Review Article

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Teeth, a Chemical Record of the Owner – Amino Acid Racemization, Radiocarbon Analysis: A Review

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Abstract

There are three main elements in the procedure of anthropological investigation and the identification of exhumed human remains: race determination, sex determination and age determination at the time of death. The most difficult one to determine is the age. Patterns of aging are detected by direct observation and radiological examination. Simple methods can provide data about age at death for large number of individuals, but with less accuracy. More complex methods which require qualified and trained personnel can provide data about age for a smaller number of individuals, but with more accuracy. Using different age calculation methods for archaeological samples can raise the level of confidence and percentage of success in determining age. Analysis based on morphological methods can yield age estimation error margins of greater than 10 years, whereas precise methods such as aspartic acid racemization and radiocarbon analysis report accuracies of 3 and 1–2 years.

Key words: Age estimation; Amino acid racemization; Radiocarbon analysis.

Introduction

There is no better judgment of a society than dissecting the fervor in which it pursues truth and justice. The age and the sex of the victim constitute important information that can limit the search for possible matches amongst a large number of alternatives. Whereas the gender usually can be determined by morphological methods or by DNA analysis of the remains, most methods for age determination suffer from poor estimate precision. Since teeth are highly resistant to decomposition, chemical degradation and heat, they constitute a particularly valuable material for forensic analysis. The observation of a gradual conversion of L-aspartic acid to its D-form in teeth led to the development of aspartic acid racemization analysis as a tool for age estimation.[1]

Radiocarbon is incorporated into all living things which therefore form an isotopic chronometer of the past 60 years. In 2005, a novel method was proposed to determine the "bomb" radiocarbon activity in the carbonate component of tooth enamel; it is used to predict when a person was born. Although the method is simple in principle, the level of ^{14}C is low and very sensitive radioactive counters are necessary to measure the activity rendering this method as one requiring skilled

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operators.[2]

Amino Acid Racemization

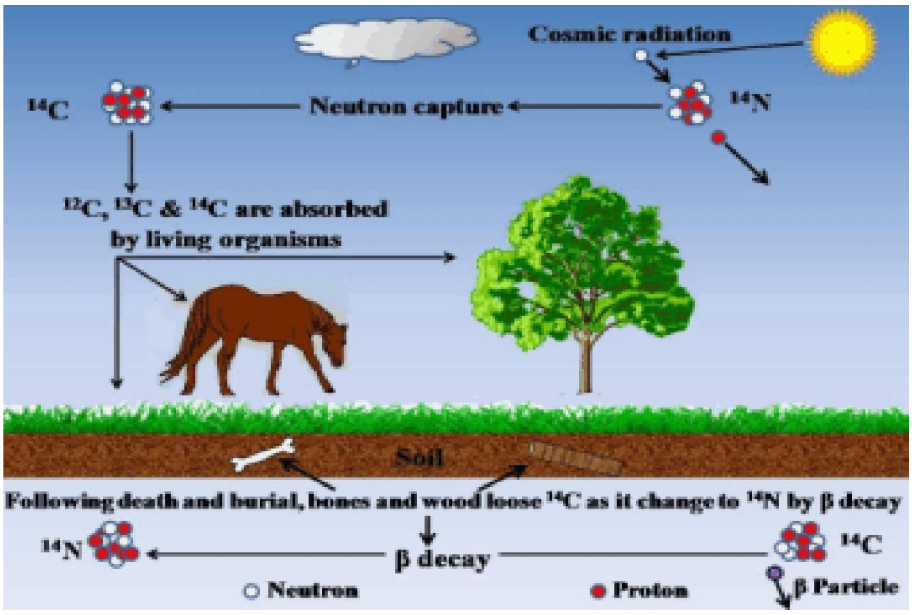
There are several age related changes that occur in proteins, like oxidation, isomerisation & racemization. Among these changes, racemization is the first order chemical reaction from levo or L-form to dextro or D-form and highly correlates with age of the protein.[3] The principle behind racemization is that D and L amino acids have same molecular and structural formula. These D and L forms are the mirror images of each other but cannot be superimposed like right and left hands.(Figure 1) Molecules of amino acids having carbon atom with four different groups, when subjected to plane polarized light, rotates the light in both right and left directions at equal degrees making amino acids optically inactive. In some cases, an enantiomer (L form or D form) is produced in excess because of the hindrance on one side of the molecules. Such a mixture of two enantiomer, unlike a racemic mixture shows a net optical rotation and thus, it is said to be optically active. The rate at which molecules rotate light, forms the basis of study done for estimation of age.[2] Amino acid racemization is an age dependent, nonenzymatic changes of L-form amino acid to D-form amino acids and is considered to be one of the most reliable and accurate method.[3]

In the living body, newly synthesized proteins are normally composed of L-form amino acids, although there are some exceptional peptides that are biologically synthesized using D-form amino acids. At the human body temperature of approximately 37 °C, amino acid residues in tooth enamel protein undergo racemization from L- to D-residues at a rate of approximately 0.1% per year.[2]

Racemization is a natural process which will eventually convert optically active compounds into a racemic mixture but would take about 100,000 years at 25 °C for all L amino acids present in living systems to undergo complete racemization to an equilibrium mixture.[2]

It provides information about the chronological age of an individual at death because the chemical conversion from the L-enantiomer to the D-enantiomer will typically stop completely after death. Thus, analysis is independent of the calendar years in which the person was born and died, to determine these calendar years, other methods must be used.

Figure 1: Picture showing mirror images of Enantiomers (Levo and Dextro forms) of an amino acid.



Helfman and Bada in 1976 focused on aspartic acid racemization as these exhibited the most rapid racemization among the amino acids. They correlated the ratio of L-amino acid & D-amino acids (D/L ratio) in dentine to age and obtained excellent results.[4]

Other amino acids like alanine, serine, and glutamic acid were also evaluated as possible age identification markers. Alanine and serine proved to be inadequate as age identification markers whereas aspartic acid and glutamic acid were established to present a tendency toward linearity with high correlation coefficients.[2]

Ogino and Ogino examined racemization on unerupted and supernumerary teeth, while Ohtani and Yamamoto studied racemization of enamel in addition to dentine. Analysis of crown dentin yielded more accurate age estimation than dental enamel.[5,6]

Racemization process is basically a function of temperature and time; teeth are exposed to different ambient temperatures depending on their location. Ohtani et al[7] demonstrated that racemization rates differ between the same tooth in middle-aged versus elderly individuals. Teeth that have been situated deep in the oral cavity for a long time (and thus are exposed to higher ambient temperatures) are more influenced by the environment than by the period of tooth formation. Teeth best suited for racemization analysis are single rooted teeth such as mandibular incisors or mandibular premolars. Analysis using whole dentin yields a more accurate age estimate than analysis using only part of the dentin. Burned remains will show high D/L ratio because the temperature in the fire will enhance the conversion rate of L-amino acids to the D-form. As dentine forms from the crown toward the root apex, the D/L ratio should be higher in the crown and decreases toward the root apex.[6]

Rate of change of L-form amino acids to D-forms are influenced by various factors, such as temperature, humidity, p^H, etc. Because of the continuous formation and removal or degradation of amino acids, tissues with low metabolic rates provide better age estimate than those with high metabolic rate.

At present, based on accuracy, simplicity, and the time required, teeth are the best organ for estimating age. However, depending on the circumstances, one can choose from other organs such as bone, cartilage, brain cells or the eye lens for estimating the D/L ratio. Among dental tissues, enamel, dentine, and cementum may be used for racemization.[3]

Sample handling is vital since fixation influences racemization. Ohtani *et al*[8] worked with fixatives such as ethanol and formalin and have recommended these fixatives as the standard for dental age estimation. Ethanol provided the highest recovery as well as baseline resolution of the amino acid peaks. Waite *et al*[9] used tooth specimens which were fixed in formaldehyde. This is known to cross-link proteins, particularly at amino, amide, and guanidine side chains and has the potential to fix soluble proteins to the insoluble (collagen–rich) matrix. Therefore, formaldehyde fixation influences racemization in human dentine. Although these authors were unable to obtain reproducible results from the storage of dentine in formaldehyde, they suggest that the racemization induced during acid hydrolysis is altered in the acid-soluble extract of formaldehyde-stored dentine and therefore the use of formalin fixatives is not advisable. There are some enzymes which influence racemization, such as staphylococcal hydrolyse-V8. This enzyme splits only peptide bonds shared by L-glutamic or L-aspartic acid, which leads to more amounts of D-aspartic acid residues at advanced ages.[10]

Andrea Lee Toll[2] in her study showed that the amino acids are soluble in water and capillary electrophoresis would prove to be a more "Green Chemistry" method using aqueous solutions instead of toxic organic solvents used in gas chromatography. Capillary Electrophoresis also provides comparable resolution to capillary gas chromatography as well as typically faster elution times.

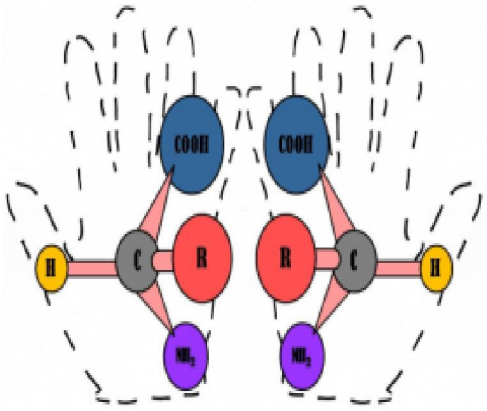
One advantage of aspartic acid racemization analysis is that it is independent of the bomb spike and hence can be used for age determination of subjects born long before the beginning of aboveground nuclear weapon testing.[11] However, since this method is temperature dependent, it cannot be used in cases exposed to extreme temperatures, such as the analysis of teeth from fire victims. In addition at least 4 teeth of the same type, from the same geographical location should be run in parallel with the test tooth, to ensure accurate age estimation.[12] Lastly, one should also keep in mind that teeth from persons residing near or at the surface of a hot climate such as dessert can experience accelerated conversion and appear erroneously aged.

Radiocarbon Analysis

Radiocarbon dating is a radiometric dating method that uses carbon-14 (^{14}C) to determine the age of carbonaceous materials. The technique was developed by Willard Libby and his colleagues in 1949.[13] Libby estimated that the radioactivity of exchangeable ^{14}C would be about 14 disintegrations per minute (dpm) per gram of pure carbon, and this is still used as the activity of the modern radiocarbon standard.[14]

It is relatively a new technique (1st time described in 2005) in dentistry and yet to be significantly tested by the forensic community. There are three main isotopes of carbon on earth. The carbon-12 (^{12}C) isotope makes up 99% of all carbon on earth, carbon-13 (^{13}C) accounts for almost 1%, and ^{14}C is found in trace amounts. ^{12}C and ^{13}C are stable isotopes, but ^{14}C is unstable and is radioactive. ^{14}C is continually produced in the upper atmosphere as neutrons, by-products of cosmic rays and are absorbed by nitrogen atoms. ^{14}C atoms react with oxygen to form carbon dioxide, and ^{14}C is incorporated into the food chain when plants use carbon dioxide for photosynthesis.[2]

Figure 2: Carbon cycle



Living things contain ^{14}C and ^{12}C in a ratio which is same as present in the atmosphere at that time. When the organism dies, the ratio of ^{14}C to ^{12}C decreases, as ^{14}C decays and is no longer incorporated into the organism. (Figure 2)

Above-ground nuclear weapons testing in the 1950s and 1960s resulted in a dramatic increase of ^{14}C in the atmosphere. This is known as the ^{14}C bomb spike. Nuclear tests released lots of neutrons into the atmosphere. Some of these neutrons hit nearby nitrogen atoms, turning them into atoms of ^{14}C. The rates of disintegration of potassium-40 and ^{14}C in the normal adult body are comparable (a few thousand disintegrated nuclei per second).[15] The beta-decays from external (environmental) radiocarbon contribute approximately 0.01 millisievert [mSv/year (1 mrem/year)] to each person's dose of ionizing radiation.[16] This is small compared to the doses from potassium-40 (0.39 mSv/year) and radon (variable).

Radiocarbon dating can date samples up to 50,000 years old. Samples older than that contain so little ^{14}C that the dating process is inaccurate. Enamel isolated from human teeth is processed to form graphite and ^{14}C levels are measured using accelerator mass spectrometry. Since there is no turnover of enamel after it is formed, ^{14}C levels in the enamel represent ^{14}C levels in the atmosphere at the time of its formation.

In addition to radiocarbon dating of enamel, ^{13}C levels in tooth enamel can be used as a predictor of the geographical origin of an individual.[1] ^{13}C is a stable isotope that constitutes about 1.1 % of all carbon. Plants,

to a variable degree, can discriminate between ^{12}C and ^{13}C, resulting in differences in the levels of this isotope between different types of plants. Differences in the fixation of CO_2 during photosynthesis distinguish the more common C3 plants from C4 plants. C4-plants have a double fixation step for CO_2 and their photosynthetic pathway is located deeper in the leaves. Isotope fractionation in C4 plants is primarily limited by diffusion.[17] This is in contrast to C3 plants which can better discriminate between these isotopes and reduce the binding of ^{13}C and more readily make ^{13}C diffuse out through the stomatal pores to the ambient air.[17] As a result, C4 plants (corn and sugar cane) contain higher amounts of ^{13}C than C3 plants (potato, sugar beet, and wheat). In general, C4 plants tend to grow in hotter or drier climates than C3 plants whose open stomata lose too much water to thrive. This in turn means that animals, including humans, having a diet based mainly of C4 plants, or animals feeding on such plants, will incorporate more ^{13}C than those that have C3 plant based diets. Recently, analysis of ^{13}C (along with other stable isotopes) was applied on hair samples from subjects of different geographical origin and large differences between certain populations were observed.[18]

Rational for Using Enamel rather than Collagen

Bone is a preferred sample matrix for traditional radiocarbon dating. Bone's ability to resist rapid decay while containing a relatively high concentration of carbon makes it a desirable material for traditional dating. Traditional radiocarbon bone dating dissolves the mineral component of bone in acid and retains collagen to avoid potential complications with mineral exchange of carbonates in the environment over thousands of years. Collagen is a protein and is not affected by environmental carbonate exchange like the mineral component of bone. However, they had limited success. Bone and cartilage are living tissues and exhibit low but variable turnover, depending on age, activity and type of bone. Older individuals tend to lose more bone than they replace during the bone recycling process.[19]

Although dental enamel is the hardest substance in the body, teeth are not routinely used in traditional radiocarbon dating due to fear of carbonate mineral exchange during centuries of burial. After being produced in childhood or adolescence, there is no turnover of enamel throughout life. It is known that enamel is more susceptible to alteration by weathering than the dentine, which is on the interior of the tooth, and therefore an enamel date is less reliable and likely to be younger than reality.[19]

Processing of enamel samples is different than soft tissues because the carbon resides in a mineral matrix rather than protein. Enamel samples must be dissolved in acid and the liberated CO_2 must be trapped for isotopic analysis. In collagen separations, the mineral phase is dissolved and discarded and the protein not affected by the acid is retained for isotopic analysis. The live part of the tooth, principally dentin, is similar to bone, with high collagen content and slow turnover and recycling of the carbon. Its collagen provides little information from ^{14}C other than whether an individual was alive during the pulse.[20]

If it is not obvious whether an individual is born before or after the peak of the bomb tests, then two teeth with different enamel laydown times need to be analyzed which will distinguish whether the ^{14}C measurement relates to the rising or falling part of the curve.[21]

Ubelaker DH and Parra RC conducted radiocarbon analysis on dental enamel, cortical bone and trabecular bone. The results showed that dental enamel can provide useful information on birth dates because of known formation times of the specific teeth examined if the radiocarbon values fall within the modern bomb-curve period. Trabecular bone radiocarbon values are closer to the tropospheric values at the date of death than are values from cortical bone reflecting relative differences in the rate of bone remodeling in those tissues.[22]

Radiocarbon analysis gives an estimated year of birth where as aspartic acid racemization analysis indicates the chronological age of an individual at the time of death. The combination of these methodologies can help the forensic dentist in identification of the individual.

Both methods have strengths and limitations. The radiocarbon birth dating method can tell the birth date of the person regardless of the time of death. However, the time window for this analysis is limited to subjects born after the early 1940s because the calculations are based on the measurement of bomb pulse-derived ^{14}C. For older subjects, analysis of third molars (if available) may be necessary to detect bomb carbon because of the delay in their enamel laydown time.[11]

A Lawrence Livermore National Laboratory researcher and a team of international collaborators have discovered a multidisciplinary approach. Using "bomb pulse" radiocarbon analysis with recently developed anthropological analysis and forensic DNA techniques. DNA analysis indicates the sex of the person from mitochondrial profile that can be matched to a living maternal relative. Lawrence Livermore and Simon Fraser University multidisciplinary analyses highlight the enormous potential of combining these methods.[23]

Oxygen isotope analysis of dental enamel can assist in determining a person's place of origin.

Tooth enamel's chemical composition is primarily calcium, phosphorous and oxygen with trace amounts of other elements including strontium and lead. Of these elements the isotopes of oxygen and strontium are the strongest independent indicators we have of the local natural environment. Nearly all of the oxygen that goes into the formation of tooth and bone comes from the water we drink and virtually all the water we drink is ultimately derived from precipitation as rain or snow. Knowing the age of the individual and the place of origin can allow narrowing on fewer potential identities.[24]

The power of radiocarbon analysis is likely to improve only in the coming years. The reasons for this are (i) Improved status of the teeth in the population, particularly because of more effective control of caries and interventions to treat paraodontological problems. (ii) The population exposed to bomb spike radiocarbon rapidly increases for every year. This means that a growing number of unknown dead bodies are expected to have remaining teeth displaying bomb spike derived ^{14}C. (iii) The precision of Accelerator Mass Spectrometry (AMS) analysis improving, implying that a lesser amount of intact enamel will be necessary for analysis. (iv) and the increasing availability (especially of smaller 'table-top' AMS models).[25]

Conclusion

An accurate evaluation of the age of living or deceased individuals is an important aspect of forensic sciences. Even though amino acid racemization and radiocarbon dating provides promising results for age estimation, they should be supplemented by other methods to narrow down the search and obtain accurate and precise result.

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