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Rates of Putrefaction of Dental Pulp in the Northwest Coast Environment

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ABSTRACT: Cytological stability is of interest to criminal investigators in instances where remnants of soft tissue have been preserved, since such tissue can aid in the identification of human remains, helping to determine either the sex of the individual or his or her identity. This study based on seven experiments shows that, in Northwest coast outdoor environments in both summer (three experiments) and winter (three experiments), the stability of dental pulp nuclei ranges from 4 days to 2 weeks. The seventh experiment serves to describe the morphological sequence observed in nuclear putrefaction. The specimens included human and pig extracted teeth and unextracted pig teeth. Deposition of the specimens was made both on the surface and in the subsurface (30-cm depth), and the environmental variables were recorded.

KEYWORDS: odontology, dental pulp, pigs, putrefaction, human identification, Northwest coast

Determination of sex can be accomplished from sex chromatin evaluation, as was the case in the present research. Sex diagnosis can also be made by the application of Y deoxyribonucleic acid (DNA) probes, and individuals can be identified by DNA typing [*1–3*]. Knowledge of cellular decomposition rates in cadavers deposited in outdoor environments is therefore of considerable interest. Moreover, decomposition rates vary with the regional environment. This study provides data on putrefaction rates in human and pig dental pulp nuclei deposited in surface and subsurface (30-cm depth) environments in coastal British Columbia, Canada, together with the related climatic and depositional information. Although this study was undertaken to evaluate the stability of sex chromatin [*4*], its findings could have implications for potential DNA analysis as well. Badly damaged DNA may make the application of polymerase chain reaction procedures impossible [*5*].

Materials and Methods

Dental Samples

Maxillofacial surgeons in the vicinity of Vancouver, British Columbia, Canada, donated human teeth, which had been stored at −20°C. Local meat packers provided fresh jaws

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and heads from 5 to 6-month-old pigs (*Sus scrofa*). Deposition of single human teeth is not typical of the forensic situation. In order to record any differences between extracted and unextracted teeth in the rates of putrefaction, the specimens included extracted human teeth, extracted pig teeth, and unextracted pig teeth, either in jaws or in heads. The teeth were retrieved daily, weekly, or monthly until cytological examination showed autolysis. The number of teeth in the individual samples varied between 3 and 20.

Site Deposition

The samples were deposited in Vancouver, either on the ground surface or in the subsurface at a 30-cm depth. Seasonal variation data were obtained by depositing the samples at different times of year (October/December 1987, June/July 1988, October/January 1988, and November/December 1988).

Preparation of the Pulp

Anterior pig teeth were extracted using a peridontal membrane elevator and a molar extractor (forceps). All pulp extraction was accomplished by placing the individual teeth in polythene bags to prevent fragment scattering and then placing the bag on a wooden block in which shallow depressions had been whittled to accommodate the tooth shape. Each tooth was fractured by a sharp blow with a steel chisel and a wooden mallet, exposing the pulp chamber. The pulp tissue was gently detached from the walls of the chamber with a needle and removed with a pair of forceps. The putrefactive pulps retrieved were prepared for histological examination by fixation in Bouin's fluid, paraffin embedding, and staining with carbol-fuchsin. Six of the experiments correlate the seasonal environmental variables that affect pulp putrefaction. The seventh experiment studied the general process of putrefaction.

Environmental Data

The Northwest coast experiences mild temperatures year-round and has a high rainfall: the mean daily temperature in January is 5 to 0°C and that in July is 16 to 18°C, and the mean annual precipitation is 150 to 200 cm [*6*]. The climate data (ambient temperature and precipitation) were furnished by Atmospheric Environment Service, Environment Canada, from a weather station located about 3 km from the site. The soil temperature and soil pH (obtained using a Green Valley color-coded soil test kit, (from Sudbury Laboratory, Sudbury, Massachusetts) was recorded at burial depth at the outset of each experiment and at sample retrieval. The site elevation was less than 150 m above sea level, and the soil type was humic.

Results

Seasonal Putrefaction Rates

Autolysis of pulp nuclei occurred in all the samples from all the experiments (summer Experiments 1 through 3 and winter Experiments 4 through 7) within a time range from 4 days to just over 2 weeks. Data on the three summer experiments (Experiments 1 through 3) and on the three winter experiments (Experiments 4 through 6) are given in Tables 1 and 2, respectively. Data on the nuclear stability in these same experiments are summarized in the form of histograms in Figs. 1 and 2, respectively.

In the course of cellular disintegration of dental pulps in outdoor environments, it was noted that fibroblast nuclei tended to disintegrate more rapidly than white blood cells.

TABLE 1—*The summer experiments.*

	Experiment 1
Sample	40 human molars (10 retrieved per week, surface and buried)
Time period	2 weeks (26 June–10 July 1988)
Ambient temperature	
Average	15°C
Standard deviation	5.3°C
Subsurface temperature	
Weekly average	13.3°C
Rainfall	
Average	1.1 mm
SD	2.3 mm
Soil pH, weekly average	6.0 pH
	Experiment 2
Sample	30 anterior pig teeth (15 retrieved surface, 15 buried)
Time period	1 week (11–17 July 1988)
Ambient temperature	
Average	15.1°C
SD	5.4°C
Subsurface temperature	
At burial	11.0°C
At 1 week	13.0°C
Rainfall	
Average	1.6 mm
SD	2.6 mm
Soil pH	
At burial	6.0 pH
At 1 week	6.0 pH
	Experiment 3
Sample	10 pig jaws (6 buried, 4 surface)
Time period	2 weeks (11–24 July 1988)
Ambient temperature	
Average	15.0°C
SD	7.3°C
Subsurface temperature	
Average	12.0°C
Rainfall	
Average	0.8 mm
SD	2.0 mm
Soil pH, average	6.0 pH

This phenomenon was particularly apparent in Experiment 6 in pulps from unextracted pig teeth. Pulps retrieved from burial environments showed that, after cellular decay had occurred, the fibrous tissue matrix degraded to a jelly-like substance, which eventually decomposed to leave an empty pulp chamber. Acellular pulps from teeth exposed on the ground surface, in contrast, tended to dehydrate, leaving a fibrous mass or a papery layer on the wall of the pulp chamber. These descriptive comments apply to both extracted and unextracted pulps. In pig jaws exposed on the ground surface in summer (Experiment 3), adipocere formed under the skin by one month and fly puparia were noted.

TABLE 2—*The winter experiments.*

Experiment 4	
Sample	37 human molars (20 buried, 17 surface)
Time period	2 weeks (26 Nov.–11 Dec. 1988)
Ambient temperature	
Average	5.9°C
SD	3.6°C
Subsurface temperature	
Weekly average	7.3°C
Rainfall	
Average	6.28 mm
SD	6.6 mm
Soil pH, weekly average	6.5 to 7 pH
Experiment 5	
Sample	32 anterior pig teeth (15 buried, 17 surface)
Time periods	1 week surface (5–12 Oct. 1988)
	1 week buried (10–17 Oct. 1987)
Ambient temperature	
Buried	
Average	13.5°C
SD	5.0°C
Surface	
Average	9.9°C
SD	5.0°C
Subsurface temperature	
At burial	19.0°C
At 1 week	15.0°C
Rainfall	
Buried	0.0 mm
Surface, trace	0.2 mm
Soil pH	
At burial	5.6 pH
At 1 week	5.6 pH
Experiment 6	
Sample	1 pig head buried, 7 jaws surface
Time periods	1 week buried (10–17 Oct. 1987)
	9 days surface (6–14 Nov. 1988)
Ambient temperature	
Buried	
Average	13.0°C
SD	5.0°C
Surface	
Average	9.9°C
SD	5.0°C
Subsurface temperature	
At burial	19.0°C
At 1 week	15.0°C
Rainfall	
Buried	0.0 mm
Surface	7.2 mm
SD	7.2 mm
Soil pH	
At burial	5.6 pH
At 1 week	5.6 pH

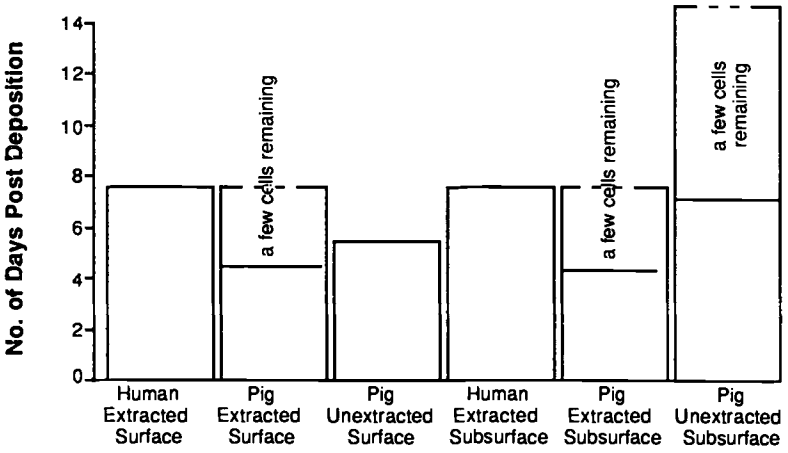

FIG. 1—*Time elapsed to total cell decomposition in summer coast (Vancouver) surface and subsurface (30-cm depth) experiments using human and pig pulp tissue (July 1988), weekly intervals.*

Experiment 7

Histology of Nuclear Putrefaction in Human Dental Pulps in Outdoor Environments

The process of cellular disintegration, accelerated by the release of endogenous hydrolytic enzymes, and the process of putrefaction, accelerated by the invasion of external and internal microorganisms, are impossible to separate histologically [7]. The combined effects of these two processes are described here as putrefaction. Eighteen human teeth deposited on the surface were retrieved daily (6–12 Oct. 1988) for six days (three pulps per retrieval) and sectioned for analysis of the necrosis/putrefaction process (Figs. 3 through 7).

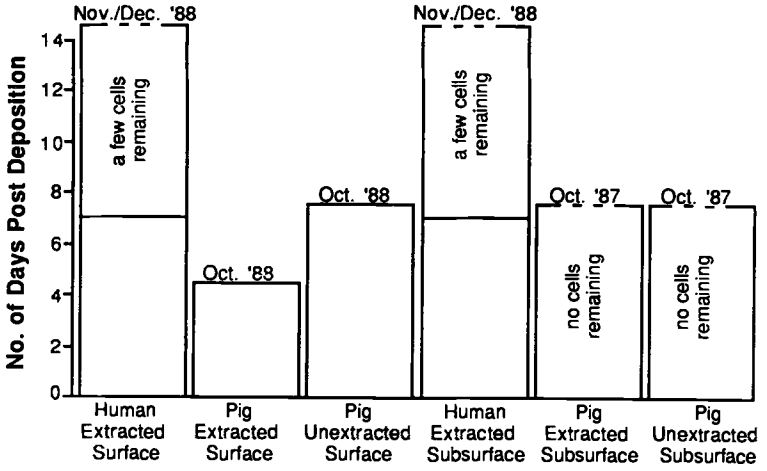

FIG. 2—*Time elapsed to total cell decomposition in winter coast (Vancouver) surface and subsurface (30-cm depth) experiments using human and pig pulp tissue, weekly intervals.*

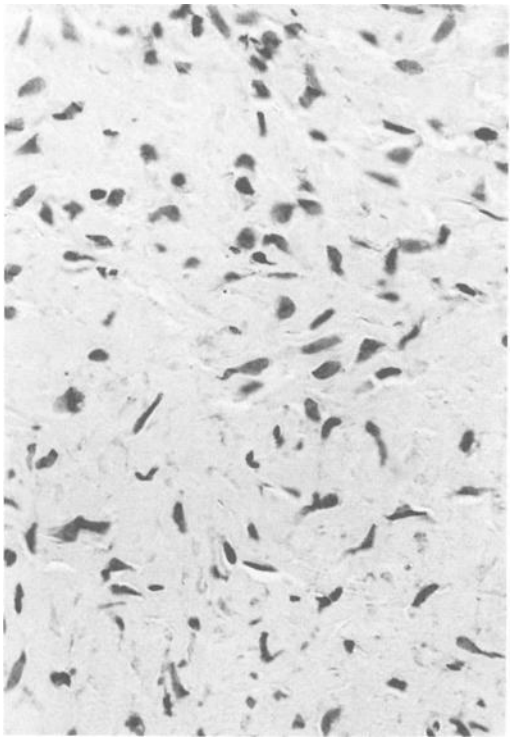

FIG. 3—*Human dental pulp fibroblasts deposited in an outdoor environment after one day. The nuclei appear nearly normal (carbol-fuchsin stain; original magnification, ×400).*

At the end of the first day after deposition, many odontoblast nuclei were still intact; some exhibited pyknosis or karyolysis. Some fibroblast sheets appeared substantially intact, but chromatin margination was noted in certain nuclei, as was nuclear vacuolation. Other cell sheets revealed nuclear ghosts.

On the second day, odontoblast nuclei showed a degenerative mosaic from a virtually undamaged chromatin arrangement to chromatin margination and karyolysis. Fibroblast cells were relatively intact in a few areas, but in many areas, cell sheets exhibited only nuclear ghosts. The fibrous intercellular matrix appeared vacuolated.

By the third day, both odontoblast and pulp cell nuclei appeared either as nuclear debris or as ghost outlines. The fibrous intercellular matrix looked vacuolated.

Days 4, 5, and 6 presented much the same picture as Day 3. One pulp on Day 6 still contained a few intact nuclei. Chromatin in these cells was marginated, and the nuclei showed vacuolation.

Conclusion and Discussion

There was no difference in the putrefaction rate between the extracted human and extracted pig pulps and the unextracted pig pulps. In winter, the longest observed nuclear stability (beyond 2 weeks) occurred in human teeth buried and surface deposited in November/December 1988 (Experiment 4, Table 2, Fig. 2). In summer, the longest period of nuclear stability (beyond 2 weeks) was observed in subsurface pig jaws deposited July 1988 (Experiment 3, Table 1, Fig. 1). However, extracted pig teeth, buried during the same time period and at the same depth, exhibited extensive nuclear lysis by 1 week.

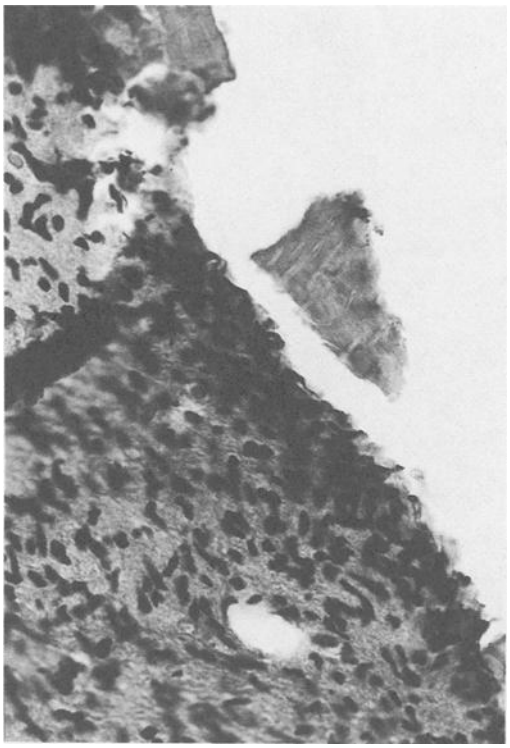

FIG. 4—*Human dental pulp fibroblasts deposited in an outdoor environment in an early stage of putrefaction. The nuclei appear pyknotic (carbol-fuchsin stain; original magnification, ×400).*

Reports of pulp cell stability have been made beyond the 2-week constraint recorded here (only extracted teeth have been tested). Dixon and Torr [*8*] buried human fetal tissue in England at 60 cm and found intact cells at 4 weeks. The soil type and climate were not recorded. Yamamoto [*9*] in Japan found that pulp cell fibroblasts, but not odontoblasts, survived intact for 15 days in the open air and for the same length of time buried beneath 50 cm of sand. The surface teeth were sheltered in a slatted box on a raised stand, and the buried sample was interred at 50 cm. No climate data were provided. More recently, Seno [*1*], also in Japan, found intact cells in pulps after 1 month of burial in a can of mud and sand at a 25-cm depth, with the can placed at the front of the house for 1 month (no climate data were provided). Finally, Ionesey [*10*] in the Union of Soviet Socialist Republics (USSR) reported intact pulp cells in teeth buried in soil up to 20 days (no burial depth or environmental data were given).

The rate of tissue decay is affected by a host of variables. The deposition environment of the cadaver, however, is a pivotal factor in the decomposition rate. Deep burial delays decomposition [*11*]. The shallow depth of 30 cm selected for this study was based on the premise that, in forensic cases, burial is usually hasty. The soil type also affects decay rates. Moist clay, clay-loess, porous sand, dry wood humus, and acidic soils speed decay, whereas lime-soil retards the process [*12*]. The ambient temperature, seasonal temperature changes, altitude, and humidity are also major contributors to the decomposition mosaic. High temperatures and moist or humid conditions are conducive to necrotic autolysis by endogenous hydrolytic enzymes and to putrefaction, in that they favor bac-

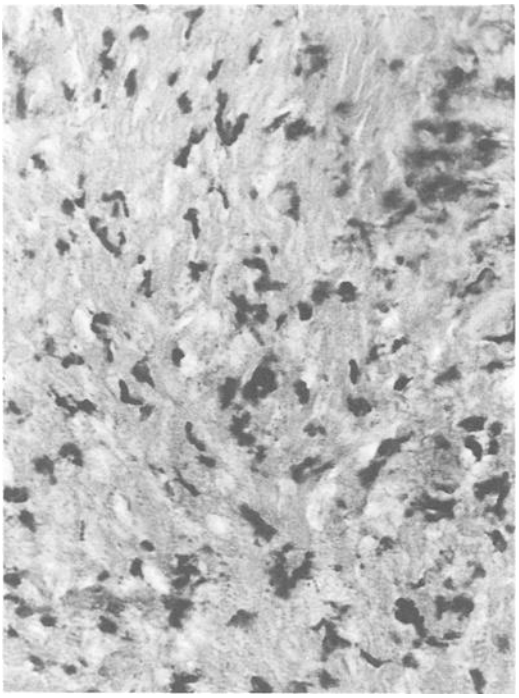

FIG. 5—*Human dental pulp fibroblasts deposited in an outdoor environment after autolysis has converted the nuclei to debris (carbol-fuchsin stain; original magnification, ×400).*

terial and fungal proliferation. Cold temperatures inhibit microbial activity, as does dessication of tissue [*13*].

The literature cited above refer to research conducted in widely disparate regions of the world, and no environmental information other than the burial depth and/or the soil type was provided. In the absence of data on the climate, altitude, or soil pH, it is impossible to make a meaningful comparison with the present study on the influence to be accorded to particular environmental variables. It seems likely, however, that the more rapid decomposition rate observed on the Northwest coast can be attributed largely to the damp environment (the rainfall is 150 to 200 cm per year) and acidic soil (pHs of 5.6 to 7 were recorded in this study).

The longest period of tissue stability (slightly beyond 2 weeks) occurred during the coldest period recorded in these experiments, when the average temperature was 5.9°C. Bacterial and mycotic activity was probably inhibited by the low temperatures. In interpreting the anomaly, described above, of prolonged stability of pulp nuclei in buried jaws in comparison with the stability in extracted pig teeth, it is tempting to infer that the prolonged nuclear stability resulted from the additional protection afforded the pulps by the surrounding jaw tissue. This hypothesis appears not to be supported by the winter deposition of 10–17 Oct. 1987 (Experiments 5 and 6, Tables 1 and 2, Fig. 2), when extracted and unextracted pig pulps under the same burial conditions, and over the same time period, deteriorated at very similar rates (about 1 week). However, the interplay of several environmental variables requires consideration.

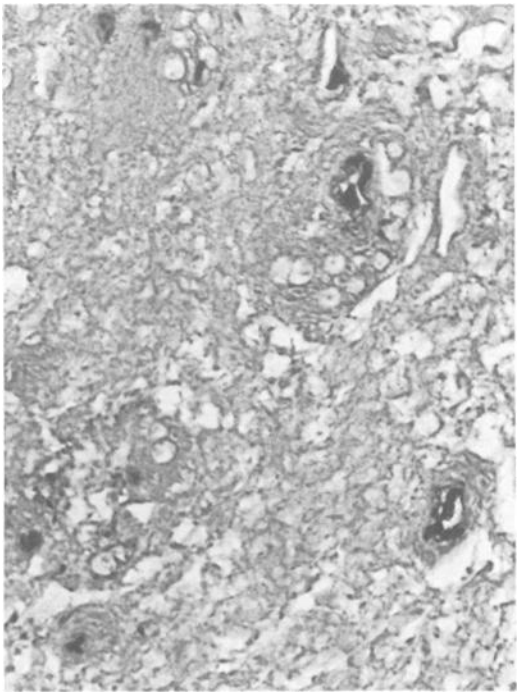

FIG. 6—*Human dental pulp fibroblasts deposited in an outdoor environment in which cell putrefaction has created lacunae or "cell ghosts" (carbol-fuchsin stain; original magnification, ×400).*

In the October 1987 experiment, the soil temperature at burial depth was relatively high, an average of 17°C (the average ambient temperature was 9.9°C) in comparison with the average soil temperature of 12°C at burial depth in the July 1988 experiment (the average ambient temperature was 17.5°C). The rainfall was low (0.2 mm). The high temperature at burial depth may have been the major catalyst in the pulp deterioration rate, since higher temperatures are known to increase microbial activity. In the July experiment, in the first week the rainfall recorded was 11.3 mm. The subsurface was cool (12°C). If the rain penetrated the soil to burial depth, the pulps in the extracted teeth may have been more vulnerable to its hypotonic affects than those encased in the jaw. In the second week, the rainfall was 0°C; the lack of moisture may have then contributed further to nuclear stability by inhibiting putrefaction activity.

Most experimental studies on decay rates of human remains have been designed for prediction of the time elapsed since death [*14–17*]. The variations noted have been in the macroscopic stages of decay and in the carrion insect microseres present, not in the cytology of the putrefactive process. However, if soft tissue derived from human remains is in a good state of preservation, it can yield much useful information to forensic investigators. Decomposition rates vary geographically, correlating with environmental variables. Studies such as this can provide background data for determining the parameters for application of sex chromatin analysis to forensic investigations and can be set up to provide similar relevant information on the application of DNA analysis as well.

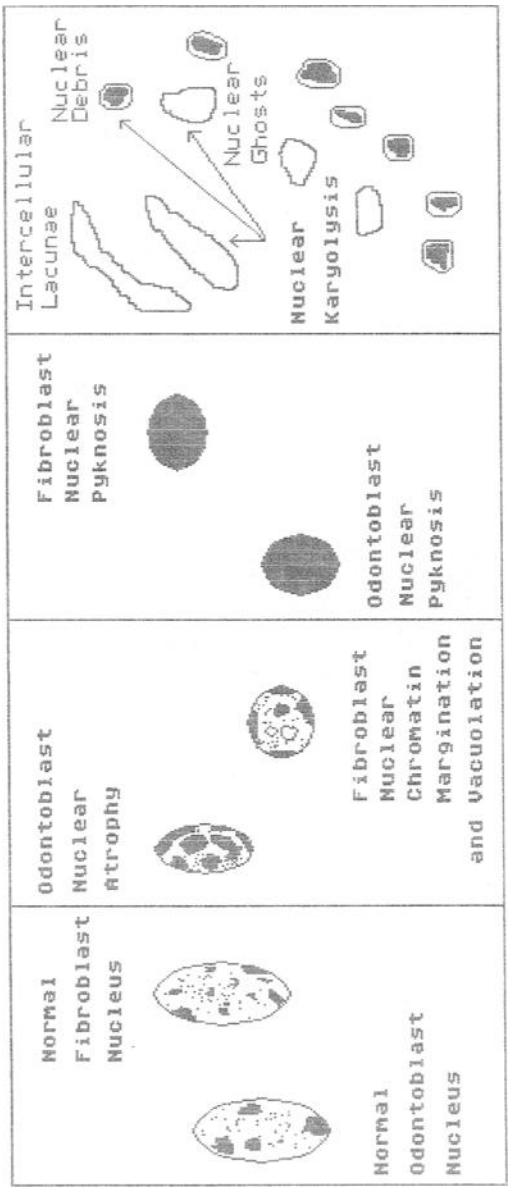

FIG. 7—*Morphological sequence of nuclear destruction as cellular disintegration and putrefaction progress.*

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