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Detection of Dietary Changes by Intra-tooth Carbon and Nitrogen Isotopic Analysis: An Experimental Study of Dentine Collagen of Cattle (*Bos taurus*)

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Hypsodont teeth potentially contain a record of dietary or environmental changes occurring during their growth. The aim of our study is to understand how a dietary change is recorded in dentine collagen. Analyses were conducted on five steers (*Bos taurus*) raised in an experimental farm. From birth until weaning the steers were fed on a C_3 diet; after weaning they were fattened on a C_4/C_3 mixed diet until slaughter. Dentine collagen was sampled on demineralized molars from top to bottom. The change from the C_3 to the C_4/C_3 diet and weaning are both reflected in intra-tooth variations in $\delta^{13}C$ and $\delta^{15}N$ values, respectively. The abrupt change in carbon isotopic composition of the diet is reflected by a progressive change of the dentine collagen $\delta^{13}C$ values. The gradual change may reflect sampling strategy and/or gradual turnover of the metabolic nutrient pool. The weaning process is reflected by a decrease in $\delta^{15}N$ that exactly coincides with increase in $\delta^{13}C$. This demonstrates that when steers are weaned to a protein-poor diet, $\delta^{15}N$ traces the cessation of suckling. Archaeological applications of this study are considered, including determination of the duration of lactation in prehistoric herds, and detection of residential mobility in cattle herders.

Keywords: DIETARY CHANGE, WEANING, NITROGEN AND CARBON ISOTOPES, INTRA-INDIVIDUAL VARIATION, DENTINE COLLAGEN, *BOS TAURUS*.

Introduction

Numerous studies dealing with palaeodietary issues are based on data obtained from bone collagen and carbonate hydroxylapatite analysis (for a review see Ambrose, 1993). Enamel has also been widely studied, usually for environmental and climatic reconstruction (Bryant *et al*., 1996*a*, *b*; Fricke & O'Neil, 1996; Fricke *et al*., 1998). Dentine collagen and apatite are rarely used for dietary and environmental reconstruction. Some studies (Koch *et al*., 1989; Bocherens *et al*., 1994, 1995, 1997; Koch *et al*., 1995; Sealy *et al*., 1995; Hobson & Sease, 1998; Balasse *et al*., 1999; Wright & Schwarcz, 1999) exploit the fact that dentine, once formed, is not replaced (Gage *et al*., 1989) and preserves the isotopic composition from its formation time. In brachyodont species, teeth are formed over a short time and record an isotopic signature of diet and environment during the very beginning of the individual's life. Bocherens *et al*. (1992, 1994, 1995, 1997) have shown that in adult brachyodont mammals a consistent difference exists between the nitrogen isotopic composition of dentine collagen and bone collagen, most likely resulting from dietary change during weaning. Sealy *et al*. (1995) have

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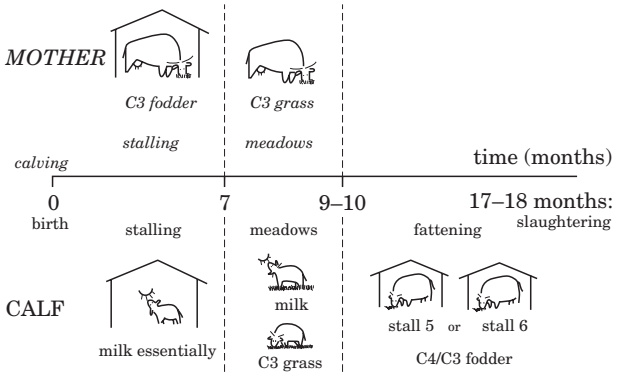

Figure 1. Chronology of the dietary changes in the steers, from birth to death.

also suggested that a difference between the isotopic composition of bone and dentine may reflect a change of diet and/or place of residence during lifetime.

Comparison of the isotopic compositions of dentine and bone may reveal a dietary or environmental change between childhood and adulthood. However, while the age at which dentine forms can be accurately determined, that of bone cannot because it continues to be remodelled and replaced throughout life. Because of its variable turnover rate, bone does not provide accurate information about the time when this change occurred. Information on the chronology of isotopic changes can be obtained with a study at the inter- and intra-tooth scale. Sampling of small growth increments in proboscidean tusks (Koch *et al.*, 1989) and marine mammal teeth (Hobson & Sease, 1998) demonstrate that dentine preserves a high resolution isotopic record of physiological, environmental or dietary events throughout life.

Sampling of small growth increments is unfortunately not applicable to the majority of terrestrial mammals, either because they do not show any clear cyclic growth patterns in dentine, or because their teeth are too small. Sample size becomes particularly problematic when the methodology is applied to ancient material. In such cases, low collagen contents and the dissolution of apatite during purification must be compensated by increased sample size. We tested another sampling procedure on modern bovine tooth dentine: following the tooth growth in length rather than in thickness.

Experimental Design and Materials

The study was performed on five steers (*Bos taurus*) raised in Vendée (France) and slaughtered in 1996, on an experimental farm, where their diet was controlled from birth to slaughter (Figure 1). During gestation, the mother cows grazed on meadows of grass following the C_3 photosynthetic pathway. From birth up to the age of 7 months, the calves were kept with their mothers in stalls. The mothers were fed with C_3 plants (grass, silage, hay, wheat and soy bean meal), and the calves were raised mainly on milk. From 7 to 9–10 months after birth, the calves followed their mothers to pasture where both ate C_3 grass. During this period the calves were still suckling, but were progressively weaned. The weaning process was achieved with the separation of the calves from the mothers when they were around 9–10 months old. After weaning they were kept in stalls and fattened with a C_4/C_3 mixed food until death. The individuals labelled V2, V4 and V6 were kept in stall 6, where they were raised on a mixture of maize silage (C_4 plant), soybean meal and wheat (C_3 plants). The individuals labelled V3 and V5 were kept in stall 5, where wheat was replaced by maize grain (Table 1). The steers were slaughtered 8 months after the dietary change, when they were 17 to 18 months old.

Milk consumption puts the young suckling mammal in a position of consumer relative to its mother: the young is one level higher in the trophic web than its mother. A step-wise increase in $\delta^{15}N$ values of 3–3·5‰ has been observed between trophic levels (DeNiro & Epstein, 1981; Minagawa & Wada, 1984; Schoeninger & DeNiro, 1984). Weaning involves descending to the same trophic level as the mother, resulting in a change in the nitrogen isotopic composition of the synthesized

Table 1. Carbon and nitrogen content (%), carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic composition of each ingredient of the fattening diet

	Component	Weight (kg)	% C	% N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Prot. contribution
Meadow	Grass	*ad lib.*			**−26·5**	**+1·0**	C_3=100%
Stall 5	Maize silage (C_4)	7·0	44·6	1·1	−12·1	+4·4	C_4=36·6%
	Maize grain (C_4)	2·5	44·6	1·4	−11·6	+5·0	C_4=16·6%
	Soybean (C_3)	1·2	43·5	8·2	−25·1	+0·7	C_3=46·8%
	Total				**−13·4**	**+2·8**	**$\delta^{13}C_{prot}$ = −18·1‰**
Stall 6	Maize silage (C_4)	7·0	44·6	1·1	−12·1	+4·4	C_4=34·5%
	Soybean (C_3)	1·2	43·5	8·2	−25·1	+0·7	C_3=44·0%
	Wheat (C_3)	3·0	43·8	1·6	−25·4	+4·2	C_3=21·5%
	Total				**−17·0**	**+2·7**	**$\delta^{13}C_{prot}$ = −20·8‰**

The carbon isotopic composition of the protein part of the whole diet ($\delta^{13}C_{prot}$) is an approximation (see the text)

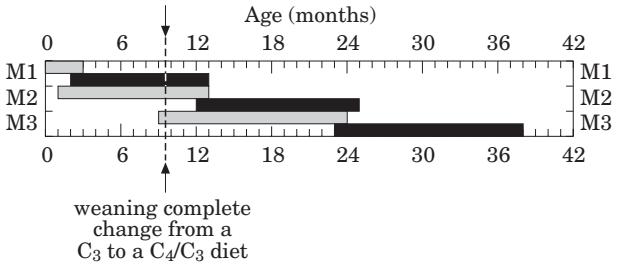

Figure 2. Chronology of development of mandibular molars in cattle (after data from Brown *et al*., 1960). The data were acquired by radiography on 75 animals of different breeds and nutritional background, but of known ages, which were equally distributed from birth to slightly older than 2 years. The first part of the study by Brown *et al*. (1960), which presents the chronology of crown (▨) and root (■) formation for the incisor and canine teeth in 869 animals, shows that no significant difference in the chronology of tooth development can be found in the different breeds or sexes.

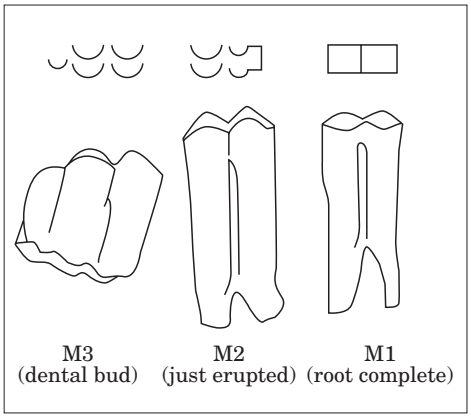

Figure 3. Development stage and crown attrition (code after Payne, 1973) of the steers' molar teeth at the time of death. The first molar's (M1) growth is complete and its two lophs are worn. The second molar (M2) is erupting, its root is half formed and attrition of the anterior loph has begun, but the posterior one is not yet worn. The third molar (M3) is still a dental bud.

collagen (Fogel *et al*., 1989; Katzenberg *et al*., 1996). This nitrogen isotopic change should be recorded in the dentine of teeth whose growth spans the weaning period. Similarly, the change from a pure C_3 to a C_4/C_3 mixed diet should be reflected in the collagen carbon isotopic composition, because C_3 plants and C_4 plants have different carbon isotopic compositions linked to their different photosynthetic modes (Deines, 1980; O'Leary, 1981). It is important to note that the weaning dietary change was progressive and gradual, but the change in plant-food diet was sudden. There was no interference with the weaning process, which occurred gradually from the first months of life until the separation from the mother. The change from a pure C_3 to a C_4/C_3 mixed diet was, however, very abrupt.

These sequential changes in diet were expected to be reflected in intra-tooth variation patterns of the dentine isotopic composition. Numerous histological studies conducted on marine and terrestrial mammals' teeth have shown that when a tooth is growing in length it also gains in thickness, due to the fact that dentine is laid down in a succession of stacked cones (for a review see Hillson, 1986). The teeth to be analysed were chosen according to the chronology of mandibular tooth development in cattle (Figure 2). At age 9–10 months, when the diet was changed, the first (M1) and second (M2) molars were growing fast, and third molar (M3) growth was just beginning. The second molar is especially useful for this study: its growth begins soon after birth and continues beyond the age at which the steers were slaughtered. Moreover, its crown was scarcely worn: the tooth was erupting, but not completely erupted. This tooth should thus preserve a record of diet isotopic composition from near birth to almost 1 year after weaning. The dietary change was expected to be detectable in the last third of the M2 crown. The first molar should also record the change of diet in its roots. The third molar, present as a dental bud 17–18 months after birth, formed mainly after the dietary change: the C_3-milk signal should only be detectable in the very upper part of its crown. All three molars were analysed: M2 seems to offer the best recording conditions; M1 growth in length was almost complete when the diet change occurred, but the tooth has since this time accumulated some material in thickness; M3 should show almost no isotopic composition variation in its length, providing an internal reference for intra-tooth variability after dietary change.

Methods

Each ingredient of the post-weaning diet (maize silage, maize grain, soy bean and wheat) was sampled, as well as some grass from meadows. The samples were dried (60°C for 24 h) and homogenized by grinding to less than 0·3 mm. A few milligrams (4 to 6 mg) of each ingredient was analysed for carbon and nitrogen content and isotopic composition (Bocherens *et al*., 1997). The values shown in Table 1 are means of five analyses of each sample.

The three left molars of each steer were extracted from jawbones. First and second molars have anterior and posterior lophs of equal size; third molars have three lophs. The development stage of the teeth at time of the steers' death is shown in Figure 3. Each first and second molar was cut in half lengthwise. The posterior half, which is less worn than the anterior one, was cut lengthwise again in two parts: only a quarter of the tooth was treated. For the third molars (dental buds), the anterior loph was analysed. Teeth were demineralized in EDTA (0·5 M, pH=7·4) at room temperature (Tuross *et al*., 1988). The solution was renewed every 3 days. Complete demineralization took 2 months. The collagen isomorph was thoroughly rinsed with distilled water and then cut into 4-mm sections with a scalpel from the top to the bottom of the tooth. The M3 from

individuals V6 and V3 were only sampled every 8 millimeters. These samples were homogenized by solubilization (HCl 10^{-2} M, 100°C, 17 h), filtered and freeze-dried. Nitrogen and carbon isotopic composition of the collagen was measured by isotope ratio mass spectrometry as previously described (Bocherens *et al.*, 1997). The analytical precision is 0·2‰ for $\delta^{15}N$ analysis and 0·1‰ for $\delta^{13}C$ analysis.

Results

The carbon content, nitrogen content, and carbon and nitrogen isotopic compositions of each component of the diet are presented in Table 1. The isotopic composition of the bulk post-weaning diet mix was calculated according to the proportional contribution of each ingredient to the total carbon and nitrogen contents of the diet. We are aware that the collagen isotopic composition mainly reflects that of the protein fraction of the diet (Ambrose & Norr, 1993; Tieszen & Fagre, 1993). The estimated $\delta^{13}C$ values of the protein part of the whole diet (Table 1) are based on the assumption that the isotopic composition of protein is not radically different from that of the whole plant. The aim of our study is to detect the change of the diet isotopic composition, rather than to determine the absolute diet-tissue isotopic difference. The difference in the carbon isotopic composition of C_3 and C_4 plants is so large that even if most proteins still come from C_3 plants after the dietary change (46·8% of proteins are from C_3 plants in stall 5, and 65·5% in stall 6), the $\delta^{13}C$ of the post-weaning diet should be different enough from that of the pure C_3 food for the dietary change to be clearly detectable.

The organic matter extracted from dentine is identified as collagen by the carbon content (mean ± 1 σ = 42·2 ± 1·7%; min. = 37·6%; max. = 44·3%), the nitrogen content (mean ± 1 σ = 15·5 ± 1·3%: min. = 13·2%; max. = 16·5%) and the atomic C/N ratio (from 3·1 to 3·3) (data not shown). The isotopic values are presented in Table 2 and in Figures 4 & 5. The sample numbers have been assigned from the top—formed the earliest in life—to the bottom of the tooth—formed latest.

The change from the pure C_3 to the C_4/C_3 mixed diet and weaning are recorded in the carbon and nitrogen isotopic compositions of the first and second molars' dentine (Figures 4 & 5). The dentine isotopic composition from the top of these teeth reflects a C_3-milk based diet (M1: $\delta^{13}C = -23{\cdot}4$ to $-20{\cdot}7$‰ and $\delta^{15}N = +7{\cdot}8$ to $+8{\cdot}9$‰; M2: $\delta^{13}C = -21{\cdot}2$ to $-19{\cdot}7$‰ and $\delta^{15}N = +6{\cdot}9$ to $+8{\cdot}4$‰). The isotopic composition of the samples from the bottom of the teeth reflects a C_4/C_3-herbivorous diet (M1: $\delta^{13}C = -14{\cdot}5$ to $-10{\cdot}9$‰ and $\delta^{15}N = +5$ to $+5{\cdot}8$‰; M2: $\delta^{13}C = -14{\cdot}2$ to $-10{\cdot}5$‰ and $\delta^{15}N = +5{\cdot}2$ to $+5{\cdot}7$‰). The isotopic composition of the third molars' dentine refers mainly to the C_4/C_3-herbivorous diet.

The pattern of change in the dentine isotopic composition is similar from one individual to another. The $\delta^{13}C$ range of variation is slightly higher in individuals V3 and V5 because of the higher proportion of C_4 ingredients in their diet. The isotopic composition of the upper dentine samples from the first and second molars is relatively stable—however, in the first molar crown, this stability seems to be less clear for $\delta^{13}C$ values, where a very smooth, continuous increasing gradient can be observed: this point will be discussed later. The isotopic composition of the successive dentine samples changes systematically to higher $\delta^{13}C$ values and lower $\delta^{15}N$ values. In the first molar, this change begins midway along the length of the tooth and continues to near the bottom of the root where the values become stable again. Stabilization occurs higher in the second molar, in the lower zone of the crown. Isotopic values from the third molar are stable below the top, which seems to be influenced by the C_3-milk based diet. The small variability of the isotopic composition of this tooth shows the stability of the isotopic composition of the post-weaning diet.

Discussion

$\delta^{13}C$ values record change from a pure C_3 to a C_4/C_3 mixed diet

The sudden change from the pure C_3 to the C_4/C_3 mixed diet is reflected by a gradual ^{13}C-enrichment in dentine samples (Figure 4). However sudden a dietary change may be, it would likely never be reflected by an abrupt change of isotopic composition of the synthesized tissues, because of an equilibration period during which turnover of the metabolic pool occurs. Recycling of nutrients from the breakdown of tissues and stored metabolites results in a mix of both ante- and post-dietary change isotopic signals in the newly synthesized collagen. A study conducted by Jones *et al.* (1981) on steers fed on C_4, C_3 and C_4 diets in sequence gives a quantitative estimation of this equilibration time. During the experiment, the steers hair was regularly clipped and the regrown hair isotopically analysed. Isotopic equilibrium was progressively reached in hair around 50 to 65 days—depending on the steer—after the first dietary change, and around 70 days after the second dietary change (ibid.: figure 1). Considering the time lag for the new portion of the hair to reach the skin surface, we roughly estimate the equilibration time to be 1·5 to 2 months. These data were obtained by Jones and colleagues on still-growing individuals ("steers") and can be applied to our individuals.

If what is recorded in dentine between points A and B of the isotopic curves (Figures 4 & 5) really corresponds to an equilibration period, it would mean that the dietary change occurred at point A and isotopic equilibrium was reached at B. If each 4-mm dentine sample represents roughly 1 month's growth in length, then the transition would have lasted from

Table 2. Carbon and nitrogen isotopic composition of collagen extracted from each dentine sample

Stall 6									Stall 5					
Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
V2M1-1	−20·7	+8·0	V4M1-1	−22·0	+8·7	V6M1-1	−22·4	+8·9	V5M1-1	−21·0	+8·6	V3M1-1	−23·4	+7·8
2	−21·3	+7·9	2	−21·8	+8·8	2	−22·0	+8·6	2	−21·1	+8·8	2	−22·9	+7·8
3	−21·4	+7·9	3	−21·6	+8·5	3	−21·8	+8·7	3	−20·4	+8·5	3	−22·6	+7·8
4	−21·1	+7·9	4	−21·4	+8·8	4	−21·6	+8·6	4	−20·5	+8·4	4	−22·3	+8·1
5	−20·6	+8·0	5	−21·2	+8·4	5	−21·4	+8·5	5	−19·8	+8·4	5	−22·0	+8·1
6	−20·0	+7·9	6	−20·9	+8·4	6	−21·2	+8·5	6	−19·7	+8·2	6	−21·2	+8·1
7	−19·3	+7·5	7	−20·2	+7·9	7	−21·2	+8·4	7	−19·2	+8·1	7	−21·3	+7·7
8	−18·6	+7·1	8	−19·3	+7·4	8	−20·5	+8·2	8	−18·9	+7·7	8	−20·5	+7·5
9	−17·9	+6·8	9	−18·5	+7·0	9	−20·0	+7·8	9	−19·1	+7·5	9	−19·9	+7·3
10	−17·2	+6·7	10	−17·6	+6·4	10	−19·1	+7·4	10	−17·8	+7·4	10	−18·7	+7·2
11	−16·9	+6·4	11	−17·2	+6·5	11	−18·4	+7·1	11	−18·0	+7·8	11	−18·7	+6·8
12	−16·9	+6·6	12	−16·9	+6·2	12	−18·3	+6·9	12	−15·8	+7·4	12	−19·0	+6·6
13	−15·9	+6·3	13	−13·9	+5·2	13	−18·5	+6·9	13	−12·8	+5·9	13	−18·2	+6·9
14	−13·7	+5·5	14	−12·6	+4·8	14	−17·5	+6·4	14	−11·8	+5·5	14	−16·8	+6·1
15	−13·5	+5·1	15	−13·5	+5·0	15	−14·4	+5·6	15	−10·9	+5·8	15	−13·9	+5·3
16	−14·5	+5·3				16	−13·1	+5·4				16	−13·5	+5·0
						17	−14·1	+5·1						
V2M2-1	−19·7	+6·9	V4M2-1	−21·1	+7·8	V6M2-1	−20·6	+8·0	V5M2-1	−21·2	+8·1	V3M2-1	−21·1	+8·4
2	−18·7	+7·1	2	−19·9	+7·8	2	−19·1	+7·8	2	−20·7	+8·0	2	−19·7	+7·9
3	−18·9	+7·1	3	−19·6	+7·7	3	−19·5	+7·7	3	−20·5	+7·6	3	−18·3	+7·4
4	−18·7	+7·0	4	−18·9	+7·5	4	−18·7	+7·6	4	−20·8	+8·1	4	−19·0	+7·9
5	−18·7	+7·1	5	−18·0	+7·0	5	−18·0	+7·1	5	−20·4	+8·0	5	−18·4	+7·9
6	−18·3	+7·1	6	−17·2	+6·9	6	−17·6	+6·9	6	−20·1	+8·0	6	−18·5	+7·3
7	−17·9	+7·1	7	−16·7	+6·8	7	−17·0	+6·7	7	−19·4	+8·0	7	−18·1	+8·0
8	−16·8	+6·7	8	−16·2	+6·6	8	−15·9	+6·6	8	−18·0	+7·8	8	−17·7	+8·3
9	−15·3	+6·2	9	−15·3	+6·3	9	−14·5	+6·3	9	−15·9	+7·4	9	−15·5	+7·5
10	−13·8	+5·7	10	−13·9	+6·0	10	−13·4	+5·5	10	−14·1	+6·7	10	−12·9	+6·9
11	−13·2	+5·3	11	−12·8	+5·6	11	−12·6	+6·0	11	−12·0	+6·2	11	−13·0	+6·0
12	−13·3	+5·5	12	−12·7	+5·8	12	−13·6	+5·8	12	−11·3	+6·1	12	−13·0	+5·8
13	−13·3	+5·7	13	−12·7	+5·9	13	−13·9	+5·8	13	−10·9	+6·0	13	−11·9	+5·3
14	−13·7	+5·8	14	−13·3	+5·7	14	−13·8	+5·8	14	−10·5	+6·2	14	−12·1	+5·4
15	−14·0	+5·7	15	−13·6	+5·4	15	−14·1	+5·7	15	−10·5	+6·2	15	−11·2	+5·3
16	−14·1	+5·6	Δ	8·4	2·4	Δ	8·0	2·5	16	−10·5	+5·7	16	−12·3	+5·2
Δ	6·5	1·8							Δ	10·7	2·4	Δ	9·9	3·2
V2M3-1	−13·3	+5·7	V4M3-1	−17·7	+6·5	V6M3-1	−15·7	+5·9	V5M3-1	−17·2	+7·6	V3M3-1	−12·6	+6·8
2	−13·1	+5·7	2	−14·2	+5·9	2	−12·6	+5·2	2	−13·1	+6·1	2	−11·4	+5·6
3	−13·2	+5·5	3	−12·7	+5·6	3	−13·1	+5·6	3	−11·8	+5·6	3	−11·1	+6·0
4	−13·3	+5·6	4	−12·4	+5·6	4	−13·5	+5·6	4	−11·5	+5·7	4	−10·9	+5·6
5	−13·4	+5·8	5	−12·5	+5·6	5	−14·1	+5·7	5	−11·3	+6·1	5	−10·3	+6·1
6	−13·6	+5·9	6	−12·6	+5·7	6	−14·4	+6·1	6	−10·9	+6·1	6	−10·8	+6·7
7	−14·0	+6·0	7	−12·8	+5·8				7	−10·7	+6·1			
8	−14·0	+6·1	8	−13·2	+5·9				8	−10·6	+6·3			
9	−14·1	+6·1	9	−13·6	+6·0				9	−10·6	+6·5			
10	−14·1	+6·2	10	−13·6	+6·0				10	−10·6	+6·7			
11	−14·1	nd	11	−13·6	+6·3				11	−10·6	+6·9			
			12	−13·4	+6·6									

Sampling was done from the top (sample 1) to the bottom of each tooth, at right angles to the long axis of the tooth.

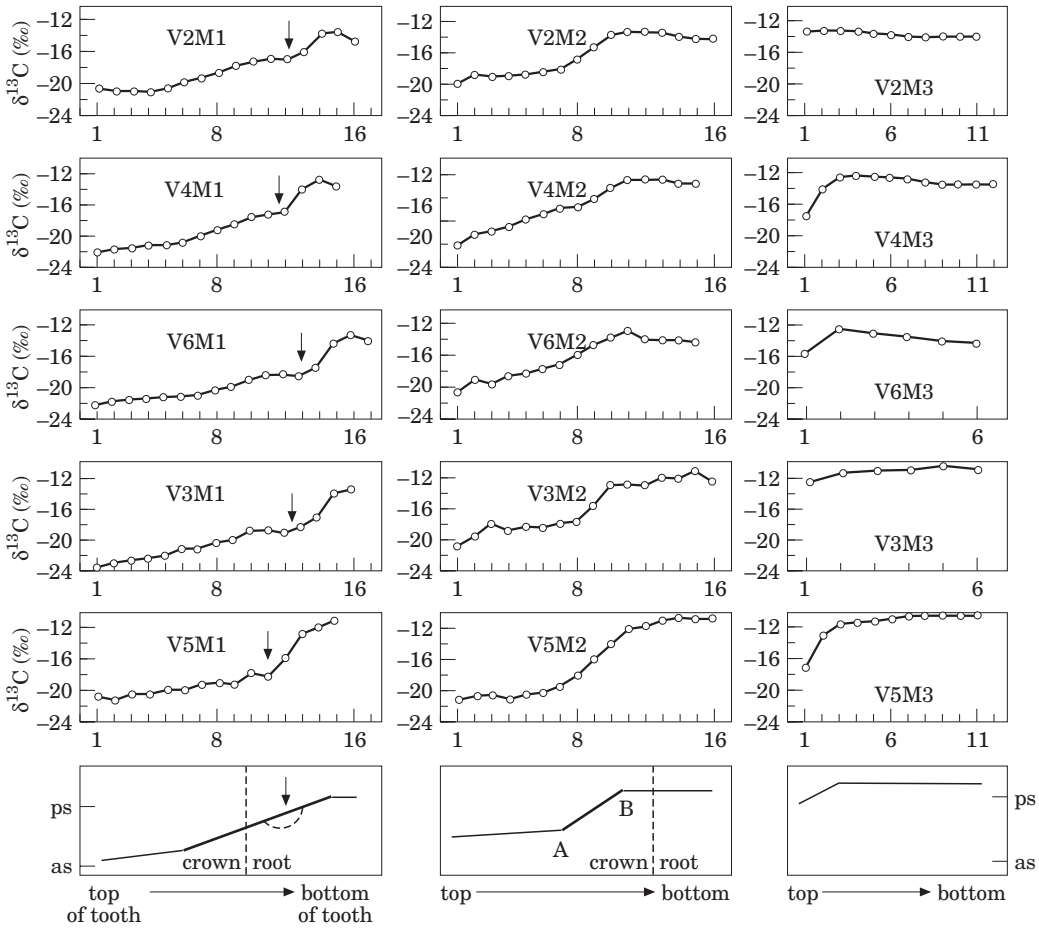

Figure 4. Carbon isotopic composition of the dentine collagen from the first (M1), second (M2) and third (M3) molars of the steers. In each tooth, sample 1 was formed earliest in life. The idealized pattern for each tooth was drawn after superposition of the five individual curves obtained for each tooth. The arrow indicates a shift in the isotopic composition of the first molar dentine, which could reflect a physiological stress resulting from the change from stalls to pasture. Point B marks the tooth length when the dietary change occurred. Point A marks the zone where the isotopic change begins to be detected, due to superposition of new dentine layers on previously formed dentine. as: ante-dietary change signal; ps: post-dietary change signal.

1 to 4 months, which is comparable to the results of Jones *et al.* (1981).

However, point A corresponds to an early growth stage—in length—which refers to a time before the dietary change was initiated. According to the chronology of development of lower molars in cattle, the dietary change should have been recorded in the last third of the second molar crown (Figure 2), but the change in carbon isotope ratios begins in the middle of the crown. What is even more surprising is that the isotopic change is detectable in the first molar crown (Figure 4), although it was expected in the second half of its root. Is there a real disparity between the data shown in Figure 2 and the real timing of the dental development of our steers, which would be later than expected? This is unlikely because the dental development of the steers at death is consistent with the model illustrated in Figure 2 for individuals between 17 and 18 months old.

Turnover of the metabolic pool may have been masked by an artefact due to the use of a sampling procedure that does not accurately follow the geometry of dentine growth (Figure 6). When the isotopic composition of the synthesized collagen changes, some dentine with a new isotopic signal is laid down on dentine synthesized earlier. Because our sampling method cross-cuts growth layers, superposition creates a zone where the two signals are mixed (Figure 6). This would partly explain the gradual change of the dentine isotopic composition even when the record of the dietary change is sudden, and would also explain the fact that the isotopic change appears earlier than expected in the crown of the first molar. This would mean that point B (Figures 4 & 5) actually marks the dental stage (the tooth length) at the time when the isotopic equilibrium was reached, not very long after the dietary change for these fast-growing individuals.

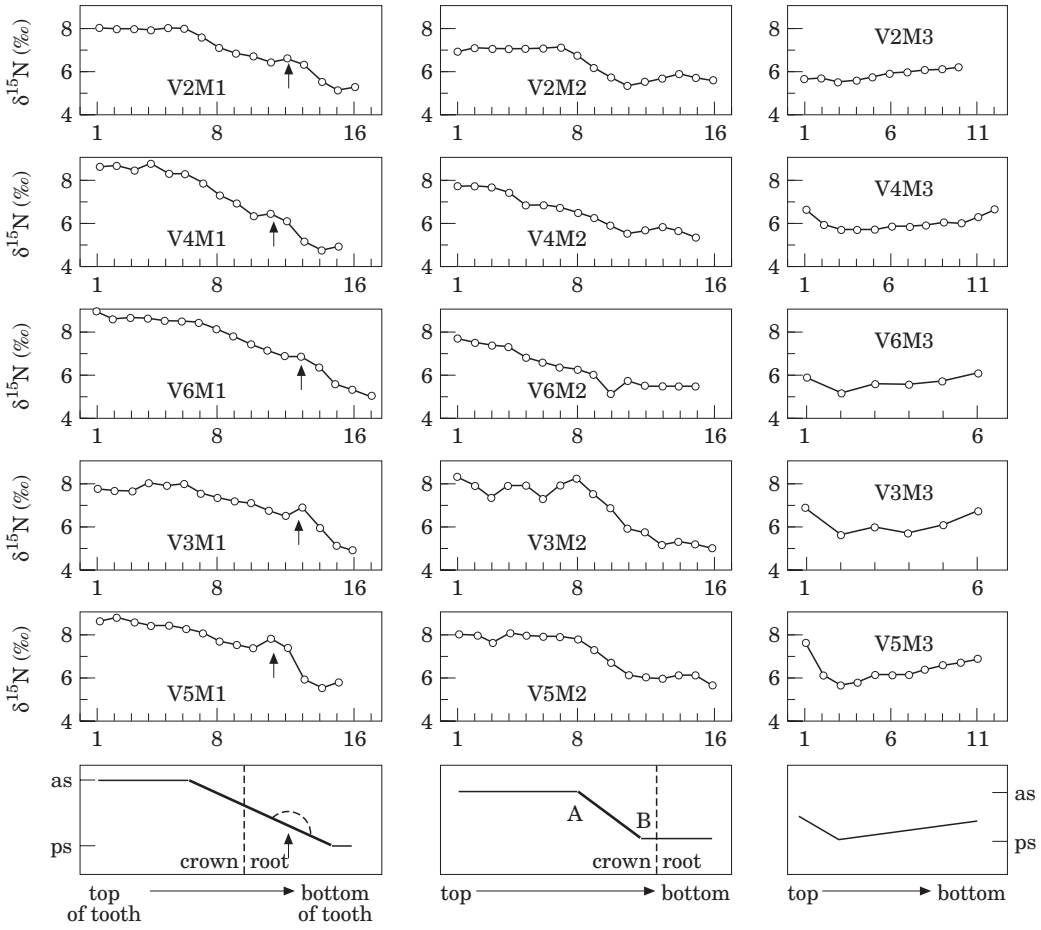

Figure 5. Nitrogen isotopic composition of dentine collagen from molars of steers (see legend for Figure 3). as: ante-dietary change signal; ps: post-dietary change signal.

The complexities of accurate sampling of growth layers in dentine collagen have several consequences. First, using the sampling procedure we tested in this study, it may be difficult to identify an abrupt change of diet: an artefact due to the dentine growth pattern is added to the necessary time for equilibration of the metabolic pool with the new diet isotopic composition. Consequently, a progressive change in dentine isotopic composition must not be interpreted as the record of an equally gradual dietary change. Second, a dietary change will not be reflected by the same pattern of isotopic variation depending on the growth stage of the analysed tooth at time of the individual's death (Figure 6 (b), (c)). The addition of dentine in thickness (growth layers oblique to the growth axis on the side of the loph) could be such that after a certain time the dietary change could appear as having occurred earlier than it really did (Figure 6(c)).

The first, second and third molar teeth were grown in partially overlapping times (Figure 2). Despite that, attempts to superimpose the isotopic curves show that the values are non-coincident (data not shown). This non-coincidence can be easily explained: first, by differences in the rhythm of growth of the different parts of the tooth (for example, the six dentine samples from the M1 root were formed during the same time as were the 12 samples of the M2 crown); second, by the different states of growth of the molar teeth at the time of the diet change—because of the subtle play between the growth in length and in thickness, the variation pattern of isotopic values is the same in the first and the second molars, but the absolute values can be shifted.

$\delta^{15}N$ values record the weaning process

Variation pattern in the first molar. The pattern of variation in $\delta^{15}N$ values is similar to that observed for $\delta^{13}C$ values. However, $\delta^{15}N$ values are more stable than $\delta^{13}C$ values in the first samples of the first molar crown. The change in the dentine carbon isotopic composition in this part of the tooth is probably due to a superposition of dentine synthesized after the dietary change on dentine laid down earlier. In this part of the first molar crown, the deposition of newly synthesized dentine would have been voluminous enough to influence the $\delta^{13}C$ values, whose range of increase was high (mean 8·7‰ between the first and last samples of M2), without influencing the $\delta^{15}N$ values, whose range

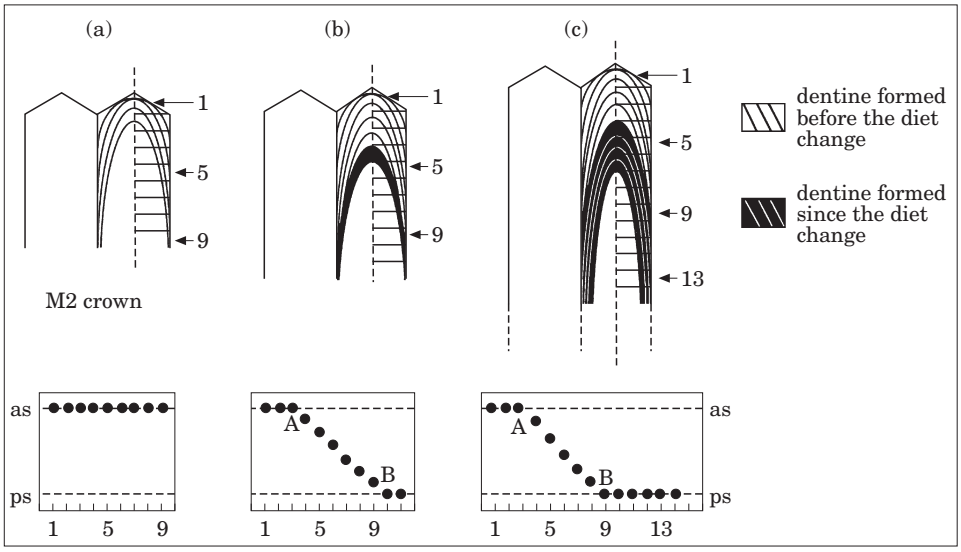

Figure 6. Schematic representation of dentine growth pattern and consequences for the interpretation of data obtained with our sampling procedure. The dietary change occurred between dental stages a and b. Point B (sample 19) marks the growth stage of the tooth when the change occurred. When subsequent dentine layers are superimposed (stage c), point B may be moved, leading to a misinterpretation of the age of the dietary change. as: ante-dietary change signal; ps: post-dietary change signal.

of decrease was much lower (mean 2·5‰ between the first and last samples of M2). An alternative explanation would be that a gradual change occurred in the $\delta^{13}C$ of collagen synthesized during uterine life. As the cows did not undergo any change of diet during pregnancy, the isotopic change could be interpreted as reflecting a shift in the nutritional relationship between the pregnant cow and the calf foetus.

The upper part of the first molar crown was partly formed before birth (Figure 2). The dentine collagen $\delta^{15}N$ values are as high in this part of the tooth as that of collagen synthesized during suckling period. It has been demonstrated that the $\delta^{15}N$ value of a human newlyborn's tissues is similar to that of its mother, with ^{15}N-enrichment occurring later with suckling (Fogel *et al.*, 1989). No similar study was made on modern cows. However, it seems unlikely that the high values measured on the upper part of the first molar crown of the calves correspond to that of the mothers, who spent the greatest part of their gestation in meadows (grass $\delta^{15}N$ values around +1·0‰, see Table 1). Either the measured ^{15}N-enriched signal of the M1 tip is really that of antenatal collagen, which would mean that in *Bos taurus* the collagen synthesized *in utero* is ^{15}N-enriched compared to that of the mother, or, alternatively, the measured signal may not really be an *in utero* signal, because it has been buffered by the superimposition of a new layer of dentine containing collagen synthesized during the suckling period. Dentine is very thick in this upper part of the crown, a lot of dentine has been accumulated since birth, and the tip of the crown is worn. However, we cannot settle this question. The isotopic composition of the antenatal collagen has to be studied in mammals other than humans, because different placental systems may lead to different mother/foetus isotopic relationships.

The variation pattern of $\delta^{15}N$ values in the first molar root is characterized by a small shift towards higher values in the middle of the root (indicated by an arrow and dashed line in Figure 5). A shift towards lower $\delta^{13}C$ values occurs exactly at the same location in the root (Figure 4). The fact that this irregularity appears in both $\delta^{15}N$ and $\delta^{13}C$ values, and that it is present in all five individuals in the same place, suggests that it is not an artefact. This irregularity might reflect the occurrence of a physiological stress when the calves were brought to pasture and first exposed to outside conditions after having spent their entire infancy in stalls. A ^{15}N-enrichment in tissues has been found as a result of water and/or nutritional stress (Ambrose & DeNiro, 1986, 1987, 1989; Sealy *et al.*, 1987; Ambrose, 1991; Hobson *et al.*, 1993). However, no similar influence has ever been observed in the fractionation of tissue carbon. What is observed in these steers could be a remobilization of reserves stocked during the first part of life from the C_3-milk based diet. Such a remobilization might be caused by a stress due to a physiological adaptation to new environmental and nutritional conditions, perhaps with a difference in water balance (Ambrose, 1991, 2000) or catabolism rate linked to the intensification of physical activity and energy expenditure when changing from stalling to grazing.

Variation pattern in the second molar. The range of decrease in $\delta^{15}N$ in the second molar in response to diet change is around 2·5‰ (from 1·8‰ to 3·2‰). This value is close to that observed between human mothers

and nursing offspring (+2·4‰) by Fogel *et al.* (1989). However, this range seems smaller than expected considering the 3–3·5‰ ^{15}N-enrichment observed in other studies as characterizing each trophic step (DeNiro & Epstein, 1981; Minagawa & Wada, 1984; Schoeninger & DeNiro, 1984) and the 3·2–3·6‰ ^{15}N-enrichment actually measured between cow's milk and their diet (Steele & Daniel, 1978; Koyama *et al.*, 1984). We did not analyse milk from the cows nor the fodder they were fed during lactation. Nevertheless, the δ^{15}N value of the grass from the meadows where the calves and mothers spent 2 months is low (1‰), and lower than that of the fattening diet (2·8‰ in stall 5 and 2·7‰ in stall 6). The lower-than-expected range of δ^{15}N values may thus be explained by the fact that the steers were not weaned with the diet from which the mother's milk had been synthesized.

We were interested in observing the way the weaning process, which was initiated earlier in life and occurred gradually over several months, would be isotopically recorded, compared to the very sudden change from the C_3 to the C_4/C_3 mixed fodder. A more accurate comparison of δ^{15}N and δ^{13}C variation patterns was performed after the δ^{15}N and δ^{13}C values from each analysed sample were transformed into amount (%) of collagen synthesized since weaning or the change from the C_3 to the C_4/C_3 diet (Figure 7). The change in isotopic composition, from 100% of collagen synthesized before the dietary change to 100% of collagen synthesized since the dietary change, is the same for δ^{15}N and δ^{13}C values. The two dietary changes are reflected by the same isotopic variation pattern: the progressive weaning process was recorded in dentine as an abrupt event, and only at the time when the calves were separated from the mothers. This demonstrates that δ^{15}N values do not record the progressive lowering of the amount of milk consumed, but the cessation of milk consumption. This is easily explained by the fact that our calves have been weaned to grass, which contains lower amounts of protein—and nitrogen—than milk. Milk will thus make the largest contribution to collagen nitrogen. This also explains why the very upper part of the third molar crown, formed during the 10th month of the steers' life, at a time when the cows lactation was nearly finished, still has a high δ^{15}N value (Figure 5), despite the fact that at this time the calves drank small amounts of milk.

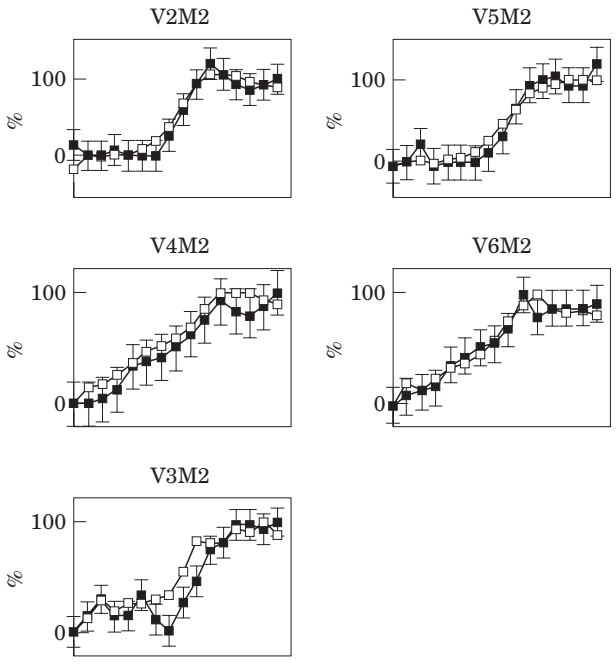

Figure 7. Comparison of the variation pattern of δ^{15}N (■) and δ^{13}C (□) values in M2. Each value was calculated as amount (%) of collagen synthesized since the dietary change in each dentine sample. For individuals V2 and V5, the value 0% (no collagen synthesized since the dietary change in this sample) was estimated by averaging of the δ values of the first samples of the crown (samples 1 to 5); the value 100% (no collagen synthesized before the dietary change in this sample) was estimated in the same way by averaging the δ values of the last samples of the root (samples 11 to 16 in V2M2, samples 12 to 16 in V5M2). For individuals V4, V6 and V3, the extreme values of the variation were chosen as values 0 and 100%.

Conclusions

The sampling procedure applied in this study allows not only the detection of a change in the isotopic composition of the synthesized collagen, but also the determination of the dental growth stage of the tooth—and the age of the individual—when this change occurred. However, the dentine growth pattern is such that it may be difficult to accurately assess the rate of a change of diet with the sampling strategy used in this study. A sudden dietary change should never be reflected by an abrupt change in the dentine isotopic composition because of the turnover rate of metabolic precursors of protein. Moreover, because our sampling procedure did not accurately follow the dentine growth increments in thickness, the apparent time of change appears earlier than it really occurred, and its duration is overestimated. Thus any gradual change of the dentine isotopic composition must not be interpreted as reflecting a progressive dietary change. Strategies for more precise sampling of growth increments in dentine should be developed.

These results also suggest that it should be difficult, using collagen δ^{15}N values, to distinguish between a relatively abrupt and a more gradual weaning when weaning was made to a low-protein food—as is the case in herbivorous diets, since in this case the lowering of the δ^{15}N values would mean the end of suckling. As Katzenberg *et al.* (1996) pointed out, studies on weaning practices should ideally couple δ^{15}N value analysis, which traces the significant decline and the end of suckling, with Sr/Ca ratios analysis, which traces the beginning of supplementation by food other than milk (Sillen & Smith, 1984).

This methodology could be applied in a fruitful way to archaeological material. The results of this study show where a dietary change occurring around 9–10 months is reflected in the second molar dentine of a steer slaughtered 8 months later. It would be too hasty at the moment to extrapolate to individuals who died at an older age: the dentine growth in thickness could complicate the interpretation of results. However, by analysing of second molars from cattle slaughtered when they still were young (maximum 18–24 months old), it should be possible to determine when the individuals were weaned. This information is not trivial since it could provide evidence for the length of cattle lactation (the minimal length if forced weaning is envisaged) in prehistoric pastoral societies. Knowledge of the duration of lactation may permit a more accurate estimation of the amount of milk potentially available for human consumption. Weaning age is also an aspect of domestic mammal growth, development and nutrition that can be controlled by humans, and therefore might reflect the orientation of the production strategy of the first farming communities.

There are many potential archaeological applications of the study of intra-individual variation in tooth dentine collagen isotopic composition. For example, Bogucki (1987) proposed a residential mobility strategy for the cattle herders of the Early Neolithic *Linearbandkeramik* culture of Central Europe. Gifford-Gonzalez (1998; Gifford *et al.*, 1980) and others (Marshall, 1990; Ambrose, 1984) have proposed models of seasonal transhumance between pastures with potentially different proportions of C_3 and C_4 plants for Neolithic pastoralists in East Africa. Smith (1986) has proposed models of seasonal transhumance between marine coastal and interior pastures for pre- and protohistoric Khoi pastoralists in the Western Cape, South Africa, and seasonal use of coastal sites has been documented by Klein & Cruz-Uribe (1989). Whenever this mobility is accompanied by changes in the isotopic composition of the cattle fodder, it may be detected by such intra-tooth sampling. The analysis of the dentine from the three mandibular molars would permit one to follow such mobility for the first 3 years of the animal's life.

Potential applications in mammalian ecology and palaeoecology should also be considered. For example, seasonal, altitudinal and latitudinal gradients in the distribution of C_3 and C_4 grasses have been documented in North America (Teeri & Stowe, 1976; Ode *et al.*, 1980; Epstein *et al.*, 1997) and Africa (Vogel *et al.*, 1978; Tieszen *et al.*, 1979). Previous studies of the carbon isotopic composition of whole teeth of prehistoric bovids and equids have provided evidence for past environmental changes and seasonal migration patterns (Chisholm *et al.*, 1986; Tieszen, 1994; Vogel, 1983). Isotopic analysis of small growth increments in the teeth of prehistoric migratory, grazing bovids and equids could provide a more detailed record of differences in the past position of altitudinal or latitudinal clines in C_4 grasses or seasonal changes in the abundance of C_4 plants in response to prehistoric environmental changes.

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