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States of Preservation of Bones from Prehistoric Sites in the Near East: A Survey

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A survey of the states of preservation of organic material in 30 fossil bones from 16 different prehistoric sites in the Near East shows that whereas almost all the bones have little or no collagen preserved, they do, with few exceptions, contain non-collagenous proteins. These macromolecules, therefore, represent an important reservoir of indigenous fossil bone constituents.

Keywords: FOSSIL BONE, FOSSIL PROTEINS, DIAGENESIS, COLLAGEN, MINERAL CRYSTALLINITY, STATE OF PRESERVATION.

Introduction

Bones are usually the most common biologically-formed materials in archaeological sites. If the bones are sufficiently well preserved to still contain some of the indigenous organic macromolecules, they can potentially be used to provide information on the age of bone by carbon-14 dating (Arnold & Libby, 1951), the diet of the animal by stable isotopic analysis (DeNiro & Epstein, 1978; van der Merwe, 1982), and some information on genetics using remnants of intact proteins (Hedges & Wallace, 1978) or polynucleotides (Pääbo, 1987). Information of this type is of particular importance in prehistory where relevant supplementary information is very limited. We therefore surveyed the relative states of bone preservation from a sample of prehistoric sites in the Near East to assess the potential both for applying currently available technology for studying these bones, and, even more important, for better defining the types of problems that need to be solved for future applications.

The methods used in the survey follow the study by DeNiro & Weiner (1988*a*) in which an assemblage of modern and fossil bones from marine and terrestrial mammals with well defined dietary habits were studied. The fossil bones that produced the expected isotopic composition of their so-called "collagen" fraction* based on the known diet of the animal were classified as well preserved, and those that did not were referred to as poorly preserved. It was noted that the C/N ratios of the former group were characteristic of collagen, whereas those of the latter were not (DeNiro, 1985). In a follow-up study of the same bone samples, DeNiro & Weiner (1988*a*) showed that the well preserved bones and

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*Referred to as the fraction that shares the same solubility characteristics as collagen in fresh bone. Note that in some fossils this fraction may not be composed of collagen.

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the poorly preserved bones could be differentiated by obtaining the infrared spectra and/or amino acid compositions of the organic fractions that are soluble and insoluble in 1N HCl. As these procedures are fairly quick and simple to perform, they can conveniently be used to survey the states of preservation of fossil bones. The reader is referred to DeNiro & Weiner (1988*a*) for details of methods, representative infrared spectra and amino acid compositions.

The basic trends in the diagenesis of the organic fraction recognized by DeNiro & Weiner (1988*a*) confirm those reported earlier by Masters (1987). The best preserved bones have relatively large amounts of collagen (more than 2% by weight as compared to about 20% by weight for modern bone). This collagen is predominantly in the HCl-insoluble fraction. The HCl-soluble organic fractions of these bones are also composed mostly of collagen, that is most probably the breakdown product of the insoluble collagen fraction. The more poorly preserved bones with less than roughly 2% by weight protein, have little or no HCl-insoluble protein, and the predominant components of the HCl-soluble fraction are often non-collagenous proteins (NCPs).

Here we report a study of some 30 bones from 16 different prehistoric sites in the Near East using primarily amino acid contents and compositions of the HCl-insoluble and soluble fractions and to a lesser extent infrared spectroscopy. The results show unequivocally that almost all the fossil bones are poorly preserved and only a minority have significant amounts of protein in the insoluble fraction. Almost all of them do have high molecular weight proteins in their soluble fraction. Clearly, the indigenous macromolecular components still preserved in this reservoir should be used for future studies of fossil bone constituents.

Materials and Methods

Samples

The bone samples, besides the two recent ones, were collected from various prehistoric sites in the Mediterranean Levant, including Sinai (Table 1 and Figure 1). The bones were primarily splinters of the long bones of middle sized ungulates. The samples represent both cave sites (Kebara, Hayonim, Nahal Hemar and Wadi Makukh) and open air sites. In Table 1 the samples are ordered according to their place in the chronology of the Near East beginning with the Chalcolithic/Early Bronze Age (5500/5000 BP) and ending with an early phase of the Upper Palaeolithic (*c.* 30,000 BP). The dates are mostly based on ^{14}C determinations (the original data can be found in the cited references, Table 1) while the ages of some sites are based on typological correlations with radiocarbon dated assemblages.

The samples are from diverse environments that more or less characterize this region. Early Neolithic open air sites are located in granitic valleys of southern Sinai (Abu Madi I, Wadi Tbeik and Ujrat el Mehed). Natufian and Neolithic samples are from the Lower Jordan Valley where a mixture of allochthonous red soils with Lisan marls occurs (Salibiya I, IX and Netiv Hadgud). The Hula Valley (the Upper Jordan Valley) is represented by several stratified samples from Ein Te'O, a well-buried small village which was occupied from the Pre-Pottery Neolithic B period through the Early Bronze Age. The samples from Yiftahel, (a stratified village with PPNB, Pottery Neolithic and Early Bronze I layers), Hayonim Terrace (an Early Natufian site), Nahal Zehora and Ein Hashofet (two Chalcolithic sites in Mt. Carmel) and Hatula (a stratified Late Natufian through Pre-Pottery Neolithic A or Sultanian site in the Judean Hills) are all from various wadis descending from the central hilly range to the coastal plain.

The four caves in the sample fall into two categories: (1) Prehistoric caves with large open chambers regularly influenced by atmospheric conditions, dripping water and infiltration of colluvial deposits (Kebara in Mt. Carmel and Hayonim in the Western Galilee).

Table 1. Provenance of fossil bone samples

Sample no.	Site	Prehistoric Period of Culture	Reference
3	Wadi Makukh	Chalcolithic	Y. Patrick, Hebrew University, Jerusalem (pers. comm.)
4,5,6	Ein Te'o	Chalcolithic	E. Eisenberg, Dept. of Antiquities, Israel (pers. comm.)
7	Nahal Zehora	Chalcolithic	A. Gopher, Tel Aviv University (pers. comm.)
8	Ein Hashofet	Chalcolithic	A. Gopher, Tel Aviv University (pers. comm.)
9	Yiftahel	EB–PPN	E. Braun, Dept. of Antiquities, Israel (pers. comm.)
10,11,12	Yiftahel	Pre Pottery Neolithic B	Garfinkel (1987)
13	Nahal Hemar	Pre Pottery Neolithic B	Bar-Yosef & Alon (1988)
14	Ujrat el Mehed	Pre Pottery Neolithic B	Dayan *et al.* (1986)
15	Wadi Tbeik	Pre Pottery Neolithic B	Tchernov & Bar-Yosef (1982)
16	Abu Madi I	"PPNA"	Bar-Yosef (1985)
17, 18	Netiv Hagdud	Sultanian (PPNA)	Bar-Yosef *et al.* (1980)
19	Hatula	Sultanian (PPNA)	Lechevallier & Ronen (1985)
20	Hatula	Khiamian (PPNA)	Lechevallier & Ronen (1985)
21	Hatula	Late Natufian	Lechevallier & Ronen (1985)
22,23	Salabiye, I, IX	Late Natufian	Valla (1984) Schuldenrein & Goldberg (1981)
24,25	Hayonim Terrace	Early Natufian	Henry *et al.* (1981) Valla (1984)
26	Urkan ErRubb IIa	Kebaran	Hovers *et al.* (1989)
27	Nahal Hadera V	Kebaran	Hovers *et al.* (1988)
28	Hayonim Cave	Levantine Aurignacion	Belfer-Cohen & Bar-Yosef (1981)
29	Kebara Cave	Upper Paleolithic	Bar-Yosef *et al.* (1986)

(2) Closed, dry caves, often with stable temperatures (Nahal Hemar cave and Wadi Makukh). These are located in the Judean Desert which lies in the "rain shadow" and is not part of the planetaric desert belt.

Experimental methods

Only superficial adhering sediments were mechanically removed from the bone. The sample was ground to a fairly fine powder. Eight millilitres of 1N HCl was added to a weighed aliquot (about 200 mg) of the powder and after dissolution the sample was transferred to a dialysis bag (Spectrapor 3 with 3500 Da cutoff) and dialysed against two changes of 4 l of deionized water. The contents of the bag were centrifuged at about 15,000 *g* and the supernatant ("HCl-soluble fraction") was separated from the pellet ("HCl-insoluble fraction"). Both fractions were lyophilized and if possible, weighed. An aliquot of each was placed in a glass tube with 0·5 ml 6N HCl at 144 °C for 40 min to hydrolyse the protein. The tube was evacuated after flushing twice with nitrogen prior to hydrolysis. Amino acids were analysed on a Durrum D-500 amino acid analyser. Protein contents were calculated from the yields of amino acids. The detection limit is about 5 μg amino acids per 100 mg bone powder and reproducibility varies considerably depending upon the concentration of protein in the bone.

Infrared spectra of the bone powder, as well as of the HCl-soluble and HCl-insoluble fractions were obtained using KBr pellets and a Nicolet-2 Fourier transform infrared spectrometer. The infrared spectra of the bone powder provide information on the crystallinity of the carbonate apatite crystals. This is a function of the extent of splitting of the two absorptions at 603 and 565 cm^{-1} (Termine & Posner, 1966) and reflects a

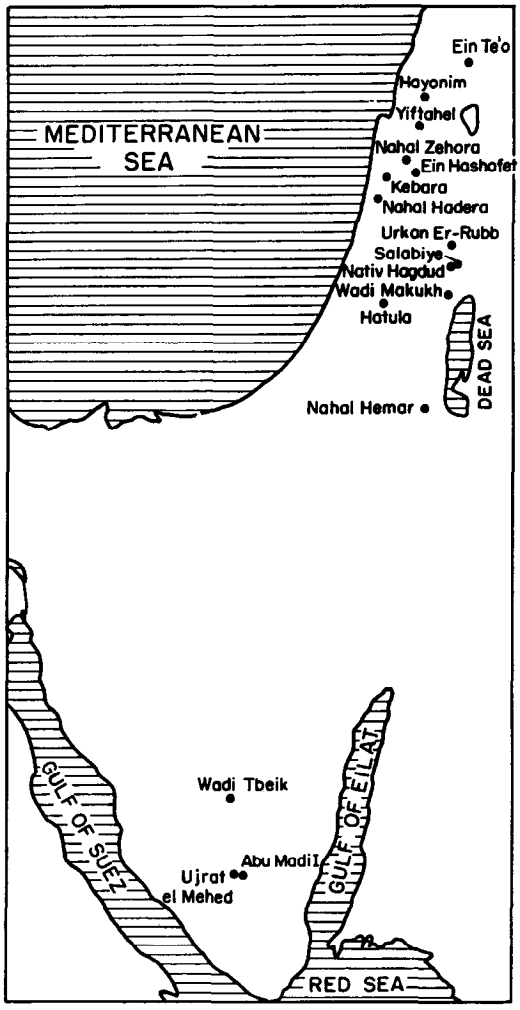

Figure 1. Map showing the locations of the sites sampled in this survey.

combination of the relative sizes of the crystals as well as the extent to which the atoms in the lattice are ordered. The higher the value the larger and/or more ordered are the crystals. The degree of splitting ("crystallinity index") of the bone powder was estimated by drawing a baseline from 750–495 cm^{-1} and then measuring the heights of the absorption peaks at 603 cm^{-1} (a) and 565 cm^{-1} (b) as well as the distance from the baseline to the lowest point between them (c). An arbitrary value was calculated from the formula $(a+b)/c$ and is referred to as the crystallinity index (Termine & Posner, 1966). This is illustrated in Figure 2.

Results

Table 2 lists the protein contents and compositions and mineral crystallinity indices of representative bones from the sites studied, together with the approximate ages of the sites and the associated sediments in which the bones were buried. Almost all the bones examined from prehistoric sites have less than 2% by weight protein, with one unusual

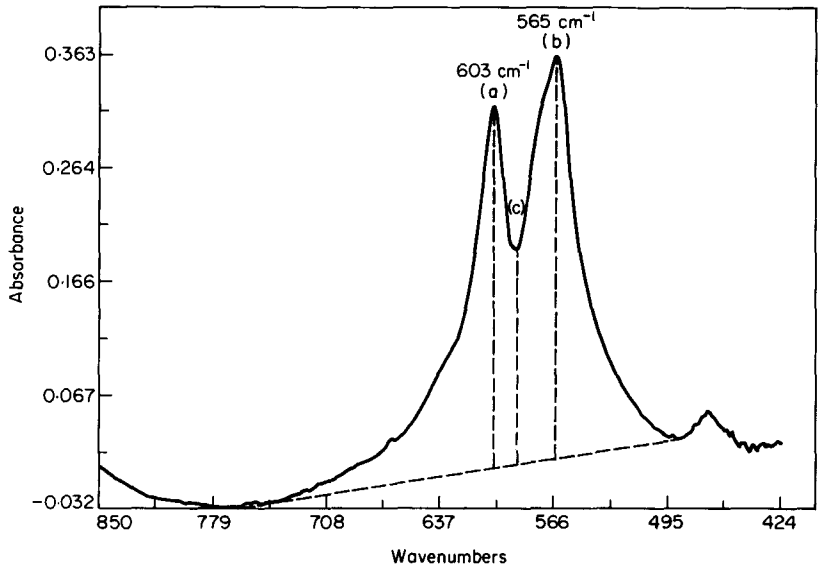

Figure 2. Portion of the infrared spectrum of sample 25B used for calculating the crystallinity index. A baseline is drawn from approximately 495–750 cm $^{-1}$. The heights of the 603 cm $^{-1}$ and 565 cm $^{-1}$ absorptions are summed, and then divided by the height of the valley between them. ((a + b)/c = crystallinity index or splitting factor).

exception (sample 3) (Table 2). By this criterion (DeNiro & Weiner, 1988*a*) they are poorly preserved. Eight of the bones do, however, have collagen in their insoluble fractions (Gly/Asp ratios greater than 6–7) and these significantly are the ones with the highest total amino acid contents. Note that pure collagen has a Gly/Asp ratio of close to 7. They are, therefore, the best preserved bones in the survey. A few other bones have detectable amounts of amino acids in the HCl-insoluble fraction but these do not have a collagen-like amino acid compositions, as indicated by the Gly/Asp ratio usually being less than about 6 (Table 2). A third category of even less well preserved bones have no protein in their HCl-insoluble fraction and their HCl-soluble fractions are generally non-collagenous. One exception (sample 4) has a collagenous HCl-soluble fraction. Finally the worst preserved bones have amino acid contents in both fractions below the detection limit (about 5 μg 100 mg^{-1} bone), that is due to background contamination in the analytical system. Five of the bone samples fall into this category.

The infrared spectra of the HCl-insoluble fractions of almost all the samples contain little or no protein and are dominated by clays and sometimes humic acid-like organic material (Figure 3). The only exception being samples from the two Judean Desert caves (samples 3 and 13) which have predominantly collagen-type spectra (Figure 4). The infrared spectra of the HCl-soluble fractions are all consistent with the bones being poorly preserved as described by DeNiro & Weiner (1988*a*), with the exceptions of samples 3, 13, 6 and 24 which showed collagen and/or NCP type infrared spectra. The infrared spectra thus confirm the fact that almost all these bones are poorly preserved.

The infrared spectra of the bone powder provide information on the crystallinity of the carbonate apatite crystals. Almost all the bones have crystallinity indices greater than modern bones (Table 2), presumably due to diagenetic processes involving the growth of larger crystals at the expense of smaller ones. There is no obvious correlation with the

Table 2. Protein contents and compositions, and mineral crystallinity indices of bones analysed in this study

Sample no.	Site	Approximate age BP	Associated sediment	Mineral crystallinity index	HCl–insoluble fraction			HCl–soluble fraction		
					Amino acid content*	Gly/Asp‡	Designation§	Amino acid content*	Gly/Asp‡	Designation
1	Modern bone	0	—	2·8	16,120	6	C	270	5	C, NCP
2	Recently buried bone	~40	—	3·0	5260	7	C	1630	5	C, NCP
3	Wadi Makuk (cave)	5500	Organic rich sediment	5·0	7460	7	C	2880	6	C
4	Ein Te'o	6000	Colluvium	3·5	tr	—	—	160	7	C
5	Ein Te'o	~5000–6000	Colluvium	4·1	tr	—	—	10	4	NCP
6	Ein Te'o	~6000	Colluvium	3·4	tr	—	—	25	6	C, NCP
7	Nahal Zehora	~6500–7000	Anthropomorphic accumulation beneath clay layer	3·2	80	7	C	130	3	NCP
8	Ein Hashofet	7000	Anthropomorphic accumulation beneath clay layer	2·8	360	8	C	55	2	NCP
9	Yiftahel (EBI)	5200	Terra rossa (colluvial clay)	3·0	1160	7	C	30	1	NCP
10	Yiftahel (St. III)	~6500	Terra rossa	3·6	30	3	NCP	15	1	NCP
11	Yiftahel (St. IV)	~7000	Terra rossa	3·2	tr	—	—	15	1	NCP
12	Yiftahel (PPNB)	8500	Terra rossa	3·3	110	9	C	20	3	NCP
13	Nahal Hemar (cave)	8500	Organic rich sediment with authigenic cave minerals	3·1	785	5	C, NCP	145	9	C
14	Ujrat El Mehed	8200	Granitic calcareous arkose	3·3	tr	—	—	15	2	NCP
15	Wadi Tbeik	8500	Sandstone, metamorphic arkose	3·5	tr	—	—	15	1	NCP
16	Abu Madi I	10,000	Granitic arkose	3·9	tr	—	—	10	2	NCP
17	Netiv Hagdud	9700–9400	Marls admixed with organic matter	3·6	tr	—	—	15	5	NCP
18	Netiv Hagdud	9700–9400	Marls admixed with organic matter	5·3	tr	4	—	10	2	NCP
19	Hatula	10,000	Calcareous gritty clay	3·1	75	4	C, NCP	20	2	NCP
20	Hatula	10,500	Calcareous gritty clay	3·1	15	2	NCP	35	2	NCP
21	Hatula	11,000–12,000	Calcareous gritty clay	3·2	10	1	NCP	25	2	NCP
21B†	Hatula	11,000–12,000	Calcareous gritty clay	3·4	10	2	NCP	25	3	NCP
22	Salabiye IX	~10,500	Reworked calcareous marl 0·8% organic matter	3·6	tr	—	—	tr	—	—
23	Salabiye I	11,000	Reworked calcareous marl 0·8% organic matter	3·6	tr	—	—	tr	—	—
24	Hayonim Terrace	~12,000	Terra fusca, limestone	3·1	10	3	NCP	10	4	NCP
25	Hayonim Terrace	~12,000	Terra fusca, limestone	4·3	tr	—	—	tr	—	—
25B†	Hayonim Terrace	~12,000	Terra fusca, limestone	3·7	tr	—	—	tr	—	—
26	Urkan ErRubb IIa	14,500	Calcareous clay loam	3·6	tr	—	—	tr	—	—
27	Nahal Hadera V	15–17,000	Sand	4·1	95	1	NCP	25	1	NCP
28	Hayonim Cave	~30,000	Aggregated silty loam with organic material	3·3	25	6	NCP	95	15	NCP
29	Kebara Cave	~30,000	Reworked terra rossa with calcareous cement	3·4	110	7	C	10	2	NCP

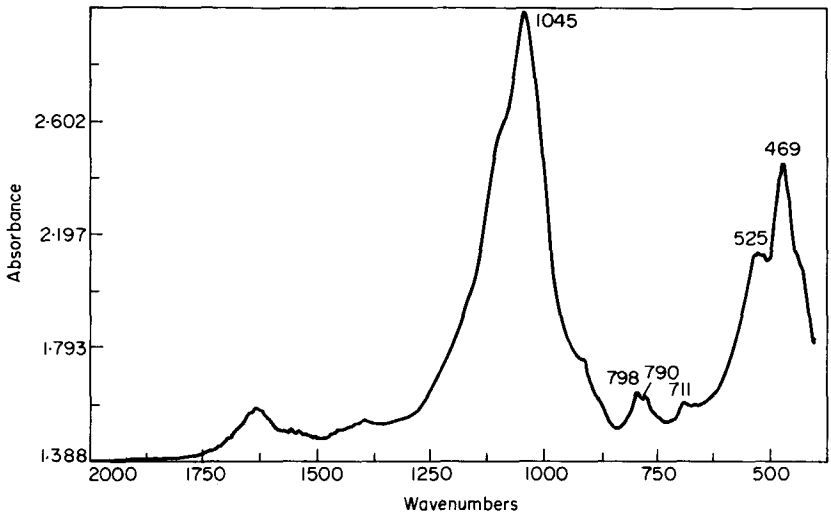

Figure 3. Infrared spectrum of the HCl-insoluble pellet of sample 5. The spectrum is characteristic of clay and most closely resembles illite. The absorption around 1650 cm^{-1} is due to water. There is no evidence of protein in this sample.

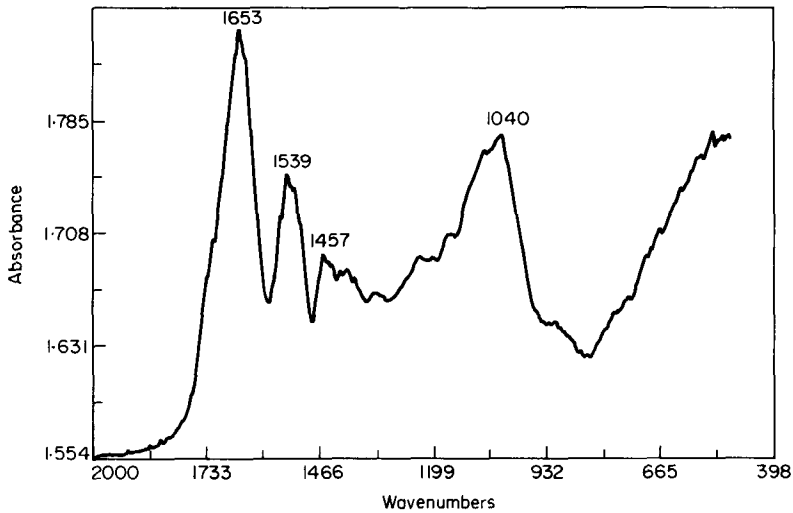

Figure 4. Infrared spectrum of the HCl-insoluble fraction of sample 13. The absorption peaks at 1653, 1539 and 1457 cm^{-1} are characteristic of collagen. The broad absorption around 1040 cm^{-1} could be due to polysaccharides or possibly to humic acid-like materials.

states of preservation of the organic fraction. Surprisingly the values are, with few exceptions, relatively low, especially when younger bones from arid environments can have measured indices as high as 7 (unpubl. data).

Table 2 also shows that there is no correlation between the states of preservation of the bone organic constituents and their age. There is also no straightforward correlation with sediment type, although we do note that six of the eight best preserved bones are in one

way or another associated with clay deposits. Four of them are buried in a clay-rich sediment (terra rossa) and two are buried below a clay horizon (samples 11 and 12). The remaining two better preserved bones (samples 3 and 13) are both from the caves located in an arid environment close to the Jordan Rift Valley. Nahal Hemar cave also yielded very well preserved organic artefacts, including wood, cordage, basketry and fabrics (Schick, 1988). The sediments in both caves are rich in organic material in the form of faecal pellets.

Discussion

This survey shows that the bones excavated from prehistoric sites in the Near East are generally poorly preserved. Most, however, do still contain some high molecular weight proteinaceous material in the HCl-soluble fraction, which if not contaminated, can be used for dating, palaeodiet reconstruction, probing the genome and so on. This will require the development of new techniques for purifying and handling this material, because to date the most widely used methods exploit only the HCl-insoluble organic fraction (Gurfinkel, 1987). Two new developments in this regard are the use of collagenase to purify collagen fragments, in both soluble and insoluble forms (DeNiro & Weiner, 1988*b*), and the prior treatment of bone powder with sodium hypochlorite. The latter removes most contamination and leaves only the relatively well preserved aggregate-derived organic fraction for further analysis (DeNiro & Weiner, 1988*c*).

Our observations are consistent with earlier reports (Cohen-Solal *et al.*, 1987; Masters, 1987) in that the most labile organic fraction in bone is the HCl-insoluble fraction (essentially all collagen). In less well preserved bones only the HCl-soluble fraction contains protein that is usually composed primarily of NCPs. The most poorly preserved bones have only NCPs or no proteinaceous material at all. Analogous diagenetic processes have also been observed in fossil mollusc shells (Weiner & Lowenstam, 1980), and the explanation proposed is that the acidic constituents of the matrix are the most intimately associated with the mineral phase and as a result are in some way partially protected. In fact, in sea urchin mineralized tissues some of the acidic macromolecules can actually be occluded within the crystals themselves (Berman *et al.*, 1988). Proteins such as these may well turn out to be the best preserved of all the macromolecules in fossil skeletal material.

The bones analysed in this survey were chosen so as to cover the range of states of preservation of the bone assemblage at a given site. It would, however, be advantageous to choose only the best preserved bones at a site for further analysis of their organic constituents. This survey shows that neither age nor the state of preservation of the mineral phase (at least with respect to the crystallinity index) are reliable guidelines for choosing the best preserved material. We do, however, note that at sites such as Yiftahel and Hatula, the youngest bones are better preserved than the older ones. The type of sediment in which the bones are buried is also not a useful indicator of state of preservation, with the possible exception of the presence of a high proportion of clay. We also note that some, but not all, the better preserved bones (those with collagenous HCl-insoluble fractions) are somehow associated with clays. This has also been observed for other very well preserved fossils (Weiner & Lowenstam, 1980) and is presumably related to the limited water permeability of clays. It should be stressed that in most of these sites a large portion of the bone deposits resulted from human activities. In these anthropogenic sediments the bones appear to be, morphologically at least, well preserved and taxonomic definitions are often easily made (Tchernov, pers. comm.).

The two best preserved bone samples in this study are both from caves located in an arid environment. Furthermore, the sediments are rich in organic material. As water and oxygen are the two major agents involved in the breakdown of organic material, the

unique combination of aridity and a large reservoir of potentially oxidizable organic material other than bone matrix, probably accounts for these caves having such well preserved bones. The 5500 year old Wadi Makuk sample (no. 3) is truly extraordinary in that its matrix amino acid composition and content is similar to modern bone, even though the mineral crystallinity is very unlike modern bone.

Concluding comment

Fossil bones represent a potentially invaluable reservoir of preserved macromolecules. Although in the Near East they are usually relatively poorly preserved and can be assumed to be contaminated by extraneous material, this survey shows that some of the macromolecules, and in particular remnants of the noncollagenous components, are often preserved. Efforts to more fully exploit this resource should, therefore, be focused on the HCl-soluble fraction of organic macromolecules.

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