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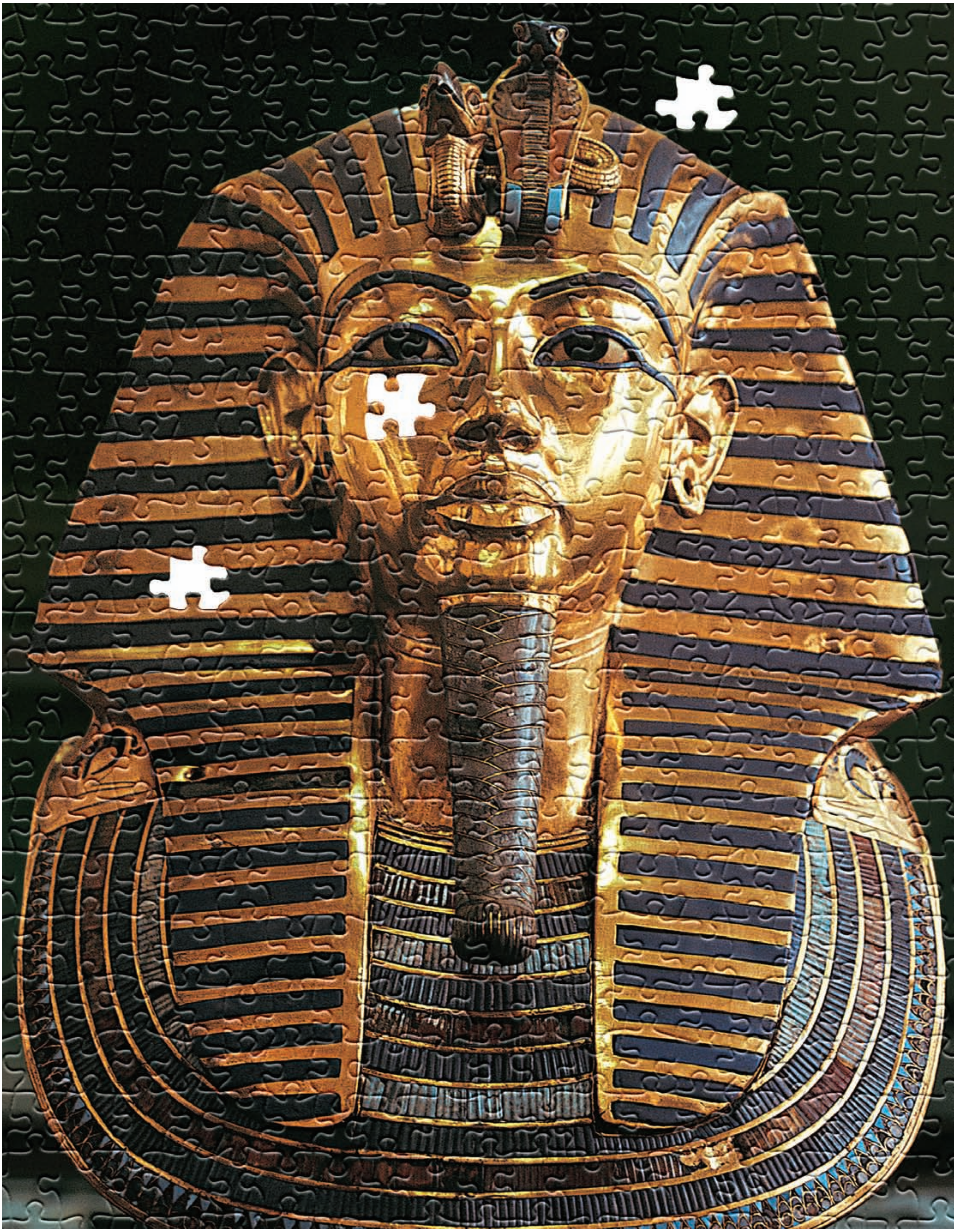


# Solving an ancient puzzle

Analytical chemistry is revolutionising archaeological study – as well as igniting some controversy. Simon Hadlingon meets some of the chemists piecing together the ancient world

Archaeologists are the world's greatest jigsaw puzzlers – piecing together tiny fragments to create a coherent picture of the past. For more than 200 years, analytical chemistry has been an invaluable part of their toolkit. As modern analytical techniques become more sophisticated and sensitive, the chemist is able to provide increasingly accurate and more detailed information.

'The archaeological community has not always been quick to embrace chemistry, especially in the States,' says Patrick McGovern, an archaeological chemist from the University of Pennsylvania Museum, US. 'Traditionally, the community has focused on the study of culture, but we're now able to reconstruct humanity's organic past in exquisite detail from extremely small samples. It has changed the face of the field.'

### Going on a date

Perhaps the best-known analytical method in archaeology is radiocarbon dating. It has been used for the past 50 years to date samples based on the decay of the naturally occurring radioactive isotope of carbon, $^{14}C$. The isotope is constantly formed in the atmosphere by cosmic radiation, and is incorporated into plants and ultimately every living tissue. When an organism dies and stops taking up $^{14}C$, the extent of its radioactive decay – with a half-life of 5730 years – can be used to determine the time at which it died. The technique can date carbon-containing materials up to about 60 000 years with an accuracy of around 1–200 years.

Measuring $^{14}C$ in a sample originally involved converting the carbon to benzene and then using liquid scintillation counting – whereby each radioactive particle emitted can be measured as a pulse of light. This process is still used where extremely precise measurements are needed, but it requires large samples, and is usually replaced by accelerator mass spectrometry (AMS).

AMS is essentially similar to conventional mass spectrometry but, because the proportion of radioactive isotope to non-radioactive is so low in these samples – of the order of $1:10^{12}$–$10^{15}$ – it is difficult to discriminate on mass alone. Fragments of $^{12}CH_2$ and $^{13}CH$ can be formed, which are very nearly the same mass as $^{14}C$, the isotope being measured. AMS gets around this by accelerating the carbon ions produced from the sample to high energies. Multiple collisions between these ions leave all the carbon in a +3 charge stage – with none of it remaining bound to hydrogen.

A major issue for radiocarbon dating is that samples must be completely free from contamination. 'Every trace of non-contemporaneous carbon must be removed,' Christopher Ramsey, Director of the Oxford Radiocarbon Accelerator Unit, explains. 'The classic pre-treatment is acid–base–acid, in which carbonate is removed by acid treatment, followed by an alkali treatment to remove any humic acid – the complex organic acid mixture found in soil. A further acid treatment takes out any carbon dioxide that might have become absorbed from the atmosphere.'

But whilst radiocarbon dating is widely accepted as providing accurate and reliable data, it has caused controversy. 'Occasionally we find that radiocarbon data does not match well with good chronological data from sites in Egypt,' says Ramsey.

The biggest single area of

**In short**

- **Analytical chemistry has become a valuable part of the archaeological toolkit**
- **As techniques advance, artefacts have been more definitively dated, but chemical analysis doesn't always agree with accepted timelines and some results have caused controversy**
- **Some chemists are reinventing techniques that have previously been discredited – achieving good results by applying the right technique to the right type of sample**

PASCAL GOETGHELUCK / SCIENCE PHOTO LIBRARY

controversy relates to the eruption of Santorini, creating the volcanic islands in the Aegean Sea. Groups working on carbon dating gave a date for the eruption that was 100 years earlier than the accepted archaeological findings suggested.[1]

'Meetings are now being organised between members of both communities to try to resolve this,' says Ramsey.

### Rehabilitating the past

At the University of York, Matthew Collins and Kirsty Penkman are in the process of rehabilitating an archaeological dating technique that had a dramatic fall from grace. It was first demonstrated around 30 years ago only to be discredited a few years later. The method is based on measuring racemisation of amino acids. Amino acids exist in two chiral forms, D and L. For reasons that are not fully understood, proteins contain only L amino acids.

But, when an organism dies, temperature and time act upon the amino acids resulting in their racemisation towards, ultimately, a 50:50 mixture of the two chiral forms. Different amino acids racemise at different rates, and the extent of racemisation can give a good indication of how long the organism has been dead. Crucially, racemisation can date a specimen back several hundred thousand years – far beyond the 50–60 000 possible with radiocarbon dating.

The idea is sound and the method has been demonstrated, so why did it fall out of favour? Collins explains. 'In the 1970s a group from Washington DC demonstrated that amino acid racemisation was able to date seashells. A second group then applied the method to the protein collagen in ancient bone samples and came up with a date for when humans first arrived in North America.' The results caused a sensation, but subsequent carbon dating of the samples showed that the racemisation age estimate was wrong. 'The original paper on racemisation dating of collagen was published in 1974[2] but overturned in 1984,' says Collins. 'Sadly, after that, for most archaeologists, the whole technique was discredited.'[3]

Collins' studies have shown that racemisation is indeed a viable dating technique – but not for every protein sample. Hannah Koon in the York lab has shown that collagen is packed so densely and tightly, as fibrils within the mineral matrix of the bone, that it is essentially protected from degradation, and therefore racemisation. If any damage occurs, the collagen expands out of this protective matrix, but the collagen fibrils then collapse and the protein is lost. The upshot of this is that any surviving collagen fibrils closely resemble new collagen – with little racemisation – and cannot be dated in this way.

**The improvement of analytical techniques has allowed smaller samples to be studied and dated**

**'Occasionally we find that radiocarbon data does not match well with good chronological data from sites in Egypt'**

But, by choosing her protein samples carefully, Penkman has been able to use it as a dating technique. 'The trick is to find something that is fairly ubiquitous, is biological and contains surviving protein in which racemisation has occurred.' The unlikely candidate that the researchers have hit upon is the tiny trapdoor that is found at the opening of the shell of freshwater snails, an 'opercula'. The structure is made of calcite, a form of calcium carbonate, is about the size of a sesame seed and contains a closed system of protein whose amino acids undergo racemisation.

'We take samples, grind them and bleach them for 48 hours to destroy any contamination,' says Penkman. 'The protein is then digested with acid into its constituent amino acids. We tag the amino acids with a chiral derivative which discriminates between the chiral forms, and fluorescently label them. When we subject the mixture to reverse high-performance liquid chromatography, the different chiral forms of each amino acid separate out and can be detected, in picomolar concentrations.'

## Ashes to ashes

DAVID A. HARDY / SCIENCE PHOTO LIBRARY

**The eruption of Mount Vesuvius destroyed and preserved the city of Pompeii**

Dramatic volcanic eruptions, such as that at Pompeii, have frozen entire communities in time, and volcanic ash from such well documented eruptions can be used to date other sites.

Mark Pollard from the University of Oxford, UK is developing new ways to study tiny particles of volcanic ash, or tephra, that are carried hundreds of miles from the site of an eruption. 'We recently found volcanic glass from Iceland in a lake in Switzerland,' he says. 'If you can identify the volcano from which the tephra came, you know that any two sites containing the same volcanic glass will be contemporary. And if you know the date of the eruption you can use the presence of the particles as a dating technique.'

The particles are usually smaller than 100 micrometres and can be chemically interrogated by electron microscopy in its chemical probe mode to provide an analysis of the major and minor elements present – typically silicon, aluminium, potassium, sodium, calcium, iron, magnesium and manganese. In this way a chemical fingerprint of the glass can be obtained and compared with material from other sites and from the parent site.

One drawback of this technique of tephrostratigraphy – linking two different sites in time due to the presence of similar tephra – is that major volcanoes can erupt relatively frequently, perhaps once every 200 years, and produce chemically similar ash. For this reason Pollard is investigating finer techniques for characterising volcanic glasses, looking at trace element compositions and isotopes of rarer elements such as strontium and neodymium. Accurate measurement of these elements would provide a more detailed chemical fingerprint of the glass, distinguishing between different eruptions. 'This is theoretically feasible, but technically quite tricky. We aim to carry out single grain analysis, which is hindered by the microscopic size and texture of the glass grains – very little surface area is available to analyse,' says Pollard.

Having analysed numerous samples from sites across the UK the researchers have shown the method to be very reliable – samples of the trapdoors found at a given site can even provide a date for the site, and therefore other archaeological samples present. The work has provided a key piece of evidence for dating the earliest humans in Northern Europe. 'We believe the method can date sites that are more than 600 000 years old,' says Penkman. 'No other technique can date biological samples over this range.'

### What's in the pot?

But chemical analysis can do more than just date a sample. Recently, techniques have allowed a glimpse into the way people lived, right down to the food they consumed – a key clue about early civilisations.

The average fragment of pottery obtained from an archaeological site contains subtle clues about its use – and by extension the diet of the person using the pot – within chemical residues residing in the clay matrix. Richard Evershed of the University of Bristol has pioneered the analysis of organic residues in pottery samples to reveal compelling evidence relating to the diet and agricultural practices of ancient communities.

'Most archaeological pottery is unglazed so has an amazing capacity to absorb organic materials coming into contact with it from cooking or storage,' Evershed says. The most important residues are lipids which become trapped in the sub-micron pores within the clay, out of the reach of bacteria. And the lipids' hydrophobicity makes them less likely to be washed away.

By grinding a small, cleaned fragment of pottery and extracting organic residues with a solvent, it is possible to separate up to milligram quantities of compounds with gas chromatography, then identify them with GC–mass spectrometry (GC–MS). Importantly it is possible to identify, within limits, the origin of the fatty acid depending on its ratio of the two stable isotopes of carbon, $^{13}C$ and $^{12}C$. About one per cent of naturally occurring carbon is $^{13}C$, and its relative proportion in biochemicals can vary depending on the biosynthetic origin of the compound. Significantly, Evershed has shown that fatty acids from dairy products can be distinguished in this way from fatty acids derived from animal carcasses.

**'This method can date sites that are more than 600 000 years old – no other technique can date biological samples over this range'**

'A big question that archaeologists have asked is when did man change from being a hunter-gatherer to farming? And what were domesticated animals originally used for – were they just for traction and meat, or for milk as well?'

By analysing pottery fragments from many sites around the British Isles, Evershed has demonstrated that milk fats as well as animal carcass fats are present since the beginning of the development of agriculture, at around 4000 BC, thereby answering a key question. The first farmers were indeed milking their livestock.

More recently the researchers have shown that certain key fatty acids found in pottery are of marine origin. Both isoprenoid carboxylic acids, and those possessing aromatic rings that form from

DARRYL DE RUITER, TEXAS A&M UNIVERSITY, US

certain marine polyunsaturated fatty acids during cooking, can provide further detail about the diet of various ancient communities.

'Essentially we are interested in applying the rigours of organic chemistry to this field by trying to look at substances at the molecular level and developing a proper understanding of how these molecular and stable isotope signatures arise and survive chemically,' Evershed says.

### Ancient business

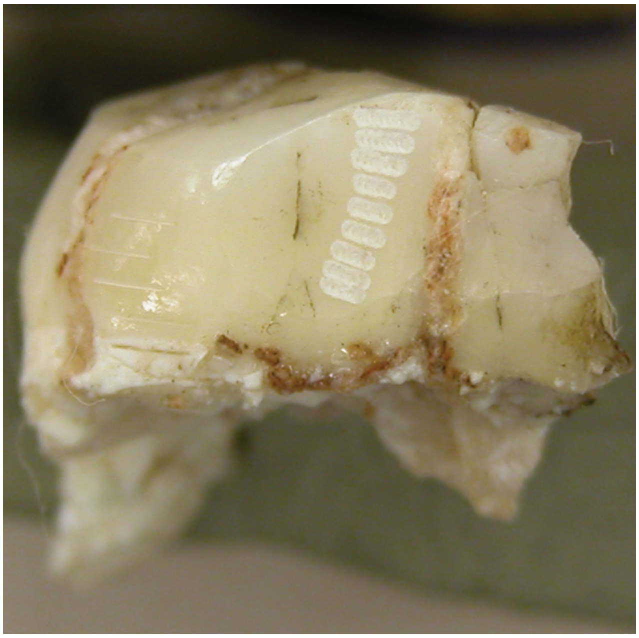
Study of our ancestors' food can also reveal much about their society. McGovern's research into ancient foods and drink, particularly wine, has provided evidence of early industry and trade.

'One of the most interesting examples of trade in wine are the jars found in the tomb of one the first pharaohs of Egypt, Scorpion I, at Abydos,' he says. Using neutron activation analysis of the pottery, his team discovered that the jars had been made in the Jordan Valley and the adjoining hill country of Palestine and Transjordan.

'Assuming that the jars were made in the same places that the wine was produced, as established by our chemical analysis of the residues inside the jars, it's clear that the wine in the tomb was transported some 500–700 miles, overland by donkey caravans, on a route that included the Sinai stretch – the so-called "Ways of Horus" – and then probably by boat up the Nile. This scenario makes sense, since we know that the Levantine winemaking industry in that region had already been in existence for a thousand years, whereas the domesticated grapevine had not yet been transplanted to the Nile Delta.'

With techniques such as GC–MS and liquid chromatography-mass spectrometry (LC-MS), McGovern has unravelled lists of ingredients and natural products from ancient civilisations. 'We've found that Egyptian and Chinese wines, dating from around 3150 BC and 1200 BC respectively, contain herbs such as mint, rosemary Chinese fir resin, and Artemisia argyi, which is a member of the wormwood family, and a plant source of the anti-malaria and anti-cancer compound artemisinin. This suggests that, rather than being simple, rice-based beverages, these were early medicines.'

His current research includes a study of the world's earliest chocolate, discovered in Honduras and dating back to around 1150 BC.



**A two million year old tooth still contains evidence of its owner's diet**

**'A big question for archaeologists is when did man change from being a hunter–gatherer to farming?'**

### A very early diet

Delving even further into the history of our eating habits can tell archaeologists how early humans and their close relatives – collectively termed hominins – lived and behaved when they first appeared several million years ago. Knowledge of the diet of these communities is central to answering this question, and at the University of Bradford Julia Lee-Thorp works on ways to obtain evidence about diet from fossilised remains of human skeletons millions of years old.

The older the fossil material, however, the more difficult it is to extract useful information. For really ancient material, often all that is available is fossilised bones and teeth. One way to obtain information about diet in these remains resides in the stable isotopes of elements such as carbon. At the base of the food chain, plants can be broadly divided into two types, $C_3$ and $C_4$, depending on their photosynthetic pathways. Grasses that grow in tropical environments such as savannahs are $C_4$ plants, while all trees, herbs and temperate grasses are $C_3$ plants. The different photosynthetic pathways result in a differential accumulation of the $^{13}C$ isotope of carbon in the plant tissue, which is passed up the food chain and is ultimately detectable in, for example, bones.

'One of the big debates is about whether early humans in Africa lived primarily among trees, or whether the opening up of the savannahs coincided with the emergence of bipedalism – walking on two legs rather than four,' says Lee-Thorp, who has shown that it is possible to measure the carbon isotope ratio from the enamel on teeth from early homonin specimens. 'The carbonate within the tooth enamel has been derived from the diet and is well preserved within the tightly packed crystalline structure of the enamel,' she says. 'From carbon isotope studies on enamel we were able to show that roughly a third of the carbon in the hominins' diet in what is now South Africa must have come from $C_4$ sources – the grassland. This could have been grass, including seeds, or animals that themselves lived on the grass – or both.' These results suggest that hominins obtained at least some of their foods from grassy areas even if they still lived in more closed woody habitats.

More recently Lee-Thorp and her colleagues have demonstrated that seasonal information about diet can be obtained from a single tooth by measuring carbon isotope ratios at different points along the depth of the tooth. 'It takes more than a year for a tooth to develop fully, so the top of the tooth is roughly a year older than the bottom of the tooth,' she says. By using a laser ablation technique to vaporise samples of the enamel at different points and measure the carbon isotope ratios of the vaporised samples with a mass spectrometer, the researchers found that in both species there were strong seasonal differences in diet.

Gathering detailed and definitive information about ancient samples is not easy, but Lee-Thorp has shown what can be made possible with the right approach and technology. 'Using this method is it possible to obtain information about a year in the life of these individuals which would be difficult to gather in any other way,' she says.

'These techniques make it possible to shed new light on our genetic heritage, cuisines, pharmacopoeias, clothing and so on,' says McGovern. 'I think this is the wave of the future in archaeology.'

*Simon Hadlington is a freelance writer based in York, UK*

*Additional reporting by Victoria Gill*

**Further reading**

1 G Bonani *et al*, *Radiocarbon*, 2001, **43**, 1297
2 J L Bada *et al*, *Science*, 1974, **184**, 791
3 J L Bada *et al*, *Nature*, 1984, **312**, 444
4 P E McGovern, *Ancient Wine*, 2003, New Jersey, US: Princeton University Press