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Studies on Age Estimation Using Racemization of Aspartic Acid in Cementum

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ABSTRACT: An attempt was made to estimate the age of cementum using the racemization method, which focuses on the ratio of D- and L-aspartic acids (referred to as the 'D/L ratio'). Using the whole cementum, the correlation coefficients between the D/L ratio and age were $r = 0.984$ to 0.997. When the use of incisors and premolars was compared, the former gave better results than the latter. We then made a comparison of cementum, enamel and dentin, using a first premolar from the same individual. For each component tissue the correlation of D/L ratio with the actual age was calculated and the highest value was shown for dentin at $r = 0.992$, followed by cementum at $r = 0.988$ and enamel at $r = 0.961$. These results show that the racemization reaction in cementum proceeds at a constant rate, and confirm that cementum, like dentin, is a tissue with very low metabolism. Therefore, it is clear that accurate estimation of age is practically possible using the amino acid racemization method for cementum.

KEYWORDS: odontology, racemization, cementum, age, aspartic acid

The racemization of amino acids is a reversible first-order reaction, which is relatively rapid in living tissues that have a slow metabolic rate. The amino acids composing proteins are L-enantiomers. However, over the course of time, amino acids undergo racemization with an increased ratio of D-enantiomers, metamorphosing into a racemate [*1*]. Aspartic acid shows a high racemization reaction rate and is considered to provide useful information on changes occurring in living tissues over time. Masters et al. [*2*] investigated aspartic acid in the lens, which has one of the slowest metabolic rate of all living human tissues. They reported that aspartic acid racemized with time and that D-aspartic acid tended to accumulate. Man et al. [*3*] focused on the white matter of the brain, which is rich in myelin that forms the medullary sheath of the nervous system, and found that D-aspartic acid in this tissue increased with age.

The tooth is the hardest of all human tissues, and is highly resistant to physical and/or chemical influences. The tooth retains its form long after death, and is slow to metabolize.

The precise estimation of age contributes greatly to the solution of criminal cases. Helfman and Bada [*4*] carried out an elegant study on the racemization of amino acids in the crown region of dentin, and obtained a correlation of $r = 0.979$ with age. Their studies were repeated and the results were confirmed by Shimoyama and Harada [*5*], and by Ogino and Ogino [*6*]. Meanwhile, Ohtani and Yamamoto [*7–9*] conducted various basic experiments in an attempt to obtain further and more detailed findings using the methods employed in the previous studies. They demonstrated that better correlations were obtainable ($r = 0.991$) using whole dentin, but not the crown region (referred to as 'dentin' hereafter), from longitudinal sections. However, there have been no reports on the estimation of age from cementum using the racemization method.

Against this background, we studied the estimation of age from teeth applying the amino acid racemization method using cementum, which is believed to have a relatively high content of water and a high environmental temperature in the oral cavity, paying close attention to the correlation of the ratio of D- and L-aspartic acids (referred to as the 'D/L ratio' hereafter) with actual age, as well as the rate of the racemizing reaction. In addition, we compared the value obtained from cementum with those obtained from enamel and dentin.

Materials and Methods

Teeth were extracted from living human subjects and preserved in a dry state for between 1 month and 4 years after extraction. The teeth studied were extracted became of periodontal disease or for orthodontic reasons from individuals of known age and sex. Treated teeth and teeth with caries extending to the dentin were excluded.

The 32 specimens, all from different individuals included eight central incisors, eight lateral incisors, eight first premolars and eight second premolars. After they had been rinsed overnight in running water, they were air-dried and the sediment retained was carefully removed with a scaler. Then, 1-mm-thick longitudinal sections were prepared from their central parts using a cutter (ISOMET, type 11-1180, BUEHLER, U.S.A.). The sections were then washed with 0.2 N HCl, pure water (three times), ethanol, and ether, using ultrasonication at each step for 5 min, and then air-dried. The cementum layer was isolated from each section with a surgical knife under a stereomicroscope ($\times 10 - \times 20$). After preparation of the cementum sample, the same procedure was also applied for the enamel and dentin. Quantitative analysis of the D/L ratio and estimation of age were carried out mostly according to the method described by Ohtani and Yamamoto [*7–9*].

Results and Discussion

As samples of the whole cementum, a central incisor, lateral incisor, first premolar, and second premolar were selected for investigation. The correlation of the D/L ratio determined from

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the cementum of these teeth with the actual age was examined using longitudinal sections. Table 1 shows measured D/L ratio and estimated ages. As shown in Fig. 1, there was no marked difference between the front central incisor (r = 0.991) and the front lateral incisor (r = 0.997), and between the first premolar (r = 0.988) and second premolar (r = 0.984). The incisors gave slightly higher correlation values than the premolars, suggesting that the amount of addition of cementum differs from one individual to another because of the presence of more intense stimuli from occlusion with molars than with the front teeth [*10,11*].

The D/L ratio was determined from the cementum, enamel, and dentin of the first premolar from eight individuals (Table 2), and then compared with the actual age. Dentin showed the highest correlation at r = 0.992, followed by cementum and enamel at r = 0.988, and 0.961, respectively (Fig. 2). In comparison with cementum and enamel, dentin is exposed to constant environmental conditions throughout life and also during its formation process, and the D/L ratio in dentin is assumed to increase linearly with aging. Although the environment of the cementum is assumed to remain constant, the relative irregularity in the increase of the D/L ratio in cementum with age in comparison with dentin is presumed to reflect variations in the composition of the cementum from site to site as well as the irregular timing of the addition of cementum resulting from various kinds of stimulation. In addition, the enamel is presumed to have a less stable or constant environment than dentin or cementum.

The racemization rate was studied using the cementum, enamel, and dentin of first premolar of the same individual, and revealed that cementum had the fastest reaction, followed by dentin, whereas enamel had the slowest reaction, with reaction constants (kyr^{-1}) of $k = 9.1800 \times 10^{-4}$, $k = 6.4950 \times 10^{-4}$, and $k = 8.7500 \times 10^{-4}$, respectively (Fig. 2). The cementum showed the fastest racemization rate because it is surrounded by periodontal tissue

TABLE 1—*Estimation of age from cementum using the amino acid racemization method.*

Central incisor				Lateral incisor				First premolar				Second premolar			
Individual No.	Actual age	D/L	Age estimated	Individual No.	Actual age	D/L	Age estimated	Individual No.	Actual age	D/L	Age estimated	Individual No.	Actual age	D/L	Age estimated
1	32	0.0716	31	9	32	0.0714	30	17	16	0.0410	15	25	13	0.0428	16
2	37	0.0878	40	10	37	0.0810	36	18	43	0.0990	45	26	26	0.0576	24
3	41	0.0868	39	11	41	0.0894	41	19	45	0.1024	47	27	41	0.0812	37
4	47	0.0996	46	12	43	0.0964	45	20	47	0.1014	47	28	50	0.1126	54
5	54	0.1126	54	13	51	0.1096	52	21	51	0.1032	48	29	53	0.1048	50
6	55	0.1130	54	14	56	0.1138	55	22	55	0.1182	56	30	56	0.1198	58
7	62	0.1290	63	15	66	0.1330	66	23	59	0.1206	57	31	59	0.1238	60
8	62	0.1252	61	16	83	0.1598	81	24	59	0.1202	57	32	67	0.1304	64
r = 0.991 S.D. = ±1.7				r = 0.997 S.D. = ±1.3				r = 0.988 S.D. = ±1.9				r = 0.984 S.D. = ±3.1			

NOTE: D/L, ln[(1 + D/L)/(1 − D/L)]; r, Correlation coefficient; S.D., Standard deviation.

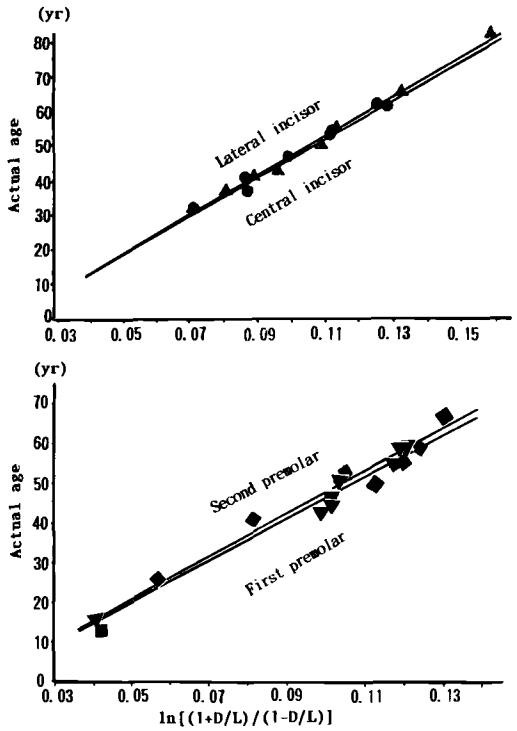

FIG. 1 - Estimation of age from cementum using the amino acid racemization method.

● = Central incisor, Y = 556.72X-8.70, r = 0.991, δ = ±1.7;

▲ = Lateral incisor, Y = 580.58X-10.88, r = 0.997, δ = ±1.3;

▼ = First premolar, Y = 531.64X-6.69, r = 0.988, δ = ±1.9;

◆ = Second premolar, Y = 547.43X-7.27, r = 0.984, δ = ±3.1;

Y = Age; X = ln[(1+D/L)/(1-D/L)]; δ = Standard deviation;

r = Correlation coefficient; k = Reaction constant (yr^{-1}).

FIG. 1

TABLE 2—*The D/L ratio was calculated from the cementum enamel, and dentin of the first premolar from eight individuals.*

		Cementum		Enamel		Dentin	
Individual No.	Actual age	D/L	Age estimated	D/L	Age estimated	D/L	Age estimated
17	16	0.0410	15	0.0324	17	0.0426	17
18	43	0.0990	45	0.0684	43	0.0826	40
19	45	0.1024	47	0.0748	47	0.0902	44
20	47	0.1014	47	0.0804	51	0.0952	47
21	51	0.1032	48	0.0746	47	0.0996	49
22	55	0.1182	56	0.0744	47	0.1060	53
23	59	0.1206	57	0.0928	60	0.1202	61
24	59	0.1202	57	0.0916	59	0.1174	59
		r = 0.988 S.D. = ±1.9		r = 0.961 S.D. = ±3.7		r = 0.992 S.D. = ±1.6	

NOTE: D/L, ln[(1 + D/L)/(1 − D/L)]; r, Correlation coefficient: S.D., Standard deviation.

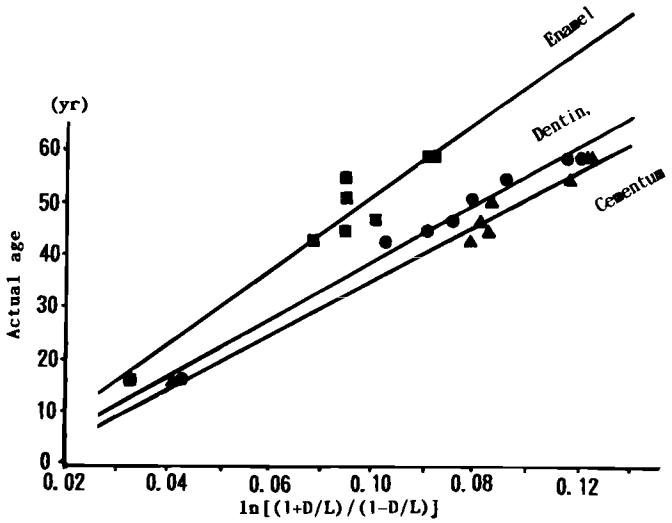

FIG. 2 - Comparison of age estimation based on the cementum, enamel and dentin from the same teeth.

■ = Enamel, Y = 710.54X-5.47, r = 0.961, δ = ±3.7, k = 6.4950×10⁻⁴;

● = Dentin, Y = 562.44X-6.12, r = 0.992, δ = ±1.6, k = 8.7500×10⁻⁴;

▲ = Cementum, Y = 531.64X-6.69, r = 0.988, δ = ±1.9, k = 9.1800×10⁻⁴;

Y = Age, X = ln[(1+D/L)/(1-D/L)]; δ = Standard deviation;

r = Correlation coefficient; k = Reaction constant (yr^{-1}).

FIG. 2

and dentin, and therefore has a high environmental temperature with a high water content. In contrast, dentin is probably influenced by air in the oral cavity, even though the part that constitutes the crown is surrounded by enamel. Moreover, the difference in racemization rates among the above three dental domains suggests that dentin and cementum are built up independently.

All these findings confirm that in cementum the racemization reaction proceeds in a relatively linear manner and that the cementum, like dentin, is a tissue which has a low rate of metabolism. As a result, the amino acid racemization method based on cementum is, like that with dentin, sufficiently useful for precise estimation of an individual's age.

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