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Skin and Bones

March 25-27, 1999

DAY 1: Bones

The following article was put together from the notes made for a talk at the 1999 Biology Curators Group Meeting on the 25th March 1999 at The Natural History Museum, London. The aim of the talk was to provide a brief overview of the structure and chemistry of bone, and then to explore how bone deteriorates with methods of preparation and conservation. The article describes these concerns with reference to modern bone.

What is bone?

Bone in common with other connective tissue consists of cells, fibres and ground substance, but unlike other tissues its extracellular components are calcified, making it a hard unyielding substance ideally suited for its supportive and protective function in the skeleton. In addition bone plays a physiological role in body functions, particularly as a storehouse of minerals. In life bone is a dynamic living material that is constantly being renewed and reconstructed. As a result it is surprisingly responsive to metabolic, nutritional and endocrine factors, to the extent that the skeleton of an animal can be used to piece together the history of the animal in life.

Bone has a distinct chemical and structural make up (Bloom and Fawcett 1968; Romer and Parsons 1977; von Endt 1979; Page 1982). Fundamentally the gross structure of bone has two forms;

- Spongy (substantia spongia) – a 3D lattice of branching bony spicules.
- Compact (substantia compacta) – a solid continuous mass.

At the microscopic level, uniformly spaced through the interstitial substance of bone, are lenticular cavities, or lacunae. Each is completely filled with a bone cell or osteocyte. From these, radiating in all directions, are very slender branching tubular passages or canaliculi, which penetrate the interstitial substance of the lamellae. These form a continuous network of cavities interconnected by an extensive network of minute canals.

The chemical matrix of bone consists of collagenous fibres embedded in an amorphous ground substance consisting of bone mineral and polysaccharides. The mineral phase consists of submicroscopic crystals of an apatite of calcium and phosphate, commonly called hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). The mineralisation of bone is under the control of living bone cells. The mineral hydroxyapatite is deposited in the form of slender crystals within both the substance of the collagen fibres and the organic matrix. As a result these crystals are in close packed association with each other, the surface of the microfibrills, and within the microfibrils themselves. This strong association between mineral and protein provides the strong mechanical structure associated with the bone.

Collagen is an interesting molecule in itself – a three stranded thread that is wound about itself into a helix. Each strand consists of covalently linked amino acids, a third of which is glycine – the smallest amino acid. Of the rest about 20% will be alanine with 10% each of aspartic, glutamic and proline (figure 1). The amino acids form a regular repeating triplet in which every third amino acid is glycine (figure 2). This superhelix molecule is procollagen. Enzymatic action assembles the procollagen into microfibrills, which

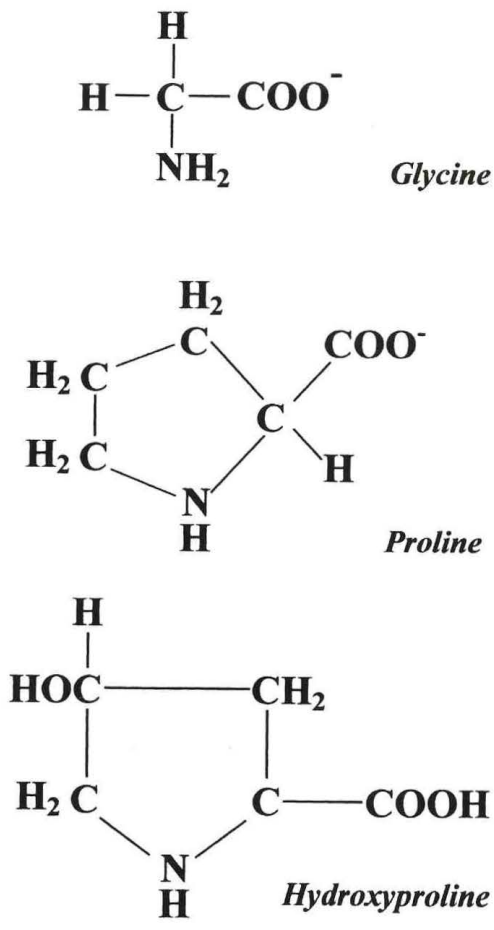


Figure 1: Some key amino acid structures that form Collagen

chemically cross-link in a regular fashion through specific amino acid residues to other microfibrills, thus strengthening the aggregate. Hydrogen bonding within the trimeric helix is probably the single most important factor in the stabilisation of collagen. Ultimately sheets of protein form, which overlay each other.

Non collagenous organic molecules, such as proteoglycans and glycoproteins, are in intimate association with the collagen molecules and these are thought to maintain the three dimensional, or steric, integrity of the collagen. It is also considered that these proteins also aid in the deposition of the mineral phase. Overall the weight of bone is approximately 25% protein and 75% hydroxyapatite mineral.

Living cells are also required to maintain the biochemical structure and relationship of the materials from which bone is composed. The presence of cells provides the presence of an important component in bone – DNA. The result is that bone has become an important resource for use in biomolecular studies (e.g. see Cooper 1994; Richards and Sykes 1995).

The deterioration of Bone

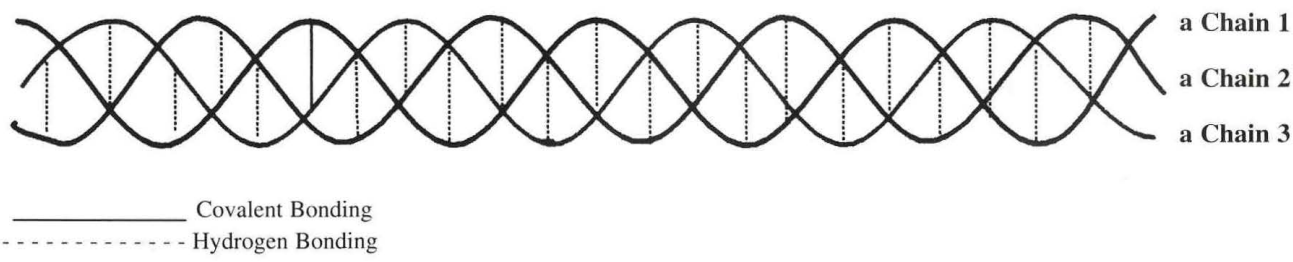
Despite its hard and solid appearance, bone is susceptible to deterioration because of its porous and microcrystalline structure. Moreover, bone must initially be extracted from the other body components of an animal, and therefore has undergone various types of chemical reactions in its removal and cleaning, making it more susceptible to further deterioration (von Endt 1979; Williams and Rogers 1989; Shelton and Buckley 1990; Williams 1992).

The composition of bone will start to alter as soon as the animal dies. On death, post mortem changes can cause the spontaneous re-ordering of the crystalline matrix and the presence of internal water can act on the bone proteins. Cellular metabolites and enzymes can also remain active and, once out of the control of the life processes, can cause degradation reactions. Overall two main events contribute significantly to the degradation of bone;

* Protein hydrolysis
* Crystal matrix modification

As the mineral-protein bonding becomes disrupted by these processes, the bone becomes softer and more friable. The degradation of the protein involves the unwinding of the helix in conjunction with the breaking of the polymer chains from the addition of water. This hydrolytic action breaks down the collagen into smaller polypeptide units and amino acids. The constituent amino acids are further open to steric changes such as racemization that will alter bonding relationships. If the disintegration of the protein goes far enough then the friable organic ghost of the bone will be all that remains. During the hydrolytic processes other reactions may proceed which can further accelerate the degradation of the bone. Oxidation (especially of lipids); decarboxylation (removal of the carboxyl group); deamination (removal of the amine group) and 3 dimensional changes such as

Figure 2: A simple representation of the collagen triple helix. Each collagen fibril is linked by hydrogen and covalent bonding



epimerization and racemization. Some amino acids resulting from the hydrolysis of bone proteins can undergo decomposition reactions that result in other organic chemicals and ammonia. These acid and amine products are themselves reactive and can further degrade bone.

Many of these degradation processes can be driven from the existing biological components of the bone. However, if water is introduced, the bonding between the mineral and protein portions of the bone is further weakened. This can cause the interior portions of the bone to become more accessible to these chemical processes increasing degradation. Steric changes and substitution can also occur within the crystals, further weakening the bonding between the mineral and protein portions of the bone.

Environmental factors can further affect the bone in a numbers of ways. Temperature will alter the rate of reaction, the higher the temperature, the faster the rates of hydrolysis and other chemical reactions. The relative humidity can have both a gross structural affect as well as a chemical effect through the introduction of water at high humidities.

Atmospheric pollutants can also have an effect, particularly acids that can combine with water to form hydronium ions. These can promote hydrolysis and increase the degradation of bone, through both the destruction of the protein and by dissolving the apatite mineral. The same can occur with bases such as hydroxide, which can also increase the rate of protein hydrolysis. The more porous the bone the greater the surface area, the greater the reaction rates.

The long-term degradation of modern bone is not clearly understood. Work by von Endt and Hare (1996) has looked at the degradation products from modern bone at high temperature and relative humidity. Their work found significant degradation in the amino acid pattern in the bone, and the production of the major deterioration product of the organic component of bone, ammonia. At low humidities, but high temperatures volatile oxygen containing hydrocarbons were found to be released, probably from heat activated reactions from the lipid portion of the bone. These were followed by cyclic organic molecules containing nitrogen, pyrrolidines and their derivatives, which are formed from the amino acid precursors that make up the organic component of bone.

With all these factors acting on the bone, it is surprising that bone is as durable a material as it is. It is thought that the presence of the mineral phase retards denaturation of the collagen (Collins at al 1995). This could be due to mineral-collagen bonding via calcium bridges. Also, despite the porous structure of the bone, the collagen still forms dense bundles which can help resist the effect of enzymatic dissolution, especially into the mineral-protein composite where it is difficult for molecules larger than water to penetrate. The result is that the collagen in mineralised tissue is more resilient to degradation than non-mineralised collagen tissues such as the skin.

Biochemical changes with the preparation of bone

When preparing bone it will be important to consider the potential uses of the specimen, and this may influence the choice of preparation method. Ideally we are aiming to maintain the bone in as natural a condition as possible for technical research; biochemistry; genetics; environmental chemicals. Introduction of reactive substances will alter natural components, leave chemical residues or reaction by-products (Matienzo and Snow 1986; Shelton and Buckley 1990; Williams 1991; Williams 1992). The introduction of oestological materials to adverse environments (e.g. solutions, fumigants) will compromise structural and material stability, conflicting with current museum conservation ideals. However if the specimen has limited data and is required for teaching or display then the ethics of preparation could be considered more 'flexible'.

The previous brief review of the deterioration of bone now puts us in a position to consider the effects of preparation methods on bone. Skeletal preparations can be prepared from the whole animal in a number of ways (Wagstaffe and Fidler 1968; Hounsome 1988; Davis and Payne 1992; Hendry 1998);

- Cold water maceration – possibly with the addition of enzymatic detergent.
- Warm water maceration – again with possible addition of enzymatic detergent
- Hot water maceration
- Chemical – sodium and potassium hydroxide; ammonia solution, sodium perborate (also consider final degreasing treatments).
- Scavenging organisms e.g. *Dermestes* spp
- Burying in sand

Generally the most commonly used method is some form of maceration with the addition of some enzyme activity by using a biological washing powder. This is due to basic maceration methods being easier to maintain and run than less invasive methods such as using *Dermestes* beetles.

However as has been previously discussed, the important aspect in maintaining the structure of the bone is the state of the protein – mineral interaction. Degradation of this will deteriorate the bone. Bone is a porous material open to the introduction of water and other aqueous borne compounds. Thus any process involving water and indeed heat must be considered destructive (Williams 1992). Combined with this bone is hygroscopic and anisotrophic in nature, and essentially such methods as maceration (with or without enzymes) can considerably alter the chemistry of the bone for biochemical and genetic investigations as well as promoting the rates of deterioration. Also the use of enzymes can also have a long term damaging effect on the bone, causing gradual and continual degradation of the bone protein. If using enzymes it is very important to consider a neutralising step.

Many institutions use methods that employ scavenging organisms such as Dermestes beetles, which overall damages the bone very little, although the subsequent washing procedures or treatments (bleaching, alkaline solutions, chemicals for pest control) can be detrimental. Alkaline solutions are particularly damaging to collagenous material, especially >pH9. Broadly it can be considered that cleaning solutions will be deleterious to proteinaceous materials, acidic solutions can dissolve the mineral component, and fumigants or pest control chemicals have the potential to degrade collagen and lipids. Combined with this will be the

effects of environmental factors, specifically through excessive temperature and RH. Essentially the elimination (as much as possible) of fumigants and aqueous solutions should improve the long-term stability of the bone, as well as promote a healthier work environment.

How the specimen has been previously stored prior to preparation of the bone material may also have effects that require consideration (e.g. Williams and Rogers 1989);

- Freezing – may promote desiccation.
- Alcohol – desiccant, may promote mobilisation of lipids and possibly cause material reactions with the ultrastructure of bone. The result is a whitening of the bone, which may cause later problems with desiccation and embrittlement or be beneficial through the removal of these substances, as they will not contribute to the deterioration through oxidation or the formation of acid by-products.
- Formaldehyde – can cause a staining of the bone probably through the fixation effect on the protein component.

Chemical effects on other skeletal elements

The skeleton consists of more than bone;

* Teeth – enamel; dentine; cementum
* Cartilage – connective tissue
* Ivory.

This results in a complex mix of materials that potentially respond differently to chemical treatments and environmental influences. e.g. with the dentine in teeth. This is hygroscopic and, as it absorbs water, internal stresses develop that cause internal stresses (Williams, 1991). As the crack develops the surface area increases, subsequently increasing the rate of deterioration. Connective tissue can be damaged by bleach where the pH is above 12. Ivory has been found to be extensively altered by treatments such as HCl due to formation of amino acid salts. Such ionizable residues make the material more hygroscopic (Matienzo and Snow 1986).

Conservation treatments – cleaning and consolidation

When considering the role of consolidation and cleaning treatments it must be questioned whether the work is necessary. The role of cleaning must be carefully considered as the addition of water can be particularly damaging to the protein-mineral interactions in the bone. Treatments with other standard organic solvents can also bring more organic matter to the surface of the bone.

However bone will need cleaning, whether for display, teaching or to remove the grime of time which in itself could be damaging the bone. The main recommendation is not to overtreat the bone whatever method is chosen. If using aqueous solutions do not over wet the bone surface and remove the solution as quickly as is possible. Some general recommendations can be:

- 10% sodium bicarbonate solution – can be very good (Photo 1). Has also be used to help degrease bone (Horie 1988).
- Synperionic N – non-ionic detergent, with the addition of very small amounts of EDTA and PVP to help chelate and remove dirt (Horie 1988; Jaeschke and Jaeschke 1992).
- Decon 90 – can be useful with very greasy material but is a very alkaline solution and must be used with caution.
- Organic solvents, e.g. Acetone. Consider their use with care and in relation to health and safety practice in your work environment (Horie 1988; Jaeschke and Jaeschke 1992).

But what if the bone requires repair or consolidation?

This is a tricky area. Past treatments with consolidants have caused problems in museum collections. The consolidant has started to degrade over time causing damage to the specimen. Often the consolidant has become chemically changed and the treatment becomes difficult to reverse. Materials such as shellac have caused particular problems. Whenever considering using a consolidant, take time to research the material and to assess its possible long-term stability and potential reversibility. There are a number of consolidants around with a proven history, and some are mentioned below. However it must be remembered that such polymers used in conservation are commercial compounds, and their properties can vary between batches.

- Paraloid B72 – an acrylic polymer miscible with solvents such as toluene or acetone (e.g. Koob 1984; Horie 1987; Jaeschke and Jaeschke 1992). This is useful as a surface consolidant, or for basic repairs of breaks, although the strength of the repair will not be very high. B72 is also useful as a filler (photo 2) when mixed with glass micro-balloons (for further details on fill materials in conservation see Craft and Loew 1998). The advantage of B72 is that it is very stable and reversible. It is however difficult to mix and tends to work best with fast evaporating solvents such as acetone which can make it difficult to work with. To reduce the rate of solvent evaporation and increase the penetration of the polymer a 50:50 mix of acetone to ethanol is useful.

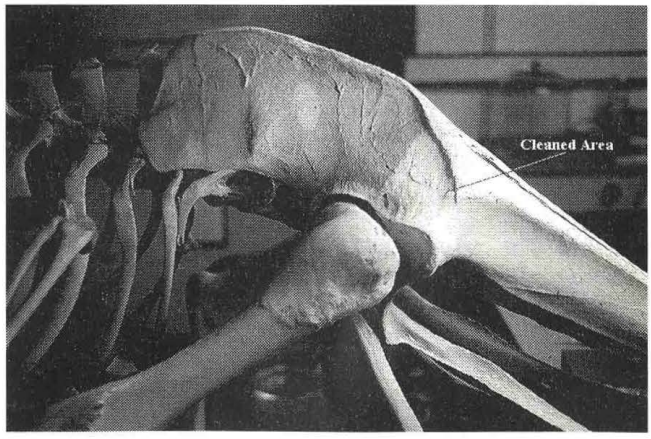


Photo 1: Ostrich skeleton, partially cleaned with IOY sodium bi-carbonate solution

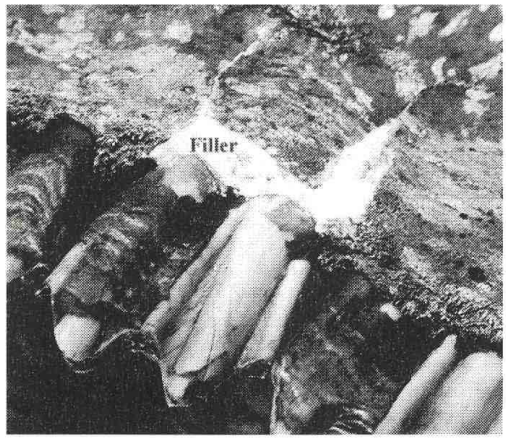


Photo 2: Repair using Paraloid B72 mixed with glass micro balloons

- Primal WS24 – this is a short chain acrylic dispersion miscible with water (Koob 1984; Horie 1987). This is very useful in the consolidation of weak friable bone as it is readily absorbed, and appears to be stable. However due to its ease of absorption it is not reversible, and it will introduce water to the bone with some degree of biochemical change.
- PVA's – Polyvinyl acetate (Horie 1987). Popular with paleontological material but tend to have a high degree of plasticer added, although plasticer free PVA is apparently becoming available.

If a strong repair is required then epoxy resins will need to be considered. However these are not reversible, and often stronger than the repair surface (it is recommended to coat the repair surfaces with a polymer such as B72 prior to epoxy repair). They are also non-reversible, can shrink a lot on drying pulling the bone surface with it and can have acidic off-gassing products on curing.

Storage Effects

We may think that our collections are safe once stored away in our collection areas. But think again. Many potential problems await. Some notable concerns are;

- Acidic offgasing products from storage units. Many materials will release corrosive chemicals that can react with stored object. Thus the storage environment of the collection must be evaluated (see von Endt et al, 1995).
- Temperature and humidity fluctuations. Inappropriate storage temperatures and humidities levels can significantly aid the deterioration of an object (Mathias 1994) More importantly if these factors are allowed to fluctuate then this can cause continual structural alterations in the object as it responds to the environmental changes. The result is the degradation of the object as cracks open up and increases the surface area for degradation reactions.
- Insect pest damage. A subject which now has a great deal of information, especially in relation to Integrated Pest Management (e.g. Pinniger 1994; Linnie 1996). Bone is open to insect damage (e.g. Carter 1995), especially if there is available moisture.

These problems can often be dealt with quite simply e.g. if you keep the doors on a storage cupboard closed, the humidity and temperature fluctuations that occur in the room as a whole are markedly reduced within the storage unit. This can be further improved with good enclosed storage boxes for the specimen. Secure storage units can also help deter insect visitors, although always keep a monitoring programme going, a good storage unit can also keep insect visitors in. Good hygiene will also greatly deter any insects, as will regular disturbance. If your existing storage furniture has an off-gassing problem, and replacement costs are prohibitive then some form of barrier lining may be possible (Thicket, 1998; Ganiaris and Sully, 1998). For a good review on aspects of preventative conservation see Rose et al 1995.

Bone is an important element in many areas of museum collection; natural history, ethnographic; art; archaeology; palaeontology. How we prepare, store and subsequently conserve our specimens will all have an effect on the biochemistry of the bone. It is important that we continue to care and research our collections, thus gradually improving our knowledge and understanding for this remarkable material.

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Moving Large Articulated Skeletons

In the current climate of building projects and ever-changing gallery displays, all museums eventually have the problem of moving collections, either internally or to off-site storage facilities. The skills required to move collections, whether large in numbers of specimens or large in terms of the size of individual objects, is rarely present in-house. External contractors will be required in many cases.

We have a Museum Services Division with a dedicated team of operatives experienced in the handling and transport of museum collections. We understand the museum ethos and we believe in solving problems in conjunction with curators and conservators, to ensure the safety of collections. The need to protect specimens and provide a sealed environment to minimise changes during movement, are central to the way we work.

I'd like to talk to you today about how we move specimens, the problems that occur and some of the ways we have developed to overcome them I will be concentrating on a number of moves which we have undertaken here in the Natural History Museum.

When faced with moving specimens like the articulated skeletons of fully-grown elephant and giraffe, the first question to be asked is 'can this be moved complete or does it need to be sectioned?' The answer to this question is always 'is there enough room to physically and safely move the specimen from A to B?' Assuming a complete physical move cannot be achieved, the next stage is to stay the construction of the specimen from the point of view of its constructor. If you study the method of securing the joints, it becomes apparent which parts can be easily separated and which, if possible, should be left intact. Our policy has been to section a specimen as little as possible to minimise disruption to the specimen.

We were recently tasked to relocate six articulated elephant skeletons from this museum to a new storage facility. These specimens have been in store for many years and building work, principally ducting and fire-door systems, had severely reduced the available headroom. Therefore, the specimens needed to be sectioned prior to removal.