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Abstracts

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Energetics of amino acid synthesis in hydrothermal solutions

The amino acid synthesis pathways in microorganisms, though diverse, share several noteworthy features; the nitrogen source for α-amino groups of amino acids is NH_4^+, and the skeletal carbons come from intermediates of the tricarboxylic acid cycle and other major metabolic pathways. Although the corresponding mechanisms, including the specific functions of essential enzymes, are well understood, the energetics of amino acid biosynthesis in the context of the natural environment of the host cell have not received their due. These energetics can be determined by combining standard state Gibbs free energies of synthesis reactions (ΔG_r°) with *intracellular* activity products. Values of ΔG_r° as functions of temperature and pressure for amino acid synthesis can be computed from recently published thermodynamic properties [Shock EL et al. (1989) *GCA* 53: 2157–83; Shock EL et al. (1997) *GCA* 61: 907–50; Amend, Helgeson (1997), *J Chem Soc Faraday Trans* 93: 1927–41; *GCA* 61: 11–46]. However, the intracellular concentrations of the essential reactants and products are only poorly understood. Nevertheless, energetic demands that organisms place on their geochemical environments can be evaluated using *extracellular* activity products, which we use to constrain the amounts of energy released by or required for amino acid synthesis from CO_2, H_2, NH_4^+, and H_2S by autotrophic microorganisms in two diverse ecosystems, hydrothermal solutions and surface seawater.

Chemical disequilibria in hydrothermal solutions due to subsurface mixing of seawater and thermal vent fluids may provide energy for amino acid synthesis. Thermodynamic calculations at 100 °C show that the formation from inorganic precursors of 11 of the 20 naturally occurring amino acids are exergonic in these highly reduced fluids. By comparison, similar calculations indicate that the synthesis reactions of all 20 amino acids are strongly endergonic in cooler, relatively oxidized surface seawater. Activities of CO_2, H_2, H^+, NH_4^+, H_2S, and amino acids in hydrothermal solutions can be determined from mixing calculations involving thermal vent fluid and seawater; corresponding activity products in surface seawater are calculated from tabulated measurements [McCollom, Shock (1997) *GCA* 51: 4375–91; Keil R. (1998) pers. comm.].

We find that although values of ΔG_r° for these reactions are strongly temperature dependent, it is the hydrogen activity (a_{H2}) in both the mixed hydrothermal solutions and in surface seawater that dictates the reaction energetics; the differences in a_{H2} values between a 100 °C hydrothermal solution (~$5 \times 10^{-4} m$, McCollom & Shock, 1997) and surface seawater ($1–2 \times 10^{-9} m$, Lilley, 1998, pers. comm.) can be five orders of magnitude or more. As a result, the synthesis reactions in a 100 °C hydrothermal solution are exergonic for the ten most reduced amino acids (leu, ile, val, lys, phe, met, pro, tyr, trp, ala) and endergonic for nine of the ten most oxidized amino acids (his, asn, asp, gly, cys, ser, gln, arg, thr).

We conclude that the energetics of amino acid synthesis are strongly favoured in hydrothermal systems over surface seawater. For example, preliminary computations show that the overall Gibbs free energy (ΔG_r) for the reaction

$$3CO_2(aq) + NH_4^+ + 6H_2(aq) \rightarrow Ala(aq) + H^+ + 4H_2O$$

equals –5.9 kcal mol^{-1} in the mixed hydrothermal solution at 100 °C and +50.6 kcal mol^{-1} in surface seawater. Similar comparisons of ΔG_r for the other amino acids show differences ranging from approximately 50 to 200 kcal per mole of amino acid produced. Combined with the conclusion that peptide bond formation is energetically favoured with increasing temperature [Shock EL (1992) *GCA* 56: 3481–91], it can be argued that thermophilic autotrophs expend less energy for the synthesis of macromolecules, such as proteins, that do their mesophilic counterparts. In fact, depending on the amino acid composition of the protein, the synthesis of its monomers from CO_2, H_2, and other inorganic precursors in hot, reduced aqueous solutions may provide substantial surplus energy that can be harnessed to drive intracellular synthesis of enzymes and other biopolymers.

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Osteocalcin – an unusually stable bone protein?

Osteocalcin (OC) is the 7th most common protein in the human body. It is a small, highly negatively charged protein associated with the mineral phase of bone. This close association is linked to the interaction of three g-carboxyglutamic acid (Gla) residues with the surface of calcium hydroxyapatite. It has been claimed that OC survives in dinosaur bones. We have investigated the preservation potential of this protein using monoclonal antibodies, which detect different re-

gions of the molecule. The various regions of the OC molecule demonstrate different survival rates; the best preservation is seen in the a-helix, the worst in the N-terminus. This same survival pattern is seen in archaeological bones. All three Gla residues lie in the region we call „a-helix", and this region is well-preserved in bone, but there is little preservation potential seen when the extracted helix and mineral are mixed together as a „reconstituted" bone. Indeed, there is little advantage for helix preservation when reacted with mineral compared to that seen in water.

This may be due to damage during extraction and purification procedures. High temperature studies show that at temperatures of less than 95 °C, we could not demonstrate any reduction in signal for the Gla region, thus we cannot predict accurately the likely survival time for this REGION. On the basis of our kinetic analyses, we are not able to refute reports of OC in dinosaur bones. Can we use OC to assess the level of microbial degradation? Having a strong indicator of this alteration would be desirable, since we can then determine which bones are more or less likely to be useful for archaeometric studies. Archaeological bone samples from Bercy (Paris) have shown that OC survival rates can be closely linked to the microbial re-working of bone (macro-porosity).

The correlation between macro-porosity and OC content is better than that between macro-porosity and nitrogen content or collagen yield. OC content, therefore, becomes an ideal tool for predicting the degree of microbial degradation.

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Dating of intertidal flat sedimentation of aspartic acid racemization – First results

The present study examines the D/L aspartic acid ratio of bivalve shells (*Cerastoderma edule* and *Mya arenaria*) in sediments below a mussel bed *(Mytilus edulis)* on a tidal flat in the German Wadden Sea. The aim of the present work is to investigate the applicability of amino acid dating to highly dynamic environments. Analysis of D/L aspartic acid allows to date recent (late Holocene) sediments with high resolution. Additionally the age distributions may be used to elucidate transport processes of the shells. Sediment core MK3 contained two pronounced shell layers: one from 8 to 20 cm depth (*M. edulis, M. arenaria* in life position and *C. edule*) and a second from 67 to 87 cm with only juvenile articulated *C. edule* of one size class (ca. 1 cm). Some isolated shells of *C. edule* were found between these shell layers. Two shell layers were also found in core MK4 (13–18 and 24–36 cm). Both contained *M. edulis* and *C. edule*. A slight condensation of shells occurred at a depth of 53–65 cm. The D/L aspartic acid ratio, analyzed by gas chromatography, was used to determine the age of *C. edule* and *M. arenaria* shells. Ages ranged from 0 to 2000 yr B.P. and generally increased with sediment depth. Exceptions included three very young *C. edule* shells at 24–36 cm depth (16–22 yr B.P.) and one extremely old shell of this species (2000 yr B.P.). Linear regression analysis yields a sediment accumulation rate of 34 ± 4 cm/100 years ($R^2 = 0.91$). This compares favourably to the 25–30 cm/100 years reported for the Wadden Sea by other authors. It is not conceivable that the young shells in deeper sediment layers were buried by bioturbation, because they were too big (2.5–2.8 cm). Sediment reworking by e. g. storms up to this depth is also feasible. However, the occurrence of older shells which do not fit the linear age trend with depth can be explained by reworking. As the energy for the transport of shells must have been very high, it is assumed that storm events are responsible for this. From these preliminary results it can be concluded that aspartic acid dating can be applied to dynamic depositional environments at time scales from decades to millennia in the study region. The occurrence of significantly older or younger shells, which do not fit a linear trend with depth, indicates that these shells have been transported during high energy events.

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Paleoenvironmental reconstruction using amino acid racemization in bones and teeth

In modern bones and teeth from saline lakes in Australia and Canada amino acid racemization analyses combined with mineralogical and geochemical analyses have demonstrated that bone and tooth fossilization causes significant disruption in the „normal" racemization process. In these samples, time and temperature are definitely not the controlling factors for racemization. In these samples, some amino acids have racemization ratios that greatly exceed those seen in samples as old as 200 ka.

Bone and tooth samples that had live algal filaments within their trabeculae also had much slower alanine racemization, but phenylalanine racemization. Normal alanine concentrations coincide with the presence of algae, but proline, phenylalanine, aspartic and glutamic acid concentrations were reduced compared to bone not affected by algae.

Amino acids are leached from fossils when secondary mineralization occurs. Proline leaching is correlated with high proline racemization rates ($R^2 > 99.5\%$), as is the ubiquitous glutamic acid leaching. Increased alanine racemization occurs when alanine concentrations increase relative to other amino acids. In associated sediment samples, low glutamic acid concentrations are associated with slow racemization rates. Aspartic acid racemization rates also correlate with aspartic acid leaching.

Under reducing conditions, secondary minerals such as siderite, vivianite, kutnahorite, and hydromagnesite form. Trace elements, such as Mn, Fe, Co, Sc, Zn, Ni, and U increase simultaneously. Reducing conditions do not promote as rapid nor as marked changes in cortical bones biogeochemistry as do oxidizing conditions. Relative concentrations of most amino acids change little from those seen in modern tissues, and racemization rates are only moderately increased in reducing conditions. In cancellous bone, however, the changes are more rapid and pronounced than in cortical bone or dentine.

Under oxidizing conditions, secondary carbonate mineralization is favoured in the fossils. Aragonite, calcite, and dolomite may all co-occur, but monohydrocalcite, and other carbonates may also form secondarily. Faster alanine racemization, enriched alanine, and severely reduced proline concentrations are correlated with secondary carbonate mineralization, especially with dolomite formation ($R^2 > 99\%$). Effects are more rapid in cancellous bone than in cortical bone.

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Time and place in aminostratigraphy

As a means of correlating land sequences with marine oxygen isotope stratigraphy aminostratigraphy contributes to understanding the ice-age climate system.

A test of high resolution D/L data is provided by a geochronological framework based on magnetostratigraphy, tephrochronology and first and last appearance datums of marine foraminifera is presented back to ca. 1 Ma in the Wanganui Basin, New Zealand. The venerid marine bivalves Tawera spissa and Austrovenus stutchburyi provide an effective D-alle/L-Ile framework for New Zealand that is consistent with both lithostratigraphy and geomorphology. At higher latitudes (Baffin Island, Spitsbergen and Alaska) regional marine correlation is possible although not at such high resolution. In the mid-latitude UK epimerization of terrestrial molluscs provides an independently calibrated geochronological scale back to ca. 650 ka. This allows testing, and upholding, the hypotheses of Shackleton (1987) and Raymo (1997) that only four major glaciations occurred in the last ca. 800 ka instead of nine inferred from oxygen isotope stratigraphy. Thus D/L data contributes to the theory of ice-age climate system variability that is this case supports a theory (Raymo), using new calculations for orbital forcing, predicts that the present interglacial is nearly at an end.

It is concluded from D/L and U-series data from global locations and the east and west coast U.S.A. that the relatively high sea-level 80,000 years ago is a function of uplift; and that notions of „stable" coastlines anywhere are no longer valid.

The concept of climate amelioration mid-way through the last ice-age (stage 3) requires revision forced by data from the Greenland ice-sheet and high sedimentation cores showing repetitive ice-rafting events correlated with climate variability on a global scale. Palaeothermometry derived from aminostratigraphic data indicates temperature depression, below those predicted by CLIMAP (1976) in Australia and by extension in the Mississippi Valley. This sipport the notions of Rhodes Fairbridge (1971) about a cold and dry ice-age world; and also suggests that one of the „smoking guns" of climate change is water vapour.

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Prebiotic amino acid synthesis pathways via α-keto acids: An alternative to the Strecker synthesis

Prebiotic synthesis of amino acids is considered one of the essential requirements for the genesis of life. The overwhelming majority of amino acid synthesis experiments involve reactions of HCN, formaldehyde and ammonia (Strecker synthesis). However, this reaction, while capable of producing a variety of amino acids, bears little resemblance to amino acid synthesis pathways in living organisms. Recent theories by Wächtershäuser and others have suggested that biochemical pathways employed by modern life had their beginnings in abiotic reactions within hydrothermal systems. Little work has been done so far to investigate the viability of chemical reactions similar to those utilized by modern organisms. One pathway for the incorporation of reduced nitrogen into cells proceeds via reductive amination of α-keto acids such as pyruvate and α-keto glutarate. We examined the potential for this reaction to occur in a non-catalytic environment using sealed gold tube reactors incubated at pressures and temperatures relevant to hydrothermal systems. The results indicate that ammonium quickly reacted with pyruvate in solution via a self-catalyzed reductive amination to form the amino acid alanine. Given a source of alpha-keto acid, this reaction provides a prebiotic route for nitrogen incorporation into organic molecules to form amino acids, and mimics a pathway used by bacteria in nitrogen replete environments. The possible metal sulfide-catalyzed generation of these acids within hydrothermal systems will be discussed. The results indicate that at least one primary cellular biochemical pathway could have been generated in prebiotic hydrothermal systems. Because of the ease of nitrogen incorporation into these molecules, generation of amino acids may have been one of the first biochemical pathways, and may have predated other more complex protein-mediated biosynthesis pathways in the earliest life forms.

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The isotopic integrity of α-carboxyglycine (aminomalonate) in fossil bone based on ^{14}C data

Radiocarbon determinations have been obtained on α-carboxyglycine (aminomalonate) [Am] isolated from a series of fossil bones. As far as we are aware, neither has Am previously been reported in fossil bone and nor has Am ^{14}C values have previously been measured. Unfortunately, the isotopic integrity of Am in the bones examined was significantly compromised. Even for bones retaining significant amounts of collagen, Am extracts yielded ^{14}C values discordant with their expected age and with ^{14}C values obtained on total amino acid fractions isolated from the same bone sample.

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Protein decomposition

Over the past three years our group has been investigating various processes which impact upon protein diagenesis over archaeological and geological time. As the costs of laboratory investigation rise, falling computing costs are shifting our emphasis towards modelling rather than analysing the processes of decomposition – with variable degrees of success.

The difficulty of modelling complex systems and undefined biomolecules is considered in a study of mechanisms of long-term diagenesis of macromolecules in brachiopod shells. The advantages of studying a semi-closed system are contrasted with the limitations imposed on systems in which the components and chemistry are poorly characterised.

The one study of invertebrate skeletons is contrasted with three investigations of bone proteins. Advances in the understanding of the chemistry of aspartic acid racemization enables a more detailed kinetic model to be developed. Free solution experiments appear to provide reasonable predictions of the rates in both phosphatic and carbonate skeletons. Upon closer inspection of the mechanism, however, the results appear to be somewhat less predictable. Attempts to model the rates of reaction are hampered by the sheer diversity of reactions which must be considered; a case of more is less.

In contrast, the melting of collagen is a complex phenomenon which we have caricatured in a simple model. We are attempting to investigate the apparent diversity of survival of collagen using this model, but even this appears to add unnecessary complexity. A straightforward relationship emerges between temperature and loss of collagen which then describes a simple bounding plane controlling the upper limit of collagen survival over a wide range of temperatures. We are now testing this against data on collagen survival from radiocarbon dated bone.

Finally, the survival of another bone protein, osteocalcin, seems to resist attempts to explain it in terms of predictive models. Attempts to relate the decomposition of this molecule to either laboratory estimates of hydrolysis or decarboxylation of the unusual γ-carboxyglutamic acid residues in this protein have failed. Molecular models do not seem to conform to experimental observations, and the reasons for this are considered.

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The use of a kinetic model to predict collagen survival

The survival of collagen in archaeological bones seems bewildering in its ranges and quality. We have attempted to describe the preservation of collagen in terms of yields *vs.* ^{14}C age, but without success. At the extreme, it does appear that collagen survival is prolonged in cool climates when contrasted with warm ones, but this appears to be the limit of observable trends. We have previously proposed a simple model which described the loss of collagen by a process of hydrolysis leading to its transition to soluble gelatin. We speculated that collagen degradation (i. e., melting) would prove very temperature sensitive due to a combination of thermally rate-controlled processes.

Laboratory experiments have been used to determine the rate of collagen melting over a range of temperatures (95 °C–55 °C). As previously predicted, the rate of melting is highly temperature sensitive, but a sigmoidal pattern of weight loss (as predicted by the model) was not observed. The rate of melting displays an unexpected simple relationship to temperature; we are now to use this simple kinetic model to explore the survival of collagen in archaeological bones.

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The fate of proteins on ceramics during simulated cooking experiments

Recent studies by the authors and other researchers have revealed that only very low amounts of amino acids are detectable on archaeological and recently used cooking pots. These results contradict proposed mechanisms which suggest that proteinaceous material will become bound and stabilised to the clay surface and protected from enzymolysis by its porous structure. Is it the case that the methods used for extraction are inappropriate on tightly bound material or is protein derived material not surviving, even over short time scales in this context?

To answer these questions, a series of simulated Iron Age pots have been constructed in order to assess pyrolysis screening techniques as well as amino acid and protein extraction methods. The simulated ceramics have been used in a series of laboratory and field experiments involving different foodstuffs, cooking methods and on pots with different pore size distributions, thus allowing assessment of protein accumulation during a range scenarios. To understand the fate of any protein residues during deposition, sections of pot have been buried under well defined environments and other test pieces are to be used in artificial diagenesis experiments. Once the extraction has been optimised, a series of monoclonal antibodies, which have been raised against degraded and intact proteins, will be used to assess the survival in each of the different simulated contexts.

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Stereoisomerism of meteoritic amino acids

Analyses of the molecular and isotopic composition of the organic matter of CM carbonaceous chondrites, for example, the Murchison meteorite, can provide unique insight to the process(es) of chemical evolution. The amino acids are an interesting case in point. They comprise a mixture that is quite different, when viewed in terms of isomerism, from those found in terrestrial materials. For example, within the four structural classes of amino acids found in meteorites, all of the constitutional (structural) isomers appear to be present, i. e., all of the chain and amino position isomers. This isomeric diversity persists at the level of stereoisomerism. When examined from the standpoint of symmetry, all enantiomers and diastereomers of the amino acid structural isomers are found. In contrast to the specificity of biosynthetic processes, structural diversity appears to be a hallmark of chemical evolution.

Enantiomer ratios of meteorite amino acids have been of interest for many years because they offer a way to assess terrestrial contamination. Although it has been generally believed that the indigenous amino acids occur as racemates [Kvenvolden K et al. (1971) Nature 228: 923], the significance of a convincing demonstration of even small enantiomeric excesses (ee) not related to contamination has motivated further analyses. Such a finding would signify the operation of an asymmetric influence during chemical evolution and suggest the possible origin of biological homochirality in chemical, rather than biological, evolution.

We recently approached the question of meteoritic ee by analysis of 2-amino-2,3-dimethylpentanoic acid (2a23dmpa) [Cronin J R, Pizzarello S (1997) Science 275: 951], a meteorite amino acid with two chiral centers and thus four stereoisomers, alternatively named DL-α-methylisoleucine and DL-α-methylalloisoleucine. This amino acid was of particular interest in view of a current hypothesis for meteorite amino acid synthesis. Accordingly, the α-amino acids were formed in the primitive carbonaceous chondrite parent body by a Strecker synthesis from HCN, ammonia, and carbonyl compounds of interstellar origin. If formed in this way, 2a23dmpa, which would be derived from a chiral ketone, would be sensitive to an asymmetric influence exerted either in the interstellar medium (ketone) or in the parent body (amino acid). These different possibilities would produce, in principle, different ratios among the four 2a23dmpa stereoisomers. When analyzed by chiral GC-MS, L-ee were observed in both diastereomer pairs, a result favouring an asymmetric effect on the amino acid, per se, rather than the precursor ketone.

L-Ee have been observed in several other α-methyl amino acids from Murchison and confirmed in preparations of these amino acids from the Murray meteorite. The hypothesis [Rubenstein E et al (1983), Nature 306: 118] that ee might come about from exposure of planetary or interstellar organic matter to a flux of circularly polarized light of a specific handedness produced as synchrotron radiation by neutron stars provides an attractive possible explanation for the ee observed in these meteorite amino acids.

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Applications of stable isotopes for determining the origins of amino acids in extraterrestrial materials

Amino acids are essential components of all living systems and it is therefore commonly assumed that their occurrence in our solar system was a precondition for life's origin. Given the likelihood that life already existed on Earth at the time of formation of the oldest known crustal rocks (~4.0 Ga), the search for primordial, abiotic amino acids has focused on the analysis of ancient, extraterrestrial materials, i. e. carbonaceous meteorites. Stable isotope analyses of bulk organic matter in, for example, the Murchison meteorite, indicate an interstellar source for elemental constituents that predates the formation of our solar system. However, the fact that all cabonaceous meteorites have experienced brief to extended residence times on Earth presents a serious challenge for distinguishing indigenous amino acids from terrestrial overprints. This is particularly true with respect to the common amino acids that are the building blocks of all proteins in living organisms.

Elemental (C, H, N) constituents of organic matter in the Murchison meteorite are enriched in ^{13}C, ^{15}N, and D relative to terrestrial organic matter of biological origin. We hypothesized that it could be possible to determine the indigeneity of amino acids in carbonaceous meteorites based on their respective stable isotope compositions. The challenge has been to make these isotopic measurements on compounds typically present at $nmolg^{-1}$ levels. We have developed gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) methods to address this challenge and our results confirm an extraterrestrial origin for amino acid enantiomers in the Murchison meteorite. In addition to isotopic assessments of the origins of organic compounds in other meteorites, it may be possible to use this approach to determine the source(s) of exotic amino acids in sediments that have been attributed to the bolide impact at the K/T boundary.

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Food web dynamics of the juvenile blue crab, Callinectes sapidus: Whole tissue and compound-specific stable isotope techniques

As the blue crab migrates inland within the estuarine ecosystem of the Delaware Bay, metamorphosing from its larval stage to its early juvenile stage, we hypothesize that the crab shifts from a phytoplankton-based diet to a Spartina alterniflora-based diet. This dietary shift occurs during a critical period in the lifecycle of the blue crab, during which marsh ecosystems might play a key role in the development of juvenile crabs. The purposes of this study are to isotopically characterize the estuarine food web, determine if marsh ecosystems support the growth of juvenile blue crabs, and examine the usefulness of stable carbon isotopes of amino acids in food web studies.

We have measured, by mass spectrometry, the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of representative members of the blue crab food web at each trophic level, including potential diets such as phytoplankton, zooplankton, meiofauna, and Spartina, as well as experimentally-cultured and field-collected samples of Callinectes sapidus. In addition, we have analyzed the $\delta^{13}C$ of individual amino acids in these samples by gas chromatography/combustion-isotope ratio mass spectrometry (GC/C-IRMS). Whole tissue measurements indicate that early juvenile blue crabs in the bay feed primarily on zooplankton. Marsh-dwelling crabs, which are isotopically heavier in carbon than those in the bay, appear to utilize marsh-derived carbon in some form. The blue crab fractionates dietary carbon and nitrogen in a way that depends on the net growth of the crab over time. This, in turn, suggests that metabolic factors complicate whole animal measurements and that amino acids might be helpful in elucidating pathways responsible for isotope fractionation.

The trend in $\delta^{13}C$ of amino acids in estuarine organisms such as zooplankton and marsh organisms such as the fiddler crab (Uca pugnax) mirrors that of terrestrial organisms. Essential, less abundant amino acids such as valine, leucine, isoleucine, and phenylalanine are the lightest amino acids while non-essential, relatively abundant amino acids such as glycine, proline, alanine, glutamic acid, and aspartic acid are heavier. Juvenile blue crabs appear to fractionate the carbon in essential amino acids differently from non-essential amino acids, illustrating that these two groups might be used to trace diet and reflect the degree of metabolic stress of blue crabs.

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Discerning the diet of humans from stable isotope ratios of individual amino acids

Stable C and N isotopes in the collagen of prehistoric human skeletal materials are valuable tools for determining human diet. Carbon isotopes of collagen are used to estimate percentages of C_3 and C_4 based foods, whereas N isotopes are utilized to predict trophic levels. We have undertaken a study of isotopes in individual amino acids in collagen isolated from the bones of paleohumans and their potential food sources in order to search for additional dietary prediction potential. Ten possible dietary sources were compared with eleven humans who had diets ranging from primarily maize to C_3-based hunter-gatherers. Humans with C_4 sources of dietary carbohydrates had $\delta^{13}C$ values of the nonessential amino acids, glutamate, aspartate, proline, and hydroxyproline, that were 3–5‰ enriched relative to the total $\delta^{13}C$ of collagen, but those with C_3 or seafoodenhanced diets deviated by +1 to –4‰ from total collagen. The $\delta^{13}C$ of lysine in humans ranged from –10 to –23: the most enriched values occured in humans known to eat extensive amounts of seafood and the most depleted, in hunter-gatherers. The essential amino acids, leucine and phenylalanine, were always the most isotopically-depleted amino acids with a range of –18.6 in the Indians with a maize diet to –30 in C_3-based hunter-gatherers. Humans with dietary meat sources influenced by injestion of C_4 grasses, e. g. rabbits and bison, had more depleted isotopic compositions of leucine and phenylalanine, which matched that in potential diets. There was no difference between the $\delta^{13}C$ of lysine and these two essential amino acids (Δ) in corn, and in only one human with enriched $\delta^{13}C$ in collagen was this pattern detected, the Paleoindian with a known maize diet. In all other humans, the Δ was 7–10‰. These findings were used to determine paleodiets of individuals with more mysterious diets: an Indian Chief with an enriched $\delta^{13}C$ value living before maize was introduced into North America and Easter Islanders.

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Applications of amino acid racemization to detailed chronostratigraphic analysis of Holocene sedimentary sequences

Although amino acid racemization has traditionally been applied primarily to dating and correlation of Pleistocene sequences, the technique also is very useful in carrying out detailed chronostratigraphic analyses of Holocene sequences. For much of the Holocene and for most materials, racemization dating does not provide dating as precise as that possible with radiocarbon. However, because racemization analyses are relatively easy and inexpensive to carry out, the method lends itself to very detailed studies that would be not possible with radiocarbon because of budget limitations. Two examples of applications of racemization dating to Holocene sequences are presented: shallow marine core chronostratigraphy in the southern Gulf of California (Y. Halfar) and assessment of the stratigraphy and integrity of archaeological sites in southern Texas (with Mike Quigg, TRC-Mariah Associates).

An initial radiocarbon date on core 5 from the Gulf of California gave a surprising late Pleistocene age, whereas a Holocene age was expected. Racemization analyses were carried out on bivalves (mostly *Chione*) and rhodoliths (calcareous red algae). These are apparently the first studies of racemization in calcareous algae. Racemization analysis confirmed an old age for this lower part of the core (2–4 m), but low A/I (D-alloisoleucine/L-isoleucine) values, consistent with a Holocene age, were found in the upper part. With a series of further analyses, we were able to locate the Pleistocene-Holocene boundary within the core. This stratigraphic break is not apparent from visual inspection of the core. Rhodolith A/I values were very close to those for *Chione* at each level where these were compared, indicating a similar rate of racemization. Holocene sedimentation in this core appears to be episodic. However, racemization analyses of core 3 indicate that sediments accumulated relatively continuously and there is little evidence of redeposited material at any level. This core is entirely of Holocene age. Like core 5, core 4 penetrates into Pleistocene sediments in its lower part. The area where cores 4 and 5 were taken apparently represents a Pleistocene bench or terrace, covered by a couple meters of Holocene sediments. Preliminary analyses of two cores from a second location show a chaotic pattern of racemization in relation to depth. Disturbance of the sediments by hurricanes is a possible cause.

In Zapata Co., Texas, several test pits at two archaeological sites are being evaluated for chronostratigraphy and integrity. Racemization analyses of shells of the land snail *Rabdotus alternatus* have been carried out on ca. 8 shells per level at several levels within the test pits. Reworking of material (indicated by a range of A/I values within a level) was found to be common, although certain levels show high integrity (tight clustering of shell A/I values).

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Origins and reaction histories of organic matter in Andean tributaries of the Amazon River: An amino acid perspective

Over the last decade we have found that coarse (> 63 μm), fine (0.5–63 μm) and dissolved (< 0.5 μm) organic materials transported by the lower (Brazilian) Amazon mainstream and its major tributaries are ultimately derived from leaves and woods of trees in the surrounding forests. Each of these size fractions, however, is compositionally distinct throughout the lower Amazon system. One outstanding contrast is that the coarse, fine and dissolved fractions of each water sample are progressively enriched in nonprotein amino acids (β-alanine and γ-aminobutyric acid), suggesting that smaller materials are more degraded. A second texturally-related trend is that organic matter in the dissolved fraction is depleted in nitrogen, amino acids and basic amino acids (lysine and arginine) versus the corresponding coarse fraction, which in turn is depleted versus the nitrogen-rich fine fraction. These two patterns have been hypothesized to result from microbial breakdown of coarse plant debris to soluble remnants, among which the more nitrogen-rich components are preferentially sorbed to mineral grains prevalent in the fine size fraction.

To address the question of where and how the compositional patterns characteristic of the lower basin are locked in, we have analyzed organic matter from tributaries (e. g. Rios Achumani and Beni) in the Bolivian Andes that flow via the Madeira into the Amazon River. Laboratory experiments also have been carried out in which dissolved organic materials from Bolivian waters and plant leachates are partitioned with riverine or organic-free mineral particles. The field samples demonstrate that the pattern of elevated nonprotein amino acids in smaller size fractions is also evident even in the highest reaches (~4,000 m) of the sampled Bolivian tributaries. Although dissolved and coarse particulate organic matter in the Andean tributaries are as depleted in nitrogenous materials as counterparts from the lower basin, the fine particulate fraction is not as rich in amino acids or basic amino acids as in the lower mainstream Amazon. Thus, diagenetic alteration of nitrogenous materials appears to occur extensively throughout the Andean and lower Amazon reaches, whereas partitioning is more pronounced downstream outside the Madeira basin. Laboratory experiments with natural materials demonstrate, as hypothesized, that nitrogen-rich organic materials are preferentially concentrated on mineral surfaces versus in the dissolved phase. Additional simulations are underway to test the mechanism of this fractionation, as well as the origins of nonprotein amino acids in Amazon River waters.

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The characterisation of fossil bone collagen using animal species affinity purified polyclonal antibodies

We have been investigating the immunological properties of both fresh and archaeological collagen using polyclonal antisera and the enzyme-linked immunoassay system. Collagen is an evolutionarily conserved molecule. Consequently, antisera produced against one animal species cross-react with collagen from other species. The degree of cross-reactivity is dependent upon several factors, one of which is the degree of evolutionary relatedness of the animals from which the target antigens are derived. We investigated the use of animal species collagen-Sepharose absorption chromatography to reduce the level of cross-reactivity in a polyclonal anti-bovine type I collagen antiserum. The method involved preabsorption of the antiserum on a column containing collagen from a heterologous species and then capturing the unbound antibody that remained on a bovine collagen-Sepharose column. Whereas the anti-collagen antibody recovered from the preabsorption column reacted with collagens from a wide variety of species (mammals, birds, and fish species), the antibody recovered from the second column identified epitopes

shared only within bovidae. We labelled this antibody „bovine-specific" for convenience, however its reactivity spanned collagens from several genuses (e. g. bovine and ovine collagen competed equally well for antibody binding).

These two antibody fractions (non-species specific, and „bovine-specific") were used to characterise collagen extracted from a series of archaeological cow bones of increasing age. The picture emerged that the epitopes identified by the two fractions decayed at different rates. The non-species specific epitopes were remarkably stable, whereas the „bovine-specific" epitopes disappeared with time. Competition experiments between fresh and archaeological collagen suggested that a portion of the bovine-specific epitopes were missing from the archaeological collagen. Additional experiments showed that the „bovine-specific" antibody bound to modern or archaeological collagen (approx. 120 ka) with a similar pattern of sensitivity to increasing concentrations of salt. We interpreted this as indicating that high affinity binding sites survived in old collagen, and therefore information obtained by antibody binding to ancient samples was reliable even though the magnitude of the signal small.

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70,000 years of vegetation change in the Australian Outback: Implications for the Australian monsoon

P. Edgar Hare and his colleagues were the first to discover the extraordinary potential of amino acid racemization (AAR) in ostrich and emu eggshell for providing reliable geochronologies and paleotemperatures through much of the late Quaternary in Africa and Australia (Hare et al 1984; Brooks et al 1990; Miller et al 1992; Miller et al 1997). These studies demonstrated that eggshell retains most of its original proteinaceous residues for 10^6 years or longer. As a result of these findings, the stable isotope geochemistry of the organic fraction of ostrich eggshell has been used for paleodietary and paleoenvironmental reconstructions in Africa (Johnson et al 1993, 1998). In this paper, we continue to expand on the pioneering work of Hare and others by deriving paleoenvironmental information from the stable carbon isotope composition of emu eggshell collected from central Australia.

Lake Eyre, South Australia, is located in the diffuse boundary between the summer and winter rainfall regions in the arid zone of the Australian Outback. Currently, precipitation to the north of the boundary primarily occurs during the Australian monsoon in the summer months, and precipitation to the south primarily occurs from seasonal northward migration of the Westerlies during the winter months. In this paper, stable isotopes in emu eggshell from Lake Eyre are used to track shifts in the Australian monsoon and position of the Westerlies over the last 70 ka. Stable carbon isotopes in eggshell laid by the flightless bird emu *(Dromiaus novaehollandiae)* are used to reconstruct diet (i. e., percentage of C_3 and C_4 plants consumed) and ambient vegetation, and subsequently to reconstruct large-scale changes in the seasonality of rainfall through the Australian arid zone.

The $\delta^{13}C$ values of 41 modern emu eggshell (EES) sampled across a N-S transect through Australia reflect regional changes in the dietary uptake of emus. Modern EES collected from summer rainfall regions are generally isotopically enriched (due to a diet dominated by C_4 plants) relative to those samples collected from winter rainfall regions (where diets are dominated by C_3 plants). The $\delta^{13}C$ values of nearly 300 dated fossil eggshell collected from Lake Eyre reflect large-scale shifts of dietary input and ambient vegetation over the last 70 ka. Between 70 and 35 ka, the $\delta^{13}C$ values of EES reflect a variable ecosystem and the most diverse dietary intake over the last 70 ka, where emus consumed between 95% C_3 and 100% C_4 plants. Such quantities of C_4 plant consumption are not seen in any of the modern settings, and indicate different climatic conditions than present. We hypothesize that this variable ecosystem resulted from enhanced monsoonal rainfall throughout more of the year than present. At 25 ka, the $\delta^{13}C$ values of EES are more depleted, and the diet more restrictive where emus consumed between 50 and 100% C_3 plants. The depleted $\delta^{13}C$ values of EES at the Last Glacial Maximum (LGM) reflect an ecosystem dominated by C_3 plants due to an increase in the duration of winter precipitation. This interpretation of the EES isotope data is in agreement with the palynological record from Lake Frome, and may be attributed to northward migration of the Westerlies storm track during the LGM. Beginning at 20 ka, the $\delta^{13}C$ values become increasingly enriched through most of the Holocene. We believe this trend in $\delta^{13}C$ values to represent the on-set of present day rainfall patterns leading to a more mixed vegetation assemblage. Emu eggshell collected over the last 150 years are isotopically depleted relative to the rest of the Holocene, possibly due to the selective removal of C_4 grasses by cattle and sheep introduced by European pastoralists.

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Racemization in fossil and laboratory-heated ostracodes measured by reverse phase liquid chromatography

Amino acid geochronology and paleothermometry are based largely on the extent of racemization in fossils as measured by the ratio amounts of D- and L-isomers. Recently, Kaufman and Manley (1988) developed a reverse phase HPLC method for simple stereoisomeric separations. The fully automated procedure separates DL pairs of at least nine amino acids with baseline resolution in 75 minutes using commercially available reagents and equipment. Experimental conditions were optimized for resolution of the most abundant amino acids in mollusc and ostracode fossil protein, including: aspartic acid, glutamic acid, serine, alanine, valine and isoleucine. Analytical uncertainty for nine DL ratios in four fossils spanning a broad range of ages averages 7% (n = 14 to 28). Aspartic acid and glutamic acid DL ratios are the most consistently well resolved and reproduced, with analytical variations of < 3%. Ratios in three fossil molusc samples (ILC standards; Wehmiller, 1984) analyzed by the new method and measured previously by GC-based laboratories overlap in 17 out of 18 cases, when considering the $\pm$ 1σ analytical interlaboratory errors. The new procedure reduces sample size requirements by an order of magnitude compared with ion-exchange chromatography, making analyses of sub-milligram quantities of ostracodes practical. Because ostracodes are common in lake deposits, the technique can be integrated into studies of lake cores to derive more complete time series.

To assess the integrity of the new technique applied to ostracodes, we measured the extent of racemization in both fossil and laboratory-heated shells. Late Holocene *Candona* from the floor of Bear Lake, Utah/Idaho was heated to determine the rate or racemization at elevated temperatures (140°, 110° and 80° C) for up to 50 days (longer-term heating is in progress). The results show the expected differences in the relative rates of racemization between amino acids (Asp > Ala > Glu > Val > Ile). D/L aspartic acid, the most abundant amino acid (next to glycine), increases as a power function of time in both the heated Holocene ostracodes (Fig. 1a) and in a down-core sequence of well-dated Plio-Pleistocene fossil ostracodes recovered from the Burmester core, Bonneville

basin, Utah (Fig. 1b). The mean aspartic acid D/L measured in four species of *Candona* heated at 140° differ by 5 to 9%, with *C. caudata* consistently racemizing slower than an unidentified species of the *C. Rawsoni* group at three time steps.

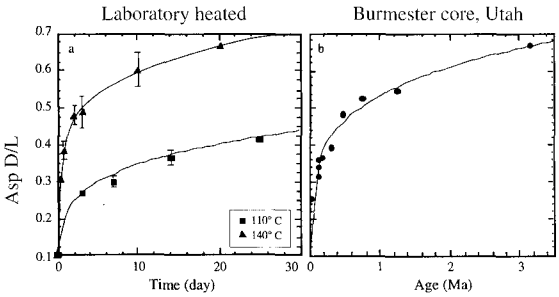

Fig. 1. Extent of aspartic acid racemization (Asp D/L) measured in: (a) laboratory-heated, late Holocene *Candona caudata* from Bear Lake (Utah/Idaho); and (b) ostracode *Candona* spp from Burmester core, Utah. Error bars in (b) are ± 1σ of 2 to 6 subsamples (typically 4 or 5), each comprising 0.1 to 0.2 mg of ostracode valves and each analyzed twice. Ages for core samples are based on tephrochronology and magnetic stratigraphy of Williams (1994); samples provided by R. Thompson and J. Oviatt.

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Degradative history of amino acids in sediments underlying oxic and suboxic waters of the Pacific coast of Mexico

An oxygen-deficient water mass currently impinges along the coast of the eastern tropical north Pacific continental margin. Sediments deposited within this oxygen-deficient environment (oxygen not measurable in the bottom waters by Winkler titration) typically have organic matter contents that are elevated over similar sediments deposited under more oxic conditions. We evaluated the amino acid content and composition of sediments from a transect off the Mexican coast near Mazatlan, as well as a gravity core taken in the center of the current oxygen-deficient zone. Organic matter contents in these cores range from 1–11% OC and amino acid contents range from 8–24 mg AA/100 mg OC. Amino acid contents (either carbon- or mass-normalized) are inversely related to oxygen content of the bottom water and more strongly to the oxygen exposure time of the sediments (calculated as the quantity of time sedimentary organic matter is exposed to oxygen prior to burial). The positive correlations between carbon-normalized amino acid contents and overlying water oxygen content or oxygen exposure time suggests that amino acids undergo diagenesis at an accelerated rate relative to the bulk organic matter in the sediments.

Amino acid compositions also correlate with environmental parameters. Within the oxygen-deficient zone, amino acid contents are very similar to that of fresh plankton samples or fecal materials. This suggests that the amino acids present in these sediments have undergone relatively little diagenetic alteration. Conversely, the non-protein amino acids β–alanine, γ-aminobutyric acid and ornithine, which are not commonly found in planktonic sources, are present in elevated quantities in sediments having higher ambient oxygen concentrations or longer oxygen exposure times. Elevated quantities of non-protein amino acids in marine sediments result from diagenetic reactions, and thus indicate an increased relative level of diagenesis. Mole percentages of non-protein amino acids are low (< 2.5%) in sediments taken from within the oxygen-depleted zone, and higher (3–6 mole %) in sediments taken from areas with longer oxygen exposure times. These latter levels (3–6%) are typical of continental margin sediments that are not oxygen-deficient. The correlations of mole percent non-protein amino acids with ambient oxygen levels in overlying water masses or oxygen exposure times suggest that the amino acids present in sediments form under the oxygen-deficient waters have undergone less diagenesis than those deposited under more oxic conditions.

Similar trends are inferred in the gravity core sample taken from within the present-day oxygen-deficient zone. In sediments deposited during the Holocene, at which time oxygen-deficient waters are thought to have been continuously present, amino acid contents and compositions are consistently elevated and plankton-like in composition. Before the Holocene-Pleistocene transition, approximately 11,000 years ago, the amino acid contents and compositions were consistent with those observed under the present day oxic conditions (lower yields, altered compositions). These data, along with other (^{15}N isotopic compositions, organic carbon to mineral surface area ratios) suggest that the oxygen minimum zone was not present (or was not as intense) during the last glacial period.

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Chemical composition and bacterial utilization of dissolved protein in the Sargasso Sea

The chemical form and bacterial degradation of dissolved amino acids were investigated in surface and intermediate waters (0–900 m) in the northern Sargasso Sea. Dissolved free amino acid (DFAA), dissolved combined amino acid (DCAA) and protein (using the BCA assay) pools were measured and their utilization by bacteria monitored. In all waters, DCAA dominated over DFAA. In surface waters (< 150 m), DCAA : DFAA ratios were ~150 and in deep waters DCAA : DFAA averaged 350. DCAA in surface waters was largely identifiable as protein, but most of the DCAA at depth is not identifiable as protein. A kinetic approach was used to estimate protein and DCAA turnover. In surface waters, protein was the dominant form of organic nitrogen used to support bacterial growth, supporting 20–65% of the calculated bacterial N-demand. With increasing depth (150–900 m), less-labile forms of protein dominated microbial use of the DCAA pool, supporting an average of 40% of the observed bacterial growth. Within these deeper waters, free and combined amino acids did not meet the full bacterial N-demand and other, unmeasured nitrogen sources were required. Similar to trends in utilization, kinetically-estimated maximum uptake velocities (Vmax) for fresh protein (RuBPcarboxylase) and slowly

degraded protein (glucosylated RuBPcase) showed peaks at the surface and at depth, respectively. Thus, the forms and reactivities of amino acids in northern Sargasso Sea water change between the surface waters and the deeper waters. This may be due to either shifts in the relative importance of various biological processes that release amino acids to sea water, or may be due to non-biological processes in deeper waters. Regardless of cause, bacterial assemblages appear capable of utilizing the recalcitrant forms of protein that dominate the protein pool at depth.

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Amino acid racemization rates in degraded and non-degraded agricultural soils

It is recognised that the D/L ratios used in the AAR dating technique are calculated from the D- and L-forms of amino acids derived from mixtures of many types of peptides of differing chain lengths. Variations in these parameters, as well as age, determine the degree of racemization measured. Interpretation of the data can therefore be complex even with single entities such as shell, coral or bone. In soils clearly the complexity and therefore the potential usefulness of the technique for dating purposes would seem to be reduced further due to the presence of both living and dead microorganisms, as well as plant material. In addition, in most soils organic matter is continually being added ensuring that, at least in the top soil, most residual organic material is modern and has low D/L values.

Laboratory heating experiments on soils used for long-term continuous crop rotations of wheat/fallow (WF) and permanent pasture (PP) showed a dramatic difference in rate kinetics. The WF soil that had been „mined" for nutrients for 60 years generally showed a much higher apparent racemization rate than the PP soil (40 years) where loss of nutrients was minimal due to the ready on-going supply from the pasture material. Treatment of the soil residues remaining after traditional HCl hydrolysis (and removal of the solute – HCl fraction) with HF released more peptide material (up to 10%). D/L ratios of amino acids in this „entrapped" fraction determined following further HCl hydrolysis (HF fraction) were lower than those from the HCl fraction. The PP soil contained mainly modern organic matter and gave very low D/L ratios, even when the soil had been heated. This applied both to the HCl and HF fractions. The WF soil that had been continually degraded due to the continual „mining" of nutrients produced high D/L values.

These results indicate a close relationship between D/L ratio and accessibility to peptides that can rapidly be hydrolysed to smaller units and in the process encourage rapid racemization of the amino acids formed. Soil particles that have been „mined" for nutrients from entrapped organic matter seem to allow for easier access to the protein and peptide moieties at the same time producing more rapid racemization. In „mined" WF soil the HCl fraction being more easily accessible is more easily broken into small chain peptide fragments during heating than the more protected HF fraction.

Valine, allo/isoleucine and alanine had similar patterns of D/L ratio change with time when the soil was held at 140 °C. This was for both the HCl and HF fractions in permanent pasture and wheat/fallow soils, directly reflecting the above hypothesis. The atypical nature of aspartic acid, however, as observed previously reflects preferential rapid loss of the more highly racemized low molecular weight peptide species leaving behind less racemized material – hence producing a reversal of racemization.

We suggest that the AAR technique used in this way provides a method of differentiating between degraded and non-degraded soils with implications for farm management strategies.

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Fate of dissolved protein in the pelagic zone of marine ecosystems

The input of protein into the upper layer of oceans is potentially very large since protein comprises over 50% of planktonic organisms. Recent evidence suggests that at least some of this material may be preserved in the dissolved organic matter pool and may comprise a large fraction of dissolved organic nitrogen (DON) in the oceans. This presentation will review the current understanding of what happens to protein as it is released into seawater. It is well known that protein must be hydrolyzed by extracellular proteases to free amino acids and oligopeptides roughly smaller than pentamers which can be transported into microbes. Some of this low molecular weight material is released and used by other bacteria. Our experiments and that of others have shown that „soluble" protein, i. e. not associated with cell membranes, is used readily by heterotrophic bacteria and sometimes can support much bacterial growth. In contrast, it appears that degradation of „insoluble" or membrane-associated protein is slower than that of soluble protein, for reasons that are still not entirely clear. Membrane-associated protein may be simply less available to proteases because of steric hindrance by lipids and other membrane or cell wall material. If so, the steric hindrance mechanism alone would not explain the apparent preservation of membrane proteins in seawater. Particularly intriguing is the work of E. Tanoue and colleagues who found a bacterial membrane protein (a porin) widely distributed in the surface and deep ocean. We hypothesize that membrane proteins are generally found in liposome-like particles consisting of membranes surrounding an aqueous core. Protein inside these liposomes are degraded more slowly than freely dissolved protein. In addition to delaying microbial attack, liposomes may be „reaction chambers" and make possible abiotic reactions that require high concentrations not present in the dissolved pool. Abiotic reactions that modify proteins, such as condensation and Amadori rearrangement to form melanoidins, can inhibit degradation by bacteria. The formation of these liposomes and subsequent abiotic modifications of liposome protein may be the first step in the formation of refractory DON found in the oceans.

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Stratigraphic analysis of White Paintings Shelter, Botswana utilizing isoleucine epimerization in ratite eggshell

White Paintings Shelter, Botswana is one of the few sites in sub-Saharan Africa which provides evidence for very early (ca. 30,000 yrs bp) use of fishing technology. Numerous examples of both single and double barbed harpoons have been discovered at this rock shelter. Isoleucine epimerization analysis has been combined with radiocarbon dating of ostrich eggshell to provide both an absolute chronology as well as a determination of the extent of age-mixing within each stratigraphic level at this site.

Allo-isoleucine/Isoleucine (Alle/Ile) ratios have been ob-

tained for more than 60 individual fossil ostrich eggshell fragments. Radiocarbon determinations were made on eight of these samples. The epimerization values correlate well with the radiocarbon ages. Although this site was originally thought to consist of a single component, the AIle/Ile ratios cluster around three different sets of values, suggesting three different archaeological horizons. The radiocarbon determinations have provided the following ages for these 3 periods: Historic, 3.5–3.9 ky, and 28.5–33.5 ky (the harpoon bearing levels).

The distribution of the AIle/Ile values within each excavation square also suggest that stratigraphic mixing occurred within the upper levels at this site.

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A review of the discovery of extraterrestrial amino acids in the Murchison meteorite

The finding of amino acids in meteorites has always excited scientific imaginations, sometimes leading to observations suggesting evidence for extraterrestrial life; however, results of meteorite investigations before 1969 often could be explained in terms of terrestrial contamination. But in 1969 the Murchison meteorite fell in southeastern Australia, and this event changed the course of studies in organic cosmochemistry. Samples from this meteorite provided material for the first unambiguous identification of extraterrestrial amino acids. Acid-hydrolyzed water extracts were examined in 1970 by ion exchange chromatography, gas chromatography, and combined gas chromatography-mass spectrometry. These procedures, developed in preparation for the analyses of returned lunar samples from the Apollo program, first established unequivocally the presence of six amino acids (glycine, alanine, valine, proline, aspartic acid, and glutamic acid), commonly found in protein of living systems along with twelve non-protein amino acids (N-methylglycine, β-alanine, N-methylalanine, N-ethylglycine, α-aminoisobutyric acid, α-amino-*n*-butyric acid, β-aminoisobutyric acid, β-amino-*n*-butyric acid, isovaline, norvaline, and pipecolic acid).

The presence in this meteorite of all the amino acid structural isomers with two and three carbon atoms and with all but two of the isomers with four carbon atoms strongly suggested a mixture created by random, abiotic syntheses. Later work by others showed that all amino acid structural isomers with two to seven carbon atoms are present, lending further support to the suggestion of random syntheses. Each of the amino acids with an asymmetric centre and whose diastereoisomeric derivatives could be separated by the procedures available at the time (alanine, valine, proline, aspartic acid, glutamic acid, α-amino-*n*-butyric acid, norvaline, β-aminoisobutyric acid, and pipecolic acid) had approximately equal amounts of D and L isomers. Recent analyses in 1997 of portions of the same meteorite sample analyzed in 1970 have confirmed the racemic nature of the stereoisomers, although others have now reported non-racemic mixtures of amino acids in other samples of the Murchison meteorite. The distribution of structural isomers and stereoisomers of the meteorite amino acids first analyzed suggest that they are products of extraterrestrial, abiotic, random syntheses. These discoveries provided a relatively uncomplicated basis for understanding cosmochemical evolution; in contrast, the later results showing non-racemic mixtures of amino acids in some samples of the meteorite require much more complex explanations.

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Stable carbon and nitrogen isotopes of individual amino acids: Clues to origin and history

A significant challenge in amino acid geochemistry is the identification of potential sources of the amino acids in a fossil or other geological material. This identification could include the authentication of the amino acids presence in the fossil since the time of deposition, or the contribution of contaminating amino acids which may have been added to the fossil through contact with the environment. The only mechanism presently available for distinguishing separate sources of chemically identical compounds is through the stable isotope characterization of the component. Initially, through the development of preparative scale liquid chromatographic techniques, it was possible to establish that there existed little fractionation during racemization of amino acids under mild temperature regimes. Furthermore, amino acids exhibited isotopic compositions that reflected biochemical pathways during synthesis, with products of the reactions being substantially depleted in the heavy isotope. More recent advancement in the coupling of a gas chromatograph, through a combustion interface, to an isotope ratio mass spectrometer (GC/C/IRMS) have allowed for the analysis of nanomol levels of individual amino acids in fairly complex mixtures for either their carbon or nitrogen isotope compositions. Additionally, this novel system has allowed for the isotopic characterization of individual amino acid stereoisomers, which are separated using a chiral phase.

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Aspartic acid racemization in arctic marine bivalves: Improved age resolution of Quaternary glacial and sea-level histories at Brøggerhalvøya and Kapp Ekholm, Western Svalbard

D/L Aspartic acid ratios in *Hiatella arctica* and *Mya truncata* provide increased age resolution for glacial and raised marine deposits related to fluctuations of the NW Barents Ice Sheet. Fast-racemizing Asp provides greater resolution [1] for Quaternary deposits at high latitudes relative to D-alloIsoleucine /L-Isoleucine (A/I). Total D/L Asp ratios were quantified [2] with a Reverse Phase Liquid Chromatograph (hydrolysis: 110 °C for 6 hr). Free D/L Asp results are pending.

At Kapp Ekholm, four deglacial emergence cycles are associated with shallow marine sand of Formations B, D, F, and H. Mangerud and Svendsen [3] correlated Fm. B with the last interglacial, Fms. D and F with early and middle Weichselian interstadials, and Fm. H with isostatic emergence 11–9 ka following late Weichselian advance across the site. Total A/I ratios were important for correlating among sections, for age control, and for paleotemperature considerations relating to duration of ice cover. However, variation about the mean A/I ratio for each Fm. is high (average c. v. = 17%), and results for Fms. B and D overlap at ± 1 sigma (Table 1). In contrast, variation about the mean D/L Asp ratio for each Fm. is low (average c. v. = 4%), and D/L Asp results for Fms. B and D are distinct at ± 1 sigma. D/L Asp better separates the deposits into age groups, and more clearly identifies periods of racemization associated with marine submergence or ice cover (warm relative to annual subaerial temperatures ≤ –5 °C).

At Brøggerhalvøya, at least five deglacial emergence cycles are preserved [1,4], termed Episodes H, D, C, B, and A. The oldest, Ep. H, is early or middle Pleistocene in age. Ep. C is correlated to the last interglacial, and Ep. A is tied to isostatic uplift following the Late Weichselian. We reanalyzed 111 shells from 15 collections for both A/I and D/L Asp ratios (Table 1). The latter displayed significant variation for EP. A, but variation about mean ratios is less for D/L Asp (7%) than for A/I (11%). In particular, D/L Asp better separates EP. C from Ep. B. Both measures indicate that Brøggerhalvøya has experienced lower temperatures since the last interglacial period than has Kapp Ekholm, apparently due to greater subaerial exposure. Pyrolysis studies underway for both Total and Free D/L Asp will quantify temperature kinetics, and will clarify the temperature histories of both sites.

Table 1. A/I and D/L aspartic acid ratios for two sites on western Svalbard

Unit	A/I	D/L Asp
	Kapp Ekholm *M. t.*	
Fm. H	0.015 ± 0.003 (8)	0.123 ± 0.007 (10)
Fm. F.	0.026 ± 0.004 (26)	0.205 ± 0.008 (16)
Fm. D	0.063 ± 0.013 (6)	0.279 ± 0.009 (11)
Fm. B	0.069 ± 0.008 (14)	0.297 ± 0.006 (16)
	Brøggerhalvøya *M. t.* and *H. a.*	
Ep. A	0.017 ± 0.002 (17)	0.156 ± 0.033 (17)
Ep. B	0.027 ± 0.004 (33)	0.183 ± 0.011 (33)
Ep. C	0.038 ± 0.006 (22)	0.213 ± 0.009 (22)
Ep. D	0.063 (14)	0.283 ± 0.007 (19)
Ep. H	0.104 ± 0.001 (20)	0.333 ± 0.005 (20)

Values are mean ± 1 s. d. of collection averages (number of shells analyzed).

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Amino acid enantiomeric and isotopic signatures of oceanic dissolved organic matter: Evidence for remnant microbial cell wall material as major component of high molecular-weight dissolved organic nitrogen in the sea

The majority of dissolved organic nitrogen (DON) in the sea is relatively refractory, remineralized on time scales slow enough to allow both the advected export of fixed nitrogen from the euphotic zone (Jackson and Williams, 1985), as well as persistence in the deep sea over multiple ocean mixing cycles (Hedges, 1992). The biochemical identity as well as the processes controlling the long-term persistence of this enormous fixed nitrogen reservoir remain largely unknown. Recent work with high molecular-weight fraction of DON has indicated that while only a minor portion can be identified as hydrolyzable amino acid (McCarthy et al., 1996), nevertheless essentially all is functionally composed of amide nitrogen (McCarthy et al., 1997). This comparison suggests that much of this material may be hydrolysis resistant, of substantial age, or composed of non-proteinaceous amide biochemicals.

We report here new evidence based on amino acid enantiomeric ratios in high-molecular weight DON suggesting that peptidoglycan remnants directly constitute a considerable portion of this nitrogen pool. High D/L ratios of Ala, Glu, Asp, and Ser were found to be extremely consistent in both surface and deep ocean DON isolates from three ocean basins. Initial molecular-level C and N isotopic signatures for similar oceanic samples have also been conducted, and generally support the enantiomeric data. These results indicate that enantiomeric and isotopic signatures of amino acids may prove to be powerful new tools for tracing sources of nitrogenous organic matter within the oceanic water column. Overall, our observations suggest that processes which directly introduce relatively refractory microbial cell wall components into sea water, and not age or fundamental abiotic structural alterations, are central to the longer-term cycling of organic nitrogen in the sea.

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A model for $\delta^{15}N$ changes caused by weaning in humans

Detection of weaning in the archaeological and palaeoanthropological record is an important issue in assessing patterns of fertility and fecundity of past populations. In the past most attempts to obtain information on this have used physical examination of skeletal material and making deductions from infant mortality patterns, or enamel hypoplasias. However, these have recently been shown not to be reliable indicators of weaning practices (Katzenberg et al., 1996), and various workers are now turning to nitrogen isotope measurements to investigate the trophic level shift associated with weaning.

A recent paper by Schurr (1997) has suggested a mathematical model for isotopic changes in infants during weaning, which is an improvement on previous purely descriptive models. However, in this presentation I develop a critique of this model, showing that the two saturating exponential functions used by Schurr are appropriate only for modelling an instantaneous switch in diet whilst maintaining a constant body weight. Weaning is far from instantaneous, and infancy is the time of most rapid growth in humans (relative to body weight), which suggests that a more realistic model could be sought. This paper develops such a model, taking account of the effect of changing body mass, and an extended weaning period on the trajectory of isotopic change within juveniles.

The model includes (a) typical growth patterns for human infants, based on longitudinal studies of child growth, and (b) a function which represents a gradual shift from sole dependence on the mother's milk to complete weaning. These are incorporated into the isotope mass balance equations, which are then solved numerically. Comparison with longitudinal isotopic data from modern populations allows estimates of the

age for start of weaning and its duration. Comparison with archaeological population data gives less precise estimates of the mean values of these parameters in the population.

In principle this model can be extended to other isotopic and elemental indicators of weaning, or other age-dependent dietary change. An indication of the possibilities will be given.

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Quaternary coastal aminostratigraphy – Australian data in a global context

Since the early 1980s amino acid racemization (epimerisation) reactions have been applied to the dating of Quaternary marginal marine successions in Australia. Deposits relating to OI Stage 9 or younger have received the greatest attention and in particular, OI Substage 5e, and Stages 7, 3, 2 and 1. Molluscs of Plio-Pleistocene age from the Perth Basin, Western Australia and from several deposits in Tasmania and the Bass Strait Islands have also been studied. The relative tectonic stability of the Australian continent, conferred by its intra-plate setting and extensive distribution of Precambrian Cratons, accounts for the general absence of emergent marine shell beds of OI Substages 5c (ca. 105 ka) or 5a (ca. 82 ka) amenable to dating.

The most reliable results are from replicate analyses of the hinge region of well-preserved and diagenetically, relatively unmodified (e. g. generally greater than 98% primary aragonite present), fossil bivalve moluscs from well-buried contexts (i. e. >1 m). Analyses using gas chromatography have permitted the quantitation of a range of amino acid D-L isomers that include alanine (ALA), aspartic acid (ASP), glutamic acid (GLU), leucine (LEU), phenylalanine (PHE), proline (PRO), valine (VAL) and with optimum column performance, isoleucine (ALLO/ISO). Results for the amino acids leucine and isoleucine provide a framework to compare with data sets published from other countries.

Mollusc species that have been analysed by amino acid racemization principally include the bivalves *Anadara trapezia, Katelysia* sp., *Donax (Plebidonax) deltoides, Glacymeris* sp., *Mactra australis, Fulvia tenuicostata,* and *Pecten* sp., in view of their common occurrence in marginal marine successions in Australia. Opercula of the gastropod *Turbo undulatus* from archaeological and geological contexts have also proved an important medium for dating.

Calibration of Australian amino acid racemization data by direct comparison with other methods (e. g. radiocarbon, uranium-series disequilibrium, luminescence, and electron spin resonance dating) and selection of suitable samples within a set of stringent criteria (e. g. depth of burial, preservation state, genus and hence general racemization rate as well as reproducibility of measurements) provides a preliminary framework to evaluate Australian amino acid data in a global context. Australian data reaffirm the strong temperature dependence of amino acid racemization, as reflected in a temperature (latitude) plot of the extent of racemization in last interglacial molluscs (OI Substage 5e). The data also compare favourably with fossils of equivalent age from a range of northern hemisphere sites, providing additional evidence for the potential application of amino acid racemization (epimerisation) as a tool to complement other methods in global correlation.

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Protein preservation during early diagenesis in marine waters and sediments

The fate of protein was followed during phytoplankton decay in flow-through laboratory incubations and with depth in the sediments of Mangrove Lake, Bermuda, which recieves principally algal input. Two-dimensional electrophoresis and amino acid analysis suggest that while most proteins are degraded during early diagenesis, a significant fraction is preserved. Although >92% of the initial particulate nitrogen was lost during the oxic water column decay of the diatom *Thalassiosira weissflogii,* proteins remained a significant fraction of total amino acids and of the residual nitrogen (83% and 48%, respectively). Hydrolyzable amino acids associated with a >2 kDa molecular weight fraction accounted for 78 to 98% of total amino acids and 56 to 63% of total nitrogen in Mangrove Lake sediments. Two-dimensional electrophoresis revealed the preservation of few discrete, acidic protein species, some of which were common to both water column and sediment samples. Amino acid analysis of low (3.5–18 kDa), mid (18–43 kDa), and high (43–200 kDa) molecular weight protein fractions observed a shift toward high molecular weight proteinaceous material after extensive algal decay and with increasing sediment depth, compared to fresh algal material or recently deposited organic matter. These results suggest that small amounts of discrete proteins survive early diagenesis, with most proteins retained in the residual organic matter as extensively modified and cross-linked, acidic species.

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Separation, isolation and isotopic analysis of individual amino acids from archaeological bone collagen: a new method using RP-HPLC

This paper presents a new method for the separation, isolation and isotopic analysis of individual amino acids from proteins. The technique and its constituent steps are discussed, then isotopic analyses of amino acids from several samples of bone collagen from the Late Roman site of Poundbury, Dorset, UK are presented.

The applications of the method are discussed, as well as some advantages of this technique relative to other methods. Although developed for use with archaeological bone collagen, the technique is equally applicable to other proteinaceous materials. The use of reversed-phase HPLC avoids problems of isotopic fractionation inherent in using ion exchange HPLC. Amino acids are separated preparatively allowing both carbon and nitrogen isotopic values to be measured on a single sample using cf-irms. Since amino acids are isotopically analysed in an underivatized form (unlike GC-C-IRMS), the method also presents the possibility of collecting the CO_2 generated during cf-irms: this would allow the subsequent dating by ^{14}C-AMS of individual amino acids isolated from archaeological samples.

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Amino acid geochronology of Delaware Bay Quaternary coastal terrace deposits: Morie Pit, Southern New Jersey, U.S.A.

A chronologic sequence of deposition for Quaternary sea-level highstand deposits on the northern margin of Delaware Bay was derived through an amino acid racemization (AAR) analysis of fossil molluscs from dredge spoil and vibracores collected in the Morie Company sand and gravel pit on the western flank of the Maurice River in southern New Jersey. AAR analyses of total amino acid content were performed on 20 *Mercenaria mercenaria* samples after acid hydrolysis, using a high-pressure liquid chromatograph. Data are reported for D-alloisoleucine/L-isoleucine (A/I). Two populations of fossil *Mercenaria* were identified, above and below a prominent unconformity, interpreted to be a transgressive ravinement surface, recognized in ground penetrating radar profiles, vibracores and split-spoon cores. The two populations yielded A/I mean values of 0.367 and 0.365, above and below the unconformity, respectively.

Age estimates for the fossil *Mercenaria* were modeled using calibration A/I values of 0.24, 0.22 and 0.20 and a calibration age of 100 ka, from data collected on marine oxygen isotope substage 5e-equivalent deposits exposed in Gomez Pit, Virginia (Mirecki et al., 1995). A temperature correction of 8%/°C (Kaufman and Brigham-Grette, 1993) was applied to age estimates to compensate for the 2 °C difference in current mean annual temperature between the latitudes of southeastern Virginia and southern New Jersey. The three parabolic kinetic curve models suggest correlation of the Morie Pit deposits with isotope highstand stages 7 to 9, 9 to 11 or 9 to 13. While not resolvable geochemically, the two *Mercenaria* populations are interpreted to be chronologically equivalent to oxygen isotope stages 9 and 11, above and below the prominent unconformity, respectively, on the basis of stratigraphic position and elevation in relation to a nearby coastal escarpment of probable substage 5e (minumum) age.

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New perspectives on ancient proteins: The application of MALDI-MS for characterization of modern and ancient osteocalcin protein sequences

We have begun to evaluate diagenetic changes in the structure and preservational potential of the bone protein, osteocalcin (OC), using matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). This approach is rapid, can identify very small quantities of protein (pmoles), does not exhibit interferences from most salts or solvents used for protein purification and is unaffected by amino-terminus blockage. We have purified OC from 0.5 M EDTA and 1 N HCl extracts from crude extracts of modern and ancient bones by demineralization, dialysis, and reversed-phase HPLC and subjected the extracts to SDS-PAGE, radioimmunoassay (RIA) and MALDI-MS. The sequence of modern bovine osteocalcin was obtained by peptide mass mapping and by direct MS sequencing using a novel peptide derivatization approach that produces a mass spectra containing easily interpretable fragment peaks indicative of amino acid sequence. Analysis of ancient OC by SDS-PAGE produces a band near 7 kDa consistent with that observed for modern OC standards. The difference between the observed and expected molecular weight of OC (expected = 5200 to 5900 kDa) is due to polyelectrolytic effects. The presence of OC in these bones and other fossils as old as 450,000 years was confirmed by RIA in diminishingly small quantities (less than 0.2 ng/mg). In initial studies we have isolated OC from fossil material and have observed peaks with molecular masses consistent with OC from several 800 year old to 53,000 year old bones.

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Separation of intra $\delta^{18}O$ stage 5 glacial and sea level events in the Bering strait region: HPLC vs. GC aminostratigraphy

The stratigraphy and geochronology of Pliocene and Pleistocene high sea level events studied in coastal deposits on both sides of the Bering and Chukchi seas over the last 18 years has been dramatically strengthened by the use of aminostratigraphy on enclosed fossil mollusks (Kaufman and Brigham-Grette, 1993; Brigham-Grette et al, in press). Traditional use of HPLC to measure the extent of L-isoleucine epimerization in genera such as *Mya, Hiatella, Macoma,* and *Astarte* has allowed several workers to recognize at least 6 Pre-Holocene high sea level sequences. In places where marine deposits are found interbedded with glaciogenic sediments, aminostratigraphy has allowed reinterpretation of the timing and extent of glacial vs. interglacial periods. Limiting the usefulness of stratigraphic applications, however, is the fact that at sustained ground temperatures well below 0 °C, the rate of isoleucine epimerization to alloisoleucine is impeded to the point that we cannot clearly distinguish deposits less than roughly 50 ka–100 ka years apart. In particular at a few sites, we seem to have stratigraphic evidence for changes in eustatic sea level as well as the rapid buildup of local mountain glaciers that may record a complex series of paleoclimatic events within marine isotope stage 5 (132,000 to 72,000 years BP). To improve age resolution and answer these new science questions, it is now to our advantage to utilize faster stereochemical reactions such as the racemization of aspartic and leucine to distinguish depositional events that differ in age by much less than 50 ka years. We are particularly interested in determining whether the sedimentological evidence at two sites along the Russian coast are recording climatic and sea level changes at the Substage 5e/5d boundary or at the Substage 5a/4 boundary within the Bering Land Bridge.

To test our hypothesis concerning Stage 5 and early Wisconsin events, we have analyzed using HPLC a series of bivalves from sections interpreted to represent the last interglacial substage 5e (Pelukian/Val'katlen Transgression, 120 ka to 132 ka) and stratigraphically younger samples thought to record substage 5a (Simpsonian transgression, 72 ka–85 ka) from both the Alaskan and Russian coast of the Bering strait region. The results from these deposits show virtually no separation of stratigraphic events. We now have begun analyses of the same samples for D/L aspartic acid and D/L leucine using GC separations and expect a clear separation of at least one high sea level event around 80 ka. Published results (Goodfriend et al., 1996) are encouraging as they demonstrate nearly an order of magnitude difference in D/L ratios between modern samples and those 125 ka years old even, at 71° N where modern ground temperatures are −11° C.

Further analysis of mollusks from chronosequences found at other high latitude sites, e. g. those on Ellesmere Island and across the Russian Arctic, are planned for study to further enhance our ability to evaluate oceanic/cryospheric/atmospheric interactions at times of paleoclimatic transitions.

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Carbon and nitrogen isotopic variability of different protein fractions from modern and fossil shells

Molecular geochemical characteristics from individual shell matrix proteins from modern organisms can provide a baseline for comparing origin and history of fossil organic material and for potentially understanding the function of these components in a modern system.

Shell matrix proteins from modern Mercenaria and Polinices were isolated and characterized by reverse phase HPLC. Individual proteins were then analyzed for carbon and nitrogen isotopic values. The carbon isotopic composition of amino acids resulting from the hydrolysis of the proteins were also determined.

At the bulk protein level, we observed significant isotopic differences between hydrophobic and hydrophilic proteins of modern shells. Variability was also expressed at the molecular level. Differences in $\delta^{13}C$ values of individual amino acids between hydrophilic and hydrophobic protein fractions were as large as 10‰. Such differences could be related to the functional attributes of the proteins. Consequently, we are currently investigating the relationship between biomineralization and environmental conditions and isotopic composition of individual proteins. This information would be particularly useful in understanding molecular biogeochemistry in the fossil record.

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Patterns of intramolecular isotopic heterogeneity within the amino acids of animals and plants

The existence of large intramolecular variations in the carbon isotopic composition of amino acids has been known since the pioneering work of Abelson and Hoering at the Carnegie Institution in 1961. They found that the carboxyl carbons of amino acids isolated from photosynthetic algal cultures were enriched by 10.5 ± 2.9‰ relative to the other carbon atoms of the molecules. Amino acids isolated from heterotrophic cultures displayed little or no ^{13}C enrichment of their carboxyl carbons.

We have surveyed the $\delta^{13}C$ of the amino acid carboxyl carbons of a variety of field-collected autotrophic and heterotrophic organisms. In general, autotrophs displayed a significant ^{13}C enrichment in the carboxyl carbons of their amino acids, although the magnitude of the enrichment was about one half that observed by Abelson and Hoering for algal cultures. Multicellular heterotrophs did not conform to the pattern observed for unicellular cultures. Amino acid isotopic heterogeneity for heterotrophs was not significantly different than that measured for autotrophs. The biochemical basis for the results will be discussed, and potential applications of the technique to biogeochemistry will be presented.

P. A. St. John

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The amino acid analyzer in a suitcase: A high sensitivity ion exchange chromatograph for laboratory or field analyses of trace amino acids

The Model 2000 amino acid analyzer is the commercial embodiment of P. E. Hare's legendary „Analyzer in a Suitcase". This tough, compact little analyzer features a short, narrow-bore cation exchange column and gas-pressure delivery of ophthalaldehyde fluorophore. The design resulted from Dr. Hare's desire to produce an ion exchange chromatograph with high speed and sensitivity that could be taken into the field for on-site analyses. Model 2000's have seen duty in diverse locations around the globe, from Spitsbergen to the Amazon Basin. Today they are primarily found in the lab, dwarfed by their ancillary autosamplers and data processors, producing results that continue to reaffirm the genius of the designer.

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Assessment of diet composition through stable isotopic analysis of collagen amino acids from bone cell cultures

Ancient diets can be examined using the stable isotopic composition of collagen from fossil bones. Current models for how isotopic values are translated from diet to bone protein have been previously investigated using whole animal feeding experiments, but many questions remain unanswered. Experiments therefore have been designed to examine the stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$) in collagen produced by osteoblastic cell cultures. Cultured collagen provides a clearer understanding at the molecular level of isotopic fractionation during the biosynthesis of collagen than is possible with whole animal studies. In addition, this *in vitro* system allows easy manipulation and control of diet variables through the culture growth media. Culture media has been formulated to address the specific question of the relative contributions of diet protein and carbohydrate to bone protein synthesis. Furthermore, isotope measurements on the individual amino acids are expected to allow discrimination in more complex dietary situations as well analysis of older and more degraded fossil bones.

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Photoinduction of UV-absorbing mycosporine-like amino acids in marine phytoplankton

Production of ultraviolet (UV)-absorbing compounds may improve the ability of some algal populations to acclimate to variations in the radiation environment. High fluence PAR (photosynthetically active radiation, 400–700 nm), UV-A (320–400 nm), and UV-B (280–320 nm) radiation were tested for their ability to stimulate the production of UV-absorbing mycosporine-like amino acids (MAAs) in six diverse species of marine phytoplankton. *Dunaliella tertiolecta, Thalassiosira, weissflogii, Pyramimonas parkeae, Pavlova gyrans* and *Isochrysis* sp. were grown under 1) low fluence

PAR (LL, 25–75 μmol photons $m^{-2}s^{-1}$), 2) high fluence PAR (HL, 255–290 μmol photons $m^{-2}s^{-1}$), 3) PAR + UV-A (240–268 μmol photons $m^{-2}s^{-1}$ and 4910 mW m^{-2}, respectively) and 4) PAR + UV-A + UV-B (103, 206, or 304 mW m^{-2} weighted UV-B).

In vivo absorption spectra indicated that only *Pavlova gyrans* has a pronounced UV maximum. Of the three MAA compounds detected in *P. gyrans,* only one with peak absorption in the UV-A range was photoinducible. The other species examined exhibited relatively small *in vivo* UV absorption peaks. In these species only one MAA was detected, a compound with peak absorption in the UV-B range and limited inducibility. UV-B radiation more effectively induced MAAs than UV-A or HL in 4 of the 6 species. In *P. gyrans* large increases in the concentration of the inducible MAA were obtained with HL and with UV-A + UV-B radiation. Relative to LL cells, UV-B-exposed *P. gyrans* exhibited a 145-fold MAA increase, accompanied by an 11-fold increase in the *in vivo* UV absorption. In all other species HL had minimal or no effect on MAA production. UV-A radiation effectively increased the chl *a*-specific MAA content in *Isochrysis* sp. (77%), *T. weissflogii* (73%), and *P. parkeae* (43%), and UV-B supplementation increased it by a further 141% in *Isochrysis* sp. and 95% in *P. parkeae.* On a cell volume basis, UV-B also approximately doubled the MAA concentration in the latter two species. We conclude that although MAAs may be commonly present in phytoplankton cells, an ability to produce significant amounts of these compounds through photoinduction is limited to certain species or taxa.

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Aspartic acid racemization in teeth from late Holocene burial caves in Jerusalem

Amino acid racemization reactions may, in principle, be used for dating of teeth, if the tooth proteins are well preserved and either the effective average temperature is known or some other means exists to calibrate the rate of racemization. In this study, we evaluate the potential and applicability of aspartic acid racemization dating of human tooth dentine from archaeologically-dated Jewish burial caves in Jerusalem. Based on associated cultural artefacts, these burials date primarily to the latter half of the 7th century BC, just prior to the Babylonian conquest and destruction of the First Temple, with possible reuse of the burial caves during the Second Temple period, ca. 100 BC [1]. Several milligrams of dentine of teeth of various types and ages (at burial) were analyzed by gas chromatography of N-TFAA isopropyl ester derivatives using a capillary Chirasil-val column. To obtain the net racemization since the time of burial, we subtracted the racemization induced by the preparation procedures and the *in vivo* racemization, as estimated from the age at burial (through analysis of abrasion) and the rate of *in vivo* racemization reported in the literature [2].

The net D/L aspartic acid values fall into two groups. Based on the reported kinetics of aspartic acid racemization [3], as determined by heating experiments and the *in vivo* rate, the expected rate of racemization at ambient temperatures could be calculated. When these values are used in the first-order kinetic equation with a rate corresponding to the effective average temperature measured inside the burial cave (18.0 °C), the estimated ages of these groups are consistent with the archaeologically-determined ages, viz. 2550–2650 yr BP and 2000–2050 yr BP. These results confirm the later reuse of the caves for burials. The close correspondence of the racemization ages to the archaeological ages indicates that racemization in these late Holocene teeth proceeds in a quantitatively predictable way.

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Preservation of biosignatures in museum herbarium collections

Throughout the museums of the world there exist extensive collections of plant specimens. These herbarium specimens are a potential reservoir of information of changes in climate and environments throughout the world over the past two centuries. These samples are well documented, dated and geographically constrained. In order to fully realize the usefulness of the information contained within herbarium specimens we examined plant specimens from the National Museum of Natural History. Although the macro-integrity of the plants survive storage and preservation under herbarium conditions, it is unknown whether organic material such as proteins survive during the storage of plant samples.

Concentrations of individual amino acids, C/N ratios and whole tissue $\delta^{13}C$ and $\delta^{15}N$ of a suite of plant samples, dating back to 1849, were determined. The stable carbon isotopic composition of plant tissue was progressively more enriched in ^{13}C with increasing age. Indeed, this pattern of enrichment closely followed observed changes in $\delta^{13}C$ of atmospheric CO_2 over a similar time period. C/N ratios showed little consistent changes over time indicating preservation of organic matter. Preservation was further confirmed by the detection of suites of amino acids in the oldest samples similar to those of modern plants. These results indicate that herbarium collections will be a useful source of molecular information of changes in plant ecology and environmental changes in the recent past.

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Aminostratigraphy of the Middle-Upper Pleistocene marine and continental deposits of the Cape of Huertas on the Mediterranean coast of Spain

The Pleistocene history of the Mediterranean coastal line of Spain has been controlled by consecutive sea level high standing/falling periods. This produced stepped terrace systems. In some places a red soil was developed on the top of the terrace deposits. In other cases loess-like silt and aeolian sand appeared on the topmost of the marine terraces. Because of neotectonics the real coastal evolution was much more complex than described above, acting as a local forcing controlling sedimentation and geomorphological evolution. Tyrrhenian Cycle (Last Interglaciar 5th oxygen isotopic stage or older) coastal deposits are characterized by abundant fossil mollusca that indicate Mediterranean Sea water warming. This fauna has been named the „Senegalian Complex".

According to different authors, Thyrrhenian evolution occurred in three different ways: (i) a single long still-standing sea level (ii) a slow but continuous sea level change, and (iii) two or three periods of rapid sea level change. In fact in the Balearic Islands Tyrrhenian deposits had recently been interpreted as result of ii) and iii), depending on the viewpoint of the author of the paper in question. The aim of this paper is to add information on the Pretyrrhenian and Tyrrhenian history of the Mediterranean coast of Spain, based on amino acid racemization analysis of faunal remains from the Cape of Huertas (C. H.) (Alicante).

The oldest Pleistocene marine deposits (terraces) from the C. H. out of the range of the $^{234}U/^{234}Th$ dating method > 350 ka, are placed 30 m a.s.l. and consist of biomictric algal limestone measuring 1.5 m in thickness. 38 samples of sea urchin spicules (Echinoidea), fragmenta indet. *Patella* sp., *Spondylus* sp., and *Glycymeris* sp. have been analysed. The average amino acid racemization ratios of 11 samples of *Glycymeris* sp., the most frequent Pelecypoda of the Mediterranean Quaternary record are as follows: A/I = 101.10%; LEU = 81.72%; and GLU = 65.39%. These marine deposits interfinged landwards with yellow silty (aeolian?) sediments containing land snail shells. Their amino acid racemization ratios (8 samples) do not differ significantly from those of the marine terrace fauna.

The higher Tyrrhenian terrace appears 2 m a.s.l. and consists of bioclastic sand and gravel measuring 2 m in thickness, at the top of this terrace aeolian sand with land snail shell appears. 95 samples of *Monodonta* sp., *Gibbula* sp., *Patella* sp., *Helix* sp. (from the topmost of the marine deposits strata), *Arca* sp., *Ostrea* sp., *Pecten* sp., *Venus* sp., *Spondylus* sp. and *Glycymeris* sp. have been analysed. The average amino acid racemization ratios from 15 samples of *Glycymeris* sp. are as follows: A/I = 54.65%; LEU = 37.86%; GLU = 48.30%. The amino acid racemization ratios in aeolian sand land snail shells do not differ significantly from those of the *Helix* sp. from the top strata of the marine terrace which has been $^{234}U/^{234}Th$ dated (91 ± 6 ka).

The lower Tyrrhenian terrace in the C. H. section is placed at the sea level covered during storms and high tides. It consists of bioclastic sand and gravel measuring 35 cm in thickness. 35 samples of *Monodonta* sp., *Ostrea* sp., *Pecten* sp., *Venus* sp. and *Glycymeris* sp. have been analysed. The amino acid racemization ratios from 11 samples of *Glycymeris* sp. are as follows: A/I = 42.35%; LEU = 35.80% and GLU = 36.71%.

It seems that two rapid sea level changes took place during the Tyrrhenian in the C. H. area.

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Aspartic acid racemization in the dentine of cave bears (*Ursus deningeri* von Reichenau and *Ursus spelaeus* Rosemüller-Heinrooth)

Cave bears inhabited Europe during Middle and Upper Pleistocene times. Because they were cave hibernants, thousands of bones and teeth were preserved, along with other mammal and even hominid remains („Sima de los Huesos"). *Ursus deningeri* was a typical representative of the Middle Pleistocene and *Ursus spelaeus* lived from the uppermost part of the Middle Pleistocene until the end of the Upper Pleistocene, colonizing both lowlands (coastal and continental) and highlands. In some cases it has been possible to establish subspecies on the basis of skeletal or dental perculiarities. In spite of the striking differences detected, our less creative colleagues have argued that both species (*U. deningeri* and *U. spelaeus*) and the subspecies were an extreme case of polytypism.

The Madrid School of Mines has a very large collection of European Pleistocene fossil bears from fifteen palaeontological localities in Spain, excavated by one of us (T. T.) since 1976. Dating cave bear populations seemed to be an interesting objective. Our first results are presented below.

A sample of five teeth was selected from each of four different localities, the selection being made on the basis of condition of conservation and age of death; the teeth of immature animals were rejected because they have hollow roots which might be an entry route for contamination. From each tooth an 80 milligram sample of dentine was obtained from the innermost part of the crown. After light attack with HCl the sample was dialyzed (Spectra/Por mnco 3500 membrane) to remove free amino acids (of lower racemization velocity) and the dialyzates were prepared according to the usual protocol. Analyses were carried out using a GC-HP 5890 with a 25 m long Chirasil L-Val column and NP detector.

Comparison of recent analytical data with some old results obtained in our laboratory some years ago, without previous sample dialysis, confirms the need to work with bounded amino acid fraction (dialyzate), our former results being erratic. We have also confirmed that *Ursus deningeri* and *Ursus spelaeus* are not only morphologically and morphometrically different species, but are in clearly different aminozones. The *ASP racemization averages* obtained are as follows:

4.2% *ASP r. a.* in two samples from a canine of recent *Ursus americanus* Pallas from USA. This is the racemization of the sample preparation method but a little additional amino acid racemization in the Museum deposit cannot be neglected. On living mollusca shells the induced sample preparation method amino acid racemization is 3%.

19.4% *ASP r. a.* of ten samples from teeth of *U. spelaeus* from two different caves: the Reguerillo Cave (20.3% *ASP r. a.*) in the central part of Spain and the Arrikrutz Cave (18.5% *ASP r. a.*) in the vicinity of the Atlantic Ocean shore line. *Ursus spelaeus*, the „true" cave bear, probably inhabited the Iberian Peninsula (the species border) during the last interglacial period (Eem).

39.0 *ASP r. a.* of ten samples from teeth of *U. deningeri* from two different caves: the „Sima de los Huesos" Cave (38.15.3% *ASP r. a.*) in the central part of Spain and the Santa Isabel Cave (39.87% *ASP r. a.*) near the Iberian Peninsula's Atlantic Ocean Coast. A 320 ka age was recently obtained from „Sima de los Huesos" bear remains (ESR and U-series).

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Racemization reconsidered: The potential use of the insoluble extracellular matrix of vertebrate calcified tissue

It has been over twenty-five years since bone was proposed, utilized, challenged and, by some, rejected as a tissue source for racemization studies. When used as a relative chronometer, whole bone aspartic acid racemization values often did not agree with the radiocarbon ages of the fossils, and a range of aspartic acid racemization values were documented as a function of molecular weight in the soluble protein of one bone by Hare and Kimber. In this study, the racemization of aspartic acid in whole bone is compared to that of well characterized collagen from both experimentally high temperature aged cow bone and in two fossil assemblages that range over thirty-five thousand years in time and were utilized previously in paleodietary investigations. As has been previously reported, the difference in both the experimental and fossil samples D/L Asp values in whole bone versus collagen is large. In the fossil bone collections, the

collagen D/L Asp values were much lower and more consistent than those observed in the whole bone from the same sample. Further, the D/L Asp values in the collagen from both the 40,000 year old faunal collagen and the much younger human bone collagen agrees well with the depositional history of the assemblages. It is proposed that the insoluble gel-like state of the collagen molecule imposes constraints on the racemization rates of the constituent amino acids, and as in the ratite eggshell, it is the physical state of the extracellular matrix that termines the utility of the tissue in racemization studies.

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Biogeochemistry and Paleoceanography: An amino acid perspective

Isotopic analysis of bulk organic matter in foraminifera have been used to estimate the isotopic composition of nutrients in ancient oceans. The isotopic compositions of the nutrients, however, are altered by processes associated with fixation and assimilation by phytoplankton and may not be accurately recorded by foraminifera. In addition, the isotopic composition of the total organic matter in foraminifera is a complex mixture of proteins, carbohydrates, and lipids, all of which have distinct isotopic compositions relative to that of the entire organism. Our knowledge of the synthesis and fate of organic matter within individual foraminifera is limited and may be important to the interpretation of this data.

Through the use of compound specific stable isotope analysis, it is possible to trace elements through complex flow paths as the $\delta^{15}N$ and $\delta^{13}C$ of a molecule records its biochemical history in the food chain and its signature may reveal evidence of the isotopic fractionation between the individual amino acids and the diet. Consequently, isotopic analysis of individual amino acids in foraminifera provides a method to assess the $\delta^{13}C$ and $\delta^{15}N$ of the protein consumed by foraminifera, provided that the isotopic effects associated with the incorporation of protein from the diet to the foraminifera can be assessed.

Previous studies have reported trends in the stable carbon and nitrogen isotopic compositions of individual amino acids which reflected known biosynthetic pathways. The majority of those studies focused on modern and fossil organisms. This work extends those findings with data on the amino acid isotopic compositions of foraminifera as well as bulk isotope analyses on the organic matter of the organisms and their diets.

Specimens of juvenile *Orbulina universa* and *Globigerina bulloides* were collected by SCUBA divers off the coast of Santa Catalina Island, California, during the summers of 1994 and 1996. These specimens were cultured in the laboratory in sea-flow tank under ambient light conditions. The foraminifera were grown in individual glass jars in filtered sea water and fed on a daily basis *Artemia* nauplii of a known stable carbon and nitrogen isotopic composition. In addition, specimens of *O. universa* were recovered from a sediment core, the amino acids were isolated and isotopically analyzed by GC/IRMS. The acidic amino acids were typically more enriched in ^{13}C, whereas the neutral amino acids were depleted, leucine being the most depleted in ^{13}C. The exception to this was glycine which was the most enriched in ^{13}C. These data are consistent with $\delta^{13}C$ values reported for the modern foraminifera as well as modern and fossil mollusk shells.

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The stability of collagen and keratin in museums: Some nitrogen-containing heterocycles found in bone, and the preservation of keratin in ethanol and formalin

Vertebrate collections are stored in museums under a variety of conditions, ranging from dried skins and skeletons on shelves or in drawers, to their storage in fluid preservatives such as 70% ethanol. We have decided to study the stability of these specimens in museums by examining the organic materials (primarily structural proteins) common to various taxa under simulated museum storage regimes.

To follow the effect of water (relative humidity, RH) on some of the organic reactions of bone deterioration, we conducted accelerated aging experiments, and heated bone beginning at 45% RH, and at 100% RH for 24 hours at 160 °C, 190 °C and 220 °C. At the end of the experiment, the 100% RH bone heated at 220 °C had lost about 50% of its weight, and the amino acids of the bone proteins had been altered to varying degrees. Aspartic acid, threonine and serine were partially or completely destroyed, while glutamic acid and glycine were the most stable. Hydrolysis, deamination, and decarboxylation reactions also occurred as evidenced by the production of organic acids and amines.

Heating bone beginning at 45% RH (dry) for the same time and temperatures produced a 20% loss of weight and less destruction of the protein as evidenced by amino acid patterns. Dry-heated bone also released a series of volatile, nitrogen-containing cyclic deterioration products under these conditions. These findings will be used to point to deterioration products that should be investigated in the breakdown of bone under varying museum treatment and storage conditions.

Another group of structural biopolymers is the keratins, a closely related family of chemically stable proteins composing mammalian hair, horn, hooves, quills and avian feather. Sheep hair (wool) has been much studied chemically because of its economic value. To our knowledge, no studies have been conducted on the long-term stability of the other keratins from a museum perspective. Feather and hair were heated dry, in 70% ethanol, and in 70% ethanol plus 1% formalin at 180 °C for periods of one and two days. Feather keratin was approximately 50% less stable in ethanol than was hair keratin, as evidenced by the amount of amino acids lost from the sample and appearing in the solution. Feather and hair, heated for the same periods of time under dry conditions, exhibited the same pattern of stability. Ethanol containing a small amount of formalin (to simulate residual fixative) produced even more deterioration than did ethanol alone. As a corollary, the amino acid patterns of fresh hair and feather from different species were found to be distinct, and indicative of their originating taxon.

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Improving the analysis of aspartic acid racemization in human dentine for use in age and death determinations

The racemization of aspartic acid in human dentine proteins has been used in both archaeological and forensic contexts to determine age at death. This approach appears quite successful in some forensic studies, having aged unidentified individuals to within ± 3 years of their actual age. The mechanisms of protein degradation in metabolically isolated

dentine are poorly understood but will include deamidation and isomerization reactions as well as epimerization. We have attempted to model this complex series of reactions and use the model to re-examine the data from these earlier studies.

The model has highlighted a number of inter-laboratory differences, notably in the amount of racemization induced during sample preparation. We have used the model to track down the causes of this variation. Notable findings have included the impact of the method of long-term storage and somewhat unexpectedly the extent of pulverisation of the dentine.

Further undocumented analytical problems have arisen using the standard forensic method of gas chromatography and separation on a chiral capillary column. The presence of an interfering peak was noted, which has not previously been reported in the forensic literature. This was identified as a monoacetylated derivative of hydroxyproline by mass-spectrometry. The peak must be well resolved from L-Asp for accurate DL Asp ratios, particularly from collagen-rich samples.

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The thermal environment of fossils: Measurement of modern ground temperatures

The use of diagenetic amino acid racemization (AAR) for chronostratigraphic purposes requires understanding not only a variety of geochemical reactions but also the thermal history of analyzed samples. Measurements of ground temperatures over extended periods at selected sample sites are a necessary component of this understanding, although modern thermal records cannot unambiguously characterize the entire temperature history, especially for Pleistocene samples or any samples with complex burial histories. Here we report results from a program of ground temperature observation for sites along the US Atlantic coastal plain from Maine to Florida, a region where contrasting thermal histories have been invoked as explanations of conflicting AAR age estimates. Data collection began in August, 1994 and now continues at 10 sites that range in mean annual air temperature from 7 to 23 degrees C. Data from 1994–1995 were obtained using Ambrose diffusion cells that yield a single effective temperature for the period of observation. Data from 1995 to the present have been obtained using electronic recorders. Both methods have yielded results from multiple depths (to 2 meters or more) and for a variety of exposure conditions. From these data, estimates of the modern effective ground temperature (MEGT) for AAR (assuming an activation energy of 27 kcal/mole) can be calculated. These effective ground temperatures can be compared with mean annual air temperatures (MAT), the parameter most frequently used in aminostratigraphic comparisons. Although the relations between MEGT and depth, or MEGT and MAT, are consistent with theory and regional trends, local variability in MEGT (explained by exposure and/or soil-moisture differences) can be large enough to introduce at least at 10% variability in AAR results for sites with the same MAT value. The consequences of annual freeze-thaw cycles for comparison of MAT and MEGT have implications for modelling of Quaternary temperature histories. Exposure variability appears to have a more significant impact on higher latitude sites than lower latitude sites.

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Mollusk shell formation

The pioneering studies of Gregoire and his colleagues in the 1950s revealed the complexity of mollusk shell formation, and in particular the organic matrix. The importance of the organic matrix proteins in controlling the mineralization processes was highlighted by the study of Hare in 1960, which showed that the proteins are different in shell layers composed of aragonite and calcite, and that they may therefore function in polymorph determination.

In the intervening years, we have learned that mollusk shells contain a relatively insoluble component composed mainly of silk fibroin-like proteins and beta-chitin, as well as a complex assemblage of relatively soluble glycoproteins some of which are rich in aspartic acid. The amino acid sequence of a major insoluble protein shows that it is similar to spider silk, and a soluble protein contains a Gly-X-Asn repeating sequence, where X is Asp, Asn or Glu. Furthermore, this sequence is flanked by segments of carbonic anhydrase, an enzyme that may be involved in concentrating the anions.

The importance of the matrix in controlling crystal nucleation was evidenced by the well defined spatial relationship that exists between the orientations of the insoluble protein and chitin components and the *a* and *b* crystallographic axes of the associated aragonite. This was further demonstrated in nucleation experiments in which analogues of the matrix components were assembled in vitro and crystals were induced to form. If the soluble proteins from an aragonite layer were added, aragonite crystals formed. The same was observed for calcite. Fractionation of the glycoproteins from an aragonitic layer of the bivalve *Atrina* by anion exchange chromatography, followed by assaying their nucleation potential in vitro, showed that one fraction, composed mainly of a single protein, is capable of inducing aragonite formation with high efficiency. These observations confirm Hare's prediction.

A large proportion of the soluble acidic proteins are located within the crystals. In vitro experiments involving the growth of calcite crystals in the presence in solution of matrix glycoproteins showed that some of these are able to interact preferentially with certain crystal faces and in so doing change the shape of the growing crystal. Once adsorbed they are overgrown and end up inside the crystal. Their presence also alters the mechanical properties of the crystal. The shell is thus a composite material at the level of single crystals, as well as at the level of crystal-matrix relations.

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Application of amino acids racemization in East China

This study reports amino acid racemization (AAR) on the fossil bones and teeth (lacustrian), shells (marine), and in-situ megaspores (terrestrial) in China (Table 1).

Preliminary analyses of amino acids using gas chromatography has been obtained for the in-situ megaspores of a fossil lycopsid, *Pleuromeio epicharis,* which is one of the most distinctive plants in the early Triassic floras in North China. Through comparison with that of a modern lycopsid, i. e. herbaceaous *Lycopodium,* the results are considered to indicate indigeneity of amino acids, at least in part, through a rather low total quantity of amino acids, the general trend of decreasing amounts with age, in having a high D/L value (Leu: 0.88; D-Allo/L-Iso: 0.54), and in the similarity of

Table 1. Case study of the AAR in China

Locality and stratigraphy	Material dated			D-Allo/L-Iso	Age (BP)
1.Qikou,	*Arca sp.*				
Chenier II (beach ridge)		52-1		0.31	3650 a.
		53-1		0.26	2860 a.
		54-1		0.14	2240 a.
	Corbulidae				
		52-2		0.26	2860 a.
		53-2		0.16	1330 a.
		54-2		0.14	1030 a.
2. Nihewan					
Bed 12 Pulu	Clam	N-3		0.37	2.70 Ma.
Bed 5 Danangou	Bone	N-5		0.38	2.78 Ma.
3. Yuanmou					
Bed 26 Yuanmou	Teeth *Cervus sp.*		87994	1.16	1.54 Ma.
4. Wushan					
Bed 8 Longgupo	Teeth of horse		D85.b	0.77	2.39 Ma.

The amino acid analysis was carried out using a gas chromatograph [GC: HP5890A (USA); capillary column: Chirasil-Val. ID. 0.32 mm x 25 m. (USA)].

amino acid compositions that can be recognized from the results of the two samples. The high concentrations of the acidic and aromatic amino acids of the modern sample might be a result of an adaptation to strong acidic soils in which the plant lives. In contrast, the higher concentrations of alkaloidal components in the fossil lycopsid could be produced under reductive taphonomic conditions, and probably, are not indigenous.

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Evaluation of ancient diet: Dietary tracing by stable isotope analysis of consumer tissues

The reconstruction of past human diets through isotopic analysis of archaeological human skeletons has been successfully used for two decades in tracking the spread of maize (a C_4 plant) into the C_3 biomes of the Americans. Contradictions between isotope data and other evidence suggest that the relationship between the dietary importance of maize and the carbon isotope ratios of consumer bone collagen (or hair, muscle, etc.) is not linear, as the initial model (the „scrambled egg" model) proposed. Recent experiments with rats showed consumer apatite carbonate to reflect whole diet, rather than only energy components, and consumer collagen to be dominated by, but not entirely formed from, dietary protein. This demonstrates the failing of the alternative „routing" model. A correct model requires an appreciation of biochemistry.

In order to provide basic metabolic information for future archaeological interpretations of human diets, experimental diets in this study have been designed to simulate the introduction of maize to C_3 biomes in the Americas. We measured natural stable isotope ratios in the tissues (bone, muscle, hair) of pigs (large omnivores with the same essential amino acids as humans) in order to study the biochemical routing from dietary components to consumer tissue. We raised pigs on 13 different diets, using foods that are naturally labelled with stable carbon and/or nitrogen isotopes. Their diets are designed to identify the dietary components used in the synthesis of pig protein tissues, particularly where more than one protein source (animal and/or plant) is available, and also to determine whether dietary carbon is averaged during the formation of mineral bone carbonate. The full set of results for the first generation of pigs will be presented. Requirements for a new biochemically informed model will be discussed. Final results will make it possible to interpret isotopic dietary signatures in archaeological human skeletons with greater accuracy in most dietary situations.

This study was inspired, in part, by work done with pigs by P. Edgar Hare and co-workers (Hare et al, 1991).

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