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A preliminary investigation of the application of differential scanning calorimetry to the study of collagen degradation in archaeological bone

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Abstract

The study investigated the potential application of differential scanning calorimetry (DSC) to archaeological bone collagen deterioration. The thermal transition of collagen was compared with the preservation state of the bones. Methods of sample preparation were shown to have a significant effect upon the ability to extract reproducible, reliable thermal data from the collagen. Three main protocols were examined, but the optimal method of collagen extraction (10%, w/v ethyldiamine tetraacetic acid demineralisation of bone shards) was slow, reducing the overall utility of DSC for archaeology. Comparison of the T_{max} (the maximum temperature of thermal transition) with the diagenetic state of the bone revealed no correlation with histological deterioration or alterations to the bone mineral or organic components. A correlation was observed, however, in young bone samples between T_{max} and age. This correlation was improved when thermal age, a parameter that integrates thermal history with the temperature dependence of collagen gelatinisation, was used. In thermally older bones T_{max} displayed little variation.

Keywords: DSC; Archaeological bone; Collagen; Diagenesis

1. Introduction

Differential scanning calorimetry (DSC) has been successfully used as a tool for investigating collagen degradation by correlating changes in thermal transition (T_{max}) with the deterioration of historic parchments and leather [1]. The method is particularly appropriate to the study of collagen-based tissues and materials, because of an unusually large endotherm related to denaturation of type I collagen [2]. This irreversible transition is a feature of the collagen triple helix, although there is disagreement over what the transition represents. In the Flory and Garret model [3], the transition is considered as a melting phenomenon, Miles et al. [4] argue that it is an irreversible rate process; while Boghosian et al. [5] argue, using results from Raman spectroscopic analysis, that denaturing does not actually lead to denaturation. Lowering of the T_{max} occurs if collagen fibres are swelled [6], or if the collagen deteriorates [1].

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Dehydration [6], cross-linking [7] and mineralisation [8] can all increase T_{max}. Elevation of T_{max}usually occurs without a concomitant increase in the enthalpy of transition ΔH. In such circumstances this implies a decrease in ΔS the entropy of transition ($T = \Delta H/\Delta S$) [7]. The idea has been developed by Miles and Gelashvilli [6] to argue that increase in thermal stability of dehydrated collagen is due to the reduced free-volume available for denaturing α-chains (the polymer-in-a-box model). Their model would explain why mineralisation of collagen fibres has such a dramatic effect on the transition temperature [8]. Mineralisation within gap and overlap zones will further increase the confinement of the collagen molecules.

1.1. The 'enzyme exclusion' hypothesis

We have argued that mineralised collagen is physically protected from enzymolysis (autolysis and microbial decay) by the presence of apatite [9] (Fig. 1(a)). Partial loss of the mineral phase will expose collagen to collagenolytic enzymes (Fig. 1(b)), which will in turn increase the solubility and thus sensitivity to proteolysis. We anticipate that loss of 'exposed' collagen will be absolute and complete (Fig. 1(c)). It follows that any residual collagen detected in archaeological bone has been 'protected' by the mineral phase from enzymatic attack and has suffered only chemical modification. The 'enzyme exclusion' hypothesis would predict that the diagenetic state of residual collagen is related, not to the state of bone preservation, but instead to thermal history.

DSC enables us to test this prediction, extracted collagen from bones of the same thermal age will have the same state of collagen preservation; ΔH of bone collagen (per unit weight bone) will be unpredictable, but all collagens will have similar T_{max}.

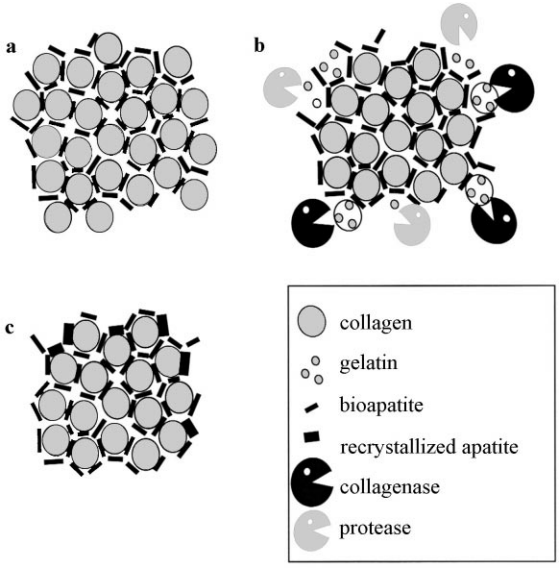

Fig. 1. The enzyme-exclusion hypothesis. Most collagen in bone is protected from enzymolysis by physical exclusion of enzymes (a). Any exposed collagen is susceptible to attack from collagenase (b), and following gelatinisation less specific proteases (many of which are smaller than matrix metalloproteases. No insoluble collagen will remain in the exposed fraction. All insoluble collagen remaining in the bone (c) is assumed to remain intact and only degrades by slow and predictable chemical decomposition.

2. Materials and methods

Archaeological bones displaying variable preservation states were obtained from a range of UK sites (Table 1). The state of diagenesis of each bone was determined using a range of so-called 'diagenetic parameters' [10,11]. The parameters represent simple methods of rapidly characterising aspects of the diagenetic state of the organic and mineral components of bone. Nitrogen content (%N) of powdered whole bone was measured using a CHN analyser (Europa ANCA Roboprep). The histological preservation was determined using the histological index (HI), a simple graded system of 0–5 as measured and described in [10]. Porosity of the bone was estimated from measurements of the water content in bone in equilibrium with a controlled water vapour pressure: microporosity (μ porosity) (i.e., the volume of bone retaining water up to a water vapour pressure of 75% of saturation which corresponds to a nominal pore radius of ≥ 4 nm), and macroporosity (M porosity), which includes pores of radii > 4 nm. Mineral alterations were measured using Fourier transform infrared spectroscopy (FTIR). Crystallinity was assessed using the index based upon the splitting of the IR phosphate doublet at 567 and 603 cm^{-1}; this 'splitting factor' (SF) corresponds to a generalised 'degree of crystallinity' of the bioapatite [12–14]. The SF was determined using the simple measurement described by

Table 1
Sample details and results of diagenetic analyses (L. mamm: large, unidentified mammal; M porosity: macroporosity (pore radii > 4 nm); μ porosity: microporosity (pore radii < 4 nm); thermal age: a parameter that integrates thermal history with the temperature dependence of collagen gelatinisation)

Samples	Species	Bone	Age BP	Thermal age BP	%N	HI	SF	C/P	μ Porosity	M porosity
Modern mineralised	Bovine	Femur	0	0	4.8	5	2.7	0.36	0.0597	0.0674
Modern collagen	Bovine	Femur	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Modern collagen*	Bovine	Femur	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Brean Down										
6422	Cattle	Metatarsal	3700	6360	0.8	0	3.3	0.29	0.1080	0.0200
6121	Cattle	Humerus	3700	6360	0.8	1	3.2	0.22	0.1068	0.0260
4291	Cattle	Radius	3000	4469	0.9	1	3.7	0.25	0.1833	0.0414
3840	Cattle	Metatarsal	3200	5500	1.3	4	3.0	0.33	0.1072	0.0368
6205c	Cattle	Radius	3300	6016	1.5	0	3.3	0.32	0.2562	0.0200
4568	Cattle	Humerus	3000	4469	1.7	0	3.3	0.24	0.2330	0.0328
4503	Cattle	Ulna	3000	4469	2.2	0	3.2	0.26	0.3237	0.0363
6205e	Cattle	Humerus	3300	6016	4.3	n.a.	3.3	0.39	0.0490	0.0515
5799	Sheep	Radius	3200	5500	3.3	5	2.7	0.48	0.0364	0.0471
5757	Sheep	Metatarsal	3200	5500	3.8	n.a.	3.1	0.34	0.0360	0.0411
5755	Sheep	Metatarsal	3200	5500	3.9	4	2.8	0.42	0.1145	0.0431
6205a	Sheep	Metatarsal	3300	6016	3.8	5	2.8	0.45	0.0431	0.0445
Yarnton										
7058c	Cow	Femur	2700	5011	1.0	4	3.0	0.30	0.0510	0.0326
7058a	Cow	Femur	2700	5011	1.1	0	3.6	0.22	0.1160	0.0277
7058b	Pig	Femur	2700	5011	1.1	0	3.2	0.25	0.0781	0.0275
7554a	Cow	Femur	2300	2412	3.4	4	2.7	0.35	0.0309	0.0541
7554e	L. mamm	Femur	2300	2412	0.9	0	3.3	0.19	0.1866	0.0234
7554c	L. mamm	Femur	2300	2412	1.2	0	3.0	0.30	0.1512	0.0412
7875b	L. mamm	Femur	3000	5568	1.4	3	3.5	0.18	0.3643	0.0279
7875e	Cow	Femur	3000	5568	1.4	3	3.5	0.16	0.3758	0.0273
7875a	L. mamm	Femur	3000	5568	2.7	0	3.0	0.30	0.2270	0.0302
7875f	L. mamm	Femur	3000	5568	0.3	3	3.4	0.21	0.2962	0.0303
7870a	Cow	Femur	1800	3341	0.8	0	3.1	0.24	0.0979	0.0283
7870b	Cow	Femur	1800	3341	0.41	0	3.1	0.248	0.1306	0.0299
7870c	L. mamm	Femur	1800	3341	2.6	1	2.9	0.30	0.0608	0.0399
7870d	Sheep/goat	Femur	1800	3341	1.9	0	3.2	0.22	0.1025	0.0375
7870e	L. mamm	Femur	1800	3341	1.4	0	3.3	0.22	0.1695	0.0303
5506a	L. mamm	Femur	4650	8630	0.1	0	3.3	0.31	0.7821	0.0305
5359a	L. mamm	Femur	4650	8630	0.1	0	3.4	0.29	0.5970	0.0341
5359c	L. mamm	Femur	4650	8630	0.1	0	3.4	0.30	0.5888	0.0336
19	Cow	Femur	3000	5568	3.1	5	2.9	0.38	0.1101	0.0467
32a	Cow	Femur	3000	5568	3.9	3	2.7	0.38	0.0647	0.0533
33	Horse	Femur	3000	5568	4.7	5	2.6	0.47	0.0785	0.0620
41a	L. mamm	Femur	3000	5568	4.3	4	2.6	0.42	0.0803	0.0632
Poundbury										
Po1357	Human	Femur	1700	3237	1.4	1	3.1	0.37	0.2414	0.0363
Po1351	Human	Femur	1700	3237	1.4	1	3.0	0.41	0.2324	0.0355
Po1348	Human	Femur	1700	3237	1.6	0	3.2	0.45	0.2154	0.0370
Po1403	Human	Femur	1700	3237	2.5	2	2.8	0.98	0.3325	0.0400
Po1409	Human	Femur	1700	3237	1.0	0	3.2	0.76	0.3244	0.0360
Roman										
Carlisle			1800	3094						
Sergontium			1700	2210						

Table 1 (*Continued*)

Samples	Species	Bone	Age BP	Thermal age BP	%N	HI	SF	C/P	μ Porosity	M porosity
Bercy										
B8600	Ox	Metapodium	6000	18037	3.8	5	2.8	0.40	0.0668	0.0660
B8900	Ox	Tibia	6000	18037	3.1	5	2.7	0.36	0.0385	0.0549
B2000	Aurochs	Radius	6000	18037	1.0	5	3.0	0.57	0.0898	0.0548
B2200	Aurochs	Tibia	6000	18037	3.5	5	3.2	0.26	0.1264	0.0509
B8400	Ox	Metapodium	6000	18037	0.7	0	3.2	0.32	0.2856	0.0329
Pleistocene samples										
GTW auroch	Aurochs	Tibia	30000	12000	3.2	4	2.9	1.18	n.a.	n.a.
Illford type mammoth (surface)	Mammoth	Scapula	200000	40000	0.3	n.a.	3.7	0.26	n.a.	n.a.
Illford type mammoth (internal)	Mammoth	Scapula	200000	40000	2.3	3	3.2	0.36	n.a.	n.a.
Gough's Cave										
M49911	Equus ferus	Phalanx	14500	12481	4.6	5	2.7	0.51	0.1156	0.0607
M49949	Equus ferus	Phalanx	14500	12481	4.1	5	2.6	0.58	0.1990	0.0614
M49737	Equus ferus	Phalanx	14500	12481	4.5	5	2.7	0.49	0.0891	0.0521
M49834	Equus ferus	Phalanx	14500	12481	4.2	5	2.7	0.89	0.0795	0.0591
GC8741	Equus ferus	Phalanx	14500	12481	0.9	3	3.4	0.46	0.3387	0.0249
M49805	Equus ferus	Phalanx	14500	12481	0.7	5	3.0	0.44	0.2396	0.0456
Palace Lees										
Cooked	Bovine	Femur	1	1						
Uncooked	Bovine	Femur	1	1						
Wood Hall										
2.1A	Cattle		600	777		4	3.1	n.a.	n.a.	n.a.
2.1B	Cattle		600	777		5	2.7	n.a.	n.a.	n.a.

Weiner and Bar-Yosef [13]. The carbonate/phosphate ratio (C/P) was also measured using FTIR to assess biogenic carbonate loss and/or diagenetic carbonate inclusion in the archaeological samples. In this project the method employed by Wright and Schwarcz [15] has been followed. Altered bones may show both elevated and depleted C/P ratios in comparison with fresh bone.

For DSC analysis, bone and collagen samples were prepared using five alternative methods, in order to find an optimal balance between reproducibility, speed, safety and cost.

1. no extraction (whole bone powder);
2. extraction with 0.6 M HCl, 4°C, bone shards (0.36 g sample to 12 ml acid);
   - 2.1. extraction with 0.6 M HCl in the presence of counter ions (10%, w/v NaCl) to reduce swelling, 4°C, bone shards;
3. extraction with 10% (w/v) ethyldiamine tetraacetic acid (EDTA), pH 8.0+protease inhibitors (*p*-hydroxy-mercuri-benzoic acid, benzamidine, ε-amino caproic acid and *N*-tosyl-L-phenylanine chloromethyl ketone), 4°C, bone powders;
   - 3.1. extraction with 10% (w/v) EDTA, pH 8.0+protease inhibitors, 4°C, bone shards;
   - 3.2. extraction with 10% (w/v) EDTA, pH 8.0, 4°C, bone shards;
   - 3.3. extraction with 10% (w/v) EDTA, pH 8.0, 4°C, bone powders.

For analysis the samples were encapsulated in stainless steel sample pans in the presence of excess water to ensure complete hydration. Samples were run on a Perkin-Elmer Pyris1 DSC at a scan rate of 10°C/min.

3. Results and discussion

3.1. Extraction methods

The most simple preparation method was the using of otherwise untreated, powdered bone (Method 1).

Previous studies of mineralised collagen had reported extreme elevation in the T_{max} relative to non-mineralised protein collagen [8]. Despite being able to reproduce these results on modern bone, we were unable to obtain satisfactory signals in archaeological bone, despite the use of large stainless steel pans and upwards of 100 mg of sample.

Further investigations therefore focused upon extractable collagen. All results from the DSC analyses of extractable collagen can be seen in Table 2. Mineral acid dissolution (Methods 2 and 2.1) gave high yields of collagen. Bone shards were used in preference to powders as it is has been observed that powdering bone damages the collagen [16]. Acid extraction resulted in inevitable swelling of the collagen. The T_{max} values ($45 \pm 5°C$, $n = 40$) were higher than tendon (e.g. [2]), due either to more cross-linking or incomplete demineralisation. The

Table 2
Results of DSC analyses for all sample preparation techniques[a]

Samples	T_{max}				ΔH			
	HCl	HCl+NaCl	EDTA	EDTA*	HCl	HCl+NaCl	EDTA	EDTA*
Modern mineralised								
Modern collagen			69.5				3.90	
Modern collagen*				68.7				10.93
Brean Down								
6422	47.27				33.03			
6121	47.80			52.7	37.45			2.80
4291	45.17			47.8	6.91			4.33
3840	48.20				18.87			
6205c	40.43				30.09			
4568	45.47			55.4	4.68			5.62
4503	47.80				4.22			
6205e	50.53			47.3	32.05			4.80
5799	42.80				16.23			
5757	45.57				16.51			
5755				49.3				4.89
6205a	45.37			48.2	14.88			7.45
Yarnton								
7058c	43.70				14.21			
7058a	45.63		56.9		3.82		2.84	
7058b	46.43				4.85			
7554a	49.07				25.94			
7554e	42.83				9.69			
7554c	47.00				8.79			
7875b	45.30				3.84			
7875e	43.23				4.51			
7875a	45.17				7.99			
7875f			51.2				1.40	
7870a			52.8				2.65	
7870b			52.9				1.12	
7870c	44.80				14.32			
7870d	46.93				9.50			
7870e	49.30				8.55			
5506a	No T_{max}				No ΔH			
5359a								
5359c	No T_{max}				No ΔH			
19	48.17				27.99			
32a	50.53				32.05			
33			52.2					
41a	41.47				4.43			

Table 2 (*Continued*)

Samples	T_{max}				ΔH			
	HCl	HCl+NaCl	EDTA	EDTA*	HCl	HCl+NaCl	EDTA	EDTA*
Poundbury								
Po1357								
Po1351	51.80				18.27			
Po1348	48.90		64.2		9.75		1.29	
Po1403	55.43				4.10			
Po1409								
Roman								
Carlisle			52.0					
Sergontium			58.0					
Bercy								
B8600			50.5				6.68	
B8900								
B2000								
B2200			52.6				5.47	
B8400			51.9				3.37	
Pleistocene samples								
GTW auroch				No T_{max}				No ΔH
Illford type mammoth (surface)				No T_{max}				No ΔH
Illford type mammoth (internal)				48.75				5.64
Gough's Cave								
M49911	No T_{max}		No T_{max}		No ΔH		No ΔH	
M49949	No T_{max}			No T_{max}	No ΔH			No ΔH
M49737	No T_{max}				No ΔH			
M49834	No T_{max}				No ΔH			
GC8741				No T_{max}				No ΔH
M49805				No T_{max}				No ΔH
Palace Lees								
Cooked			61.7				3.15	
Uncooked			67.9				4.14	
Wood Hall								
2.1A			61.4				9.86	
2.1B			59.9	62.1			8.60	10.25

[a] All samples were run by PETA except * where sample was run by Dr. M. Odlyha.

T_{max} values correlated neither with physical appearance, nor diagenetic indicators, although there was a slight ($p < 0.05$) correlation with age (Table 3). The 23°C lowering in T_{max} of swelled (HCl extracted) bone collagen, when compared with EDTA extracted samples, was similar to that observed for rat-tail tendon following swelling in acetic acid [2]. The ΔH of the EDTA extracted fully hydrated bone collagen ($\Delta H = 4.7 \pm 2.7$ J/g, wet weight insoluble extract; $n = 17$) was only one-third that of HCl preparations ($\Delta H = 14.7 \pm 10.6$ J/g wet weight insoluble extract, $n = 29$). The low values and large errors obtained with both estimates are in part due to variable degrees of hydration but the values are nevertheless well below typical measurements (70 J/g dry weight).

Comparison of T_{max} values of collagen extracted from two medieval bones using three separate methods (Methods 2, 2.1 and 3) confirmed that only the more time consuming method of EDTA demineralisation avoided swelling of the collagen

Table 3
Pearson's correlation coefficients, r, for T_{max} against measured diagenetic parameters for HCl and EDTA demineralised archaeological bones (age < 6K yr)

	%N	SF	M porosity	μ Porosity	HI	C/P	Age	Thermal age
T_{max} HCl	0.09	−0.18	0.21	0.07	−0.05	0.51	−0.42*	−0.41*
T_{max} EDTA	−0.35	−0.12	−0.37	0.40**	−0.63**	0.01	−0.82**	−0.79**

* $p < 0.05$; ** $p < 0.001$.

(Fig. 2). Method 3 was not only slow, but also dangerous and costly (due to the use of protease inhibitors). Simplified versions of the EDTA method (Methods 3.1–3.3) were therefore examined. As all three protocols produced the same T_{max} within experimental error (±2°C), the most straightforward (3.3) was used for all remaining analyses.

3.2. Correlation with state of bone diagenesis

Current studies on archaeological bone use a variety of simple parameters to determine the preservation state of both mineral and organic phases [13–15,17,18]. Some of these measure change in the mineral structure, notably the infrared SF and changes in the carbonate/phosphate ratio of mineral (C/P). The organic nitrogen content reflects changes in the overall protein abundance. In modern bone measured using the same instrument nitrogen content represented 4.8 wt.% of total bone. Porosity changes correlate with the loss of nitrogen and an increase in the crystallinity. Histology is a gross examination of microscopic alteration to the physiological microstructure of bone, caused primarily by microbial attack.

There was no significant correlation between any of above diagenetic parameters and T_{max} (see Table 3 and Fig. 3). Despite the fact that some bones display evidence of considerable diagenetic change, these did not correlate with the results from the DSC analyses. The only correlation was between T_{max} and the thermal age, calculated using site temperature data (Table 4). However, the correlation is only with bones of less than 6K $yr_{10°C}$ (see Table 3 and Fig. 4). Older samples had low, but consistent, transition temperatures ($T_{max} = 51 \pm 2°C$, $n = 4$) with the exception of a well-preserved horse metatarsal from Gough's Cave (14.5K yr calibrated BP, thermal age of 12.5K $yr_{10°C}$ no measurable T_{max}).

Miles and Gelashvilli [6] have argued that thermal stability of collagen and other proteins is related to

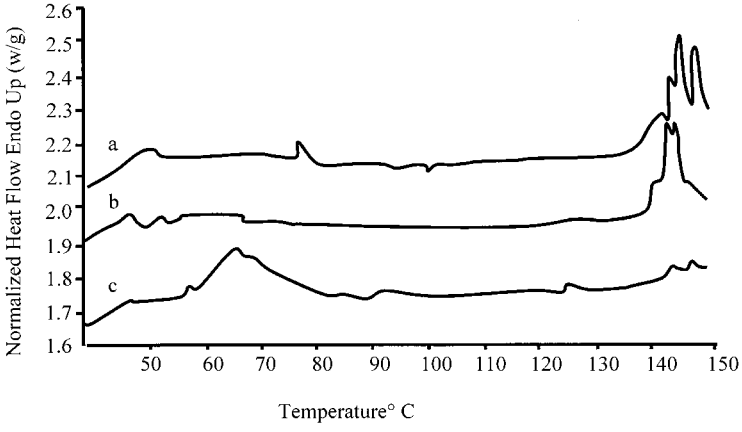

Fig. 2. Comparative DSC analysis. Mediaeval bone from Wood Hall prepared for analysis using three different demineralising agents: (a) HCl; (b) HCl+NaCl; (c) EDTA. Note the high temperature T_{max} values of (incomplete?) HCl demineralised bone collagen (cf. [10]). Only the EDTA demineralised samples gave satisfactory, reproducible, results. Filled points are modern bone.

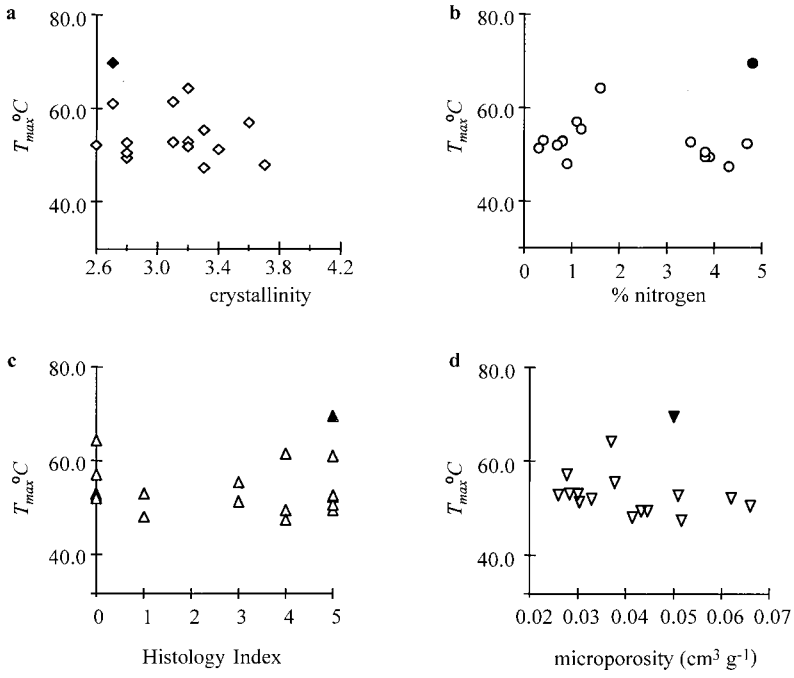

Fig. 3. T_{max} plotted against a variety of parameters that are used to assess the diagenetic state of bone. There is no obvious correlation between T_{max} and any of these parameters. The parameters are: (a) crystallinity of the bone mineral as measured by the infrared SF; (b) nitrogen, a measurement of the protein content; (c) histology index, a visual assessment of the state of bone preservation; (d) microporosity (measured by water hydration), which is assumed to represent the total volume of pores of less than 4 nm radii (see text for further details). Filled points are modern bone.

changes in the configurational entropy of the uncoupled, random-coil state. They argue that confinement of this thermally labile domain increases the temperature of thermal transition. The volume of water in a fibril is directly related to the inter-axial spacing of collagen molecules, and the authors have been able, in-part, to predict the increase in thermal stability upon progressive dehydration. Mineralization of collagen has also been observed to increase the transition temperature, and a similar argument has been employed although the process has not been examined quantitatively [8].

In our studies the transition temperature of the archaeological bone collagen fell rapidly with thermal age, much more rapidly than the predicted rate of collagen loss (extrapolated from heating experiments using an empirical rate law [18]). However, the polymer-in-a-box model would anticipate this relationship between collagen loss and transition temperature if the loss of collagen molecules occurred within fibres. This

Table 4
Temperature data for weather stations near to sites[a]

Site	Weather station	Time series	Mean	Maximum	Minimum	T_{eff}	Factor
Wood Hall	Leeming	1971–1993	9.2	18.4	−0.8	11.0	1.3
Brean Down	Cardiff airport	1961–1993	9.9	16.3	−2.1	12.1	1.7
Yarnton	Oxford	1828–1980	9.7	19.7	−3.0	12.4	1.9
Poundbury	Yeovelton	1981–1990	10.2	20.3	−0.1	12.5	1.9
Bercy	Paris Le Borget	1757–1993	10.6	24.6	−7.9	14.3	3.0

[a] The T_{eff} is estimated using an activation energy for gelatinisation of 173 kJ/mol, yielding a multiplication factor against which the ages of each site are corrected to a nominal 10°C.

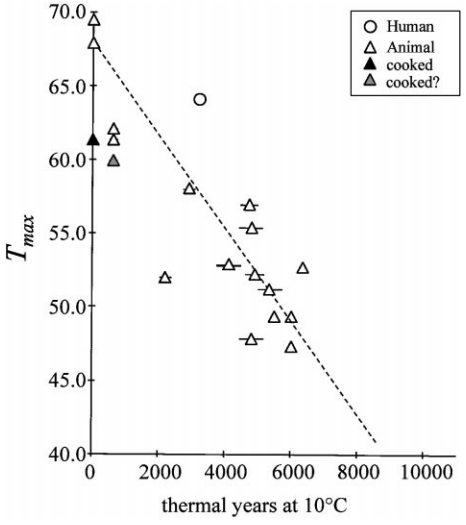

Fig. 4. T_{max} vs. thermal age (BP) for Holocene samples. Error bars represent the probable age range of the contexts from which the bones were recovered. All the bones, with the exception of one human burial (○) may possibly have been cooked. A laboratory cooked bone (▲) and one probable 'cooked' bone from Wood Hall (△) both display relatively low T_{max}.

does appear to be the case, all archaeological bones so far display increases in porosity at the sub-fibre scale (< 50 nm radius, see [18]). Losses from within the fibres will increase inter-axial spacing of collagen up

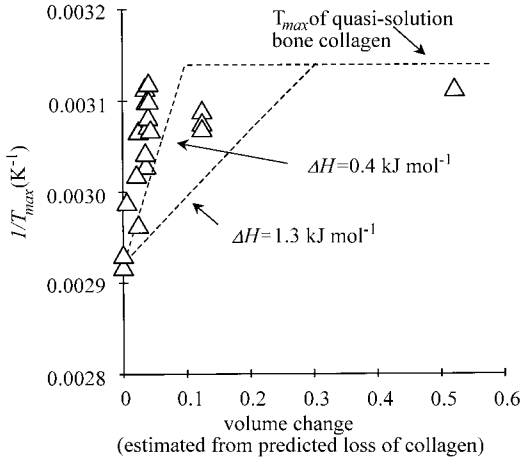

Fig. 5. $1/T_{max}$ (K^{-1}) plotted against estimated increase in volume of the collagen fibres as a consequence of thermal age. Trend lines fits taken from Eq. 13 of [6] using values given in Appendix A. T_{max} of the quasi-solution state is given from the values for HCl swelled modern bone collagen.

to the point at which the collagen molecules attain a quasi-solution state (the T_{max} of swollen collagen fibres). The low but consistent T_{max} values of bones $> 6\text{K yr}_{10^\circ\text{C}}$ (with the exception of Gough's Cave) may reflect this stage of decomposition.

The rate of decline in T_{max} is faster than is predicted from collagen volume changes alone (Fig. 5). Alternative explanations could include changes in the effective size of the cooperative unit (due to chain scission) or a lower enthalpy of transition. If the rate is modified to account for the low enthalpy of transition measured in EDTA demineralised samples, the fit is improved.

4. Conclusion

The finding of a strong correlation with age in thermally young samples and no correlation with other diagenetic parameters does not contradict the enzyme exclusion hypothesis. In histological section, highly altered bones display regions of apparently unaltered bone. It is in these unaltered regions where we suspect that collagen is preserved, its state of degradation a function of its integrated thermal history. However, despite the significance of the correlation, it should be reiterated that the sample size is small and the number of sites limited. A better understanding of the relationship between T_{max}, ΔH and collagen loss is required from a wider range of archaeological sites. The polymer-in-a-box model appears to be a promising avenue worth exploring further, particularly if measurements could be made on mineralised samples.

One problem with the use of thermal history is the impact of cooking; cooking would significantly alter the thermal history of a sample and therefore lower T_{max}. In a preliminary experiment, boiling for 7 h lowered the T_{max} by 7°C (J.P. Roberts, Unpublished data). It is therefore possible that DSC could detect prolonged boiling in selected samples.

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Appendix A. Polymer in a box calculation

Miles and Gelashvilli [6] relate the change in the transition temperature of collagen T_0 at a particular volume fraction of water ϵ_{w0} (where T_m is the temperature of denaturation 69.5°C and ϵ_w the fraction of water in the fibre) to the increase in volume for destabilisation to occur. In the simplified version of the polymer-in-a-box model only increases in lateral dimensions of the 'box' are considered:

$$\frac{\Delta H^{\Updownarrow}}{R}\left(\frac{1}{T_{max}}-\frac{1}{T_0}\right)=3\alpha_0 f\frac{C_N N b_0^2}{\Psi n d^2}(\epsilon_w-\epsilon_{w0}) \quad (A.1)$$

The space surrounding a thermally labile domain consisting of 65 residues is calculated from estimates of the packing density of the collagen within the fibre. α_0 (=3.29) is a numerical factor related to the cross-section of the box and f (=0.5) and n (=3) reflect the constraints placed by different packaging arrangements upon the spaces that can be occupied by denatured α-chains. The volume occupied by the collagen molecule is a function of the diameter of the collagen molecule d ($=1.2\times10^{-9}$ nm), the actual bond length b_0 ($=0.38\times10^{-9}$ nm) the sum of the number of links in the labile domain N (=65), and a bond stiffness coefficient C_N (=9.4). The hypothetical proportion of a fibre occupied by collagen molecules Ψ in a hexagonally packed quarter is 88% (i.e. 4.4/5). R is the universal gas constant (=8.314 J/(mol K)).

In our application of this model to degrading archaeological collagen, the loss of collagen (estimated from high temperature heating experiments to be 7.4×10^{-6} per year at a constant 10°C; Collins et al., Unpublished data) leads to a proportional increase in ϵ_{w0}.

Bailey and Miles [2] estimate that the enthalpy of transition of the thermally labile domain to be 1.306×10^6 J/mol (70 J/g dry weight). Our measurements produce much lower enthalpies ($H=11$ J/g wet weight for HCl extraction, $H=4.9$ J/g wet weight EDTA extraction), but the HCl data is consistent between two laboratories. The 80% reduction in H of EDTA extracted material relative to HCl extraction is one possible explanation for the more rapid decline in T_{max}.

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