# Water – the 'sore decayer'



This beautiful skeleton of a bison was found in the tar pits at Rancho La Brea where, in the absence of water, collagen and bone are beautifully preserved.

Collagen from human or animal bone is a valuable archaeological resource. It provides information about diet, the age of a sample and how well other molecules such as DNA might be preserved in ancient bones. Our work has unexpectedly led to an improved method for extracting proteins from old bones.

Ancient bones are among the most common archaeological remains. Yet why and how some bones survive and others decay is a frustrating question which has echoed down the centuries.

**'How long will a man lie i' th' earth ere he rot?'** (Hamlet Act V, scene 1).

Today's scientist can give an answer little better than Shakespeare's grave digger:

**'He will last you some eight year or nine year. A tanner will last you nine year... his hide is so tann'd by his trade he that he will keep out water a great while, and your water is a sore decayer of your whoreson dead body.'**

Our investigations show that Shakespeare got it right when he identified water as the 'sore decayer'. Water is indeed an important component of protein decomposition, breaking the chemical bond that links the amino acids together.

Collagen is the major protein found in bone, comprising some 18% of the dry weight. It is made of three protein chains wound together in a helix. Three of the main building blocks (amino acids) which go to make this helix are repeated many times. Because the amino acid sequence in collagen is so repetitive and regular it seemed a good system in which to study protein decay. Applying a few basic rules, we have put together a simple mathematical description of what happens to collagen as it decays in the presence of water.

Our mathematical model has identified a number of surprising features. In particular, we found that the way the bone sample is prepared will affect the state of the collagen. Simply grinding the sample causes much of the collagen to change from its normal insoluble form into gelatin (soluble collagen). Since only insoluble

It is very rare to find leather artefacts like this archaeological shoe, because collagen is decayed by bacteria. In this case, the shoe was found in waterlogged conditions with low levels of bacterial activity. This, and the presence of tanning agents, have lengthened its survival.

collagen is used when carbon-dating old bones, grinding may destroy most of the sample. Grinding also increases the rate of conversion amino acids to a different chemical form (a process called racemisation). Racemisation is a measure of ageing in bone, so misleading results will be obtained from ground bone.

**An important result of our work is to suggest improved methods for preparing bone collagen for analysis: don't grind it to powder, and keep it cold while you extract the minerals from the bone.**

The Ancient Biomolecules Initiative is a five-year programme to understand the fate of biological molecules in archaeological and fossil materials, and to explore the applications of this new knowledge.
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# Water – the 'sore decayer': the science in detail

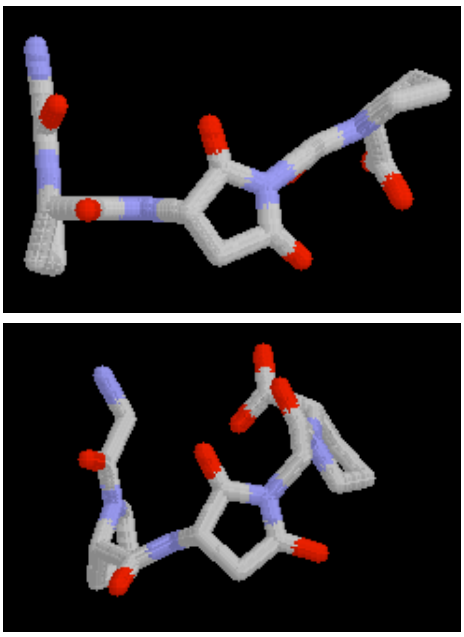
What is the predicted rate of loss of extractable 'collagen' in bone over archaeological time? No simple mathematical function can describe the disappearance of insoluble bone protein as it is influenced by so may factors (such as burial environment, and the extent of enzymatic and chemical degradation), but perhaps one factor (chemical attack by water) may explain a significant part of this loss.

Since it is only the insoluble collagen residue remaining after demineralisation of the bone which is used in carbon dating and isotopic analysis, then the following basic model is required. *k* is the rate of 'melting' of the collagen, which is governed by how rapidly the collagen gets chopped into smaller pieces.

collagen (insoluble) —*k*→ gelatin (soluble)

Spinning off from this 'melting' model has been the realisation of the importance of the triple helix to the racemization of aspartic acid and other amino acids. Racemisation of aspartic acid has been widely studied in fossil bones originally as a dating or stratigraphic tool and more recently as a measure which can serve as a proxy for the state of preservation of DNA

Most of the bone's aspartic acid occurs within collagen and nearly one in three of these residues occurs linked to sequences which are particularly prone to rapid racemisation, We think that racemisation is closely related to melting of collagen, and have used molecular dynamic simulations of short peptides in constrained and unconstrained conformations to test this idea. Our results indicate that the ordered helix of collagen dramatically reduces (by about 10,000 fold) racemisation in comparison with randomly coiled polypeptide.



Molecular dynamic (MD) estimations of the heat of formation of the cyclic (Asu) intermediate in aspartic acid racemization, is 7 kcal mole$^{-1}$ higher in the (upper) extended collagen peptide than in the (lower) flexible (i.e. gelatin) peptide of the sequence Gly-Pro-Asp-Gly-Pro. This difference suggests that the intermediate is 10,000 times less likely to form in the constrained collagen peptide than in the highly flexible gelatin peptide.

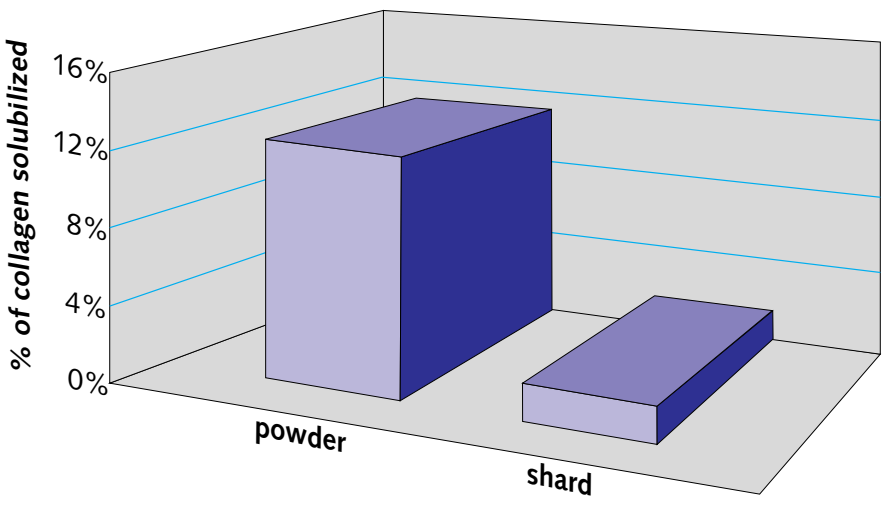
Collagen is extremely hard to solubilise (which is why the number of scission events is so hard to determine). Great care has to be taken to avoid damage to the protein (using enzyme inhibitors, slow demineralisation at neutral pH using organic chelators, etc.) but a common feature of all preparations was that samples had been ground to speed up the rate of demineralisation. Laboratory experiments have now established that powdering a sample leads to a significant solubilisation of collagen, prompting us to develop an improved method for the preparation of bone collagen using low temperature acid demineralisation of bone shards. The method has the advantages of speed and simplicity and avoids significantly adding to the archaeological damage already wrought on the sample.

## Methods

To investigate the relationship between the rate of melting and the rate of hydrolysis we simulated the processes using a large number of idealised collagen molecules. The simulation assumes long-polypeptide chains held together with hydrogen bonds, the extent of melting being controlled by the rate of chain scission, the number of hydrogen bonds required to hold the strands together and the minimum size of an insoluble triple helical fragment. However it is difficult to relate to the more commonly measured rate of peptide bond hydrolysis due to the problem of counting the number of scission events within the insoluble collagen mass (as the analysis can only be conducted on soluble protein).

## Future work

**We need reliable rate data for hydrolysis of mineralised collagen to test and improve the accuracy of the model. We then hope to analyse archaeological bones for a range of different parameters to determine whether the patterns of decomposition in a range of samples is consistent with hydrolysis as the driving force of diagenesis. This will give us the first crude predictions of the long-term fate of archaeological bone collagen at different latitudes under optimal circumstances - against which the apparent bewildering diversity of actual collagen yields can be compared.**




The impact of powdering on the collagen yield is very dramatic. Powdering modern bone to speed up the rate of demineralisation causes chain scission which results in loss of over 10% of the collagen by solubilisation.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