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Dating Methods of Pleistocene Deposits and Their Problems: IV. Amino Acid Racemization Dating

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Article abstract

Amino acid racemization dating is used in Pleistocene stratigraphie studies as a tool for correlation and relative age dating of equivalent strata or for the absolute dating of deposits. The method is based upon detection of changes in amino acid isomer distributions that accompany fossilization.

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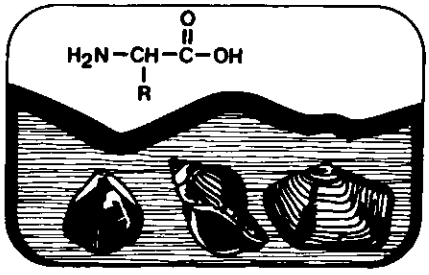
A number of complications present themselves in amino acid racemization dating Sample contamination is a concern The validity of certain assumptions required for data manipulation may be questioned. The requirement of a knowledge of the precise temperature history of the fossil severely restricts application of the uncalibrated method, particularly m areas such as Canada where wide temperature fluctuations have occurred. Nevertheless, the method has already proven its usefulness and offers so much potential that it cannot be disregarded In Canada. amino acid racemization dating has been applied extensively in the eastern and western Arctic



Dating Methods of Pleistocene Deposits and Their Problems: IV. Amino Acid Racemization Dating

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Summary

Amino acid racemization dating is used in Pleistocene stratigraphic studies as a tool for correlation and relative age dating of equivalent strata or for the absolute dating of deposits. The method is based upon detection of changes in amino acid isomer distributions that accompany fossilization.

The study of amino acids from a geochemical dating perspective began about 25 years ago with the investigations of Abelson (1954) and gathered considerable momentum in the late 1960s after development of high resolution gas chromatographic (GC) techniques made possible the accurate and rapid determination of amino acid isomer distributions. During the last decade, over 200 publications have dealt with various aspects of the method. Dating studies have been carried out with Pleistocene bones, teeth, wood, seeds, coral, foraminifera, clay minerals, marine and fresh-water sediments, and with marine, freshwater and terrestrial molluscs.

The method is particularly useful for correlation and relative age dating of equivalent strata which have experienced similar temperature histories and diagenetic conditions.

There are two approaches to absolute age dating – an *uncalibrated* and a *calibrated* method. The uncalibrated method requires a knowledge of the precise temperature history of the fossil. A small error in temperature would lead to a large error in absolute age estimation. This severe limitation is ameliorated by application of the calibrated method.

A number of complications present themselves in amino acid racemization dating. Sample contamination is a concern. The validity of certain assumptions required for data manipulation may be questioned. The requirement of a knowledge of the precise temperature history of the fossil severely restricts application of the uncalibrated method, particularly in areas such as Canada where wide temperature fluctuations have occurred. Nevertheless, the method has already proven its usefulness and offers so much potential that it cannot be disregarded. In Canada, amino acid racemization dating has been applied extensively in the eastern and western Arctic.

Introduction

Dating Pleistocene deposits beyond the range of radiocarbon dating, currently about 75,000 years, has always been a problem. Even with new and perfected absolute methods such as fission track, potassium-argon and uranium disequilibrium, the difficulty persists because all of these methods require special circumstances not found in the majority of Pleistocene deposits.

A comparatively new method, based upon amino acid racemization, promises to alleviate the problem. The method has been applied widely in the last decade for absolute dating, but its major value at present, particularly in Canada, is in correlation and relative age dating.

A number of fossil materials have been employed with the method. Bone has been used extensively; as a consequence, much is known about the diagenesis of amino acids in bones (Bada, 1972, Bada and Helfman, 1975, Bada *et al.*, 1973, Bada *et al.*, 1974; Bender, 1974; Dungworth *et al.*, 1976; Hare, 1974a, Ho, 1965, 1967). Correlation and relative age dating of marine, freshwater and terrestrial molluscs have been carried out (Miller and Hare, 1975, Miller *et al.*, 1977; Mitterer, 1974, 1975; Wehmiller *et al.*, 1977; Rutter *et al.*, in press). Wood offers potential, but has been used only rarely (Lee *et al.*, 1976, Rutter *et al.*, in press). Teeth offer potential, with amino acid racemization occurring at different rates in the enamel and dentine fractions (Rutter *et al.*, in press).

In addition, investigations involving amino acid racemization age dating have been carried out with marine sediments (Bada *et al.*, 1970; Kvenvolden *et al.*, 1970), foraminifera (Wehmiller and Hare, 1971) and coral (Wehmiller *et al.*, 1976; Wehmiller and Hare, 1970).

Historical Review

Geochemical studies of proteins, peptides and amino acids have been carried out since the turn of the century. However, it was not until 25 years ago that investigations by Abelson (1954) pointed to the significance of amino acids in fossil shells and bones as age indicators. Abelson's pioneering work encouraged others to investigate further until, today, some 15 laboratories in North America are actively engaged on one aspect or another of amino acid geochemistry.

Probably the most influential laboratory, and one that has provided impetus for others, is operated by E. Hare at the Carnegie Institution (Washington, D.C.). Even though Hare and Mitterer (1969) provided the first demonstration of the potential for dating old deposits by determining the extent of isoleucine epimerization of fossil *Mercenaria* shells, Hare exercises caution in applying the method to absolute age dating in general. On the other hand, J. Bada of the Scripts Institution of Oceanography (San Diego, California), claims success in assigning absolute ages to many deposits. Thus, both cautious and speculative interpretations persist, but most workers will tend to be cautious until more is known of the details of the diagenetic processes involved in amino acid racemization.

The purpose of this paper is to provide an overview of the method and its applications. More exhaustive treatments may be found in a number of recent review articles: Williams and Smith, 1977; Davies and Treloar, 1977; Dungworth, 1976; Schroeder and Bada, 1976; Bada and Helfman, 1975; Bada and Schroeder, 1975; Kvenvolden, 1975; Hare, 1974b; Hare, 1969.

Amino Acid Racemization and Analytical Methods

Amino acids are low molecular weight, non-volatile, crystalline compounds. They are present - as building blocks of proteins and as structural components - in all living organisms. Structurally, all amino acids except glycine contain a "central" tetrahedral carbon atom which is attached to four different atoms or groups of atoms (Figure 1): a carboxylic acid group (-COOH), and amino group ($-NH_2$), a hydrogen atom (-H) and a hydrocarbon group (-R). In glycine, two hydrogen atoms are attached to the tetrahedral carbon atom (i.e., R=H). Although the number of different hydrocarbon groups - and, hence, the number of possible amino acids - is almost limitless, only 20 commonly occur in the protein of organisms (Table I).

A tetrahedral carbon atom to which *four* different atoms or groups of atoms are attached is a chiral or asymmetric carbon atom. Molecules such as glycine, having no chiral carbon atoms, are said to be achiral. Molecules containing one chiral carbon atom are chiral molecules, and exhibit a common feature - they exist in two stereoisomeric forms. The two stereoisomers differ in the same manner as the left and right hands - i.e., they have a mirror image relationship to each other. The stereoisomers are called enantiomers, optical isomers or optical antipodes. Enantiomers of aspartic acid are shown in Figure 2. The relative configuration of enantiomers is designated by the symbols D and L, the absolute configuration by (R) and (S). L-Amino acids usually, but not always, have the (S) configuration. In this paper, the D/L convention is used. Two protein amino acids, isoleucine and threonine, contain two chiral carbon atoms each. As a consequence, these amino acids may exist as four stereoisomers - a set of enantiomers (mirror image isomers) and a set of diastereomers (non mirror image isomers).

Only glycine and L-amino acids are incorporated into the high molecular weight protein molecules of living organisms. As the organisms pass from the biosphere to the lithosphere, these protein-bound amino acids undergo a slow interconversion in which L-amino acids are changed into D-amino acids. Eventually, by the Miocene (roughly), a 50:50 "racemic" mixture of D- and L-stereoisomers results. This process is called racemization; it occurs slowly at ambient temperatures but is greatly accelerated by acids, alkalis or by elevated temperatures. Since L- to D-conversion is time dependent, the D/L ratio of a given amino acid, which ranges from zero for living organisms to one for Miocene (roughly) fossils, may be used as an indicator of fossil age.

The above model applies to amino acids having only one chiral carbon atom. In amino acids having two such centers, such as isoleucine, each chiral carbon atom can, in principle, undergo interconversion to produce all four stereoisomers. Under diagenetic conditions, however, only one of the two chiral carbon atoms undergoes interconversion so that only one other isomer, D-alloisoleucine, a non-protein amino acid, is formed. In this case, the interconversion process is called epimerization. L-isoleucine and D-alloisoleucine are diastereomers (Fig. 2).

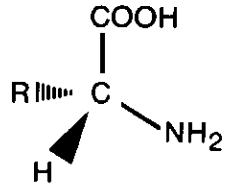
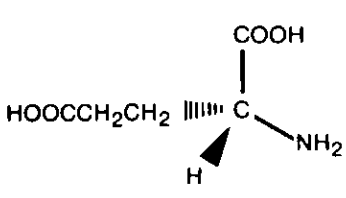


Figure 1
A three dimensional representation of the general structure of amino acids: the COOH and NH_2 groups are in the plane of the paper, the R group projects behind the plane and the -H projects in front of the plane of the paper.

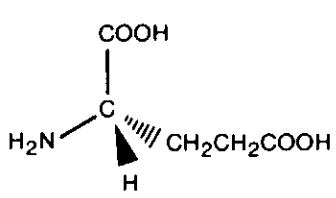
Table I
The protein amino acids: glycine has no chiral carbon atom; isoleucine and threonine each contain two chiral carbon atoms, the others contain only one chiral carbon atom.

alanine	leucine
arginine	lysine
aspartic acid	methionine
asparagine	phenylalanine
cysteine	proline
glutamic acid	serine
glutamine	threonine
glycine	tryptophan
histadine	tyrosine
isoleucine	valine

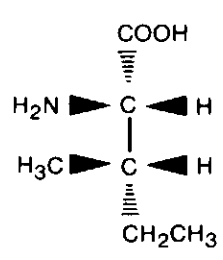
Figure 2
A three dimensional representation of the enantiomers of aspartic acid, L-isoleucine and its diastereomer, D-alloisoleucine. Substituents attached to chiral carbon atoms project behind (dotted lines), lie in (solid lines) or project in front (wedge lines) of the plane of the paper.



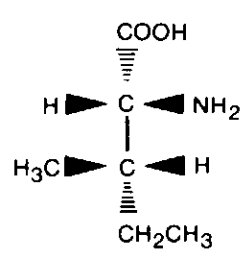
D-aspartic acid
(an enantiomer)



L-aspartic acid
(an enantiomer)



L-isoleucine
(a diastereomer)



D-alloisoleucine
(a diastereomer)

Racemization (or epimerization) of amino acids in geologic specimens is a complex process involving a number of factors. The rate of these interconversion processes are especially dependent upon ambient temperatures, types of matricies in which the amino acids are imbedded, and state in which the amino acids occur - i.e., whether they are protein-bound or "free". In addition, other external diagenetic factors such as moisture content, acidity (or pH), and oxidation-reduction conditions play a role. In general, interconversion rates double for approximately each 5°C increase in temperature, are faster in going from shells to bones to wood, and are faster for free amino acids than for protein-bound amino acids. In view of the latter observation, it is desirable to isolate one particular state of the amino acid for analysis. Optimally, protein-bound amino acids are used since they are less susceptible to contamination and are probably least affected by trace metal catalysis, a factor which apparently accounts for the fast racemization rate of free amino acids. However, in many cases, analytical work is carried out with the "total" amino acid fraction containing both protein-bound and free amino acids. Usually, the proportion of free amino acids is small compared to protein-bound amino acids, and decreases with increasing fossil age.

An accurate determination of D/L ratios of low concentrations of amino acids in fossil specimens is, of course, the key to success regardless of how the data is to be utilized. Methods of extracting amino acids vary from laboratory to laboratory depending upon the preference of the worker and the type of fossil to be analyzed.

A common procedure, and the one used at the University of Alberta, is as follows: a small amount of fossil specimen (ca. 200 mg.) is cleaned by sonication with cold 2N HCl and distilled water, and then heated in a sealed tube with *ca* 5.5 N ("constant boiling") hydrochloric acid. This latter step decomposes the fossil matrix and causes the imbedded protein to be broken down ("hydrolyzed") into individual amino acids. Inorganic salts are then removed from the solution, either by ion exchange methods, (our preference) or by HF precipitation, and the solution is evaporated to dryness.

At this point, it should be noted that enantiomers (mirror image isomers) of a given amino acid have the same physical properties (except for the direction in which they rotate plane-polarized light) and cannot be separated by conventional chromatographic techniques, diastereomers, on the other hand, have different physical properties and can, therefore, be resolved by simple chromatographic methods. Further, a mixture of enantiomers, in a chemical reaction with an enantiomer of a different compound, will produce a mixture of diastereomers, providing the configuration of all chiral carbon atoms is retained.

The mixture of amino acids (above) is then analyzed for D/L ratios either by ion exchange or by gas chromatography. In either method, the ratio of the diastereomers, D-alloisoleucine/L-isoleucine (allo/iso), may be determined directly using conventional methods. The D/L ratios of other amino acids may be determined by chiral GC methods or, after appropriate derivatization, conventional ion exchange or GC (our preference) methods.

Gas chromatographic analysis requires that non-volatile amino acids are converted into volatile derivatives. This is normally accomplished by using a two-step chemical procedure: in the first step, the dry amino acids are heated with an anhydrous, acidic alcohol reagent. The resulting amino acid esters are then acylated by heating with pentafluoropropionic anhydride (PFPA). The N-acylated amino acid esters are then analyzed by GC.

Some laboratories employ an achiral alcohol in the esterification step, and analyze the resulting mixture of volatile enantiomers using a GC equipped with a glass capillary column coated with a chiral stationery phase. Hare, for example, at the Carnegie Institution currently uses this method. Other laboratories, including the University of Alberta, have found it convenient to carry out the esterification step with a chiral alcohol, (+) 2-butanol, and to analyze the resulting mixture of volatile diastereomers using a GC equipped with an analytical column coated with an achiral stationery phase. A flowchart summarizing our analytical procedure is shown in Figure 3. The former method is most elegant since it avoids the use of a chiral reagent which, if not optically pure, can itself introduce error. Both techniques, however, are sufficiently refined that D/L ratios should be reproducible and accurate to *ca* 5 to 7 per cent.

In the latter method of GC analysis, the mixture of amino acid diastereomers, in the gaseous state, is propelled through the GC column by helium, an inert carrier gas. Owing to differences in polarity and volatility, each of the diastereomers is eluted from the column after a different time interval. Volatile diastereomers of valine and alanine, for example, are eluted rapidly (10 to 15 min.) whereas less volatile diastereomers of glutamic acid and phenylalanine are eluted slowly (35 to 45 min.). In all cases, diastereomers derived from D-amino acids elute slightly faster than those derived from L-amino acids. As diastereomers are eluted, they are detected and recorded as peaks on chart paper. Areas under the peaks are determined by electronic integration and, thanks to new microprocessor technology, are automatically reported at the end of the run. D/L ratios of diastereomers are proportional to those of the original amino acids. Abundances of the various amino acids may be estimated by the addition of a known amount of an internal standard, commonly DL-norleucine, to the original amino acid solution. No single GC stationery phase is capable of resolving

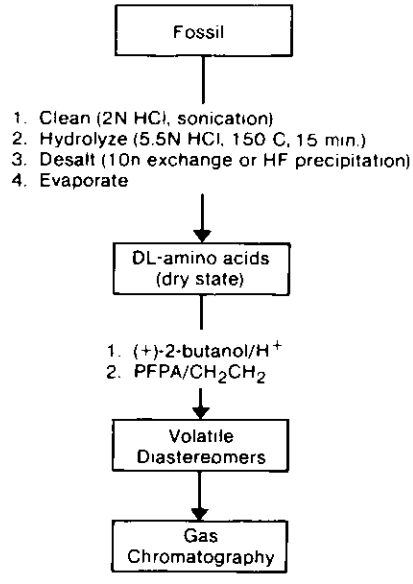


Figure 3
A flow chart of the analytical method used for amino acid determination in fossils at the University of Alberta.

all diastereomers of all protein amino acids. Adequate resolution of the diastereomers of valine, alanine, leucine, proline, aspartic acid, phenyl alanine and glutamic acid is routinely achieved in our laboratories using columns coated with carbowax 20 M and/or stabilized EGA. An example of the output of our GC system is shown in Figure 4 and Table II.

Correlation and Relative Age Dating

D/L ratios of amino acids from fossils of various stratigraphic horizons can be used to correlate equivalent beds and to relative age date the beds providing they have been subjected to similar thermal histories. Correlation studies are most successful where there is a wide variation in age - and hence a wide variation in fossil amino acid D/L ratios - between the units to be correlated. Units of nearly the same age, in which the range of values of D/L ratios from each unit overlap, cannot be correlated with assurance. The resolution with which D/L ratios from various fossils and conditions can bracket, or separate, time intervals can only be speculated upon from data currently available.

In principle, D/L ratios of any protein amino acid would suffice for these studies; in practice, owing to ease of detection and to adequacy of racemization rates, two amino acids - aspartic acid and isoleucine - have found the greatest applicability. Aspartic acid undergoes racemization rapidly and, hence, is well suited for studies involving

Mammoth Bone Report

Run # 135 Area %		Apr. 28, 1977	Time 17:51:28	
	RT	Area	Area %	D/L
VAL	11.28	1867	2.136	
	13.00	152	0.174	
	13.82	157	0.180	
ISO	13.73	984	1.126	
ALA	14.17	6992	7.875	
LEU	16.50 (D)	142	0.162	0.024
	17.11 (L)	5885	6.734	
NORLEU	18.36 (D)	3088	3.534	
	18.89 (L)	3173	3.631	
	19.39	132	0.151	
	24.07	194	0.222	
GLY	25.38	21930	25.095	
PRO	27.65 (D)	583	0.667	0.030
	28.13 (L)	19120	21.879	
ASP	35.79 (D)	1063	1.216	0.140
	36.28 (L)	7526	8.612	
PHE	49.11	5253	5.954	
GLU	50.03 (D)	399	0.457	0.045
	51.23 (L)	8908	10.194	

Table II

Mammoth bone report produced by gas chromatograph microprocessor and printer (Hewlett-Packard Model 5840). Amino acid names are added, and D/L ratios are computed from peak areas.

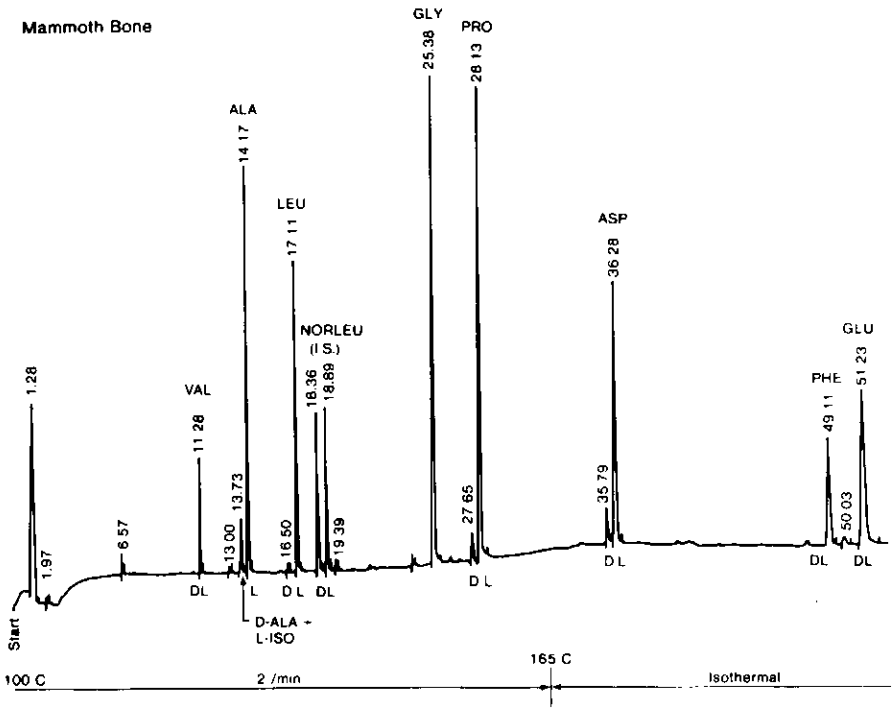


Figure 4

Gas chromatogram of the N-PFPA(±)-2-butyl esters of 9 amino acids occurring in Pleistocene mammoth bone and norleucine (added as a internal standard). Column: 50 meter glass capillary column cooled with carbowax 20 m.

fossils from the northern latitudes. The half-life for aspartic acid racemization in bone over the temperature range 10°C-20°C varies between 30,000 and 15,000 years. Isoleucine, which epimerizes about four times slower, can be used to date material so old that aspartic acid racemization would have been essentially completed. Isoleucine epimerization is more suited for dating studies involving fossils obtained from warm regions.

Racemization rates of amino acids are strongly dependent upon the matrix in which the amino acids are imbedded. Thus, a given amino acid would be expected to undergo racemization at a different rate in shell than in bone or in wood, etc. Further, racemization rates for a given fossil material may be species dependent. Miller and Hare (1975) for example, have observed such an effect with isoleucine epimerization in mollusc shells. In their study, three species of molluscs from the same stratigraphic layer gave allo/iso ratios varying between 0.09 and 0.21. Other fossil materials might exhibit this effect as well, and so correlations and relative age dating should be done with the same type and species of fossil.

The following examples illustrate some of the potential of correlation and relative age dating by this method. In Baffin Island, N.W.T., under cold climatic conditions, Miller *et al.* (1977) correlated four members of the Clyde Foreland Formation using allo/iso ratios obtained from *Hiatella arctica* (marine molluscs). Ratios and estimated ages of the members are: 0.02 (8-10,000 yrs. B.P.), 0.045 (40-48,000 yrs. B.P.), 0.10 (48,000-130,000 B.P.) and 0.15 (*ca.* 130,000 yrs B.P.).

In correlating marine sequences in Florida, under relatively warm conditions, Mitterer (1974) used allo/iso ratios from *Mercenaria* (marine molluscs). Ratios and ages of sediments from which the molluscs were taken are: 0.2 (*ca.* 4,500 yrs. B.P.), 0.4 (*ca.* 34,000 yrs. B.P.) and 0.6 (*ca.* 110,000 yrs. B.P.). Although epimerization rates may have been different for the different genera used in these two studies, the large differences in allo/iso ratios for valves of about the same age is best explained by the large differences in temperature histories of the two sets of valves.

Miller and Hare (1975) measured allo/iso ratios in molluscs from sections of fossiliferous units on Baffin Island,

N.W.T. Their data was used to correlate units from sections four km apart. Lateral correlation between the units was hampered by a major outlet. In the northern Yukon, wood (*Salix* and *Populus*) yielded D/L aspartic acid ratios averaging about 0.06 from Holocene deposits less than 9,190 years B.P. and about 0.24 for *Picea* from deposits greater than 53,000 B.P. but probably less than 100,000 B.P. (Rutter *et al.*, in press). Thus, it appears that wood of different taxa can be used for relative age dating without undue concern for an effect analogous to the species effect. That such a small D/L ratio increment (0.24 - 0.06 = 0.18) separates deposits of such large age difference probably reflects the cold climatic conditions and, hence, retarded racemization rather than the different varieties of wood employed.

Absolute Age Dating

Geochronological determinations have been carried out mainly with fossil shells (Hare and Mitterer, 1969) and bones (Bada, 1972; Dungworth *et al.*, 1974) and with deep sea sediments (Bada *et al.*, 1970). The method has been more successful with shells and bones. In addition, some work has been conducted with wood, coral, woodrat middens and teeth.

There are two methods for utilizing amino acid racemization data for determination of absolute age - the *uncalibrated* (or "extrapolation") and *calibrated* methods. The former approach has been applied far more extensively although its application requires a precise knowledge of the temperature history of the fossil. The calibrated method partially overcomes this limitation and, where calibration standards are available, is expected to give more accurate results.

To apply the uncalibrated method, it is necessary to know the precise temperature history of the fossil, the racemization rate constant of the pertinent amino acid at the time - average temperature of the fossil, and the D/L (or allo/iso) ratio of the amino acid in the fossil. The age of the fossil is then calculated by substitution of these values into an appropriate integrated rate equation, which has been derived on the basis that the amino acid racemization process follows reversible first order kinetics. The integrated rate expression for an amino acid having one chiral carbon atom is:

$$\ln\left[\frac{1 + D/L}{1 - D/L}\right] = 2\,kt + \text{constant}$$

where k = racemization rate constant; t time or, in this case, age, and "constant" is a constant of integration which would be zero if the reaction starts with pure L-isomer. In practice, it has a small, finite value since some D-isomer is both detectable in modern specimens and induced by the anlytical methods. The integrated rate expression is slightly more complicated for isoleucine epimerization. The racemization rate constant for geological temperatures, which is required by the above equation, is obtained by extrapolation (hence the term "extrapolation" method) of an Arrhenius Plot (logarithm of the rate constant versus the reciprocal of the absolute temperature). Data required for the construction of an Arrhenius Plot are obtained through elevated temperature kinetic studies with modern or "young" material of the same type which is to be dated. This latter procedure requires rigorous experimentation and is time-consuming, but needs only to be carried out once for each type (and species) of material. Parameters needed for the construction of an Arrhenius Plot are already known for a number of amino acids from bone, shell and deep sea sediments.

In the calibrated method (Bada and Protsch, 1973), which avoids some of the temperature problems inherent in the uncalibrated method, the D/L ratio of an amino acid, commonly aspartic acid, is obtained for a fossil which has been radiocarbon dated. From the D/L ratio and the radiocarbon date, an in situ racemization rate constant is calculated (above equation). This rate constant is then applied to other fossils from the same site which are outside the radiocarbon range. The assumption implicit in the use of this method is that the time-average temperature history of the calibrated fossil is the same as that of the fossil which is to be dated.

The following examples are illustrative of absolute age dating studies which have been carried out with shells and bones.

Historically, it was a study of the D-alloisoleucine content of progressively older shells that first indicated a correlation between amino acid racemization and age (Hare and Abelson, 1968). The first application of the dating method came when Hare and Mitterer (1969) used the uncalibrated method to estimate an age of *ca.* 70,000 years for an upper Pleistocene *Mercenaria* shell having an allo/iso ratio of 0.32 and a probable average temperature history of 11 to 12°C. Mitterer (1975) has dated *Mercenaria* shells of late Holocene age and of known diagenetic temperature by the uncalibrated method.

Fossil bone has received the greatest attention in the application of amino acid racemization to geochronology. Studies on bones date back to 1972 when Bada (1972) used the uncalibrated method and allo/iso ratios to estimate ages of a fossil goat bone from Muleta Cave, Isle of Mallorca, Spain, and a fossil shark vertebrate from inside a manganese nodule found on the ocean floor *ca.* 1,000 km west of Hawaii. The age of the goat, based on an allo/iso ratio of 0.083 and a temperature history of 19°C, was found to be 26,000 years. This date agrees well with the radiocarbon date of 28,000 years. The shark vertebrate, which had an allo/iso ratio of 0.78 and an average temperature history of 3°C, was 8.7 million years old. In both cases, the fossil bones were taken from sites having stable temperature histories.

Dungworth *et al.* (1974) used the uncalibrated method to estimate the age of a fossil walrus femur dredged from Pleistocene estuarine sediments at a depth of 18 to 20 metres. A fossil age of 300,000 years was determined from D/L ratios of four amino acids - alanine, valine, isoleucine and leucine - and agreement between the ages was good. Some amino acid dating results on bone have led to considerable controversy. For example, Bada and Helfman (1975) have put forward racemization dates of 40-50,000 B.P. for human remains from the San Diego area and a possible date of 70,000 B.P. for the Sunnyvale skeleton. Previously, on the basis of a radiocarbon date for the "Los Angeles Man", it was thought that man's earliest presence in North America was *ca.* 25,000 B.P.

As an example of the application of the calibrated method, Bada and Deems (1975) used a calibrated bone from

Nelson Bay Cave, southern Cape Province, South Africa, to determine a regional in situ racemization rate constant for aspartic acid. The rate constant was then applied to the dating of a bone from nearby Klasies River Mouth (KRM) caves. The age of the KRM bone was found to be 110,000 B.P. An age deduced from radiometric dating of fossil corals in the area was in close agreement.

Extensive dating studies have been carried out on deep sea sediments, but results have been less successful than was earlier anticipated. There are a number of complications, including lack of sample homogeniety, increasing extent of isoleucine epimerization with increasing depth and, most significantly, a change in isoleucine epimerization rate at an allo/iso ratio of *ca.* 0.3 (see Schroeder and Bada, 1976).

Problems and Limitations in Amino Acid Dating

There are a number of complications inherent in amino acid dating and, as a consequence, caution must be exercised throughout. A major consideration is the possibility of sample contamination. Analytical methods are generally well worked out, and should be accurate for most amino acids to *ca.* 5 to 7 per cent. The correlation and relative age dating approach appears to be sound as long as caution is exercised with regard to the species effect. Certain assumptions and laboratory methods – the validity of which remains uncertain – must be employed in absolute dating by the uncalibrated method. The most severe limitation of this method, the requirement of a knowledge of the temperature history of the fossil, is partially offset by application of the calibrated method.

Several factors are involved during the history of a fossil which effect the rate of racemization (or the observed D/L ratios), and should be considered in an amino acid dating study. For example, some protein hydrolysis occurs under diagenetic conditions to produce peptides and free amino acids. The rate constants for racemization of protein-bound and free amino acids differ. Thus, the apparent kinetic pattern is complicated and depends on the relative amounts of each in a particular sample. The problem can be avoided by using only the protein-bound amino acid fraction. The importance of hydration in racemization dating has been considered (Hare, 1974a). Whether the presence of water effects racemization rates, or whether it simply effects D/L ratios by leaching out highly racemized free amino acids is uncertain. Racemization rates may be pH-dependent, although studies in bone suggest that little variation in rate occurs between pH 5 to 9. The species effect occurs in foraminifera and shells, and may be important in other fossils as well.

Contamination of the fossil by amino acids other than those originally present at the time of death of the organism would lead either to under - or over-estimation of absolute age. Incorporation of free amino acids or of older protein material into the fossil specimen would lead to a high D-amino acid content, and an over-estimation of age. Conversely, contamination by recent protein would lead to a high proportion of L-amino acids and a low age estimate. The older the fossil, the lower the concentration of original amino acids and, consequently, the greater the potential for erroneous D/L ratios. Contamination by bacteria, fungi or ground water are possible.

The major problem with the uncalibrated method stems from the fact that the racemization process is highly temperature dependent. With isoleucine epimerization, a relatively small temperature uncertainty of ± 2°C may yield an estimated age whose uncertainty is *ca.* ± 50 per cent. Further, higher temperatures disproportionately influence amount of racemization. With aspartic acid, for example, 1000 years at 25°C produces ten times as much racemization as 2000 years at 12.5°. Although the earth's general climate has been relatively constant for the last 10,000 years, present day average temperatures can give only a rough overall average for a particular site. The problem is less significant for fossil specimens gathered from deep ocean sites or from deep caves where long term temperature variations would have been minor. Consequently, application of the uncalibrated method is limited to fossil specimens taken from such sites.

A second major difficulty with the uncalibrated method concerns determination of the racemization rate constant for an amino acid at geologic temperatures. This is done using data obtained by elevated temperature kinetic experiments carried out with the same type of material. Whether or not the changes which occur at these elevated temperatures accurately reflect changes which occur during low temperature diagenesis is not known with a high degree of certainty. The validity of the extrapolation procedure itself must also be assumed.

The calibrated method may be used with fossils occurring at sites which have undergone relatively wide temperature fluctuations. Since the method partially allows for these temperature fluctuations, it is expected to be more accurate than the uncalibrated method. However, the method is not without criticism: use of a calibrated fossil having an age of 20-25,000 years will give an erroneously low age for an older fossil, since higher postglacial racemization rates are not accurately reflected by a calibrated bone of this age. It has been suggested that two calibrated fossils be used - one of postglacial age and one of preglacial age (Davies and Treloar, 1977).

Concluding Remarks

Dating by amino acid racemization is a relatively new technique. The problems, which include temperature dependence and contamination, are considerable. However, the method is potentially so useful that it cannot be ignored. Results obtained during the last decade are probably highly significant. The method is still in its infancy. Two major advantages are that it extends beyond radiocarbon limits, and that it requires only very small amounts of fossil specimen.

Correlation and relative age dating studies of equivalent beds by amino acid racemization have shown their worth in many Pleistocene stratigraphic investigations. In Canada, the method has been employed mainly in the eastern and western Arctic regions, although studies are now being undertaken in Pleistocene deposits on the west coast, archeological sites in Saskatchewan and Holocene deposits in Ontario. The major limitation at this time centers around the lack of similar types of fossil material in the deposits. However, many

areas of Canada have this requisite and, therefore, offer potential.

Determination of absolute dates by amino acid racemization dating requires a knowledge of the temperature history of the specimen. While such a knowledge may be possible for fossils gathered from the ocean bottom or from deep tropical caves, fossils from most Pleistocene deposits in Canada have been subjected to wide temperature variations throughout their diagenetic history. Therefore, absolute age dating, except perhaps by the calibrated method, will be restricted in Canada

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References

Abelson, P.H., 1954, Organic constituents of fossils: Carnegie Institution of Washington Yearbook, v. 53, p. 97-101.

Bada, J.L., 1972, The dating of fossil bones using the racemization of isoleucine: Earth and Planetary Science Letters, v. 15, p. 223-231.

Bada, J.L. and L. Deems, 1975, Accuracy of dates beyond the ^{14}C dating limit using the aspartic acid racemization reaction: Nature, v. 255, p. 218-219.

Bada, J.L., and P.M. Helfman, 1975, Amino acid racemization dating of fossil bones: World Archaeology, v. 7, p. 160-173.

Bada, J.L., K.A. Kvenvolden, and E. Peterson, 1973, Racemization of amino acids in bone: Nature, v. 245, p. 308-310.

Bada, J.L., B.P. Luyendyk, and J.B. Maynard, 1970, Marine Sediments: Dating by the racemization of amino acids: Science, v. 170, p. 730-732.

Bada, J.L., and R. Protsch, 1973, Racemization reaction of aspartic acid and its use in dating fossil bones: Proc. Nat. Acad. Sci. U.S.A., v. 70, p. 1331-1334.

Bada, J.L., and R.A. Schroeder, 1975, Amino acid racemization reactions and their geochemical implications: Naturwiss., v. 62, p. 71.

Bada, J.L., R.A. Schroeder, and G.F. Carter, 1974, New evidence for the antiquity of man in North America deduced from aspartic acid racemization: Science, v. 184, p. 791-793.

Bender, M.L., 1974, Reliability of amino acid racemization dating and palaeotemperature analysis on bones: Nature, v. 252, p. 378-379.

Davies, W.D., and F.E. Treloar, 1977, The application of racemization dating in archaeology - a critical review: The Artefact, v. 2, p. 63-95.

Dungworth, G., 1976, Optical configuration and the racemization of amino acids in sediments and the fossils - a review: Chemical Geol., v. 17, p. 135-153.

Dungworth, G., A.W. Schwartz, and L. Van De Leempart, 1976, Composition and racemization of amino acids in mammoth collagen determined by gas and liquid chromatography: Comparative Biochemistry and Physiology, v. 53b, p. 473-480.

Dungworth, G., N.J. Vincken, and A.W. Schwartz, 1974, Composition of fossil collagen, analysis by gas-liquid chromatography: Comparative Biochemistry and Physiology, v. 47b, p. 391-399.

Hare, P.E., 1969, Geochemistry of proteins, peptides and amino acids: Org. Geochem., p. 438-463.

Hare, P.E., 1974a, Amino acid dating of bone the influence of water: Carnegie Institution of Washington Yearbook, v. 73, p. 576-581.

Hare, P.E., 1974b, Amino acid dating - a history and an evaluation: MASCA Newsletter, v. 10, p. 4-7.

Hare, P.E., and P.H. Abelson, 1968, Racemization of amino acids in fossil shells: Carnegie Institution of Washington Yearbook, v. 66, p. 516-528.

Hare, P.E., and R.M. Mitterer, 1969, Laboratory simulation of amino acid diagenesis in fossils: Carnegie Institution of Washington Yearbook, v. 67, p. 205.

Ho, T.Y., 1965, Amino acid composition of bone and tooth proteins in late Pleistocene mammals: Proceedings of the National Academy of Sciences, U.S.A., v. 54, p. 26-31.

Ho, T.Y., 1967, The amino acids of bone and denture collagens in Pleistocene mammals: Biochimica et Biophysica Acta, v. 133, p. 568-73.

Kvenvolden, K.A., 1975, Advances in the geochemistry of amino acids: Annual Review of Earth and Planetary Sciences, v. 3, p. 183-212.

Kvenvolden, K.A., E. Peterson, and P.S. Brown, 1970, Racemization of amino acids in sediments from Saanich Inlet, British Columbia: Science, v. 169, p. 1079-82.

Lee, C., J.L. Bada, and E. Peterson, 1976, Amino acids in modern and fossil woods: Nature, v. 259, p. 183-186.

Miller, G.H., and P.E. Hare, 1975, Use of amino acid reactions in some arctic marine fossils as stratigraphic and geochronologic indicators: Carnegie Institution of Washington Year Book, v. 74, p. 612-617.

Miller, G.H., J.T. Andrews, and S.K. Short, 1977, The last interglacial-glacial cycle, Clyde foreland, Baffin Island, N.W.T., stratigraphy, biostratigraphy and chronology: Can. Jour. Earth Sciences, v. 14, p. 2824-2857.

Mitterer, R.M., 1974, Pleistocene stratigraphy in southern Florida based on amino acid diagenesis in fossil Mercenaria: Geology, v. 2, p. 425-428.

Mitterer, R.M., 1975, Ages and diagenetic temperatures of Pleistocene deposits of Florida based on isoleucine epimerization in Mercenaria: Earth Planet Sci. Lett., v. 28, p. 275-282.

Rutter, N.W., R.J. Crawford, and R. Hamilton, in press, Correlation and relative age dating of Quaternary strata in the continuous permafrost zone of northern Yukon with D/L ratios of wood, freshwater molluscs and bone: Proceedings Volume, 1st International Conference in the Biogeochemistry of Amino Acids.

Schroeder, R.A., and J.L. Bada, 1976, A review of the geochemical applications of the amino acid racemization reaction: Earth Science Reviews, v. 12, p. 347-391.

Wehmiller, J.F., and P.E. Hare, 1970, Amino acid diagenesis in fossil carbonates: Geol. Soc. Amer. Abst., v. 2, p. 718.

Wehmiller, J.F., and P.E. Hare, 1971, Racemization of amino acids in marine sediments: Science, v. 173, p. 907-911.

Wehmiller, J.F., P.E. Hare, and Kujala, 1976, Amino acids in fossil corals: Racemization (epimerization) reactions and their implications for diagenetic models and geochronological studies: Geochim. Cosmochim. Acta., v. 40, p. 763-776.

Wehmiller, J.F., R. Lajoie, K.A. Kvenvolden, *et al.*, 1977, Correlation and chronology of pacific coast marine terrace deposits of continental United States by fossil amino acid stereochemistry - technique evaluation, relative ages, kinetic model ages and geologic implications: U.S.G.S. Open File Report, v. 77, p. 680.

Williams, K.M., and G.G. Smith, 1977, A critical evaluation of the application of amino acid racemization to geochronology and geothermometry: Origins of Life, v. 8, p. 91-144.

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