Dating techniques

History revealed from bones

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As Mark Antony pointed out[1], most of what we should like to know about our forebears lies interred with their bones. But it is no easy task to reconstruct the cultural life of our ancestors from the fragmented evidence that their bones (and other remains) provide. Bones are very complex structures and contain potentially a wealth of information about, for example, an individual's genetic constitution, medical history, environmental influences and cultural practices. They are also far from chemical equilibrium, and information is inevitably lost with the passage of time. The effect of the burial environment, in terms of diagenetic changes both to the organic and inorganic components of bone, is even more marked, the remaining information being overwritten and further scrambled. Nevertheless, progress continues in sifting out the effects of environmental contamination and in deciphering what remains. Some new papers in *Geochimica Cosmochimica Acta*[2-4] exemplify this, and reinforce research reported at a recent workshop[5].

Analytical work on ancient bone ranges from isotope studies to immunochemical detection of antigens, taking in trace-element measurements, amino-acid racemization, protein identification and the search for DNA. So far, isotope methods promise the most straightforward results. These include dating (^{14}C and, much more arguably, $^{230}Th/^{234}U$), and dietary information (^{13}C and ^{15}N). But even here the state of preservation of the bone is paramount.

Radiocarbon dating of bone, especially in the critical period of the transition of man from Neanderthal to modern, thought to have ended about 30,000 years ago, can produce dates far too young because of a small admixture from younger environmental contamination. How this admixture might be separated depends on our understanding of the way in which unknown contaminant molecules can crosslink to degraded bone collagen. The same problems apply to stable isotope results; as carbon and nitrogen atoms pass along the varied food chains of man's diet, the different small degrees of isotope fractionation can be recognized. Distinctions between marine and terrestrial animals, leguminous and non-leguminous plants, as well as plants growing by different photosynthetic pathways, are easily made, and more subtle influences of environment (such as climatic stress or the effect of famine) are now being explored. But the measurements are subject to the relatively inscrutable effects of bone diagenesis.

In something of a technical *tour de force*, Stafford *et al.*[4] have now isolated individual amino acids from bones in varying states of decay and have measured their radiocarbon age by accelerator mass spectrometry. As expected, the results are complicated, but the admixture of environmental amino acids can now be clearly followed. In a mammoth bone known to be about 11,000 years old, the dates obtained for some of the amino acids were only 3,000–4,000 years old.

The new work of De Niro and Weiner[2,3] aims to develop strategies to recover collagen fragments free from contamination. One approach is to exploit subtleties in the biomineralization of the hydroxyapatite/collagen matrix of the bone, in which a small fraction is in the form of multicrystalline arrays. This fraction is preserved after the treatment of the matrix with sodium hypochlorite, and in fossil bone still retains isotope signatures much closer to those expected for the original bone. Non-collagenous proteins and lipids are also apparently better preserved in these 'aggregates'.

A second approach that is being explored is to take advantage of the characteristic sequential structure in collagen: glycine–Y–X, where Y is often proline or hydroxyproline and X is any residue. This structure is specifically cleaved by the enzyme collagenase into fragments of relative molecular mass typically 500–700, which can then be extracted and purified. Although yet to be proved, the benefits for the radiocarbon dating of bone, particularly from hotter regions than northern Europe, are potentially very significant. Much Middle Eastern chronology, for example, rests on dating of unsatisfactory charcoal samples; the collagen levels surviving in bone are usually too low to provide reliable dates by existing techniques. Reliable dates obtained by these new techniques could transform our understanding of some of the archaeology of the region.

Other dating methods are less likely to evade the effects of environmental influence[5]. Uranium-series dating, for example, depends on modelling the uptake of uranium ions by buried bone from the ground water. Even the basic question of why some bones seem better preserved than others found in the same stratum on a site is not yet easy to answer. Perhaps the first steps are being taken in the continuing study of the disintegration of macromolecules from naturally weathering bones in the Amboseli National Park of East Africa. The first few years of a bone's death may be crucial for preserving its information for posterity. □

1. Shakespeare, W. *Julius Caesar* Act 3, Scene 2.
2. De Niro, M. & Weiner, S. *Geochim. cosmochim. Acta* **52**, 2415–2424 (1988).
3. DeNiro, M. & Weiner, S. *Geochim. cosmochim. Acta* **52**, 2425–2432 (1988).
4. Stafford, T.W., Brendel, K. & Duhamel, R.C. *Geochim. cosmochim. Acta* **52**, 2257–2268 (1988).
5. Hedges, R.E.M. *et al.* (eds) *Appl. Geochem.* (in the press).

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Circadian rhythms

Mutant hamster in a hurry

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THE golden hamster (*Mesocricetus auratus*) is to chronobiologists what the fruitfly is to geneticists. Hamsters occupy this niche because their circadian rhythms of wheel-running activity are easy to measure, easy to quantify and highly predictable. At a recent meeting*, Martin Ralph (previously at the University of Oregon, now at the University of Virginia) reported his new work[1] on how the circadian-activity rhythms of hamsters can be experimentally manipulated by transplanting a part of the brain.

When the light–dark (LD) cycle is 14–10 hours, wheel-running in this nocturnal species is neatly confined to the hours of darkness. An occasional individual displays a sloppy or unusual rhythm, and such animals are usually discarded from experiments. But when Ralph noticed that one hamster started to run in its wheel several hours before the onset of darkness in a 14–10-hour LD cycle (see figure), he placed the animal in constant darkness, reasoning that the early activity onset in the presence of a LD cycle might reflect a circadian pacemaker with an unusually fast frequency. In this condition the hamster's activity free-ran with a periodicity of only 22 hours. The shortest free-running rhythm previously recorded[2] by Ralph and Menaker, out of more than 1,000 hamsters tested, was 23.5 hours.

Tests on these offspring revealed that there is a trait, an abnormally short free-running circadian period (*tau*), at a single autosomal location[2]. The *tau* mutant can thus be used to tackle unresolved questions about biological rhythms. For example, it is thought that the supra-

* Society of Neurosciences, Toronto, 13–18 November 1988.