1974

Effects of Aging on Human Dentin, as Related to Age Determinations

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EFFECTS OF AGING ON HUMAN DENTIN, AS RELATED TO AGE DETERMINATIONS

by

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A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

May

1974

ACKNOWLEDGEMENTS

I wish to express my appreciation and sincere thanks to:

Dr. Paul Goaz, for acting as my advisor. Without his guidance and advice, this work would have never been initiated or completed.

Drs. Michael Kiely and James Jensen, for serving on my committee and for aiding my education in varied facets.

Dr. John Madonia, who for six years has been my teacher and friend.

The many people on the staff of Loyola Dental School who have made my life a little easier because they were here.

DEDICATION

to my Parents -

Always a source of strength and

guidance and goodness.

LIFE

William D. Petty was born November 24, 1946, in Rockford, Illinois. He received his secondary education in the Rockford Public School System, and graduated in 1964 from Rockford West High School. He attended the University of Illinois, in Urbana, Illinois, from which he received a Bachelor of Science degree in 1968. He graduated from Loyola University School of Dentistry, Chicago College of Dental Surgery in 1972, with a Doctor of Dental Surgery degree. In June 1972, he enrolled in a graduate program at Loyola University School of Dentistry, Chicago College of Dental Surgery, leading to a degree of Master of Science in Oral Biology and a certificate of specialty training in orthodontics.

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CHAPTER I

INTRODUCTION

Age determinations are a difficult responsibility for an individual charged with the identification of human remains. Teeth are frequently the major clue to the identity of bodies badly decomposed or mutilated at or after the time of death.

Historically many techniques have utilized teeth for the determination of age. Yet these techniques for determination of age are limited in their applications and reliability. The ideal technique would be a simple, reproducable, and quantitative one. The purpose of this study was to find such a technique utilizing changes in the chemical composition of dentin.

CHAPTER II

REVIEW OF THE LITERATURE

Age determinations play an important role in forensic medicine. Skeletal material yields a number of features for estimating age. When appropriate skeletal material is unavailable, such as when bodies have undergone extensive damage as in air disasters, the teeth are often the principle means of identification.

Where antemortem records can be compared with the postmortem dentition identification is no problem. That is to say; the investigators have ten bodies and ten dental records belonging to the same ten bodies. The task is to match the dentitions to the proper record and thereby arrive at the identifications.

All cases are not this simple. In many instances there are no records with which to match the dentition. No clue may exist to tell the investigators where to look for records to compare the dentition with, as would be the case in utilizing the crew and passenger list in an airliner crash. These cases, where he must start from scratch test the mettle of the forensic scientist. Age determination and sex determination are frequently the keys to the identification process.

Furuhata and Yamamoto (1967), Knott (1968), Brues (1958), Johnson (1969), Scott (1963), Jerman and Tarsitano (1969), and Gustafson

(1950), advocate the use of the dentition in making identifications and particularly in age determinations. Johnson (1969), Knott (1968), and Jerman and Tarsitano (1969), refer to incidences when the remains were so badly incinerated or mutilated that the teeth yielded the only clues to positive identification.

Historically age determinations from the dentition have encompassed many procedures. The oldest method in use is probably the eruption patterns of human teeth. Miles (1963), in the "Journal of Dental Research" referred to the use of eruption patterns for age determination as far back as 1836 in Great Britain. Furuhata and Yamamoto (1967), Miles (1963, 1963), Brues (1958), Keiser-Nielsen (1968), and Demirjian, Goldstein and Tanner (1973) indicate the use of eruption and/or calcification patterns of the dentition in making age determinations. Age determinations through these means can only be utilized during the period of development, calcification, and eruption of the dentition. Keiser-Nielsen (1968), considers the use of eruption patterns useful from one to fourteen years of age. Demijian, Goldstein and Tanner (1973), have used a system utilizing panoramic X-ray studies of the calcification pattern of teeth to determine age from three to seventeen years of age. Furahata and Yamamoto (1967), describe the use of root calcification for age determination from three to twenty-nine years of age. Miles (1963, 1963), states the limit of age determinations by eruption patterns to be eighteen, while Furuhata and Yamamoto (1967), and Brues (1958), indicate the usefulness of eruption patterns

extends into the twenties. If these contradictions in limits of usefulness are not enough, the number of different tables of tooth eruption available should lead one to question the reliability of tooth eruption patterns in age determinations.

If age determinations in young individuals is difficult then age determinations in adults must be nearly impossible. In discussing age determination by present methods, Keiser-Nielsen (1968), states "In the adult ages, estimation is difficult and can hardly be better given than within ten year intervals."

Furuhata and Yamamoto (1967), list various phenomenon that may be useful in determining age. After twenty-five years of age, they related an increase in the specific gravity of teeth with age. They also pointed out the process of attrition progresses with age, and the components of the tooth, enamel, dentin, and cementum, harden with increasing age. These investigators also described the diminishing size of the root canals with age.

The use of changes in the specific gravity of whole teeth (excluding the pulp) for age determinations shows some promise. It is a quantitative test which should make determinations by its use more reliable and reproducable as opposed to the subjective systems used by some investigators. Unfortunately, Furuhata and Yamamoto (1967) illustrate only one table representing data from twenty-eight human incisors, excluding the pulp tissue, for which the specific gravity was determined and compared with age. The possible usefulness of this phenomenon for

age determination from human teeth was illustrated by its successful use in a homicide case recounted by Furuhata and Yamamoto.

Changes in the hardness of permanent teeth as described by Furuhata and Yamamoto (1967), are of questionable value in age determinations. The hardness of tooth components may increase with age yet there is such a large overlap of hardness values between the different age groups that the usefulness of this technique is questionable.

Estimation of age by reference to the attrition of teeth is another questionable technique. Attrition varies according to sex, nature of diet, occupation, and the presence of an occluding tooth. With this many variables involved it is doubtful whether dependable estimates of age can be made through the consideration of attrition.

The narrowing of the root canal with age is a well documented phenomenon. Utilizing this age change is another example of a quantitative estimate. This technique is described as a quantitative estimate because no one uses a more quantitative measurement than to say, "one third or one half the pulp chamber is filled with secondary dentin." This is to say, this is still a very subjective factor for the determination of age.

Butler (1972), presented a study of the occlusal wear patterns on human teeth that were associated with age. For individuals up to eighteen years of age, occlusal wear planes of the mandibular teeth are lingually sloped and those of the maxilla are bucally sloped, the only exception being the maxillary premolars. In persons eighteen to

thirty, the mandibular first molar becomes buccally sloped and the maxillary first molar becomes lingually sloped. With advancing age the mandibular premolar becomes buccally sloped and the maxillary premolar lingually sloped. Butler questioned the general forensic usefulness of his research when he spoke of the unknown cultural values, "such as how the teeth were used as tools and how food is prepared, may turn out to be of equal significance in influencing or modifying overall pattern of wear."

Gustafson (1950), presented a method of age determination utilizing six biological changes that are apparent in ground longitudinal sections of teeth:

1. Attrition: wearing off of the incisal or occlusal surface
2. Paradontosis: apical migration of the epithelial attachment
3. Secondary Dentin: diminishing size of the pulp cavity
4. Cementum Apposition: increase in cementum thickness
5. Root Resorption: cementum and dentin resorption at the root tip
6. Transparency of the Root: extends crownward with age

Each classification is ranked on a zero to three scale. The points for each classification are added together and the total compared to a standard curve to determine the estimate of age. Gustafson illustrated the standard curve for individuals of Swedish ancestry. It is obvious that for each population a standard curve would have to be constructed and prior knowledge of the population the unidentified person belonged

to is necessary. The second obvious shortcoming of this frequently used method is that it is primarily a subjective non-quantitative approach.

Miles (1963, 1963), compared the ability of trained observers estimating age by intuition and the Gustafson method, allowing a plus or minus three years tolerance. By the intuitive method 33% were correct while 38% were correct by the Gustafson method. Miles states, "Much good would occur if scoring in the Gustafson system could be based on actual measurements instead of subjective evaluation. Of the six criteria used by Gustafson, translucency of the root is the one that most lends itself to measurement." On the other hand, Johnson (1968), studied ninety-three teeth and attempted to correlate age and root transparency. He found no statistically significant relationship between the two. This is just one report contradicting the usefulness of root transparency, but it does emphasize the greater problem; that a clear cut, quantitative method of age determination from tooth material is not available today.

Reasoning would tell one to review the literature to find a possible key to the development of a quantitative method of age determination. When considering the components of teeth, dentin the major part of the human tooth seems to be the logical material to investigate to find a quantitative change that occurs in it with increasing age.

Inasmuch as dentin comprises the bulk of the tooth, and that this tissue does show progressive changes with age, suggested that an examination of dentin may well reveal quantitative changes that could be

critically measured and would show close correlation with advancing age.

References disagree as to the percentage composition of dentin, possibly because the age of the tooth analyzed was not considered nor was the area of the tooth from which the sample was obtained. Mjor and Pindborg (1973), state the composition on a wet weight basis as: 70% inorganic, 18% organic, and 12% water. Arey (1963), and Bloom and Fawcett (1968), place the inorganic material at a slightly higher percentage. Many writers, Beust (1931), (1931), (1934), Bradford (1958), Fish (1933), Arey (1963), Bloom and Fawcett (1968), Sicher (1962), and Mjor and Pindborg (1973), have noted changes in the dentin's physical and chemical properties with advancing age.

Before considering the changes in dentin with age, the structure and composition of dentin will be considered in detail. The inorganic portion, as in all other mineralized tissues of the human body, consists mainly of hydroxyapatite crystals. The smallest repeating part of these crystals has the formula $Ca_{10}(PO_4)_6(OH)_2$, the unit cell of the hydroxyapatite. The mineralizing crystals are made up of thousands of unit cells. They are plate-like in shape and viewed edgewise will have a needle like appearance. Mjor and Pindborg (1973), list the crystals as 50-60 millimicrons in length and possibly as long as 100 millimicrons with a width of 3-5 millimicrons. The crystals are similar to those found in bone and cementum but smaller than those found in enamel. Other inorganic salts are found in trace amounts. Carbonates and calcium phosphate are found as well as traces of fluoride, copper, zinc and iron.

The hydroxy group within the hydroxy apatite may be replaced by fluoride, which yields a fluorapatite. This is of clinical significance, since fluorapatite is less soluble than hydroxyapatite.

The organic portion consists primarily of collagen. Collagen occupies one half the total volume of the dentin, yet it is only 17% of the total weight or 93% of the organic material (Mjor and Pindborg (1973). The collagen is typical of collagen found elsewhere in the body. The 640 Angstrom banding of the fibrils and their 0.02 to 0.05 thickness typifies collagen. According to Mjor and Pindborg (1973), fractions of proteins, mucopolysaccharides, and lipids each constitute approximately 0.2% of the organic portion. Citric acid accounts for slightly less than 1%.

The structural entities of dentin are: the odontoblast, with the odontoblastic process, the dentinal tubule, the periodontoblastic space, the peritubular dentin, and the intertubular dentin. Odontoblasts are specialized cells found within the dental pulp, lining the inner boundry of the dentin. The odontoblasts have long cytoplasmic processes which extend through the dentin, within the dentinal tubules, very close to, but not usually through the dentino-enamel junction. (Sicher 1962)

The dentinal tubules, in which the odontoblastic process lie varying in volume and lumen depending upon location within the dentin. Sicher (1962), states they are 2-3 microns wide near the pulp and about 1 micron wide near the enamel. Mjor and Pindborg (1973), state that as much as 80% of the dentin volume near the pulp may be dentinal tubules

whereas near the enamel they may occupy as little as 4%. This change in density can be explained because the pulpal surface area is so much less than the enamel surface area.

The periodontoblastic space surrounds the odontoblastic process within the tubule. This space contains tissue fluid and a few organic constituents, such as collagen. This space is important because the soft tissue changes in dentin occur here.

The mineralized tissues of dentin are the peritubular and intertubular dentin. The highest mineralized area is the peritubular dentin. Upon demineralization only a scanty organic matrix, mainly collagen fibers, remains of the peritubular dentin. These collagen fibers are continuous with the abundant collagen fibers of the matrix of the intertubular dentin. According to Mjor and Pindborg (1973), and the evidence from electron microscopy, Sicher (1962), the sheath of Neumann around the peritubular dentin does not exist.

All of dentin is not evenly mineralized. There is a layer of dentin lying next to the odontoblastic cell layer, the predentin, which is not mineralized throughout life. For the most part the intertubular dentin is evenly mineralized, with limited areas being of lesser mineral content. The mantle dentin adjacent to the enamel is of diminished mineral content. Isolated areas of less mineral content can be found within the intertubular dentin. These areas are called globular dentin, since in demineralized sections they appear as voids or vacuoles. Tomes layer adjacent to the cementum has the characteristics of globular

dentin.

In cross sections of dentin, incremental lines are visible. These lines are lines of von Ebner which resemble the lines of Retzius found in the enamel. The lines are an index of dentin production. When disturbances in dentinogenesis occur accentuated lines are visible, the contour lines of Owen. Bloom and Fawcett (1968), Arey (1963), Sicher (1962), Mjor and Pindborg (1973), Nalbandian (1960), have noted a change in mineral content and in the width of the peritubular dentin with age to the point of obliteration of the tubule.

The dominant inorganic substance of dentin, collagen, is found primarily in the intertubular dentin. Mjor and Pindborg (1973), record that less is known as to the distribution of the other dentinal organic fractions: lipids, polysaccharides, proteins, and citric acid. Citric acid has been linked to the hydroxyapatite unit cell. Acid mucopolysaccharides have been associated with the process of mineralization. Two primary zones of acid mucopolysaccharide concentration have been found. The predentin and dentin boundry, and the periphery of the dentinal tubules, where secondary peritubular dentin is formed, (Mjor and Pindborg, 1973). Mjor and Pindborg (1973), also report that this pattern may depend upon the age of the teeth. Glycogen has also been noted to be more prevalent in mature teeth.

The odontoblasts are well suited for the process of dentinogenesis. They are characterized by: rough endoplasmic recticulum cytoplasmic ribosomes, a well developed Golgi apparatus, mitochondria, and other

organelles, which make them well prepared for active matrix production. Initially the dentin matrix is formed by the subodontoblastic cells of the dentinal pulp. After the initial matrix formation, the major part of the organic matrix is formed by the odontoblasts.

The layer of unmineralized matrix, the predentin, always lies between the odontoblasts and the dentin. This layer can be divided into a young and old layer, which indicates changes occur in the matrix when it is being mineralized, Mjor and Pindborg (1973). Predentin is not found only during dentinogenis, but is found throughout the life of the tooth, since dentin formation occurs slowly throughout life.

Once the full thickness of the predentin is formed, mineralization begins. Platelike crystals appear in the matrix close to and even in the collagen fibers. These crystals aggregate into spherical groups called calcospherites. These aggregates grow and eventually fuse. Interglobular dentin results when there isn't complete fusion. Periods of mineralization are evidenced by the lines of von Ebner. This process occurs within the intertubular and peritubular dentin simultaneously.

According to Mjor and Pindborg (1973), at or approximately the same time as completion of the crown formation there is a line formed of reduced mineral content. Dentin produced after this line is formed is called "secondary dentin". Secondary dentin usually has less tubles with more tortuous paths than the primary dentin. Since secondary dentin production continues throughout life, it has the effect of reducing the volume of the pulp chamber.

Reparative dentin results if extensive wear, erosion, caries, or operative procedures assaults the tooth. Damaged odontoblasts may continue to function or degenerate and be replaced by undifferentiated cell in the pulp. Damaged cells stimulate the production of hard tissue to seal off the area of insult. This hard tissue, reparative dentin, is evidenced by twisted tubules of a reduced number. "Dead tracts" empty spaces formally occupied by soft tissue or fluid are frequently found peripheral to reparative dentin in ground sections.

Transparent or sclerlotic dentin is a result of the dentinal response to adverse stimuli. As previously noted, calcium salts may be deposited around degenerating odontoblastic process and may eventually obliterate the tubule. Frank (1959), reports that this process may occur without noxious stimuli. The transparency results when the refractive indices of the dentin and the former dentinal tubule are equalized. Transparent dentin has frequently been reported under slowly advancing caries and in the root dentin of the elderly.

Age changes in dentin are of critical interest to this paper. Previously we have noted: a gradual mineralization of the peritubular dentin that may obliterate the tubule, continued production of secondary dentin throughout life, changing staining properties for acid mucopoly saccharides with age. Historically, changes in the dentition and in the dentin particularly have been the subject of many studies.

Beust in 1931 and 1934 noted age changes in the human dentition.

Particularly the formation of translucent zones in areas of caries or irritation. These areas were evidenced by increased density and hardness. Beust called these changes "sclerosis". He ascribed the transparency as a result of the dentinal canal obliteration. He also stated this changed the normally "sieve-like" dentin into a structure that was resistant to acids and stains. He surmized that the deposits that caused this change in permeability were "calcific in nature". In the summary of his 1931 paper, Beust stated that this was a gradual and physiologic process occuring with increasing age. In 1934 Beust used diffusion studies to further illustrate maturation changes in human teeth.

Bodecher and Lefkowitz (1937 and 1946), used permeability and x-ray studies to show maturational changes in human teeth. They believe their studies indicated an increased calcification of the dentin with age. Fosdick (1963), found the prime variable in the rate of penetration through dentin of water, ions, and small molecules was the age of the tooth donor.

In 1967, Witte and Fullmer reported a variation in the staining of dentin with azure A relative to age. Their studies were conducted on decalcified dentin sections. Azure A binds to anionic groups. The stain was therefore assumed to bind to acid mucopolysaccharides not the predominant collagen which is acidophilic. The decrease in basic dye uptake with the increasing age of the sample would tend to indicate a decrease in mucopolysaccharides with age.

Nalbandian (1959), used light microscopy, microradiography, and electron microscopy to study changes in root dentin. In transparent or sclerotic dentin he noted: with light microscopy the tubules were difficult to visualize, with microradiographs the tubules appeared to be virtually obliterated by a highly radiopacque substance. With electron microscopy sclerotic dentin is found to be of relative homogeneous electron density as compared to the irregular pattern found in young opacque dentin. Electron diffraction patterns of obliterated tubules were identical to those of intertubular dentin. This would indicate crystals deposited within sclerotic tubules of the root are the same as crystals found in the intertubular dentin.

Throughout the review of literature, references have been noted to: changes in permeability, changes in hardness, changes in specific density, and tubule obliteration in the dentin of human teeth with age. Yet no quantitative evidence of the change in inorganic as opposed to organic content of human dentin with age could be found. Evidence, such as the increase in sclerotic dentin with age, suggests that there is a parallel increase in the inorganic component of dentin with age. It was the hypothesis of this research that such a positive relationship exists and that this relationship might be useful in age determinations.

CHAPTER III

MATERIAL AND METHODS

This study was conducted upon randomly selected extracted human teeth from Caucasian donors. Upon extraction, the teeth were washed and placed in numbered vials. The age, sex, race and occupation of the patient as well as the identification of the teeth were recorded on a correspondingly numbered paper. Selected teeth were restoration free and had no carious insult to the dentin as determined by visual inspection.

The teeth were divided into two groups according to age. The younger group was from thirty years and younger, while the older was forty-five years or more. After air drying for a minimum of seventy-two hours the teeth were sectioned by using dental separating discs. Teeth from thirty-eight separate individuals were used. Since it was necessary to remove the pulps, single rooted biscuspids and anterior teeth were utilized to insure easier access to the pulp. After midline sectioning the enamel and cementum were ground off. The pulp chambers were abraded to insure thorough pulp tissue removal. Abrasive dental green stones were utilized for these processes.

The remaining material, dentin, was washed with deionized water and air dryed for twenty-four hours. The samples were placed in

numbered, previously weighed plastic vials. A torbal EA-1 electrical balance with a readability and precision of ± 0.003 mg. was used throughout the research to determine weights. The plastic vials and samples were never touched by human hands or left uncovered unless absolutely necessary throughout the research. The samples were placed in the numbered vials without regard to group in order to prevent experimentor bias in reading and in recording the weights. Forty-five vials were used for thirty-eight samples. Seven vials were treated as the sample containing vials in order to act as a control group.

Reagent alcohol, 95% ethyl alcohol denatured with 5% propyl alcohol, was added to each vial to adsorb the free water from the samples. The alcohol was left in the vials for three hours and then drawn off with an aspirating syringe, taking care not to touch the samples. The residue alcohol was evaporated by placing the vials in a Forma anaerobic incubator at 32 degrees centigrade and a negative pressure of eighteen pounds per square inch for twenty-four hours.

Upon removal from the incubator, the vials were placed in a covered bell jar. Calcium sulfate and calcium carbonate crystals were placed in the jar to adsorb hydrogen sulfide and water respectively. The vials were left in the bell jar and allowed to cool to room temperature. Upon cooling, the vials were removed from the bell jar one at a time and weighed. After the first weighing, the vials were stored in the bell jar for 24 hours and the weighing procedure was repeated.

The next step was to decalcify the dentin samples. "Decal", a commercial mixture of chelating agents and hydrochloric acid decalcifying media was used. "Decal" is produced by The Omega Chemical Company, Cold Spring on the Hudson, New York. The samples were immersed in the decalcifier for one hundred and sixty eight hours (7 days). Gustafson (1950), refers to six or seven days being necessary to decalcify teeth. New decalcifying solution was added periodically to assure the samples were completely immersed, since the decalcifying solution tended to evaporate.

At the end of the decalcification period the samples were washed three times with deionized water. The water was added to the vials from a wash bottle and drawn off with an aspirating syringe. At this time the same drying procedures as were used for the original samples, were used to dry the decalcified samples.

The same procedures were also repeated for weighing the decalcified samples. After weighing the four largest samples, highest initial weight, were x-rayed to determine whether thorough decalcification had occured. The samples were x-rayed using a dental x-ray unit and standard film at 90 KVP, 15ma., for one-tenth of a second. The x-ray films were examined for any traces of mineral content.

The weight of the dried undecalcified dentin sample was determined by subtracting the weight of the empty vial from the weight of the vial plus the dried sample. The weight of the dried decalcified sample was determined by subtracting the weight of the empty vial from the weight

sample from the weight of the dried sample. The percentage loss in weight was determined by dividing the loss in weight by the weight of the original dried sample and multiplying by one hundred. All weights referred to are an average of the two weights taken at each stage of the research.

The results were statistically analyzed utilizing the Student t-test. Values above the 5% level of probability were considered significant. A regression line was calculated from the data. The significance of this line was analyzed by means of the Student t-test. A coefficient of correlation was calculated, in order to determine whether the data corresponded to this regression line.

CHAPTER IV

RESULTS

For the purpose of comparison, the data was divided into two groups, young and old. These two groups were further divided as to sex. The young group consisted of nineteen teeth from individuals eight years of age to thirty years. There were twelve males and seven females in this group. The older group consisted of nineteen teeth from individuals forty-five years of age to sixty-nine years. There were nine males and ten females in the older group.

The young male group of twelve teeth showed a range of weight loss upon decalcification of 71.08% to 84.36% with a mean of 77.34%. The young female group of seven teeth had a weight loss range of 70.66% to 81.62% with a mean of 79.24%. The total young group had a range of weight loss from 70.66% to 84.36% with a mean of 78.04%.

The older male group of nine teeth had a range of 75.54% to 86.85% with a mean weight loss of 81.55%. The older female group of ten teeth had a weight loss range of 69.88% to 89.82% with a mean loss of 81.77%. The range for the total older group was the same as for the older females with a mean of 81.67% weight loss.

The standard deviation for the young group was 3.59%. The standard deviation for the young male teeth was 3.38% and the standard deviation for the young female teeth was 3.89%. The standard deviation for the old group was 4.62% with a standard deviation for male teeth of

3.22% and 5.73% for female teeth. The Student t-test was utilized to statistically analyze the results. The percentage of weight loss between the total young group and the total old group was found to be statistically significant at the 0.02 level of probability. The difference in weight loss between the young males and old males was also found to be statistically significant at the 0.02 level of probability. The differences in weight loss between young females and old females, old males and old females, and young males and young females were not statistically significant. The statistical analysis of the weights of the control vials at the three weighings showed no statistical significance to the variations in weight.

A regression line can be drawn for the data with a y intercept of 75.44% weight loss and a slope of .115. To test the significance of this line it was compared to a line with a slope of zero. A line with a zero slope would show no relationship between weight loss and age existed. The regression line was found to be statistically significant from a line of zero slope at a 0.01 level of probability utilizing the student t-test.

A coefficient of correlation was also calculated for this data. The r value was .455. The r value can range from a value of zero to plus or minus one. A value of zero showing no correlation and a value of plus or minus one showing direct correlation. An r value of .455 at thirty-six degrees of freedom is significant at the 1% level.

Table #1

Percentage of Weight Loss Through Demineralization of Dentin Samples

Young Males

Age	Percentage of Weight Loss
8	80.18
13	77.51
13	77.80
13	72.96
17	77.40
21	76.40
21	79.84
22	77.95
23	71.08
26	75.94
28	76.64
30	84.36

Young Females

12	79.27
22	79.85
24	80.48
25	70.66
27	81.62
28	81.28
30	81.54

Table #2

Percentage of Weight Loss Through Demineralization of Dentin Samples

Old Males

Age	Percentage of Weight Loss
45	79.49
45	84.17
49	82.17
52	81.48
52	78.50
52	83.30
53	82.48
54	75.54
69	66.85

Old Females

45	78.77
52	83.08
55	69.88
55	86.71
56	76.70
58	84.04
61	89.82
62	84.95
65	79.32
67	84.46

Table #3

Cummulative Percentage of Weight Loss Through Demineralization of Dentin Samples

	Average Age	Average Percentage of Weight Loss
Young		
Males	19.6	77.34
Females	24.0	79.24
Total	21.2	78.04
Old		
Males	52.3	81.55
Females	57.6	81.77
Total	55.1	81.67

Table #4

Distribution of Weight Lost Through Demineralization of Dentin Samples and the Regression Line Calculated from the Experimental Data

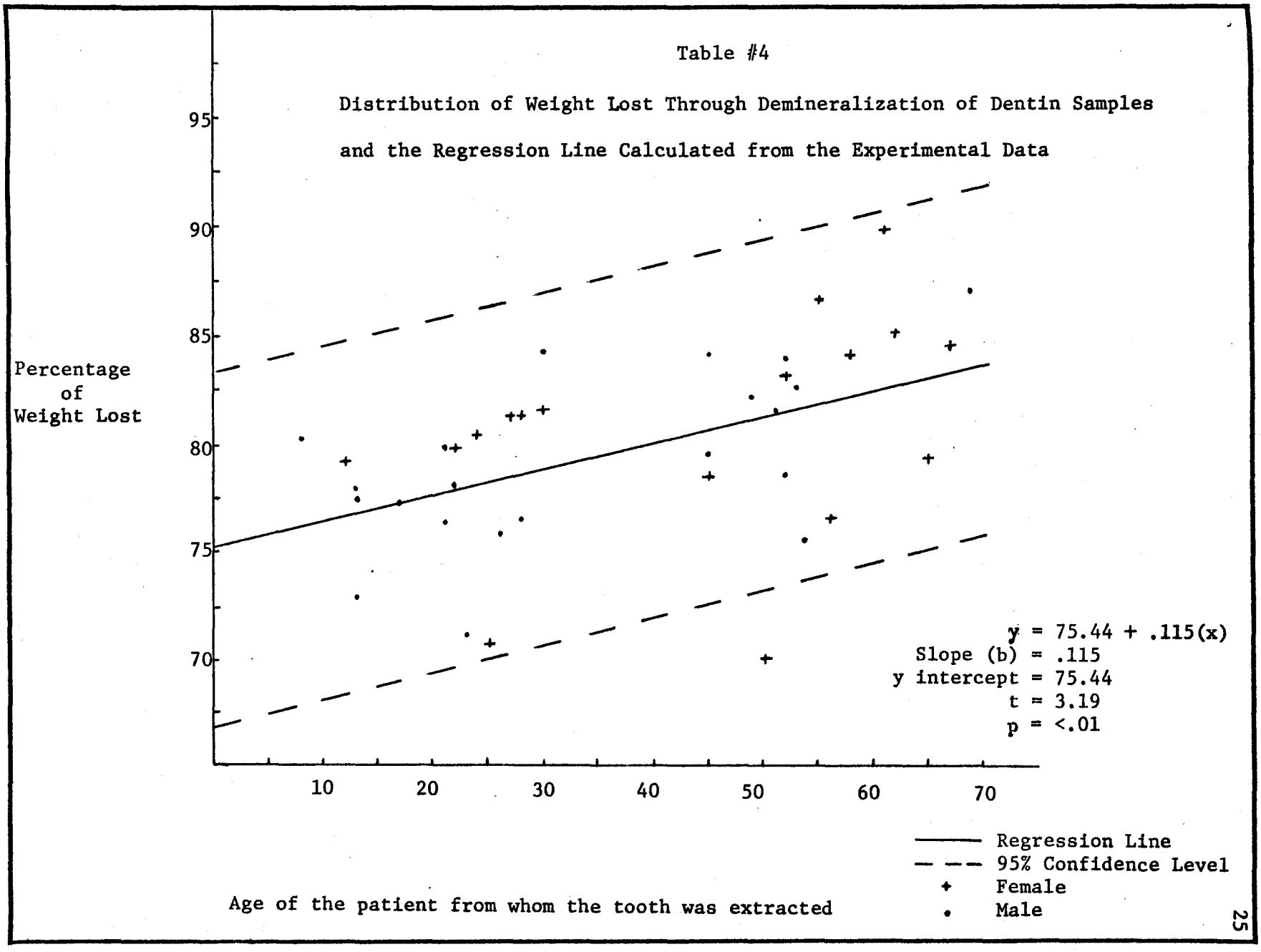

Table #5

Statistical Analysis of Weight Loss Through Demineralization Data

Comparison of Tooth Samples	Number of Observations	Mean	Standard Deviation	t value	Probability Level	Statistical Significance
Total Young	19	78.04	3.59	2.71	< .02	Yes
va.						
Total Old	19	81.67	4.62			
Young Males	12	77.34	3.38	2.84	< .02	Yes
vs.						
Old Males	9	81.55	3.32			
Young Females	7	79.24	3.89	.95	> .05	No
vs.						
Old Females	10	81.77	5.73			
Young Males	12	77.34	3.38	1.12	> .05	No
vs.						
Young Females	7	79.24	3.89			
Old Males	9	81.55	3.32	.10	> .05	No
vs.						
Old Females	10	81.77	5.73			

Table #6

Statistical Analysis of the Variations in Vial Weights Using Paired Sample t Test

Comparison of Vials	Number of Observations	Mean	Standard Deviation	t value	Probability Level	Statistical Significance
Empty	7	.7165	.0032	.84	> .05	No
vs.						
After Drying	7	.7228	.0198			
Empty	7	.7165	.0032	.41	> .05	No
vs.						
After Decalcification and Drying	7	.7218	.0336			
After Drying	7	.7228	.0198	.07	> .05	No
vs.						
After Decalcification and Drying	7	.7218	.0336			

CHAPTER V

DISCUSSION

The data from this research showed a statistically significant difference in weight loss by demineralization of dentin from young teeth (8-30 years of age) and dentin from old teeth (45-69 years of age). According to Mjor and Pindborg (1973), this difference in weight loss upon decalcification can be attributed to a difference in inorganic content. Mjor and Pindborg (1973), state that if demineralized sections of dried teeth are studied, the inorganic fraction is removed leaving only the organic portion.

Duyvejonck (1968), utilizing incineration at 700^{0}C of pooled samples of human teeth found a decrease in organic material with increasing age upon a percentage of weight basis. Mjor and Pindborg (1973), state, "The ingress of mineral salts occurs primarily at the expense of the water content. Duyvejonck (1968), and Kastelic (1968), support this statement. They found a decrease in the water content of human dentin (Duyvejonck) and the whole tooth (Kastelic) with age. Kastelic (1969), also found evidence that seemingly contradicts Duyvejonck results. Kastelic found through incineration studies, an increase, upon a percentage basis, in the organic content of the whole human tooth with age. It is difficult to explain this contradiction.

Possibly the increase in organic content of the dental pulp is of such magnitude to offset the fact that the pulp becomes smaller with age and thereby offsets the change in the hard tissues of human teeth. This seemingly contradictory evidence and questions as to the mechanisms of the changes illustrate the need for further study of this subject.

The difference in weight loss by demineralization of dentin from young males and old males was statistically significant. There was no significance between the weight loss by demineralization of dentin from young females and old females. This would tend to indicate a sex related mechanism for, or rate of, demineralization of young male and female teeth in either the young or old group. In other words, how can there be a sex related mechanism if there is no statistical significance between the age groups.

In considering the data in general, the control vials showed no significant change in weight. Indicating if there was a change in weight, it was a result of changes in sample, not vial weights. The variation in weights between the first and second weighings on the average was less than one percent, a very small variation considering the time period involved and the sensitivity of the balance.

We have quantitatively shown an increase in the inorganic content of human dentin with age. The question remains whether this phenomenon is useful in determining age. In its present state, the technique is probably no better than many techniques in use today. Although it is an improvement over the subjective techniques, in that it utilizes

measurable results. The magnitude of change in inorganic content was not great enough to yield critical age determinations.

There is still the possibility of utilizing the basic principle of this study in making age determinations. We considered the inorganic content in general, possibly a more effective means would be to consider the individual components of the inorganic portion of dentin. We also used the whole longitudinal section of dentin. It may be more effective to utilize just the coronal, root or possibly just the secondary dentin.

Quantitative assays of the components of dentin may yield data that shows such a magnitude of change with age that could be effectively utilized in determinations of age. Newesely (1970), stated that ions can replace each other isomorphically in the crystal lattice of the hydroxy apatite, if the difference in ionic size ratio is less than fifteen percent. This is further evidence of the variability of dentin composition. Further research may delineate a change in dentin composition truly useful in the determination of age.

CHAPTER VI

SUMMARY AND CONCLUSIONS

An attempt was made to develop a quantitative technique for age determination from human dentin. The percentage of weight loss in human dentin upon demineralization with a mixture of chelating agents and hydrochloric acid was determined for thirty-eight samples of dentin. The results were compared in two groups, young (8-30 years old) and old (45-69 years old) according to the age of the donor. They were also compared by sex.

There was a statistically significant difference in weight loss by decalcification of dentin from young donor teeth as compared to old donor teeth. The inorganic content was found to increase with age. Although there was a significant difference in weight loss between dentin from young male donors and old male donors, no other sex related significance was found in the analysis of the data. With this contradictory evidence, it is difficult to state conclusively whether a sex related difference in inorganic content of dentin or change in inorganic content with age exists. The majority of the data does make it doubtful whether either relationship exists.

Where as this paper delineates a definite trend of increase in inorganic content of human dentin with age, it does not indicate that

the magnitude of this change is great enough to lend itself to usefulness in the determination of age. Further research is indicated to possibly find a component of human dentin or area of the tooth from which the dentin could be removed that indicate a magnitude of change with age sufficient to be effectively used in age determinations.

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APPENDIX

Vial and Sample Distribution

Vial #	Age	Sex
1	8	Male
2	27	Female
3	49	Male
4	17	Male
5	45	Male
6	58	Female
7	52	Female
8	21	Male
9	22	Male
10	24	Female
11	12	Female
12	45	Male
13	52	Male
14	69	Male
15	28	Female
16	30	Female
17	13	Male
18	65	Female
19	62	Female
20	45	Female
21	54	Male
22	30	Male
23	28	Male
24	13	Male
25	21	Male
26	61	Female
27	55	Female
28	52	Male
29	13	Male
30	22	Female
31	26	Male
32	23	Male
33	53	Male
34	52	Male
35	56	Female
36	25	Female
37	67	Female
38	55	Female

Vial and Sample Distribution

Vial #	Age	Sex
39	Control	
40	Control	
41	Control	
42	Control	
43	Control	
44	Control	
45	Control	

Weight of the Vial with the Dried Sample

Weight in Grams

Vial #	I	II	Average
1	.7973	.8027	.8000
2	.9199	.9277	.9238
3.	.9254	.9463	.9358
4.	.9790	.9584	.9687
5	1.0288	1.0247	1.0268
6	1.0172	1.0255	1.0214
7	.7945	.8133	.8039
8	.9703	.9675	.9689
9	.8670	.8735	.8702
10	.9770	.9700	.9735
11	.8947	.8790	.8868
12	1.0064	1.0084	1.0074
13	.8999	.9070	.9034
14	.9923	.9720	.9822
15	.9313	.9170	.9242
16	.9136	.9183	.9160
17	.9770	.9752	.9761
18	.7872	.8075	.7974
19	.8777	.8760	.8768
20	1.0450	1.0420	1.0435
21	.9321	.9752	.9536
22	.9520	.9765	.9642
23	1.0976	1.0778	1.0877
24	1.2162	1.1970	1.2066
25	1.1487	1.1527	1.1507
26	.8118	.8094	.8106
27	.9328	.9334	.9331
28	.9736	.9417	.9576
29	1.0543	1.0200	1.0372
30	.9906	1.0040	.9973
31	.9902	1.0232	1.0067
32	.8489	.8233	.8361
33	.9046	.9360	.9203
34	.9423	.9355	.9389
35	.9855	.9489	.9672
36	.8515	.8228	.8372
37	.8622	.8490	.8556

Weight of the Vial with the Dried Sample

Weight in Grams

Vial #	I	II	Average
38	.9142	.9102	.9122
39	.7329	.7259	.7294
40	.7224	.7355	.7290
41	.7192	.7190	.7191
42	.6800	.6847	.6824
43	.7380	.7823	.7602
44	.7083	.7030	.7056
45	.7326	.7360	.7343

Weight of the Empty Vials

Weight in Grams

Vial #	I	II	Average
1	.6344	.6469	.6406
2	.7307	.7210	.7258
3	.6875	.6780	.6828
4	.6962	.6960	.6961
5	.7133	.7107	.7118
6	.6974	.6999	.6986
7	.7045	.7040	.7042
8	.7031	.6951	.6991
9	.6671	.6832	.6752
10	.7278	.7284	.7281
11	.6994	.6999	.6996
12	.6104	.6140	.6122
13	.7135	.7000	.7068
14	.6925	.6893	.6909
15	.7040	.7010	.7025
16	.7018	.7000	.7009
17	.7288	.7262	.7275
18	.6767	.6714	.6741
19	.6973	.7040	.7007
20	.7069	.7075	.7072
21	.7089	.7035	.7062
22	.7130	.7115	.7122
23	.7245	.7128	.7186
24	.7356	.7298	.7327
25	.7188	.7111	.7150
26	.6440	.6414	.6427
27	.7161	.7150	.7156
28	.6798	.6875	.6836
29	.7233	.7283	.7258
30	.7173	.7185	.7179
31	.7110	.7104	.7107
32	.6701	.6640	.6670
33	.6595	.6571	.6583
34	.7319	.7398	.7359
35	.6846	.6824	.6835
36	.7121	.7019	.7070
37	.7016	.7008	.7012

Vial #	I	II	Average
38	.7142	.7209	.7176
39	.7270	.7309	.7290
40	.7230	.7205	.7218
41	.7153	.7130	.7142
42	.6853	.6811	.6832
43	.7409	.7420	.7414
44	.6916	.6940	.6928
45	.7316	.7345	.7331

Weight of the Vial and the Dried
Decalcified Sample

Weight in Grams

Vial #	I	II	Average
1	.6685	.6760	.6722
2	.7620	.7623	.7622
3	.7258	.7300	.7279
4	.7564	.7590	.7577
5	.7778	.7749	.7764
6	.7546	.7455	.7501
7	.7177	.7245	.7211
8	.7552	.7518	.7535
9	.7206	.7158	.7182
10	.7750	.7770	.7760
11	.7371	.7396	.7384
12	.6715	.6781	.6748
13	.7406	.7458	.7432
14	.7340	.7245	.7292
15	.7540	.7340	.7440
16	.7454	.7359	.7406
17	.7910	.7759	.7834
18	.6963	.7028	.6996
19	.7286	.7258	.7272
20	.7793	.7780	.7786
21	.7647	.7687	.7667
22	.7558	.7474	.7516
23	.7978	.8117	.8048
24	.8351	.8401	.8376
25	.8205	.8150	.8178
26	.6615	.6582	.6598
27	.7448	.7442	.7445
28	.7395	.7455	.7425
29	.8110	.8090	.8100
30	.7761	.7722	.7742
31	.7858	.7780	.7819
32	.7153	.7165	.7159
33	.6965	.7120	.7042
34	.7690	.7707	.7698
35	.7401	.7592	.7496
36	.7555	.7350	.7452
37	.7241	.7262	.7252

Weight of the Vial and the Dried
Decalcified Sample

Weight in Grams

Vial #	I	II	Average
38	.7703	.7821	.7762
39	.7310	.7340	.7325
40	.7240	.7375	.7308
41	.7174	.7235	.7204
42	.6885	.6821	.6853
43	.7365	.7460	.7412
44	.7064	.7006	.7035
45	.7379	.7397	.7388

Calculation of Weight Loss

Vial #	Dried Sample	Decalcified Dried Sample	Percentage of Weight Loss
1	.1594	.0316	80.18
2	.1980	.0364	81.62
3	.2530	.0451	82.17
4	.2726	.0616	77.40
5	.3150	.0646	79.49
6	.3228	.0515	84.04
7	.997	.0169	83.05
8	.2698	.0544	79.84
9	.1950	.0430	77.95
10	.2454	.0479	80.48
11	.1872	.0388	79.27
12	.3952	.0626	84.17
13	.1966	.0364	81.48
14	.2913	.0383	86.85
15	.2217	.0415	81.28
16	.2151	.0397	81.54
17	.2486	.0559	77.51
18	.1233	.0255	79.32
19	.1761	.0265	84.95
20	.3363	.0714	78.77
21	.2474	.0605	75.54
22	.2520	.0394	84.36
23	.3691	.0862	76.64
24	.4739	.1052	77.80
25	.4357	.1028	76.40
26	.1679	.0171	89.82
27	.2175	.0291	86.71
28	.2740	.0589	78.50
29	.3114	.0842	72.96
30	.2794	.0763	79.85
31	.2960	.0712	75.94
32	.1691	.0489	71.08
33	.2620	.0459	82.48
34	.2030	.0339	83.30
35	.2837	.0661	76.70
36	.1302	.0382	70.66
37	.1544	.0240	84.46
38	.1946	.0586	69.88

Calculation of Weight Loss

Vial #	Dried Sample	Decalcified Dried Sample	Percentage of Weight Loss
39	.0004	.0035	
40	.0072	.0090	
41	.0039	.0062	
42	-.0008	.0021	
43	.0188	-.0002	
44	.0128	.0107	
45	.0012	.0057	

APPROVAL SHEET

The thesis submitted by Dr. William D. Petty has been read and approved by three members of the Graduate School faculty.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with references to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

5/17/74
Date

Signature of Advisor