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Characterization of Deoxyribonucleic Acid (DNA) Obtained from Teeth Subjected to Various Environmental Conditions

REFERENCE: Schwartz, T. R., Schwartz, E. A., Mieszerski, L., McNally, L., and Kobilinsky, L., **"Characterization of Deoxyribonucleic Acid (DNA) Obtained from Teeth Subjected to Various Environmental Conditions,"** *Journal of Forensic Sciences*, JFSCA, Vol. 36, No. 4, July 1991, pp. 979–990.

ABSTRACT: This study was designed to determine the effects of various environmental factors on the deoxyribonucleic acid (DNA) obtained from dental pulp. Extracted teeth were subjected to the following conditions: varying pH (3,7,10); temperature (4°C, 25°C, 37°C, incineration); humidity (20%, 66%, 98%); various types of soil (sand, potting soil, garden soil); seawater; burying the teeth outdoors, and aging (one week to six months). In addition, teeth that had been extracted and held at room temperature for 16 and 19 years were also examined. Following isolation of DNA, the samples were analyzed on yield gels to determine the concentration and integrity of the recovered DNA. Restriction digestion with Pst I was followed by electrophoresis of the generated fragments, Southern transfer to nylon membranes, and hybridization to both human and bacterial probes. It was determined that, aside from soil, the environmental conditions examined did not affect the ability to obtain high-molecular-weight human DNA from dental pulp. Restriction fragment length polymorphic (RFLP) analysis of selected samples was performed. Dental pulp patterns were compared with bloodstain exemplars, revealing matching patterns, although an increase in band-shifting was observed with extended exposure to elevated temperatures.

KEYWORDS: odontology, dentition, deoxyribonucleic acid (DNA), serology, genetic typing, restriction fragment length polymorphisms, band-shifting, monomorphic probe

The forensic odontologist is often called upon to examine the dentition of human remains. Because the enamel of a tooth is the hardest substance of the human body, teeth are sometimes the only remains available for an identification. Generally, a comparison is made between the dentition of the body and antemortem records of the deceased. There are instances, however, where an identification such as this is impossible

Presented at the 42nd Annual Meeting of the American Academy of Forensic Sciences, Cincinnati, OH, 19–24 Feb. 1990. Received for publication 3 Aug. 1990; revised manuscript received 5 Nov. 1990; accepted for publication 17 Dec. 1990.

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for various reasons, such as an inadequate number of teeth in the bodily remains or the unavailability of dental records.

It has been demonstrated that deoxyribonucleic acid (DNA) restriction fragment length polymorphism (RFLP) analysis can be performed on various human tissues to reliably identify an individual [*1–3*]. A tissue sometimes overlooked is the dental pulp, which is housed in the pulp cavity in the center of the tooth. This tissue provides the tooth with nutrients and contains nerve cells which gives the tooth sensation. It is also a rich source of DNA.

An important aspect of DNA analysis as applied to forensic science is how environmental factors effect the quality and quantity of DNA obtained from a sample. To date, environmental studies of casework specimens have been conducted only in bloodstains [*2*]. This study was designed to determine the effects on the DNA obtained from dental pulp of teeth subjected to aging and various environmental factors. The conditions studied included exposure to varying conditions of pH, temperature, humidity, soil types, seawater, and aging. For all samples, yield gels were performed to determine the relative amount of high-molecular-weight (HMW) DNA obtained, and test gels were run to detect the presence of human and bacterial DNA. Additionally, RFLP analysis was performed on some of the aging study samples and the patterns obtained were compared with bloodstains from the appropriate individual.

Materials and Methods

Samples

Teeth were obtained from oral surgeons, who, upon extraction, placed them directly into a −20°C freezer. Because extracted teeth which had been placed into hydrogen peroxide were found to be lacking in native, HMW DNA and were therefore unsuitable for this study, the surgeons were requested to avoid exposing extracted teeth to any chemical treatment prior to freezing. In some cases, blood was obtained by wiping the patient's mouth with a sterile gauze pad following the extraction procedure. The pad was air dried and placed into the freezer together with the appropriate tooth or teeth.

Aging Study

Teeth were removed from the freezer and maintained at 4, 25, or 37°C for periods of time ranging from 1 to 24 weeks. These teeth were used to obtain dental pulp for subsequent RFLP profiling analysis. One tooth maintained at ambient conditions of temperature and humidity was 16 years old, and two maintained under the same conditions were 19 years old at the time of analysis.

Conditions of Exposure

In all cases, each tooth was placed into a separate glass jar containing the appropriate environmental material. Samples were tested in triplicate whenever possible.

1. pH—Standard pH calibration buffers (Fisher Scientific, New Jersey) of pH 3.0, 7.0, and 10.0 were utilized. Teeth were maintained in each of these buffers, at 4, 25, and 37°C, for one and three weeks.
2. Soil—Beach sand, commercially made potting soil, and garden soil were used, each at 4°C, 25°C, and 37°C. Soil samples were dry and non-sterile. This study was set up for both one and three weeks.
3. Seawater—Teeth were submerged in 20 mL of ocean water at 4°C for three weeks, 25°C for four weeks, and 37°C for two weeks.

4. Humidity—Humidity chambers were prepared using saturated aqueous solutions maintained at 20°C inside closed containers. Teeth were placed on a small ring-stand inside the containers. Humidities studied were 20% potassium acetate ($KC_2H_3O_2$), 66% sodium nitrite ($NaNO_2$) and 98% lead nitrate [$Pb(NO_3)_2$]. Samples were maintained for one and three weeks.

5. Temperature—A total of 75 teeth were maintained at either 4°C, 25°C (average daily room temperature), or 37°C for varying periods of time prior to analysis. We incinerated five teeth by holding each one approximately 2.5 cm above the top of a bunsen burner flame for 2 min.

6. Burial—Seven teeth were placed approximately 5 cm into the ground. Four of these were maintained for one week and three were maintained for three weeks under these conditions. The teeth were buried in July in Little Falls, New Jersey.

In all cases described above, the storage period is considered to begin at the time of exposure of teeth to a particular environmental condition.

Sample Preparation and Analysis

After being exposed to the appropriate condition, the teeth were rinsed with distilled water and dried. We obtained the dental pulp by breaking open the teeth with a hammer, then manually retrieving the pulp with fine forceps. DNA was prepared as described by McNally et al. [2].

One percent of the total DNA isolated from each sample was loaded onto a 0.8% agarose gel. Ethidium bromide (0.5 mg/mL) was included in the gel buffer. Along with the dental pulp samples, several samples of serially diluted Lambda DNA (200, 100, 50, 25, and 10-ng aliquots) were run to determine the approximate quantity of HMW DNA present in each dental pulp sample.

DNA samples were digested with Pst I (Bethesda Research Laboratory [BRL], Maryland) and precipitated with ethanol. Test gels of the DNA in each sample were then set up. A portion of the DNA (1/10 of the sample) was loaded onto a 0.8% agarose gel in TAN (40-m*M* Tris, 4-m*M* sodium acetate, 1-m*M* ethylenediaminetetraacetate [EDTA]) buffer, pH 7.9. Electrophoresis was carried out at 50 V for 4 h. When the run was completed, the DNA fragments were transferred to a nylon membrane by Southern blotting [4] and hybridization was performed with both human and bacterial specific probes [2]. After hybridization, the membranes were washed free of excess probe and autoradiography was performed to visualize the positions of the hybridized fragments. Some of the samples used in the aging study were then further analyzed on analytical gels for RFLP analysis. The procedure used for RFLP analysis was as described previously [5]. The loci examined were D2S44, DXYS14, D18S27, and DXZ1.

Results

A total of 75 teeth were sampled in the temperature-aging study. Extracted teeth were maintained at 4°C, 25°C, or 37°C for varying periods of time ranging from 1 to 24 weeks. In addition, one 16-year-old tooth and two 19-year-old teeth maintained at ambient room temperature (estimated to average 25°C) were included in this study. Table 1 summarizes the results of the yield gel and test gel analyses. The number of teeth with a sufficient amount (approximately 100 ng) of human DNA for RFLP analysis (X) relative to the total number of teeth sampled (Y) is expressed as a ratio (X/Y) and is used in Tables 2–4. These results indicate that HMW DNA can be isolated from teeth stored at 4°C up to 6 weeks; at 25°C, HMW DNA can be isolated after 19 years; at 37°C, teeth can yield HMW DNA following storage for 6 months. In addition to the 75 teeth included in this study, 5 sample teeth were incinerated for 2 min as described in Materials and Methods.

TABLE 1—*Extracted teeth maintained at 4°C, 25°C, or 37°C for up to 19 years. Ratio X/Y represents number of teeth containing HMW DNA relative to the total number tested.*

Temperature, °C	Exposure Time								
	1 wk	2 wk	3 wk	4 wk	6 wk	12 wk	24 wk	16 yr	19 yr
4	1/2	3/3	0/3	0/3	1/3	. . .	. . .	. . .	. . .
25	4/5	2/3	3/6	2/3	4/6	3/3	3/3	1/1	2/2
37	4/5	3/3	3/6	0/3	1/6	3/3	1/3	. . .	. . .

TABLE 2—*Extracted teeth exposed to solutions of pH 3, 7, or 10 for up to three weeks at 4°C, 25°C, or 37°C. Ratio is same as in Table 1.*

pH	Temperature, °C	Exposure Period, Weeks	
		1	3
3.0	4	2/3	1/3
	25	0/3	0/3
	37	2/3	3/3
7.0	4	3/3	2/3
	25	0/3	1/3
	37	3/3	1/3
10.0	4	1/3	2/3
	25	2/3	2/3
	37	2/3	0/3

TABLE 3—*Extracted teeth exposed to various sand and soil types for up to four weeks at 4°C, 25°C, or 37°C. Ratio X/Y is as described in Table 1.*

Exposure Medium	Temperature, °C	Exposure Period, Weeks		
		1	3	4
Potting soil	4	1/3	1/3	. . .
	25	. . .	. . .	0/3
	37	0/3	0/3	. . .
Garden soil	4	2/3	2/3	. . .
	25	. . .	. . .	3/3
	37	2/3	3/3	. . .
Sand	4	3/3	2/3	. . .
	25	. . .	. . .	2/3
	37	2/3	1/3	. . .

TABLE 4—*Extracted teeth exposed to various levels of humidity for one and three weeks at 25°C. Ratio is as described in Table 1.*

Humidity, %	Exposure Time, Weeks	
	1	3
20	3/3	3/3
66	2/3	3/3
98	3/3	3/3

Incineration results in complete loss of HMW and low-molecular-weight human DNA.

In the pH study, DNA was extracted from teeth which had been exposed to solutions maintained at pH 3, 7, or 10 at three different temperatures (4°C, 25°C, 37°C) for one and three weeks. As Table 2 illustrates, HMW DNA can be found in dental pulp of teeth exposed to a solution which is pH 3.0, or pH 7.0 at 37°C for up to three weeks. Exposure of teeth to a solution which is pH 10.0 for three weeks at either 4 or 25°C does not destroy the HMW DNA.

Table 3 gives the results of DNA analysis of dental pulp obtained from extracted teeth exposed to potting soil, garden soil or sand for periods up to four weeks and at temperatures of 4°C, 25°C, or 37°C. HMW DNA could not be isolated from any tooth which had been exposed to potting soil except for those exposed at 4°C for one and three weeks. Surprisingly, no similar affect was observed for dental pulp of teeth exposed to garden soil or sand, regardless of exposure temperature. The affect of potting soil on the ability to isolate HMW DNA has been reported previously [*2*]. Burial of teeth in soil outdoors, where temperature, humidity, and soil microbes are uncontrolled variables during the month of July, for periods up to three weeks resulted in complete loss of HMW human DNA from isolated dental pulp.

The results of DNA analysis of dental pulp obtained from extracted teeth which were exposed to either 20%, 66%, or 98% humidity at 25°C for one and three weeks are presented in Table 4. HMW human DNA was obtained from 17 of 18 teeth used in this study. Thus, humidity alone does not appear to play a significant role in the destruction of HMW DNA from dental pulp.

Table 5 gives the results of DNA analysis of dental pulp obtained from extracted teeth which were exposed to seawater maintained at temperatures of 4°C, 25°C, or 37°C for up to four weeks. Despite the limited number of teeth sampled, it can be shown that, at 25°C, HMW DNA can be isolated after one month and, at 37°C, HMW human DNA can be isolated after two weeks of exposure.

TABLE 5—*Extracted teeth exposed to seawater maintained at temperatures of 4°C, 25°C, or 37°C for up to four weeks. Ratio is as described in Table 1.*

Temperature, °C	Exposure Time, Weeks			
	1	2	3	4
4	. . .	. . .	0/2	. . .
25	. . .	. . .	. . .	1/3
37	. . .	1/3	. . .	. . .

Autoradiograms showing the RFLP patterns of selected aging samples, along with the corresponding blood samples are shown in Figs. 1–5. The RFLP banding pattern obtained from dental pulp samples matches the pattern obtained from the corresponding blood exemplar, even after extracted teeth are aged for six months at 25°C (Fig. 1).

Similarly, the RFLP patterns of extracted teeth maintained at 37°C match the exemplar pattern; however, at 12 weeks, the banding pattern of the DNA extracted from the dental pulp begins to shift anodally relative to the matching exemplar pattern (Fig. 2). At 24 weeks of exposure, the "downward" shift is even more pronounced.

The membrane used to produce the autoradiogram shown in Fig. 2 was washed free of labeled probe and rehybridized with a probe detecting monomorphic fragments at the DXZ1 locus. This technique is used to document electrophoretic mobility shifts between sample lanes (Fig. 3). Hybridization with this probe produces two bands (4.1 and 2.0 kb) appearing at the same position in the autoradiogram for all individuals tested [6]. It

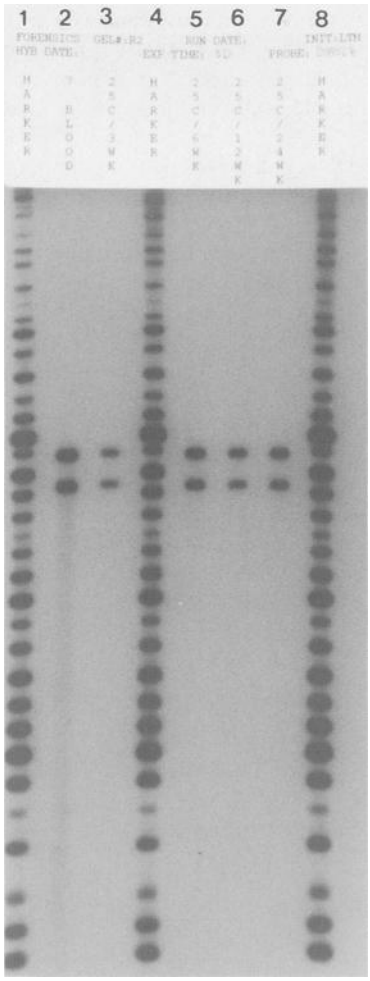

FIG. 1—*The D18S27 RFLP patterns from a blood exemplar (Lane 2) and dental pulp of four teeth from the same individual, exposed to 25°C at ambient humidity. Aging times are 3, 6, 12, and 24 weeks, shown in Lanes 3, 5, 6, and 7, respectively. Molecular weight markers are in Lanes 1, 4, and 8.*

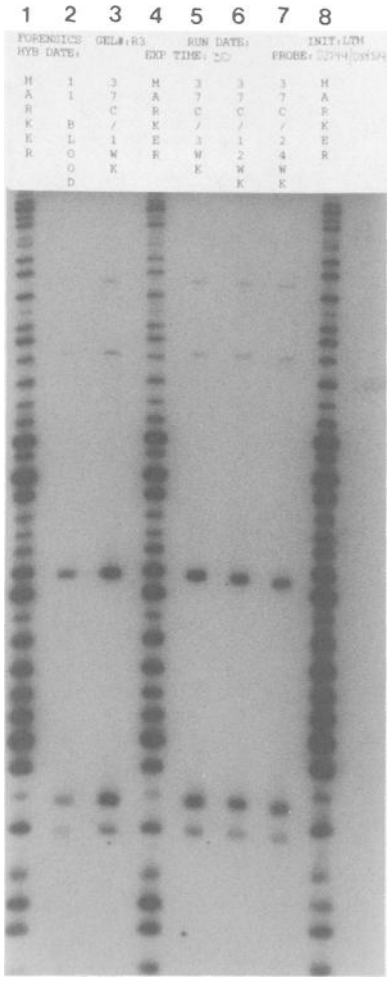

FIG. 2—*The D2S44 and DXYS14 RFLP patterns of DNA from dental pulp of four teeth from the same individual, exposed to 37°C at ambient humidity for up to 24 weeks, and a blood exemplar (Lane 2). Aging times are 1, 3, 12, and 24 weeks, in Lanes 3, 5, 6, and 7, respectively. A downward shift in the banding pattern at 12 and 24 weeks can be observed relative to the matching blood exemplar.*

can be seen that the monomorphic markers are shifted anodally in position in the samples exhibiting an electrophoretic mobility shift.

The autoradiogram in Fig. 4 illustrates the RFLP pattern of DNA extracted from the 16 and 19-year-old teeth described in Table 1. DNA extracted from blood samples obtained from each donor was used as "control samples" and run alongside the dental samples. Although the test gel revealed the presence of human HMW DNA, no RFLP pattern was obtained from the 16-year-old tooth. However, RFLP analysis of the DNA isolated from the two 19-year-old teeth and the blood exemplar resulted in matching patterns, although the dental samples exhibited an exaggerated downward shift.

The membrane used to produce the autoradiogram shown in Fig. 4 was washed free of labeled probe and rehybridized with "monomorphic probe" DXZ1 (Fig. 5). The resulting autoradiogram illustrates the downshifted DNA pattern.

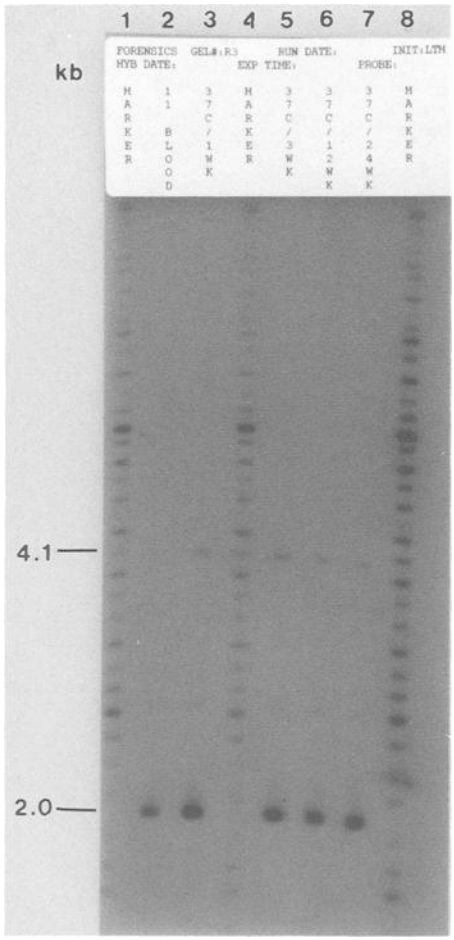

FIG. 3—*The monomorphic bands generated by hybridization with DXZ1. The probe was hybridized to the same nylon membrane shown in Fig. 2. A downward shift of the banding pattern of the 12- and 24-week samples is apparent.*

Discussion

We have found that dental pulp obtained from various types of extracted teeth which have been exposed to a variety of different environmental conditions contains sufficient DNA for RFLP analysis in most instances. As much as 15 to 20 μg of HMW DNA can be isolated from an average-sized tooth in good condition.

It should be noted that, in this study, variables other than environmental factors existed that could have affected the quantity and quality of the isolated DNA. These factors include (1) type of tooth, that is, incisor, canine, premolar, or molar, (2) condition of the tooth prior to extraction (degree of decay or other pathology), (3) condition of the tooth following extraction (anatomical trauma), (4) period of time from extraction to DNA isolation, (5) age of the donor, and (6) amount of pulp present in different teeth. Some of these factors may have a significant effect upon the researcher's ability to isolate HMW DNA. Unfortunately, in a study such as this it is not possible to fully control the samples and, therefore, conclusions regarding the results must be drawn with caution.

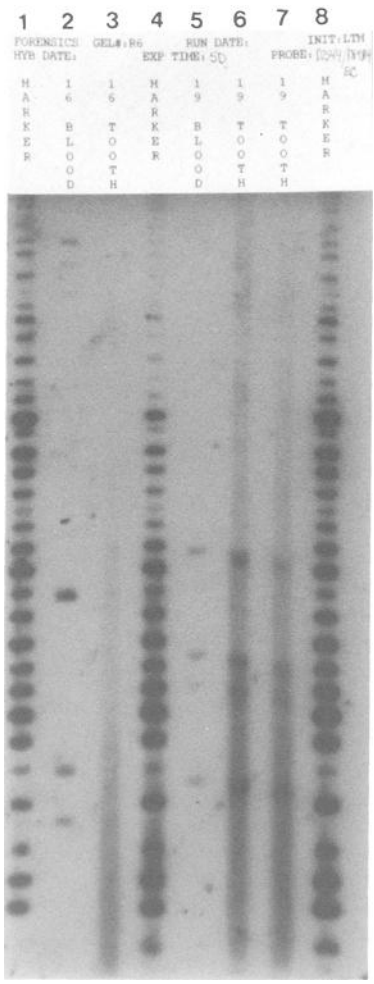

FIG. 4—*The D2S44 and DXYS14 RFLP patterns of DNA from dental pulp of one 16 and two 19-year-old teeth, with corresponding blood exemplars (Lanes 2 and 5). No RFLP pattern was observed in the 16-year-old tooth (Lane 3), and a similar but downshifted pattern was observed in the two 19-year-old teeth (Lanes 6 and 7).*

Despite these variables it appears, based upon our observations, that aging under certain circumstances may cause a complete loss of HMW DNA. In approximately 5% of the teeth which lacked HMW DNA, the lower-molecular-weight forms of DNA could not be detected on the yield gels. However, the overwhelming majority of teeth lacking HMW DNA did contain degraded DNA to various extents. Although it is possible that the degradation of dental pulp DNA may result from nonspecific nuclease activity, other factors may also be responsible.

It is apparent from the aging study that HMW human DNA can be isolated from some 19-year-old teeth. The surprising stability of DNA in extracted teeth is, in part, due to the well-protected position of the dental pulp within the pulp cavity. Bacterial access to dental pulp in a relatively intact tooth is limited, and contamination of dental pulp appears not to be a serious problem. Furthermore, environmental factors such as temperature between the limits of 4 and 37°C, humidity between the limits of 20 and 98%, pH between

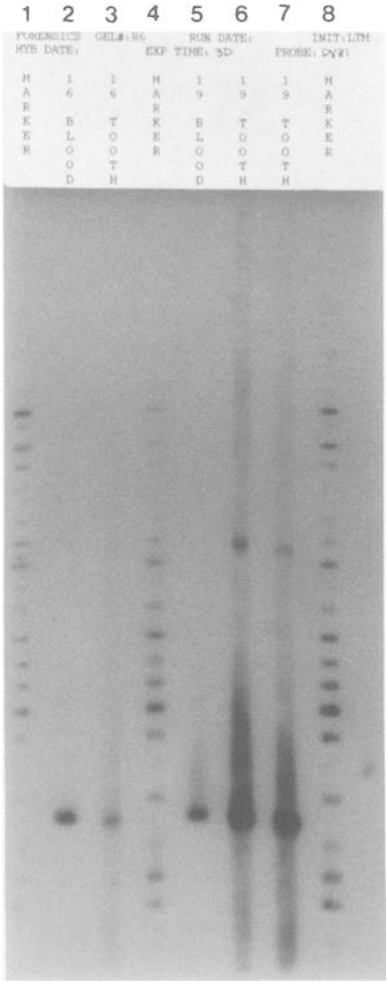

FIG. 5—*The monomorphic fragments generated by hybridization with DXZ1. The probe was hybridized to the same nylon membrane shown in Fig. 4. As in Fig. 4, the same downward shift can be seen in the two 19-year-old teeth (Lanes 6 and 7), as well as a shift in the 16-year-old tooth (Lane 3).*

the limits of pH 3.0 and 10.0, and exposure to seawater do not create significant difficulties in the isolation of HMW human DNA under the conditions described in Materials and Methods.

It should be noted that, in the temperature study, only approximately one third of the teeth stored at 4°C yielded HMW DNA, whereas three quarters of the teeth stored at 25°C and about one half of the teeth stored at 37°C provided HMW DNA. This observation is surprising in that biological specimens are generally more stable when stored at lower temperatures. Despite these results, we do not believe that one can or should infer that lower temperatures adversely affect the isolation of HMW DNA. Several factors may be responsible for the observations described in this preliminary study, including (1) the relatively small number of teeth (n = 14) stored at 4°C as compared with 32 at 25°C and 29 at 37°C and (2) the absence of samples stored longer than six weeks at 4°C.

On the other hand, the results seen in Table 3 indicate that exposure of extracted teeth to certain soil types can result in the loss of HMW DNA. At this time, it is unclear if chemical components of soil or microbes present in the soil or if both factors combined cause the observed degradation of dental pulp DNA. This problem is now under study.

The observed band-shifting of dental pulp DNA relative to the matching exemplar seen in Figs. 2 and 4 is an indication that DNA present in dental pulp may progressively change over time, causing an increased electrophoretic mobility with extended exposure to environmental insult. These observations are consistent with previous reports about DNA isolated from other tissues [*6*]. Although the reason for this type of increased electrophoretic mobility has not been determined, it is known that large differences in salt concentration or sample overloading can result in an increased electrophoretic mobility of the restricted fragments [*6*]. This can be problematic when the researcher is determining if two DNA samples match. Irreversible chemical modification of the DNA resulting from environmental exposure might account for the altered mobilities observed in the restriction fragments of the treated samples. As a result, charge or conformational changes, or both, that may be induced in the extended exposure conditions may modify the electrophoretic behavior of the DNA, thus increasing the migration rate of the treated sample relative to the untreated exemplar. Additional study is needed to determine the nature of the observed phenomenon.

One way to document electrophoretic mobility differences is to include the use of monomorphic markers in the analysis. These markers, which detect monomorphic fragments at a given locus (such as DXZ1), provide information as to whether samples have altered electrophoretic mobilities. Since differences in mobility may not be of the same magnitude throughout the gel, it is important for the researcher to be able to document differences throughout all regions of the gel. In the case of the DNA samples obtained from two 19-year-old teeth, as seen in Fig. 4, the mobility of all bands was increased uniformly approximately 2.5% and 5.0%, respectively, as compared with the mobility of the corresponding blood exemplar bands. It is clear that individual teeth obtained from the same person are not identical and that, if band-shifting occurs in various samples exposed to identical environmental and storage conditions, the degree of the shift can be somewhat different. The use of DXZ1 appears to work well in identifying the presence of electrophoretic mobility shifts in dental pulp DNA between the 2 and 4-kb regions of the gel.

Acknowledgments

Parts of this paper were submitted in partial fulfillment of the requirements for the M.S. degree in Forensic Science at John Jay College of Criminal Justice by Laura Mieszerski. We would like to thank Dr. Ivan Balazs of Lifecodes Corporation for his valuable comments and review of this paper, Essex Oral Surgery Associates (Bloomfield, New Jersey) for providing extracted teeth for this project, and those individuals who donated their extracted teeth for our long-term aging study. We would also like to acknowledge the support of the Department of Education, MSIP, Grant No. G008641165 and NSF-ILI Grant No. USE 885-1684.

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