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Restriction Fragment Length Polymorphism (RFLP) Analysis on DNA from Human Compact Bone

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ABSTRACT: DNA typing techniques primarily identify specific genetic markers that are highly polymorphic within a population and have found great utility in forensic science. The established DNA identification protocol, termed restriction fragment length polymorphism (RFLP), has been admitted as physical evidence in the investigation of crimes such as assault, sexual assault, and homicide. The limitation associated with this procedure concerns the integrity of the genetic material. This study sought to evaluate human bone as a source material for DNA identification following exposure to common forensic field conditions. Often, with the onset of decomposition and eventual disarticulation of a body, soft tissues, hair and teeth may not be recovered. The significance of this study lies in the fact that, within forensic anthropology, human bone represents the most biologically stable evidence and is sometimes all that remains after periods of exposure. Genomic DNA was extracted from human bone following exposure to surface deposit, shallow burial, and fresh water immersion. Samples were collected over a three month time course and analyzed by spectrophotometry and agarose gel electrophoresis as well as RFLP analysis. The data suggest that high molecular weight DNA may indeed be extracted from human bone and typed by RFLP analysis for use in forensic identification. Under simulated forensic field conditions, the severity of DNA degradation was in the order of fresh water immersion > shallow burial > surface deposit. Genomic DNA from bone deposited on the desert surface for up to 4 weeks was detected by RFLP analysis. No spurious bands were detected in any specimens, and to the extent that bands were still present, the RFLP patterns matched. These findings demonstrate that human bone can be a reliable source of genomic DNA, and that bone recovered from surface deposit is the most desirable for use in forensic identification.

KEYWORDS: forensic anthropology, DNA, Restriction Fragment Length Polymorphism, human cortical bone, forensic science, criminalistics, bone DNA, human identification

In the past decade, forensic identification based upon deoxyribonucleic acid (DNA) analysis has been used as physical evidence in the investigation of crimes such as assault, sexual assault, and homicide (1). For both psychological and legal reasons, positive identification of a decedent is desired and often may be accomplished via the traditional scientific methods of comparative dental radiography, friction ridge analysis (fingerprint matching), and/or thorough osteological examination (including comparative radiography). However, because of extensive putrefaction of soft tissues, disarticulation and destruction of key skeletal landmarks, and unavailable pre-mortem records, these traditional methods may not be useful (2). Recent developments in DNA analyses and their application to forensic identification of biologic evidence have revolutionized the possibility of identifying human remains (3).

At this writing, the most widely applied genetic forensic identification or exclusion diagnosis is achieved using the established protocol for genomic DNA analysis, termed restriction fragment length polymorphism (RFLP). This kind of analysis utilizes radiolabeled human-specific probes which recognize and bind to restriction endonuclease-digested sample DNA that has been size-separated and transferred onto a nylon membrane. Each probe detects the variable number of tandem repeats (VNTR) polymorphism within a hypervariable region of the human genome (3–6). The polymorphic nature of these VNTR are visualized as a band pattern on an autoradiograph and in this way demonstrate the capability of virtually individualizing human DNA (7,8).

The DNA identification currently employed in crime laboratories has to this point been mainly confined to body fluids and soft tissue samples. Under laboratory conditions, blood and semen stains in a protected dry environment have yielded DNA still suitable for testing after three years (9–11). Teeth and hair also may serve as excellent sources of DNA and are biologically very stable. Schwartz et al. determined that, aside from burial in various soil types, many environmental conditions do not affect the ability to obtain high molecular weight human DNA from dental pulp (12). However, it is the authors' experience that many forensic case remains are often only fragmentary, where teeth and hair may not be recovered after protracted exposures, especially if scavenger activity is involved. We thus propose to use human compact bone samples as a source of DNA for RFLP analysis.

The use of human bone as a source for DNA typing is a relatively recent development in forensic science. Studies which explore the utility of this technique include DNA extraction from Civil War bones, identification of a murder victim, and the analysis of ancient human remains (13–15). A common problem that plagues this type of analysis is the preservation of the DNA. Degradation is the limiting factor in DNA recovery. We know that exposure to simulated environmental insult results in the degradation of DNA in postmortem tissues (16,17). As yet, however, there has not been shown a strict correlation between the degree of DNA degradation and the time since death (2). Some early studies of DNA stability

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have shown recovery of typable DNA out to three weeks postmortem in brain cortex, lymph nodes, and psoas muscle (18). A study on recovery of DNA from post mortem blood, heart muscle, chest muscle, liver, spleen, hair and bone tissues indicated that liver DNA tends to be most quickly degraded, while DNA from heart muscle, spleen, bone and hair tends to be more stable (19). Predictably, variability in bacterial growth and autodegradation due to putrefaction during decomposition appear to have major effects on DNA recovery from these tissues. Hochmeister et al. demonstrated the feasibility of extracting high molecular weight DNA from bone samples exposed to various temperate environmental conditions, including outdoors (25°C), water immersion and shallow burial (20). They noted that forensic-use DNA could be recovered from bone under these conditions which proved too severe for quality DNA recovery from soft tissue.

In general, it appears that all biological samples will demonstrate significant loss and degradation of DNA upon exposure. Thus, in order to develop a DNA typing procedure for use as a sensitive and valid analysis, we must first evaluate the effect of typical local environmental conditions found in many forensic cases on recoverable DNA from bone. We assume that these effects are condition, location, and time dependent. In this study, we tested the hypothesis that DNA may be recovered from human bone exposed to the Sonoran desert and typed for use in forensic identification.

Materials and Methods

Treatment of Bone Samples

One midshaft femoral section was removed, fresh at autopsy, from each of three individual cadavers where disease was not associated with death. One section of each was immediately frozen at −80°C as a set of controls (time zero). The remainder of each sample was exposed, respectively, to the three exposure conditions of surface deposit, shallow burial, and fresh water immersion. For the surface deposit, the sample was left fully exposed on the desert floor. The shallow burial sample was buried 0.5 meters below the surface. The samples treated with these exposures were protected from scavenger activity by a bottomless 2 × 2 cm wire mesh cage. For fresh water immersion, a femoral section was fully enclosed in a 2 × 2 cm wire mesh cage and immersed in a local spring-fed pond. Bone samples were collected at week 2, 4, 8, and 12 for each exposure condition. At each time point a 3 gram section of bone was removed and immediately stored at −80°C prior to extraction of genomic DNA.

DNA Isolation

All soft tissue, including any marrow that might remain, was removed from the bone and all surfaces were ground to a depth of approximately 1 mm. Each sample was then surface-sterilized with 70% ethanol, wrapped in clean bench paper and fragmented with a mallet. Dehydration and desiccation were carried out with sequential washes of sterile deionized water, 100% ethanol, and ethyl ether shaken at 4°C for 20 min each. When dried, the sample was frozen in liquid nitrogen and ground to a fine powder using a Waring blender fitted with a special stainless-steel specimen cup (Fisher Scientific). Two grams of bone powder were digested with 3 mL of 10 mM Tris, pH 8.0, 10 mM EDTA, 0.1 M NaCl, 2% SDS, and 0.5 mg/mL Proteinase K at 42°C for 16 hours in a shaking water bath. The Proteinase K concentration was then brought up to 1 mg/mL and incubation continued 5 additional hours (21). Each sample was centrifuged at 5,000 × g for 3 min at 4°C; the supernatant was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (1:1:0.04). The DNA was precipitated with 2.5 volumes of cold 100% ethanol. The DNA pellet was recovered by centrifugation at 12,000 × g for 12 min at 4°C and resuspended in 10 mM Tris, 0.1 mM EDTA, pH 8.0.

DNA Quantitation

DNA recovered from bone samples was quantitated by 1) absorbance at 260 and 280 nm; 2) agarose gel electrophoresis alongside known quantitative standards (15–500 ng DNA, Gibco BRL) and 3) Southern transblot (22) of the agarose gel followed by hybridization analysis using the [^{32}P]-labeled, human-specific probe V1 (locus D17S79) (Lifecodes) (see RFLP analysis for probe labeling and washing conditions). Isolated human DNA was detected by autoradiography. Transblots were also hybridized with two oligodeoxynucleotide probes, p11E and p13B, which were derived from conserved gene sequences for the 16S ribosomal RNA found in eubacteria (23). These probes were labeled at the 5′end with [^{32}P]ATP (5000Ci/mmol, Du Pont, NEN) and hybridization was carried out overnight at 42°C in 30% formamide/6 × SSC/5 × Denhardt's solution/0.1 mg/mL herring sperm DNA/0.5% SDS supplemented with 500,000 dpm/probe/mL. The membrane was washed sequentially with 2 × SSC and 0.2 × SSC, 15 min each at room temperature, blotted dry and analyzed by autoradiography.

RFLP Analysis

RFLP analysis was carried out according to the method described by Budowle and Baechtel (24). Briefly, the isolated DNA was digested with 5 times excess of the restriction endonuclease HaeIII (Boehringer Mannheim) overnight at 37°C, extracted with phenol/chloroform/isoamyl alcohol, and the DNA precipitated with ethanol. The DNA sample was then electrophoresed through a 1% agarose gel alongside size markers (λDNA, 0.5–22.6 kb, Gibco, BRL) and a predigested positive control (DNA from the human cell line K562 (Lifecodes). After electrophoresis, the DNA was transblotted onto a BiodyneB membrane (Pall Biosupport) under alkaline conditions (22) and covalently linked to the membrane in a CL-1000 ultraviolet crosslinker (UVP) at 254 nm with 120,000 μJ/cm^2. Each membrane was hybridized with a human-specific DNA probe and a DNA probe for the size markers, both labeled with [^{32}P]dCTP (3000Ci/mmol, Du Pont, NEN). Two human-specific probes were employed: V1 and CMM101 (locus D14S13) (Promega). Hybridization was carried out at 65°C for 16 hr in 10% polyethylene glycol/1.5 SSPE/7% SDS supplemented with 500,000 dpm/mL for each probe used. Following high stringency washes, the membrane was exposed to Kodak X-AR 2 film at −80°C.

Quantitative Analysis of Autoradiograms

Autoradiograms from RFLP analyses were analyzed with the Bio Image Electrophoresis Analyzer (Millipore). The intensities of the fragments observed for the time zero controls and the exposed samples were quantitated by two-dimensional digitization based on whole band scanning analysis. The intensity of the signal observed for each treatment time point was normalized against that of its time zero control and expressed as a percentage of the intensity of the control. The size of the fragments was determined based on the migration of the molecular size standards. For all

treatments, the size of each restriction fragment observed at any particular time point was compared to that of its respective time zero.

Statistical Analysis

To compare the intensities of the signals detected at the various time points for each treatment, the normalized values for all the fragments detected by each of the human-specific probes were pooled, based on the assumption that any particular exposure condition has the same (random) effect on each of the probed loci. The data were then analyzed by repeated measures ANOVA.

Results

The results of this study indicate the following: 1) two grams of femoral cortical bone were sufficient to extract quality, typable genomic DNA from relatively fresh cadavers (hours to a few days old), 2) under the simulated Sonoran desert forensic field conditions, the severity of DNA degradation was in the order of fresh water immersion > shallow burial > surface deposit, and 3) in the case of surface deposit, DNA bands could be detected at up to 4 weeks of exposure based on the use of 5 μg of DNA and a 6 day exposure for autoradiography. No spurious bands were observed in any specimens, and to the extent that bands were still present, the RFLP patterns matched.

To quantitate the DNA recovered from the bone extracts, the absorbance of the extracts was measured at 260 nm and 280 nm wavelengths (Table 1). Based on the 260 nm/280 nm ratios, these data suggest that the surface and water exposed samples give the highest quality DNA yield, while those from the shallow burial samples diminish after two weeks.

Figure 1*A* shows the electrophoretic analysis of the DNA extracted from bone samples that had been subjected to surface exposure. Each sample contains an intense band of high molecular weight DNA (co-migrated with the quantitative DNA standards) as well as a smear of lower molecular weight DNA, indicated by the spread of the ethidium bromide staining on each lane. The presence of a significant amount of lower molecular weight products (located between 5.5 cm and 6.5 cm on the scale) in each sample suggests that the extracts may also contain RNA. Figure 1*B* shows the results of Southern analysis of these DNA extracts

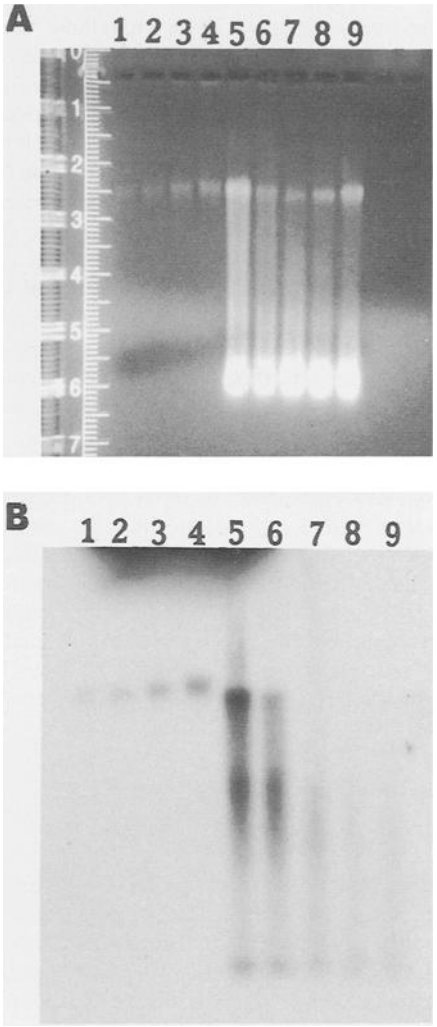


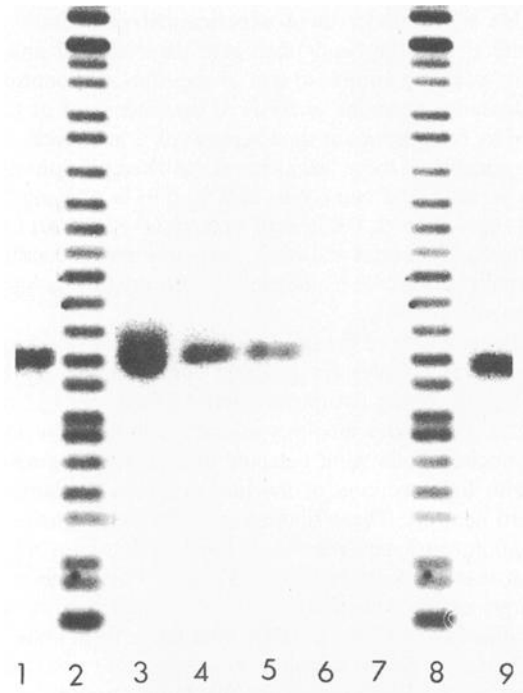
FIG. 1—(A) *1% agarose gel electrophoresis of DNA from control and surface exposed bone. Lanes 1–4, 63, 125, 250 and 500 ng of human DNA quantitation standards; lanes 5–9, 5 μg of DNA from time 0, week 2, 4, 8 and 12.* (B) *Southern hybridization analysis of the DNA samples in (A) with probe V1.*

TABLE 1—*Spectrophotometric determination of DNA recovered from control and treated human compact bone.*

Exposure	Time	260 nm/280 nm	DNA(μg)/g tissue
Surface Deposit	Control	1.79	63
	2 wk	1.86	96
	1 mnth	1.90	78
	2 mnth	1.93	90
	3 mnth	1.84	101
Shallow Burial	Control	1.85	82
	2 wk	1.85	160
	1 mnth	1.45	35
	2 mnth	1.33	23
	3 mnth	1.54	20
Water Immersion	Control	1.81	73
	2 wk	1.69	46
	1 mnth	1.59	38
	2 mnth	1.67	54
	3 mnth	1.75	51

by their hybridization to the human-specific probe, V1. The time zero control showed a substantial spread of radiolabeled signal. This could be due to overloading of the sample, or may indicate some degree of degradation of the DNA. Radiolabeled signal was also detected for all the time points, yet high molecular weight signal was only present in the time zero control and week 2 extracts. Furthermore, at week 2, the high molecular weight signal was estimated to be similar to the intensity of the signal for the 500 ng quantitative standard, suggesting that after 2 weeks of surface exposure, only 10% (5 μg of DNA was loaded on each sample lane) of the DNA extract consisted of high molecular weight DNA of human origin.

DNA extracts from the surface exposure, shallow burial and water immersion samples were further analyzed by RFLP using V1 and CMM101 (Figs. 2, 3 and 4). All three time zero controls, as well as the K562 controls, show a clear restriction pattern with both probes. Band signals could also be detected by image scanning analysis after up to 4 weeks of surface exposure for both V1 and CMM101. Samples from all longer exposure did not show



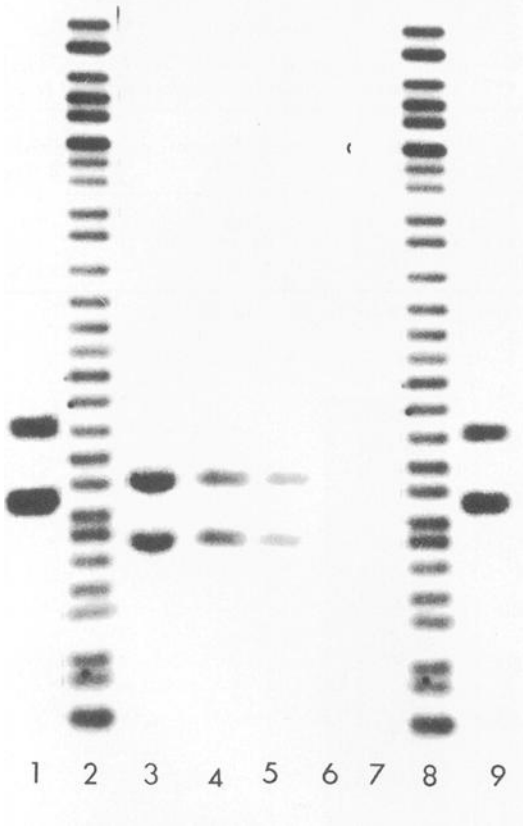
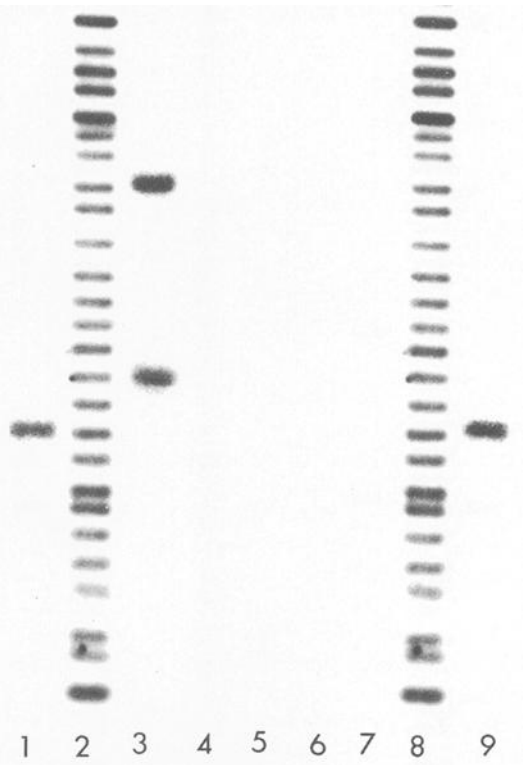


FIG. 2—*RFLP analysis of DNA from control and surface deposits.* (Top) *CMM101;* (Bottom) *V1. In each panel, lane 1, positive control (K562, 1 μg); lane 2, molecular size markers; lanes 3–7, time 0, week 2, 4, 8 and 12; 5 μg per lane; lane 8, molecular size markers; lane 9, positive control (K562, 1 μg).*



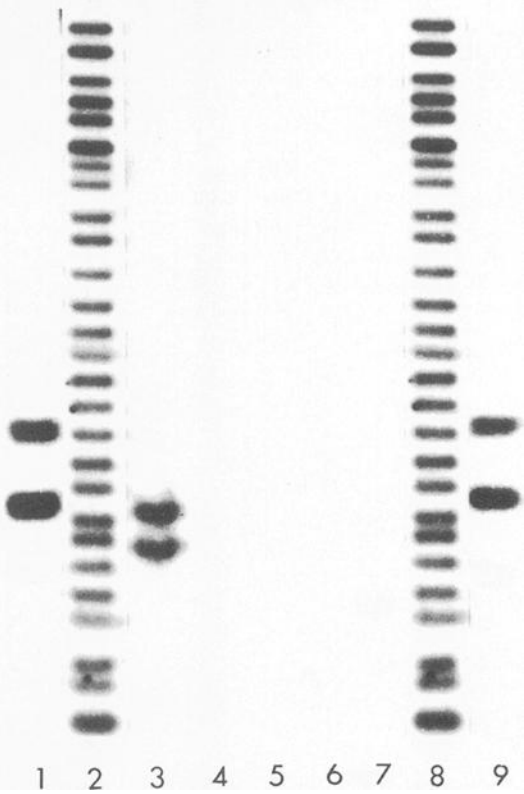


FIG. 3—*RFLP analysis of DNA from control and shallow burial.* (Top) *CMM101;* (Bottom) *V1. In each panel, lane 1, positive control (K562, 1 μg); lane 2, molecular size markers; lanes 3–7, time 0, week 2, 4, 8 and 12; 5 μg per lane; lane 8, molecular size markers; lane 9, positive control (K562, 1 μg).*

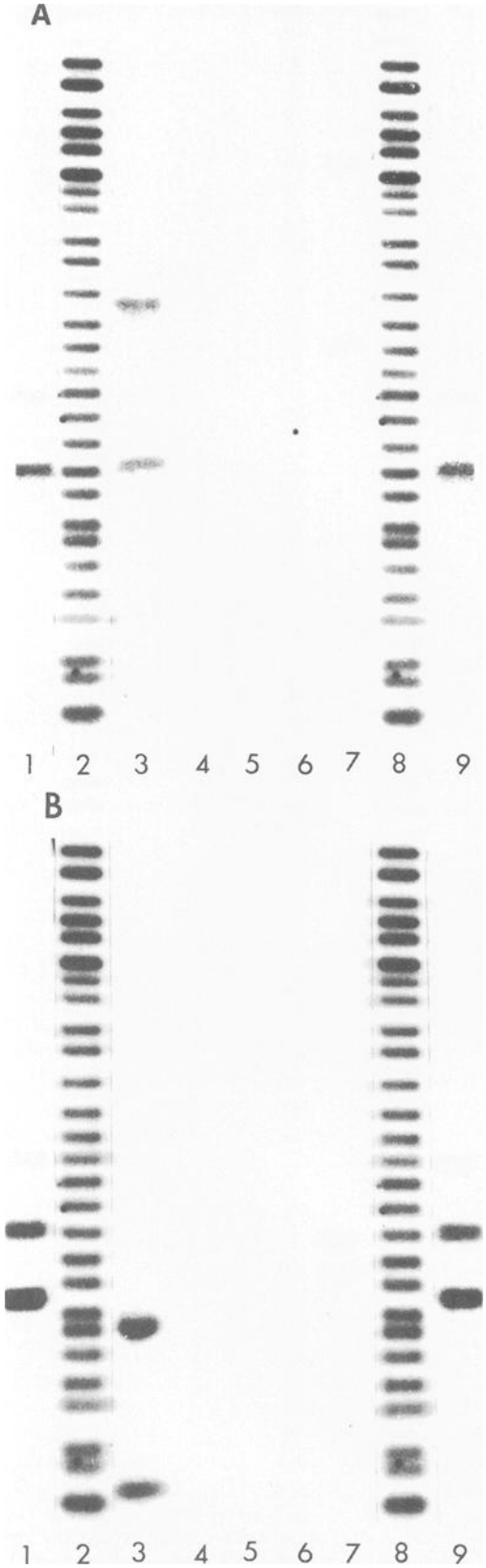


FIG. 4—*RFLP analysis of DNA from control and fresh water immersion. (A) CMM101; (B) V1. In each panel, lane 1, positive control (K562, 1 μg); lane 2, molecular size markers; lanes 3–7, time 0, week 2, 4, 8 and 12, 5 μg per lane; lane 8, molecular size markers; lane 9, positive control (K562, 1 μg).*

detectable signal under these experimental conditions. Furthermore, the size of the bands that were detected at 2 and 4 week exposure was very similar to that of the time zero control (Table 2). Quantitative scanning analysis of the intensities of the bands detected by both probes at time zero, week 2 and week 4 showed that the quantity of these fragments diminished precipitously, such that by week 2, the level was 18.9 ± 5.91% of control and at week 4, only 0.66 ± 0.096% of control ($P < 0.0001$) (Fig. 5). For both shallow burial and fresh water immersion conditions, no signal could be detected by the probes from any time points beyond the time zero control.

We also re-analyzed the DNA extracts from surface deposit and shallow burial samples for possible contamination by bacterial DNA (Fig. 6). Using two probes derived from the 16S rRNA of eubacteria, significant amounts of both high and low molecular weight nucleic acids were detected upon autoradiography in all lanes with the exception of the human DNA standards and the time-zero controls. These findings support the diagnosis that the spectrophotometric analysis was skewed by non-human DNA and RNA, at least partially of bacterial origin. Furthermore, extracts from bone exposed to shallow burial contained a substantially greater quantity of bacterial DNA than those from bone exposed to the surface. These findings are consistent with the results observed upon RFLP analysis of the same samples, in that the rapid loss of RFLP signal from shallow burial exposed samples correlates with a marked increase in microbial contamination.

TABLE 2—*Restriction fragments[a] detected by V1 and CMM101 from DNA extracted from control and surface exposed bone.*

Probe	Control (kbp)	Treated week 2 (kbp)	Treated week 4 (kbp)
V1	1.61	1.63	1.63
	1.24	1.27	1.27
CMM101	1.72	1.71	1.72

[a]Determined from autoradiogram shown in Fig. 2.

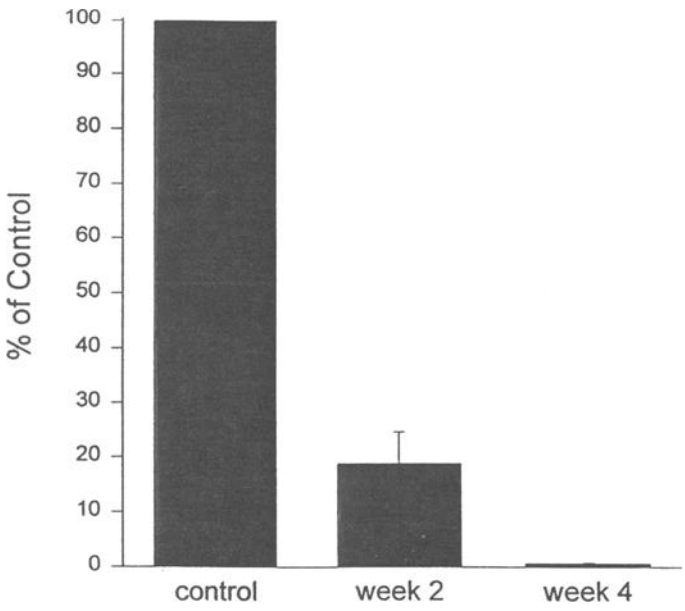
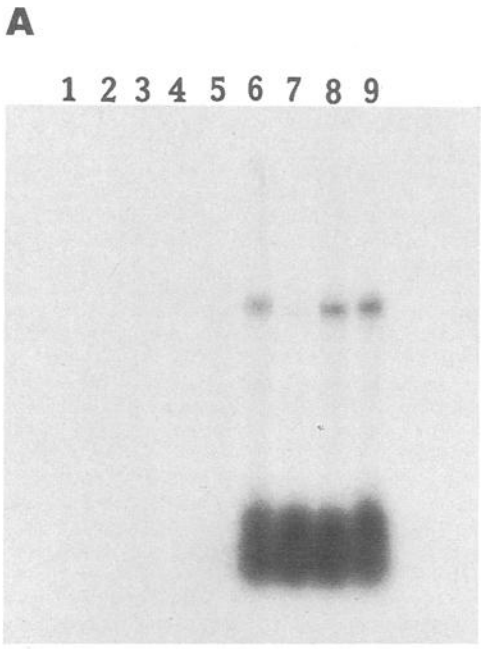


FIG. 5—*Quantitative analysis of the intensities of the RFLP fragments from surface deposits.*



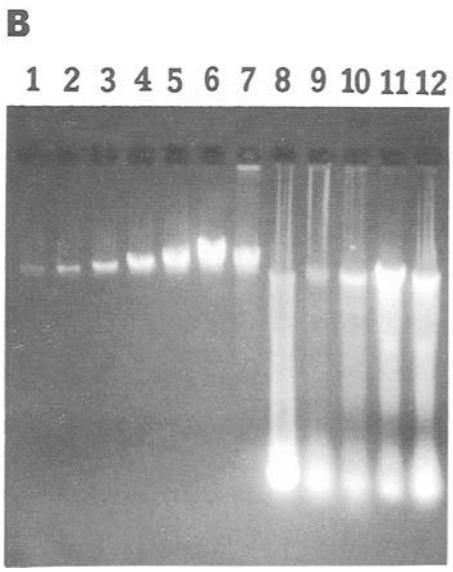


FIG. 6—(A) *Southern hybridization analysis of DNA from control and surface exposed bone with p11E and p13B; the order of samples is the same as that in Fig. 1A.* (B) *1% agarose gel electrophoresis of DNA from control and shallow burial. Lanes 1–6, 15, 31, 63, 125, 250 and 500 ng of λ DNA quantitation standards; lane 7, 250 ng of K562 DNA; lanes 8–12, 5 μg of DNA from time 0, week 2, 4, 8 and 12.*

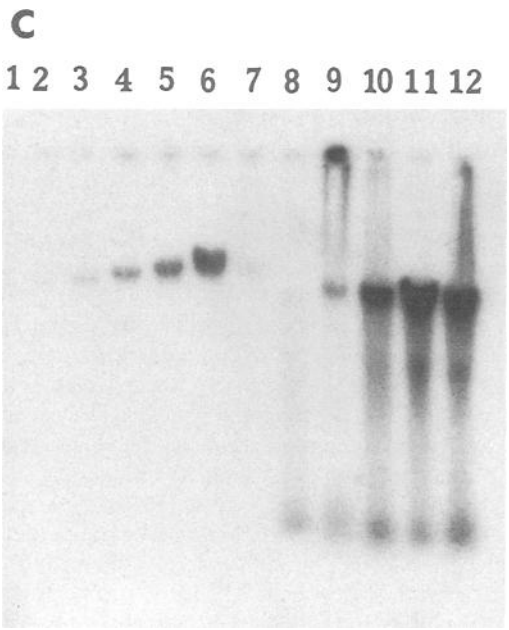


FIG. 6—*Continued,* (C) *Southern hybridization analysis of the DNA samples in* (B) *with p11E and p13B.*

Discussion

The main goal of this study is to establish human bone as a valid and reliable source for use in DNA testing in forensic casework. The majority of the forensic cases in the Sonoran desert can be identified by traditional anthropologic methods, yet in many cases only estimations of age, race, sex, and stature may be made. The methods employed in this study are not only significant when there is direct ante-mortem evidence for DNA comparison, but may also be used in cases where the separation of comingled evidence is problematic, when the relationship between spatially disparate fragmentary remains is in question, or when first order living relatives of the victims are available for reverse-paternity testing.

Based on our initial analysis of the DNA extracted from various bone specimens (time zero controls), it is clear that bone can be used as a reliable source of DNA. An average of 73 μg per gram of bone was recovered from three separate specimens (time zero controls) (Table 1). Although the quality of the DNA may vary, RFLP analysis of the three bone specimens suggests that they yield high quality restriction patterns (Figs. 2, 3 and 4). Exposure of these bone samples to various environmental insults indicates that the DNA is susceptible to the prolonged treatments of shallow burial (Fig. 3) and water immersion (Fig. 4). After 2 weeks of exposure, the DNA demonstrated extensive degradation upon RFLP analysis. This was despite an apparent good recovery of DNA material for both treatments at week 2 (Table 1, 160 μg/g for shallow burial and 46 μg/g for water immersion). We consistently found that a large amount (μg quantities) of DNA was required from these bone samples for our RFLP analysis, even though in typical forensic RFLP analyses, only nanogram quantities of DNA are employed (1). The Southern analyses performed on unrestricted DNA with the human specific probe V1 (Fig. 1*B*) and the eubacteria-specific probes, p11E and p13B (Fig. 6, *A* and *C*), provide strong evidence that the human DNA degrades rapidly upon exposure to the experimental conditions. It also demonstrates that much of the nucleic acid in the extracts was of non-human origin. These results are consistent with the RFLP data from all exposure conditions, showing a rapid loss of sample signal over time when compared to the respective controls. It must be noted that the three experimental conditions in this study were carried out using three unrelated bone specimens, and for this reason individual variability must be taken into consideration. We believe, however, that the effects of the various exposure conditions overwhelm these considerations. Thus, bone specimens that have been subjected to the various environmental conditions typically found in forensic casework in this locale appear to be limited in overall utility for DNA typing. The sensitivity of the RFLP analysis

depends largely on the preservation of the high molecular weight human DNA, and contamination by microbial DNA and RNA in the bone extracts limits the sensitivity of the this analysis. Conceivably, the sensitivity of the RFLP analysis may be enhanced by separating out the human DNA from the microbial contamination prior to restriction of the sample, particularly in the case of surface deposits.

Our results also support the contention that microbial activity and/or moisture in the soil may be more important factors for DNA degradation in bone than radiation and heat (9,12). This would explain the relatively intact DNA in the samples subjected to surface exposure. Longer exposure time for autoradiography or increasing the amount of total DNA loaded may enhance the sensitivity of detection. Of the three simulated forensic exposures, bone from the surface deposit is the most desirable source of DNA for RFLP analysis. It is also important to note that the relative stability of the surface exposed sample DNA and its potential for improved detection are significant not only because the majority of our forensic cases fall into this category, but many if not most of the shallow burial cases are unearthed by opportunistic predators within hours post-mortem and thus become surface exposed cases.

Acknowledgments

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References

(1) Kelly KF, Rankin JJ, Wink RC. Method and applications of DNA fingerprinting: a guide for the non-scientist. In: Criminal Law Review 1987:105–10.
(2) Gaensslen RE, Lee HC. Genetic markers in human bone tissue. Forensic Sci Rev 1990;2(2):126–46.
(3) Gaensslen RE, Berka KM, Pagliaro EM, Ruano G, Messina D, Lee HC. Studies on DNA polymorphisms in human bone and soft tissues. Anal Chimica Acta 1994;288:3–16.
(4) Fowler JCS, Burgoyne LA, Scott AC, Harding HJW. Repetitive deoxyribonucleic acid (DNA) and human genome variation—a concise review relevent to forensic biology. J Forensic Sci 1988;33(5):1111–26.
(5) Huang PL, Huang PL, Lee-Huang S. DNA polymorphism and forensic identification. In: Lee HC, and Gaensslen RE, editors. DNA and other polymorphisms. Yearbook Mosby, Chicago: 1990;1–25.
(6) Odelberg S, White R. Repetitive DNA: molecular structure, polymorphisms, and forensic applications. In Lee HC, and Gaensslen RE, editors. DNA and other polymorphisms. Yearbook Mosby, Chicago: 1990;26–44.
(7) Jeffreys AJ, Wilson V, Thein SL. Hypervariable 'minisatellite' regions in human DNA. Nature 1985;314:67–73.
(8) Jeffreys AJ, Wilson V, Thein SL. Individual-specific 'fingerprints' of human DNA. Nature 1985;316:76–9.
(9) McNally L, Shaler RC, Baird M, Balazs I, DeForest T, Kobilinsky, L. Evaluation of deoxyribonucleic acid (DNA) isolated from human bloodstains exposed to ultra-violet light, heat, humidity, and soil contamination. J Forensic Sci 1989;34(5):1059–69.
(10) Kuo M, Bashinski J, Vukovich K, et al. {California Association of Crime Laboratory Directors} DNA Committee Report #6, 1988.
(11) Madisen L, Hoar DI, Holroyd CD, et al. DNA banking: the effects of storage of blood and isolated DNA on the integrity of DNA. Am J Med Genet 1987;27:379–90.
(12) Schwartz TR, Schwartz EA, Mieszeraki L, McNally L, Kobilinsky L. Characterization of deoxyribonucleic acid (DNA) obtained from teeth subjected to various environmental conditions. J Forensic Sci 1991;36(5):979–98.
(13) Fisher D, Holland M, Mitchell L, Sledzik P, Webb Wilcox A, Wadhams M, Weedn VW. Extraction, evaluation and amplification of DNA from decalcified and undecalcified United v States civil war bone. J Forensic Sci 1993;38(1):60–8.
(14) Hagelberg E, Cray IC, Jeffreys AJ. Identification of the skeletal remains of a murder victim by DNA analysis. Nature 1991; 352:427–9.
(15) Pääbo S. Molecular genetic investigations of ancient human remains. *Cold Spring Harbor Symp Quan Biol* 1986;51 pt. 1:44–6.
(16) Perry WL, Bass WM, Riggsby WS, Sirotkin K. The autodegradation of deoxyribonucleic acid (DNA) in human rib bone and its relationship to the time interval since death J Forensic Sci 1988; 33(1):144–53.
(17) Ludes B, Pfitzinger H, Margin P. DNA fingerprinting from tissues after variable postmortem periods. J Forensic Sci 1993;38(3): 686–90.
(18) Bär W, Kratzer A, Mächler, M, Schmid W. Postmortem stability of DNA. Forensic Sci Int 1988;39:59–70.
(19) Swarner S, Reynolds R, Sensabaugh GF. A comparative study of DNA extracted from several post-mortem tissues. Proceedings of the International Symposium on the Forensic Aspects of DNA Analysis. U.S. Government Printing Office, Washington DC: 36, 1990.
(20) Hochmeister MN, Budowle B, Borer UV, Eggmann U, Comey CT, Dirnhofer R. Typing of deoxyribonucleic acid (DNA) extracted from compact bone from human remains. J Forensic Sci 1991;36(6): 1649–61.
(21) Holland MM, Fisher, DL, Mitchell LG, Rodriguez, Canik JJ, Merril CR, Weedn VW. Mitochondrial DNA sequence analysis of human skeletal remains: Identification of remains from the Vietnam War. J Forensic Sci 1993;38(3):542–53.
(22) Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975;98(3): 503–17.
(23) Chen K, Neimark H, Rumore P, Steinman CR. Broad range DNA probes for detecting and amplifying eubacterial nucleic acids. FEMS Microbiol Lett 1989;48:19–24.
(24) Budowle, B, Baechtel, FS. Modifications to improve the effectiveness of Restriction Length Polymorphism Typing. Applied and Theoretical Electrophoresis 1990;1:181–7.

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