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Chemical signals and reconstruction of life strategies from ancient human bones and teeth – problems and perspectives

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ABSTRACT Chemical analyses of historical and prehistoric bone material provide us with a complex body of knowledge in bioarcheological studies. These can be used for reconstructing diet, migration, climate changes and the weaning process. The analysis of enamel, dentin and bones allows researchers to gather data on life strategies of an individual by retrospectively tracing his ontogenetic phases. This is made possible through knowledge of the mineralization periods of permanent and deciduous teeth while simultaneously taking account of differences between enamel, dentin and bone remodelling rates, dependent on the age of the individual. Yet, the large interpretative potential of isotope analyses of bone material is severely limited by diagenesis. The accurate recording of diagenetic changes in historical human bone material is a current main trend in bioarcheological research. Today, a highly specialised set of research tools is used for verifying whether bones unearthed at archeological sites are suitable for isotope tests. Isotope determinations are pivotal in this research as reconstructions of paleodiets or migrations of our ancestors can be based only on material that has been maintained intact in sufficient proportions post mortem.

KEY WORDS: bone chemistry, diagenesis, stable isotopes, diet, migration

Archeological bone chemistry

Chemical tests in bioarcheology focus primarily on the analysis of human and animal remains, which originate at different points in time and space. These are mainly bones and teeth but also snail- and cockleshells as well as the remnants of soft tissues, if preserved, at archeological sites. This branch of science has been developing rapidly for over ten years. Initially, the researchers centred their interest on the reconstruction of paleodiets of our ancestors by studying trace elements. Currently, we notice the dynamic development of methodology based on the application of stable carbon, nitrogen, oxygen and strontium isotopes in examining the diet, migrations

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and life histories of specific individuals in relation to population studies. Continuing improvement of research methods and implementation of new technologies has made it possible to analyse increasingly older (in chronological terms) remains, including fossilised material. Stable isotopes of carbon traditionally used in relation to diet were originally isolated from preserved bone collagen. At present, an alternative source of carbon isotopes is carbonates connected with the inorganic bone component (bone apatite). Chemical analyses of fauna contemporaneous to specific archeological sites, but also of contemporary fauna present at explored sites are an essential factor supporting the interpretations related to the reconstruction of both paleodiet and migration movements of our ancestors. From these we can access the trophic, geological and climatic background of a given environment. Such data are essential and frequently used in interpretation of dynamics and, most importantly, directions in which historical and prehistoric human groups propagated. A number of model studies carried out on animals with controlled diets allow us to explore physiological ways in which stable isotopes and trace elements became integrated into apatites and bone collagen, having huge interpretative and cognitive significance for bioarcheological research. In recent years considerable emphasis has been also put on studying the nature of diagenetic processes altering the biogenic structure of bones and teeth in diagenetic (geological) structure. Chemical and biochemical signals from archeological bone material provide us with the knowledge of life strategies of our ancestors only if we deal with biogenic research material, which is chemically unchanged post mortem. Thus, a substantial amount of work is devoted to controlling ion exchange processes in bones and teeth. As necessary background for these studies, soil analyses must include samples not only from the gravesite but also samples from the areas surrounding the gravesite: the above-mentioned issues were presented by Sillen *et al.* [1989]. The authors suggested focusing on experimental work enabling researchers to fully grasp the connection between the diet of an individual and chemical constitution of their bones. Moreover, Sillen *et al.* [1989] paid special attention to the application of appropriate types of bones in the research with a view to unifying the results in various research centres. They also pointed out the need for studies on nitrogen isotopes in various natural environments and the use of carbon isotopes from bone apatites. This triggered the appearance of new trends in the 1990s. In consequence, archeological sites were manned by interdisciplinary research teams consisting of: archeologists, anthropologists, paleobotanists, archeozoologists, geologists, geochemists, ecologists and biochemists, all cooperating with each other.

Research trends

Diet

For the purpose of reconstructing paleodiets, contemporary studies use the analyses of stable carbon and nitrogen isotopes, including isolation from the preserved bone of collagen (C and N) and bone apatite (C), and such elements as strontium, barium and macroelements like Ca and P. Analyses of Ba, Sr and Ba/Sr, Ba/Ca and Sr/Ca ratios are used for reconstructing the weaning process and for determining the difference between marine and land diet. The usefulness of strontium and barium in paleoanthropological analyses stems from the fact that they meet the requirements of a theoretical

model confirmed by experimental studies. At the same time, their bio-purification has unanimously been substantiated. Owing to the fact that skeletal concentrations reflect their quantity in various foods and that they are accumulated in bone apatites (by substitution of calcium), strontium and barium are employed as a tool for reconstructing the paleodiets of human populations. Several model conditions which a chemical element needs to fulfil in order to be a diet indicator, particularly in reference to population studies, were presented by Szostek [2006]. An element should display clear diversification on relevant trophic levels, its level in the body should be related both to its content in food and its physiological assimilability, its final concentration in bones should be known as overall content in the body and, so as to be subject to homeostatic control in the minimum extent possible or entirely free from homeostatic control, it should permanently combine with the bone mineral (hydroxy- or fluoroapatites) by ion substitution in lieu of calcium (discrimination), thus permitting chemical analysis of its concentration, and, finally, it should be relatively resistant to diagenetic changes. It has to be concluded that, currently, unlike strontium and barium, zinc does not meet the ideal model requirements of a quantitative indicator of diet. Zinc is subject to intra-body regulation and its final bone concentrations do not reveal its total content in the body. In addition, its level may be concealed by those nutrients, which are rich in calcium. An attempt to verify the relationship between the accumulation of zinc in deciduous tooth enamel in children and the diet of their mothers during pregnancy and breastfeeding was recently made by Dolphin and Goodman [2009]. The results unanimously demonstrate that in the analysis of tooth enamel from both prenatal and postnatal period no relationship was found between the diet of the breast-feeding mother and final Zn concentration and Zn/Ca ratio values in their children's tooth enamel. A quite unanimous conclusion drawn from this study is that the Zn level does not reflect a diet rich in animal proteins. These results concern prenatal (enamel before the neonatal line) as well as postnatal period (enamel beyond the neonatal line). Two questions, however, arise from these findings: First, the work demonstrated the transfer of maternal food (mother's milk) and consequences resulting from the final Zn concentrations in the child's enamel. The authors did not investigate real concentrations of this element in mother's milk and assumed that the entire potentially available zinc obtained from the mother was also present in her milk. Since, as we now know, zinc is subjected to strict homeostatic control, a straightforward relationship between its content in a mother's body and in her milk is disputable. The second question concerns the presentation of average values of Zn/Ca ratio in children's enamel before ($x = 0.02$, $SD = 0.082$) and after birth ($x = 0.05$, $SD = 0.852$). If the results are correctly reported, the coefficients of variation (CV) are respectively 430% and 1700%. Even for such tests, which generally show a high variation, it is unlikely. If data had been misplaced, the reported variation would be higher before rather than after birth, which would alter the entire interpretative context. Notwithstanding the observed discrepancies, it must be concluded that an understanding of the complex processes of zinc metabolism is extremely difficult to achieve even in model studies carried out on contemporary human populations. Therefore, on account of the possibility of over interpretation of results, it has been proposed that Zn be excluded from standard analyses of diets of historical human populations.

Migrations

Initially, chemical methods of analysing archeological bone material focused mainly on reconstructing diets of prehistoric human communities. Only recently has it been discovered that the analysis of stable isotopes may have a broader application. Longinelli [1984] reported a correlation between the proportion of stable oxygen isotope ($\delta^{18}O$) obtained from bone phosphates of mammals and the level of $\delta^{18}O$ in consumed environmental water from the area inhabited by them. This led to the conclusion that the oxygen incorporated while tissues are formed and re-built has an isotopic concentration determined by the isotope composition of drinking water. Since this finding was published, the method of analysing stable oxygen isotopes of human bone tissue has been invaluable for determining, among others, the origins of examined individuals and their migrations [White *et al.* 1998, 2004*b*; Dupras and Schwarcz 2001; Hoogewerff *et al.* 2001; Evans *et al.* 2006; Knudson and Price 2007; McGlynn 2007; Prowse *et al.* 2007]. The identification of migrants, individuals from areas other than the one in which their remains were found, is highly significant for estimating the amount of migration movements, mixing ratio and dynamics of prehistoric and historical human populations, etc. Evidence for a different origin of a given individual, such as archeological artefacts (weapon, ceramics), untypical burial rites, etc., cannot always be found on the archeological site. Therefore, biochemical tests of osteological and odontological material as well as the analysis of stable isotopes may be of valuable assistance in this matter. In line with the foregoing, analyses of oxygen isotopes, strontium isotopes, lead isotopes and sulphur isotopes were performed. The tests were based on animal models, and anthropological applications were administered in regard to sex, age and socio-economic status.

History of life strategies

An important part of isotope tests is how short time periods (life periods) can be recorded from analyses of different bone fragments (enamel, dentin, bone). Many publications were based on knowledge of bone re-modelling and circulation rate. They retrospectively reconstructed life histories of individuals against the studied group both in terms of diet and migration [Stuart-Williams and Schwarcz 1997; Wright and Schwarcz 1998, 1999; Price *et al.* 1998, 1994*a*, 2001; Dupras and Schwarcz 2001; White *et al.* 2004*a*; Lee-Thorp and Sponheimer 2006; Humphrey *et al.* 2008]. Owing to a varied rate of bone remodelling, the compact substance of bones shows information dating back to ca. 10 years before the individual's death [Longinelli 1984, Lee-Thorp and Sponheimer 2006]. For permanent and deciduous teeth and their different parts (enamel, dentin), we may retrace life strategies of a given individual in relation to their childhood and adolescence.

Among various bone fragments used in chemical analyses, enamel proves the most stable in physical and chemical terms [Lee-Thorp and Sponheimer 2007]. Despite the application of diverse cleansing techniques for bones and dentin, their chemical composition is often so distorted that they are not suitable for analysis [Sillen 1986, Budd *et al*. 2000, Hoppe *et al*. 2003, Lee-Thorp *et al*. 2003]. Using, as far as possible, various sections of odontological material (different tooth types reveal different ontogenetic periods) and bones, we may retrace the activity of an individual over a period of time. Humphrey *et al.* [2008] showed that

microspatial analysis of enamel chemistry using laser ablation inductively coupled plasma mass spectrometry across thin sections of enamel, demonstrated differences in calcium-normalized strontium intensities across each tooth. Trace elements variation implies that the sampling location (variation in tooth mineralization) must be taken into account in interpreting results for example of the reconstruction weaning effect.

Isotopes

Carbon

Studies by Ambrose [1993] and Tieszen and Fagre [1993] explicated the differences between the signal from the carbon isotopes sent by preserved organic collagen structures and those attributed to bone apatite carbonates. The carbon absorbed with food can be incorporated into bone or tooth tissue in three ways: as a component of the organic bone (primarily of collagen, less frequently fats), structural carbonates of apatite (bioapatite) or soluble unsteady carbonates bonded to the crystalline surface of apatite [Wright and Schwarcz 1996, Gibson and Bonfield 2002]. In comparison, nitrogen is a structural material of bones and teeth found only in collagen. For a number of reasons, most studies of the reconstruction of diet focus on isotope analyses of collagen: the first and foremost reason is the possibility of a complex analysis of stable isotopes of carbon and nitrogen. In addition, collagen is relatively insoluble and highly stable, which makes it resistant to diagenetic factors. Therefore, $\delta^{13}C$ measurements in bone or tooth apatites are used less frequently and usually when collagen is totally degraded *post mortem* or as a result of bone material incineration [Wright and Schwarcz 1996, Koch *et al.*1997, Munro *et al.* 2007].

Anthropological applications using isotopes of carbon originating from apatites were presented by Krueger [1991] as well as by Lee-Thorp and van der Merwe [1991], pointing out the importance of the laboratory preparation in extracting carbonate radicals from the bone mineral. Consequently, it was proved that nutrients other than collagen are the source of carbon in bone and tooth carbonates. Tests have subsequently confirmed Krueger and Sullivan's [1984] that the carbon forming collagen comes mainly from consumed proteins, whereas the carbon contained in the apatite reflects the entire diet [McGlynn 2007]. This can be explained by the fact that collagen is composed of endogenous and exogenous amino acids. Exogenous amino acids come from consumed proteins, while the endogenous ones come either from consumed protein substances or other nutrients and products of metabolism. Furthermore, carbonates in bones are formed by bicarbonates diluted in blood, which are created from hydrocarbons, lipoids as well as proteins. Therefore, carbonates are the reflections of diet in its entirety, while collagen reflects mainly protein intake [Katzenberg 2000]. Recent research suggests that in the case of fossil or sub-fossil remains it is mainly tooth enamel that should be analysed. Not only is it thicker, but it also has fewer organic substances and is more crystallised, which makes it more resistant to post mortem changes [Le Geros 1991, Elliot 1994]. It also shows the most accurate picture of diet in relation to trophic fractionation (ca. 13 promille), although some results of experiments on rodents with controlled diet reported 10 promille [Ambrose and Norr 1993, Tieszen and Fagre 1993]. Studies conducted over a number of years have contributed to the formulation of a model showing the fractionation of carbon and nitrogen in individual trophic networks (Fig. 1).

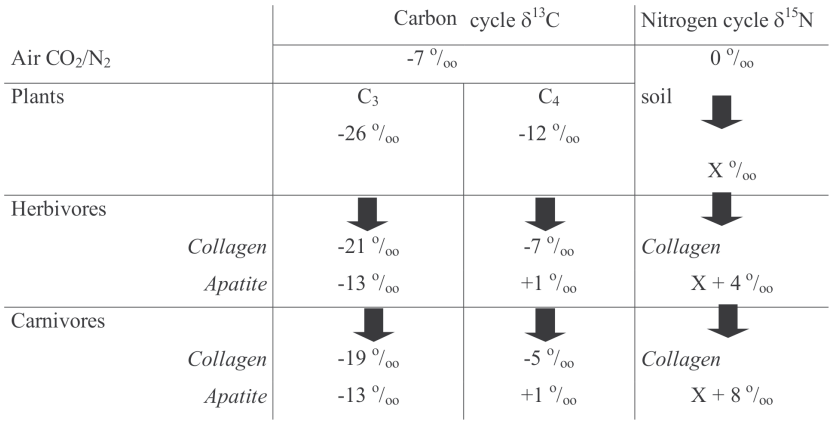

Fig. 1. Patterning of stable carbon (d13C) and nitrogen (d15N) isotopes in typical foodwebs. Tissues (collagen, apatite) are shown for herbivorous and carnivorous animals.

When interpreting the results of analyses, one should also consider the fact that $\delta^{13}C$ level is 5 promille higher for collagen and 12 promille higher for carbonates, than for the entire body. The reasons for this phenomenon are not fully known. In regard to collagen, it is expected that its increased level is caused by fractionation during deamination, transamination and synthesis of amino acids. For carbonates, the increased level is probably due to mechanisms governing their incorporation into the apatite structure [Katzenberg 2000]. Such studies were based on experimental models carried out on animals with known diets. Using this knowledge, Ambrose [1993] proved that reconstruction of the period during which low-protein maize was introduced into the human diet in South America may only be undertaken on the basis of bone apatite analyses.

Currently, in investigations of stable isotopes of carbon and their bioarchelogical interpretations, complex analyses of collagen, as well as enamel, dentin and compact substance apatites, are performed simultaneously. Thus, it was possible to conclude that well-off individuals from the Ecuadorian uplands did not eat maize, but the meat of maize-eating animals. This finding was related to the fact that the research also included remains of contemporaneous animals discovered at the investigated site [Ubelaker *et al.* 1995].

Carbon isotopes incorporated during an individual's lifetime into mineral structures of enamel may remain unchanged for several millions of years. This affords researchers the potential opportunity to analyse the fossilised remains [Lee-Thorp and van der Merwe 1987, Lee-Thorp *et al.* 1989]. However, as further demonstrated, carbonates are more exposed to diagenetic changes than phosphates [Kohn *et al.* 1999]. In addition, one should not forget that enamel provides accurate information on the diet, whereas bone and, to a lesser extent, dentin, yields more inconsistent results [Lee-Thorp and van der Merwe 1991]. From the enamel tests it was possible to verify the theory of herbivory, proposed by Robinson [1954], of *Australopithecus robustus* from the Swartkrans site. It was reported that tooth enamel contained carbon isotopes

characteristic of the C4 photosynthetic cycle in plants which, however, were not directly consumed by the australopithecines (from tooth attrition analysis) but indirectly supplied by animals feeding on those plants (grazers). An important achievement was the analysis of stable carbon isotopes by Lee-Thorp *et al.* [2003] which used carbonates from hominid remains *(A. africanus*, *A. robustus* and *Homo ergaster* (Fig. 2).

It has been shown by Lee-Thorp *et al.* [2003] that early hominids also fed on ^{13}C-rich grass and sedges and/or animals that graze on such plants, findings which suggest that hominidae migrated to open spaces (outside woods) in order to search for food. Research on carbon isotopes contained in enamel apatites may also indirectly be used for reconstructing paleoclimatic changes. Using the changes in biomass of aquatic and terrestrial animals living during the Permian period, Thackeray *et al.* [1990] demonstrated that the isotope method may also be used for long-term analyses of climate changes.

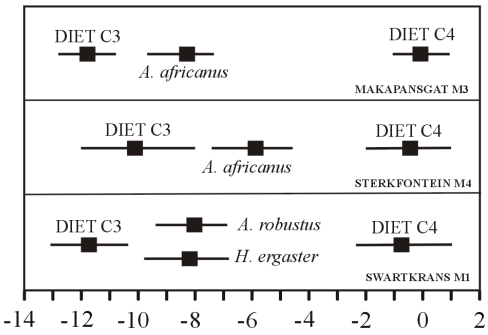

Fig. 2. Hominid diet according to δ13C level (modified from Lee-Thorp and Sponheimer [2003]).

Nitrogen

In addition to specifying which ecosystem the sustenance originates from, it is also important to study the type of food and locate it in the trophic network of a specific individual, population, or species. A small increase in the value of the δ^{13}C index (+1 promille) along with the subsequent, higher level of the trophic pyramid of a given ecosystem was revealed, which hinders or even makes it impossible to reconstruct the trophic network only on the basis of the analysis of stable carbon isotopes [Bocherens 1997]. As a result, the analysis of trophic networks also involves studying the proportions of stable nitrogen isotopes. Stable isotope concentrations range from 3 to 5 promille on consecutive trophic levels. These relationships concern both terrestrial and marine environments [Minigawa and Wada 1984, Schoeninger and DeNiro 1984, Sealy *et al*. 1987, Ambrose 1993, Richards and Hedges 1999, Katzenberg and Weber 1999, Katzenberg 2000]. This phenomenon is related to preferential excretion of the lighter nitrogen isotope (^{14}N) compared with the heavier ^{15}N isotope, which is incorporated in tissues, including bones and teeth. Along with the step-up of trophic level, the consumer's intake includes food increasingly enriched with the heavier nitrogen isotope, in addition to his own metabolic processes increasing its accumulation in tissues [Katzenberg 2000] (Fig. 3).

This makes it possible, to reconstruct the process of feeding on material of marine origin [Richards and Hedges 1999, Fisher *et al.* 2007]. Prowse *et al.* [2004] inferred that the diet of the individuals from Isola Scara (Imperial Roman-age cemetery) included a significant proportion of marine foods. Despite total dietary carbon intake was dominated by terrestrial food (apatite δ^{13}C values); the distribution patterns of δ^{13}C and δ^{15}N suggest that people from Isola Scara obtained their nitrogen from a mixture of marine and terrestrial proteins. Fisher *et al.* [2007] suggested that Middle and Late Mesolithic humans and

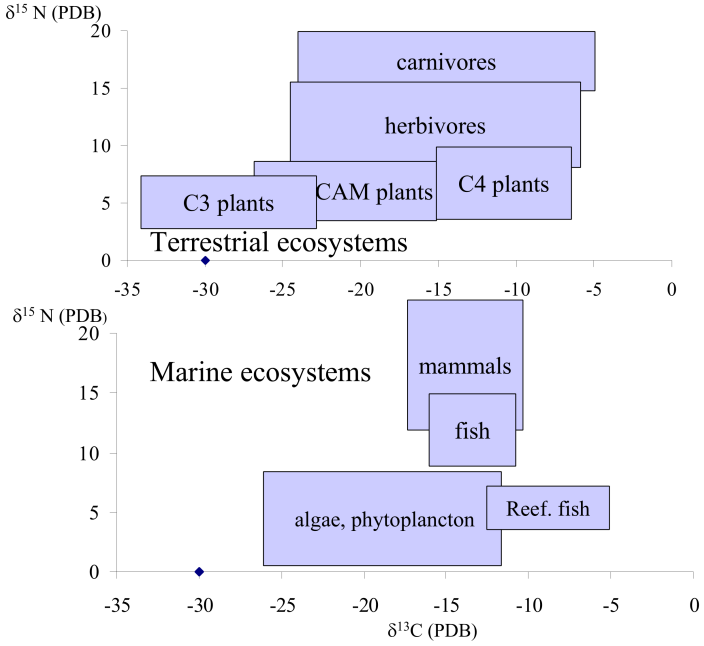

Fig. 3. The distribution of stable carbon and nitrogen isotopes in terrestrial and marine ecosystems (modified from Ambrose [1993]).

dogs found in the Danish interior consumed large amounts of marine food and must have spent long periods at the coast.

Nitrogen isotopes facilitate the understanding of climate changeability. Sealy *et al.* [1987] carried out analyses of nitrogen isotopes within various animal species inhabiting different climate zones. He demonstrated the importance of local variation of nitrogen isotopes in ecosystems of diverse historical and pre-historic human populations. In recent years the concentration of stable nitrogen isotopes has repeatedly been analysed. Such isotopes were extracted from isolated collagen of the Neandertals and compared with Upper Paleolithic human groups [Sponheimer and Lee-Thorp 2006, Lee-Thorp and Sponheimer 2006]. Apparently, no difference between these isotopes was reported; in addition, both studied groups had significantly higher concentrations than most fauna of that period (even in comparison to typical carnivores). Detailed analyses proved that the only (albeit not entirely satisfactory) explanation is that the populations may have fed exclusively on mammoth meat, which, despite their herbivorousness, have the highest concentrations of the nitrogen isotope. Such interpretative difficulties confirm the need for experimental and model studies (relationships between diet, physiology and accumulation) [Iacumin *et al.* 1996, 1997, 1998; 2000; Ostrom *et al.* 1997; Ballasse *et al.* 1999, 2001; Surovell and Stiner 2001; White *et al.* 2001; Peters and Vogel 2005; Honch *et al.* 2006; Knobbe *et al.* 2006; Ovalle *et al.* 2006; Dubois *et al.* 2007; Hart *et al.* 2007; Hedges and Reynard 2007; Pearson *et al.* 2007; Riehl *et al.* 2007; Tutken *et al.* 2007; Munro *et al.* 2008; Schurr *et al.* 2008].

Analysis of different parts of the tooth, provide the resolution needed to reconstruct detailed records of an individual's life history. Van der Merve *et al.* [2003], measured stable carbon and nitrogen ratios for tooth enamel apatites and dentine collagen obtained from the Moatfield ossuary (Iroquoian community), NY Ontario. The results showed that the Iroquoian diet included selected fish species and a substantial maize component. Comparison between different parts of tooth showed that maize consumption occurred between 20–29 years of age.

Nitrogen and carbon isotopes are also used to reconstruct social paleostratigraphy and gender status. Ambrose *et al.* [2003] showed that high and low status individuals from Cahokia Mound (1050–1150 AD) of the Mississippian period had significantly different diet compositions and nutritional qualities. Nitrogen isotope ratios of bone collagen show that high status individuals ate more animal protein, but carbon isotope analysis suggested that they ate only 10% less maize than lower status individuals; in addition, carbon isotope analyses showed females to be of low status.

Another anthropological application is the use of nitrogen isotope analyses in respect of the weaning process (analyses of breastfeeding time). Along, with the rising position in the trophic network, the ^{15}N level increased by 3–4 promille. Excreted metabolic products are rich in a lighter nitrogen isotope ^{14}N, while ^{15}N is incorporated into tissues; it is also a component of mother's milk [Wright and Schwarcz 1998, 1999]. A breast-fed child is on a higher trophic level than its mother, which is expressed by a higher final level of $\delta^{15}N$ in the child. Such observations are used for determining the age in which the child was taken from the breast, which is the moment when the child's $\delta^{15}N$ level equals that of its mother. For this purpose, the researchers compare the $\delta^{15}N$ level from bone collagen of adult individuals and youngsters in a given population, deceased at different ages [Katzenberg 2000].

In their model studies, Fuller *et al.* [2006*a*] performed an analysis of stable isotopes in nail and hair collagen obtained periodically over a total of 100 days from women and their children of the current UK population. It was proved that together with a gradual introduction of external sustenance – first complementing, then replacing mother's milk – a decrease in the child's $\delta^{15}N$ level is noticeable, until it reaches a value identical with the mother. Conversely, no difference between, mother's and child's $\delta^{15}N$ levels was reported for a child fed only with synthetic milk (Fig. 4).

Fuller *et al.* [2006*b*] also investigated the diet of a Late Roman period population located in the upper Thames region in Britain using isotope analysis of carbon and nitrogen. It was discovered that weaning took place at different ages for many individuals but, as demonstrated by $\delta^{15}N$ values (see Fig. 5A), mother's milk was gradually replaced with non-maternal food in children aged 2–4 years. In particular, children aged between 2 to 4 years show different ^{13}C concentration compared to the adult mean (see Fig. 5B). This result represents a different childhood (weaning) diet based more on terrestrial carbon dietary sources such as cereals.

Oxygen

Theoretical and empirical models of the application of oxygen isotopes are based on the fact that oxygen concentration in phosphates and carbonates of bone apatite demonstrates a positive linear relationship with oxygen content in the surrounding

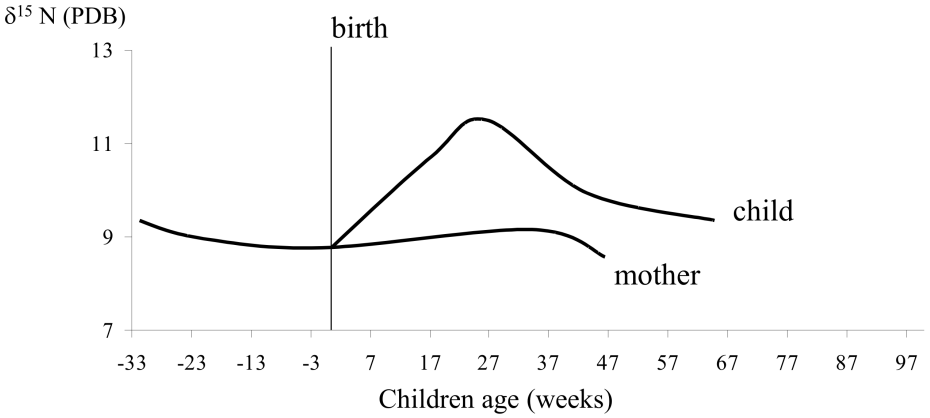

Fig. 4. Level of δ15N in collagen of mothers and children since birth to the age of 66 weeks (modified from Fuller *et al*. [2006]).

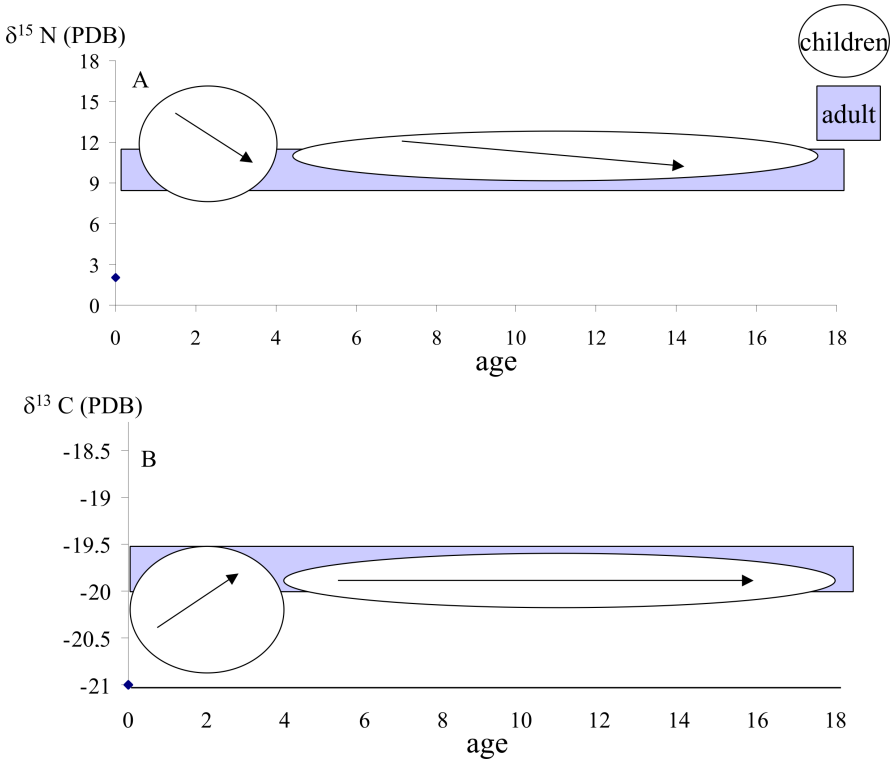

Fig. 5. Weaning age according to δ15N (A) and δ13C (B) concentrations (modified from Fuller *et al*. [2006]).

environmental water. Furthermore, there is a considerable variation of oxygen isotopes across different natural environments. Consequently, organisms inhabiting a given area have almost identical oxygen levels in the bone mineral relative to its characteristic fraction in their habitats irrespective of the quality and manner of feeding [White *et al.* 1998, 2004*a*; Dupras and Schwarcz 2001]. As early as in the 1990s Luz *et al.* [1990], studying deer in various environments in North America, reported a close relationship between habitats of herds, characteristics of oxygen isotopes in a given region and its final bone concentrations. The latter are determined by a set of such factors as: temperature, ambient humidity, distance from large basins and altitude above sea level. Bryant *et al.* [1994] were among the first ones to use horse tooth enamel from one site in Nebraska and found that for over 10 million years the level of oxygen isotopes in bones has been falling due to climate changes in this area (decreasing humidity and temperature). By applying a laser ablation technique to different enamel layers, Ballasse [2003] demonstrated the possibility of tracing short-term changes in the composition of oxygen isotopes, which reveal alterations in the seasonal activity of an individual. This model was designed on the basis of various species of contemporary animals.

In addition to studies on paleoclimatic changes, oxygen isotopes are used on a large scale in analyses of migrations of entire groups as well as individuals. Analyses of various bone sections enable us to retrospectively reconstruct the dynamics of an individual's movements in their lifetime. Determination of the presence of migrants is essential for evaluating the number of migration movements, mixing ratio and the dynamics of prehistoric and historical human populations.

In research practices, the $^{18}O/^{16}O$ ratio is specified without considering the ^{17}O isotope, since it is only present in nature in vestigial quantities. Fractionation processes of oxygen isotopes depend largely on physical properties of water and changes of weather conditions. Water which contains the heavier isotope ^{18}O - takes longer to evaporate than $H_2{}^{16}O$, because it requires the supply of a greater amount of energy [Mays 1998, Katzenberg 2000, McGlynn 2007]. The level of $\delta^{18}O$ measured both in carbonates and phosphates, reveals a complex isotope ratio in the body. The $^{18}O/^{16}O$ ratio in the body is determined by means of assimilated (food, drinking water, atmospheric oxygen) to released (exhaled CO_2, sweat, urine) oxygen ratio. The composition of stable oxygen isotopes originating both from food and drink is determined by the level of $\delta^{18}O$ in environmental water for example, atmospheric water (rainfall, snow) and circulating water (springs, rivers, lakes) [White et al. 2004*b*]. Luz *et al.* [1984], showed a correlation between $\delta^{18}O$ in bone phosphates and environmental water, while Iacumin *et al.* [1996] demonstrated a relationship between the $\delta^{18}O$ content in bone apatites and carbonates.

Oxygen isotope $\delta^{18}O$ level in metabolically inactive tissues such as tooth enamel reflects the isotopic image of the geographical area where synthesis of the tissue in an individual's childhood took place. The isotope ratio of metabolically active tissues represents that in a given environment within a specific time interval depending on the remodelling rate of a given tissue. The time span necessary for rebuilding and changing of the isotope ratio of oxygen in the mineral fraction was established as 10 years. Consequently, the method reflects water consumption by a given individual over the last 10 years of their life [Katzenberg

2000]. Tooth enamel composition after its complete formation practically does not change during an individual's lifetime. This allows us to draw conclusions on changes in an adult individual's locations during the last 10 years of their life on the basis of the analysis of stable oxygen isotopes from bones and dentin. On the other hand, by studying $\delta^{18}O$ level in enamel we obtain data on the individual's migrations in their childhood. The chronology of tooth enamel formation allows us to make a more precise estimation of the age of the subject's when they changed their location. Unfortunately, this method fails to trace short-term seasonal migrations of an adult individual [White *et al.* 2004*a*].

The following environmental factors influencing $\delta^{18}O$ level in the bone mineral need to be taken into consideration during archeological interpretations:

- $\delta^{18}O$ precipitation decreases along with increasing distance from the ocean, altitude above sea level and fall in temperature;
- low air humidity causes a rise in $\delta^{18}O$ (e.g., $\delta^{18}O$ increases in water contained in leaves, since it is mainly ^{16}O that is released during transpiration); this is the reason why $\delta^{18}O$ level in the water contained in bodies of herbivorous mammals, which predominantly obtain water from food, increases with falling air humidity;
- low ambient humidity increases human perspiration, which leads to loss of the lighter oxygen isotope and accumulation of the heavier one; this may have an impact on test results;
- $\delta^{18}O$ level in bone tissue depends not only on the level of environmental water, but also on temperature and humidity [White *et al.* 2004*a*, McGlynn 2007].

Current studies of prehistoric migrations of humans frequently use oxygen isotopes [Dupras and Schwarcz 2001, White *et al.* 2004*a*, McGlynn 2007]. Most frequently, $\delta^{18}O$ level tests employ phosphates isolated from bones and teeth. Phosphates are far more resistant to diagenesis than carbonates. In their oxygen isotope analyses, Dupras and Schwarcz [2001] studied the population from Dakhleh Oasis (Egypt) in order to establish the presence of migrants. It was shown that one of the subjects, who did not display genetic similarity to individuals in other graves in earlier mtDNA studies, exhibited differences in both $\delta^{18}O$ and $\delta^{15}N$ levels. The level of $\delta^{18}O$ was identical with that of the Egyptian population from Nubia on the Nile, suggesting that the 'alien' might have migrated from that location less than 10 years before death. Comparison of oxygen isotope proportions in M1 and M3 teeth in the Isola Sacra population (Italy, 1st–3rd century AD) made it possible to isolate individuals who had spent childhood in a place different from the location in which their remains were found [Prowse *et al.* 2007]. Schwarcz *et al.* [1991], used the method to identify the geographic origin of soldiers who fought in the war of 1812. White *et al.* [1998] studied migrations of Mexican population.

Stable isotopes of oxygen and nitrogen are also used in describing the weaning process. As mentioned before, the oxygen used for tissue synthesis (unlike carbon or nitrogen) comes mainly from water. Lighter ^{16}O oxygen isotopes are lost during respiration and perspiration, so body fluids (urine, blood, milk) contain more of the heavier isotope ^{18}O in comparison to drinking water [Stephan 2000]. A child who consumes only mother' milk receives more ^{18}O than its mother who drinks water. The above observation is useful in analysing stable oxygen isotopes in tests on 'weaning stress' [Wright and Schwarcz 1999]. Dupras and Schwarcz

[2001] investigated the isotopic level of oxygen, nitrogen and carbon in mummies found in Nubia (today's northern Sudan) on the Nile, dating back to 350–1400 AD. It was found that in 3–4 year-old children the level of $\delta^{18}O$ and $\delta^{15}N$ increased, reaching the highest values at that age and gradually falling afterwards. This proves that children in that community were breast-fed for a long period. Wright and Schwarcz [1999] studied the weaning process by analysing levels of $\delta^{18}O$, $\delta^{15}N$ and $\delta^{13}C$ in enamel and dentin of individuals from Kaminaljuyu in Guatemala (400–600 AD). Their work was based on the finding that the time of transition from mother's milk to non-maternal food overlaps with the period of tooth budding and mineralization, and that the isotopic composition of fully developed teeth does not change significantly over the life span. Three permanent teeth were extracted from each individual: M1, P3 and M3. The results revealed an increase in the level of $\delta^{13}C$ between (M1→P3→M3) teeth for every individual, with a greater difference (approx. 1 promille) between M1 and P3 than between P3 and M3. Moreover, the studied individuals displayed a decrease in the level of $\delta^{18}O$ between teeth (M1→P3→M3). It was concluded that non-maternal food was incorporated into child's diet at the age of 2 years and that milk was the main source of water until age 4–5 years.

Strontium

Strontium, like oxygen isotopes, demonstrates high territorial diversification. Its levels are a product of the geological structure of relevant world regions. However, unlike oxygen isotopes, it is supplied to the body together with food. The same species of plants and animals inhabiting different regions have specific levels of strontium isotopes characteristic of local parent rocks, so these differences accurately reflect separate habitats. Prince *et al.* [1994*b*] were one of the first to compare the composition of strontium isotopes in tooth enamel and bones from the same individuals. The analysis of individual variation allowed them to prove that historical human populations inhabiting the southwest coast of North America travelled over long distances during their lifetime. Similar results were obtained by Sealy *et al.* [1995], when analysing human remains in South Africa. Comparative studies of the level of strontium isotopes in bone and tooth apatites are considered a valid and direct measure of migration. They make it possible to identify directions and locations to and from which individuals migrated relative to analysis of geologically diverse environments [Price *et al.* 1994*a*, 1998, 2000; Ezzo *et al.* 1997; Grupe *et al.* 1997]. Comparisons of isotope composition of strontium in enamel and bones of skeletons from several Neolithic sites in Germany indicate that most individuals in the studied groups changed location during their lives [Bentley *et al.* 2004]. An analysis of isotope proportions of strontium in human remains from Grasshopper Pueblo (AD 1300) in central-north Arizona, Price *et al.* [1994*b*] and Ezzo *et al.* [1997] reported a high percentage of migrations in this population. Price *et al.* [2004] showed that isotope-based research also confirmed a very high mobility of the Bell-Beaker culture in Central Europe, supplying new information on directions and extent of the spread of this culture in Europe. Price *et al.* [2006] analysed skeletal remains individuals from Early Neolithic period (Linearbandkeramik) found near Talheim in Germany. Strontium isotope ratios in enamel and bone showed that several of the individuals from the mass grave were

born in a different geological location. These results help us to understand complex matter of migration and community structure in Early Neolithic of prehistoric Europe. Such analyses are based on measurements of the heavier-to-lighter strontium ratio ($^{87}Sr/^{86}Sr$). Strontium is supplied to an organism along with food, which comes from different trophic networks. Fractionation of strontium isotopes in individual trophic chains is not observed, due to large atomic mass of strontium [Hurts and Davis 1981]. It is substituted in the bone apatite for calcium, permanently replacing it in the crystalline lattice.

Strontium isotopes incorporated in enamel during childhood do not change concentration during the remainder of the ontogenetic development of an individual. Because of remodelling, their concentration in bones changes and is characteristic of a given habitat. Individuals who move across geologically different areas incorporate and retain their unique isotope proportions. This can be observed while comparing the isotope composition of enamel and bones or dentin in the same individual [Price *et al.* 1994*a*, 1998]. On the basis of concentration of strontium isotopes in teeth and bones and analysis of the geochemical composition of parent rocks of a given area we may draw conclusions about the dynamics and activity of individuals as well as entire human groups. Price *et al.* [2001] proposed an outline for interpreting isotope signals, which discriminates between local or non-local origin of individuals (Table 1).

While investigating strontium or oxygen isotopes it is essential to analyse in detail the remains of historical fauna discovered on relevant archeological sites. When comparing stable strontium isotopes across different animal species, Bentley *et al.* [2004] reported that the remains of less mobile animals (snail shells), animals which do not graze freely in open spaces (pigs) and those that are closely connected with human activity (dogs), constitute the best achaeozoological background for comparing local geochemical conditions of the investigated area since they are able to reflect subtlest variations in relation to the local environment. It should be noted, however, that theoretically the dog reveals isotope ratios reflecting native geophysical conditions but, being a mobile species migrating together

Table 1. Possible outcomes of the analysis of bone and enamel from a single individual (modified from Price *et al.* [2001])

		Bones	
Teeth		local signal	non-local signal
	local signal	Either 1/ lifelong resident 2/ teeth are diagenically contaminated 3/ the region is geologically homogeneous	Unusual: Possibly a locally born individual spent the last years of life in a different region and then return home shortly before burial
	non-local signal	Immigration some time after the adult teeth were formed	Either 1/ recent migrant 2/ eater of non-local foods or seafood 3/ lifelong seasonal mobility over different geological areas

with humans, it may demonstrate isotope signals other than local ones (Fig. 6).

Schweissing and Grupe [2003] verified migration processes of group individuals from the Roman period from Neuburg/Donau site (330–400 AD) on the basis of the analysis of archeological artefacts found in the graves. Analysis of stable strontium isotopes in tooth enamel and bones revealed that the number of immigrants described by means of classical archeological methods was over represented by more than 20 percent. This concerned all studied age groups and analysed sexes, with greater differences for men. In an analysis of migration both methods appear to be appropriate, since they allow researchers to draw more complete conclusions. Evans *et al.* [2006] studying the oxygen – strontium isotope ratio, confirmed that a group of individuals buried at the Lankhills cemetery in southern England (Late Roman period) according to rites different from the local population did not come from the said location. It was also revealed that the group was heterogeneous and that individuals had arrived from geographically diverse locations. Both methods were used also to recreate the origin of a mummified individual found in the Ötztal valley in Tyrol Alps on the Austrian-Italian border. Tests carried out on water from nearby rivers both to the north and south of the mountain ridge and their comparison with results of isotope ratio analysis of Ötzi's bone phosphates demonstrated that these proportions were similar to the ones obtained for rivers located on the southern (Italian) side of the Alps. Analysis of strontium isotopes in geological and osteological material from these areas narrowed Ötzi's reported place of origin down to the area adjacent to the contemporary town of Merano [Hoogewerff *et al.* 2001, Müller 2005].

Diagenesis

Diagenesis in relation to analyses of stable isotopes should be considered in terms of the preservation of organic components of bones and teeth (collagen) as well as the mineral part (hydroxyapatite). As previously stated, stable isotopes of C and N are obtained from collagen. In the case of oxygen, strontium, and also carbon, the mineral fraction (phosphates and/or carbonates) of the bone is isolated. As a result, the control of the extent to which the samples are

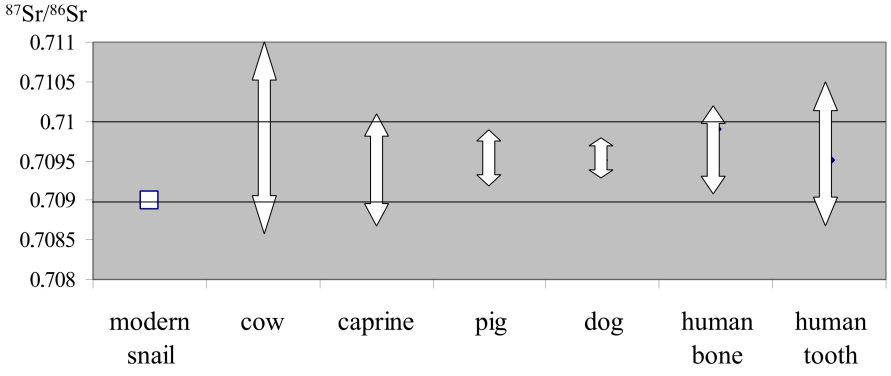

Fig. 6. $^{87}Sr/^{86}Sr$ variations in neolithic human enamel and bone from Vaihingen in comparision to the excavated neolithic animals from the same place (modified from Bentley *et al*. [2004]).

preserved should be specifically adjusted to the research problems postulated by scientists. There are differences in susceptibility to diagenesis between the bone organic and the bone mineral fractions. Generally, collagen is less susceptible to post mortem changes than bone apatites [Pearsal 2008].

Collagen

There are, however, many factors which have a destructive impact on collagen. Its preservation depends largely on the activity of microorganisms as was confirmed experimentally by Grupe *et al.* [1989, 1993] and Child [1995]. It is therefore absolutely necessary to check the preservation of the organic fraction before analysing the stable C and N isotopes. After thoroughly cleaning bones or teeth of the remains of organic substance adsorbed during the deposition, the quality of preserved collagen is tested in three ways:

(1) Calculation of the ratio of C/N atom concentrations [DeNiro 1985, Ambrose 1990, Ambrose and Norr 1992]. Contemporary animal bones demonstrate small variability of this index, ranging from 2.9 to 3.6. A result obtained from archeological bone material outside the above range suggests diagenetic changes in the osteological material (organic phase of the bone). The affected section or individual should not be subjected to isotope analysis, because doing so does not guarantee reliable results [Schoeninger and Moore 1992].

(2) Checking the percentage of collagen isolated from the bone in proportion to its dry mass. It was proved that a low percentage of collagen in the bone is strongly correlated with distorted proportions of carbon and nitrogen isotopes originating from the bone. Collagen normally constitutes more than 2% of the mass of a well-preserved bone [Jørkov *et al*. 2007]. In many cases, the proportion of collagen should not be less than 1% [Schoeninger and Moor 1992, Ambrose 1993].

(3) Analysis of the C/N concentration ratio in the isolated collagen. During diagenetic processes, one of the amino acids constituting collagen, hydroxyproline or glycine, may be lost. Selective, significant loss of any of the amino acids may have an impact on the proportion of C and N isotopes obtained from collagen. Thus, apart from data on collagen percentage proportion in bones, the percentage proportion of carbon and nitrogen in collagen is given, and should not be less than C 6.6%, N 1.9% [Pearsal 2008]. Results of isotope analyses of carbon and nitrogen from the organic bone should be complemented by the above data.

Bone apatites

The inorganic fraction of bone is more susceptible to post mortem diagenetic changes than collagen [Krueger 1991, Lee-Thorp and van der Merwe 1991, Pearsal 2008]. There is therefore emphasis put on detailed descriptions of any post mortem changes relating to the bone mineral. At present, verification of diagenetic changes in the apatite involves the application of a set of special, complementary techniques and methodology from detailed analyses of the soil from the grave and determining its physical and chemical properties with the surroundings, studying the Ca/P ratio, to the crystallinity index of phosphates and carbonates (CI). Such tests concern the relevant sections of the skeleton in relation to contemporaneous fauna from specific archeological sites [Bentley *et al.* 2004, Sponheimer and Lee-Thorp 2006]. Crucially, it is the enamel that is the part of the skeleton most resistant to diagenetic changes [Lee-Thorp and van der Merwe 1991, Ambrose and Norr

1993, Kohn *et al.* 1999, Ballasse 2003, Lee-Thorp and Sponheimer 2006]. Studies by Sponheimer and Lee-Thorp [2006] revealed that chemical analyses of fossilized enamel of various animal species dating back millions of years reflect biogenic concentrations of trace elements. Owing to the fact that enamel, in specific circumstances, also undergoes post mortem changes, the verification process in the diagenetic scale should be carried out on as many sections of the skeleton as possible (enamel, dentin, compact substance). In other words, the fact that, for example, enamel does not undergo diagenetic changes does not mean that other parts of the skeleton reveal the ante mortem biogenic chemical composition of an individual. As previously stated, only after verifying that all parts of the skeleton varied in terms of mineralization age and remained unchanged in respect of remodelling rate (intra vitam biogenic signal), is it possible to reconstruct the life strategies of an individual. Interpretation of chemical signals from enamel and dentin in relation to their mineralization age and low remodelling enable us to perform a retrospective reconstruction of the life history of an individual with great accuracy (accurate to several months). In the case of bones, remodelling and turnover run at varying rate depending on an individual's age. Longinelli [1984] demonstrated experimentally that this rate decreases with age. Consequently, signals from skeletons of children or adolescents reveal relatively short time periods – about 3 years; 5–10 years before death for adults.

Acidity and chemical composition of soil

Verification of skeletal material for analyses should begin at the archeological site. Soil samples and pH tests should be taken both within and outside the grave environment. Chemical analyses of soil for bone element concentrations may reveal potential knowledge of ion exchange in the bone-soil phase. Such analyses are based on pH gradient and ion motion theory, which states that the direction of ion flow occurs from locations of higher concentration to places of lower concentration [Edward and Benfer 1993, Farnum *et al.* 1995].

Ca/P ratio

Around 70% of bones and dentin and nearly 98% enamel is made of mineral compounds, mainly calcium phosphate, in the form hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] crystals. Tooth enamel has large phosphate crystallites (>1000nm long), which form a tighter structure (small lacunae) and provide greater hardness than in the case of bone or dentin, where hydroxyapatite crystals are <100nm long with greater empty spaces between them [Koch *et al.* 1997, Niedźwiecki and Kuryszko 2007].

Carbonates constitute approx. 2–5 promille of the hydroxyapatite part by weight and may be substituted for PO_4^{3-} and/or OH (type A carbonates) or bounded on the surface of hydroxyapatite (type B carbonates) [Katzenberg 2000]. Studies by Molleson [1987] reported high variability of trace elements connected with apatite in enamel. During the apposition of enamel with time, significant declines in concentration of e.g., lead and fluorine in its layers are observed, although no changes are reported for strontium, cuprum or calcium. Since such studies demonstrated a differentiation in the quality and quantity of individual elements in teeth of contemporary subjects, tracing such records of historical material combined with Ca/P ratio analysis supplies us with additional information about any changes in the surface layers of enamel,

which are most exposed to direct contact with post mortem external environment.

Hydroxyapatite present in tooth enamel is not metabolically active and, following its formation, its components are not replaced *intra vitam*. However, because carbonates on the surface of bones and dentin are connected with bicarbonates in blood, their quantity is subject to variation. The main source of contamination in bone carbonates is calcium carbonate, frequently depositing in empty spaces and on the surface of the crystalline structure of the bone [Koch *et al.* 1997, Mays 1998]. These properties are conducive to exogenous pollution, which may affect the level of analyses of stable isotopes.

During an individual's lifetime elements such as Sr and Ba are substituted for calcium. Oxygen isotopes are incorporated in phosphates and carbonates, carbon into collagen and carbonates, and so on. As can be seen, apatite stoichiometry is highly complex and each individual is represented by a unique apatite chemical composition. The Ca/P ratio in contemporary bones and teeth is approx. 2.2. However, its high variability is marked, as shown in Safont's *et al*. [1998] work. During the complex and unpredictable ion exchanges following an individual's death the value of Ca/P constituted *intra vitam* may be altered. As a consequence of a possible loss of calcium or phosphorus, information on intra vitam concentrations of various trace elements and stable isotopes is also lost (Fig. 7).

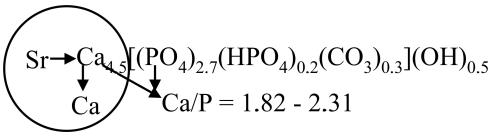

Fig. 7. Elemental exchanges inside hydroksyapatite.

These changes may be confirmed by a decrease in ratio value (loss of mineral fraction) or its increase (exogenous contamination). The range of variability of the Ca/P ratio in contemporary bones and teeth extends from 1.8 to 2.4 [Sillen 1981, 1989, 1992; Gawlik *et al.* 1982; Bisel 1988; Grupe and Pipenbrink 1988; Hancock *et al.* 1989; Ezzo 1992; Sanchez-Quevedo *et al.* 2001]. Outside of this range, potential diagenetic changes in bones and teeth may occur. There are many methods of determining the above-mentioned ratio, e.g., Atomic Absorption Spectoscopy (AAS), Inductively Coupled Plasma Spectrometry (ICP), Particle Inducted X-ray Emission (PIXE), Energy Dispersive X-ray Spectroscopy (EDS-SEM). Classical analytic quantitative methods using atomic absorption spectrophotometers are based on mineralised bone samples. Consequently, when solubilized in acids, the material is irrevocably lost. This has considerable negative impact when analysing odontological material (enamel, dentin), because the weight of the output material is small. The mineralised material may not therefore be re-used in other studies of this type. Other, non-invasive methods, permitting a further analysis of data from bone sections, are postulated. These include, among others, LA-ICP, PIXE, EDS-SEM. In this paper, preliminary results obtained from the analysis by a scanning microscope equipped with EDS X-ray microanalysis system (energy dispersive X-ray spectroscopy) are presented, demonstrating its usefulness in such research and analyses as performed by Fourier Transform Infrared Spectrometry.

Energy Dispersive X-ray Spectroscopy

The method is presented in the diagram below. If one of the electrons is knocked

out by another electron from the beam with which the carbon- or gold-sprinkled sample (e.g., a tooth) is bombarded, an electron from an orbit further away from the nucleus will 'leap' to fill the empty space. The difference in energy between the two levels of the atom will be radiated as an X-ray quantum. The energy of this quantum for a given element is strictly defined and is thus a diagnostic feature. The spectrometer counts the X-ray pulses (X-ray intensity) and sorts them according to energy – which forms the basis for determining the spectrum.

A preliminary analysis was performed on polished sections of permanent teeth from contemporary and historical subjects. Figure 8 presents measurement points on the enamel and the dentin and the spectra created by the analyses. For each point on the enamel and the dentin a spectrum was determined, on the basis of which a % share of Ca and P was calculated for contemporary and ancient teeth (Table 2).

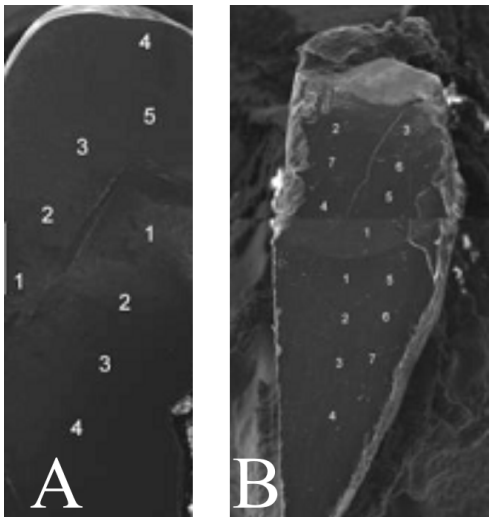

Fig. 8. Analysed places by EDS system in contemporary (A) and ancient teeth (B).

Fourier Transform Infrared Spectrometry

The verification of diagenetic processes while analysing stable isotopes from apatite phosphates and carbonates involves determining the extent to which the bones are crystallised. Despite the fact that phosphate groups are less susceptible than carbon groups to post mortem changes, it does not mean that such changes cannot occur. Post mortem substitution of phosphorus with ions concerns primarily Si, Al and B [Kohn *et al.* 1999]. Currently, the most frequently used reference method for studying changes in bone apatite crystallisation and their diagenetic changes, namely of the phosphate group (PO_4), is Fourier Transform Infrared Spectrometry (FTIR) [White *et al.* 1998]. The spectrometer analyses the spectrum of the powdered bone and allows us to specify the crystallinity index (CI), reflecting changes in bone structure. For contemporary bone, CI ranges from 2.50 to 3.3 [Thompson *et al.* 2009]. For osteological material, these values should not exceed 3.5 (preferably <3.3). This gives us the basis for establishing the extent of any diagenetic changes connected with the destruction of phosphate radicals in the apatite. Three recorded sets of absorbance for three enamel sections from chronologically varied subjects are shown in the diagram below (2.1 contemporary, 3.1 medieval, and 10.3 Neolithic enamel). It can be seen that the archeological samples do not deviate from the contemporary model. The sets are almost identical – the CI indexes are respectively (CI 2.1 = 3.21, CI 3.1 = 3.22, CI 10.3 = 3.06) (Fig. 9).

The results, which conform to standard values, allow us to conclude that the above samples are suitable for further research on oxygen isotope analysis. As stated before, despite the fact that phosphates are resistant

Table 2. Data from EDS examination

Part of tooth	Material	P (wt. %)*	Ca (wt. %)*	Ca/P
Enamel	Contemporary tooth	17.45 ±0.29	37.27±0.28	2.14
	Ancient tooth	18.78±0.94	38.28±1.05	2.04
Dentine	Contemporary tooth	15.96±0.17	32.32±0.18	2.03
	Ancient tooth	16.96±0.23	34.59±0.46	2.04

* wt. % – weight percent

to diagenetic processes, they may undergo changes (substitution of elements, e.g., Si). The diagram below shows the absorbance line obtained during an analysis of a Neolithic femur of an individual discussed earlier (enamel analysis 10.3). In the vicinity of the phosphate groups a peak connected with silicates or exogenous carbonates is observed. This is highly significant even though the CI index conforms to the standard. Obviously, oxygen isotopes connected with exogenous minerals may distort the final results of analyses. Thus, apart from quantitative analyses (calculating the CI), qualitative analyses are recommended (observation of the absorbance line), as well as specifying the extent to which a sample is contaminated on the basis of any additional, non-biogenic peaks. In these particular cases, an individual's enamel does not demonstrate any diagenetic changes. On the other hand, the bone, in spite of a good CI index, suggests diagenesis has occurred (Fig. 10).

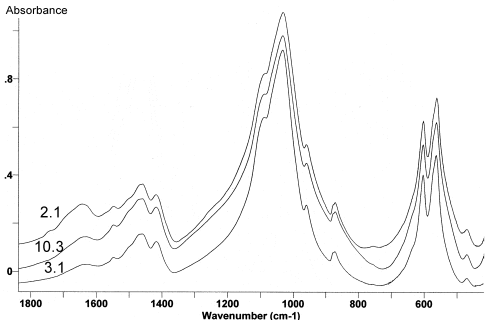

Fig. 9. States of preservation of contemporary (2.1), medieval (3.1) and Neolithic (10.3) enamel.

Conclusions and prospects

Chemical analyses of historical and prehistoric bone material provide us with complex knowledge in bioarcheological studies. They can be used for reconstructing diet, migration, climate changes or the weaning process. Importantly, the analysis of enamel, dentin and bones allows researchers to gather data on life strategies of an individual by retrospectively tracing his various ontogenetic phases. This is made possible by knowledge of the mineralization periods of individual permanent and deciduous teeth while considering differences between enamel and dentin as well as different bone remodelling rates depending on the age of the individual. Yet, the large interpretative potential linked to isotope analyses of bone material is severely limited by unpredictable diagenesis. Consequently, the research employs a specialised and complex set of techniques and methods by which we may verify post mortem changes in the chemical structure of the material. Only after precise quantification of any possible diagenetic changes can the results be interpreted. In chemical procedures of isotope analysis, the final result is always complicated by greater or smaller error. This uncertainty

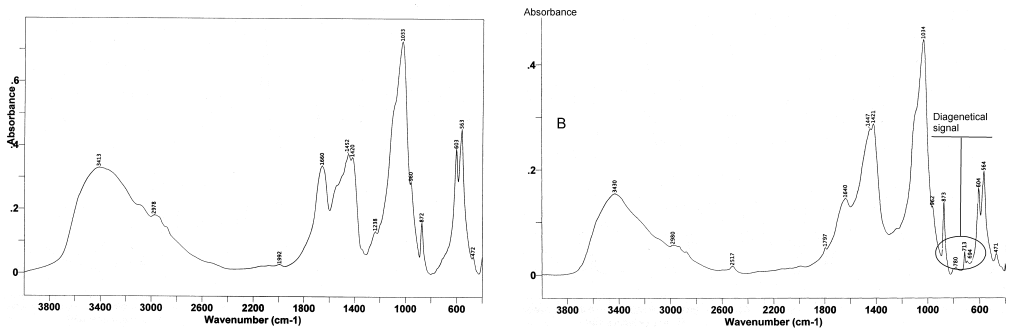

Fig. 10. Differences between preservation of enamel (A) and cortical bone (B) in the same individual from the Neolith period. Graph B shows a peak connected with silicates or exogenous carbonates in the vicinity of the phosphate groups.

is connected with potential errors made at different stages of experiments. Potential sources of error and their relative proportions are as follows: data processing 10%, measurement and calibration 30%, and collecting and preparing samples 60%. Bioarcheological studies also involve the resolution of diagenetic processes. Results in the form of diagenetic, but qualified as biogenic signals, provide geochemical information (relevant for geologists) and not biochemical information (relevant for anthropologists), and no manipulation of the data can alter this fact. This is the reason that for anthropological applications in elemental and isotope tests it is necessary to thoroughly analyse material, starting from site work (taking soil samples and bones), to assessing the organic and inorganic structure of investigated bones and teeth. Archeological data, water samples (oxygen isotopes) and environmental soil/rock samples (strontium isotopes) are essential for determining the archeological, geophysical and trophic background. Note that there is a close relationship between the loss of the organic bone (% collagen, C/N) and its crystallisation (Ca/P, CI). Generally this is a straight positive relationship [Lee-Thorp and van der Merwe 1991, Ambrose and Nor 1993, Stephan 2000]. The most reliable research strategy connected with isotope analyses is to use apatites from enamel and dentin or bones containing large amounts of original collagen [Pearsal 2008]. It is impossible to accurately estimate the quality of preserved bone apatites if the concentration of collagen is low. However, bones deprived of organic components (with higher CIs) are more exposed to post mortem isotopic and elemental changes. Another important issue is that high organic structure content hinders the analytical process of obtaining phosphates and carbonates from the bones. Therefore, efficient laboratory procedures, which yield as large quantity of non-organic material for analysis as possible should be used [Stephan 2000]. The crystallinity (CI) of the bone does not always conform to the standard of modern material (2.5–3.3) [Thompson *et al.* 2009]. For archeological material these values should not exceed CI = 3.5 [White *et al.* 1998]. It frequently happens that, despite results higher than the above-mentioned, the material is suitable for further analyses. This stems from the fact that, for example, loss of a certain phosphate fraction is not always connected with changes in isotope

ratios of oxygen. If the material does not demonstrate a significant correlation between oxygen isotopes and the CI index (as for contemporary bones and teeth), this means biogenic (unchanged) isotope composition [White *et al.* 1998]. Unfortunately, this knowledge is accessed only after all possible analyses. Therefore, biological interpretation of data from chemical analysis of bones and teeth should be preceded by a verification of any diagenetic changes in the investigated material.

Experimental research is carried out in multiple directions. Gaining new, non-invasive techniques makes it possible to reuse the analysed material, whose weight loss is currently not more than 10–20 mg. Creating theoretical and experimental models gives us the potential to verify the usefulness of the analysed chemical elements for bioarchaoelogical research (e.g., exclusion of zinc as diet index). On one hand, such models allow us to understand the complex nature and physiology of isotope and element fractionation in reference to diet and habitat and, on the other, to evaluate their susceptibility to taphonomic changes. New applications, which lead to comprehending the history of life strategies of an individual involve the use of various bone and tooth sections. This entails the reconstruction of diet (including the weaning process) as well as migrations. Reduction of the weight of samples used for analyses allows us to study tooth enamel in relation to the neonatal line, and thus to observe the period before birth. Enamel analyses shifts the limits of studying human biology at the earliest stages of human ontogenesis and also the age of the investigated remains. It is then possible to study fossilised bone sections, which breaks new ground for research into human evolution. As a result, isotope analyses may reveal a hitherto unknown world of our ancestors.

Notes

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Streszczenie

Analizy chemiczne historycznego i przedhistorycznego materiału kostnego dostarczają kompleksowej wiedzy w badaniach bioarcheologicznych i mogą być wykorzystane do rekonstrukcji diety, migracji, zmian klimatycznych czy też procesu odstawienia od piersi. Ważnym aspektem tych badań jest możliwość uzyskania wiedzy o strategiach życiowych osobnika dzięki śledzeniu retrospektywnemu różnych jego faz ontogenetycznych. Jest to możliwe dzięki znanym okresom mineralizacji poszczególnych typów zębów stałych i mlecznych (z uwzględnieniem różnic pomiędzy szkliwem a zębiną), a także odmienności tempa remodelingu kostnego w zależności od wieku osobnika. Ponieważ duży potencjał interpretacyjny, który niosą ze sobą analizy izotopowe materiału kostnego podlega jednak znacznym ograniczeniom, związanym z nieprzewidywalną diagenezą, w badaniach wykorzystuje się specjalne metody pozwalające weryfikować pośmiertne zmiany struktury chemicznej materiału. W procedurach chemicznych analiz izotopowych efekt końcowy badań jest zawsze obarczony większymi lub mniejszymi błędami wpływającymi na ostateczny wynik. Ich źródła można przedstawić następująco: obróbka danych 10%, pomiar i kalibracja 30%, pobieranie i przygotowanie próbki 60%. Uzyskane wyniki będące efektem zmian diagenetycznych dostarczają wiedzy geochemicznej (interesującej geologów) a nie biochemicznej (interesującej antropologów), w związku z tym późniejsze próby interpretacji danych diagenetycznych nie są zasadne i nie wnoszą również żadnej istotnej wiedzy biologicznej. Dlatego gdy przedmiotem badań antropologicznych są pierwiastki i ich izotopy, niezbędna jest kompleksowa analiza materiału, uwzględniająca ocenę organicznej i nieorganicznej struktury kości i zębów.

Niezbędne są również dane archeozoologiczne oraz próbki wody środowiskowej (izotopy tlenu) i skał rodzimych (izotopy strontu) w celu określenia tła archeologicznego, geofizycznego i troficznego. Należy pamiętać, że istnieje prostoliniowa dodatnia zależność pomiędzy utratą frakcji organicznej kości (% kolagenu, C/N) a stopniem jej krystalizacji (Ca/P, CI). Najlepszą strategią badawczą związaną z analizami izotopowymi jest wykorzystanie apatytów pochodzących ze szkliwa i z zębiny lub kości zawierających dużą ilość oryginalnego kolagenu. Kości pozbawione składników organicznych są bardziej narażone na pośmiertne zmiany izotopowe oraz pierwiastkowe. Należy zwrócić także uwagę, że duża zawartość struktur organicznych utrudnia pozyskiwanie fosforanów i węglanów z kości. Nie zawsze uzyskane wyniki stopnia krystaliczności kości (CI) mieszczą się w zakresie odpowiadającym współczesnym materiałom (2,5–3,3). Dla materiału archeologicznego wartości te nie powinny przekraczać CI = 3,5. Zdarza się jednak, że pomimo wyższego CI materiał jest przydatny do dalszych analiz, ponieważ np. utrata pewnej części fosforanów nie zawsze jest związana ze zmianami stosunków izotopowych tlenu. Jeżeli materiał nie wykazuje istotnej korelacji pomiędzy izotopami tlenu a wskaźnikiem CI (tak jak we współczesnych zębach i kościach), oznacza to biogenny (niezmieniony) skład izotopowy. Podsumowując należy stwierdzić, że przed biologiczną interpretacją danych uzyskanych z analiz chemicznych kości i zębów trzeba zawsze zweryfikować stopień ewentualnych zmian diagenetycznych w badanym materiale.