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A HISTOLOGICAL PROCEDURE TO DETERMINE DENTAL AGE

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ABSTRACT

Sclerosis of dentine has become one of the well established indicators of ageing and age determination. In this study a new technique was reported where a photomicrographic image of a cross section of sclerotic dentine was converted to a grey scale of 256 tones and then reduced to black and white and read by computer using specially developed software. A regression analysis was applied to a sample of 62 teeth (age range 17-84 years) and an age determination within an error limit of 11 years was obtained. Using a Neural Network software however the error was reduced to 8 years. **(J. Forensic Odontostomatol 2000; 18:1-5)**

Key words: Dental ageing, histology, optic microscope, computer elaboration, Neural Network

INTRODUCTION

Age determination by examining the teeth is one of the useful functions of medico-legal practice in person identification. It is particularly useful as the teeth are scarcely affected by exogenous factors, which, in other procedures, can make the age determination difficult and inaccurate. As a result there has been much research into methods to link age with relevant tooth characteristics.

A milestone in the field of age determination by tooth analysis was the work of Gustafson[1] which takes into account six factors: abrasion, level of periodontosis, secondary dentine shape, cementum apposition, resorption of the root and translucency. These criteria are classified according to particular indices and then combined in a formula which is linked with age. The error of age derived by this method is within 10 years.

Almost half a century later Gustafson's work is still relevant, not only because his methods anticipated modern techniques such as that based on the fuzzy approach[2] used for classifications in various fields of interest, but also because many authors[3,4] have derived their own methods from Gustafson's work.

Generally speaking research has evolved along two routes: a) the search for more objective and measurable parameters and b) the establishment of techniques to preserve the specimens as far as possible.

Thus, Calonius *et al.*[5] analysed bony tissue formation, Boyde[6] observed the increment lines in the dental enamel, Ito[7] proposed that age be determined by a purpose built formula based on the ratio between the sum of the tooth enamel with the pulp cavity thickness and the dentine thickness.

Other papers[8,9,10,11,12,13] report that the increasing transparency of the dentine is associated with increasing age which is a phenomenon caused by the deposition of calcium salts in and around the dentinal tubules, leading to an obliteration of the tubules themselves, and consequently a reduction in their number and an increase of dentinal sclerosis. In this way, the mineralised tubules assume the same refractive index as the dentine and the section appears more transparent.

MATERIALS & METHOD

Sixty-two caries-free, non-endodontically treated teeth with up to 3 roots each were obtained from subjects in the age range 17-84 years (Tables 1 & 2).

They were decalcified, embedded in paraffin wax and the roots sectioned at four points between the lower limit of the crown and the apex of the root, giving four equal parts (Fig. 1). The root portions were then microsectioned at a thickness of 4-5 micrometres at points P1, P2 and P3 (Fig. 1). For two and three rooted teeth the slices were through only one root (a future project will compare observations between the different roots of the same tooth) and the sections for microscopic analysis were all horizontal, following the original cuts.

The sections were then stained by haematoxylin and eosin and observed by optical microscope at 1000x magnification, photographed and processed with a standardized enlargement.

The photographic prints were then transformed into bitmap images and scanned into a PC with software that converts colours into 256 grey tones (Fig. 2).

Gender	Tooth type	Age	RST
M	M	17	6.69
M	C	17	6.53
M	M	17	6.42
F	C	17	4.61
M	PM	19	5.15
M	PM	21	6.96
F	M	21	7.17
M	PM	21	5.02
F	M	24	6.5
F	C	25	7.11
M	C	26	9.17
M	M	27	6.55
M	PM	28	10.22
M	PM	28	10.05
F	M	28	11.22
M	M	28	10.32
F	PM	31	7.76
M	C	32	9.23
F	PM	34	9.12
M	M	35	8.17
F	M	38	8.46
F	PM	39	11.56
M	M	40	9.74
F	M	45	10.9
M	M	46	10.6
M	C	46	10.7
F	M	48	7.97
M	PM	54	9.52
F	C	54	9.5
M	C	54	12.12
M	PM	54	9.55

Gender	Tooth type	Age	RST
M	PM	55	12.24
F	PM	55	10.98
F	M	55	9.27
F	M	56	11.97
F	PM	56	10.29
M	C	57	12.76
M	PM	57	13.15
F	C	57	9.25
F	M	58	14.21
M	M	59	11.77
F	PM	60	12.78
M	C	60	13.65
F	C	60	13.26
M	PM	60	12.04
M	M	61	11.11
F	PM	62	13.42
M	M	64	12.18
M	PM	65	11.24
F	M	66	12.78
M	M	66	12.49
F	M	66	10.56
M	M	67	10.57
M	PM	67	11.96
F	C	69	12.02
F	M	70	8.13
M	C	70	11.34
F	PM	70	11.45
M	M	74	13.01
F	M	77	12.23
F	M	84	11.46
F	PM	84	13.12

RST = Ratio of Sclerosis to Tubules; M = Molar; C = Canine; PM = Premolar

Table 1: *Subjects and specimen teeth*

	Male	Female
Molar	13	14
Premolar	12	9
Canine	8	6

Table 2: *Distribution of cases*

This means that the image becomes black and white through a computerized mathematical procedure using special software. This phase consists of a calibration procedure where some parameters are set according to reference diagrams. In Fig. 3 the operation of recognising what is sclerosis and what is dentinal tubule is depicted. After trials by a team of observers, it was possible to establish a threshold at a particular grey tone for what the different observers agreed distinguished a tubule from sclerosis. It is obvious that this threshold mainly depends on a strictly standardized method of photographing, processing and observing the images.

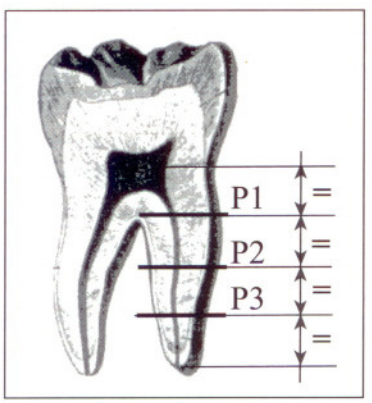

Fig.1: *The root was sectioned at four points and the microsections were obtained at P1, P2 and P3.*

The final result was a black and white image in which what appeared black was sclerosis, and what appeared white was tubule (Fig. 4). The software then automatically read the number of black pixels (representing the dentinal sclerosis area) and the white ones (representing sections through tubules).

RESULTS

The contingency coefficient is a measure of the degree of association of dependence or independence of two samples. The larger the value of this coefficient, the greater the degree of association, but if the coefficient tends to zero, the distribution of cases is random, which suggests that the cases are

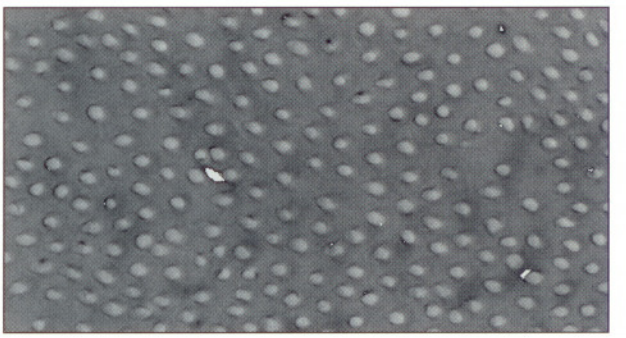

Fig.2: *A colour micrograph of dentine is converted to 256 grey tones.*

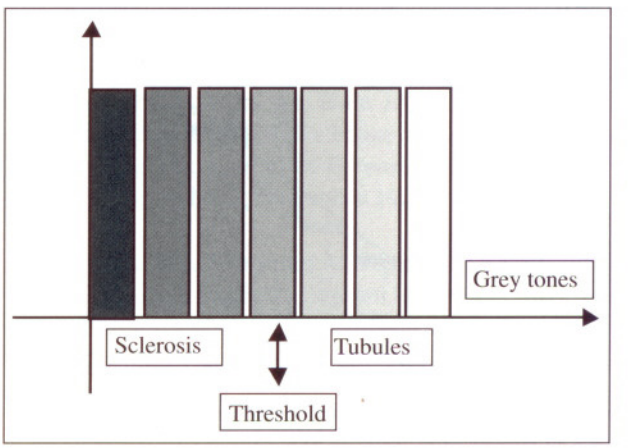

Fig.3: *Using a filter the software user must select the tones that indicate sclerosis or dentinal tubule.*

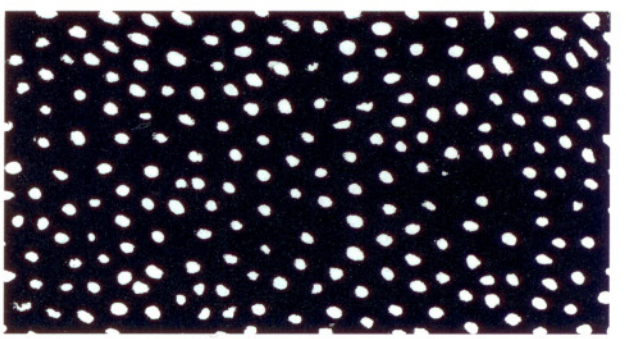

Fig.4: *A micrograph after completion of the filtering procedure.*

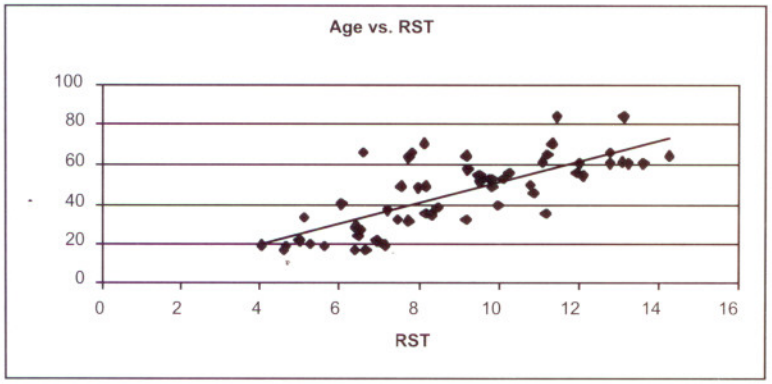

Fig.5: *Distribution of age to RST*

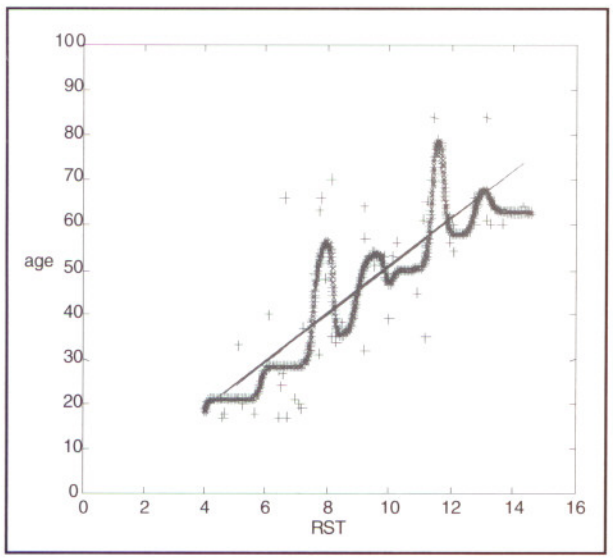

Fig.6: *Distribution of age to RST using the Neural Network software*

well distributed. In this study, the distribution of the two gender groups was well randomized as demonstrated by the contingency coefficient of 0.078.

Dentinal sclerosis was found to increase with age at a statistically significant correlation as the ratio of sclerosis to tubules (RST), representing the average sclerotic area per unit area of tubular dentine, correlated quite strongly with age (r = 0.752). See Fig. 5.

The equation Age = RST x 5.3 – 2.28 displayed the link between age & RST which then delivered an error limit of 11 years.

There was no meaningful difference in the findings between genders. By analysing them separately we found that males had a correlation coefficient r = 0.865 and females had r = 0.699. Comparing the two correlation coefficients statistically, weighted by the number of subjects, a non-statistically significant difference was found (p = 0.0975) and the two groups were therefore considered as uniform.

Three separate analyses were carried out on the three different types of teeth and correlations between RST and age for canines was found to be r = 0.846, for premolars it was r = 0.837 and for molars it was r = 0.721. Once again the differences were very small and the sample could be considered as uniform. The size of the sample however would affect the reliability of the results.

In answer to critics of the linear regression test who question its indiscriminate use in all situations we applied the Neural Network[2] (NN) approach. This is based on a computer programme which is able to interpolate limited data (named "examples" in the NN special language), and then being able to predict additional, collocated data and creating a more complete set. This method then delivered a reduced limit of error for age determination by RST of 8 years.

In addition, the Neural Network calculations gave an indication of the sensitivity of the RST in relation to age. The resulting curve (Fig.6) had horizontal and vertical components and where the vertical showed a wide age range determination the horizontal components delivered more accuracy with less sensitivity in the RST.

DISCUSSION

Initial results have demonstrated that the proposed method appears quite reliable when compared with other methods either reported or directly tested by the authors. In preliminary work [14,15] we compared the present method with two others investigating "tooth translucency" and the "analysis of the cementum thickness" [16,17] and found weaker correlations regarding RST/age determination.

While the results appear good, there is no doubt that the method is difficult to perform, mainly because of the complex procedure including cutting the roots, treating the sections, microscopic observation of the

slices, photographic processing, scanning, computerisation (after parameter calibration). It is furthermore a reality that the correlation is strengthened with an increase in the sample size and that the more frequent the performance of the procedure the more it becomes reliable because of growing confidence in the operators and the growing efficiency of the technique.

The success of the technique has therefore inspired us to pursue this research, not only to consolidate the statistics by increasing the sample size, but also to make efforts to simplify it. For example, to be able to dispense with photography entirely by allowing image scanning directly from microscope into computer and to allow automatic calibration of the grey scale and estimation of age by the Neural Network software should make age estimation simpler, quicker and more accurate.

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USE OF BUCCAL EPITHELIAL CELLS FOR PCR AMPLIFICATION OF LARGE DNA FRAGMENTS

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ABSTRACT

The analysis of human DNA is widely employed in the genetic studies of families and populations, and in most cases is performed with samples obtained from peripheral blood. The use of buccal epithelial cells as a source of DNA for PCR amplifications has several advantages over blood sampling but has only been used to amplify small fragments of DNA. Its use in forensic analysis has been limited to cases where the sampling of peripheral blood is not feasible. In the present study we show that buccal epithelial cells are a reliable source of DNA for the PCR amplification of high molecular mass fragments, which could be used in large-scale population sampling. Since most PCR gender-typing systems rely on the amplification and electrophoretic separation of the amelogenin gene, our results show that buccal epithelial cells may be the preferred source of DNA for gender -typing analysis. **(J Forensic Odontostomatol 2000; 18:6-9)**

Keywords: PCR, buccal epithelium, amelogenin.

INTRODUCTION

The analysis of human DNA is widely employed in genetic studies of families and populations and in most cases is performed with samples obtained from peripheral blood. Blood sampling is however an invasive procedure and sample collection may involve ethical problems in cases such as extreme illness, elderly persons and babies. Additionally, it requires medical supervision and specific equipment, which contribute to the increased overall costs of the procedure.

The polymerase chain reaction (PCR) provides a rapid and sensitive approach for the analysis of polymorphisms and mutations of the human genome. The use of buccal epithelial cells as a source of DNA for PCR reactions has been limited to studies of infectious agents present in oral mucosa;[1,2] and in forensic analysis, where the sampling of peripheral blood is not feasible.[3,4] Invariably, these studies report the amplification of small fragments of DNA (< 300 base pairs), which are suitable for the identification of viral and bacterial sequences, as well as the analysis of highly polymorphic loci in the human genome. However, amplification of larger DNA fragments may be desirable in some instances such as PCR-RLFP,[5] mismatch cleavage mutation analysis,[6,7] cloning, and sequencing of amplified sequences.[8] In the present study, we show that buccal epithelial cells are a reliable source of DNA for the PCR amplification of high molecular mass fragments, which could be used in large-scale population sampling and epidemiological studies, as well as in forensic analysis. As will be seen our results show that buccal epithelial cells may be the preferred source of DNA for gender-typing analysis.

MATERIALS AND METHODS

Sampling

A group of 83 consenting female subjects undertook a mouthwash containing 5 mL of 3% sucrose rather than water in order to prevent osmotic imbalance that would cause rupture of cells and loss of genomic DNA, for 2 min. A sterile wood spatula was then

used to scrape the buccal oral mucosa and the tip of the spatula shaken into the retained mouthwash solution. Buccal epithelial cells were pelleted by centrifugation at 2000 rpm for 10 min, the supernatant was discarded and the cell pellet resuspended in 500 uL of extraction buffer [10 mM Tris-HCl (pH 7.8), 5 mM EDTA, 0.5 % SDS] with antibiotics. The samples were then frozen at –20°C until used for DNA extraction.

DNA extraction

After defrosting, the samples were incubated at 37°C with 100 ng/mL proteinase K*, and agitated overnight. DNA was then purified by sequential phenol/chloroform extraction and salt/ethanol precipitation and dissolved in 70 µL TE buffer [10 mM Tris (pH 7.8), 1 mM EDTA]. The concentration was estimated by measurements of OD 260.

Polymerase chain reaction

Amplification reactions were performed with 300 to 700 ng DNA in a volume of 50 µL in reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM $MgCl_2$, deoxyribonucleotides (200 µM each), 1 µM primers, 2U *Taq* DNA polymerase** and five pairs of primers were used. The primer sequences are as follows:

AMX1- 5' GGATTGGTTGTTACAGATGCC 3'
AMX2- 5' TTACTCACAGGCATGGCAAAAGCTGC 3'
AMX3- 5' CATTTCAGAACCATCAAGAAATGGG 3'
AMX4- 5' CTTTACAGAGCCCAGGGCATTG 3'
AMX5- 5' CCTCCCTGTAAAAGCTACCACC 3'
AMX6- 5' AATGTCTACATACCGGTGGCC 3'
AMX7- 5' GTAGAACTCACATTCTCAGGC 3'
AMX8- 5' GGCTTCAAAATATACTCACCACTTCC 3'
AMX9- 5' CCAGCCCCAGCCTGTTCAGCCAC 3'
AMX10- 5'TGTCTGCTAATGGTACTTTTTTAG 3'

Samples were heated initially to 95°C for 5 min, each cycle comprising denaturation at 95°C for 50 sec, primer annealing at 67°C for 1 min and polymerization at 72°C for 2 min. Samples were subjected to 35 cycles of amplification followed by a final extension of 72°C for 7 min. Amplification was carried out in a *Perkin-Elmer GeneAmp 2400 thermal cycler*#. Amplification products were visualized by electrophoresis on vertical 5% polyacrylamide gels in 1 X TBE (89 mM Tris-Borate, 89 mM boric acid, 2 mM EDTA), followed by silver staining¶.

RESULTS AND DISCUSSION

The use of buccal epithelial cells as a source of DNA for PCR amplifications has several advantages over blood sampling. The collection of material is fast and inexpensive, buccal samples can easily be obtained from people who are reluctant to donate blood, consent becomes simplified, there is no need of medical supervision during sampling, and the risk of contamination is reduced.[9,10]

The amplifications produced specific reaction products of 1690 base pairs [bp] (AMX1-AMX2), 1986 bp (AMX3-AMX4), 1550 bp (AMX5-AMX6), 879 bp (AMX7-AMX8), and 2039 bp (AMX9-AMX10), spanning the whole amelogenin X gene.

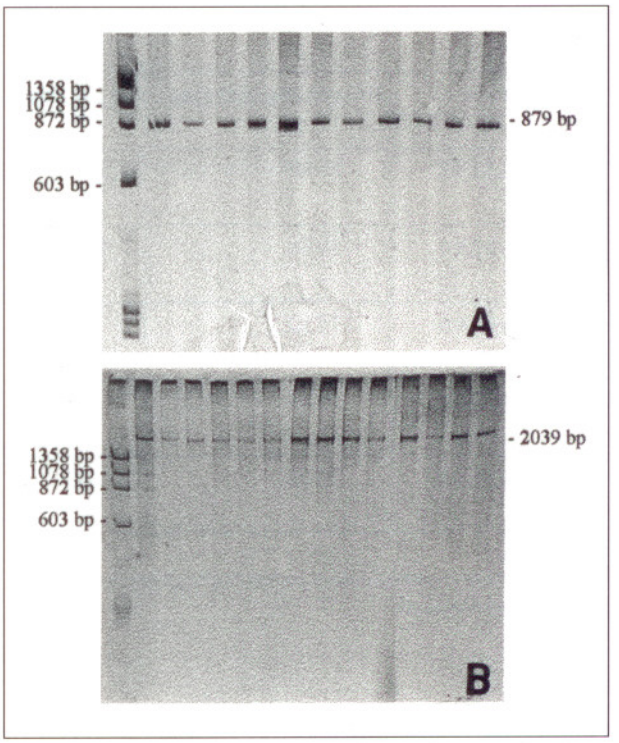

Figure 1. *Polyacrylamide gel electrophoresis showing PCR products from total genomic DNA derived from buccal epithelium. A: 879 bp product of amplification with primers AMX7 and AMX8. B: 2039 bp product of amplification with primers AMX9 and AMX10. Lane 1- ϕX-174-RF DNA Hae III digest molecular weight marker (Pharmacia).*

* Sigma Chemical Co., St. Louis, MO, USA
** Amersham Pharmacia Biotech, Uppsala, Sweden
# Perkin-Elmer, Perkin-Elmer Co., Norwalk CT 06859, USA
¶ Bio-Rad Silver Stain Kit, Bio-Rad Laboratories, 200 Alfred Nobel Dr, Hercules CA 94547, USA

Fig. 1 illustrates typical PCR products from total genomic DNA derived from buccal epithelial cells showing that buccal epithelial cells can be used for the PCR amplification of large DNA fragments. The amount of DNA extracted ranged from 2.1 µg to 360 µg, which is sufficient to enable PCR amplification, with a success rate of identification of the gender gene, in this case the female, of around 90% of large fragments ranging from 879 to 2039 base pairs (Table 1). Failure to amplify DNA may result from degradation or from the presence of inhibitors which interfere with PCR reaction and can in most cases be overcome by adding bovine serum albumin to the PCR reaction,[11-13] or by repurification of DNA with Chelex extraction.[14] The use of small volumes of DNA extract is also recommended in order to reduce the amount of inhibitors.[3,14]

Primers	Fragment size (bp)	% positive
AMX1-AMX2	1690	91.5
AMX3-AMX4	1986	92.5
AMX5-AMX6	1550	88
AMX7-AMX8	879	88
AMX9-AMX10	2039	88

Table 1: *Positive amplification percentages of amelogenin X gene of 83 individuals.*

Gender determination can be a valuable piece of information in forensic investigations. Most PCR gender-typing systems rely on the amplification and electrophoretic separation of the amelogenin gene which produce small PCR products with 106-112 bp[15,16] or 330-218 bp[14] from the X and Y chromosomes respectively. However, the main difference between X and Y loci is a 177 bp insertion in the X gene[17] and the X and Y sequences can be amplified using a single set of primers which produce a 977 bp and 780 bp fragments,[18] which can be clearly distinguished in agarose or polyacrylamide gels. The results presented here show that buccal epithelial cells may be the preferred source of DNA for gender-typing analysis. Finally, it is worth mentioning that DNA extracted from this source has been routinely used in our laboratory for RFLP and direct sequencing of PCR products.

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BITEMARKS IN CHOCOLATE: A CASE REPORT

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ABSTRACT

Police investigating a theft from a chocolate factory recovered three pieces of chocolate with irregular fractured surfaces displaying a pattern of marks made by human teeth. A highly accuracy dental impression material was used to prepare casts of these marks which were examined and photomicrographed, confirming that they had in fact been produced by human teeth.

Casts and photomicrographs of the suspect's teeth were made in order to record the fine details of the casts of the dentition. Unique characteristics evident on these casts included a small notch on the incisal edge of the upper right lateral incisor, wear facets on the incisal edges of the upper central incisors and on the lower right lateral incisor and a space of approximately 1.5mm between the upper left central incisor and lateral incisor which was rotated about 20 degrees distally.

Both direct and photomicrographic comparisons between the casts of the chocolates and of the suspect's dentition revealed correspondence between their unique characteristics. **(J Forensic Odontostomatol 2000; 18:10-4)**

Keywords: Foodstuffs, photomicrographs, dental characteristics, wear facets.

INTRODUCTION

In February 1996 a forced entry and burglary occurred at the confectionary manufacturing and retail premises of Haigh's Pty Ltd. in Adelaide, Australia. Entry had been obtained through the roof and a safe which was present had been overturned and a hole made in the back. An axe and jemmy bar were found nearby and a quantity of cash was missing from the safe, as well as some chocolates from a nearby display area; a number of partially eaten chocolates were recovered from the floor. During the preceding weeks there had been a number of forced entries to other properties with similar characteristics which had resulted in an accumulation of evidence. Following the break-in at the chocolate factory the police arrested a suspect who was charged and subsequently detained under the Summary Offences Act at Yatala Labour Prison. In March 1996 three pieces of partially eaten confectionary were delivered to the Forensic Odontology Unit, University of Adelaide. Three days later, at the request of the Police under Section 81, subsection 4 of the Summary Offences Act, impressions of the suspect's dentition were obtained using GC Examix.*

METHOD

Examination of the Chocolate

Each of the 3 pieces of chocolate was incomplete at one end and displayed irregular fractured surfaces which were consistent with marks made by a human bite (Figs. 1-4). The portion of chocolate honey nougat measured approximately 27.0 mm at the maximum width and 29.0mm at the maximum length. The portion of chocolate frog measured 40.0mm at the maximum width and 55.0mm at the maximum length whilst the bar of plain chocolate measured 40.0mm at the maximum width and 77.0mm at the maximum length.

*GC Examix™ GC Corporation 76-1 Hasunuma-Cho-Itabashhi-Ku, Tokyo Japan

Under microscopic examination the depressions visible in the surface of the chocolate could be identified as human tooth marks. The chocolate honey nougat had marks in both the upper and lower surfaces while the remaining two chocolate pieces displayed details in one surface only.

Casts of the marks in the chocolate surfaces were made from GC Examix impressions known to give highly accurate and stable results and were then examined and photographed microscopically (Figs. 6-7).

Fig.1: *Portion of chocolate honey nougat, lower surface.*

Examination of the Suspect's Dentition

The incisal edge configurations of the suspect's anterior teeth were examined microscopically on the stone casts and recorded as photomicrographs with several features being evident:

1. There was a space of approximately 1.5mm between the upper left central and lateral incisors with a rotation of the lateral incisor of around 20 degrees distally (Fig. 5).
2. The upper right lateral incisor demonstrated a small characteristic notch (Fig. 6a).
3. The upper right central incisor displayed occlusal wear facets (Fig. 7a).
4. The lower right lateral incisor also demonstrated wear facets (Fig. 8a).

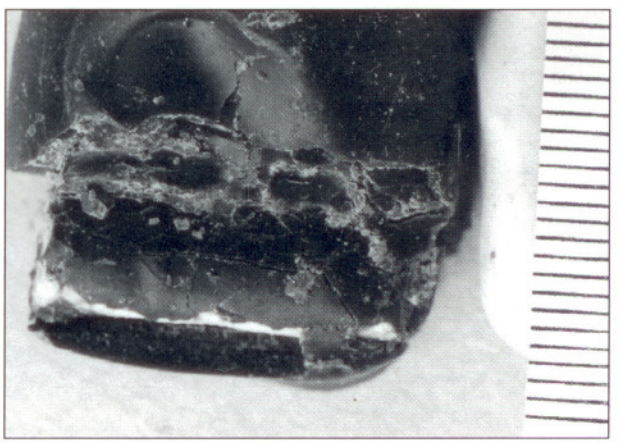

Fig.2: *Portion of chocolate honey nougat, upper surface.*

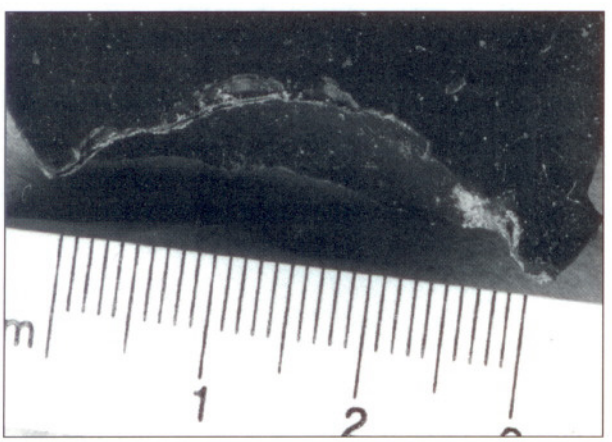

Fig.3: *Portion of chocolate frog.*

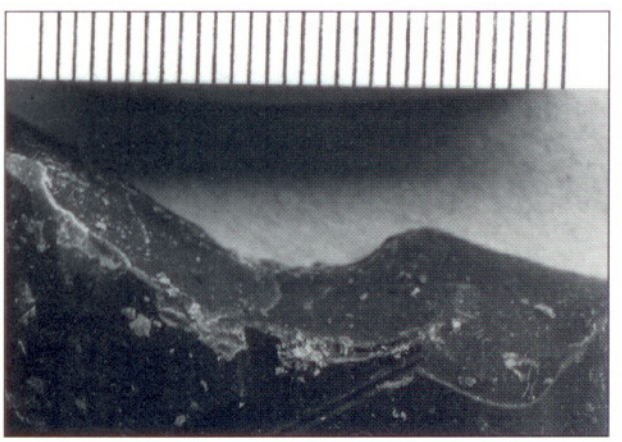

Fig.4: *Portion of dark chocolate bar.*

Comparisons between the casts of the bitemarks in chocolate and the suspect's dentition were performed by direct alignment and also by comparison of details visible on both series of photomicrographs. The features which were visible on the anterior teeth of the suspect were also apparent as features on the casts of the indentations retained in the chocolates (Figs. 6-8).

Fig. 6a displays the incisal edge of the upper right lateral incisor of the stone casts embedded in boxing wax. The labial margin is exposed adjacent to the millimetre scale, a notch is visible in the mid-portion of the labial margin. Fig. 6b displays the positive cast replication in GC Examix of one of the indentations retained in the upper surface of the chocolate honey nougat adjacent to a scale. This distinct outline delineates the same shape and general dimensions as those visible in Fig. 6a, including the labial notch. Fig. 7a shows the cast of the incisal edge of the suspect's upper right central incisor embedded in boxing wax with the labial margin exposed adjacent to the millimetre scale. Fig. 7b shows a GC Examix cast of the indentation in the upper surface of chocolate honey nougat which was adjacent to the cast shown in Fig 6b. This second indentation in the upper surface of the chocolate honey nougat displays the same shape and dimensions as those visible in Fig 7a. In particular the labial and lingual margins in Figure 7a have similarities to the margins visible in Fig. 7b. Fig. 8a demonstrates a cast of the suspect's lower right lateral incisor with the labial margin of the incisal edge adjacent to the scale. Distinctive wear facets are visible on the incisal edge. Fig. 8b depicts the GC Examix cast of the indentation in the lower surface of the honey chocolate nougat. The general outline and dimensions of the incisal edge seen in Fig. 8a are reproduced exactly.

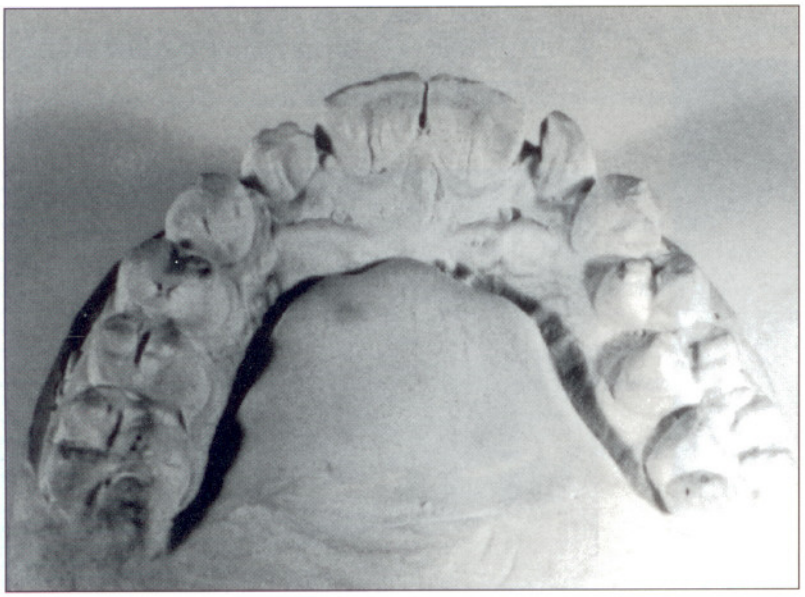

Fig.5: *Cast of suspect's upper anterior teeth*

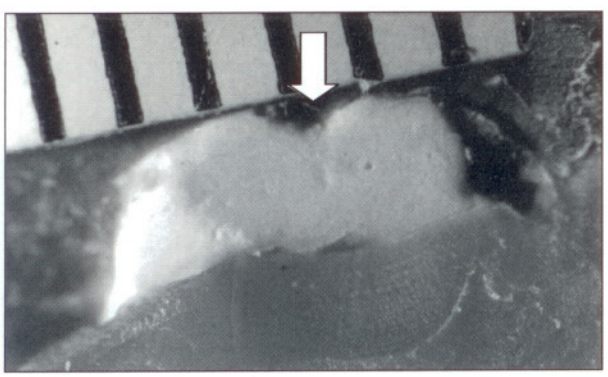

Fig.6a: *Photomicrograph of cast of suspect's upper right lateral incisor, a notch is visible in the middle third of the labial margin (arrowed).*

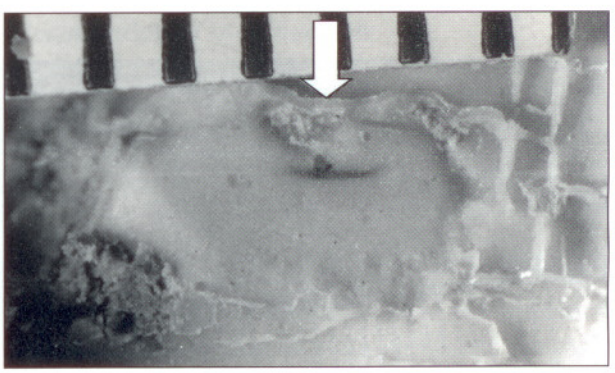

Fig.6b: *Photomicrograph of cast of one of the indentations retained in the upper surface of chocolate nougat. The outline visible is analogous with the outline of the upper right lateral incisor (arrowed).*

DISCUSSION

The degree to which dental characteristics are retained in bitemarks in foodstuffs varies greatly and obviously depends on the nature of the foodstuff involved. Evidence of bitemarks has been recovered from types of food ranging from soft cakes[1] to cheese[2]. Webster[3] classified the results of such bitemarks according to the foodstuff as follows:

Type 1 fractures with limited tooth penetration (chocolate).
Type 2 fractures with extensive pressure applied by the teeth (apples).
Type 3 fractures with complete or near complete penetration through the food substance (cheese).

Webster[3] noted that bitemarks in chocolate lead to a fracture of the material with a limited depth of tooth penetration recording possibly the most prominent incisal edges and some of the labial aspects of the upper and lower anterior teeth. This effect was visible with the marks retained in both the chocolate frog and the chocolate bar. The third type of bitemark described by Webster[3] included those where the teeth bite through or almost through a material such as cheese. The latter has provided valuable evidence previously as reported in 1906[1] and by McCullough and Layton.[4,5] The effect of a type 3 bitemark was visible in the case of the chocolate honey nougat bar. The material below the chocolate was of a different physical nature to the chocolate on the surface and was not fractured at the region of the bite. An amount

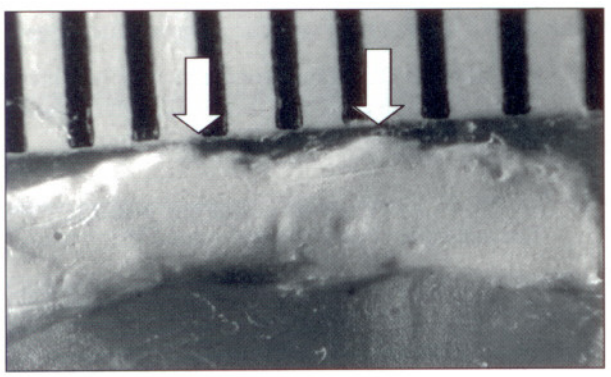

Fig.7a: *Photomicrograph of cast of suspect's upper right central incisor. The outline of the incisal edge and surface is distinctive in shape (arrowed).*

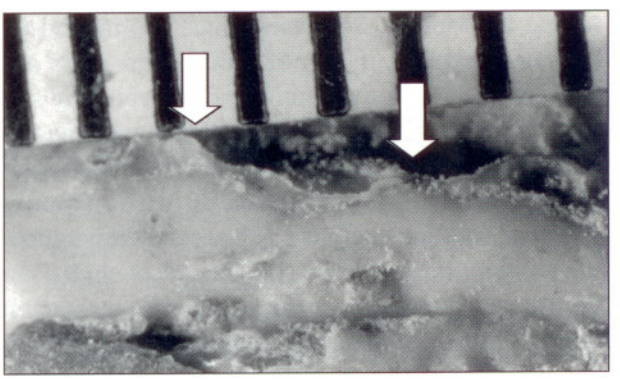

Fig.7b: *Photomicrograph of cast of another indentation retained in the upper surface of the chocolate honey nougat. A similar outline to that in Fig.7a is visible (arrowed).*

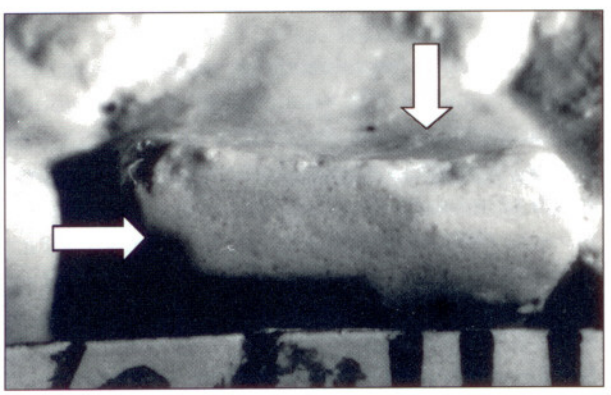

Fig.8a: *Photomicrograph of cast of suspect's lower right lateral incisor. Distinctive wear facets are visible (arrowed).*

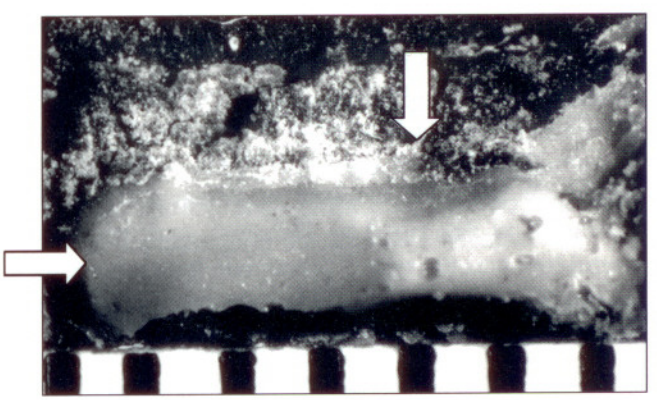

Fig.8b: *Photomicrograph of cast of indentation retained in lower surface of the chocolate honey nougat. There is correspondence between the shape of this cast and the wear facets visible in Fig.8a (arrowed).*

of chocolate had been lost from the surface and from the edge of the bar, however in the area of the bitemark the nougat had retained marks which indicated that a tooth scraping action had occurred up to an area seen as a 'stopping point' (as described by Webster[3]) which displayed an outline of the incisal edges. These details which were visible on the upper and lower surface of the honey nougat bar corresponded with details of the upper and lower anterior teeth of the suspect's dentition.

The ability to make meaningful interpretations of bitemark evidence relies heavily on both the nature of the material involved and the reproduction of unique characteristics displayed in the dentition of the perpetrator. The validity of bitemark evidence requires that each individual dentition has a combination of features relating to the size, shape, occlusion and arrangement of teeth which is unique to that individual. Sognnaes *et al.*[6] compared the bitemark pattern of monozygotic twin pairs and despite similar developmental morphology of individual teeth, significant variation was evident between twins in each pair with respect to individual tooth position and arrangement in the anterior segment. Rawson *et al.*[7] studied precise registrations of dentitions from 397 individuals and concluded that the human dentition is unique beyond reasonable doubt. These findings add weight to the generally accepted belief that dental characteristics are both distinct and unique to each individual and the possibility of mistaking an individual's bitemark for another is extremely slight.

In the case reported here the clearest evidence providing correspondence between the suspect's dentition and the bitemarks was found in the bar of chocolate honey nougat. There were additional details provided by the marks in the chocolate frog and the chocolate bar but the different nature of the foodstuffs involved resulted in different types of bitemarks as described by Webster[3] and the most useful bite was the type 3 (chocolate honey nougat) where the teeth could provide a deeper indentation and therefore more comprehensive marks.

ACKNOWLEDGEMENTS

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IDENTIFICATION CONCEPT AND THE USE OF PROBABILITIES IN FORENSIC ODONTOLOGY - AN APPROACH BY PHILOSOPHICAL DISCUSSION

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ABSTRACT

This paper questions the practitioners' deterministic approach(es) in forensic identification and notes the limits of their conclusions in order to encourage a discussion to question current practices. With this end in view, a hypothetical discussion between an expert in dentistry and an enthusiastic member of a jury, eager to understand the scientific principles of evidence interpretation, is presented. This discussion will lead us to regard any argument aiming at identification as probabilistic. **(J. Forensic Odontostomatol 2000; 18:15-8)**

Keywords: Identification, probabilities, expert witness.

INTRODUCTION

Courts are showing more and more circumspection when dealing with scientific evidence interpretation. This debate (on the way of presenting evidence) became apparent during experts' testimonies on DNA genetic evidence and could well extend to all forensic science disciplines. Despite half a century of practice vouching for its qualities, dentistry will not escape questioning as far as the scientific interpretation of evidence is concerned.

To a sensible observer considering evidence investigation practices in dentistry (for instance, the link existing between a bitemark and the dentition of a suspect), the conflicts of opinion(s) observed among practitioners worldwide can easily give rise to doubts on the foundations of the science, notably:

- Many experts consider a minimum number of characteristics; a formal identification is established only if the minimal number of corresponding characteristics between the observed mark and the image of the set of teeth or dentures from the potential source of the mark is put in evidence and no unexplained non-conformity is observed.
- Other experts exclude the idea of a minimum numerical standard. For them identification is a matter of judgement. The expert evaluates the contributions to individuality on a quantitative (number of characteristics) and a qualitative (peculiar characteristics, mark clarity, etc.) level.

Most experts refuse to give advisable opinion(s) (including experts in dactyloscopy, a science which inspired evidence interpretation in dentistry) pronouncing themselves either for an identification or an exclusion (except occasionally when no decision can be reached). They thus favour a deterministic approach to the detriment of a probabilistic one. This means in practice that a mark presenting five characteristics for example in common with a potential source may, in the end, have no conclusive value depending on the approach chosen.

This article questions the practitioners' deterministic approach(es) and notes the limits of their conclusions in order to encourage a discussion to question current practices. With this end in view, a hypothetical discussion between an expert in dentistry and an enthusiastic member of a jury, eager to understand the scientific principles of

evidence interpretation, is presented. This discussion will lead us to regard any argument aiming at identification as probabilistic.

During this debate, our two protagonists will be joined by a judge to remind us of the expert's esteemed help to Justice which allows the judge, as a last resort, to act as a decision maker based on a body of proof.

DEBATE

Juror:
In order to clear up my mind and better interpret the conclusions of your report, I would like to start with a preliminary question about numerical standards. Are the decisions which have been taken towards a minimum numerical standard based on scientific results or do they rather fulfil mandatory practices?

Expert:
I must say, that as far as identification is concerned no theory can justify[1] a fixed numerical standard. The identification process required goes beyond a mere counting of characteristics.

Juror:
Therefore I do not understand all the reticence towards qualified advice in dentistry.

Expert:
It seems impossible that the notion of probability can be applied to evidence. Experts have argued that every tiniest part of the tooth surface is strictly individual. The hypothesis that a bitemark could have several perpetrators thus appears inconceivable.

Juror:
I think I understand your argument. The information given by each part of the dental surface is complete and individual. However, can you say as much for fragmentary or poorly formed marks?

In case of a transferred mark, how do you explain the differences of interpretation methods between the mark and other biological evidence (blood, semen, etc.)? Is not any biological fluid also strictly specific to an individual when the DNA molecule is exhaustively studied? Qualified advice, i.e. the capacity to give an opinion combined with a probability seems to be an easy task for experts in genetics. So where does all this reticence come from? Does not the acceptance of qualified advice mean a questioning of the very concept(s) of identification?

Expert:
Careful! Even if we accept your argument, the absence of figures could well make experts reticent when it comes to probabilities. The provision of qualified advice implies that the expert is also able to estimate the probability of the trace in question (or the number of persons which could be taken into account as being potential suspects). However, statistical data on variability are not numerous, not to say non-existent, when compared to the individuality which results from the combination of multiple factors, for example, such as the general dental shape and outline of the characteristics.

Juror:
If I understand you correctly, the aim is thus to collect statistical data and determine a model to estimate the probability of the shape of a dental characteristic. This seems logical and conforms with Locard's[2] doctrine on fingerprints applied to the rules of the identification process. He wrote notably: "there are few characteristics: in that case (the) print(s) show(s) no certainty but a presumption proportional to the number of points and their sharpness." Locard[2] considered that there was more to the evaluation of an identification than a mere counting of characteristics.

Expert:
Exactly. It is erroneous to regard scientific evidence as dichotomous asserting only an identification or an exclusion. Given the increasing set of values between exclusion and identification such a sharp interpretation appears rather inconceivable.

Juror:
Would it therefore be reasonable to think that dental characteristics could evolve by a mere phenomenon of transfer towards "more general", characteristics? For instance, a perfectly sharp break observed directly on a tooth can be regarded as unique. However, if the same break is transferred by pressure on a surface it can, when being taken, look blurred and merge into other dental patterns.

Expert:
Yes, the idea of transfer implies necessarily a loss of information and from that moment on, the idea of

"more general" characteristics is thoroughly justified. The concept covers a continuum of values which goes from (a) "poorly/weakly descriptive" to (b) "highly descriptive" characteristic(s).

Juror:
... however judges expect scientific evidence to be one-to-one and without any compromise!

Judge :
Actually, although judges prefer indisputable evidence, they would no doubt use wisely any evidence which, without verging on certainty, would become integrated into a body of proof. It is worth remembering that the expert only brings an element of proof to Court which becomes integrated into a body of proof useful for the identification decision.

Expert :
Then, the query about "identification" must be regarded as one for the judge(s) or Court and not for the expert. In his statement/conclusion(s) the expert will just comment on the strength of the link between a mark and a tooth where the probability of casual coincidence reaches almost zero when it comes to identification.

Judge:
At Court, the identification of an individual remains a judiciary matter which calls for a group of complicated and ill-matched/dissimilar data, as, for instance, material elements, testimonies or other circumstantial evidence. Although it is not always clearly admitted, the burden of decision rests with the Court and not with the expert.

CONCLUSION

What appears clear is the need

1. to emphasise that an element of scientific proof provided by the expert is an element among others which aims at supporting (or not) the hypothesis of an identification, or more generally, at supporting (or not) the link between the mark discovered and a potential perpetrator

2. to regard considering the objective part of this type of proof the argument proposed by the expert as probabilistic, in the sense that from the characteristics observed on the mark he will exclude a certain population (to have caused it) and this argument will have to be integrated in the Court process of decision;[3]

3. to require that from now on efforts be made in the collection of data and the application of a model to describe the decision process. Both Kirk,[4] * one of the pioneers of modern criminalistics, and a famous legal expert,[5] # have already considered such a questioning/answering practice: let's just reflect and follow their advice.

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* Much of this problem [most 'expert testimony' is purely opinion testimony] would be avoided if systematic study were devoted to the development of sound probability considerations applied to evidence interpretation and also to the areas in which statistical analysis could properly contribute to correct evaluations. This is a field for combined effort by the mathematician and the criminalist. It should prove to be a most fruitful area for research- one that would strengthen the theoretical foundation on which the more practical technical structure could rest with confidence.

If it can be stated that bitemarks are due to a human bite and they show shapes which an experienced dentist can identify as having been caused by an unusual mouth pattern and there is a suspect who has that pattern then there is a probability that the bites have been caused by the suspect. The degree of probability will depend on the features of the mouth pattern and on how many of these have been transferred to the body. It is here that the evidence of the dentist becomes vital and it is also the position where the forensic medicine expert cannot give a valuable opinion. There does, however, appear to be some conflict of dental opinion on this matter. Perhaps somebody will eventually work out the mathematics of the probability involved. I believe it is necessary to render our methods more efficient by taking greater cognizance of the logical steps in our schemes of identification and not to become lost in the beauty of our instrumentation .

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AUTOPSY TECHNIQUES IN THE OROFACIAL AREA AND MACERATION USING ENZYRIM

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C. Grundmann and K. Röetzscher

ABSTRACT

The current methods of maceration are imperfect and the chemicals involved are toxic, offensive smelling, difficult to clean up and difficult to dispose of. The use of enzymes for maceration accelerates the catalytic process markedly and the method is cheap, easy to handle, non-toxic, practical and totally biodegradable. **(J. Forensic Odontostomatol 2000; 18:19-21)**

Key words: Enzyme maceration, forensic odontology, oral autopsy techniques

INTRODUCTION

The post-mortem examination is like any dental examination where teeth and oral cavity are to be investigated. If *rigor mortis* has already set in however, it will be made more difficult, particularly if the corpses are burnt or drowned, in fact the cases which most need oral autopsy as identification will depend heavily on dental data.

A dentist's emergency bag for dental identification should include magnifying glass, mirror, wedges to keep the mouth open, dividers, toothbrushes and extraction forceps for the various groups of teeth. All forensic institutes on the other hand must be equipped with the full range of sophisticated instruments for dental autopsies: bone saw, chisel, anatomical and surgical forceps, thread holder, stitching material, scissors, scalpel, curved and straight clips, gauze swabs for cleaning the teeth, strong autopsy gloves, plastic bags, photographic equipment and tape recorders.

If the investigation of the jaws has to be done *in situ*, the mouth will have to be forced open, but the best results, particularly in mass disasters are achieved if the jaws can be removed. This is strongly recommended so that forensic reports can be verified repeatedly at any time, and additional X-rays performed for comparison with an existing database of missing persons.

There can be problems with jaw removal if relatives raise an objection or want to see the body and in such cases the dentist has to have the permission of a forensic pathologist or public prosecutor to perform an oral autopsy and jaw removal.

Autopsy techniques

Depending on the merits of individual cases, the investigator can decide to cut only the cheek structure to reach the intraoral area, or they can decide to remove the jaws. The latter technique allows for more reliable examination and data collection and of course makes photography and radiography much easier.

In order to remove the jaws the muscles of mastication have to be severed and the lower jaw disarticulated. The forensic literature is well provided with advice about removing the upper jaw, sawing it horizontally, parallel to the occlusal plane but this may damage the root apices of the maxillary teeth. A better and easier way is to separate the nasal septum and the lateral sinus walls with a knife (the so called le Fort I - osteotomy) so that the maxilla separates intact. When both jaws are free they are placed in a fixing solution of formaldehyde.

Maceration using Enzyrim

The current methods of maceration using potash lye and antiformin are in some respects problematic i.e. offensive smelling, toxic, difficult disposal etc. and afterwards the preparations have to be stored in a solution of sodium hypochlorite (5%) (a bleaching agent) for several hours.

Maceration with enzymes on the other hand involves the use of catalytic reactions which are accelerated extremely rapidly, often a million to a billion times faster than the speed of a reaction without a catalyst. The enzymes themselves remain unchanged during the reaction.

The main factors necessary for an enzyme-controlled reaction are:

1. Temperature of the solution to be 55 to 60°C (130 to 150°F). Enzymes only work in the temperature range between 20 and 60°C (70 to 150°F).
2. pH-value approximately 8.5. Most enzymes operate with best results in a limited pH-range as under extreme circumstances they change their structure and lose all catalytic ability. A suitable buffer is sodiumcarbonate and the pH is measured with pH-indicators or litmus paper.

Traditional maceration method	Enzyme maceration
Potash lye	Enzyrim OSA
Antiformin	Enzyrim OSS
Putrefaction	Fluid or granular
Toxic	Non-toxic
Offensive smell	No smell
Formation of soap of potash lye-cretaceous	No destruction of bone
Difficult disposal	Simple disposal (totally biodegradable)
Problems of application	Easy to handle
Concomitant maceration of bone	Without destruction of bones
Time-consuming preparation (dissection)	No time-consuming preparation (rough dissection only)

Table 1: *A comparison of the traditional maceration method and Enzyrim OSA-OSS.*

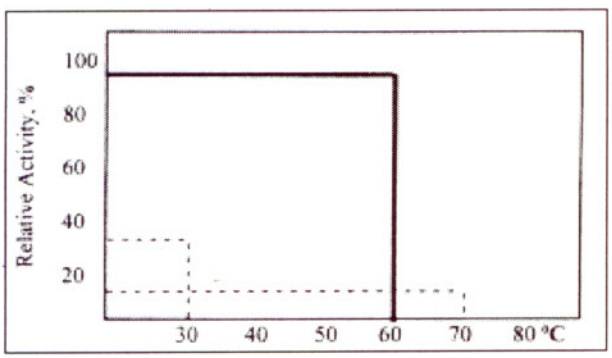

Fig.1a: *Activity of ENZYRIM-OSS at different temperatures.*

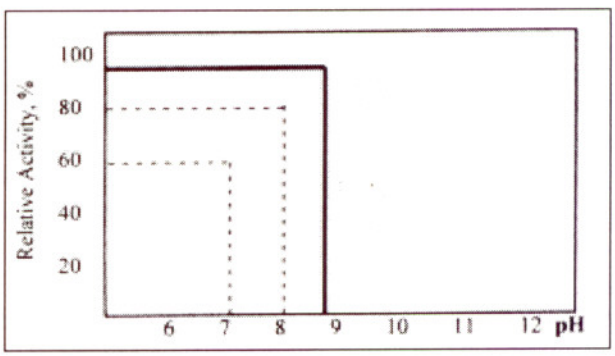

Fig.1b: *Activity of ENZYRIM-OSS at different pH-values*

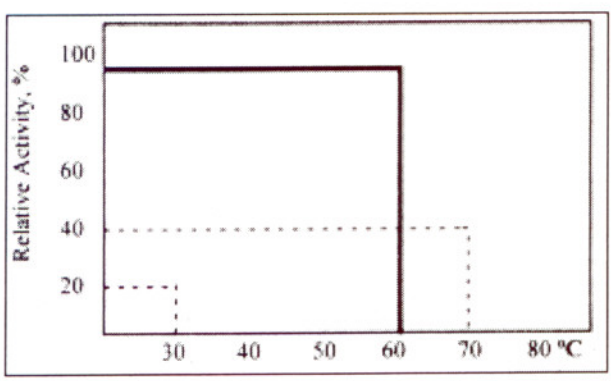

Fig.1c: *Activity of ENZYRIM-OSA at different temperatures.*

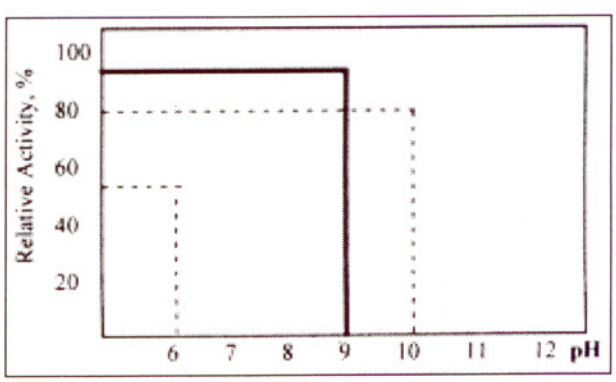

Fig.1d: *Activity of ENZYRIM-OSA at different pH-values.*

Fig.2: Lower jaw bone after dissection

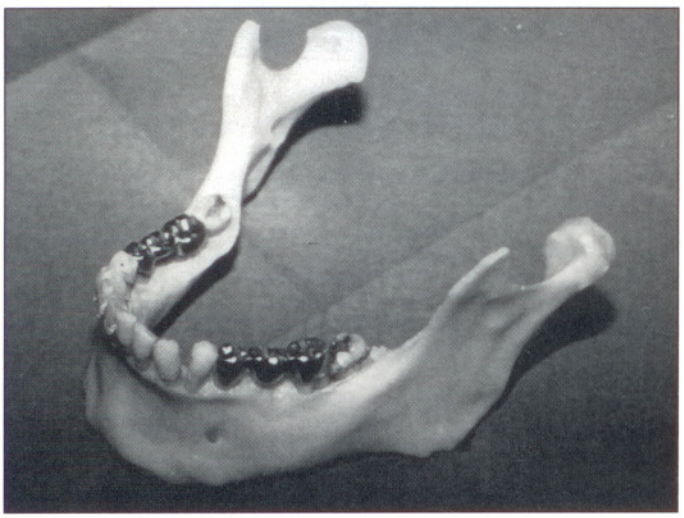

Fig.3: Lower jaw bone after maceration with Enzyrim.

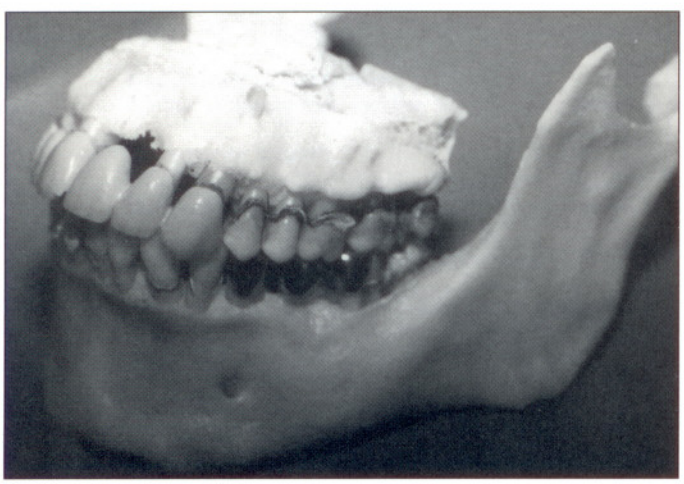

Fig.3: Both jaws after maceration with Enzyrim

A recommended solution for maceration

Two percent ENZYRIM* (granular or fluid), 1% concentrated detergent (to support the enzyme), 1 litre warm water (not demineralized) is a solution that can be used several times, but has to be kept at 60°C (150°F) otherwise it will decompose (Fig. 1a-d). The specimens shown in Figs. 2 and 3 were macerated with the ENZYRIM solution, which has already been in use generally for 6 years in the medical and biological field with excellent results.

Using the solution in an ultrasonic bath at a frequency of 35 kHz accelerates the maceration process to within 2 hours remembering that frozen or cooled material should be warmed before placing in the bath. Note that there is the possibility of teeth falling out of their sockets during maceration and a sieve should be used to catch them.

CONCLUSION

Compared with the traditional methods enzyme maceration has the following advantages:

- more cost-effective
- easy to handle
- biologically compatible
- reusable
- better results in a much shorter time without destroying the specimen and
- after only 2 hours the photographic documentation of the specimen and the specimen itself can be handed over to the dentist of the deceased person, the police or the media to allow the identification to proceed.

References may be found in the original publication.

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