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# RADIOCARBON DATING HUMAN BONE FROM NAMU, TAUMAKO, SOLOMON ISLANDS; A COMPARISON OF TWO METHODS OF BONE PRETREATMENT

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Bone as a dating material has a great deal of potential, because it is short lived usually relating directly to the event being dated, unless the organism's diet consisted mostly of marine animals. However the porous structure enables buried bones to become contaminated with mobile carbon compounds carried in ground water, while the original carbon components degrade and either combine with the exogenous carbon, or are leached from the bone. The extent of the contamination and degradation depends on the local environmental conditions and the length of time of burial. If the environment is dry with constant low temperatures bones can survive tens of thousands of years and be almost as good as new, requiring basic pretreatment. Unfortunately such conditions are rare and most ancient bones have suffered degradation and contamination by varying degrees.

Over the years various chemical pretreatments have been applied to ancient bones in an attempt to remove the contamination and extract the original carbon components. Both the mineral phase (apatite) and the organic phase (collagen) have been studied. Solutions of acetic acid or triammonium citrate have been used to remove the carbonate contamination from bone apatite (Haynes 1968, Hassan *et al* 1977, Haas and Banewicz 1980, Krueger 1991), but while some studies have been successful others have not. Therefore most radiocarbon laboratories have concentrated on extracting and purifying the organic phase.

Humic substances from the soil and the metabolic products from microorganisms are the main cause of contamination of ancient bone collagen. These compounds are not removed by the simplest pretreatment method, the 'collagen' extraction, which was designed to extract the organic phase from the mineral phase. Hence the impure collagen extracted by this method will be referred to as 'collagen' in this paper. Methods attempting to remove the contamination from the extracted 'collagen' have included alkali treatment (Berger and Libby 1966, Haynes 1967), gelatinisation (Longin 1971, Brown *et al* 1988), and a combination of the two; alkali followed by gelatinisation (Berglund *et al* 1978, Gurfinkel 1987, Redvers-Newton and Coote 1994). If the bone is well preserved a reliable date can be expected. However these methods are not sufficient for removing exogenous carbon from a poorly preserved bone, where there is an overload of contamination complicated by very low levels of original

bone protein.

These straight forward pretreatment methods were utilised by the gas counting and liquid scintillation techniques of radiocarbon dating. With the advent of the use of particle accelerators (AMS), only milligrams of sample are required rather than grams. For this reason further purification steps have been added to the standard methods in recent years (Stafford *et al* 1987, 1988 and 1991, Law *et al* 1991, Van Klinken and Mook 1990, Van Klinken and Hedges 1992). In this way more reliable dates can be obtained from less well preserved bones. However the new methods do not solve all the problems, and badly preserved bones remain undatable (Stafford *et al* 1988, Hedges and Van Klinken 1992).

For many years the standard method of bone pretreatment at the Rafter Radiocarbon Laboratory consisted of 'collagen' extraction followed by an alkali wash. The method did not change until 1993 when a gelatinisation step was added after numerous trials (Redvers-Newton and Coote 1994). In this study I have compared two methods which have both been developed to purify amino acids extracted from ancient bone for radiocarbon dating. Both methods are quite different in their approach; the method proposed by Stafford *et al* (1991) depends on the ability of Amberlite XAD-2 ion exchange resin to adsorb humic contamination, leaving amino acids in solution, whereas the method used by the Oxford Radiocarbon Unit utilises Biorad ion exchange resins to adsorb proteins and amino acids, allowing the contamination to elute from the column (Hedges *et al* 1989). The aim of this study was to determine whether the two methods were equally successful at removing the contamination, and also to assess whether purified amino acids produce more reliable radiocarbon dates than gelatinisation from a moderately well preserved bone. In order to obtain a clearer picture of the removal of contamination from the bone during the pretreatment (Figure 1) radiocarbon measurements were also taken from 'collagen', alkali washed 'collagen' and gelatin portions prior to hydrolysis and purification of amino acids.

## THE PACIFIC REGION ANALYTICAL BONE STANDARD

Numerous human bones were excavated from a burial mound at Namu, Taumako, Solomon Islands by Leach and Davidson 1977-1978 (Whitehead *et al* 1986). From the radiocarbon date of charcoal located in a hearth near the base of the burial mound, and the results of electron spin resonance (ESR) analysis on a number of teeth, the burials were estimated to have taken place over a period of 168 yrs from AD 1530 to 1698 (Whitehead *et al* 1986). The Pacific Region Analytical Bone Standard was formed from a number of bones from the Namu site and supplied by Dr Foss Leach (National Museum of New Zealand). Preparation of the standard took place in the Chemistry Department at the

University of Otago, where the sample was ground and the powder separated into 25g portions using a sample splitter. The following experiment was carried out using bone powder from bottle #9.

Analysis of the carbon and nitrogen percentages, by the Chemistry Department at the University of Otago, indicated that the bone fell into Stafford's class III category and is moderately well preserved (Stafford *et al* 1988). Initial trials extracting the gelatin produced yields of 39 ± 4 mg/g, a greater percentage than the limit of 10-15 mg/g used for Oxford's standard pretreatment (Hedges *et al* 1989). However this bone also contained large amounts of humic compounds; approximately 20% insoluble contamination was extracted by gelatinisation. Therefore reliable radiocarbon dates can only be expected after rigorous cleaning, and differences between the ensuing stages of the pretreatment should be clear. On the basis of these initial trials the bone was considered suitable for this study.

## COLLAGEN EXTRACTION

The bone powder was split into 2g portions using a sample splitter. Eight portions were labelled and used for the following experiment. Each portion was treated with 0.5M HCl at room temperature until effervescence had ceased, at which time demineralisation was complete. After washing to neutral pH, portions 9-1 to 9-4 were dried in a vacuum oven at low temperature, and portions 9-5 to 9-6 were treated in a solution of 0.1M $Na_4P_2O_7$/ 0.1M NaOH at room temperature for 1 hour. After the alkali treatment the washings were combined and the pH adjusted to 1. Precipitated humic acids were collected, rinsed once with distilled water and dried in a vacuum oven. The four insoluble residues of portions 9-5 to 9-8 required a dilute acid wash to remove any carbonates absorbed by the alkali, before being rinsed to a neutral pH and dried in a vacuum oven at low temperature.

## GELATINISATION

A small amount (50 to 80 mg) of impure collagen from each portion was dissolved in 0.01M HCl at 90°C, under a nitrogen atmosphere, overnight. The resultant impure protein solution was filtered using a glass fibre filter and 0.45$\mu$m acrodisc membrane to remove the insoluble contamination. The four gelatin samples previously treated with alkali were applied to a column of Biorad AGMP-50 resin (6$cm^3$). The column was rinsed with 18ml distilled water before eluting the protein with 18ml 1.5M $NH_4OH$. All gelatin portions were neutralised and freeze dried before continuing the pretreatment.

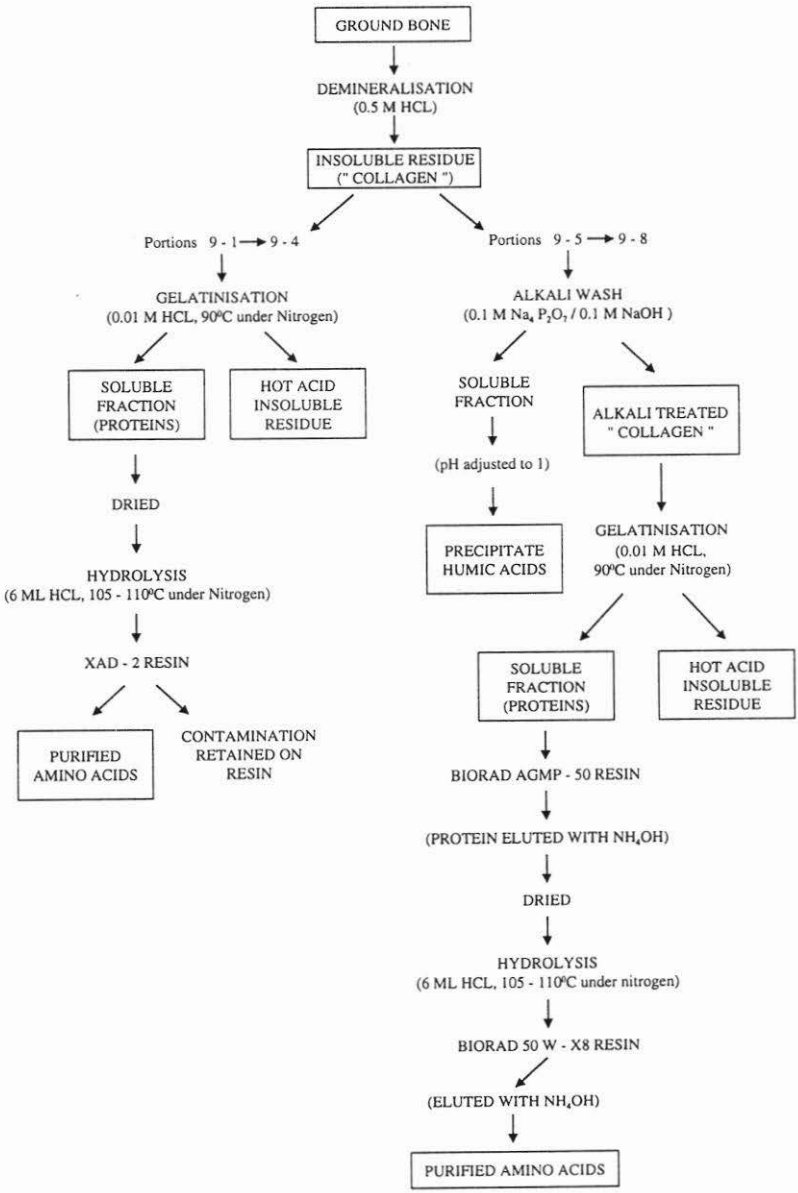


Figure 1. Flow diagram describing the two methods of bone pretreatment

## AMINO-ACID PURIFICATION

Each gelatin portion was hydrolysed to amino-acids in 3ml 6M HCl, at 105-110°C under nitrogen. The four portions previously treated with alkali and purified by the biorad resin were each passed through a Biorad AG50W-X8 column (6cm$^3$) and distilled water and 1.5M $NH_4OH$ were used as before. The other four portions were each applied to a 5ml syringe with acrodisc attached containing 3ml of XAD-2 resin. The eluate was collected and a further 9ml of 6M HCl were added to the column to flush out any residual amino-acids. All eight amino-acid portions were neutralised and freeze dried.

The Biorad resins were prepared by washing consecutively in 3M HCl, 3M NaOH, and finally 3M HCl; each wash was heated to 80-100°C and the resin was rinsed to neutral pH after each stage (Gillespie 1994). The XAD-2 resin was washed in acetone, then thoroughly rinsed in distilled water and stored in 0.5M HCl (Stafford et al 1992). All resins were stored at 4°C until used.

## C-14 DATING

At each main stage of the pretreatment fractions were removed for dating (Table 1). Samples were combusted to carbon dioxide using a sealed quartz tube method with copper oxide and silver wire after Sofer (1980) and Boutton *et al* (1983). The carbon dioxide gas was purified cryogenically before conversion to graphite with excess hydrogen, using iron powder as a catalyst, and deposited in a copper target (Lowe and Judd 1987). Graphite targets were loaded into a 12 position wheel and dated with the GNS AMS facility (Wallace *et al* 1987).

All radiocarbon dates reported are conventional ages before present (BP) after Stuiver and Polach (1977). The errors described represent one standard deviation and constitute statistical errors in the sample and standard determinations together with a systematic component of ± 0.6% associated with the measurement system.

## DISCUSSION

The results of this study show significant differences between the ages of 'collagen', gelatin and amino acid fractions from the Pacific Region Analytical Bone Standard (Table 1, Fig. 2). Changes also occur in the $\delta$13C values throughout the pretreatment. $\delta$13C values, representing $^{13}C$ content, differ between organisms because of diverse diets and/or chemistry, and can be used alongside radiocarbon dates to determine whether contamination has been removed from the dead organism. The contamination in this sample has a

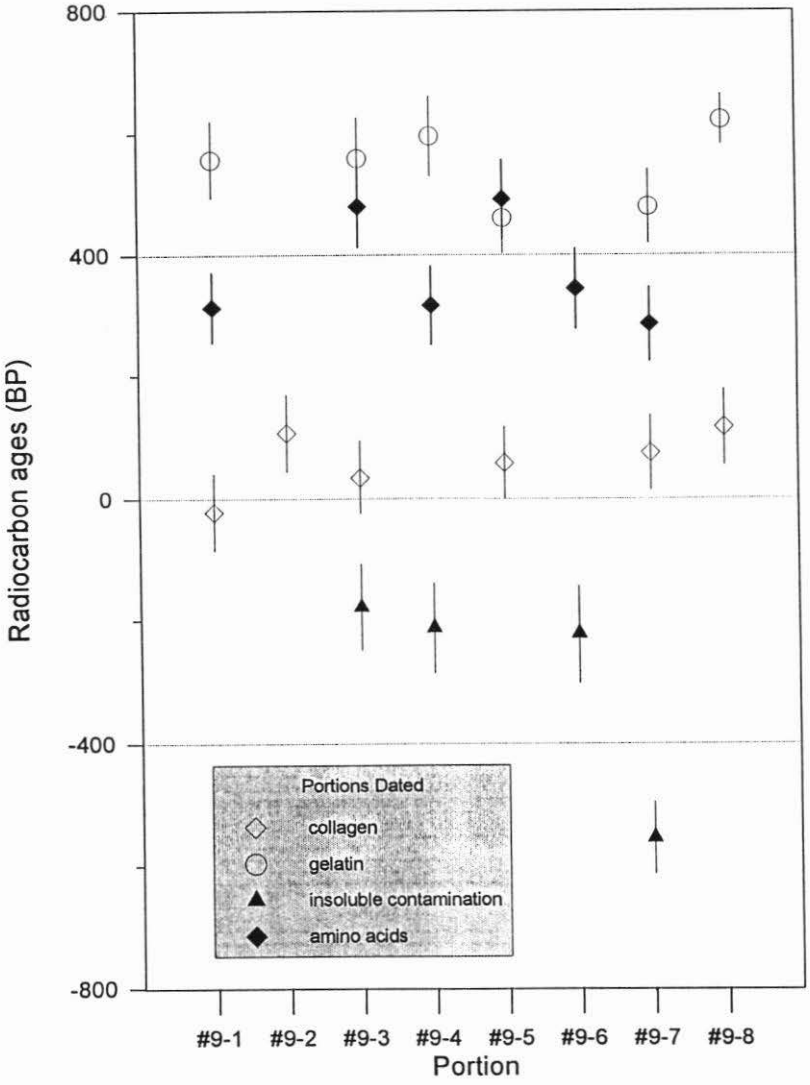


Figure 2. Radiocarbon ages of portions extracted from the Pacific Region Analytical Bone Standard.

more negative $\delta$13C value of -22.29 ± 0.21 parts per mille, compared to the bone protein and amino acids of -16.60 ± 0.08 and -16.66 ± 0.08 parts per mille respectively (Table 2).

Only a small amount of contamination was removed from the 'collagen' during the alkali wash. Approximately 2% of humic acids (the alkali soluble, acid insoluble fraction of the soil humus) were precipitated from the alkali washings with acid, and the $\delta$13C values of the alkali treated 'collagen' were less negative, -17.09 ± 0.09 compared to -17.71 ± 0.16 parts per mille. However not enough contamination was removed to have any effect on the age (Table 1 and Fig. 2). Therefore despite the large amounts of contamination present alkali was found to be an ineffective pretreatment.

Gelatinisation was more successful. A large amount of amorphous brown insoluble material was separated from the protein, resulting in a large difference between the pooled ages of 'collagen', 63 ± 25 yrs BP, and gelatin, 540 ± 26 yrs BP (Table 2). Three portions of the contamination were modern, approximately -200 yrs BP, and one portion contained bomb carbon with an age of -555 yrs BP. The amorphous material was probably composed of a mixture of humic compounds and the remains of microbes.

There was no significant difference between the pooled ages of the amino acids purified by XAD-2 resin, after Stafford *et al* (1991), or the Biorad resins, after Hedges et al (1989) (Table 2). Combining both groups of ages assigns a pooled age of 366 ± 26 yrs BP to the amino acid fraction, significantly younger than the gelatin fraction. There are two possible reasons for this outcome; addition of modern carbon during amino acid purification, or the presence of older exogenous carbon in the gelatin. The former seems highly unlikely, because over 20% of modern carbon is required to decrease a sample of 500 yrs down to 300 yrs. Such a high level of contamination would not only be visible, but would be expected to alter the $\delta$13C values; which was not the case. The most plausible explanation is that the gelatin samples were contaminated by a small amount of old carbon; only 2.5% of "dead" carbon (containing no $^{14}C$) would change the age of a sample of 300 yrs to 500 yrs.

Following this line of thought it can be concluded that the two methods of amino acid purification removed ancient carbon compounds in all but two cases; amino acid portions #9-3 and #9-5 were not significantly different from the age of their gelatin fractions (Table 1, Fig. 2). It is possible that the level of contamination in these two portions was slightly higher than in the other portions, which lead to incomplete removal by XAD-2 or Biorad resins.

The most probable source of these ancient carbon compounds are an acid soluble fraction of humic substances referred to as fulvic acid (Stevenson 1982),

because the other two main fractions of the soil humus, humic acids and humin, are insoluble in acid and would have been completely removed during gelatinisation. Also contamination from microbial activity can be ruled out because remains of microbes would be younger or contemporaneous with the amino acids. Fulvic acid consists of the smaller more mobile, less chemically stable organic molecules of the soil humus. This fraction is usually considered to be the youngest because of the assumption that age of humic compounds increases with chemical stability and decreasing mobility. However studies dating different fractions of the soil humus show that fulvic or humic acids can be older, younger or contemporary to the most stable fraction, the humin (Gillespie *et al* 1992, Scharpenseel and Becker-Heidmann 1992 and Chichagova and Cherkinsky 1993). It has also been assumed that humic compounds do not reach a great age due to constant reworking of the soil. However this is not always the case, humic compounds can be preserved to a great age in buried soils: humic acids of 18,290 ± 200 yrs BP (NZA 501) were extracted from charcoal in alluvial deposits at Lanyon, Australia (Gillespie *et al* 1992), also humic acids of > 28,000 yrs BP were extracted from wood found in Pleistocene deposits, Ob River, West Siberia (Orlova and Panchev 1993) and humic acids of > 30,000 BP were extracted from peat, Timaru Down, Canterbury, New Zealand (Goh 1978). Therefore it is entirely possible that fulvic acid released from an ancient source percolated through the Namu burial site and contaminated the bones with older carbon.

## CONCLUSION

Biorad resins (Hedges *et al* 1989) and XAD-2 resins (Stafford *et al* 1991) were equally successful in removing a small fraction of ancient fulvic acids from the gelatin portions of the Pacific Region Analytical Bone Standard. The resulting pooled age of the amino acids, 366 ± 26 yrs BP, is comparable to the charcoal date from the Namu site; 340 ± 60, NZ 4639 (Whitehead *et al* 1986).

Gelatinisation was very successful at removing a large amount (approximately 20%) of very modern contamination from the 'collagen'. This contamination was probably a mixture of acid insoluble humic compounds and microbial remains, and was quite separate from the fulvic acids removed during amino acid purification. Alkali was relatively ineffectual in removing either form of contamination present and its continued use in bone pretreatment should be questioned.

The results of this study indicate that moderately well preserved bone with high levels of contamination require more sophisticated methods of pretreatment than collagen extraction and gelatinisation. Either protein and amino acid purification using the Biorad ion exchange resins, or the simpler amino acid purification method using XAD-2 resin can be used.

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TABLE 1. **Radiocarbon ages of portions extracted from the Organic Phase of the Pacific Region Analytical Bone standard**

| | Radiocarbon Ages (yrs BP), δ13C values (parts per mille) and Laboratory number (NZA) for the Following Bone Portions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | #9-1 | #9-2 | #9-3 | #9-4 | #9-5 | #9-6 | #9-7 | #9-8 |
| "Collagen" | -22 ± 64<br>δ13C -17.62<br>(NZA 4280) | 107 ± 62<br>δ13C -17.94<br>(NZA 4281) | 35 ± 60<br>δ13C -17.57<br>(NZA 4283) | | | | | |
| Alkali Treated Collagen | | | | | 59 ± 59<br>δ13C -17.12<br>(NZA 4282) | | 75 ± 61<br>δ13C -17.04<br>(NZA 4284) | 116 ± 61<br>δ13C -17.12<br>(NZA 4285) |
| Gelatin | 558 ± 63<br>δ13C -16.56<br>(NZA 4261) | | 560 ± 67<br>δ13C -16.7<br>(NZA 4260) | 596 ± 66<br>δ13C -16.64<br>(NZA 4262) | *461 ± 59<br>δ13C -16.49<br>(NZA 4263) | | *480 ± 61<br>δ13C -16.57<br>(NZA 4263) | *623 ± 41<br>δ13C -16.67<br>(NZA 4264) |
| Hot Acid Insoluble Residue | | | -179 ± 71<br>δ13C -22.6<br>(NZA 4265) | -213 ± 74<br>δ13C -22.28<br>(NZA 4266) | | -223 ± 79<br>δ13C -22.16<br>(NZA 4267) | -555 ± 59<br>δ13C -22.13<br>(NZA 4346) | |
| A-acids (XAD-2 Resin) | 312 ± 58<br>δ13C -16.73<br>(NZA 4295) | | 480 ± 67<br>δ13C -16.74<br>(NZA 4296) | 315 ± 65<br>δ13C -16.66<br>(NZA 4299) | | | | |
| A-Acids (Biorad Resin) | | | | | 492 ± 65<br>δ13C -16.62<br>(NZA 4324) | 343 ± 67<br>δ13C -16.57<br>(NZA 4297) | 284 ± 61<br>δ13C -16.62<br>(NZA 4298) | |

* Gelatin from alkali treated collagen only, no purification with Biorad AGMP-50

TABLE 2. **Pooled radiocarbon ages and mean δ13 values of the Pacific Region Bone Standard.**

| | Pooled Age (yrs BP) | Mean δ13C (parts per mille) |
|---|---|---|
| **"collagen"** | 41 ± 36 | -17.71 ± 0.16 |
| **Alkali Treated Collagen** | 83 ± 35 | -17.09 ± 0.09 |
| **Total Collagen** | 63 ± 25 | - |
| **Gelatin** | 540 ± 26 | -16.60 ± 0.08 |
| **Humics** | - | -22.29 ± 0.21 |
| **Amino Acids (XAD-2 Resin)** | 363 ± 36 | - |
| **Amino Acids (Biorad resins)** | 370 ± 37 | - |
| **Total Amino Acids** | 366 ± 26 | -16.66 ± 0.08 |

Pooled ages were calculated after Ward and Wilson (1978) using the pooled mean and variance equations suitable for measurements on the same sample.