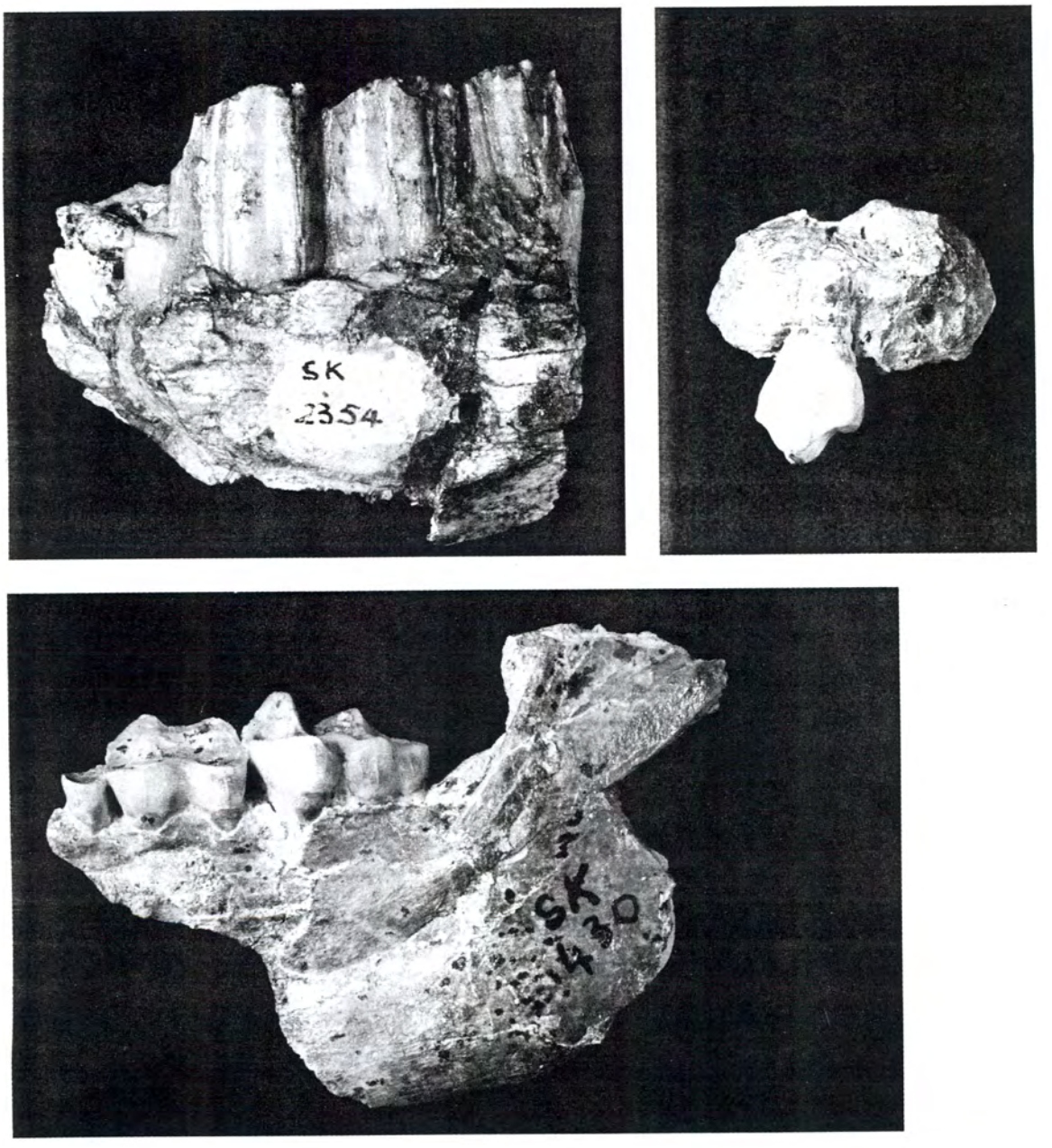

Figure 20 (a) A *Connochaetes* sp. mandibular fragment including M_2 and M_3 (UCT# 2452, Sk 2354) from Swartkrans Member 1. (b) *Theropithecus darti* maxillary fragment with P^4 (UCT# 2455, Sk 495) and (c) *Papio robinsoni* mandibular fragment with M_1, M_2 and M_3 (UCT# 2459, Sk 430).

earlier (Chapter 6), but it was the only browser available for study from Swartkrans. Some specimens of modern *P. ursinus* from the Transvaal and Cape Province were obtained for comparison with the fossil *Papio*. The methods for preparation, pretreatment and isotopic analysis of the fossil enamel specimens follow those described in Chapter 6, and, for the modern bone and tooth specimens, those in Chapter 5.

Results and discussion

The results for the fossil specimens from Swartkrans Member 1 are reported in Table 17, and those for the modern baboons in Table 18. All of these results are summarised in Figure 21. The $\delta^{13}C$ values for the fossil browsers (*T. cf strepsiceros*) are more positive by about 3.5‰ than the mean value of -14.5‰ established for modern browsers (Chapter 5). The difference represents a postmortem alteration towards matrix $\delta^{13}C$ values, which cannot be removed by chemical pretreatment. The mean value obtained for 1 breccia and 5 calcite specimens from Member 1 is -1.3‰ (Table 8), a value consistent with the dolomitic origins of the breccia. Isotopic alteration is less obvious for *Connochaetes*, because the values for grazers (modern mean $\delta^{13}C$ = -0.5‰) are very close to those of the matrix.

The fossil baboons may be compared against the background provided by the browsers and grazers. The values for *P. robinsoni* suggest that it was a consistent C_3 consumer, since they are similar to those of the kudu. Values for *T. darti* cluster at the positive end, confirming that this species was predominantly dependent on C_4 grasses. There is some spread of values for the latter, which implies some heterogeneity in the diet. Alternatively, the spread might be due to variable degrees of diagenesis producing different $\delta^{13}C$ values. This explanation seems very unlikely, however, since values for *Connochaetes* or any of the other fauna which are all intermingled in the same deposit, have not been affected in this way. The fossil *Theropithecus* seems to have had a diet very similar to that of modern *T. gelada*

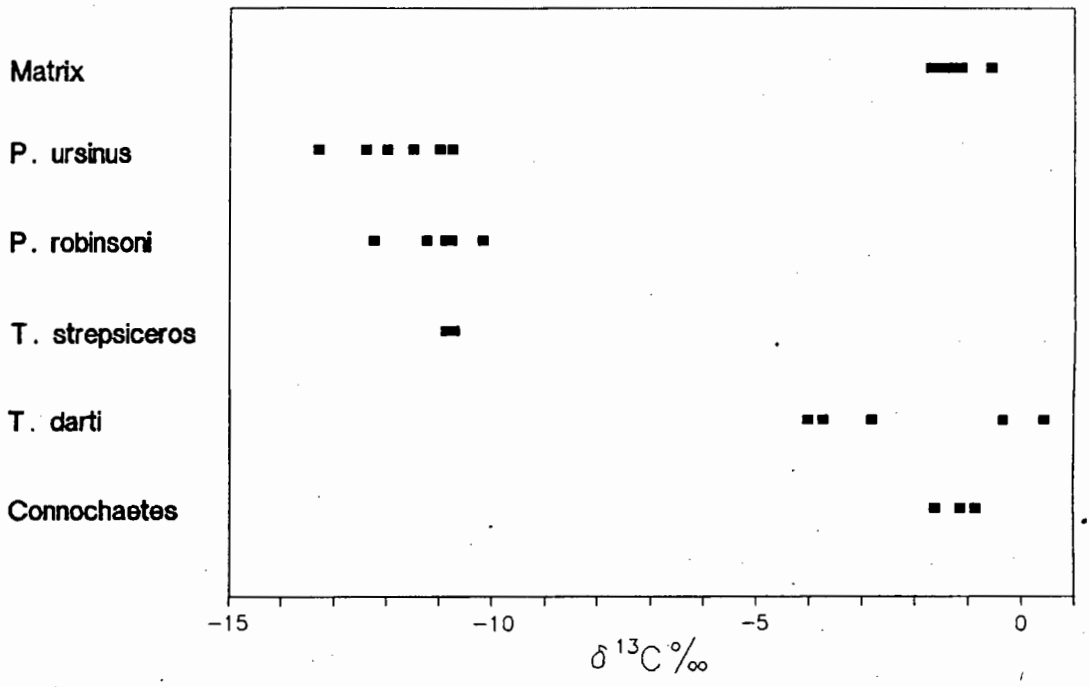

Figure 21 A comparison of $\delta^{13}C$ values for fauna from Swartkrans Member 1, including *Papio robinsoni* and *Theropithecus darti*, as well as modern *P. ursinus* specimens.

(Dunbar & Dunbar 1974), providing further support for the idea that a graminivorous dietary specialisation developed early in this genus (Jolly1972).

A small difference in diet can possibly be detected between *P. robinsoni* and *P. ursinus*. Given that the original isotopic values for the fossil enamel has been diagenetically altered by about +3.5‰, it follows that the fossil *Papio* originally had values more negative (about -14.5‰) than that of its modern counterpart in this area (mean value -12‰). This implies that modern *Papio* includes some C_4 grasses and/or grazing animals and insects in its diet, but that *P. robinsoni* did not. If this is the case, the shift might be related to sympatry of the two fossil baboon species at Swartkrans. Such an effect is described for the Ethiopian highlands, the only area where sympatry of *Theropithecus* and *Papio* still exists (Dunbar & Dunbar 1974). Here *Papio* concentrates on fleshy fruits and other tree products (Jolly 1970;

Dunbar & Dunbar 1974), which are all C_3. This tentative observation could perhaps be tested by isotopic measurement of the sympatric baboon species from the Ethiopian highlands, *T. gelada* and *P. hamadryas*, although the high altitude might preclude the existence of C_4 grasses (Livingstone & Clayton 1980), an essential element for such a test.

The results show that the diets of the two species were clearly distinct. *T. darti* depended largely on C_4 grasses, while values for *P. robinsoni* are consistent with a C_3 diet. If the effects of diagenesis are taken into account, the values for the fossil *Papio* are more depleted than those obtained for its modern counterpart in the region, *P. ursinus*. This observation implies that *P. robinsoni*, unlike modern *Papio*, lacked a C_4 grass contribution to its diet, an observation tentatively linked to niche separation of the two sympatric species in the past. The study shows that carbon isotope analysis of tooth enamel clearly distinguishes graminivorous from C_3-based diets in fossil primates as old as 1.8 m.y.

AUSTRALOPITHECUS ROBUSTUS

The successful demonstration that graminivory may be isotopically distinguished from C_3-based diets in 1.8 ma old non-human primates, provided a strong argument that such a comparative approach might be profitably applied to early hominid dietary research.

Background

Dietary reconstruction of early hominids has long been a subject of controversy ever since Robinson (1954) first proposed a niche separation hypothesis to account for the disparity in dental apparatus between the two South African australopithecine forms, *Australopithecus africanus* and *A. robustus*. His proposal became generally known as the "dietary hypothesis". Robinson believed that the two forms occupied different "adaptive zones", the gracile being a more generalized omnivore, and the

robust form specialising on very tough vegetable matter (Robinson 1954, 1963). This hypothesis has often been disputed, on several grounds. The strongest argument against the idea of niche separation is that it is no longer believed that the two species were contemporaneous, at least in South Africa (reviewed in Grine 1981). The situation in East Africa is more complex (Walker & Leakey 1978) but at present it seems unlikely that the two forms were contemporaneous. Thus the necessity for the niche separation proposal falls away.

Nevertheless, the apparent differences between the gracile and robust forms have remained the subject of controversy. It has been suggested that they are merely due to differences in body size (Brace 1973; Wolpoff 1973, 1980) and that there is a positive allometric trend between body size and molar tooth area for the two species (Pilbeam & Gould 1974), rather than dietary separation. Other modern primates, however, do not exhibit this trend (Kay 1975a, 1975b). It has been shown that there are significant differences in the anterior dentition - the incisors and canines are significantly reduced in the robust form compared to the gracile form (Wood 1979; Wood & Stack 1980), although the differences in molar size may be related to body size. In short the dental characteristics do seem to support the proposal that the two forms had different diets.

There have been numerous attempts to account for the various dental characteristics of one or the other australopithecine, and some of the results have been conflicting. Hypotheses have ranged from carnivory and bone crushing, based on the reduced anterior teeth and "incisification" of the canines (Szalay 1975) to extreme herbivory (similar to the giant panda), based on the mechanics of the teeth and masticatory apparatus and comparison with two forms of Ursidae (DeBrul 1976). On the basis of comparison with other primates, Kay has advocated frugivory for the australopithecines (Kay 1975b, 1985). Many of these arguments are based on the morphological evidence of the fossils themselves and analogy with modern animals

(usually primates), biomechanical reconstruction, or circumstantial data provided by associated remains or ecological models. While these approaches may provide valuable insights to the problem, each has its own set of drawbacks, and these have most often generated more controversy.

Scanning electron microscope studies of microwear on the occlusal facets of fossil tooth enamel (Grine 1981, 1986; Grine & Kay 1988; Walker 1981) is a more direct approach. Microwear on East African robust specimens *(Australopithecus borsei)* shows similarities with that of the chimpanzee (*Pan*) and orangutan (*Pongo*), with no evidence for grazing, suggesting a frugivorous dietary regime (Walker 1981). Studies of the two South African australopithecines indicate that the robust form exhibits more scratches and pitting on occlusal facets than does the gracile (Grine 1981, 1986). Recently Grine and Kay (1988) have been able to quantify the differences.

Current consensus seems to have returned again to Robinson's dietary hypothesis, in which *A. robustus* is seen as a specialised herbivore/frugivore. The present view, which apparently best fits evidence to date, seems to be that this hominid habitually ate small, very hard objects (Wood 1979; Wood & Stack 1980; Grine 1981, 1986; Grine & Kay 1988; Walker 1981; Kay 1985). The identity of these objects is uncertain, but seeds, hard fruits, beans and/or nuts have been proposed (Jolly 1970; Grine 1981; Walker 1981; Grine & Kay 1988; Kay 1985). None of the current hypotheses seems to doubt that *A. robustus* was an entirely herbivorous creature.

Carbon isotope analysis represents another direct approach to dietary reconstruction of *A. robustus*, since the "seed-eating" hypothesis may be clearly isotopically distinguished from the "fruit/nut-eating" hypothesis. Application to the problem has been delayed because of the controversy surrounding the use of apatite for dietary studies. Procedures for circumventing the problem of isotopic alteration, using the comparative method with fauna of known diets as internal controls, have been successfully demonstrated in the previous sections.

Method

Sampling and laboratory procedure

A similar comparative approach was used for the study of *A. robustus* specimens from Swartkrans. Hominids are found in Members 1, 2 and 3 at Swartkrans. Remains of *A. robustus* are abundant in Member 1, and are also present in Members 2 and 3, while specimens assigned to *Homo* appear in Member 2. *Homo* specimens are not found in Member 3, but the presence of this hominid has been assumed from the nature of the artefacts and food debris (Brain 1987; Brain *et al* 1988). Stone and bone artefacts are found in Members 1 to 3, while cutmarks and evidence for the controlled use of fire appear in Member 3 (Brain *et al* 1988; Brain & Sillen 1988). It seems likely that *Homo* is responsible for the cutmarks and fire, but this is not proven.

The sample consisted of eight *A. robustus* tooth fragments: 5 from Member 1, 1 from Member 2, and 2 from Member 3 (Table 19). Two fragments marked SK 879 were analysed separately (as UCT #'s 2541 and 2542) because they were considered to be from different teeth, judging by colour and appearance. Two of these specimens, UCT#'s 2689 and 2691, are illustrated in Figure 22.

Results

The results for the *A. robustus* enamel specimens are reported in Table 19. In Figure 23, they are compared with $\delta^{13}C$ values for the other fauna from Member 1 (reported in the previous section in Table 17 and Figure 21). There is no visible chronological trend between results from the Members. The $\delta^{13}C$ values for the *A. robustus* specimens (mean -8.8‰) are more positive than those of the fossil kudu (mean -10.9‰). This pattern suggests that *A. robustus* was predominantly dependent on C_3 foods, but there was a substantial C_4 contribution to the diet.

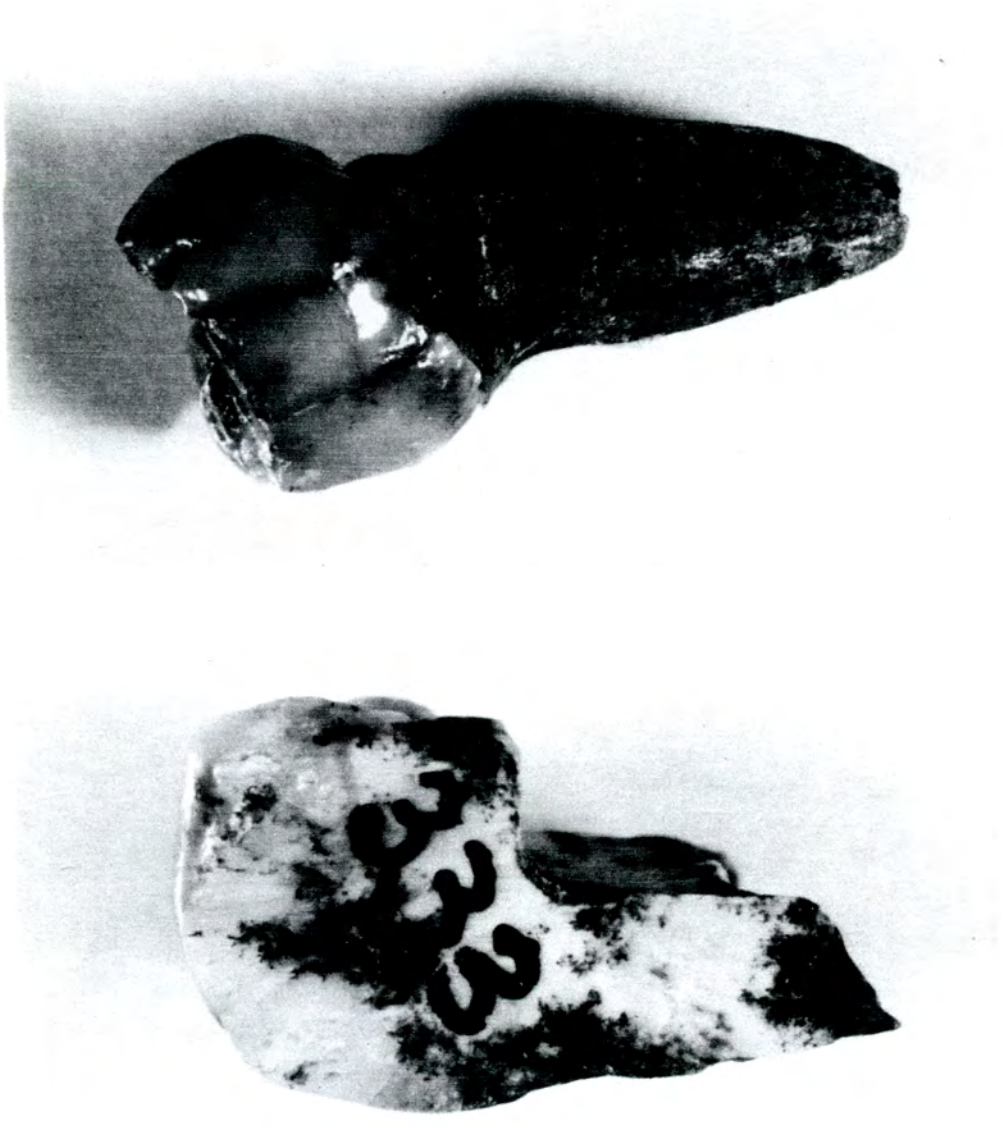

Figure 22 *Australopithecus robustus* tooth specimens (a) a complete left M_3 with root (UCT# 2689, Sk 5015) and (b) a fragment of a right M_1 (UCT# 2691, Sk 333).

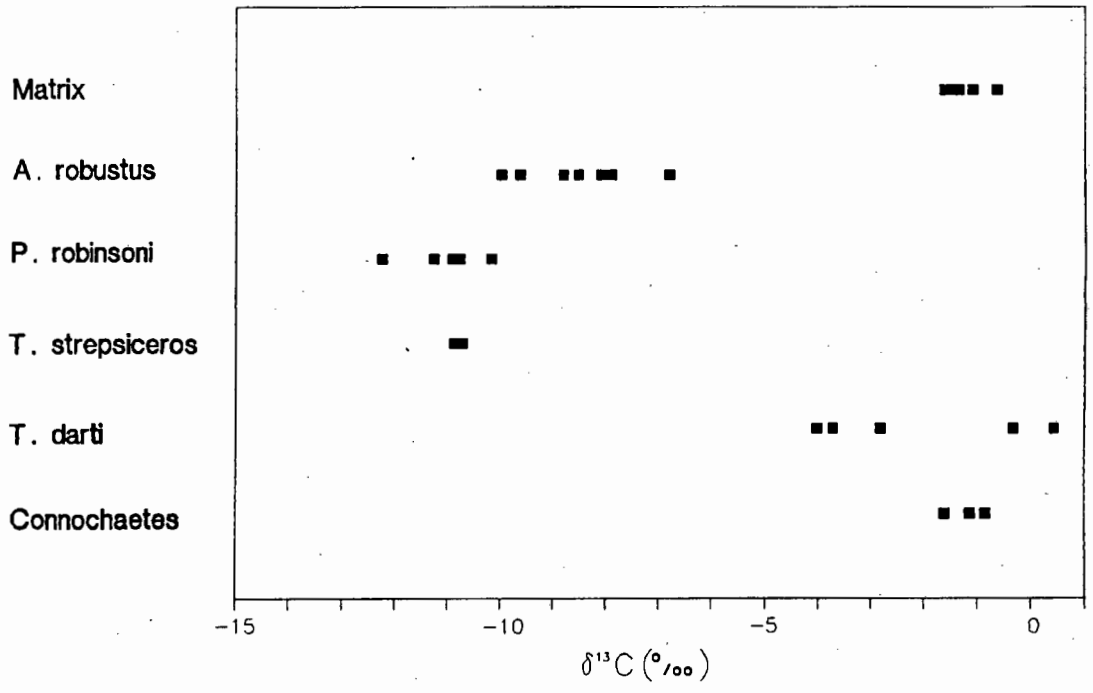

FIGURE 23 A comparison of $\delta^{13}C$ values for *Australopithecus robustus* enamel with other fauna from Swartkrans Member 1.

Discussion

Judging by the browser - grazer spectrum, roughly 20-25% of the isotopic signature of *A. robustus* enamel apatite may be attributed to a C_4 dietary component. There are several possibilities which could account for the observed C_4 contribution to the diet of this hominid. *A. robustus* may have eaten a certain amount of grass (either the blades, seeds or rhizomes or a combination of these), and/or animals which were in turn dependent on grasses. Actual grazing (i.e. eating the blades and stem material) may be discounted by the tooth structure, which is not suitable for grazing, as well by the microwear studies (Walker 1981). Grass seeds and rhizomes remain possibilities, although they seem uneconomic because of their small size. Grass seeds are limited seasonally, but rhizomes are generally available throughout the

year in the Ethiopian highlands (Dunbar 1976), and this is certainly the case in the Transvaal highlands as well (Peters & Maguire 1981). *Theropithecus gelada* is successful on its graminivorous diet because it exploits the most abundant resource (grass), differently throughout the year : blades in the wet season, seeds after that, and rhizomes in the dry season (Dunbar 1976). But *A. robustus* is obviously not exploiting such a specialized graminivorous niche, since the C_4 contribution is limited.

The alternative explanation, that *A. robustus* ate some animal food (perhaps grazing herbivores and/or insects), while at first startling, seems more likely. If this is the case, it would place *A. robustus* back into line with its omnivorous hominid "cousins". It is now understood that many primates are opportunistic feeders and will eat insects and animal food if the opportunity arises. Extreme herbivory is now considered the exception rather than the rule (Kay 1985). *A. africanus* has usually been regarded as an omnivore, if only on the basis of its (slightly) more generalized dentition. There has never been any doubt that later hominids (*Homo*) butchered and ate animal foods. *A. robustus*, however, with few exceptions (e.g. Szalay 1975), seems to have been cast as a specialised herbivore.

Whether the C_4 contribution is derived directly from grasses or indirectly from animals, or both, the isotopic evidence shows that *A. robustus* had a much less specialised diet than previously believed. The results argue against a specialised graminivorous niche as proposed by Jolly (1970). The overall reliance on C_3 foods shown here does not contradict the hypotheses based on SEM studies of enamel microwear which suggest hard fruits as the principle dietary items (Grine 1981, 1986; Walker 1981), but the isotopic evidence further suggests that this hominid was an omnivore, like most primates, rather than a specialized herbivore. Other surprising evidence has recently been produced from Swartkrans to suggest that *A. robustus* was associated with stone and bone tools (Brain *et al* 1988) and was capable of

making and wielding them (Susman 1988). Further isotopic investigation of Lower Pleistocene hominids may produce yet more unexpected results.

SUMMARY

The results for these three applied studies show clearly that stable carbon isotope analysis of fossil tooth enamel successfully distinguishes between diets based on C_4 grasses, and those based on C_3 foods. The most reliable results are obtained when comparative material, including known browsers and grazers from the same deposit, is used as an internal reference. The results for the extinct springbok, *A. bondi*, when compared with *A. marsupialis*, show that the former species was predominantly a grazer. The $\delta^{13}C$ values obtained for the two 1.8 ma old extinct baboons, *P. robinsoni* and *T. darti* from Swartkrans Member 1, show that C_3-based diets are clearly isotopically distinct from graminivorous (C_4-based) diets. The $\delta^{13}C$ results for 8 tooth enamel specimens of *A. robustus* from Members 1, 2 and 3 at Swartkrans show that the diet of this hominid was predominantly C_3-based, but there was a significant C_4 contribution. The C_4 component may be derived either directly from grasses or indirectly from animals eating the grasses. Either way, the results show that *A. robustus* had a less specialised diet than previously believed.

CHAPTER 9

CONCLUSION

A synthesis of the thesis is presented in this chapter, in which the development and applications of stable carbon isotope analysis of mineral apatite in this study is reviewed. A large number of $\delta^{13}C$ values for modern animals has established a database towards a better understanding of the isotopic relationships between diet, apatite and collagen. Although some limitations to the application of isotopic analysis of fossil apatite remain, due to post-depositional change, these problems may be at least partially circumvented using a comparative approach. Some implications of the experimental and applied studies for the patterns of diagenesis and dietary reconstructions are discussed. Once the problems and methodology have been evaluated, it is possible advance another step and consider applications of the method; here, three applied studies illustrate the significance of the method for future palaeontological and palaeoanthropological dietary studies. Finally, some possibilities for further research are suggested.

OVERVIEW

For many years it was assumed that stable carbon isotope ratios from the mineral phase were altered as a result of exchange with the matrix; an assumption based largely on radiocarbon studies which showed that the mineral phase gave highly unreliable dates. The first comprehensive attempt to test this assumption for stable carbon isotopes showed that a consistent isotopic difference of about 8‰ existed between $\delta^{13}C$ collagen and apatite values for modern and fossil bone specimens, suitably pretreated to remove normal carbonates, and therefore Sullivan & Krueger (1981) concluded that $\delta^{13}C$ values from fossil bone apatite were reliable. This conclusion was disputed (Schoeninger & DeNiro 1982), on the grounds that

archaeological faunal and human specimens from archaeological sites in Peru and Mexico did not show similar consistent differences between the two phases. Although Sullivan & Krueger (1983) proposed a modified model to account for the differences, their arguments did not convince Schoeninger & DeNiro (1983), and the debate reached a stalemate.

A review of the chemistry of the mineral phase (Chapter 3) reveals that although some of the carbonate in bone mineral is highly exchangeable and labile (adsorbed carbonate), a more stable 'compartment' of carbonate exists within the crystal structure (structural carbonate). Thus structural carbonate is not part of an open system, and thus methods should be aimed at extracting this compartment in modern and fossil mineral tissues and eliminating the more soluble one. Furthermore, since enamel is denser, more crystalline and contains less organic matrix than bone, it is less likely to change and recrystallise during fossilisation, and therefore it is a more promising candidate for isotopic analyses of fossil material. The approach followed in this thesis for resolving the debate about the reliability of fossil bone and enamel apatite values involves both a characterisation of $\delta^{13}C$ values of modern fauna towards a better understanding of the pathways of $\delta^{13}C$ values in foodwebs, and the development of a new critical test for alteration of biogenic isotopic values in fossils. The results go a long way towards resolving the problems raised in the earlier debate, but go further to illustrate the utility of the method in applied studies.

CARBON ISOTOPES IN MODERN FOODWEBS

In developing this approach, the first prerequisite was to establish the range of $\delta^{13}C$ values for modern browsers, and at the same time, to establish which species were the most reliable C_3 browsers. The species initially included *G. camelopardalis*, *T. scriptus*, *T strepsiceros*, *S. grimmia*, *R. melanotis* and *C. monticola*, but results for some *T. strepsiceros* specimens indicated that they could be opportunistic feeders in certain environments, and so this species was dropped from the list of "reliable" browsers.

The mean and range of $\delta^{13}C_{apatite}$ values for the remaining browsers was established as -14.5±1.6‰, and for $\delta^{13}C_{collagen}$ as -21.4±1.1‰ (Table 1), in close agreement with other published values (Land *et al* 1980; Sullivan & Krueger 1981). Means for $\delta^{13}C_{apatite}$ and $\delta^{13}C_{collagen}$ of grazing herbivores from habitats with C_4 grass cover were established as -0.5±1.3‰ and -8.0±1.1‰ respectively (Table 2).

The pathways of stable carbon isotopes in modern foodwebs and the isotopic relationships between diet, apatite, and collagen must be established before assessments based $\delta^{13}C_{apatite}$ values of unknown diets may be considered. Only recently has the nature of these relationships begun to emerge. Although $\delta^{13}C_{collagen}$ values have been almost routinely used to assess prehistoric diets, the nature of the isotopic relationship between diet and collagen is not clearly understood (Klepinger 1984; Sillen *et al* n.d.). It has been established that $\delta^{13}C_{apatite}$ values are substantially more positive than $\delta^{13}C_{collagen}$ values (i.e. more enriched in ^{13}C) for the same individual, and this enrichment may be attributed the well-known fractionation effects in the $CO_2/HCO_3^-/CO_3^{2-}$ system (Emrich *et al* 1970). A laboratory study reported $\delta^{13}C$ values which gave diet-apatite differences of about +9.6‰, and diet-collagen differences about +3‰, thus implying Δ (collagen-apatite) of about 6.5‰ (DeNiro & Epstein 1978a). Sullivan & Krueger (1981) estimated diet-apatite $\delta^{13}C$ differences to be +12‰, diet-collagen to be +5‰ for C_3 diets and +6‰ for C_4 diets, and obtained a mean Δ of 8‰. The mean values obtained for browsers in this study imply diet-collagen $\delta^{13}C$ differences of about +5‰, and diet-apatite of +12‰, if one assumes that, on average, the plant food base is -26.5‰. Results for the grazers indicate a diet-collagen difference of about 4.5‰ and a diet-apatite difference of 12‰. One difficulty here is that some grazers may not have purely C_4 diets. Comparison of means indicates the overall picture, but there are some disadvantages, particularly at the level of individual animals, and plants may vary considerably even between adjacent micro-habitats.

The first hint that isotopic relationships between collagen and apatite might differ between trophic levels was given in Sullivan & Krueger's (1983) reply to Schoeninger & DeNiro (1982), but their models were not published until 1984 (Krueger & Sullivan 1984). Based on data which indicated that the isotopic difference between collagen and apatite was about 8‰ for herbivores, but less (3-4‰) for carnivores, Krueger and Sullivan (1984) proposed models for carbon isotope pathways in foodwebs, in which collagen $\delta^{13}C$ values are specifically related to dietary protein (the growth aspect), and apatite $\delta^{13}C$ values are related to dietary fuel (the energy aspect). The data from modern animals with different trophic level diets from southern Africa and Malawi are used to provide an independent test of these models. Equations derived from the $\delta^{13}C_{collagen}$ and $\delta^{13}C_{apatite}$ values for these fauna confirm that the relationship between the two phases changes with trophic level. The equations have slightly different slopes to those proposed by Krueger and Sullivan (1984). The slope for the herbivore collagen and apatite relationship is slightly greater than 1.0, while that for the carnivores is slightly less than 1.0, (Chapter 5), but the two slopes are significantly different from each other. Consequently, the average difference in Δ between herbivores and carnivores is slightly less in C_3 biomes compared to the corresponding difference in C_4 biomes. In addition, Δ for each trophic level is slightly reduced in C_3 biomes. Revised foodweb models are suggested on the basis of the new data, which suggest that the diet-apatite difference is +12‰, and the diet-collagen difference is +4-5‰, whatever the trophic level. This model may be used in dietary studies of prehistoric human populations to determine the protein and fuel contributions to the diet separately. It has been applied to a study of late Holocene coastal human populations in the southwestern Cape Province (Lee-Thorp *et al* n.d.).

THE EXTENT OF ALTERATION OF BIOGENIC APATITE $^{13}C/^{12}C$ RATIOS

The test for alteration

The next step was to establish the extent of diagenetic alteration in fossil apatite, and evaluate methods for dealing with the problem. The test for alteration of fossil apatite $\delta^{13}C$ values determined whether or not a difference existed between fossil and modern browsers, since contamination with the matrix carbonates should shift the browser $\delta^{13}C_{apatite}$ values in a positive direction. The results in fact do show such a trend (Figure 10). Even recent browser apatite $\delta^{13}C$ values are more positive than most modern browsers, although some of these specimens fall within the modern range. $\delta^{13}C_{apatite}$ values become increasingly positive with time; by about 100 ka, values are 2-3‰ more positive than the modern mean, and by about 2-3 ma, about 4‰. The value of 4‰ may be a little exaggerated because the oldest site, Makapansgat, also provides the worst-case scenario for carbonate contamination and exchange. Thus the difference increases predictably with time, although the relationship is not linear. In spite of the alteration browser and grazer apatite $\delta^{13}C$ values are clearly distinct, even at Makapansgat, but only for determinations from enamel, since very old bone apatite to have been altered to a much greater degree. A consideration of other explanations for enriched fossil browser $\delta^{13}C$ values suggests that possible effects related to changes in atmospheric CO_2 $\delta^{13}C$ values are minimal compared to diagenetic exchange with the matrix.

The effect of acid pretreatment

The pretreatment procedures (1M acetic acid) were subjected to an evaluation to determine the effects on modern and fossil minerals, using isotopic measurements, CO_2 gas yields and infrared spectroscopy. Results for the modern material reveals that bone and tooth mineral differ isotopically before and after acid treatment. This implies either that biological apatites are isotopically heterogeneous, or that acetic acid fractionates apatite during dissolution. The former explanation is favoured

here, but it is not clear why the more stable phase should be so predictably enriched compared to the more soluble phase. Further experiments using synthetic apatites should provide an answer. The results also show that modern bone mineral recrystallises to brushite after a few days in acetic acid, a change clearly demonstrated in the infrared spectra. It is not clear if this phenomenon applies to enamel as well, since the experiment was only carried out for bone, but the greater crystallinity of enamel should inhibit such recrystallisation to some degree. Thus caution should be exercised in the use of acetic acid pretreatment procedure where archaeological specimens are recent and closely resemble modern mineral in structure and crystallinity.

In general, after acetic acid treatment the difference between fossil and modern browsers is only slightly reduced. Calcite was detected in only two (untreated) specimens, and all traces disappeared after acetic acid treatment. For the remaining specimens, the reduction in CO_2 gas yields and CO_3/PO_4 ratios suggests that a high carbonate apatite, which is probably diagenetic, has been preferentially leached. Although fossil specimens are much more resistant to acetic acid treatment, and show no sign of recrystallisation, CO_2 yields and CO_3/PO_4 ratios for both modern and fossil acid-treated bone or enamel specimens are comparable. A comparison of two acetic acid treatments: standard 1M acetic acid and 1M acetic acid + evacuation indicated that after the initial change between anorganic material and the standard acid treatment, there appears to be little change in the results. Therefore the standard 1M acetic acid treatment, as described here, is adequate for removing calcite and the diagenetic high carbonate apatite phase from fossil apatites. The remaining alteration cannot be removed using present chemical techniques.

Patterns of diagenesis

The patterns of isotopic alteration seen for the fossil browsers and grazers, and the results of the tests described above, have some connotations for the way in which

alteration of the calcified tissues occurs. Browser apatite values become more enriched with the passage of time, and since the difference cannot be eliminated with acetic acid treatment, it must be related to changes in structural carbonate, largely as a result of exchange with enriched groundwater carbonates. Another possible contributing mechanism is discussed below. The rate of change of browser apatite $\delta^{13}C$ values seems to slow after the initial change, suggesting that the process of exchange with matrix carbonates is slowed by some mechanism. The most likely cause is that recrystallisation and crystal growth of the apatite occurs, resulting in the formation of larger, more stable apatite crystals. Apatites have a well-known propensity for crystal growth; during life various mechanisms prevent crystal growth, but these should cease to operate after death. Apatite crystals should continue to grow, incorporating available ions from the surrounding medium in the process. When the crystals have become larger and more stable, however, this growth should slow or cease, and thereafter exchange with matrix carbonates will be limited by the slow rate of solid-state exchange (which is slower by several orders of magnitude). This explanation may account for the apparent change in the rate of isotopic alteration of browser apatite $\delta^{13}C$ values. If bone and tooth mineral is isotopically heterogeneous, as suggested above, the more labile, enriched phase should also be incorporated into the structural positions, in addition to deposit carbonates, during crystal growth. Thus, the enrichment seen in fossil browser $\delta^{13}C_{apatite}$ values may be partly biogenic in origin, although exchange with matrix carbonates is probably the main contributing factor.

Little change is seen in fossil grazer $\delta^{13}C$ values, as expected, since they are close to matrix values. Any detectable changes should be in the direction of matrix values (i.e. in the opposite direction to the browsers, since matrix values are generally slightly more depleted than grazer values), but such small changes as are detectable seem rather to be in the positive direction (enrichment), at least for some

specimens. Although this shift is barely detectable, it provides some support for the idea that alteration of fossil apatites is not simply a result of exchange with matrix carbonates. Other possibilities include the incorporation of the labile, enriched phase as discussed above; the possibility that the modern range of values for fauna which is used as the standard is slightly shifted towards depleted values as a result of *the burning* of fossil fuel; or that the physical process of fossilisation causes some fractionation of the carbon isotopes.

The mechanisms of alteration remain the subject of conjecture at this point, and require further investigation. Nevertheless, it is clear that whatever the mechanics of alteration, the results for browsers and grazers show that they may be clearly distinguished even after 3 ma using carbon isotopic analysis of tooth enamel. The distinction between the two diets should be detectable even further back in time. Preliminary results appear to show reliable dietary isotopic signatures for Miocene (van der Merwe pers. comm.) and even Oligocene faunal specimens (Krueger 1988). The applied studies described in this thesis provide further illustration that the methodology provides useful dietary information for very old fossil material.

APPLIED STUDIES

Antidorcas bondi

The feeding niche of the small extinct springbok, *Antidorcas bondi*, has been uncertain because of its unique combination of featuresincluding small body size, ruminant digestion and hypsodont dentition. It declined rapidly and became extinct at the beginning of the Holocene. Stable carbon isotope analysis provides a means of distinguishing between the two alternative diets proposed for *A. bondi*, but their age precludes the use of collagen as sample material. Bone and enamel apatite therefore provides the only means of dietary assessment. Specimens of *A. bondi* and *A. marsupialis* were compared at Florisbad and these results were compared with *A. bondi* specimens from Swartkrans Member 5. The data from Florisbad shows a

clear distinction between the two species of springbok. *A. marsupialis* $\delta^{13}C_{apatite}$ values cluster around -9.8‰ (±1.2), while the mean for *A. bondi* is -1.4‰ (±2.5). *A. bondi* specimens from Swartkrans have a mean $\delta^{13}C_{apatite}$ value of -2.5‰ (±1.6). There is a small difference of about 1‰ in the $\delta^{13}C$ means for the two occurrences of *A. bondi* at Florisbad and Swartkrans, but the bimodality seen in the Florisbad results makes small differences difficult to interpret. All the results show clearly that *A. bondi* was predominantly a grazer, and not an intermediate mixed feeder.

This finding has some implications concerning the eventual extinction of *A. bondi*, and other (larger) specialised grazers which were present in southern Africa until the end of the Pleistocene. The faunal assemblage of Florisbad contains many specialised grazers, suggesting that a highly productive grassland ecosystem once existed on the southern African plateau. It is now clear that towards the end of the Pleistocene, all of the most specialised grazers at each level of the grazing succession became extinct in this region. A reduction in primary production of grassland is likely to have been a substantial contributing factor in this event.

Papio robinsoni and Theropithecus darti

Two contemporaneous species of extinct baboons, *Papio robinsoni* and *Theropithecus darti* are found in the earlier deposits of the Swartkrans cave. Since the dentition of the modern *Papio ursinus* and the extinct species of *Papio* are closely similar, as are those of the surviving *Theropithecus gelada* and the extinct *Theropithecus*, it is generally accepted that the extinct varieties had diets very similar to their modern counterparts. This assumption may be tested using carbon isotope analysis because the *Papio* diet is primarily C_3-based, while the gelada is a graminivore, and in a C_4 grassland environment these diets are isotopically distinct. A comparative approach, in which known browsers and grazers from the same deposit provided C_3 and C_4 "references", was used to compare the diets of the two extinct baboon species, a

strategy which partly circumvents the problem caused by alteration of the biogenic isotopic signatures. In this case, the $\delta^{13}C$ values for the fossil browsers (*T. cf strepsiceros*) are more positive by about 3.5‰ than the mean value of -14.5‰ established for modern browsers. The results from 6 tooth enamel specimens of each baboon species show that the diets are clearly distinct: *T. darti* depended largely on C_4 grasses, whereas *P. robinsoni* had a consistent C_3 diet. If the effects of diagenesis are taken into account (about 3-3.5‰), the values for the fossil *Papio* are relatively more depleted than those of its modern counterpart in the region, *P. ursinus*. This suggests that *P. robinsoni*, unlike modern *Papio*, lacked a C_4 contribution to its diet, an observation tentatively linked to niche separation of the two sympatric species in the past. Whether the difference between the modern and fossil *Papio* is significant or not, the study demonstrates that carbon isotope analysis of tooth enamel clearly distinguishes graminivorous from C_3-based diets in fossil primates. This provides good reason for application of the method to hominid diets.

Australopithecus robustus

Although much attention has been paid to the specialised dentition and masticatory features of *Australopithecus robustus*, the diet of this hominid has remained something of an enigma. Analogies with modern primates and SEM studies have suggested that the diet consisted of small hard objects (Kay 1985; Grine 1981, 1986; Grine & Kay 1988), but these have not been identified. Various dietary hypotheses have been proposed, including the "seed-eating" hypothesis (Jolly 1970) and a suggestion that hard fruits or nuts were eaten (Grine 1981; Walker 1981; Kay 1985; Grine & Kay 1988). Stable carbon isotope analysis represents another avenue for addressing the problem of *A. robustus* diet. The various hypotheses may be distinguished using stable carbon isotope analysis because a frugivorous diet implies a relatively depleted C_3 isotopic signature while a graminivorous one has been shown to be enriched (near 0‰).

In this study of *A. robustus* specimens from Swartkrans, a similar comparative approach to that of the fossil baboon study was used. The $\delta^{13}C$ results for eight *A. robustus* tooth enamel specimens from Members 1, 2 and 3 cluster around a mean of -8.5‰. When compared with other fauna from the site, these results suggest a diet based mainly on C_3 sources, but a substantial C_4 contribution (perhaps 20-25%) is indicated. Bearing in mind the results obtained in Chapter 5, these would be contributions to dietary fuel which may be taken as representative of the diet for the purposes of this discussion. The C_4 contribution could be direct or indirect, i.e. derived from the C_4 grasses themselves or from animals which in turn ate the grasses. Actual grazing (eating the grass stem and leaf material) may be excluded on the dental and SEM evidence (Walker 1981), but the rhizomes and seeds remain possibilities, although seeds are seasonally restricted. Consumption of grazing animals and/or insects remains a valid alternative.

Either way, the carbon isotopic evidence argues against a specialised diet for *A. robustus*, and in particular against a specialised graminivorous niche as proposed by Jolly (1970). The isotopic evidence and hypotheses based on SEM analysis are not contradictory, since the isotopic evidence indicates an overall reliance on C_3 foods, but it does provide unique and somewhat surprising data to suggest that this hominid was an omnivore. If so, this would place the robust australopithecines back into line with its omnivorous hominid "cousins". The other hominids have generally been regarded as generalists: *A. africanus* has usually been regarded as an omnivore, and the association of early *Homo* with animal foods has never been seriously questioned. Recent evidence from Swartkrans suggests that *A. robustus* was associated with tools (Brain *et al* 1988), and was physically capable of making and wielding them (Susman 1988). In the light of these new archaeological discoveries, the isotopic evidence suggesting omnivory is perhaps not so surprising.

CONCLUSIONS AND FUTURE DIRECTIONS

The three applied studies have demonstrated that unique information about the diets of fossil hominids and fauna may be extracted using stable carbon isotope analysis of enamel. The quantification of diagenetic alteration of the biogenic isotopic signals has allowed an assessment of the problem, and avenues for *circumventing* it have been suggested. There are certain constraints on the application of this method as a result of diagenesis, but these may be at least partially avoided by the comparative faunal approach. The quality of the information from isotopic analysis of fossil apatite is not so precise as that to be gained from collagen, but at present, it is the only viable avenue for isotopic determinations of fossil species of great age.

The results provide empirical support for the method, although there are constraints in its application to fossils. Further development is required in several directions, including the mechanisms of mineralogical and isotopic alteration during the process of fossilisation, refinement of the techniques and applications. The mechanisms of alteration may be best determined in studies of more recent fossils, perhaps in the range of recent to about 20 ka BP, since it seems likely that the most critical changes take place in this time range. Studies using synthetic apatites should provide answers to the questions which have been raised in this study about the effects of acetic acid treatment. Possible fractionation of apatite carbonate during leaching with acetic acid should be clearly visible in experiments using synthetic apatites. If this is shown not to occur, the other possibilities may be considered more seriously. Possibilities exist for improving the rather crude method of extracting biogenic carbonate from fossil apatites, used here. One is the adaptation of the "solubility profile" method developed for trace element analysis (Sillen 1986). Sample size requirements must be scaled down for this reason, as well as for allowing wider

applications of the technique to palaeoanthropological dietary problems. Isotopic analysis is a destructive technique, and the reduction of sample requirements would be a substantial advantage.

Apart from these constraints, many problems involving dietary assessment and palaeoenvironmental reconstruction in the fields of palaeontology and palaeoanthropology may be addressed using stable carbon isotope analysis of fossil apatite. The palaeontological applications include assessments of the diets of extinct individual species as well as lineages. Stable carbon isotope analysis of grazer apatite in this study shows that C_4 grasses existed 3 ma ago in the Transvaal, but more detailed paleaoenvironmental assessment than this is possible. Analysis of suites of fauna from fossil assemblages provides a means for obtaining unique information about palaeoenvironments. Palaeoanthropological applications include more recent studies as well as very ancient ones. In the more recent context, paired collagen and apatite isotopic analyses are capable of providing more detailed information on human diets than is available from collagen alone. This includes separate isotopic assessment of dietary protein and fuels, as well as information about trophic level. The most exciting avenue is undoubtedly in the area of hominid diets. In line with the comparative approach outlined in this study, the diets of contemporaneous hominid species such as *Homo* and *A. robustus*, and conflicting dietary hypotheses may be isotopically evaluated.

Isotopic analysis of fossil apatite has the potential for providing unique information about ancient human and faunal diets. It promises to add a new dimension to palaeodietary studies.

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TABLE 1 $\delta^{13}C$ values for apatite and collagen of modern browsing herbivores, showing means and standard deviations.

UCT#	Species	Origin	Part	$\delta^{13}C_{apatite}$ ‰	$\delta^{13}C_{collagen}$ ‰
684	*D. bicornis*	N. Natal	bone	-15.7	-21.5
1104	*D. bicornis*	Kasungu, Malawi	bone		-21.5
1105	*D. bicornis*	Kasungu, Malawi	bone		-21.3
1989	*D. bicornis*	Kasungu, Malawi	bone		-21.8
1244	*C. natalensis*	E. Transvaal	bone	-13.6	-21.5
685	*C. monticola*	E. Cape	bone		-21.0
1138	*G. camelopardalis*	E. Transvaal	bone	-16.2	-21.7
1139	*G. camelopardalis*	E. Transvaal	bone	-13.0	-21.4
1140	*G. camelopardalis*	E. Transvaal	tooth	-16.0	-21.8
1204	*G. camelopardalis*	Okavango, Botswana	bone	-12.1	-20.5
689	*R. melanotis*	E. Cape	bone	-14.7	-20.3
692	*R. melanotis*	E. Cape	bone	-15.0	
2105	*R. melanotis*	S. Cape	bone		-21.3
2106	*R. melanotis*	S. Cape	bone		-20.3
690	*R. melanotis*	W. Cape	bone	-13.8	
1136	*S. grimmia*	Karoo	bone	-14.1	-19.2
681	*S. grimmia*	E. Cape	bone	--13.8	-22.4
693	*S. grimmia*	W. Cape	bone		-20.9
682	*S. grimmia*	Weza Forest, Natal	bone	-16.5	-21.7
863	*S. grimmia*	Gobabis, Namibia	bone	-10.7	
710	*S. grimmia*	Zimbabwe	bone	-13.9	-19.7
1128	*S. grimmia*	Kasungu, Malawi	bone		-21.3

TABLE 1 (continued)

UCT#	Species	Origin	Part	$\delta^{13}C_{apatite}$ ‰	$\delta^{13}C_{collagen}$ ‰
1984	*S. grimmia*	Kasungu, Malawi	bone		-20.4
1129	*S. grimmia*	Kasungu, Malawi	bone		-21.3
679	*S. grimmia*	Rift Valley 2190m	tooth	-12.0	
680	*S. grimmia*	Mt Elgon, Kenya	bone	-13.7	
666	*T. scriptus*	E. Cape	bone	-13.9	
1133	*T. scriptus*	E. Cape	bone	-16.8	-23.2
1134	*T. scriptus*	E. Cape	bone	-16.0	-23.3
709	*T. scriptus*	E. Cape	bone		-20.2
665	*T. scriptus*	Weza forest, Natal	bone	-17.8	-23.1
1137	*T. scriptus*	E. Transvaal	bone	-15.6	-20.2
1116	*T. scriptus*	Kasungu, Malawi	bone		-22.3
667	*T. scriptus*	Rift Valley 1920m	tooth	-12.4	
1591	*T. scriptus*	Rift Valley 2110m	tooth	-14.3	
668	*T. scriptus*	Rift Valley 2380m	tooth	-15.4	
669	*T. scriptus*	Rift Valley 2290m	bone	-16.7	-23.4
670	*T. scriptus*	Rift Valley 2460m	tooth	-14.6	-23.4
671	*T. scriptus*	Rift Valley 2230m	tooth	-13.8	-22.1
672	*T. scriptus*	Rift Valley 2000m	tooth	-14.1	
673	*T. scriptus*	Rift Valley 2600m	tooth	-13.6	-20.5
674	*T. scriptus*	Rift Valley 2360m	tooth	-15.2	-21.1
676	*T. scriptus*	Rift Valley 2560m	tooth	-14.2	
			Mean	-14.5	-21.4
			Std dev.	±1.6	±1.1

TABLE 2 $\delta^{13}C$ values for apatite and collagen of modern grazers from areas with C_4 grass cover, showing means and standard deviations.

UCT#	Species	Origin	Part	$\delta^{13}C_{collagen}$ ‰	$\delta^{13}C_{apatite}$ ‰
1121	*A. lichensteinii*	Kasungu, Malawi	bone	-6.7	+1.6
1122	*A. lichensteinii*	Kasungu, Malawi	bone	-6.2	+1.1
1994	*A. lichensteinii*	Kasungu, Malawi	bone	-6.2	-0.9
1834	*C. gnou*	OFS	bone	-7.3	+0.2
895	*C. taurinus*	Orapa, Botswana	tooth	-10.5	-0.5
1252	*C. taurinus*	Unknown	bone	-7.6	+0.8
1254	*C. taurinus*	E. Transvaal	bone	-8.5	+1.3
1835	*D. dorcas*	OFS	bone	-9.1	-0.8
1243	*D. lunatus*	E. Transvaal	bone	-6.8	-
1117	*E. burchelli*	Kasungu, Malawi	bone	-7.5	-0.2
1120	*E. burchelli*	Kasungu, Malawi	bone	-6.8	-0.4
1280	*E. burchelli*	Nyika, Malawi	bone	-8.5	-3.9
1980	*E. burchelli*	Nxai Pan, Botswana	bone	-8.6	
1143	*E. zebra*	Karoo	bone	-9.7	-2.0
1109	*H. equinus*	Kasungu, Malawi	bone	-7.9	-1.3
1999	*H. niger*	Kasungu, Malawi	bone	-7.2	-
1997	*K. vardonii*	Kasungu, Malawi	bone	-7.6	-
1981	*O. gazella*	Awarib, Namibia	bone	-8.4	-
1998	*R. arundinum*	Kasungu, Malawi	bone	-8.6	-
1246	*R. arundinum*	Unknown	bone	-8.3	-1.4
1248	*R. fulvorufula*	OFS	bone	-8.4	-
1207	*S. caffer*	Okavango, Botswana	bone	-9.1	-1.2
1977	*S. caffer*	Savuti, Botswana	bone	-9.1	-0.5
2001	*S. caffer*	Kasungu, Malawi	bone	-7.6	-
			Mean	-8.0	-0.5
			Std dev.	±1.1	±1.3

TABLE 3 $\delta^{13}C_{apatite}$ and $\delta^{13}C_{collagen}$ values for tooth and bone of the same individual modern animal.

UCT#	Species	Bone		Tooth	
		$\delta^{13}C_{collagen}$ ‰	$\delta^{13}C_{apatite}$ ‰	$\delta^{13}C_{collagen}$ ‰	$\delta^{13}C_{apatite}$ ‰
899	*T. angasii*	-15.7	-9.1	-16.4	-10.3
900	"	-16.6	-9.4	-16.7	-9.5
901	"	-15.5	-10.1	-16.4	-9.6
902	"	-17.6	-11.3	-17.4	-11.5
904	*H. africaeaustralis*	-21.0	-15.6	-20.7	-13.6
1203	*E. burchelli*	-9.3		-9.9	
1205	*P. aethiopicus*	-13.2	-7.2	-11.7	-5.1
1207	*S. caffer*	-9.1	-1.2	-9.3	+0.9
1275	*P. leo*	-10.7	-7.3	-12.9	-9.4
1276	"	-19.6	-14.5	-19.2	-14.6
1277	*F. serval*	-14.3	-10.9	-13.5	-11.2
2173	*A. marsupialus*	-14.6	-7.9	-16.4	-10.9
2175	"	-17.8	-	-16.9	-10.0
2176	"	-17.1	-	-18.8	-8.4
2424	"	-	-8.4	-	-8.0
2547	*P. ursinus*	-17.4	-12.1	-17.6	-12.0
2548	"	-17.7	-12.8	-17.2	-12.4

TABLE 4 $\delta^{13}C$ values for fauna/browsers from Klasies River Mouth Cave 1. *Raphicerus* sp.may be either *R. melanotis*, a browser, or the related species *R. campestris*, which also eats some grass, and therefore may have a C_4 component in its diet. Tooth denotes enamel and dentine, since these teeth were too small to separate the two. Layers are numbered according to the stratigraphic sequence used by Singer & Wymer (1982).

UCT#	Species	Level/Assn.	Approx age ka BP	Part	$\delta^{13}C$ (‰)
706	*T. scriptus*	Lyr 17b, MSA	115	bone	-12.0
707	*T. scriptus*	Lyr 38/39, MSA	120	bone	-12.6
708	*T. scriptus*	Lyr 14WCH, MSA	100	bone	-12.2
1031	*T. scriptus*	Lyr 13 i.c., MSA	95	tooth	-11.6
1032	*T. scriptus*	Lyr 13H, MSA	70	tooth	-12.1
1033	*T. scriptus*	Lyr 14WC, MSA	100	tooth	-12.4
1034	*T. scriptus*	Lyr 14A, MSA	100	tooth	-11.7
1035	*T. scriptus*	Lyr 15A, MSA	105	bone	-12.4
1036	*T. scriptus*	Lyr 16D+E, MSA	110	bone	-9.2
1037	*T. scriptus*	Lyr 16H, MSA	110	tooth	-13.8
1038	*T. scriptus*	Lyr 17A, MSA	114	bone	-10.6
1024	*Raphicerus* sp.	Lyr 10WC, LSA	5	tooth	-13.2
1025	*Raphicerus* sp.	Lyr 14C, MSA	100	tooth	-10.8
1025	*Raphicerus* sp.	Lyr 14C, MSA	100	bone	-10.1
1026	*Raphicerus* sp.	Lyr 15H, MSA	105	bone	-12.8
1027	*Raphicerus* sp.	Lyr 15A, MSA	105	bone	-11.3
1028	*Raphicerus* sp.	Lyr 16A, MSA	110	bone	-12.4
1029	*Raphicerus* sp.	Lyr 17A, MSA	114	tooth	-12.5
1029	*Raphicerus* sp.	Lyr 17A, MSA	114	bone	-11.8
1030	*Raphicerus* sp.	Lyr 37A, MSA	120	bone	-12.9

TABLE 5 $\delta^{13}C$ values for fossil browsers from Die Kelders. Layers are according to the stratigraphic sequence in Tankard & Schweitzer (1974).

UCT#	Species	Level/Assn.	Approx age ka BP	Part	$\delta^{13}C$ (‰)
691	*R. melanotis*	Lyr 2, LSA	1.5	bone	-12.3
695	*R. melanotis*	Lyr 12, LSA	2	bone	-13.5
696	*R. melanotis*	Lyr 12, LSA	2	bone	-12.6
697	*R. melanotis*	Lyr J. U., MSA	65	bone	-12.9
698	*R. melanotis*	Lyr J. U., MSA	65	bone	-11.8
699	*R. melanotis*	Lyr J. U., MSA	65	bone	-11.9
700	*R. melanotis*	Lyr J. U., MSA	65	bone	-12.1
701	*R. melanotis*	Lyr M.-J.A, MSA	50	bone	-12.9
702	*R. melanotis*	Lyr M.-J.A, MSA	50	bone	-13.9
703	*R. melanotis*	Lyr M/J(0),MSA	55	bone	-12.3
704	*R. melanotis*	Lyr "Tom", MSA	60	bone	-10.1
705	*R. melanotis*	Lyr "Tom", MSA	60	bone	-12.9

TABLE 6 $\delta^{13}C$ values for fossil browsers and grazers from Border Cave. Layers are numbered according to the stratigraphic sequence in Beaumont *et al* (1978).

UCT#	Species	Level/ Assocn	Approx age ka BP	Part	$\delta^{13}C$ (‰)
Browsers					
1473	*T.oryx*	1WA, LSA	37	enamel	+1.8
1472	*T. strepsiceros*	1WA, LSA	40	enamel	-11.9
1872	*P. porcus*	1WA A+B, LSA	40	enamel	-11.0
1470	*O. oreotragus*	2BS.LR, MSA	60	tooth	-11.8
1470	*O. oreotragus*	2BS.LR, MSA	60	bone	-12.8
1471	*T. oryx*	2BS.LR, MSA	60	enamel	-10.1
1469	*T. oryx*	2WA, MSA	80	enamel	-10.8
1474	*T. scriptus*	1GBS.A, MSA	95	tooth	-12.3
1873	*P. porcus*	1GBS.UP, MSA	95	enamel	-13.3
1874	*P. porcus*	1GBS.LRA, MSA	100	enamel	-13.1
Grazers					
1483	*R. fulvorufula*	Top 1WA, LSA	37	tooth	+3.3
1870	*A. bondi*	1WA.B, LSA	40	tooth	+2.3
1481	*E. burchelli*	1WA, LSA	45	enamel	1.4
1477	*E. burchelli*	45-48", LSA	47	enamel	+1.0
1480	*E. burchelli*	2BS.LR, MSA	60	enamel	+0.6
1479	*S. caffer*	2BS.LR, MSA	60	enamel	2.1
1482	*C. taurinus*	2BS.LR, MSA	60	enamel	+3.0
1478	*R. fulvorufula*	2WA, MSA	80	tooth	+3.7
1487	*R. fulvorufula*	1GBS.B, MSA	95	tooth	+0.6
1485	*E. burchelli*	1GBS.B, MSA	95	enamel	+0.5
1488	*S. caffer*	1GBS.B, MSA	95	tooth	+2.1

TABLE 7 $\delta^{13}C$ values for fossil fauna from Sterkfontein and Makapansgat.

UCT#	Species	Provenance	Approx age	Part	$\delta^{13}C$ (‰)
Sterkfontein					
1827	*T. cf. scriptus*	D16 cf. Mbr 6	150 ka	tooth	-11.2
1826	*C. taurinus*	D16 cf. Mbr 6	150 ka	enamel	+0.4
1830	*C. cf. taurinus*	D H2, cf. Mbr 5	2 ma	enamel	-1.8
1828	*T. cf. strepsiceros*	D8 cf. Mbr 4	2.5 ma	enamel	-9.7
1829	*T. oryx*	D13, cf. Mbr 4	2.5 ma	enamel	-5.7
Makapansgat					
1816	*G. camelopardalis*	Mbr 3	3 ma	enamel	-10.0
1817	*G. camelopardalis*	Mbr 3	3 ma	enamel	-10.3
1818	*G. camelopardalis*	Mbr 3	3 ma	enamel	-9.8
1815	*Sivatherium* sp.	Mbr 3	3 ma	enamel	-10.0
1819	*S. maurusium*	Mbr 3	3 ma	enamel	-10.0
1876	Giraffid	Mbr 3	3 ma	enamel	-10.4
1877	*Cephalophus pricei*	Mbr 3	3 ma	enamel	-11.5
1877	*Cephalophus pricei*	Mbr 3	3 ma	bone	-3.7
1878	*Cephalophus pricei*	Mbr 3	3 ma	enamel	-10.8
1878	*Cephalophus pricei*	Mbr 3	3 ma	bone	-7.4
1824	Equid	Mbr 3	3 ma	enamel	+0.2
1825	*Hipparion* sp.	Mbr 3	3 ma	enamel	+0.2

TABLE 8 $\delta^{13}C$ values for apatite and bone collagen of fossil browsers from Mpambanyoni, an Early Iron Age midden dated to about 950 B.P.

UCT#	Species	Part	$\delta^{13}C_{apatite}$ (‰)	$\delta^{13}C_{collagen}$ (‰)
677	*T. scriptus*	tibia	-14.7	-
678	*T. scriptus*	tibia	-12.0	-21.7
686	*C. monticola*	tibia	-13.4	-
687	*C. monticola*	tibia	-11.8	-
688	*C. monticola*	tibia	-10.9	-21.1

TABLE 9 $\delta^{13}C$ and $\delta^{18}O$ values for matrix specimens from the archaeological sites. Layer numbers at Klasies River Mouth are according to the stratigraphic sequence in Singer & Wymer (1982).

UCT#	Provenance	Material	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰
Klasies River Mouth 1				
1213	Recent, midsection	capping flowstone	-4.5	-1.8
1214	LSA, Lyr 4,midsection	shell midden	+0.7	+0.5
1215	LSA, Lyr 4-6,west cutting	shell midden	-1.2	-0.9
1216	Above Lyr 13	sandy matrix	-5.1	-3.1
1217	Lyr 13, MSA	flowstone	-5.5	-1.8
1218	Lyr 13, MSA, midsection	flowstone	-7.8	-3.7
1219	Lyr 13, MSA, midsection	sand	-2.9	-1.7
1220	Lyr 15, midsection	sand	-2.5	-1.0
1221	Lyr 17a, midsection	sand	-2.6	-0.7
1222	Above Lyr 38, rear section	cemented spall	-3.0	-2.4
1223	Lyr 38, rear sect.	cemented spall	-3.5	-2.7
1224	Above Lyr 40, below MSA	flowstone	-3.7	-1.9
1225	Lyr 40, Interglacial beach	sand	-0.4	-0.1
		Mean	-3.2	-1.6
		Std dev.	±2.1	±1.1
Florisbad				
1976	Inclusion in tooth	calcite	-7.8	+2.7
2428	Inclusion in tooth	calcite	-7.4	+2.6
Sterkfontein				
1832	Dump 13 (cf Mbr 4)	breccia	-2.1	-4.8

TABLE 9 (cont)

UCT#	Provenance	Material	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰
Swartkrans: Member 5				
2278		Sediment	-5.1	-5.7
Swartkrans, Member 1				
2449	Inclusion in tooth	calcite	-0.6	-4.4
2451	Inclusion in tooth	calcite	-1.6	-4.7
2452	Inclusion in tooth	calcite	-1.1	-4.6
2455	Attached to fossil	breccia	-1.4	-4.8
2459	Inclusion in tooth	calcite	-1.6	-4.7
2460	Inclusion in tooth	calcite	-1.6	-4.7
		Mean	-1.3	-4.7
		Std dev.	±0.4	±0.1
Makapansgat, Member 3				
1815	Inclusion in bone	calcite	-4.0	-4.5
1877	Inclusion in bone	calcite	-4.4	-4.5
1878	Inclusion in bone	calcite	-4.4	-4.3
		Mean	-4.3	-4.4
		Std dev.	±0.2	±0.1

TABLE 10 The effects of 1M acetic acid on modern bone and tooth enamel $\delta^{13}C$ values, CO_2 gas yields and carbonate/phosphate (CO_3/PO_4) ratios, calculated from the infrared spectra. The right-hand columns show results for acetic acid-treated material after 1 day, except for the last three lines, where periods were 2, 7 and 14 days respectively.

UCT#	Species	Part	Anorganic			Acid-treated		
			$\delta^{13}C$ ‰	Gas yld µmol/mg	CO_3/PO_4	$\delta^{13}C$ ‰	Gas yld µmol/mg	CO_3/PO_4
2424	*A. marsupialus*	bone	-7.6	1.2	-	-8.4	0.4	-
"	"	tooth	-7.5	0.9	0.8	-8.0	0.5	-
2290	*A. pusillus*	bone	-7.3	1.1	1.0	-10.1	0.6	0.8
2547	*P. ursinus*	bone	-9.5	1.3	1.0	-12.1	0.8	0.7
"	"	tooth	-9.3	0.9	0.7	-12.0	0.4	0.4
2548	*P. ursinus*	bone	-10.6	0.9	0.9	-12.7	0.8	0.7
"	"	tooth	-	-	-	-12.4	0.5	0.4
684	*D. bicornis*	bone	-13.3	1.1	1.3	-15.5	0.5	0.9
"	"	"			2 days:	-15.6	0.1	0.2
"	"	"			7 days:	-15.9	0.1	0.1
"	"	"			14 days:	-12.4	0.02	negl.

TABLE 11 The effects of 1M acetic acid on fossil bone, enamel and dentine $\delta^{13}C$ values, CO_2 gas yields and CO_3/PO_4 ratios.

UCT#	Species	Part	Anorganic			Acid-treated		
			$\delta^{13}C$ ‰	Gas yld µmol/mg	CO_3/PO_4	$\delta^{13}C$ ‰	Gas yld µmol/mg	CO_3/PO_4
1878	*C. pricei*	bone	-	-	0.9	-7.4	0.5	0.5
"	"	enamel	-	-	0.7	-10.8	0.5	0.4
1878	calcite	calcite	-4.2	8.1	-			
2217	*A. bondi*	bone	-1.4	1.9	-	-1.9	0.5	-
"	"	enamel	-2.1	0.8	-	-2.6	0.5	-
2450	*T. strepsiceros*	bone	-13.4	1.9	1.0	-12.8	0.5	0.4
"	"	enamel	-9.7	0.7	0.6	-10.9	0.5	0.5
"	"	dentine	-7.8	0.9	0.8	-8.0	0.4	0.4
2452	*Connochaetes* sp.	bone	-5.8	0.9	0.6	-3.6	0.4	0.2
"	"	enamel	-2.2	0.8	0.6	-1.6	0.6	0.4
"	"	dentine	-	-	0.6	-3.7	0.1	0.2
2453	"	bone	-4.7	1.2	-	-2.9	0.5	-
"	"	enamel	-	-	0.6	-0.9	0.6	0.5
"	"	dentine	-	-	0.6	-2.5	0.3	0.2
2463	*P. robinsoni*	bone	-	-	1.6	-8.9	0.4	0.3
"	"	enamel	-	-	0.7	-10.9	0.4	0.4

TABLE 12 A comparison of two pretreatment procedures - 1M acetic acid (this study) and 1M acetic acid + evacuation (Sullivan & Krueger 1981), showing the effects on $\delta^{13}C$ and $\delta^{18}O$ values and CO_2 gas yields compared with the anorganic material (treated only in $NaHCLO_3$ solution).

UCT#	Site	Species	Part	Anorganic			HAc only			HAc + evacuation		
				$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	Yield µmol/mg	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	Yield µmol/mg	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	Yield µmol/mg
1470	Border Cave	*O. oreotragus*	tooth	-	-	-	-11.7	-0.7	0.4	-12.2	+0.2	0.2
1471	Border Cave	*T. oryx*	enamel	-10.2	+3.8	0.5	-10.1	+4.6	0.2	-10.3	4.6	0.4
1480	Border Cave	*E. burchelli*	enamel	-3.4	-0.7	0.6	+0.6	+1.4	0.4	+0.9	+2.2	0.5
1482	Border Cave	*C. gnou*	enamel	-	-	-	+3.0	+3.4	0.5	+3.1	+3.8	0.6
1870	Border Cave	*A. bondi*	enamel	-	-	-	+2.3	+1.5	0.6	+2.3	+1.7	0.6
1967	Florisbad	*A. bondi*	enamel	-0.9	+0.3	0.7	-0.3	+1.5	0.6	-0.3	+1.5	0.6
1976	Florisbad	*A. marsupialus*	enamel	-9.6	+0.2	0.6	-9.7	+1.9	0.5	-9.7	+2.1	0.5

TABLE 13 A comparison of tooth enamel, dentine and bone: $\delta^{13}C$ and $\delta^{18}O$ values for specimens from Klasies River Mouth, Border Cave, Swartkrans and Makapansgat.

UCT#	Species	Enamel		Dentine		Bone	
		$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰	$\delta^{13}C$ ‰	$\delta^{18}O$ ‰
1025	*Raphicerus* sp.	-10.8	-1.4	-	-	-10.1	-2.6
1029	*Raphicerus* sp.	-12.5	-1.5	-	-	-11.8	-2.5
1470	*O. oreotragus*	-11.8	-0.7	-	-	-12.8	-0.6
1877	*C. pricei*	-11.5	+2.6	-	-	-3.7	-0.6
1878	*C. pricei*	-10.8	+1.3	-	-	-7.4	+0.7
2450	*T. cf. strepsiceros*	-10.9	+1.3	-8.0	-2.3	-13.4	-12.8
2452	*Connochaetes* sp.	-1.6	+1.0	-3.7	-0.3	-3.6	-3.7
2453	*Connochaetes* sp.	-0.9	+2.1	-2.5	-0.1	-2.9	-2.1
2454	*T. darti*	-4.0	-0.2	-	-	-6.8	-2.7
2459	*P. robinsoni*	-12.5	-2.0	-	-	-11.0	+4.7
2460	*P. robinsoni*	-10.2	+7.1	-	-	-8.9	-2.0
2461	*P. robinsoni*	-11.3	-0.1	-	-	-12.4	-9.4
2462	*P. robinsoni*	-10.8	-1.3	-	-	-6.5	-0.5
2463	*P. robinsoni*	-10.9	+0.7			-8.9	0.0

TABLE 14 Enamel $\delta^{13}C$ values for fauna from the Florisbad "Old Collection".

UCT#	Ref #	Species	$\delta^{13}C$ (‰)
1837	FLO 3100	*A. marsupialus*	-11.1
1972	FLO 3280	*A. marsupialus*	-11.3
1973	FLO 642	*A. marsupialus*	-8.1
1974	FLO 592	*A. marsupialus*	-8.5
1975	FLO 644	*A. marsupialus*	-10.7
1976	FLO 584	*A. marsupialus*	-9.7
2430	FLO 604	*A. marsupialus*	-10.2
2431	FLO 684	*A. marsupialus*	-8.2
2434	FLO 3310	*A. marsupialus*	-9.2
2438	FLO 4783	*A. marsupialus*	-10.8
1838	FLO 892	*A. bondi*	-2.6
1967	FLO 662	*A. bondi*	-0.3
1968	FLO 721	*A. bondi*	+1.2
1969	FLO 598	*A. bondi*	-0.8
1970	FLO 747	*A. bondi*	-4.4
1971	FLO 762	*A. bondi*	-0.4
2439	FLO 848	*A. bondi*	+2.0
2441	FLO 3236	*A. bondi*	-3.9
2442	FLO 3247	*A. bondi*	+1.2
2444	FLO 3377	*A. bondi*	+0.6
2445	FLO 3379	*A. bondi*	-6.0
2446	FLO 4145	*A. bondi*	-3.8
2426	FLO 1234	*T. oryx*	-11.2
2427	FLO 1261	*T. oryx*	-4.8
2428	FLO 1274	*T. oryx*	-9.9

TABLE 15 Apatite and collagen $\delta^{13}C$ values for modern *Antidorcas marsupialus* (springbok) specimens from South Africa.

UCT#	Locality	Part	$\delta^{13}C_{collagen}$ (‰)	$\delta^{13}C_{apatite}$ (‰)
821	W. Cape	bone	-21.6	-15.5
822	Kimberley	bone	-18.4	-12.9
823	Karoo	bone	-20.0	-13.9
720	Karoo	bone	-19.0	-10.9
1836	OFS	bone	-14.7	-7.7
2173	OFS	tooth	-16.4	-10.9
2173	OFS	bone	-14.6	-7.9
2174	OFS	tooth	-	-8.3
2175	OFS	tooth	-16.9	-10.0
2175	OFS	bone	-17.8	-
2176	OFS	tooth	-18.8	-8.4
2176	OFS	bone	-17.1	-
2424	OFS	tooth	-	-8.0
2424	OFS	bone	-	-8.4

TABLE 16 $\delta^{13}C$ values for *A. bondi* specimens, Swartkrans Member 5.

UCT#	Sk Ref.#	Part	$\delta^{13}C$ (‰)
2197	SkX 42522	RM_3 enamel	-3.4
2198	SkX 42531	LM_3 enamel	-12.0
2199	SkX 44229	LM_3 enamel	-2.2
2200	SkX 44246	RM_3 enamel	-1.9
2201	SkX 44729	LM_1 enamel	-3.7
2202	SkX 41545	femur	-0.1
2203	SkX 41458	radius	-2.3
2205	SkX 43624	Prox. metatarsal	-1.7
2206	SkX 43773	"	-2.6
2207	Sk 2277	LM_3 enamel	-6.0
2208	Sk 2474	RM_3 enamel	-1.2
2209	Sk 6095	M_3 enamel	+0.9
2217	Sk 5901	mandible	-1.9
2217	Sk 5901	enamel	-2.1
2219	Sk 5986	mandible	-3.1
2219	Sk 5986	enamel	-3.4
2227	Sk 9078	tibia	-1.8
2229	Sk 10774	tibia	-2.3
2230	Sk 11470	tibia	-5.6

TABLE 17 $\delta^{13}C$ values for enamel apatite of the fossil baboons, *Papio robinsoni* and *Theropithecus darti*, and comparative fauna from Swartkrans Member 1.

UCT#	Reference	Species	$\delta^{13}C$ (‰)
2448	Sk 3023	*Tragelaphus cf strepsiceros*	-10.7
2449	Sk 2304	*Tragelaphus cf strepsiceros*	-10.9
2450	Sk 2681	*Tragelaphus cf strepsiceros*	-10.9
2451	Sk 2261	*Connochaetes* sp.	-1.2
2452	Sk 2354	*Connochaetes* sp.	-1.6
2453	Sk 5946	*Connochaetes* sp.	-0.9
2454	Sk 411	*Theropithecus darti*	-4.0
2455	Sk 495	*Theropithecus darti*	-2.8
2456	Sk 507	*Theropithecus darti*	-0.3
2457	Sk 2158	*Theropithecus darti*	-0.4
2458	Sk 14162	*Theropithecus darti*	-3.7
2459	Sk 430	*Papio robinsoni*	-12.6
2460	Sk 431	*Papio robinsoni*	-10.2
2461	Sk 439	*Papio robinsoni*	-11.3
2462	Sk 497	*Papio robinsoni*	-10.8
2463	Sk 2319	*Papio robinsoni*	-10.9

TABLE 18 $\delta^{13}C$ values of bone and tooth apatite for modern *Papio ursinus* specimens from South Africa.

UCT#	Locality	Part	$\delta^{13}C_{collagen}$ (‰)	$\delta^{13}C_{apatite}$ (‰)
Transvaal				
1257	Ermelo	bone	-18.5	-13.3
2545	Messina	tooth		-12.7
2546	Ngwanetse	enamel	-17.9	-11.5
2547	Mopani	bone	-17.4	-12.1
2547	Mopani	enamel	-17.6	-12.0
2548	Saagkuil	bone	-17.7	-12.8
2548	Saagkuil	enamel	-17.2	-12.4
2549	Messina	enamel	-16.7	-10.9
2550	Messina	enamel	-16.6	-10.9
Cape				
1237	Orange River	bone	-17.1	-12.6
1236	W. Cape	bone	-20.5	-16.6
1238	W. Cape	bone	-20.6	-
1239	W. Cape	bone	-20.6	-16.3
1240	W. Cape	bone	-20.5	-
2081	W. Cape	bone	-20.0	-14.4

TABLE 19 Enamel $\delta^{13}C$ values of *Australopithecus robustus* tooth fragments from Swartkrans Cave, South Africa.

UCT no	Ref	Tooth	Provenance	$\delta^{13}C$ (‰)
2540	Sk 878		Member 1 (hanging rem)	-6.8
2541	Sk 879	M_1	Member 1, lwr breccia	-8.5
2542	Sk 879		Member 1, lwr breccia	-8.1
2543	Sk 1512		Member 1, pink breccia	-8.8
2689	Sk 5015	LM_3	Member 1, lwr bank	-9.6
2690	Sk 1312	LM^1	Member 2	-8.1
2691	Sk 333	RM_1	Member 2	-10.0
2692	Sk 35025	$RM_?$	Member 3	-7.9

Note: UCT 2540 is an unerupted molar crown, UCT 2542 and UCT 2543 are unassigned.

BROWSER -14,5 ± 1,6‰ C3 APATITE
-21,4 ± 1,1‰ C3 COLLAGEN
GRAZER -0,5 ± 1,3‰ " APATITE
8,0 ± 1,1‰ " COLLAGEN

APPENDIX

Modern faunal species from southern Africa, Malawi and Kenya, with notes on vegetation types, showing $\delta^{13}C_{collagen}$, $\delta^{13}C_{apatite}$ and $\delta^{18}O_{apatite}$ values for bone and tooth.

UCT#	Species	Site	Part	Diet	$\delta^{13}C_{coll}$ ‰	$\delta^{13}C_{ap}$ ‰	$\delta^{18}O_{ap}$ ‰
South Africa							
Eastern Cape: mixed $C_3/C_4/CAM$ bushveld							
685	*Cephalophus monticola*	Kingwilliamstown	b	B	-21.0		
692	*Raphicerus melanotis*	"	b	B		-15.0	-1.8
689	*Raphicerus melanotis*	Grahamstown	b	B	-20.3	-14.7	-1.3
681	*Sylvicapra grimmia*	Uitenhage	b	B	-22.4	-13.8	-1.3
1201	*Sylvicapra grimmia*	Grahamstown	b	B	-22.0		
666	*Tragelaphus scriptus*	"	b	B		-13.9	+3.6
1133	*Tragelaphus scriptus*	Stutterheim	b	B	-23.2	-16.8	-0.9
1134	*Tragelaphus scriptus*	Albany distr.	b	B	-23.3	-16.0	
709	*Tragelaphus scriptus*	Port Elizabeth	b	B	-20.2		
661	*Tragelaphus strepsiceros*	Fort Beaufort	b	B	-19.8	-13.8	+2.4
662	*Tragelaphus strepsiceros*	Uitenhage	b	B	-13.7	-8.2	+3.5
663	*Tragelaphus strepsiceros*	Grahamstown	b	B	-20.7	-15.9	-1.2
1194	*Tragelaphus strepsiceros*	Kirkwood	b	B	-15.8		
1195	*Tragelaphus strepsiceros*	Steytlerville	b	B	-17.3		
1711	*Tragelaphus strepsiceros*	Addo N.P.	b	B	-18.3		
1707	*Taurotragus oryx*	"	t	B/M	-18.4		
1708	*Taurotragus oryx*	"	b	B/M	-18.8		
1701	*Syncerus caffer*	"	t	G	-13.5		
1702	*Syncerus caffer*	"	t	G	-13.8		
Western Cape: C_3 macchia							
690	*Raphicerus melanotis*	Peninsula	b	B		-13.8	-1.2
693	*Sylvicapra grimmia*	Grafwater	b	B	-20.9		
821	*Antidorcas marsupialus*	Churchhaven	b	I	-21.6	-15.5	-3.4
904	*Hystrix africaeaustralis*	Boontjieskloof	b	I	-20.6	-15.6	-2.3
904	*Hystrix africaeaustralis*	Boontjieskloof	t	I	-20.7	-13.6	+1.9
1236	*Papio ursinus*	Cedarberg	b	O	-20.5	-16.6	
1238	*Papio ursinus*	Peninsula	b	O	-20.6		
1239	*Papio ursinus*	Peninsula	b	O	-20.6	-16.3	
1240	*Papio ursinus*	Peninsula	b	O	-20.5		
2081	*Papio ursinus*	Cedarberg	b	O	-20.0	-14.4	-3.4
729	*Canis mesomelas*	Hopefield	b	C/O	-17.9	-12.1	-5.0
1717	*Canis mesomelas*	Robertson	b	C/O	-19.0	-14.1	-4.3
1841	*Hyaena brunnea*	Elands Bay	b	C/O	-14.8	-11.2	-2.6
1716	*Vulpes chama*	Robertson	b	C/O	-16.9	-12.7	-2.3
841	*Otocyon megalotis*	Nardouwsberg	b	I	-19.2	-16.0	
842	*Otocyon megalotis*	Piketberg	b	I	-19.6	-15.9	
1722	*Felis lybica*	Langebaanweg	b	C	-19.0	-14.6	-5.4
1721	*Felis lybica*	Peninsula	b	C	-13.6	-8.8	-5.8
730	*Felis caracal*	Hopefield	b	C	-19.1	-14.4	-4.0
1888	*Panthera pardus*	Du Toit's Kloof	b	C	-18.5		
1889	*Panthera pardus*	Ceres	b	C	-20.2	-14.9	-6.1
1893	*Panthera pardus*	Worcester	b	C	-17.9	-14.0	-2.7
1894	*Panthera pardus*	Montague	b	C	-19.0	-14.7	-2.0

APPENDIX (continued)

Southern Cape: Forest, Mixed C_3/C_4 scrub							
1667	*Cephalophus monticola*	Knysna	b	B	-22.7		
1669	*Tragelaphus scriptus*	"	b	B	-23.0		
1670	*Potomochoerus porcus*	"	B/O		-20.3	-16.5	-5.6
2105	*Raphicerus melanotis*	Bontebok N.P.	b	B	-21.3		
2106	*Raphicerus melanotis*	Bontebok N.P.	b	B	-20.3		
2102	*Damaliscus dorcas*	Bontebok N.P.	b	G	-13.7		
1887	*Panthera pardus*	McGregor	b	C	-19.3		
1890	*Panthera pardus*	Knysna	b	C	-19.0	-13.6	-6.0
1895	*Panthera pardus*	Palmiet	b	C	-18.7	-14.2	-3.0
1719	*Felis caracal*	Caledon	b	C	-19.3	-14.5	-4.5
1720	*Felis caracal*	Caledon	b	C	-18.5	-14.0	-4.5
Northern Cape: Arid scrub							
1237	*Papio ursinus*	Orange R.	b	O	-17.1	-12.6	
872	*Otocyon megalotis*	Kenhardt	b	I	-15.7	-10.7	
873	*Otocyon megalotis*	Brandvlei	b	I	-14.6	-9.8	-2.6
1193	*Proteles cristatus*	"	b	I	-13.3	-8.2	
1891	*Panthera pardus*	Namaqualand	b	C	-13.5	-9.9	+1.6
Karoo, Cape : Karroid scrubland							
1136	*Sylvicapra grimmia*	Prince Albert	b	B	-19.2	-14.1	+3.5
659	*T. strepsiceros*	Kimberley	b	B	-11.3	-4.0	+1.9
720	*A. marsupialus*	Aspoort	b	M	-19.0	-10.9	+6.1
822	*A. marsupialus*	Kimberley	b	M	-18.4	-12.9	+0.7
823	*A. marsupialus*	Hutchinson	b	M	-20.0	-13.9	-1.1
1143	*Equus zebra*	Cradock N.P.	b	G	-9.7	-2.0	+0.9
1258	*Vulpes chama*	Karoo	b	C/O	-9.1	-6.0	
1260	*Vulpes chama*	Karoo	b	C/O	-14.2	-8.1	-5.4
840	*O. megalotis*	Nieuwoudtville	b	I	-20.8	-16.1	
875	*O. megalotis*	Aspoort	b	I	-15.2	-10.1	
Natal : Savanna/woodland							
684	*Diceros bicornis*	Mkuze Game R.	b	B	-21.5	-15.7	+1.7
682	*S. grimmia*	Weza forest	b	B	-21.7	-16.5	-1.8
665	*T. scriptus*	Weza forest	b	B	-23.1	-18.3	-1.1
Orange Free State : C_4 grassland							
1248	*Redunca fulvorufula*	Aasvogelberg	b	G	-8.4		
1834	*Connochaetes gnou*	Soetdoring N.R.	b	G	-7.3	+0.2	+2.5
1835	*Damaliscus dorcas*	"	b	G	-9.1	-0.8	+4.1
1836	*A. marsupialus*	"	b	M	-14.7	-7.7	+2.2
2173	*A. marsupialus*	"	t	M	-16.4	-10.9	+6.6
2173	*A. marsupialus*	"	b	M	-14.6	-7.9	+4.0
2174	*A. marsupialus*	"	t	M		-8.3	+5.9
2175	*A. marsupialus*	"	t	M	-16.9	-10.0	+8.0
2175	*A. marsupialus*	"	b	M	-17.8		
2176	*A. marsupialus*	"	t	M	-18.8	-8.4	+5.5
2176	*A. marsupialus*	"	b	M	-17.1		
2424	*A. marsupialus*	"	t	M		-8.0	+5.0
2424	*A. marsupialus*	"	b	M		-8.4	+4.8

APPENDIX (continued)

Transvaal : bushveld/ savanna & mopani woodland							
1244	*Cephalophus natalensis*	Klaserie	b	B	-21.5	-13.6	-5.2
1138	*Giraffa camelopardalis*	"	b	B	-21.7	-16.2	-0.3
1139	*Giraffa camelopardalis*	"	b	B	-21.4	-13.0	
1140	*Giraffa camelopardalis*	"	b	B	-21.8	-16.0	+3.2
1137	*T. scriptus*	"	b	B	-20.2	-15.6	-1.0
658	*Tragelaphus strepsiceros*	"	b	B	-19.6	-12.2	+1.6
1243	*Damaliscus lunatus*	"	b	G	-6.8		
1254	*Connochaetes taurinus*	"	b	G	-8.5	+1.3	-1.5
1262	*C. mesomelas*	"	b	C/O	-11.3	-6.6	-6.6
1141	*P. leo*	"	b	C	-9.4	-3.4	-1.1
1249	*Taurotragus oryx*	Percy Fyfe N.R.	b	B/M	-18.4		
1257	*Papio ursinus*	Ermelo	b	O	-18.5	-13.3	
2545	*Papio ursinus*	Messina	t	O		-12.7	-0.2
2546	*Papio ursinus*	Ngwanetse	t	O	-17.9	-11.5	-2.4
2547	*Papio ursinus*	Mopani	t	O	-17.6	-12.0	-0.9
2547	*Papio ursinus*	Mopani	b	O	-17.4	-12.1	-1.8
2548	*Papio ursinus*	Messina	t	O	-17.2	-12.4	+0.3
2548	*Papio ursinus*	Messina	b	O	-17.7	-12.8	-1.8
2549	*Papio ursinus*	Messina	t	O	-16.7	-10.9	-0.6
2550	*Papio ursinus*	Messina	t	O	-16.6	-10.9	+0.6
1270	*Genetta genetta*	Makapansgat	b	C	-13.2	-8.6	
1269	*Hyaena brunnea*	Potgietersrus	b	C/O	-9.7	-5.4	
Unknown locality							
1246	*Redunca arundinum*	-	b	G	-8.3	-1.4	+0.6
1252	*Connochaetes taurinus*	-	b	G	-7.6	+0.8	-2.2
1255	*Papio ursinus*	-	t	O	-11.0	-5.0	
1256	*Papio ursinus*	-	b	O	-11.2	-7.4	
1267	*Hyena brunnea*	-	b	C/O	-9.0	-6.9	
1268	*Hyena brunnea*	-	b	C/O	-9.7	-5.8	
1266	*Crocuta crocuta*	-	b	C	-10.9	-7.4	
1263	*Lycoan pictus*	-	b	C	-11.7	-5.4	
1264	*Lycoan pictus*	-	b	C	-12.1	-8.0	
1265	*Lycoan pictus*	-	b	C	-11.7	-6.4	-4.1
1273	*Panthera leo*	-	b	C	-9.6	-4.2	-1.9
1274	*Panthera leo*	-	b	C	-10.7	-7.7	
1271	*Panthera pardus*	-	b	C	-9.9	-4.1	-3.1
1272	*Panthera pardus*	-	b	C	-10.7	-6.0	
1892	*Panthera pardus*	-	b	C	-16.4	-12.8	-2.3
Namibia : Areas sampled - arid scrub/grass							
660	*Tragelaphus strepsiceros*	Keetmanshoop	b	B	-12.8	-6.3	
863	*Sylvicapra grimmia*	Gobabis	b	B		-10.7	+1.8
1981	*Oryx gazella*	Awarib	b	G	-8.4		
Botswana : Arid woodland - riverine woodland							
1204	*Giraffa camelopardalis*	Okavango	b	B	-20.5	-12.1	+4.2
1202	*Tragelaphus strepsiceros*	"	b	B	-21.4		
1203	*Equus burchelli*	"	b	G	-9.3		
1203	*Equus burchelli*	"	t	G	-9.9		
1207	*Syncherus caffer*	"	b	G	-9.1	-1.2	+1.5
1207	*Syncherus caffer*	"	t	G	-9.3	+0.9	+4.5
1977	*Syncherus caffer*	Savuti	b	G	-9.1	-0.5	

APPENDIX (continued)

1205	*P. aethiopicus*	Okavango	b	B/O	-13.2	-7.2	+0.5
1205	*P. aethiopicus*	"	t	B/O	-11.7	-5.1	+5.6
895	*Connochaetes taurinus*	Orapa	t	G	-10.5	-0.5	+2.3
1250	*Connochaetes taurinus*	Kalahari	b	G	-9.0		
1251	*Connochaetes taurinus*	Kalahari	b	G	-10.4		
1253	*Connochaetes taurinus*	Kalahari	b	G	-9.8		
1980	*Connochaetes taurinus*	Nxai Pan	b	G	-8.6		
Zimbabwe : savanna woodland							
710	*Sylvicapra grimmia*	Sabi-Lundi Jn	b	B	-19.7	-13.9	-1.2
Malawi : Kasungu - *Brachystegia* woodland, Liwonde - savanna, Nyika - savanna/alpine grassland							
1104	*Diceros bicornis*	Kasungu	b	B	-21.5		
1105	*Diceros bicornis*	"	b	B	-21.3		
1989	*Diceros bicornis*	"	b	B	-21.8		
1984	*Sylvicapra grimmia*	"	b	B	-20.4		
1128	*Sylvicapra grimmia*	"	b	B	-21.3		
1129	*Sylvicapra grimmia*	"	b	B	-21.3		
1982	*Tragelaphus scriptus*	"	b	B	-22.4		
1116	*Tragelaphus scriptus*	"	b	B	-22.3		
1115	*Taurotragus oryx*	"	b	B/M	-20.2		
1112	*Tragelaphus strepsiceros*	"	b	B	-22.4		
1121	*Alcelaphus lichtensteinii*	"	b	G	-6.7	+1.6	-3.2
1122	*Alcelaphus lichtensteinii*	"	b	G	-6.2	+1.1	-4.0
1994	*Alcelaphus lichtensteinii*	"	b	G	-6.2	-0.9	-3.9
1117	*Equus burchelli*	"	b	G	-7.5	-0.2	-4.1
1120	*Equus burchelli*	"	b	G	-6.8	-0.4	-3.6
1997	*Kobus vardonii*	"	b	G	-7.2		
1998	*Redunca arundinum*	"	b	G	-8.6		
1999	*Hippotragus niger*	"	b	G	-7.2		
2001	*Syncerus caffer*	"	b	G	-7.6		
1109	*Hippotragus equinus*	"	b	G	-7.9	-1.3	-0.9
2002	*Hippopotamus amphibius*	"	b	G/M	-11.4	-5.6	-5.7
2110	*Hystrix africaeaustralis*	"	b	M	-20.9	-16.5	-6.2
1125	*Panthera leo*	"	b	C	-6.3	-2.6	-1.3
1126	*Panthera leo*	"	b	C	-10.2	-7.3	-4.4
1127	*Panthera leo*	"	b	C	-6.1	-2.8	-2.2
2027	*Panthera pardus*	"	b	C	-17.5		
899	*Tragelaphus angasii*	Lengwe	b	M	-15.7	-9.1	+0.3
899	*Tragelaphus angasii*	"	t	M	-16.4	-10.3	-1.0
900	*Tragelaphus angasii*	"	b	M	-16.6	-9.4	-0.2
900	*Tragelaphus angasii*	"	t	M	-16.7	-9.5	-0.3
901	*Tragelaphus angasii*	"	b	M	-15.5	-10.1	-0.7
901	*Tragelaphus angasii*	"	t	M	-16.4	-9.6	+0.4
902	*Tragelaphus angasii*	"	b	M	-17.6	-11.3	+0.2
902	*Tragelaphus angasii*	"	t	M	-17.4	-11.5	+0.6
1280	*Equus burchelli*	Nyika	b	G	-8.5	-3.9	-6.2
1277	*Felis serval*	"	b	C	-14.3	-10.9	-6.1
1277	*Felis serval*	"	t	C	-13.5	-11.2	
1275	*P. leo*	"	b	C	-10.7	-7.3	
1275	*P. leo*	"	t	C	-12.9	-9.4	
1276	*P. leo*	"	b	C	-19.6	-14.5	
1276	*P. leo*	"	t	C	-19.2	-14.6	-4.7

APPENDIX (continued)

Kenya, Central Rift Valley, savanna - forest							
667	*Tragelaphus scriptus*	1920m	t	B		-12.4	+3.2
672	*Tragelaphus scriptus*	2000m	t	B		-14.1	+2.7
1591	*Tragelaphus scriptus*	2110m	t	B		-14.3	-0.2
671	*Tragelaphus scriptus*	2230m	t	B	-22.1	-13.8	+0.1
669	*Tragelaphus scriptus*	2290m	b	B	-23.4	-16.7	-1.2
674	*Tragelaphus scriptus*	2360m	t	B	-21.1	-15.2	+1.4
668	*Tragelaphus scriptus*	2380m	t	B		-15.4	-0.8
670	*Tragelaphus scriptus*	2460m	t	B	-23.4	-14.6	-0.9
676	*Tragelaphus scriptus*	2560m	t	B		-14.2	+0.5
673	*Tragelaphus scriptus*	2600m	t	B	-20.5	-13.6	+0.5
679	*Syvicapra grimmia*	2190m	t	B		-12.0	+3.0
680	*Syvicapra grimmia*	Mt Elgon	t	B		-13.7	+2.9

28 JUN 1989