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Estimation of Age from a Tooth by Means of Racemization of an Amino Acid, Especially Aspartic Acid—Comparison of Enamel and Dentin

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ABSTRACT: In a study of age estimation from teeth by means of measuring racemization of aspartic acid (Asp), a representative amino acid, the accuracy of data from enamel and dentin in the same tooth was compared.

The correlation of D/L ratio of aspartic acid with actual age gave the following parameters: $r = 0.928$, $\sigma = \pm 5.2$, $k = 4.47 \times 10^{-4}\ yr^{-1}$ in enamel and $r = 0.995$, $\sigma = \pm 1.4$, $k = 5.75 \times 10^{-4}\ yr^{-1}$. The difference in ages between one estimated by the D/L ratio and the actual one was within ± 3 years in dentin, while in enamel an error of from 2 to 11 years was observed. Reaction rate constants of the racemization in a dry postmortem state (15°C) were calculated as $k = 9.70 \times 10^{-8}\ yr^{-1}$ in enamel, and $k = 1.33 \times 10^{-7}\ yr^{-1}$ in dentin. Compared to rates determined from teeth recently extracted from living subjects, the rate was higher in dentin than in enamel. These data reconfirmed that dentin is superior to enamel in making exact age estimations from teeth.

KEYWORDS: odontology, age estimation, amino acid racemization, enamel, dentin

In a study of age estimations from teeth by means of racemization of amino acids Helfman and Bada reported results for enamel [*1*] (19 cases, $r = 0.921$ in 1975) and for dentin [*2*] (20 cases, $r = 0.979$ in 1976), showing that the correlation of D/L ratio of aspartic acid (Asp) to age is very high.

Subsequent investigations proved the reliability of age estimation by means of the D/L ratio of Asp in dentin [*3–5*]. The method has also been applied to age in estimations from teeth in unknown bodies [*6,7*].

In our study of age estimations by racemization of amino acids, we used enamel and dentin from the same tooth, then compared the correlation of the D/L ratio of Asp to actual age and the reaction velocity of racemization in dry state on the basis of Arrhenius' equation from heating experiments.

Materials and Methods

All teeth used were extracted from living subjects. Age and sex of the donor and date of extraction were recorded. The teeth showed no macroscopically severe cavities, and were stored in 10% formalin.

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The samples for age estimation were 16 upper central incisors. After each sample was sliced into 1 mm-thick longitudinal sections, chips of enamel and dentin were isolated with a cutter. These chips, after washing and drying, were powdered, and 5 mg of each was used as a specimen. The samples for determining reaction velocity were prepared by mixing samples of enamel and dentin of ten teeth respectively. After heating 5 mg of this sample in a test tube the same test was carried out, as with the sample used for age estimation. Heating was effected with an Almi-block heater at 120 to 150°C for 10 to 60 min. The D/L ratio was quantitatively measured by a standard method, using gas chromatography with a glass capillary column of 25 m length and 0.3 mm diameter, packed with optically active fixed phase [*4*].

Results and Discussion

Quantitative Determination of D/L Ratio of Asp

Figure 1 shows gas chromatograms of amino acids obtained by hydrolyzing enamel and dentin at 100°C for 6 h. In both cases, glycine, proline, alanine, and hydroxyproline were detected, showing the same proportion of amino acids as collagen. The identity of collagen is characterized by an abundance of hydroxyproline. It is generally said that organic substances in dentin account for 20% of whole tooth and 90% of them are collagen. The fact that hydroxyproline was detected in enamel suggests the possibility that substances stemming from dentin collagen may have been included. However, the amount of amino acids in whole enamel was scarce in comparison with dentin. This was to be expected,

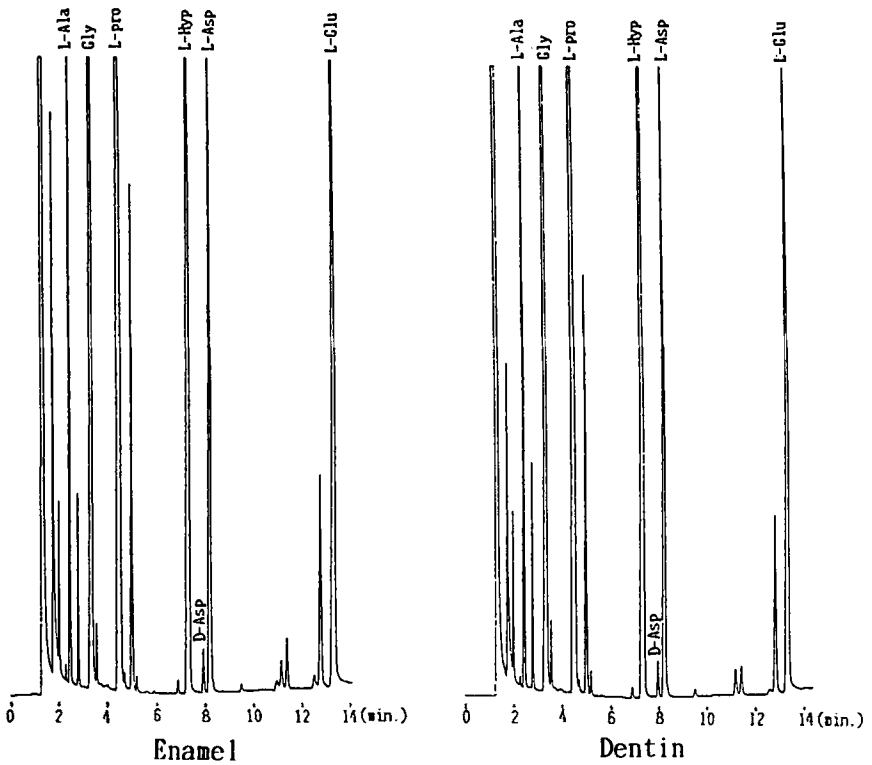

FIG. 1—*Gas chromatogram of amino acids.*

considering the assumption that the organic substances in enamel are approximately 1%. The detection of D/L ratio of Asp in both enamel and dentin, was measured at 8 min retention time. In order to measure the D/L ratio accurately, the analysis was carried out after the peak height of L-Asp on chromatogram for all specimens was adjusted to about the same level.

Age Estimation

We used 16 upper central incisors (18 to 68 years old) and investigated the correlation of actual age with the D/L ratio of Asp in enamel and dentin of the same subject (Fig. 2).

The racemizing reaction of amino acids is a reversible reaction of the first order, and if we represent the concentrations of L and D isomers as L and D, the following formula can be obtained:

$$\text{L-amino acid} \underset{k'}{\overset{k}{\rightleftarrows}} \text{D-amino acid}$$

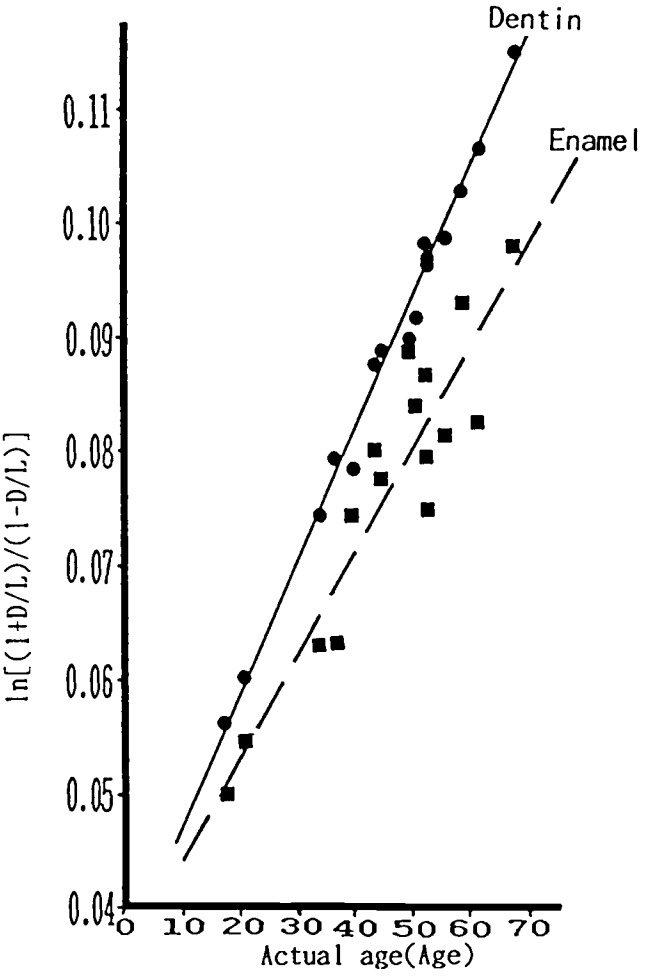

FIG. 2—*Correlation of actual age and D/L ratios of aspartic acid.*

where k and k' are reaction rate constants of racemization and its rate equation is expressed as:

$$\ln[(1 + D/L)/(1 - D/L)]_t = 2kt + \ln[(1 + D/L)/(1 - D/L)]_{t=0} \cdots\cdots \quad (1)$$

where t is elapsed time.

According to the equation (1) the rate equation, coefficient of correlation (r) and standard deviation (σ) in enamel and dentin were obtained as follows:

$$\text{in enamel: } \ln[(1 + D/L)/(1 - D/L)]_t = 0.000\,894\,t + 0.035\,454$$

$$r = 0.928 \quad \sigma = \pm 5.2$$

$$\text{in dentin: } \ln[(1 + D/L)/(1 - D/L)]_t = 0.001\,149\,t + 0.035\,356$$

$$r = 0.995 \quad \sigma = \pm 1.4$$

Calculated difference in ages between one estimated from the D/L ratio and the actual one was ± 2 to 11 years in enamel, but it was within ± 3 years in dentin. The reason why estimates from dentin showed less variation than those from enamel seems to be that dentin exists in a consistent environment, while enamel is susceptible to external influences.

In addition, the reaction rate constant of racemization in teeth from living donors from the rate equation was calculated as $k = 4.47 \times 10^{-4}\ \text{yr}^{-1}$ in enamel and as $k = 5.75 \times 10^{-4}\ \text{yr}^{-1}$ in dentin, where the speed is faster. This also may be attributed to the higher temperature and water content in dentin. Helfman and Bada, however, reported that $k = 8.29 \times 10^{-4}\ \text{yr}^{-1}$ in enamel [*1*] and $k = 7.87 \times 10^{-4}\ \text{yr}^{-1}$ in dentin [*2*], thus with the higher reaction rate in enamel. This can be attributed to a difference in washing procedure, while we washed both with 0.2 M HCl, Helfman et al. washed enamel with acetone and dentin with a weak HCl, presumably weaker than 0.2 M HCl. The authors [*8*] reported that D/L ratio from the soluble component was considerably higher than from the insoluble when dentin was extracted with 1 M HCl. This means that when a sample is washed with stronger acid, the D/L ratio of the residual sample would be lower than when the sample was washed with weaker acid. This demonstrates the importance of washing procedures in preparing samples for age estimation.

Comparison of D/L Ratio Between Labial and Lingual Sides

These measurements were carried out on both left and right upper central incisors from the same person (Fig. 3). At first, a longitudinal section was prepared and a comparison of D/L ratios between the labial and the lingual sides of the crown was made. The result showed a tendency for the lingual side to give a higher D/L ratio both in enamel and dentin. This may be attributed to the fact that the labial side is susceptible to external influence and that the environmental temperature tends to be lower. Also, the gross D/L ratio was higher in dentin; this may be due to the higher temperature and the more amount of water than in enamel. As mentioned above, the D/L ratio varied considerably, depending on the testing area chosen for the sample. This suggests that only the complete sample can give an accurate D/L ratio related to individual. Authors [*5–8*] have measured the D/L ratio of whole dentin for the last several years and estimated the age with good accuracy.

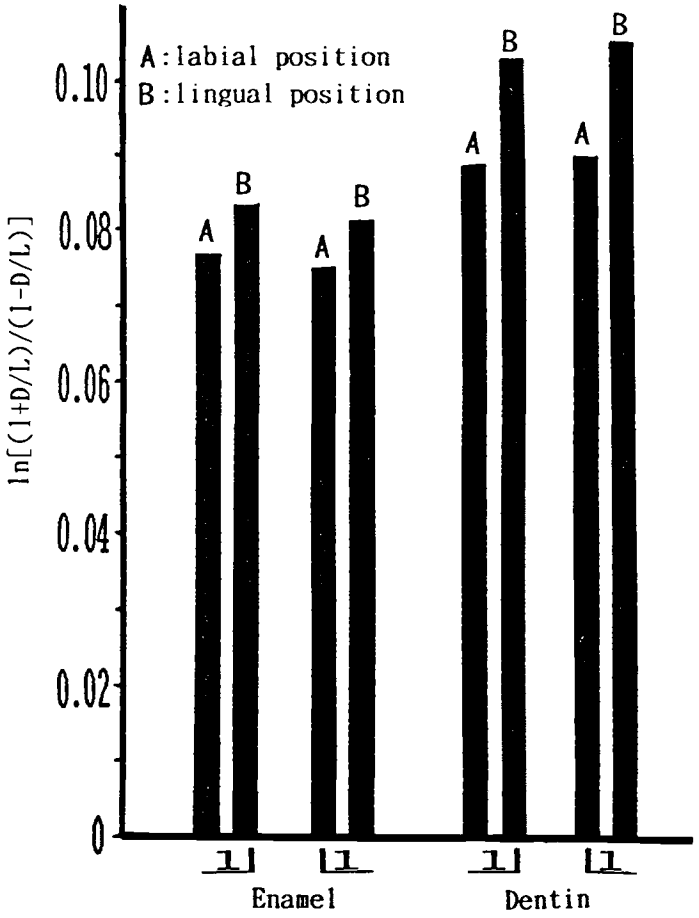

FIG. 3—*Comparison of D/L ratios between labial and lingual crown in a longitudinal section.*

Reaction Rate of Racemization

Racemization is a kind of chemical reaction; its reaction rate is influenced by environmental conditions. Particularly, the heating of a tooth markedly enhances the increases of D-isomers of amino acids. Thus, in order to determine the rate of racemization of samples in the dry state we carried out heating experiments, calculated the Arrhenius' equation and compared the reaction rates of enamel and dentin.

Figure 4 shows the change of D/L ratio of Asp by heating. The D/L ratio increases linearly with prolonged heating time. However, heating at higher temperature (160°C) for a longer time (60 min) resulted in deviations from a straight line. Thus, regarding the plot showing a coefficient of correlation (r) of 0.990 or more as a direct line, we calculated the rate equation at each temperature. The k-value at each temperature was then calculated, and the Arrhenius' equation induced. The following equations were then obtained:

$$\text{enamel: } \ln k = -11\,845\,(1/T) + 16.2$$

$$\text{dentin: } \ln k = -11\,965\,(1/T) + 16.3$$

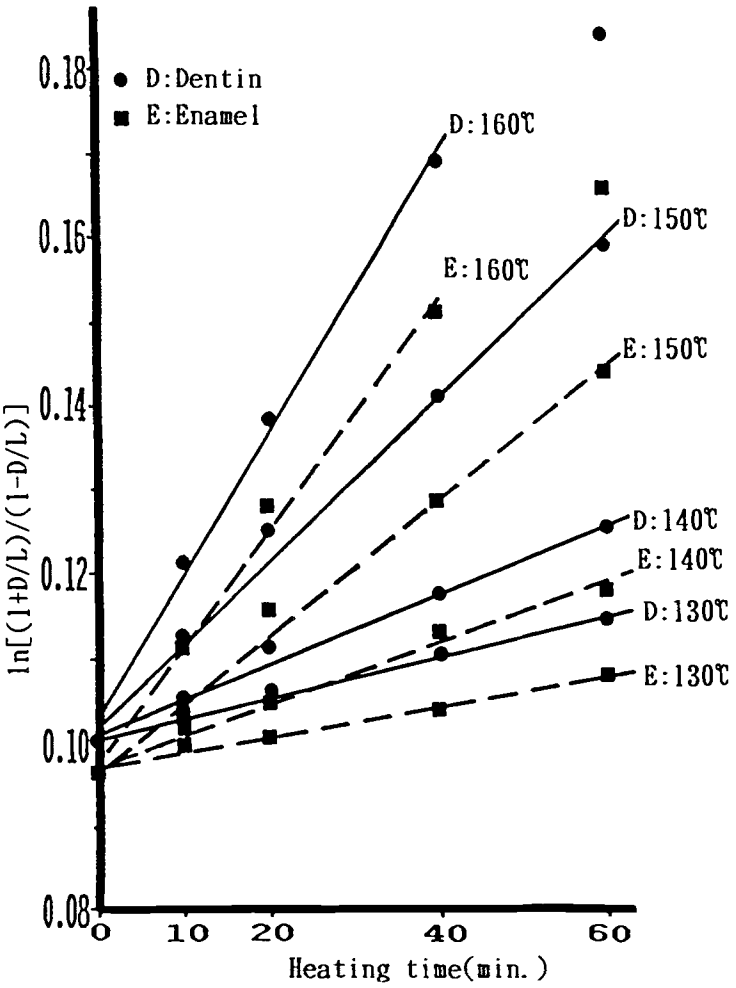

FIG. 4—*Change in D/L ratio of aspartic acid by heating.*

Supposing the average atmospheric temperature throughout 1 year as 15°C, we calculated the rate constant of racemization in the dry state (15°C) from the above equations, thereby obtaining $k = 9.70 \times 10^{-8}\,\mathrm{yr}^{-1}$ in enamel and $k = 1.33 \times 10^{-7}\,\mathrm{yr}^{-1}$ in dentin, showing a higher velocity in dentin (Fig. 5). This can be attributed to the fact that even in the dry state dentin contains more water than enamel.

Besides, the racemization rate differs considerably between the living and dry (15°C) states. In the living state, the environmental temperature is higher, there is more water, and the velocity is faster, while in the dry state this temperature is generally lower and the amount of water is much less, with hardly any increase of D/L ratio observed. These findings provide clues for interpreting estimations of age at death from teeth subjected to postmortem influences, such as exposure of a body or isolated teeth to outdoor climatic condition.

From these findings it was reconfirmed that to obtain accuracy in estimating age from tooth by measuring the racemization of aspartic acid, dentin samples are preferable to those from enamel.

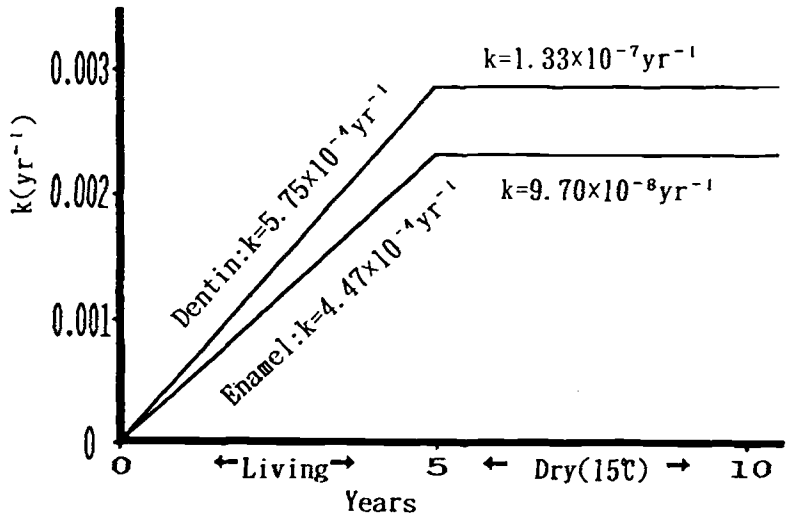

FIG. 5—*Comparison of the racemization rates between enamel and dentin.*

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