Evidence for the Use of Fire at Zhoukoudian, China

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priate, although signal can be stored as z-magnetization and read out by a FLASH sequence.

Because the susceptibility variations measured with the iZQC method depend on local tissue oxygen concentration, in vivo this parameter varies in a much more straightforward way with tissue morphology than does the contrast in normal images. This suggests a variety of applications. Transient variations in the susceptibility are believed to be responsible for functional MRI (fMRI), and thus iZQC detection might give signal enhancements, particularly at high fields. In addition, many studies have related microvessel density to tumor growth potential, so iZQC detection may also be a method to "grade" or "stage" malignancy. Finally, numerous therapeutic approaches target angiogenic factors to control tumor growth, and this might be a way to evaluate "therapeutic response" to these agents.

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Evidence for the Use of Fire at Zhoukoudian, China

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Zhoukoudian is widely regarded as having the oldest reliable evidence for the controlled use of fire by humans. A reexamination of the evidence in Layer 10, the earliest archaeological horizon in the site, shows that burned and unburned bones are present in the same layer with stone tools. However, no ash or charcoal remnants could be detected. Hence, although indirect evidence for burning is present, there is no direct evidence for in situ burning.

The use of fire was an important asset for our early ancestors, offering them protection against large carnivores, warmth, added nutrition, and light at night. The ability to make and maintain fire was probably a prerequisite for occupation of the higher latitudes of Eurasia. It is therefore important to know when humans acquired this skill. Some studies suggest that the use of fire goes back more than 1 million years (*1–3*), although the evidence presented for almost all sites older than 300,000 to 400,000 years is controversial (*4*).

The oldest reliable evidence has been thought to be from Locality 1 at Zhoukoudian (Peking Man Site) (*4–6*), which accumulated from about 500,000 to 200,000 years ago (*7, 8*). Over 60 years ago, the original investigators noted in Layers 10 and 4 the presence of "the evidently burnt condition of many of the bones, antlers, horn cores and pieces of wood found in the cultural layers, [and] a direct and careful chemical test of several specimens has established the presence of free carbon in the blackened fossils and earth. The vivid yellow and red hues of the banded clays constantly associated with the black layers is also due to heating or baking of the cave's sediments" [(*9*), p. 113]. Subsequent observations of Layer 10 as well as a few reported analyses of the bones and sediments have concurred with these early observations, although some doubts have been raised (*4, 9–14*).

The cave formed as an enlargement of a vertical fault in which silty and angular rockfall accumulated. Layer 10, the lowermost archaeological horizon, is about 50 to 65 cm thick and is composed of two lithological units. The upper part is quite compact and comprises pink to reddish-yellow silty clay, locally cemented with small rock fragments. The lower part consists of yellowish-red, dark reddish-brown, and reddish-brown silts that become increasingly well bedded with depth (*15, 16*) (Fig. 1).

We examined the sediments in Layer 10 after cleaning the exposed section in 1996 and 1997. During the cleaning, we collected 42 bones of macrofauna and a considerably larger number of microfauna. Five of the macrofaunal bone fragments were uniformly black to grey in a freshly produced fracture surface; one had a turquoise hue. We extracted insoluble residues from the black bones after dissolution of the carbonated apatite by 1N hydrochloric acid (HCl) and the adhering silicate minerals by 40% hydrofluoric acid (HF) (*17*). Infrared (IR) spectra showed that the insoluble residues are all characteristic of burned bone organic matrix (Fig. 2A). Most of the remaining bones were yellow with speckled black surface coloration. Those tested produced residues with IR spectra characteristic of oxides (Fig. 2B). There was no appreciable acid-insoluble organic residue. Only seven of the bones from the microfauna were uniformly black and hence appear burned, out of a total of 278 collected. One of these was tested and confirmed as burned. Most of the bones, burned and unburned, were derived from the upper part of Layer 10. The small fragment with a distinct turquoise color was obtained from the lower part of Layer 10. None of the bones in the upper part were turquoise in color. We have reproduced this color experimentally by heating white- to yellow-colored fossil bones from Locality 1, including some from the upper part of Layer 10, to temperatures between 400° and 800°C for 2 hours. The optimal temperature is 600°C. Fresh bones turn black to grey under these conditions, and a black fossil bone from Layer 10 also turned turquoise.

The sediments of Layer 10 have often been described as ash (*6, 9, 10, 14–16*). Fresh wood ash is composed mainly of fine-grained calcite (*18*) and a minor amount (about 2% by weight) of a relatively insoluble phase. The latter is mainly an aggregate of soil-derived minerals embedded in a biologically produced amorphous matrix rich in Si, Al, Fe, and K. These have been called siliceous aggregates (*19, 20*). In prehistoric deposits con-

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taining bones, as in the upper part of Layer 10, ash if present should occur either as fine-grained calcite or as carbonated apatite (if the calcite reacted with phosphate in the ground water), with a small amount of siliceous aggregates (*20*). The three major components of the sediments from the upper part of Layer 10 are quartz (about 45%), carbonated apatite (about 40%), and clay (about 15%). We found no evidence of siliceous aggregates, even from the lowest density fraction after centrifugation in a heavy liquid (*21*). This fraction, composing about 0.6 weight % of the sample, did contain some mineral clusters that resemble siliceous aggregates when observed in the back-scattering mode in the scanning electron microscope. They did not contain relatively large amounts of potassium, which is characteristic of other siliceous aggregates examined to date (*19*), or other properties, such as the association of characteristic phytoliths, that would reflect a biological origin. These clusters were also present in the lower part of Layer 10, in the breccia of Layer 8–9, and in Layer 4. According to micromorphological observations, they are of diagenetic origin. Infrared spectra as well as elemental analyses showed that the clays are secondarily silicified, and the aggregates are possibly a product of the silicification process. We thus infer from the above that the carbonated apatite present in the upper part of Layer 10 is not derived from ash, and that there is no evidence for the presence of wood ash in Layer 10.

The upper part of Layer 10 is rich in large bone fragments, many with sharp edges. In thin section, however, many of the microscopic pieces of bone are well rounded, possibly due to transport or to carnivore digestion, by hyenas for example. The lack of bedding and the massive loose nature of the sediments suggests bioturbation, an interpretation supported by the presence in thin section of numerous rounded silty clay aggregates (*22*).

The putative hearths in the lower part of Layer 10 are represented by (i) finely laminated silt and clay interbedded with reddish-brown and yellow-brown fragments of organic matter, locally mixed with limestone fragments, and (ii) dark brown finely laminated silt, clay, and organic matter. No charcoal was observed. The fine lamination of both sediment types, best visible in thin section (Fig. 3), is indicative of accumulation in quiet water (*23*). The cave at this time was probably the locus of ponded water and was probably more open to the atmosphere.

Fig. 1. Photograph of Layer 10 showing the upper part, comprising light to dark brown fine-grained sediments and limestone boulders and the lower part comprising red- to yellow-colored finely laminated sediments.

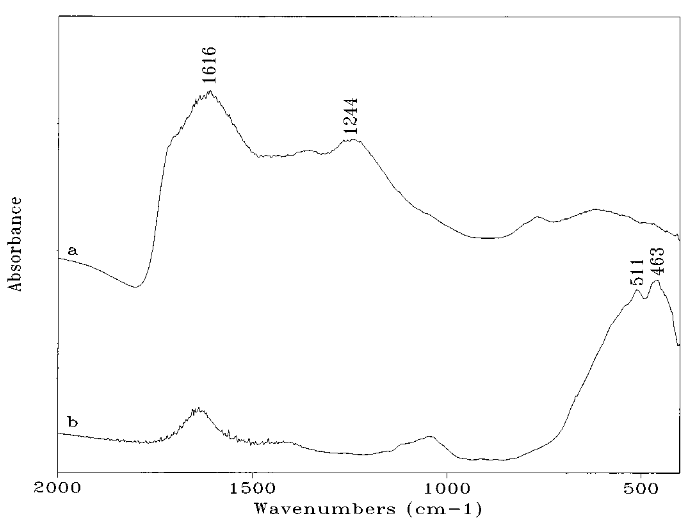

Fig. 2. Infrared spectra of the insoluble fractions of two black bones from the upper part of Layer 10 after treatment with HCl and HF (*17*). (**A**) Spectrum characteristic of burned organic matrix [see spectra in (*17*) and (*26*)]. (**B**) Spectrum characteristic of manganese and possible other oxides [see spectra in (*17*)].

The strongest evidence for fire associated with Layer 10 is the presence of burned macrofaunal bones. Layer 10 also contains an assemblage of stone artifacts composed mainly of quartzite (*9, 10*). During our examination of Layer 10, we observed several quartzite pieces, all of which came from the upper part of the section. There is thus a close association of the artifacts and the burned bones. Only 2.5% of the microfaunal bones were burned, as compared to 12% of the macrofaunal bones. These values are roughly similar to those obtained in much younger caves where fire was undoubtedly used by humans (*24*). As some of the sediments of Layer 10 were deposited under water, we cannot be sure that the bones, including the large burned and unburned bones, as well as the artifacts are in their original discard location. If fire was used at this location in the site, it is difficult to account for the absence of the insoluble fraction of wood ash.

At the base of Layer 4, there is also a close association of artifacts and macrofaunal bones, including many burned black bones. These sediments were similarly laminated and deposited under water in a low-energy environment. Here too we were not able to identify ash mineral remains. These bones, as well as many of the macrofaunal bones in the entire section, are present in a loess-like deposit (such as Layer 4) or in silt mixed with coarse angular limestone breccia (Layers 6 and 8–9). They were probably brought into the cave as runoff or in mud flows found between the breccia clasts.

The few burned bones we did observe above the base of Layer 4 and in the lower part of Layer 10 were turquoise colored, and we assume that they are fossil bones that were somehow burned by natural processes.

We conclude on the basis of the absence of ash or ash remnants (siliceous aggregates)

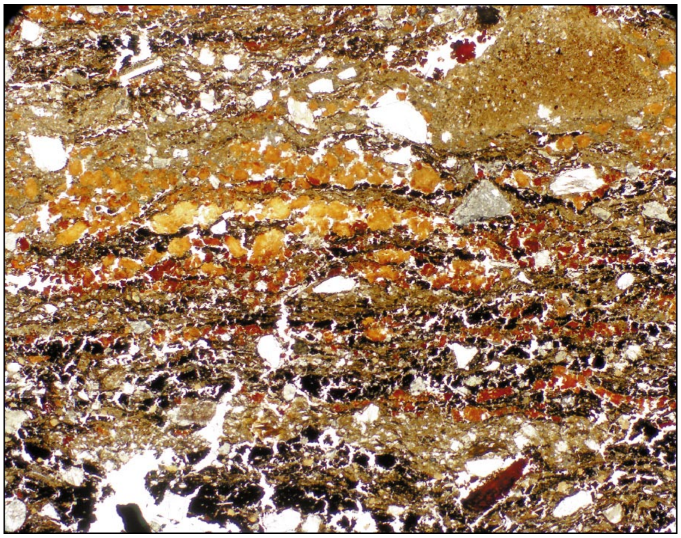

Fig. 3. Photomicrograph of Layer 10, illustrating the presence of massive sediments in the upper part and finely laminated, blackish organic matter (silts) in the lower part. The brighter orange-colored material is a secondarily precipitated phosphate, probably carbonated apatite. Regardless of whether the dark-colored organic matter in the lower part is a result of actual burning or aging, it is certainly not in its primary context but is water laid. The image was made with plane-polarized light; the width of the photograph is about 3 mm.

and of in situ hearth features that there is no direct evidence for in situ burning in Layers 4 and 10. Most of the fine-grained sediments in the site were water laid, and even if ash remains could be recognized, it would be difficult to demonstrate where they were produced. The co-occurrence of burned black bones and quartzite artifacts in the same layers is only suggestive of a cultural association, and hence of the use of fire by humans, but does not prove it. As most of the site has, however, already been excavated, it is not now possible to determine the locations of any campfires in Locality 1 at Zhoukoudian.

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21. A 0.5-g aliquot of the sediment was treated with a mixture of 3N HCl and 3N HNO_3 for 30 min at 100°C. The acid was removed by centrifugation (at 6000 rpm for 2 min), and the pellet was washed twice with water. The pellet was resuspended in 5 ml of sodium polytungstate solution (density 2.4), thoroughly dispersed by sonication, and then centrifuged as above. The supernatant was removed and diluted with 1 ml of water, vortexed, and recentrifuged. This was repeated until no mineral remained in the supernatant.
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24. In Mousterian sediments in Kebara and Hayonim caves, Israel, the overall proportions of relatively large burned bones (based on their black color) are 5.2% (*25*) and 13% (M. C. Stiner, unpublished data), respectively. In Grotta dei Moscerini and Grotta di Sant' Agostini, Italy, the proportions vary between 1 and 7%, except for two levels in which they are 32 and 52% (*26*). At the Henderson site, New Mexico, the proportion of bison and ungulate burned bones is 8.6%, whereas for rodent bones it is 1.5% (*25*).
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Design of a 20–Amino Acid, Three-Stranded β-Sheet Protein

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A 20-residue protein (named Betanova) forming a monomeric, three-stranded, antiparallel β sheet was designed using a structural backbone template and an iterative hierarchical approach. Structural and physicochemical characterization show that the β-sheet conformation is stabilized by specific tertiary interactions and that the protein exhibits a cooperative two-state folding-unfolding transition, which is a hallmark of natural proteins. The Betanova molecule constitutes a tractable model system to aid in the understanding of β-sheet formation, including β-sheet aggregation and amyloid fibril formation.

Despite the importance of β-sheet structures as regular secondary structure elements in proteins, the principles underlying their formation and stability are not well understood. A major obstacle to the study of β-sheet structures is the tendency of isolated β-sheet secondary structure elements to aggregate. Formation of amyloid fibrils mediated by the interaction of β strands is thought to be a crucial event in the progression of a wide variety of pathological disorders, ranging from Alzheimer's disease to spongiform encephalopathies (*1*). Until now, the scarce information available on the determinants of β-sheet stability has been obtained from systematic mutagenesis experiments (*2, 3*) and, more recently, through the study of de novo–designed simple β-hairpin peptides (two antiparallel β strands connected by a β turn or a short loop) (*4, 5*). This lack of knowledge is attested to by the failure so far to design an all–β-sheet protein that is soluble, monomeric, and amenable to structural characterization in atomic detail (*6*), although a nuclear magnetic resonance (NMR) model of β-sheet formation coupled to oligomerization has been reported (*7*). This contrasts with the growing number of successfully designed α-helical proteins (*8*) and α/β proteins (*9, 10*), as well as with work directed toward the modification of sequences of β proteins to cause them to adopt α-helical structure (*11*). Consequently, the design of an all–β-sheet protein is a substantial challenge and could provide insight into the pathological processes mentioned above.

A key feature for the successful design of model proteins is to make them simpler than

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