Journal of Archaeological Science 1986, **13**, 553–566

Stable Isotopic Composition of Chitin from Arthropods Recovered in Archaeological Contexts as Palaeoenvironmental Indicators

Arndt Schimmelmann,[a,c] Michael J. DeNiro,[a,d] Mathieu Poulicek,[b] Marie-Françoise Voss-Foucart,[b] Gerhard Goffinet[b] and Ch. Jeuniaux[b]

(*Received 15 July 1985, accepted 20 March 1986*)

The effects of biodegradation and heating on the stable carbon, nitrogen, hydrogen and oxygen isotope ratios of chitin in arthropods were studied. Chitinous exoskeletons from seven aquatic arthropod species were subjected to anaerobic marine biodegradation in mud, to terrestrial biodegradation in soils, and to thermal degradation under anaerobic and aerobic conditions. The isotope ratios of chromatographically separated D-glucosamine hydrochloride and derivatives from treated and untreated specimens were then compared. Carbon, nitrogen, oxygen and hydrogen stable isotope ratios were all found to be conserved during partial degradation of chitin. Micromorphological comparative studies using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) indicated that no fungal chitin or other contaminants were present in the chitins that were chemically isolated from biodegraded substrates. Our results indicate that it will be possible to use stable isotope ratios of archaeological chitin samples for environmental and climatic reconstructions. An illustration of the utility of this approach comes from the observation that the stable isotope ratios of chitin from crustacean exoskeletons recovered from archaeological sites with ages up to 1400 years bp are in good agreement with measurements on modern crustaceans from similar environments.

Keywords: CHEMICAL ARCHAEOLOGY, PALAEOECOLOGY, ARTHROPODA, CHITIN, STABLE ISOTOPES, ELECTRON MICROSCOPY, CHITIN DEGRADATION.

Introduction

Stable isotope ratios of carbon, nitrogen, oxygen and hydrogen in different types of modern, archaeological and fossil biomass have been used in ecological, physiological, environmental and climatic studies. The accuracy of these studies is increased by the

[a]Department of Earth and Space Sciences, University of California, Los Angeles, California 90024, U.S.A.
[b]Université de Liège, Institut Ed. Van Beneden, Laboratoires de Morphologie, Systématique et Ecologie Animales, Quai Van Beneden 22, B-4020 Liège, Belgium.
[c]Present address: Scripps Institution of Oceanography, Geological Research Division, Mailstop A-015, La Jolla, California 92093, U.S.A.
[d]Also Archaeology Program.

0305–4403/86/060553+14 $03.00/0

utilization of well-characterized and purified chemical substances rather than bulk biomass. Often the purified substance is an insoluble biopolymer, such as collagen (Schoeninger & DeNiro, 1984) or cellulose (Epstein *et al.*, 1976), which has no mobility into or out of the biomass.

Chitin, one of the most abundant biopolymers, would appear to be a good candidate for use in such studies. Chitin is present in the organic matrix of exoskeletons of a wide range of animal classes as well as in fungi and some other lineages (Muzzarelli, 1977). Its availability from Arthropoda, Insecta, Arachnida, Merostomata and certain Mollusca makes it a potential substrate for stable isotope studies on organisms in aquatic and terrestrial environments. Archaeological excavations in arid areas and careful analyses of peat bog cores frequently yield well-preserved chitinous arthropod remains. Chitin is also preserved in natural oil seeps such as the La Brea tarpits, Los Angeles County (Stock, 1949). Morphological evidence suggests the preservation of Devonian age arthropod exoskeletons in a black mudstone 376–379 million years old (Shear *et al.*, 1984).

In our previous isotopic studies on chitin, we focused on the stable isotope ratios of carbon, nitrogen, oxygen and hydrogen in a variety of recent arthropods and their relationship to environmental and species-specific behavioural influences (Schimmelmann & DeNiro, 1986 *b,c*). Briefly, these studies showed that the $^{13}C/^{12}C$ ratios from marine arthropod chitins are different from those of terrestrial chitins, whereas chitins from brackish or supralittoral environments fall in the middle. The $^{18}O/^{16}O$ ratios relate largely to the $^{18}O/^{16}O$ ratios of the waters available to primary biomass producers, which, in the case of terrestrial meteoric waters, can be correlated with climate (Buchardt & Fritz, 1980). The D/H ratios reflect dietary influences that, under special circumstances, may also be interpreted climatically. Finally, $^{15}N/^{14}N$ ratios are relatively independent of species and characteristic for a given environment.

The results mentioned above are only the most prominent ones obtained from studies of modern arthropod chitins. These and other results could be directly compared to stable isotope data on chitins recovered from archaeological sites, thus allowing environmental and climatic reconstructions, provided that post-mortem processes have not caused changes in the *in vivo* stable isotope ratios of chitin.

It was the objective of this study to evaluate the likelihood of such post-mortem isotopic changes in chitin. Isotopically characterized modern chitin samples were subjected to partial biological and thermal degradation to simulate processes that may occur after chitin-bearing organisms die. Isotopic analyses of the residues that remained were then performed. Some residues were also subjected to micromorphological analysis using scanning and transmission electron microscopy (SEM and TEM) in order to determine if exogenous chitin-containing contaminants were introduced during biodegradation.

Materials and Methods

Marine anaerobic biodegradation experiments were performed using fresh, muddy, near-surface sediments from Redondo Beach Harbor (Los Angeles area, California) and the Malibu Creek Lagoon (Los Angeles area), both of which represent littoral-marine to brackish-marine microbial environments. Pieces of carapace from an adult northern lobster (*Homarus americanus*; family Nephropsidae; sampled in Boston, Massachusetts), from five adult ridgeback prawns (*Sicyonia ingentis;* family Penaeidae; caught offshore near Los Angeles) and from an adult ghost crab (*Ocypode ceratophtalmus*; family Ocypodidae; from Kauai, Hawaiian Islands) were placed in nylon mesh bags and buried separately 15 cm deep in the sediments, which were held in 2 litre polyethylene laboratory bottles. The sediment surface was covered by 5 cm seawater. The containers were covered loosely by black plastic caps and were additionally wrapped in aluminium foil to simulate

aphotic conditions. Biodegradation took place during 10 weeks at room temperature, after which the nylon bags were recovered from the dark, anaerobic, hydrogen-sulphide-containing sediments.

Terrestrial biodegradation in moist soil environments was performed using ground, fresh carapace material of *Homarus americanus,* shrimp [*Penaeus (californiensis?);* family Penaeidae; from a Los Angeles fish market], and horseshoe crab (*Limulus polyphemus*; class Merostomata; Assateague Island, Maryland) in separate nylon mesh bags buried for 8 weeks 20 cm deep in garden soils in Torrance and Altadema, both in the Los Angeles area.

Thermal degradation experiments on chitin involved ground and homogenized materials from brine shrimp (*Artemia franziscana;* family Branchipodidae; commercial product from San Francisco Bay area), *Homarus americanus,* and the purple shore crab (*Hemigrapsus nudus*; family Grapsidae; caught along the North American Pacific coast at different locations). For aerobic thermal degradations, aliquots of the three crude ground chitins were placed in loosely covered Petri dishes and heated for 3 h at 275 °C in an air-convection furnace. Anaerobic carbonization at 200, 275 and 325 °C for 3 h was performed by wrapping water-soaked aliquots of the crude chitin powders loosely in aluminium foil and placing them 10 cm deep in a moistened sand bath. As the temperature rose, the water vapour in the sand bath acted as a diffusion barrier against atmospheric oxygen.

Chitin samples that were untreated, thermally degraded or recovered from the sedimentary burial environments were separated into two aliquots, which were subsequently used for the determination of stable isotope ratios and electron microscopy.

The methods for processing arthropod chitin for stable isotope analysis have been described in detail elsewhere (Schimmelmann, 1985). They can be summarized briefly as follows. Arthropod exoskeletons were decalcified and deproteinized by treatment with 2 N HCl at room temperature and with 1 N NaOH at 100 °C respectively. The resulting crude chitin was hydrolysed in 7 N HCl at 100 °C, and the deacetylated chitin monomer, D-glucosamine, was purified chromatographically in its hydrochloride form (GlcN·HCl) (Schimmelmann & DeNiro, 1986*a*). Aliquots of GlcN·HCl were combusted before mass spectrometric analysis of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios, as described by Northfelt *et al.* (1981). GlcN·HCl was pyrolysed in the presence of $HgCl_2$ before mass spectrometric determination of $^{18}O/^{16}O$ ratios, as described by Schimmelmann & DeNiro (1985). D/H ratios were determined for the carbon-bound hydrogens of GlcN·HCl, as described by Schimmelmann & DeNiro (1986*c*). The isotope ratios are expressed in the δ notation, where

$$\delta^*X = \left[\frac{(^*X/X)_{sample}}{(^*X/X)_{standard}} - 1\right] \times 1000‰$$

and $^*X/X = {}^{13}C/{}^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, or D/H. Standards are Vienna Standard Mean Ocean Water (V-SMOW) for $\delta^{18}O$ values and δD values, the Peedee belemnite (PDB) carbonate for $\delta^{13}C$ values, and atmospheric nitrogen (AIR) for $\delta^{15}N$ values. The precisions of the measurements, given as standard deviations of the means calculated for 20 replicate analyses, are ±0·2‰ for $\delta^{18}O$ values, ±0·1‰ for $\delta^{15}N$ and $\delta^{13}C$ values and ±3‰ for δD values.

For electron microscopy, chitin samples were prefixed in 2% glutaraldehyde in sodium-cacodylate buffer, pH 7·4 and 4 °C, washed in buffer and postfixed in 2% OsO_4 in the same buffer. After dehydration through graded ethanol solutions, the material was either embedded in epoxy resin and cut for TEM, or critical-point dried, sputtered with 8–10 nm gold-palladium and mounted on aluminium stubs with silver glue. The

Table 1. δ values (‰) of chitin prepared from pieces of carapaces of Homarus americanus *(H),* Sicyonia ingentis *(S) and* Ocypode ceratophtalmus *(O) before and after anaerobic biodegradation in marine sediments from the indicated localities in California. The means ± 1 S.D. of the differences between the δ values of untreated and degraded chitins are given as Δδ values (‰). The amounts of D-glucosamine hydrochloride (in mg) obtained from 1000·0 mg carapace before and after biodegradation are given as EXT*

		Untreated	Biodegraded in Redondo Beach Harbor sediment	Biodegraded in Malibu Creek Lagoon sediment	Δδ value ($N=6$)
$\delta^{13}C$					
	H	−17·6	−17·7	−17·8	+0·1 ± 0·1
	S	−20·2	−20·4	−20·1	
	O	−16·3	−16·3	−16·3	
$\delta^{15}N$					
	H	+0·5	+0·4	+0·2	0·0 ± 0·2
	S	−4·8	−5·0	−4·6	
	O	−2·3	−1·9	−2·5	
$\delta^{18}O$					
	H	+25·4	+25·0	+25·3	0·0 ± 0·5
	S	+25·2	+25·3	+24·8	
	O	+24·7	+24·6	+25·8	
δD					
	H	+59	+54	+54	+2 ± 3
	S	+73	+67	+72	
	O	+113	+115	+115	
EXT					
	H	165·3	34·1	58·8	
	S	148·0	46·1	80·5	
	O	48·2	18·1	20·8	

samples were examined with a Siemens 101 transmission electron microscope at 80 kV accelerating voltage or a Siemens ETEC Autoscan scanning electron microscope at 20 kV.

Results and Discussion

Isotopic studies on partially degraded chitins

The concentrations of D-glucosamine hydrochloride (GlcN·HCl) and the carbon, nitrogen, hydrogen and oxygen isotope ratios were obtained for chitins from seven different species of arthropods, unaltered or biologically or thermally degraded (Tables 1–3).

The amounts of extractable GlcN·HCl per gram of degraded material decrease with progressing biodegradation, indicating chitin losses of up to 75% due to microbial activity (Tables 1 and 2). This observation is in agreement with the results of a variety of similar studies. For example, the aquatic aerobic biodegradation of chitin has been studied in the ocean (Wirsen & Jannasch, 1976; Norkrans & Stehn, 1978; Okutani, 1978; Poulicek *et al.*, 1981; Poulicek & Jeuniaux, 1982; Griffiths *et al.*, 1982), in estuaries (Hood & Meyers, 1978), in limnic environments (Warnes & Randles, 1977; Brewer & Pfaender, 1979; Fjerdingstad *et al.*, 1979; Warnes & Poole Rux, 1982), and in lotic suburban waters (Streichsbier, 1982). Chitin biodegradation in the rhizosphere was studied by Mitchell & Alexander (1962), Okafor (1978) and Koval & Kharkevich (1983). Ecological and methodological aspects of chitin degradation have been reviewed by Bilai

Table 2. δ values (‰) of chitin prepared from aliquots of ground carapaces of Limulus polyphemus *(L) and* Penaeus (californiensis?) *(P) before and after biodegradation in soils in the indicated localities of California. The means ± 1 S.D. of the differences between the δ values of untreated and degraded chitins are given as Δδ values (‰). The amounts of D-glucosamine hydrochloride (in mg) obtained from 1000·0 mg carapace powder before and after biodegradation are given as EXT. Quantities that were not determined are indicated by n.d.*

		Untreated	Biodegraded in Altadena soil	Biodegraded in Torrance soil	Δδ value (*N*)
$\delta^{13}C$					
	L	−17·0	−17·6	−17·5	+0·4 ± 0·2 (4)
	P	−24·9	−25·0	−25·1	
$\delta^{15}N$					
	L	−0·7	−0·9	−0·5	−0·4 ± 0·5 (4)
	P	−1·7	−1·3	+0·5	
$\delta^{18}O$					
	L	n.d.	n.d.	n.d.	−0·6 (2)
	P	+19·7	+18·7	+21·8	
δD					
	L	+86	+89	+82	+1 ± 3 (4)
	P	+41	+41	+39	
EXT					
	L	191·7	150·1	139·0	
	P	183·0	32·2	42·0	

et al. (1984) and Muzzarelli *et al.* (1986). The consensus of these workers is that the enzymatic degradation of chitin in aquatic and moist environments occurs rapidly and leads eventually to complete destruction. The results in Table 1 indicate that this conclusion also applies to marine anaerobic biodegradation.

Apparently the biodegradation in sediment from Redondo Beach Harbor involved a higher enzymatic chitinoclastic activity than that in sediment from Malibu Creek Lagoon (Table 1). Another chitin assay based upon the enzymatic degradation of chitin (Jeuniaux, 1963, 1965) performed on the lobster materials yielded the same result (Table 4). Quantitative differences between the results of this assay and the hydrolytic GlcN·HCl yields (Table 1) may be explained by the overall heterogeneity of the sample material, for instance among body parts such as manus and carapace. The approximately constant chitin concentration in the overall organic material (Table 4) indicates that biodegradation occurs simultaneously on chitin and other (e.g. proteinaceous) components at similar rates.

Similar observations of decreasing yields of GlcN·HCl were made for chitins that were subjected to carbonization for 3 h at various temperatures (Table 3). Higher temperatures resulted in less preservation, with aerobic carbonization being even more destructive than anaerobic carbonization. Thus, several experiments did not yield enough substrate to perform any stable isotope ratio determinations, while others (the aforementioned biodegradation experiments included) yielded only enough material to allow us to perform a limited number of analyses.

The means and standard deviations of the differences in isotope ratios of chitin from untreated samples and from samples that had undergone marine anaerobic biodegradation (Table 1) fall within the respective ranges and standard deviations of natural isotopic variabilities within the carapace of an individual lobster (*Homarus americanus*) or among carapaces from populations of crustaceans sampled in one locality (Table 5).

Table 3. δ values (‰) of chitin prepared from ground whole Artemia franziscana *(A) and ground carapaces of* Hemigrapsus nudus *(h) and* Homarus americanus *(H) before and after thermal degradation for 3 h under the indicated conditions. The means ± 1 S.D. of the differences between the δ values of untreated and degraded chitins are given as Δδ values (‰). The amounts of D-glucosamine hydrochloride (in mg) obtained from 1000·0 mg ground material before and after degradation are given as EXT. Quantities that were not determined are indicated by n.d.*

		Untreated	Anaerobic 200 °C	Anaerobic 275 °C	Anaerobic 325 °C	Anaerobic 275 °C	Δδ value (*N*)
$\delta^{13}C$							
	h	−14·3	−14·2	−13·9	n.d.	−14·5	0·0 ± 0·3 (9)
	H	−17·2	−16·8	−17·0	−17·1	−17·0	
	A	−22·1	−22·5	−22·5	n.d.	n.d.	
$\delta^{15}N$							
	h	+2·1	+2·2	+2·2	n.d.	+2·7	−0·1 ± 0·5 (8)
	H	+0·4	+0·9	+0·9	n.d.	+0·2	
	A	−8·5	−9·4	−8·4	n.d.	n.d.	
$\delta^{18}O$							
	h	+25·7	+25·3	n.d.	n.d.	n.d.	−0·5 ± 0·6 (4)
	H	+25·4	+26·3	+26·0	n.d.	+26·4	
	A	n.d.	n.d.	n.d.	n.d.	n.d.	
δD							
	h	n.d.	n.d.	n.d.	n.d	n.d.	−2 (2)
	H	+59	+55	n.d.	n.d.	+66	
	A	n.d.	n.d.	n.d.	n.d.	n.d.	
EXT							
	h	55·0	32·3	13·5	0·0	0·6	
	H	165·3	162·0	79·9	0·3	11·7	
	A	9·3	6·4	3·3	0·0	0·0	

Table 4. Weight percentages of total organic matter (TOM) in Homarus americanus *carapace and of chitin (determined by enzymatic analysis of glucosamine) in total organic matter before and after anaerobic biodegradation in marine sediments from the indicated localities in California*

	TOM in carapace	Chitin in TOM
Untreated	26·6	56·3
Biodegraded in Malibu Creek Lagoon sediment	20·5	65·7
Biodegraded in Redondo Beach Harbor sediment	7·2	61·5

Thus, the isotopic variability introduced by anaerobic degradation in marine sediments is accounted for by the isotopic heterogeneity of the original substrates in combination with the analytical uncertainties inherent to isotopic analysis. This explanation cannot account for the isotopic shifts observed in ground and homogenized chitin samples subjected to terrestrial microbial and thermal degradation. Our data (Tables 2 and 3) indicate that such processes produce only small isotopic shifts, however, and do not alter the gross isotopic character of chitin.

Table 5. Ranges and standard deviation of δ values (‰) in chitins of modern arthropod individuals and populations

Substrate		Range	1 S.D.	*N*
Chitins from five body parts of *Homarus americanus* (family Nephropsidae)	$\delta^{13}C$	0·5	0·2	5
	$\delta^{15}N$	1·3	0·5	5
	$\delta^{18}O$	0·9	0·3	5
	δD	5	2	5
Chitins from individuals of a population of *Procambarus clarkii* (family Astacidae)	$\delta^{13}C$	1·5	0·4	10
	$\delta^{15}N$	1·9	0·6	10
	$\delta^{18}O$	1·2	0·4	10
	δD	17	5	10
Chitins from individuals of a population of *Ocypode ceratophtalmus* (family Ocypodidae)	$\delta^{13}C$	3·3	1·0	9
	$\delta^{15}N$	2·8	0·9	9
	$\delta^{18}O$	0·8	0·3	6
	δD	12	4	7
Chitins from individuals of a population of *Sesarma* sp. (family Grapsidae)	$\delta^{13}C$	1·5	0·5	7
	$\delta^{15}N$	2·0	0·7	7
	$\delta^{18}O$	4·5	1·3	7
	δD	25	8	7

The preservation of intact *N*-acetyl-D-glucosamine units accounts for the conservation of *in vivo* stable isotope ratios of carbon, nitrogen, oxygen, and carbon-bound hydrogen in glucosamine from chitin samples subject to bio- or thermal degradation. Isotopic exchange of oxygen and carbon-bound hydrogen in chitin with ambient water percolating through a burial assemblage, which would alter the *in vivo* isotopic record, would require thermal and pH conditions that exceed, by far, those usually encountered in depositional environments (Hoering, 1981; Wedeking & Hayes, 1983). Under these extreme conditions there would be no preservation of chitin.

Microscopic studies on partially degraded chitins

Alterations of isotope ratios of fossil arthropod chitin can be expected if secondary fungal chitin is added to the primary chitinous substrate. The magnitude of such alterations can be appreciated if one considers that the $\delta^{13}C$ and $\delta^{15}N$ values of microbial biomass from bacteria grown heterotrophically on *N*-acetyl-D-glucosamine, which is the monomer that organisms polymerize to form chitin, differ from the respective δ values of the substrate by 4·1 and 0·9‰ respectively (Macko & Estep, 1982). Fungal secondary chitin (Ruiz-Herrera, 1978) would behave as a chemically indiscriminable contaminant, thus potentially changing the overall stable isotope ratios in primary chitin if present in archaeological and fossil samples, as suspected by Hare & Abelson (1965) and Moore (1969). Scanning electron microscopy (SEM) has been used to detect fungal structures in non-fossilized materials (Poulicek *et al.*, 1981; Poulicek & Jeuniaux, 1982; Poulicek & Jaspar-Versali, 1984; Poulicek, 1985) and was used in this study to evaluate the likelihood of the presence of fungal chitin in biodegraded arthropod exoskeleton remains.

TEM and SEM studies of the various crude biodegraded substrates we prepared revealed the presence of some microbial and fungal organisms (Figures 1 & 2) penetrating and destroying primary chitinous structures, predominantly from the endocuticle side (Figure 3), while the epi- and exocuticle offered higher resistance against endolithic and chitinoclastic attack (Figure 4).

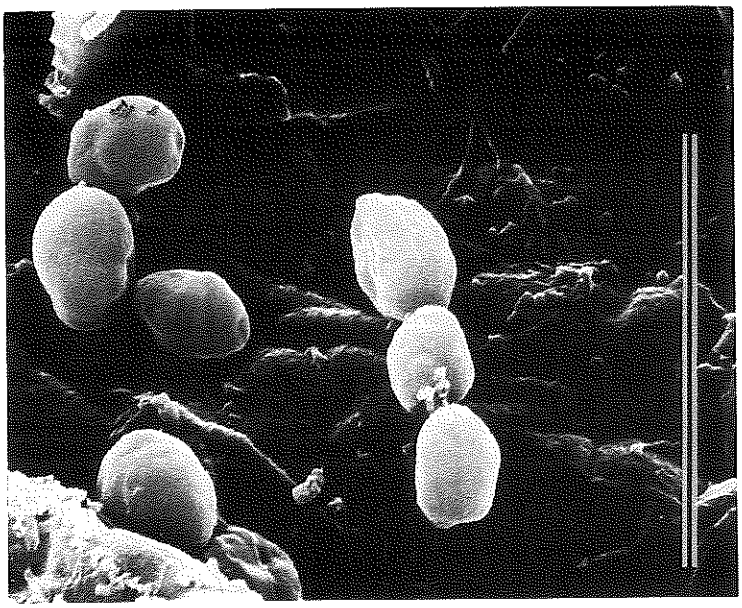

Figure 1. Surface of the inner side of a *Homarus americanus* cuticle fragment after incubation in Redondo Beach Harbor mud showing numerous bacteria and several spore-forming microorganisms (SEM, × 5600 scale bar = 10 μm).

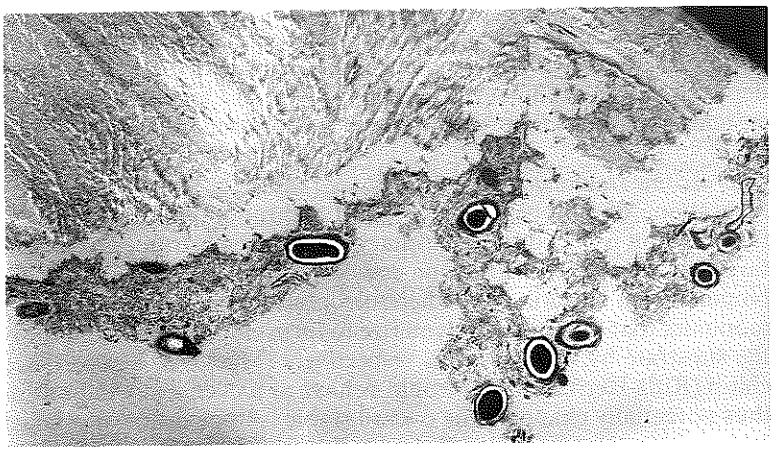

Figure 2. Ultrathin section of the same sample as in Figure 1 showing several microorganisms and borings within the biodegraded cuticle. Note the alteration of the cuticle inner layers (TEM, × 5000).

In contrast, after the chemical decalcification and deproteinization that we employed in preparing chitin samples for isotopic analysis, biodegraded substrates yielded purified chitin isolates whose SEM images (Figures 3 & 4) indicated there were no biogenic structures (such as fungal chitin) other than those related to the primary chitin. It appears that the chemical purification is also effective in eliminating other contaminants, such as soil mineral particles.

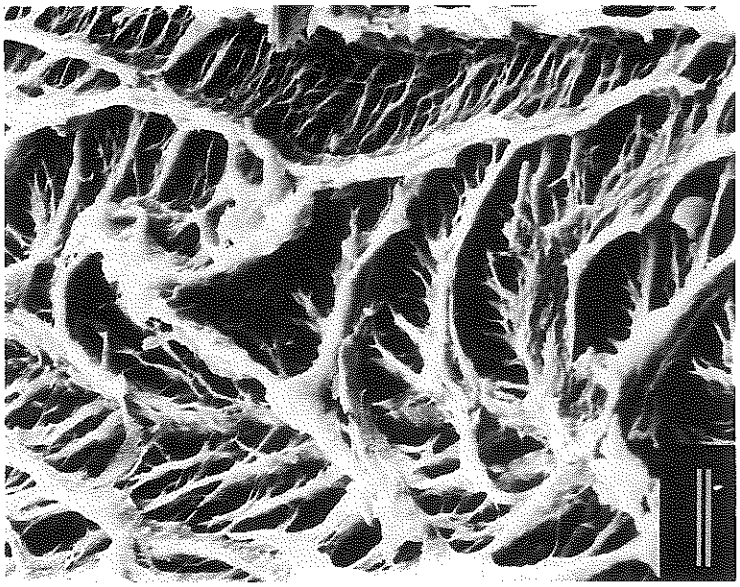

Figure 3. Detail of the surface of the inner side of the same sample as in Figure 1 showing the alteration processes (isolation of the macrofibres) after removal of the contaminating microorganisms (SEM, × 1250, scale bar = 10 μm).

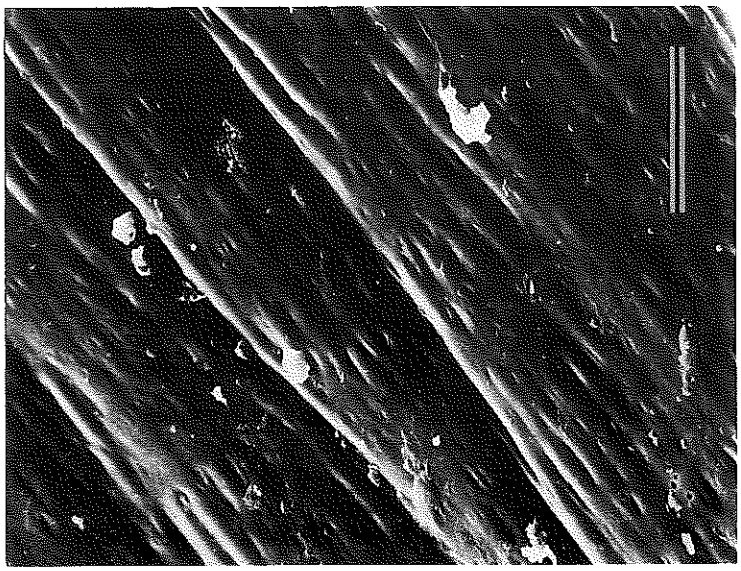

Figure 4. Detail of the surface of the epicuticular side of the same fragment as in Fig. 3 showing almost no visible alteration (SEM, × 440, scale bar = 50 μm).

Comparative measurements and observations on chitin from archaeological specimens and some modern counterparts

Our laboratory and field simulations of microbial degradation were extremely short compared to the time available for degradation of archaeological samples. The influence

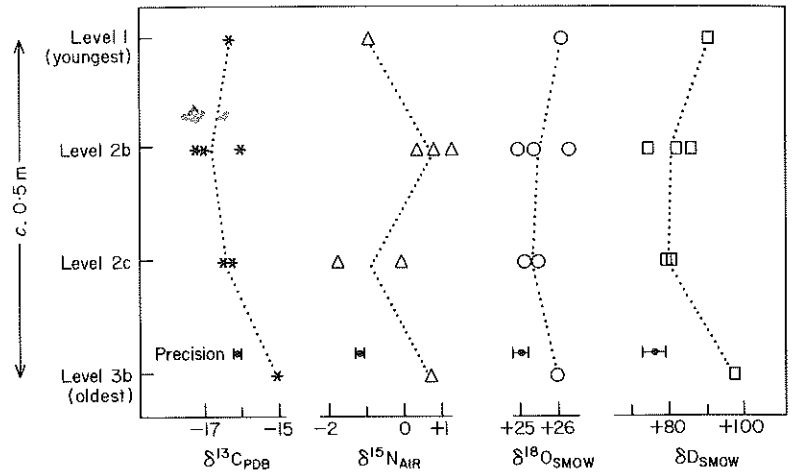

Figure 5. δ values (‰) of chitin from crabs (probably *Platyxanthus orbignyi*) excavated in Peru near the Chincha Valley (*Projecto arqueologico Pescadores de Chincha*).

of time as a factor was evaluated by analysing a variety of archaeological crustacean remains of known ages and comparing their δ values with the respective δ values of modern crustaceans caught in or near the same locations. These materials came from locations in Peru and in the Bahamas.

Parts of seven crabs were obtained from a coastal South Peruvian archaeological site close to the Chincha Valley. The specimens are probably *Platyxanthus orbignyi* (family Xanthidae) and have an approximate age of 450 years bp based on secure ceramic affiliation (D. Sandweiss, pers. comm.). *P orbignyi* is presently found along the entire Peruvian coast, living on rocky and coarse sedimentary substrates in the subtidal zone, from 0 to 27 m of depth (D. Sandweiss, pers. comm.). The results of isotopic analysis are shown in Figure 5. The means, standard deviations and ranges of the four stable isotope ratios for the samples regardless of age, are:

$\delta^{13}C = -16{\cdot}3 \pm 0{\cdot}6$‰, range 2·1‰, $N = 7$;
$\delta^{15}N = +\ 0{\cdot}1 \pm 1{\cdot}0$‰, range 3·1‰, $N = 7$;
$\delta^{18}O = +25{\cdot}6 \pm 0{\cdot}5$‰, range 1·4‰, $N = 7$;
$\delta D = +85 \pm 7$‰, range 23‰, $N = 7$.

Unfortunately, we could not obtain modern Peruvian specimens of *P. orbignyi* for comparison. The habitat of the modern Peruvian lobster *Panulirus gracilis* (family Palinuridae) is very similar and its isotopic data given in Table 6 are in good agreement with the results for archaeological *P. orbignyi*. Moreover, a comparison with the large amount of isotopic data on fully marine aquatic crustaceans given by Schimmelmann (1985) indicates the similarity of these archaeological isotopic data with modern data.

Four sets of crab remains from the coastal North Peruvian archaeological sites of Pacatnamu with approximate ages of 1000–1400 years bp (C. Donnan, pers. comm.) were measured. The poor morphological preservation did not permit the determination of species. The results of isotopic analysis are shown in Figure 6. The isotopic means, standard deviations and ranges of the samples, regardless of age, are:

Table 6. δ values (‰) of chitins from modern crustaceans sampled at the indicated locations near archaeological sites discussed in the text

	$\delta^{13}C$	$\delta^{15}N$	$\delta^{18}O$	δD
Pacatnamu, Peru				
Panulirus gracilis (family Palinuridae)	−15·5	−3·6	+25·7	+88
Pinnotherelia laevigata (family Pinnotheridae)	−15·0	+0·4	+28·7	+50
Euphylax dovii (family Portunidae)	−19·8	−7·1	+27·0	n.d.
Callinectes arcuatus (family Portunidae)	−14·2	+0·8	+26·3	+64
Mayaguana Island, Bahamas				
Cardisoma guanhumi (family Gecarcinidae)	−23·8	−2·3	+26·3	+98

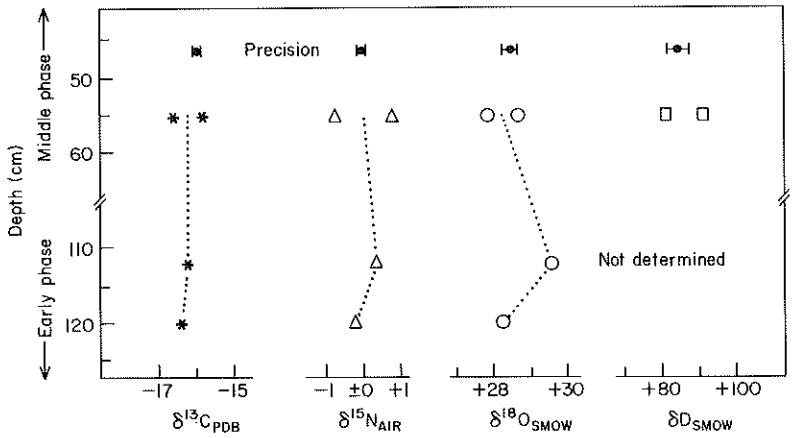

Figure 6. δ values (‰) of chitin from crabs excavated in Peru at Pacatnamu.

$\delta^{13}C = -16{\cdot}2 \pm 0{\cdot}3‰$, range 0·8‰, $N=4$;
$\delta^{15}N = +\ 0{\cdot}1 \pm 0{\cdot}6‰$, range 1·5‰, $N=4$;
$\delta^{18}O = +28{\cdot}6 \pm 0{\cdot}6‰$, range 1·7‰, $N=4$;
$\delta D = +86 \pm 5‰$, range 10‰, $N=2$.

These isotopic signatures match data on modern supralittoral crustaceans that are subject to evapotranspiration effects and thus display more positive $\delta^{18}O$ values than fully aquatic species (Schimmelmann & DeNiro, 1986*c*). In fact, the beach near Pacatnamu faces a cliff and is reported to be pebbly with abundant supralittoral crustaceans (C. Donnan and D. McClelland, pers. comm.).

Finally, we measured the archaeological remains of the claw of a land crab, either *Gecarcinus lateralis* or *Cardisoma guanhumi* (both family Gecarcinidae), excavated by Sullivan (1981) in a cave on Middle Caicos Island in the Caribbean with an approximate

age of 500–1250 years bp (E. Wing, pers. comm.). Biodegradation in this heavily calcified material had proceeded to an extent that 3·03 g of untreated exoskeleton yielded only 19 mg GlcN·HCl. The isotopic results are: $\delta^{13}C = -23{\cdot}0‰$, $\delta^{15}N = -2{\cdot}8‰$, $\delta^{18}O = +27{\cdot}8‰$, $\delta D = +86‰$. This terrestrial isotopic signature matches surprisingly well with the results (Table 6) on a modern land crab from another island in the Caribbean, Mayaguana, suggesting that the isotope ratios have been preserved in spite of extreme and longlasting biodegradation.

Conclusions

Neither biological nor thermal partial degradation produced significant changes of stable isotope ratios in arthropod chitin as measured in extracted D-glucosamine hydrochloride. Thus, archaeological and fossil chitins (for which electron microscopy indicates the absence of fungal chitin) can be used for palaeoenvironmental and palaeoclimatic studies. Isotopic ratios of ancient chitins can be interpreted in light of the corresponding values for contemporary chitins from well-characterized environments.

Acknowledgements

This work was supported by the Lerner–Gray Fund for Marine Research of the American Museum of Natural History, by the Geological Society of America grant 3216-83, by National Science Foundation grant ATM79-24591, by American Chemical Society Petroleum Research Fund grant 15582-AC2, by FRFC Belgium no 2.4506.83 and by NATO Collaborative Research Programme grant 477/84. We thank Christopher Donnan, William Keegan, Donna and Donald McClelland, Daniel Sandweiss and Elizabeth Wing for their generosity and help in making archaeological chitin samples available for this study, Bruno Marino for helpful discussions, and Dave Winter for performing the stable isotope ratio mass spectrometry.

References

Bilai, V. I., Koval, E. Z. & Kharkevich, E. S. (1984). [Ecological and methodical aspects of chitin degradation by micromycetes.] *Mikrobiologicheskii zhurnal* **46,** 97–108. [In Russian.]

Brewer, W. S. & Pfaender, F. K. (1979). The distribution of selected organic molecules in freshwater sediment. *Water Research* **13,** 237–240.

Buchardt, B. & Fritz, P. (1980). Environmental isotopes as environmental and climatological indicators. In (P. Fritz & J. C. Fontes, Eds) *Handbook of Environmental Isotope Geochemistry*. Amsterdam: Elsevier, pp. 473–504.

Epstein, S., Yapp, C. J. & Hall, J. (1976). The determination of the D/H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. *Earth and Planetary Science Letters* **30,** 241–251.

Fjerdinstad, E., Fjerdingstad, E. & Kemp, K. (1979). Further studies in Danish Lobelia-Isoetes lakes. *Archiv für Hydrobiologie* **85,** 80–83.

Griffiths, R. P., Caldwell, B. A., Broich, W. A. & Morita, R. Y. (1982). Long-term effects of crude oil on microbial processes in subarctic marine sediments. *Marine Pollution Bulletin* **13,** 273–278.

Hare, P. E. & Abelson, P. H. (1965). Amino acid composition in some calcified proteins. *Annual Report of the Director Geophysical Laboratory, Carnegie Institution, Washington, D.C.* **64,** 223–232.

Hoering, T. C. (1981). Thermal reactions of kerogen with added water, heavy water, and pure organic substances. *Annual Report of the Director Geophysical Laboratory, Carnegie Institution, Washington, D.C.* **80,** 397–402.

Hood, M. A. & Meyers, S. P. (1978). Chitin degradation in estuarine environments and implications in crustacean biology. In (R. A. A. Muzzarelli & E. R. Pariser, Eds) *Proceedings of the First International Conference on Chitin/Chitosan*. Cambridge: MIT Sea Grant MIT-SG-78-7, pp. 563–569.

Jeuniaux, C. (1963). *Chitine et Chitinolyse, un Chapitre de la Biologie Moléculaire*. Paris: Masson.
Jeuniaux, C. (1965). Chitine et phylogénie: application d'une méthode enzymatique de dosage de la chitine. *Bulletin de la Société Chimie Biologique* **47,** 2267–2278.
Koval, E. Z. & Kharkevich, E. S. (1983). [Chitin degradation by soil fungi in a wooded area.] *Mikrobiologicheskii zhurnal* **45,** 57–63. [In Russian.]
Macko, S. A. & Estep, M. L. F. (1982). Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. *Annual Report of the Director Geophysical Laboratory, Carnegie Institution, Washington, D.C.* **81,** 394–398.
Mitchell, R. & Alexander, M. (1962). Microbiological processes associated with the use of chitin for biological control. *Journal of the Soil Science Society of America* **26,** 556–558.
Moore, L. R. (1969). Geomicrobiology and geomicrobiological attack on sedimented organic matter. In (G. Eglington & M. T. J. Murphy, Eds) *Organic Geochemistry*. Berlin: Springer Verlag, pp. 265–303.
Muzzarelli, R. A. A. (1977). *Chitin*. Oxford: Pergamon Press.
Muzzarelli, R. A. A., Jeuniaux, C. & Gooday, G. W. (Eds) (1986). *Chitin in Nature and Technology*. New York: Plenum Press.
Norkrans, B. & Stehn, B. O. (1978). Sediment bacteria in the deep Norwegian Sea. *Marine Biology* **47,** 201–209.
Northfelt, D. W., DeNiro, M. J. & Epstein, S. (1981). Hydrogen and carbon isotopic ratios of the cellulose nitrate and saponifiable lipid fractions prepared from annual growth rings of a California redwood. *Geochimica et Cosmochimica Acta* **45,** 1895–1898.
Okafor, N. (1978). Chitin decomposition by microorganisms in soil. In (R. A. A. Muzzarelli & E. R. Pariser, Eds) *Proceedings of the First International Conference on Chitin/Chitosan*. Cambridge: MIT Sea Grant MIT-SG-78-7, pp. 578–581.
Okutani, K. (1978). Chitin- and *N*-acetylglucosamine-decomposing bacteria in the sea. In (R. A. A. Muzzarelli & E. R. Pariser, Eds) *Proceedings of the First International Conference on Chitin/Chitosan*. Cambridge: MIT Sea Grant MIT-SG-78-7, pp. 582–586.
Poulicek, M. (1985). Shell matrix weathering in marine sediments. *Malacologia,* in press.
Poulicek, M. & Jaspar-Versali, M. F. (1984). Biodégradation de la trame organique des coquilles de mollusques en milieu marin: action des microorganismes endolithes (I). *Bulletin de la Société Royale des Sciences de Liège* **53,** 114–126.
Poulicek, M., Jaspar-Versali, M. F. & Goffinet, G. (1981). Étude expérimentale de la dégradation des coquilles de mollusques au niveau des sédiments marins. *Bulletins de la Société Royale des Sciences de Liège* **50,** 513–518.
Poulicek, M. & Jeuniaux, C. (1982). Biomass and biodegradation of mollusk shell chitin in some marine sediments. In (S. Hirano & S. Tokura, Eds) *Chitin and Chitosan, Proceedings of the 2nd International Conference on Chitin & Chitosan*. Sapporo, Japan, pp. 196–199.
Ruiz-Herrera, J. (1978). The distribution and quantitative importance of chitin in fungi. In (R. A. A. Muzzarelli & E. R. Pariser, Eds). *Proceedings of the First International Conference on Chitin/Chitosan*. Cambridge: MIT Sea Grant MIT-SG-78-7, pp. 11–21.
Schimmelmann, A. (1985). *Stable isotopic studies on chitin*. Ph.D. dissertation, University of California, Los Angeles.
Schimmelmann, A. & DeNiro, M. J. (1985). Determination of oxygen stable isotope ratios in organic matter containing carbon, hydrogen, oxygen and nitrogen. *Analytical Chemistry* **57,** 2644–2646.
Schimmelmann, A. & DeNiro, M. J. (1986*a*). Stable isotopic studies on chitin. I. Measurements on chitin/chitosan isolates and D-glucosamine hydrochloride from chitin. In (R. A. A. Muzzarelli, C. Jeuniaux & G. W. Gooday, Eds) *Chitin in Nature and Technology*. New York: Plenum Press, in press.
Schimmelmann, A. & DeNiro, M. J. (1986*b*). Stable isotopic studies on chitin. II. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in arthropod chitin. *Contributions in Marine Science,* in press.
Schimmelmann, A. & DeNiro, M. J. (1986*c*). Stable isotopic studies on chitin. III. The D/H and $^{18}O/^{16}O$ ratios in arthropod chitin. *Geochimica et Cosmochimica Acta,* in press.
Schoeninger, M. & DeNiro, M. J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimiça et Cosmochimica Acta* **48,** 625–639.

Shear, W. A., Bonamo, P. M., Grierson, J. D., Rolfe, W. D. I., Smith, E. L. & Norton, R. A. (1984). Early land animals in North America: Evidence from Devonian age arthropods from Gilboa, New York. *Science* **224,** 492–494.

Stock, C. (1949). Rancho La Brea. A Record of Pleistocene Life in California. *Los Angeles County Museum of Natural History, Science Series* **20,** 66–67 (6th edition).

Streichsbier, F. (1982). [Ecological studies on microbial chitin degradation in suburban lotic waters.] *Wasser und Abwasser* **25,** 53–70. [In German.]

Sullivan, S. D. (1981). *Prehistoric patterns of exploitation and colonization in the Turks and Caicos Islands*. Ph.D. dissertation, University of Illinois, Urbana-Champaign.

Warnes, C. E. & Poole Rux, T. (1982). Chitin mineralization in a freshwater habitat. In (S. Hirano & S. Tokura, Eds) *Chitin and Chitosan. Proceedings of the 2nd International Conference on Chitin & Chitosan*. Sapporo, Japan, pp. 191–195.

Warnes, C. E. & Randles, C. I. (1977). Preliminary studies on chitin decomposition in Lake Erie sediments. *Ohio Journal of Science* **77,** 224–230.

Wedeking, K. W. & Hayes, J. M. (1983). Exchange of oxygen isotopes between water and organic material. *Isotope Geoscience* **1,** 357–370.

Wirsen, C. O. & Jannasch, H. W. (1976). Decomposition of solid organic materials in the deep sea. *Environmental Science and Technology* **10,** 880–886.