TECHNICAL NOTE

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Estimation of Age from Teeth by Amino Acid Racemization: Influence of Fixative

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ABSTRACT: To determine the age of a subject from teeth accurately utilizing the racemization rates of amino acids, standard samples of the same tooth species from the same jaw are necessary as controls, as well as data for identification. However, standard teeth are generally stored in fixatives such as ethanol and formalin. We investigated and compared the degree of progression of racemization of dentinal aspartic acid in teeth stored in 95% ethanol, 10% formalin, or 10% neutral formalin fixatives. The racemization rate of dentinal aspartic acid in teeth stored in 10% neutral formalin was the highest, followed by that for teeth stored in 10% formalin then that for teeth stored in 95% ethanol. Teeth stored in these fixatives at 15°C showed almost no progression of racemization. The racemization ratio (D/L ratio) in teeth extracted 10 years previously was almost unchanged from that at the time of extraction, and allowed an accurate evaluation of the subjects age at tooth extraction.

KEYWORDS: forensic science, forensic odontology, age estimation, dentin, racemization, aspartic acid, teeth, fixative, chromatography

Aspartic acid has been reported to have the highest racemization rate of all amino acids, and to be stored during aging (1–5). In particular, D-aspartic acid levels in human enamel (6,7), dentin (7–13), and cementum (14,15) have been found to increase with age, and the D/L ratio has been shown to be highly correlated with age. We have previously reported a higher correlation when using the entire dentin than when using longitudinal sections (11). Ritz et al. (13) have also confirmed this point.

We have utilized the D/L ratio to determine the age of unidentified bodies from their teeth with good results (16). The aim of age determination from teeth is usually to establish a preliminary age-calculating formula using many teeth. However, we have found that standard samples of the same tooth name (e.g., Lower Central Incisors) from mirror image jaw position (e.g., right versus left) are necessary to act as controls. In addition to data for age determination (16), because it is necessary to use the same experimental conditions for all procedures. We have reported that age can be estimated to within ±three years when at least four control teeth of known age are also used (16). However, standard teeth are frequently stored in fixatives after extraction in order to prevent various histological and chemical changes. It is not known how the process of racemization is affected when teeth are stored in fixatives.

We stored extracted standard teeth in alcohol or formalin, and investigated the rate of increase in the D/L ratio of dentinal aspartic acid on heating. The Arrhenius formula was calculated, and the values were compared. This enabled us to determine whether standard teeth stored in various fixatives are suitable for age determination.

Materials and Methods

Sampling

Teeth were extracted from living human subjects and preserved in a dry state for between three months and five years after extraction. These teeth were extracted because of periodontal disease or for orthodontic reasons from individuals of known age and sex. Treated teeth and teeth with caries extending into the dentin were excluded.

Preparation of Specimens

The teeth used in the study were 66 mandibular central incisors (left and right) from 33 subjects. From each subject, one of the two central incisors was treated by heating (heated group) and another central incisor was served as a control (non heated group). The D/L ratio was determined in each group, and the difference represented the increase in the D/L ratio. Teeth for the heated group were put into 10-mL test tubes with screw caps, to which 10-mL each of 95% ethanol (Wako, Japan), 10% formalin (pH 4, Wako), or 10% neutral formalin (1/15 M phosphate buffer pH 6.8, Wako) was added, and were heated with an aluminum block heater (YAMATO, HF-21, Japan). After heating, the teeth were ultrasonically cleaned with pure water three times, for 5 min each time. Longitudinal sections of each tooth were prepared after cleaning according to a routine method, and the dentinal fragment was isolated. After washing and drying, these were used for the investigation. The D/L ratio of aspartic acid (8,9,16) and the Arrhenius formula (12,17) were determined by previously reported methods.

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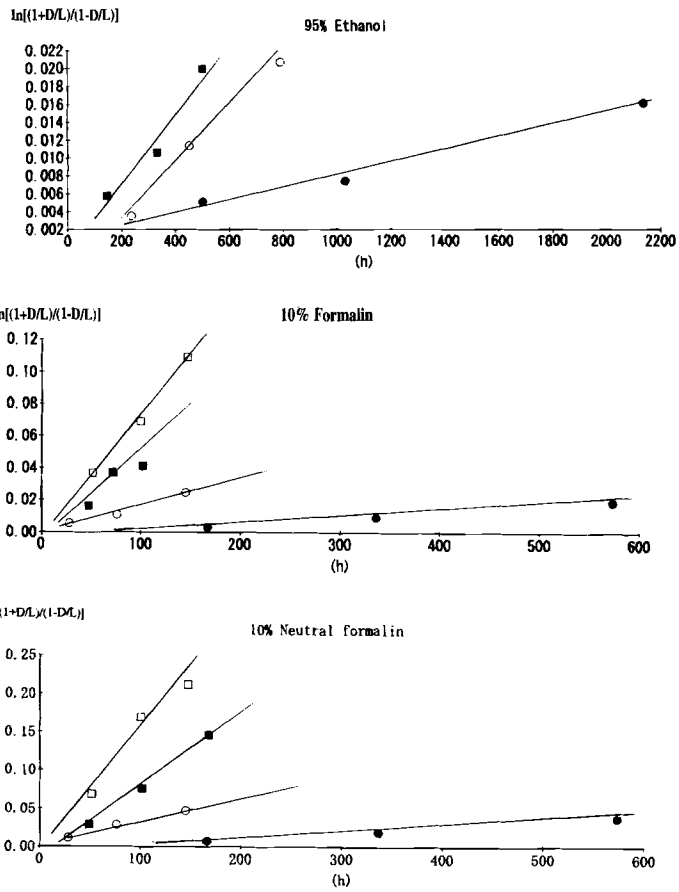

FIG. 1a), b), c)—*Heating-related changes in the D/L ratio of dentinal aspartic acid. The formula of the rate of racemization and constant of rate (kh^{-1}) for the teeth stored in each fixative were calculated as follows: 95% Ethanol,* ●, *68°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 7.3370 \times 10^{-6}t + 5.0 \times 10^{-4}$, $k = 3.6680 \times 10^{-6}$; ○, *74°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 2.7680 \times 10^{-5}t + 1.5 \times 10^{-5}$, $k = 1.3840 \times 10^{-5}$; ■, *77°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 3.8347 \times 10^{-5}t + 1.8 \times 10^{-4}$, $k = 1.9173 \times 10^{-5}$. *10% Formalin,* ●, *77°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 3.9468 \times 10^{-5}t + 3.4 \times 10^{-3}$, $k = 1.9734 \times 10^{-5}$; ○, *85°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 1.6820 \times 10^{-4}t + 2.7 \times 10^{-5}$, $k = 8.4100 \times 10^{-5}$; □, *90°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 4.4426 \times 10^{-4}t + 1.2 \times 10^{-3}$, $k = 2.2213 \times 10^{-4}$; □, *95°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 7.7236 \times 10^{-4}t + 5.6 \times 10^{-3}$, $k = 3.8618 \times 10^{-4}$. *10% Neutral formalin,* ●, *77°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 7.5500 \times 10^{-5}t + 5.2 \times 10^{-3}$, $k = 3.7750 \times 10^{-5}$; ○, *85°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 3.0085 \times 10^{-4}t + 4.5 \times 10^{-5}$, $k = 1.5043 \times 10^{-4}$; ■, *90°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 9.8792 \times 10^{-4}t + 2.1 \times 10^{-2}$, $k = 4.9396 \times 10^{-4}$; □, *95°C,* $\ln[(1 + D/L)/(1 - D/L)]_t = 1.5032 \times 10^{-5}t + 1.2 \times 10^{-4}$, $k = 7.5160 \times 10^{-4}$. *where t is the age of the dentin in hours.*

Results and Discussion

Racemization of amino acids is a chemical reaction, and is dependent on environmental temperature, humidity, and pH. Racemization has been reported to be particularly influenced by temperature (1). The quantitative relationship between reaction temperature and reaction rate has already been confirmed by the Arrhenius formula (17). This is expressed as natural logarithms as follows: $\ln k = -Ea/RT + \ln A$, where k is the rate constant, A represents the frequency factor, Ea the activation energy, R the gas constant, and T the temperature in degrees Kelvin. This indicates that the rate of racemization of aspartic acid can be calculated according to the environmental temperature by determining the k value, i.e., the reaction rate constant (kh^{-1}).

For teeth stored in 95% ethanol, 10% formalin, or 10% neutral formalin, all of which are common fixatives for teeth, we compared the degree of progression of racemization of dentinal aspartic acid according to the fixative.

Some studies have shown that the D/L ratios of teeth from the right and left central incisors in the same jaw of the same person are almost identical (18), because these teeth are formed at the same time. In our study the D/L ratio of the non heated tooth was calculated from the ratio of the heated tooth, which was taken from the mirror image position and in the same jaw, and the difference was regarded as the increase in the D/L ratio due to heating. Figures 1-a), b), and c) show the changes in the D/L ratio due to heating of the teeth stored in each fixative. The D/L ratio of dentin tended to increase linearly according to the primary reaction pattern, in parallel with the temperature and time, because the chemical reaction is activated by heating and the L-amino acid is rapidly converted to the D-isomer (1). The Arrhenius formula was calculated from the formula

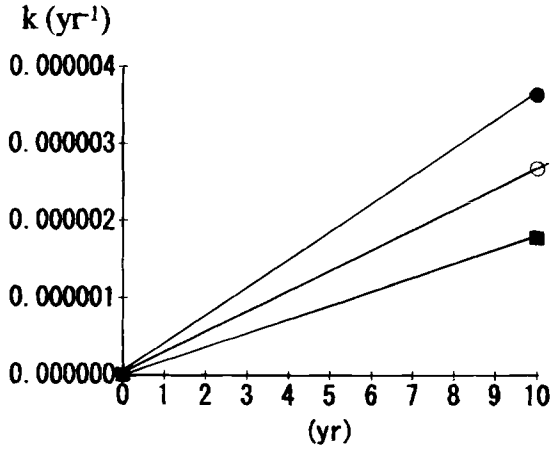

FIG. 2—*Comparison of racemization rates for dentin stored in each fixative.* ●, *10% Neutral formalin;* ○, *10% Formalin;* ■, *95% Ethanol.*

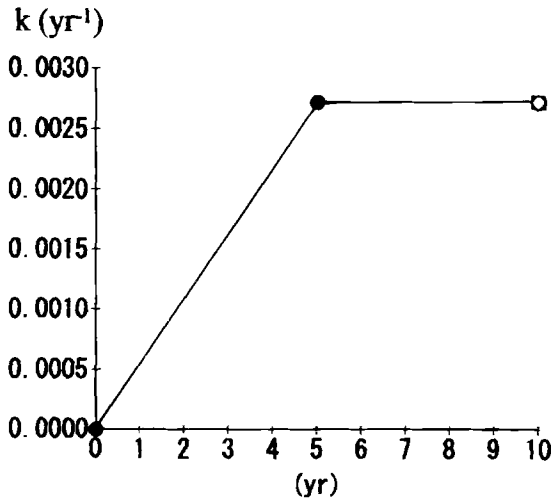

FIG. 3—*Comparison of racemization rates in the dentin of teeth stored in each fixative (15°C) and of living teeth.* ●-●, *Living teeth;* ●-○, □, ◇, *Fixative.*

TABLE 1—*Rate of racemization of the dentin and estimated age.*

Materials	°C	kasp (yr^{-1})	k fixative/k living	Estimation of Age
Living Teeth	15	5.4500×10^{-4}	1.00000	55.0000
95% Ethanol	15	1.7833×10^{-7}	0.000327	45.0033
10% Formalin	15	2.6879×10^{-7}	0.000492	45.0049
10% Neutral Formalin	15	3.6472×10^{-7}	0.000668	45.0067

NOTE—Teeth were extracted from a 45-year-old subject and stored for 10 years either in the dry state or in the three fixatives.

for the racemization rate at each temperature and rate constant (kh^{-1}) as follows:

$$95\%\ \text{Ethanol},\ \ln k = -22568\ (1/T) + 53.7$$
$$10\%\ \text{Formalin},\ \ln k = -21787\ (1/T) + 51.4$$
$$10\%\ \text{Neutral formalin},\ \ln k = -22333\ (1/T) + 53.6$$

Assuming that the atmospheric temperature over one year was 15°C, we compared the reaction rates for the three fixatives. The racemization rate constant (kyr^{-1}) was calculated as follows when the environmental temperature was 15°C (Fig. 2):

$$95\%\ \text{Ethanol},\ k = 1.7833 \times 10^{-7}$$
$$10\%\ \text{Formalin},\ k = 2.6879 \times 10^{-7}$$
$$10\%\ \text{Neutral formalin},\ k = 3.6472 \times 10^{-7}$$

The reaction rate for dentin stored in 10% neutral formalin was the highest, followed by that for dentin stored in 10% formalin then that for dentin stored in 95% ethanol, because racemization is rapid under alkaline conditions but slow under acid conditions (1,12,19). Because 10% neutral formalin has a higher pH than 10% formalin, and 95% ethanol is nonelectrolytic, this order of reaction rate was as expected (12).

The racemization formula for the 33 living non heated mandibular central incisors was as follows: $\ln[(1 + D/L)/(1 - D/L)]_t = 0.001090t + 0.0386$. When kyr^{-1} was obtained from this formula, the constant was 5.4500 by 10^{-4}. Figure 3 shows a comparison of racemization rates for dentin specimens stored at 15°C in each fixative and dentin specimens from living teeth. For teeth that had been extracted within the previous five years, the rates during their viable period are shown, whereas for teeth that had been extracted five or more years earlier, the rates after storage in fixatives are shown. The racemization rate in dentin stored in all three fixatives was found to be almost unchanged at an environmental temperature of 15°C.

Table 1 shows the racemization rate of the dentin and the age of the subject estimated from extracted teeth. In the illustrated case of subject aged 45 years at tooth extraction ten years previously, the age was estimated to be 55 years from the living teeth. However, the age determined from the teeth stored in the fixatives was almost identical to that at the time extraction. These results suggest that teeth stored in ethanol and formalin can be used as standard teeth for age determination.

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