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Two non-specific indicators of stress: Enamel hypoplasia and Harris lines

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TWO NON-SPECIFIC INDICATORS OF STRESS:

ENAMEL HYPOPLASIA AND HARRIS LINES

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ABSTRACT

Two Non-Specific Indicators of Stress: Harris Lines and Enamel Hypoplasia

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Harris lines (HL) and enamel hypoplasia (EH) are two non-specific indicators of stress, commonly used in the reconstruction of the health status in past and present populations. The aim of this study was to determine if there is a correlation between these two markers. To achieve this aim, a sample of 136 individuals from two archaeological sites (Az-71 and Az-140) from northern Chile was analyzed. HL and EH showed no correlation in terms of presence absence at the individual level. In addition, HL and EH, by age of the individual at the time of the defect formation, showed a completely different distribution. The results indicate no correlation between these two indicators at any level. Instead, the distribution of Harris lines, by age of the individual at the time of their formation, show that this indicator is associated with growth and not with arrested or slowed associated with stressful conditions.

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CHAPTER I

INTRODUCTION

Skeletal remains give researchers a unique insight into human ecology and adaptation in the past (Buikstra and Cook, 1980) and with this it is possible to reconstruct their life style and the environment that they inhabited. It is not new to say that the environment that we live in today is extremely different from the one populated by our ancestors. The environment is not different only in bioenvironmental terms; it is even more different in cultural ones. With this in mind, and considering that humans are bio-cultural beings, we can overcome the "pristine myth" that characterized ecological anthropology in the 1970's (Headland, 1997); this is, to think that "pristine societies" lived in something like a perfect equilibrium with their environment (social, biological and abiotic). An idea that is not only biased by Rousseau's concept about the "noble savage", but that is also limited as a homeostatic model. Humans are always changing, and as they change socially and culturally they impact the surrounding environment of other groups (McElroy and Townsend, 1996). By understanding that "equilibrium" with the environment is always an on-going process, we can approach the past with questions about human adaptation and the price that each group have had to pay to live the way they did.

I.1. Theoretical Orientation

In the course of human evolution different forces have shaped the human body. Natural selection has modeled human biology by affecting some physical traits that, in turn, have or have not impacted other traits. The environment where these selective forces originate include not only the biotic and abiotic aspects of the world that we inhabit, but also comprise the social conditions of human reality: humans as bio-cultural beings inhabit an environment that includes the complexity of the cultural domain (Wiley, 1992; McElroy and Townsend, 1996).

The "modeling" of the human body occurs through adaptation. Adaptation here will be understand as adjustments that at least in the short term aid the functioning, and therefore survival, of an organism (Thomas et al., 1989). Most studies about human adaptation have emphasized the concept of homeostasis as the main goal of an organism, and although it is true that internal homeostasis is essential to survival, the relationship between an organism and its environment is anything but static. Moreover, although adaptation is usually understood as beneficial (Wiley, 1992), the truth is that sometimes the long-term consequences for an individual or an entire population are not positive. Moreover, the adjustment may be beneficial for one population but it may have negative consequences in other, or it may be advantageous for one segment of the population but disadvantageous for the majority.

Thus, instead of understanding adaptation as an event we should better understand it as a process. Although adaptation has its benefits it also has costs, and what may seem beneficial at a certain moment in time, may have deleterious consequences in the future. When thinking about adaptation then, we usually focus on its positive consequences, but

adaptation, or the response to stimuli may have deleterious effects also called maladaptation.

I.1.1 Stress and Adaptation

Adaptation and stress are interrelated concepts: stress implies the existence of an environmental stimulus that produces a reaction in the organism that may or may not be favorable. Stress, however, is not the stimulus but the reaction or responses to environmental forces that deviates the organism from its original state (McElroy and Townsed, 1997). Thus, stress can be defined as the bio-behavioral response (s) to environmental conditions (Goodman et al., 1988; Little, 1995).

The environmental conditions that produce stress are called stress factors, stressors, or noxious stimuli. Thus, an environmental factor has the capacity to produce a tension in an organism that requires a response. The response can be adequate and restore the normal function of the organism; or, if it is not adequate, maladaptation occurs, resulting in precarious health (disease) and in some occasions with the death of the organism (Little, 1995).

The adaptation concept focuses in the positive consequences of stress, the survival and reproductive benefits that an adequate response to the stimuli can bring to an individual or population. Stress, however, can result in negative consequences and may weaken the individual, implying that the biological costs of the stress factors are the other side of adaptation (maladaptation).

The process of adaptation is multiple and how well adjusted a human is to its bio-cultural environment depends on a multitude of processes that are not necessarily different means to the same goal (Toulmin, 1983; Little, 1995). There are, throughout

life, different patterns of adaptation: 1) genetic (e.g. mutations), 2) developmental, 3) reversible, seasonal or flexible, 4) conscious or calculated, 5) homeostatic or autonomic, and 6) evolutive, selective or populational (Little, 1995). These mechanisms act together and can be seen in different aspects: 1) genetic success or Darwinian fitness, 2) functional morphogenesis, 3) physiological responses, 4) social flexibility, 5) cultural cohesion, 6) intellectual variability, and 7) technological refinement (Toulmin, 1983).

Consequently, the stress to which an individual is exposed is the result of different factors that include natural, cultural, social environment, and the individual's characteristics (phenotype). Accordingly, stress is the result of three factors: 1) environmental restrictions, 2) cultural systems, and 3) host resistance (Goodman et al., 1984).

Environmental restrictions include limited resources and stress factors that may or may not be filtered by the cultural system. Thus, the cultural system (e.g. taboos) can provide the means to buffer the environmental restrictions and therefore, to protect the individuals: health-enhancing behavior. The socio-cultural system, however, can also increase the existing stress factors through behavior and norms: health-lowering or ill-provoking behavior (Dunn and Janes, 1986). If the stress is not adequately buffered, its effects can only be counteracted through host resistance, which is biological (as opposed to cultural or social) and varies with age, sex, heredity, nutritional well-being and personal health (Huss-Ashmore et al., 1982; Goodman et al., 1984; 1988).

Among the individual responses there is a hierarchy: soft tissues are generally the more rapidly affected, whereas the osteological response comes later. Therefore, stress must be not only severe but also long lasting to produce a reaction in the bones (Huss-

Ashmore et al., 1982; Goodman et al., 1984; 1988). The responses at the skeletal level, however, are limited since osteoblasts and osteoclasts can deposit or remove bone respectively. Because osteological responses are limited, different pathogenic agents can produce the same bone response. In like manner, nutritional stress factors are even more difficult to diagnose. Although, some vitamin and mineral deficiencies produce specific skeletal lesions, studies in osteological collections have shown little evidence of them (Roberts and Manchester, 1995).

Several skeletal stress indicators have been used to evaluate the health of present and past populations. Some of them are the result of specific pathogens, but most of them are not. In order to reconstruct or understand the relationship between populations and their environments, however, we have to keep on asking if the indicators that we have being using to determine health status are actually the result of stressful conditions. We should also ask whether the information they provide is behavioral and not influenced or determined by other biological factors.

I.2. Intent and Relevance of the Study

In the present research, two non-specific skeletal indicators of stress are considered: Harris lines and enamel hypoplasia. These indicators show the occurrence of stress events during the growth and development of the individual. Analysis of these indicators, then, can shed light on our understanding of past and present populations' health. Interpretation of these indicators, however, can be problematic due to the absence of agreement about the method that should be used in their detection and analysis, making their interpretation

obscure (Byers, 1991 *contra* Allison et al., 1974; Ensor and Irish, 1995 *contra* Goodman and Rose, 1990).

Harris Lines and Enamel Hypoplasia, nevertheless, remain important in bioarchaeological, anthropological and clinical studies because unlike other stress markers, which give a static picture of the individual, mainly at the time of its death, they offer a retrospective record of infant or childhood stress events.

These indicators, therefore, highlight stress events that affected the individual during the early stages of its development. The incidence of these markers is an indication of health within a population, as they, apparently, respond to conditions of diminished health.

The intent of this study is to evaluate the possible existence of a correlation between these two indicators, and the alternative explanations for its presence/absence. A positive correlation between these two markers would show us that they respond to similar environmental conditions. A negative correlation might be the result of a differential response. An absence of correlation would shed light on the validity of these markers as stress indicators. As these markers are used to reconstruct past and present populations health status, their evaluation as stress markers can shed light in the validity of those studies.

In order to accomplish this, two prehistoric populations, Az-140 (Az: Azapa) and Az-71 (only the immature segment of the population), from northern Chile were selected, considering their good preservation, sample size and the large number of immature individuals with dental and skeletal elements preserved.

While this thesis will test for the coincidence of these stress markers, using two populations of the same area, future work could well include the examination of health among other populations of this area, or between populations of the same periods, that occupied other regions of the South Central Andes. Thus, the results of this study have possible wide implications for the field of Physical Anthropology and clinical studies.

I.3. Chapter Organization and Contents

This thesis is organized in six chapters. The second chapter, containing the literature review, considers the two skeletal markers under study. Bone composition, bone growth and tibial growth are explained. Harris lines are defined and their mechanism of formation, etiology and alternative methods of study are characterized. A discussion of dental development and a description of enamel deposition precede a definition of enamel hypoplasia. This section is followed by the description of enamel hypoplasia formation, its etiology, and the different methods used in its study. The chapter ends with a brief review of studies that have analyzed the correlation between these two stress indicators.

Chapter III refers to the purpose of the study, the problem, and the hypothesis under consideration. Chapter IV corresponds to materials and methods and includes a description of the area and the archaeological periods to which the skeletal sample utilized correspond. Chapter V exposes the results and is organized by indicator, Harris lines followed by enamel hypoplasia, and concludes with the statistical analysis of the correlation between these two skeletal markers. Chapter VI contains the conclusions and the implications of this study.

CHAPTER II

PURPOSE OF THE STUDY

II.1. Problem

Paleopathology focuses on the study of pathological conditions in past populations. This type of study can concentrate on one individual, with the intention of elucidating characteristics of its life, or on a population in order to identify the environmental conditions to which it was exposed and which it created. In order to do this, anthropologists must examine different pathological conditions. For this reconstruction of the environment to be reliable, though, the skeletal markers used in this endeavor must be valid and reliable. It is important then, to critically evaluate these skeletal markers and clarify, as much as possible, their etiology and relation to one another.

Two skeletal markers, enamel hypoplasia and Harris lines, are of special importance in paleopathological studies, particularly because they are some of the few that can inform us about the health status of the individuals during childhood development, even if they survived into adulthood. Although widely recognized as important, even for clinical modern studies, there is no agreement about the etiology of Harris lines. Thus, its interpretation as an stress indicator is questionable, and its utility in the reconstruction of past populations' health status remains debatable (Green et al., 1985; Baxter, 1986;

Magennis, 1990 *contra* Acheson, 1959; Garn et al., 1968; Park, 1964; Hummert and Van Gerven, 1985; Goodman, 1996).

The second marker, EH, also present some problems. Although EH etiology is well known, its interpretation as a stress marker is dubious because sensitivity to its formation is inter -and intra- tooth specific. Therefore it is questionable to what extent this differential sensitivity biases its appearance in the dental record, and, with it, our understanding of past populations' health status (Dean 1987; Goodman and Rose, 1990; Condon and Rose, 1992; Wright, 1997; Santos and Coimbra, 1999).

II. 1.1. Aim of the Study

The aim of this study is to tests the existence of a correlation between these two non-specific indicators of stress found in skeletal remains: Harris lines and enamel hypoplasia.

The correlation has to be determined at two levels:

Correlation at the individual level: Coincidence in the presence/absence of these indicators.

Correlation at time of occurrence: If the correlation is positive it would show that these two markers respond to the same conditions.

II.2. Hypothesis

In this study, a correlation between the two stress indicators is estimated by matching Harris lines with enamel. The presence/absence of a chronological correspondence, is also evaluated, according to the following hypothesis:

H0: There is no correlation.

H1: There is correlation (positive or negative) between the two indicators.

The presence of correlation may indicate that:

1) The etiology of the two indicators is similar;

2) They are equally sensitive to environmental conditions; and,

3) They are equally reliable as indicators of stress.

Whereas the absence of correlation may signify that both indicators have:

1) Different etiology;

2) Different sensitive to stressful events; and,

3) Different reliability as stress indicators.

Whereas the presence of a negative correlation may imply that:

When one developmental system is affected by environmental conditions, the other is protected.

Thus, the nature of the correlation between these two stress markers can elucidate their co-occurrence in terms of presence absence and also in chronological terms.

CHAPTER III

LITERATURE REVIEW: NON-SPECIFIC SKELETAL INDICATORS OF GROWTH DISRUPTION

Growth layers exist in the structural parts of many biological systems, and they usually reflect a rhythmic metabolism, which mirror the relationship between biological systems and the environment (Dean, 1987). Bone and teeth present these layers, however, a variety of stressors can permanently alter skeletal growth, teeth development, and they can also alter bone dimensions, as reflected by Harris lines and enamel hypoplasia.

The analyses of other skeletal lesions often show the relationship between health status and stress markers is complex, and these two indicators are no exception (Saunders and Hoppa, 1993). Harris lines and enamel hypoplasia, however, are extremely important because they may be indicative of childhood conditions (Buikstra and Cook, 1980; Corrucini et al., 1985; Hummert and Van Gerven, 1985; Saunders and Hoppa, 1993; Roberts and Manchester, 1995).

In order to understand what the presence of these indicators mean we have to analyze bone growth and enamel deposition and how these two growing processes can be altered by environmental conditions. It is also necessary to consider how the deviation form normal growth can result in the appearance of Harris lines and enamel hypoplasia.

III.1. Bone Composition, Bone Reaction and Tibial Growth

III.1.1. Bone Composition and Bone Reaction

Bone is a connective tissue composed by living cells imbedded in an extracellular matrix of collagenous fibrils made rigid by calcium salts. In fact, bone matrix is heavily calcified, which gives bone its strength. The embedded cells of the bones are known as osteocytes (Steinbock, 1976; Cormack, 2001).

Normal bone formation in the growing skeleton, a process known as ossification or osteogenesis, occurs through two different processes: endochondral and intramembranous. Endochondral ossification is the major process of bone growth until the fusion of the epiphyses occurs; it consists of the continuing replacement of the cartilage matrix at both epiphyseal plates resulting in the lengthening of the diaphysis (Steinbock, 1976; Cormack, 2001).

Long bones, then, continue to lengthen as a result of interstitial growth of their cartilaginous plates. In the growth of cartilaginous plates, the cartilage production is compensated by cartilage loss through calcification, visualization, and bony replacement on the diaphyseal side of the plate. By growing on one side and becoming replaced by bone on the other, the epiphyseal plates are progressively separated, lengthening the diaphysis. Cartilage replacement eventually supersedes cartilage production, so when bones are approaching full size their epiphyseal plates disappear (Cormack, 2001).

Bone deposition and growth, however, are not the only mechanisms required in the formation of properly proportionate bone; bone resorption is also necessary (Steinbock, 1976). Bone resorption is the dissolution of both the organic matrix and its mineral content. The osteoclasts appear to play the major role in this process. Osteoclasts secrete

acidic substances that dissolve the bone mineral and lysosomal enzymes that depolymerize the organic matrix. This extracellular digestion releases minute bone fragments, which are ingested by the osteoclasts and digested intracellulary (Steinbock, 1976).

Pathological conditions create an imbalance in the normal equilibrium of bone resorption and formation. Bone reacts then, to abnormal conditions by an increase or decrease in the normal processes of bone formation, bone resorption, or a combination of the two processes at different locations in the bone (Steinbock, 1976).

III. 1.2. Growth Plate Structure and Function

During skeletal growth, interposed between the epiphysis and metaphysis is the cartilage growth plate, which effects longitudinal bone growth (Hunzinker, 1988). The growth plate is composed of cartilage. Cartilage is a strong but slightly flexible semirigid supporting tissue that plays a key role in the development and growth of long bone. The growth plate function is to continue the cartilage growth, which in time is replaced by bone, providing the growth, at least in length, of the bone until the adult stature is obtained (Steinbock, 1976; Cormack, 2001).

The cartilage found in the growth plate corresponds to the hyaline type that contains cells called chondrocytes. The hyaline cartilage, in the growth plate, is enlarged through appositional growth, which involves the addition of new surface layers of matrix on top of the preexisting ones (Cormack, 2001).

Morphological examination of cells within the growth plate cartilage shows that the chondrocytes are arranged in axial columns separated from one another by longitudinal septa of cartilage matrix. Individual cells within columns are separated by horizontal

septa. The cells within a column represent both the histogenic (all originated from a single stem cell, and thus together comprise a clone) and functional units for longitudinal bone growth (Hunzinker, 1988).

The growth plate is a unipolar structure, meaning that it grows in one direction only. There are five zones in the growth plate: 1) reserve zone, 2) upper proliferative zone, 3) lower proliferative zone, 4) upper hyperthropic zone, and 5) lower hyperthropic zone. The reserve or germinative zone contains cells that accumulate nutrients and may serve as the stem cells for the proliferative zone. This layer is adjacent to the epiphyses, and the stem cells located in this layer present a low rate of division compared to the daughter cells that develop into proliferative chondrocytes. After having gone through a finite number of mitoses, these cells lose their potential to divide and their genetic program is abruptly switched to begin a massive hypertrophy (Acheson, 1959; Buckwalter et al., 1985; Hunzinker, 1988).

In the proliferative zone chondrocytes divide rapidly and synthesize extracellular matrix increasing the volume of the growth-plate cartilage and producing longitudinal growth. In the hypertrophic-zone the cells may participate in matrix-synthesis and help to prepare the matrix for mineralization (Buckwalter et al., 1985). Once the hypertrophied chondrocytes have disintegrated, capillaries and osteoblasts invade them, the latter laying down osteoids around the calcified remnants of the matrix (Magennis, 1990).

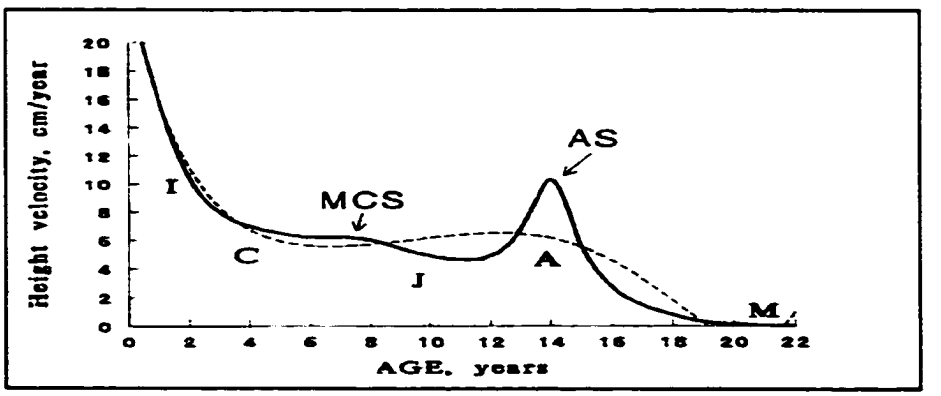
III.1.2. Tibial Growth

The human pattern of growth from birth to maturity is qualitatively and quantitatively different from the pattern observed in other mammals and even other primates. Most non-primate mammals grow in size and develop towards sexual maturity along a continuous

path from birth to adulthood, with no biological or mathematically discernible alterations, in growth trajectory. In humans, however, the pattern of growth is complex and it presents characteristics that differentiate it from the pattern observed in other animals. These differences include: 1) a delayed sexual development; and 2) a neurological development that is, about 90% before sexual maturity is achieved (Bogin, 1999).

These differences can be expressed in terms of the type and number of mathematical functions that are needed to describe human growth. The distance and velocity curves for most mammalian species can be estimated by a single function; even in monkeys and apes, with the addition of the juvenile stage, this requires no more than two formulas. In opposition, the insertion of mid-childhood and adolescent spurt into human ontogeny (see Figure 1), results in the need of at least three mathematical functions to describe the shape of the velocity curve.

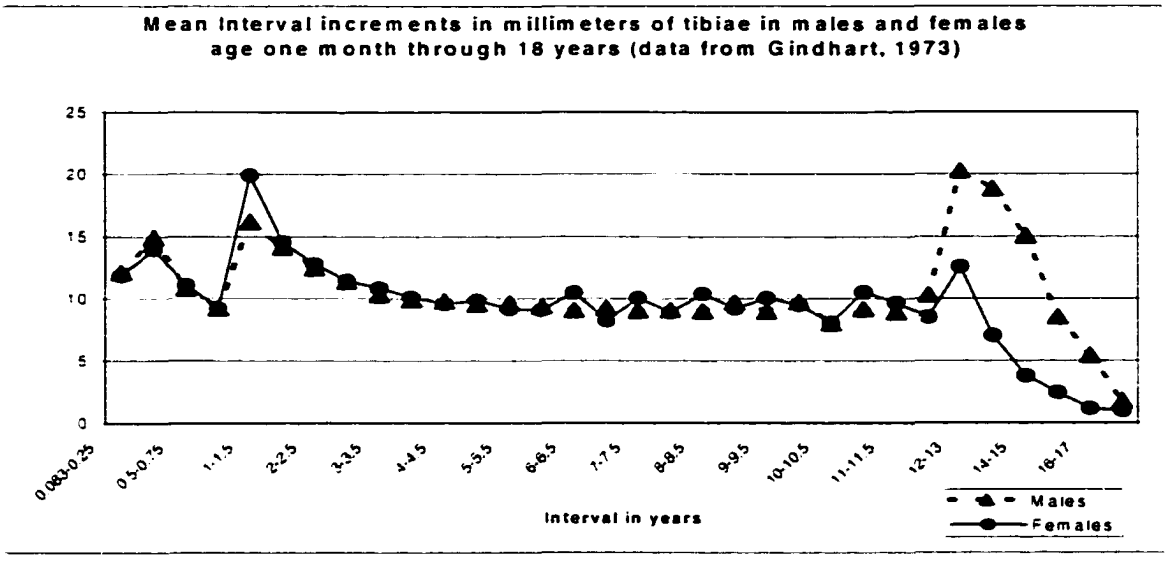
Figure 1



Using radiographs of the left limb that corresponded to a hundred individuals Gindhart (1973) determined that After Bogin, 1999:170 ollows the general pattern of

growth described in figure 1. The pattern of growth observed in the tibia, then, follows the one that characterizes the different stages of growth in humans (see graph 1 and figure 2).

Graph 1

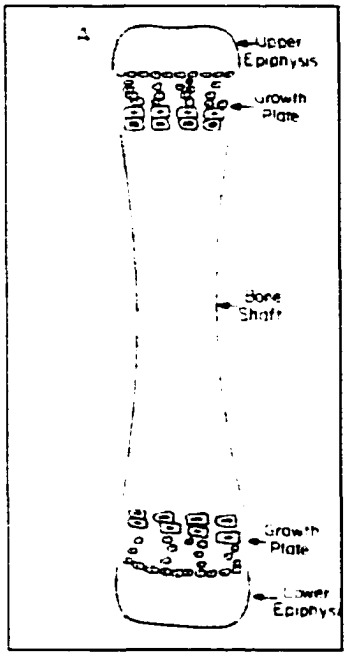


In the tibia, as in the rest of the body (see graph 1), annual rates of growth increment are high during the first year of life, after which they tend to decrease in such a way that in the interval from 6 to 9 years of age they are relatively stable. After the age of 9 years, the rates of growth in individual children become more rapid, usually reaching a maximum between 10 and 12 years of age in girls, and between 12 and 14 years of age in boys. In the following 4 years, the rates decreased rapidly until growth ceases altogether (Anderson et al., 1963).

The period from birth until about three years of age is the most sensitive to stunting. Growth during the first two years of life is very rapid with the highest growth velocities

immediately after birth until the year. Thus, the first few years of life represent a period when adverse factors can have a significant and lasting effect on growth (Saunders and Hoppa, 1993).

Figure 2



After Bogin, 1999: 94.

Fifty seven percent of the tibial growth occurs in the proximal growth plate. This information comes from Anderson et al. (1963): a study that considered 206 individuals, in whom the tibia was measured from roentgenograms. In these children, sharply delineated lines of temporarily arrested growth were seen at the end of the diaphyses. These lines persisted on consecutive roentgenograms for three years or more between 10 and 15 years of age. The growth measured from such lines indicated that, on average,

57% of the total tibial growth arose at the proximal metaphysis during this age interval (Anderson et al., 1963).

Consequently, analyses of Harris lines need to consider the percentage that each growth plate contributes, to the length of the tibia or any other long bone under study.

III.2. Harris Lines

Because bone grows and calcifies in an appositional manner, it can record, within its structure, in the form of Harris lines, the effects of disturbances in the body metabolism (Massler et al., 1941).

III.2.1. Definition

Harris lines (HL) are a non-specific condition in terms of etiology. They are identified as dense, radio-opaque transverse lines, perpendicular to the main axis of bone growth that can be seen in radiographs or using cross section samples. The lines are essentially trabecularly oriented at a right angle in relation to the diaphysis (Garn et al., 1968; Buikstra and Cook, 1980; Martin et al., 1985; Roberts and Manchester, 1995; Goodman, 1996).

Structurally, transverse lines are strata of denser and thicker bony trabeculae within the medullar cavity; these lines are radiographically visible because of the increased mineralization that results from the irregular mineral deposition in the trabeculae. Studies have shown that the mineralization value for these lines is 5-15% above normal bone. Therefore, HL involve a quantitative alteration of bone structure and do not constitute solid disc of totally different bone (Elliot, 1927; Garn et al., 1968; Buikstra and Cook,

1980; Hummert and Van Gerven, 1985; Martin et al., 1985; Roberts and Manchester, 1995; Goodman, 1996).

Lines of increased density may, on occasion, be seen on any of the tubular bones. Tibia, femur and radius, however, tend to be the most affected ones. The distal tibia shows lines more frequently than any other bone and, therefore, is the most commonly used one in this type of study. Although the lines have been more commonly studied in long bones, they have also been detected in the pelvis, the bodies of the vertebrae, the ribs, and the nuclei of the epiphyses (Elliot et al., 1927).

III.2.2. Mechanism of Formation

A transverse line results from the uncoupling of osteoblastic and chondroblastic activity where the former continues and the latter (cartilage growth) slows or stops (Magennis, 1990). As chondroblasts and osteoblasts are different cells that work under different regulatory controls, different hormones affect them (Green et al., 1985). Growth hormone (GH) and one type of somatomedin, specifically insulin like growth factor I (IGF-I), are directly involved in the growth of the cartilage plate (Baxter, 1986). Growth hormone interacts with the prechondrocytes promoting their conversion into chondrocytes, while IGF-I stimulates the mitotic division of the chondrocytes in the proliferative zone (Green et al., 1985).

An overproduction of GH, circulating levels of which tend to fluctuate, must be countered by a diminished production of IGF-1 in order to avoid chondrocyte mitoses running out of control. In that case, and because these mechanisms do not necessarily act simultaneously on the osteoblasts, HL may form because the osteoclasts continue to deposit bone (Magennis, 1990).

The mechanism then, would operate as follows: if cartilage growth slows or stops, the growth plate becomes thin and compressed, as the chondrocytes (due to a diminution in IGF-I) fail to reproduce and mature. The osteoblasts, then, are no longer capable of invading the cartilage and are displaced horizontally under the cartilage plate, resulting in a transverse stratum of bone (Magennis, 1990).

Line formation, then, can only occur while the bones are growing; as the length of the bone increases they later appear on the diaphyses (Buikstra and Cook, 1980; Hummert and Van Gerven, 1985; Roberts and Manchester, 1995). After its formation, however, a line can disappear. Indeed, lines and bands come and go so dramatically that a broad band is often reduced to a line or even vanishes completely over a period of years or, in some instances, months, although some lines do persist from childhood to adulthood. Moreover, an initially broad band may, in the course of growth, be reduced to a line of less than a millimeter in width. Most commonly, lines and bands appear in early childhood and then disappear in the course of bone remodeling as a result of subperiosteal apposition and endosteal surface resorption (Harris, 1931; Garn et al., 1968).

III.2.3. Etiology

Line formation appears to be related with an increased secretion of pituitary growth hormone and a decreased level of IGF-1 (Dreizen et al., 1964; Baxter, 1986). Growth hormone levels are very high in children suffering from kwashiorkor and other forms of malnutrition while circulating IGF-I levels are diminished by low nutrient intake. As a result of this imbalance the bone ceases to grow and a transverse line might be formed (Baxter, 1986).

"When nutritional disturbances in the young animal become so severe that nutrient material is inadequate to go around, in the economy of nature the bones are sacrificed." (Park, 1964).

Thus, under stressful conditions, then, the organs on which continued existence depends are given preferential treatment, and as a result the bones simply cease to grow. When the crisis is over the bones proceed to grow again, however, the bone arrest leaves marks on the bones identified as Harris lines. Growth arrest needs to be complete, or nearly complete, for line formation; merely slowing of growth is not sufficient (Park, 1964; Mays, 1995).

Growth retardation is widely recognized as a response to a limited nutrient supply at a cellular level (Acheson, 1959). Malnutrition, however, is not the only possible cause: infectious conditions, synergistically related to malnutrition, are another etiological factor. It is difficult to separate the impact of these two conditions on growth, as malnourished children are more sensitive to infections, due a weakened health state and reduced host resistance, and infections, in time can result in malnutrition by increasing the nutrient demands in the body (Saunders and Hoppa, 1993).

The precise etiology of these lines is varied and controversial, but their formation has often been associated with episodes of stress like nutritional deficiencies or childhood diseases. Based on experiments on animals, and observations in humans, it has been showed that HL can result from nutritional stressors, like anemia, rickets, and deficiencies in vitamin A, C and D, protein malnutrition, kwashiorkor or general defective nourishment. Harris lines have also been associated with infections such as: measles, scarlet fever, infantile paralysis, pneumonia, and immunization procedures

(Elliot, et al., 1927; Harris, 1931; Buikstra and Cook, 1980; Lobdell, 1984; Hummert and Van Gerven, 1985; Martin et al., 1985; Roberts and Manchester, 1995; Lewis, 2000). Other causes, like ingestion of heavy metals (lead, bismuth and phosphorus) and massive doses of vitamin D have also been identified (Park, 1964; Garn et al., 1968).

In addition, ethanol consumption during the growth period has being recognized as one of the triggers of HL (Gonzalez-Reimers et al., 1993). Gonzalez-Reimers et al. (1993) found that patients who confessed having consumed alcohol during growth period showed statistically significant more HL than non-consumers. Ethanol intake may cause HL by leading to an imbalance between energy intake and energy expenditure, due to, at least in part, the energy-wasteful metabolism of the microsomal-ethanol oxidizing system (MEO), linked to chronic ethanol consumption.

Harris lines, then, represent periods of stress that disrupted the normal metabolism while the bones were growing in length. In order for a stress event to cause HL it needs to arrest the growth process of the bone; HL are, then, interpreted as a growth arrest indicator (Garn et al., 1968; Hummert and Van Gerven, 1985; Goodman, 1996).

It is necessary to consider, though, that in many diseases, as in many states of malnutrition, bone growth continues even at the expense of preformed bone. Therefore, radio-opaque lines are not the invariable consequence of every impairment of health occurred during the growing period (Garn et al., 1968), and the association of HL with illness, although apparently strong, is far from being a one-to-one correspondence (Hewitt et al., 1955).

In fact, some studies have shown that a new line may appear even though no disease was reported in the previous six months. Longitudinal studies have shown that there is a

low-order association between illness or trauma and the appearance of a new line (Park, 1964; Garn et al., 1968).

A different interpretation of the etiology of HL is offered by Magennis (1990). According to this author it is true that growth is linked to the formation of transverse lines; in fact, regression analysis shows that there is a statistically significant relationship between growth increment and the occurrence of a transverse line. But, if we consider that nutritional and disease insults are commonly associated with slowed growth, then an association between slowed growth and HL should be expected. However, this association does not occur; on the contrary HL are usually formed during periods of rapid growth (Magennis, 1990). Growth alone, then, is significantly related to the formation of a HL and the results support the notion that greater increment of growth precedes line formation (Magennis, 1990). Accordingly, Magennis (1990) sustains that the generally held idea that physiological insults, including diseases and nutritional insults, are the primary underlying cause of Harris lines, is questionable.

Indeed, some studies have shown that irrespective of the presence or absence of nutritive failure, the incidence of HL decreases as the children approach adolescence, and then, nutritional status per-se is not the determining factor in susceptibility for HL, in growing children (Dreizen et al., 1956; 1964). Accordingly Magennis (1990) estimated that transverse lines reflect tissue level responses to regulation of growth rate at the epiphyseal cartilage plate and not necessarily stressful conditions.

III.2.4. Alternative Methods of Analysis

There are two basic types of Harris lines studies. In the first type of study, a total count of HL is made. The number of lines per skeleton indicates the health status of the

individual or the population, during childhood. A second type of study is characterized by the attempt made to estimate the age of the individual at the time of HL formation (Maat, 1984). As the position of the line reflects the size of the shaft at the time of the line formation, and because long bone growth-rate is known, calculation of the age of formation should be possible.

Age determination of the occurrence of these stress episodes may provide:

1) Population frequencies and distribution of lines by specific ages (that can elucidate some cultural behaviors, e.g., weaning); and,

2) Evaluation of the patterns of lines found within individuals. Indeed, within individuals, the temporal pattern may be use to analyze the existence of regular cycles of stress, that may be associated with seasonal food deprivation (Buikstra and Cook, 1980; Martin et al., 1985; Goodman, 1996; Lewis, 2000).

Different methods for the study of Harris lines have been developed. Allison et al. proposed one of the first methods in 1974. This method assumes that at birth the length of the tibia is of 90 mm. Therefore, the first step is to measure the tibia in mm, and then subtract the 90 mm. The remainder, or growth area, is divided into fifths. Three fifths are assumed to be located in the proximal end of the tibia and two fifth in the distal shaft. Then the distal and the proximal growth area are divided, respectively into 16 equal parts, equivalent to the 16 years of tibial growth. The lines located in the prenatal area are not counted because their interpretation is considered dubious (Allison et al., 1974). Once the distance between the line and the proximal or distal end of the bone has been taken, the age of its formation can be calculated. Although this method present the advantage of being simple, it has two major problems:

1) It assumes a constant rate of growth of the tibia; and,

2) It assumes an average of 90 mm for the length of the tibia at birth.

Therefore, this method does not seem the most adequate, or reliable, for the study of Harris lines.

Hunt and Hatch (1981) proposed a different method, which is based in two assumptions:

1) The relative spacing of proximal and distal transverse lines in older children and adolescents yields relative growth rates at the proximal and distal cartilages that apply to all diagnosticable younger ages; and,

2) Variations between individuals and populations in these relative rates can be neglected.

In order to apply this method it is necessary, in the first place, to determine the primary center of ossification. This center is the site where the first diaphyseal bone is formed in the embryo. Consequently, once the tibia has been measured in the radiograph k/2, or the correction factor of combined epiphyseal sizes, is subtracted from the maximum length of a long bone in order to get diaphyseal length. Then, considering that the contribution of the proximal growth plate to the total bone length in the tibia is 57%, and the distal cartilage is 43%, the researcher should be able to locate the ossification center. Then the following formulas have to be applied (see table 1):

Table 1 Formula for the Estimation of Age at the time of Formation of Transverse lines

Bone	Line Location	Formulae
Tibia	Proximal to its origin	$z(p,t) = 0.57z(t)$
Tibia	Distal to its origin	$z(d,t) = 0.43z(t)$

(After Hunt and Hatch, 1981).

Where:

z = predicted diaphyseal length of a long bone, derived from an appropriate growth equation, which is estimated through:

$$z(t)= \frac{y}{f}\ g(t)$$

$y = m-k$

m= mean maximal length.

$f= (a_1+a_2)$

a_1= upper limit of the pre-pubertal term.

a_2= upper limit of the adolescent term.

g(t)= predicted mean measurement at age t.

Once this equation has been developed for each bone in the population under study, the diaphyseal length of the immature long bones can be matched to the nearest age for the specific value of z(t). In order to make these calculations (average length for each age) a longitudinal study is necessary.

Considering that no longitudinal studies are possible in human osteological collections, it is necessary to use a growth equation already established, hopefully, for local populations, when available. The applicability of this method in osteological remains is, therefore, restricted, to say the least.

Maat (1984) established a third method. In his study only the lines that extended 50% or more across the width of the tibia were counted. Considering that 43% of the total tibial growth occurred at the distal methaphysis, it is possible to reconstruct the original

location of the primary ossification center in the tibia's shaft on the radiograph. In fact, measured from the inferior articulation, the original location can be calculated as:

$$\frac{43}{50} * \text{Tibial Length}$$

In this particular study, the curve of growth was constructed utilizing the measurements of 18 boys and 16 girls, obtained through a longitudinal study that registered their measurements between the ages of 6 month and 12 years. Using the formula expressed above, a proportional estimation of the diaphyseal length of the tibia was calculated for every age. The lines observed were divided in three types:

1) Type I: Lines that are detectable only by a careful inspection;

2) Type II: Moderate lines located in the epiphyses; and,

3) Type III: Moderate lines located in the diaphysis, plus well-marked (strong) lines located in the metaphyses.

The assumption that underlies this division is that serious incidents will generate lines of considerable density that will remain detectable in spite of subsequent bone modification. Nevertheless, Maat (1984) recognizes that this assumption needs to be tested and that only a longitudinal study can elucidate this problem.

After this, the steps demanded by this method are:

1) Determination of the location of the primary ossification center; this can be done by locating the point at 43% of the tibial length;

2) Measurement of the adult distal diaphyseal length for the primary ossification center to the level of epiphyseal fusion;

3) Measurement of the distance between the primary ossification center and a transverse line; and,

4) Using the curve of relative growth as reference, the time of causal insult, expressed in biological age, has to be determined.

This method, like one proposed by Hunt and Hatch (1981), is not only complex, but requires a control group for the growth curve. Therefore, its application in osteological samples is limited.

Byers (1991) proposed another method. According to this author the calculation of the length of the bone at the time of line formation is based in three aspects of growth and anatomy:

1) Given the fact that the lines are formed when normal growth is disrupted, transverse lines mark the position of the epiphyseal plates at the time of deposition. Therefore, when the length of the bone at the time of formation is estimated, the thickness of the epiphyseal areas beyond the lines must be taken into account;

2) Bone growth is well documented in living populations and can be used to calculate tables of growth percentage per year; and,

3) Growth at either end of a long bone is not equal. This must be considered when a line is seen at only one end.

Byers (1991) offered a table of percentage of growth per year discriminating males and females (see table 2). In fact, Byers' (1991) method for the study of Harris lines is based in the information provided by Gindart' (1973), and therefore, the advantage of this method is that it does not assume equal yearly increments of growth (*contra* Allison et al., 1974). Although the data used to reconstruct the growth pattern comes from studies of

modern populations, and the method is prone to some error owing to genetic and environmental factors that may influence the growth rate of one or all bones (Maresh, 1955), it does represent a real growth pattern.

Byers' method (1991), determines the age at the time of line formation as follow:

1) Calculating the length of the diaphysis at the time the line was deposited;

2) Correcting the epiphyseal thickness; this is subtracting the epyphyseal length from the total length of the tibia;

3) Calculating the percentage of mature bone length;

4) Comparing the percentage with the chronology of bone development; and,

5) The method requires a series of calculations that the author summarized as follow (see table 3):

Table 2 Chronology of Tibial Growth

Males		Females	
Age	Percentage	Age	Percentage
1	28.8	1	31.5
2	36.5	2	40.1
3	42.4	3	46.6
4	47.6	4	52.4
5	53.5	5	58.6
6	57.9	6	63.9
7	62.3	7	69.0
8	67.5	8	74.3
9	71.6	9	79.4
10	75.7	10	83.9
11	79.6	11	88.8
12	83.7	12	93.0
13	88.5	13	96.5
14	93.0	14	98.3
15	96.7	15	99.1
16	99.0	16	100.0
17	100.0	17	—
18	100.0	18	—

(After Byers, 1991)

Table 3 Formula for calculating percentage of mature bone Length (Pct) at the time of radiopaque line formation

Bone	End line Closest to	Formulae
Tibia	Proximal	Pct =1.15 (T-1.75P) x 100/T
Tibia	Distal	Pct= 1.15 (T-2.33D) x 100/T

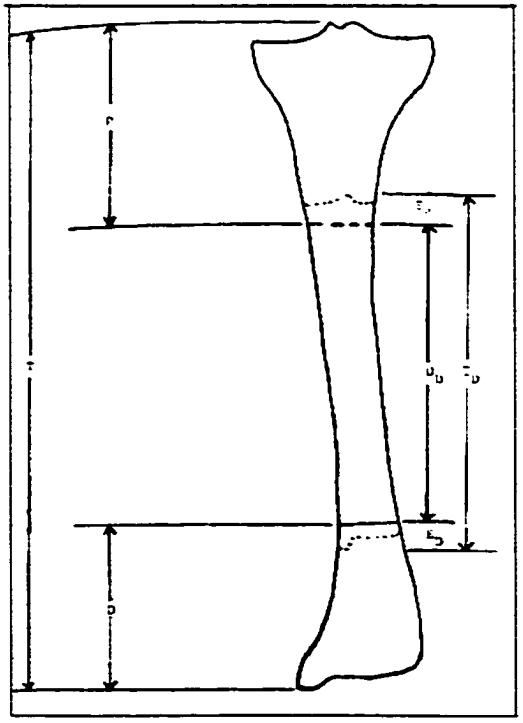
(After Byers, 1991)

Where:

T = total length of the tibia, measured in the radiograph, from the proximal to the distal end.

D, P = the distance from the transverse line to the closest end (D: Distal, P: Proximal, see figure 1).

Figure 3



After Byers, 1991; 341.

This technique has the advantage that it is simple to execute, and requires only two bone measurements. In addition, it also considers the differences between male and female, and it adapts to the variable length of bone in different populations.

Byers' (1991) method is limited in that it cannot be applied to juveniles, because their epiphyses are not yet fused to the diaphysis. Hummert and Van Gerven (1985) offered a method than can be applied to adults and juveniles. In this specific method only lines located at the distal end of the diaphysis are considered. This method then can be applied to subadult bones without fused epiphyses, but with known developmental ages. In order to determine the developmental age of an individual dental maturity must be assessed. The method can also be used in adult bones with some modifications.

In order to determine the age of the individual at the time of line formation the researcher must determine the distal tibial growth pattern for the population and then estimate the age of the individual at the time of the line formation:

In order to establish the distal tibia's growth pattern of the population the researcher must:

1) Determine the primary center of ossification, considering that 43% of the tibia's growth is distal;

2) Assume that the average length per age category is representative of the growth achieved by all children in the population at that age; and,

3) Calculate, based on the growth data, 43% of the incremental increase in millimeters for each group and these absolute values have to be converted into percentages of distal tibial growth achieved annually by each specific age group.

To estimate the age of the individual at the time of line formation the steps are:

1) For each tibia, 43% of its diaphysis length must be calculated, to determine the primary center of ossification;

2) Then the distance between the line and the ossification center must be measured. The percentage of distal growth completed when the transverse line was formed has to be calculated by dividing the distance between the line and the ossification center by the distal length of the tibia; and,

3) This value has to be compared with the percentages of distal tibial growth estimated for each specific age group of the population under study.

In order to apply this method to the adult segment of the population, the next steps have to be followed:

1) Determine epiphyseal contribution by measuring the epiphyses. This amount has to be subtracted from the total bone length to delimit the diaphyseal component of each tibia; and,

2) After that, 43% of the diaphyseal length must be calculated and then the primary ossification center has to be located. As with subadults all measurements of lines are made from this point.

This method has a series of advantages:

1) It considers the differential growth at both ends of the tibia;

2) It is population specific; and,

3) It can be applied to adults and subadults.

It is highly advantageous that this method can be applied to juveniles considering that HL are subject of resorption; therefore, if only the adult segment is analyzed in a study

the frequencies of lines formed during the early years will be misleadingly low. Consequently, stress for the early ages, based on transverse lines can only be assessed in children.

III.2.5. Scoring Harris Lines

Harris lines can be scored using sectional or radiographic records. However, Macchiarelli et al. (1994) found severe inconsistencies in both sectional and radiographic records and the morbidity index values (total number of lines/total number of tibiae). According to Macchiarelli et al. (1994), these differences are the result of the natural resorption of the lines. In fact, resorption begins shortly after the layer formation, when the vessels of the osteoblasts progressively penetrate its central portion. As a result, there will be some differences between the radiographic and sectional expression of HL. The progressive obliteration of the stratum by bone remodeling determines "migration" of the HL, and at this level, it is the radiographic record what guarantees the recognition of a higher number of residual Harris lines (Macchiarelli et al., 1994).

The score of Harris lines is difficult, as the study of Groulleaux-Raoux et al. (1997) have shown. In their study the results show that Harris lines in subjects of medium height, that is older children and adolescents, are the hardest to count.

Although counting HL may be problematic, it is not impossible and it seems reasonable to follow Clark and Mack's (1988) advice and count the lines that extend across at least 30% of the shaft, and are more than 45° and less than 135° perpendicular to the shaft.

III.2.6. Problems in the Interpretation

Harris lines are commonly used to estimate the frequency of stress episodes. This, however, is problematic because some studies have shown that the number of lines do not correlate with the number of insults the individual had suffered in the past (Marshall, 1968; Buikstra and Cook, 1980). Moreover, studies have showed that a line follows a disease in only 25% of the cases, whereas 10% of the lines occurred when no stressful episode was documented; thus, the presence of transverse lines in an adult population does not conclusively point to a rugged childhood (Garn et al., 1968; Gindhart, 1969; Lewis, 2000).

Moreover, considering that transverse lines are the subject of resorption at all ages, caution should be exercised in their interpretation; the absence of lines may either reflect that no lines had ever formed, or that previously formed lines have been resorbed (Harris, 1931; Garn and Schawger, 1967; Buikstra and Cook, 1980; Hummert and Van Gerven, 1985).

Groulleau-Raoux et al. (1997) found that there is a progressive decrease in the number of Harris lines with increasing age; the problem is that resorption is increased in sick individuals, and therefore the number of lines does not necessarily reflects the health status of the individuals under study (Hatch et al., 1983).

It is a matter of controversy whether the thickness of the line is a measurement of the severity or duration of the stress episode, or whether it relates to individual variability in the thickness of the cartilage plate and the speed of chondroblast recovery. A tentative hypothesis is that thickness may be a function of growth velocity and the length of growth disruption, while density may depend on the severity and completeness of the

disruption (Martin et al., 1985; Lewis, 2000). Thickness and degree of radiopacity, however, are apparently dependent on the quality of the radiograph and the time and rate of resorption to which they had been exposed.

Age and sex affect not only line formation but also the remodeling rates. This makes the interpretation of Harris lines even more complex because this means that, between sexes and age groups, there is a differential propensity to form lines and also a differential remodeling rate, which implies that certain age-sex groups would:

1) Form fewer lines even if they are exposed to the same number and intensity of stress events; and,

2) Remodel a greater number of lines that would be erroneously interpreted as the result of a better health and/or comparatively minor exposure to stress events.

If HL are associated with a diminished growth rate, we should expect a correlation between HL and stunting or lower height. Gindhart's study (1969), nevertheless, did not identify any stature difference between groups with different degrees of transverse line presence, and this was confirmed by the results obtained by Mays (1995). A possible explanation is that in young animals, growth has a tendency to return to its original trajectory if circumstances have conspired to push it off-course, a phenomenon called canalization. Following a period of arrest, growth will, if sufficient resources are available, restart at an accelerated rate, returning the individual to its original growth curve. Once there, growth slows back to the old trajectory once more, a phenomenon known as catch-up growth (Mays, 1995). Harris lines, moreover, are not associated with reduced tissue quality, as cortical thickness shows no correlation with HL (Van Gerven et al., 1985).

Thus, the interpretation of Harris lines is complicated not only because it has been proved that there is little association between them and childhood diseases, but also because they are more frequent during periods of rapid growth in length, and not during periods of diminished growth (Maggenis, 1990; Goodman, 1996).

III.3. Dental Development and Mineralization

III.3.1. Dental Development

There are, mainly, 3 phases in the development of a tooth, the pre-eruptive, eruptive, and intra-oral phase (Berkovitz and Moxham, 1989). The pre-eruptive phase covers the period form the initial appearance of the tooth germ to the beginning of root formation. During this phase the tooth remains in its developmental intraosseous location and grows concentrically within its follicle, and there is little active bodily movement of the tooth axially (Berkovitz and Moxham, 1989). In humans, at 6.5 weeks after conception central and lateral incisors are already developing in the embryo, and at 8 weeks all the deciduous teeth are developing. The permanent incisors, canine and premolars, repeating the developmental sequence of their deciduous predecessors have formed tooth germs by 30 weeks, but dentinogenesis and amelogenesis do not start before birth in the permanent teeth. Initially, permanent teeth occupy the same alveolus as the deciduous ones, acquiring an independent alveolus and dental follicle when the deciduous teeth erupt postnatally (Tongue, 1989).

Tooth eruption is the process whereby a tooth moves axially from its developmental position within the jaw to emerge into the oral cavity. Eruption is part of a complex system, which involves movements in other planes and which continues all through the

intraoral stage, in order to maintain the tooth in its functional position (Berkovitz and Moxham, 1989).

The eruptive phase begins with root formation and terminates when the crown first emerges through the oral mucosa. It is characterized by axial migration of the tooth, which involves its active bodily movement. If the erupting tooth has a deciduous predecessor, resorption and shedding of the deciduous tooth must take place to provide an eruptive pathway. Resorption of deciduous teeth results from the activity of osteoclast-like cells, termed odontoclasts. The origin of odontoclasts is unknown, but it is possible that like osteoclasts they arise from the fusion of precursor cells of the monocyte/macrophage type. The factors that initiate, and subsequently influence root resorption are not fully understood, but pressure from the erupting permanent tooth is generally thought to be important (Berkovitz and Moxham, 1989).

Another feature associated with the eruption of permanent teeth is the gubernaculum. Initially, each deciduous tooth and its developing permanent successor share a common alveolar crypt, the permanent tooth being situated lingually. With continued growth and eruption of the deciduous tooth, the permanent tooth comes to lie within its own crypt near to the root apex of the deciduous tooth. However, the crypt of the permanent tooth is not complete, there being a canal (the gubernacular canal) in its roof through which the dental follicle is attached to the overlying oral mucosa.

It has been suggested that the gubernacular cord plays an active role in the movements of the erupting permanent teeth through the jaws. During eruption, the gubernacular cord decreases in length but increases in thickness. Whether the gubernaculum provides an eruptive pathway or is actively engaged in pulling the tooth

out has not been established, although it cannot be implicated in the process of eruption once the tooth has reached the mucosa. Surgical removal of the cord, however, does not prevent eruption of the permanent tooth (Berkovitz and Moxham, 1989).

The third phase, the intra-oral one, is characterized by the eruption of the tooth through the oral mucosa into the oral cavity, where it continues to move axially to attain, and latter on to keep, its functional position. The maximum eruption rate is 1-2mm per month (Berkovitz and Moxham, 1989).

Tooth emergence may be facilitated by recession of the mucosa around the erupting tooth. This is termed passive eruption to distinguish it from active eruption where crown exposure is due to bodily movement of the tooth (Berkovitz and Moxham, 1989).

There is considerable controversy concerning the mechanism by which the force (s) responsible for tooth eruption is (are) generated. There are mainly 3 theories: a) Contraction of collagen in the periodontal ligament: proposes that contractional forces are set up within the oblique fiber system of the periodontal ligament during collagen maturation. However, the various explanations offered for the underlying causes of the contraction are considered biochemically untenable. Moreover, there is also evidence that some teeth can erupt in the absence of a well-organized fiber system; b) Contraction/Migration of fibroblasts in the periodontal ligaments: Evidence from tissue culture suggests that fibroblasts' traction may be important in connective tissue morphogenesis. Two cellular activities have been implicated – fibroblast contraction and fibroblast motility. It has been proposed, however, that fibroblast migration rather than contraction is responsible for tooth eruption, mainly because teeth showing differences in their eruptive behavior show no difference in their intracellular organelles. The fibroblast

migration theory claims that periodontal fibroblast ligaments near the tooth are polarized (elongated into the direction of eruption) and that eruptive force is somehow produced as a result of unidirectional cell migration in an occlusal direction. A number of authors have shown that periodontal fibroblast ligaments migrate occlusally. However, not all the cells migrate, and it is possible that cell migration is an effect of eruption and not its cause. Consequently the discussion focuses on whether or not periodontal ligament fibroblasts migrate actively; c) Vascular/Fluid pressures in the tissues around or beneath the tooth: The eruptive force is generated by blood pressure. The idea is that the eruptive force derives from the hydrostatic pressures around or beneath the tooth. Hydrostatic pressure may be exerted either by fluid, which is free in the tissue, and/or by fluid, which is bound to fibers or ground substance. Although it is known that nervous vasomotor activity or vasoactive drugs can influence the behavior of an eruptive tooth, histological studies have provided little information about the role of the vasculature during tooth eruption. Some studies have shown, however, that an increase in eruption appears to be associated with an increased vascularity (Berkovitz and Moxham, 1989). Thus, we can conclude that there is no agreement concerning the forces involved in tooth eruption.

II.3.2. Enamel Deposition and Mineralization

Dental enamel's function is to provide a hard, wear-resistant outer coating to the surface of the teeth, with which masticate food (Boyde, 1989). In human teeth the portion covered with enamel is known as the anatomical crown, which is clinically that portion of the tooth, which has erupted and is visible in the oral cavity. Enamel is thickest over biting edges and biting surface, and thinnest at the neck or cervix, of the tooth; it may be

up to 2.3mm thick over the tips of the cusps, and it is usually 1-1.3mm thick over the lateral surfaces (Boyde, 1989).

Enamel, as well as dentine, the other hard tissues of the teeth, grows by the formation of a layer of new substance on top of the old one. This is known as appositional growth and is different form proliferative or multiplicative growth. The former is the result of cellular secretion, while the latter is the result of cellular division. The appositional type of growth results in the formation of layers or rings. The formation of these layers is characterized by the regular and rhythmic manner in which it proceeds. Since the deposition of the layers is regular, their chronological sequence can be determined (Schour and Massler, 1940b; Massler et al., 1941). These incremental lines or rings are known as perykimata (Hillson and Bond, 1987; Boyde, 1989). Perykimata are overlapping layer lines. These features become increasingly common in progressing from the crown towards the cervix of the tooth (Boyde, 1989).

Formation or deposition of the enamel is different from its subsequent calcification or mineralization. The matrix is produced as an extracellular product, and calcification occurs by the precipitation of inorganic calcium salts within the deposited matrix (Massler et al., 1941). In fact, enamel is hard because of its very high proportional content of a mineral that bears a strong resemblance to hydroxiapatite, a calcium phosphate. In the mature enamel the mineral component may reach 89-91% by volume (96-98% by weight), the remaining proportion of the tissue being occupied by organic matrix and water. The proportion of mineral in the tissue increases steadily form the moment of inception of mineralization, which closely follows the initial secretion (Boyde, 1989).

Dental formation includes: 1) formation of crown and root (s); and, 2) tooth eruption. Crown formation includes the apposition of an organic matrix and its subsequent mineralization:

1) Matrix secretion: During this phase the characteristic shape of the crown is determined by the incremental apposition of enamel matrix layer upon layer, from the dentine-enamel junction to the surface. The enamel matrix consists of 65% organic material and water and about 35% of mineral salts. The ameloblasts begin to secrete the organic matrix in a thin layer close to the dentin, shortly after dentine deposition has started (Giro, 1947; Alvarez and Navia, 1989; Kelley and Larsen, 1991; Hillson, 1996).

Two principal protein groups are found within the newly secreted enamel matrix. These are known as amelogenins (the proteins found during the formative stages of enamel) and enamelins (those proteins which are retained in the mature tissue). Amelogenins are inherently unstable proteins while enamelins, on the contrary, are stable proteins. Amelogenins are the majority in young enamel and distinctly the minority in mature enamel. How amelogenins are removed from mature enamel is something that has not yet been clarified (Boyde, 1989), but some studies have shown that massive protein loss, before maturation, should be the main cause (Robinson et al., 1982).

Following the cessation of the secretory activity of the ameloblasts, they remain on the tooth surface until it acquires a high degree of mineralization. In the case of human permanent teeth, the ameloblasts may be in this functional position even years after secretory activities had stopped (Boyde, 1989).

It has been established that the normal development of the enamel is mainly linear, although some authors have questioned this (Reid and Dean, 2000). However, the major corpus of the literature indicates that:

"The normal development of dental enamel is regular and sequential /.../. Development begins with the cuspal or incisal aspect of the rudimentary dental organ, after which ameloblasts appose successive "rings" (transverse bands) of enamel downward toward the cemento-enamel junction (cervical line bordering the tooth) where enamel calcification terminates" (Blakely and Armelagos, 1985: 371).

In fact, mature enamel exhibits structural features that reflect its mode of formation: the enamel prisms outline the path followed by individual secretory ameloblasts, and the Retzius lines mark stages in the layered apposition of enamel. The incremental lines, therefore, delineate the different layers of the matrix deposited during the tooth growth. Each line outlines the surface of the enamel and the dentine deposited at a particular time. The completed incremental growth pattern is, therefore, a graphic representation of appositional growth and results from the pattern of cellular activity (Schour and Massler, 1940a; Risnes, 1986).

Each transverse band or segment of enamel represents a specific period in the individual's growth and development. Because each type of tooth develops during a different period of months or years, the ages during which transverse sections were apposed can be determined once adjustments are made for the peculiar period of development covered by each type of tooth (Sunderland et al., 1987).

2) Maturation: The change from the secretory to the maturation function, at least in other mammals (e.g., rats) is associated with a cytological revolution, which involves the death and destruction of many cells, whose remains are phagocytosed by the monocytes macrophages (Boyde, 1989).

During maturation, organic material and water are removed almost completely and replaced by mineral salts. This process starts after the enamel matrix has reached full thickness in the occlusal parts of the crown. The inception of mineralization, or maturation, occurs extremely close to the secretory front, so that there is scarcely a delay between secretion and the progress of mineralization into newly produced matrix (Giro, 1947; Robinson and Kirkham, 1986; Alvarez and Navia, 1989; Boyde, 1989; Kelley and Larsen, 1991; Hillson, 1996).

During this phase the crystal previously deposited increases its thickness and the concentration of amelogenins in the enamel is greatly reduced (Boyde, 1989). The details of calcium transportation through the secretory ameloblast are still unknown and it is not known how much enamelin is removed, if any (Höhling, 1989).

The amino-acid composition changes, reflecting the net increase in enamelin-amelogenin ratio. The mineral-to-organic ratio also increases, due to the increase in mineral and the removal of protein. The water content also reduces dramatically due to the increase in the size of the individual enamel crystallites and a reduction of the intercrystallite water and organic-matrix filled space (Boyde, 1989).

Maturation of the enamel, then, involves rapid transport of calcium and phosphate (from a rich bloody supply via the enamel organ) and early removal of the organic matrix, especially the amelogenin component, with an accompanying change in the amino-acid composition (Boyde, 1989).

There are different theories on the mechanisms of maturation. The simplest one proposes that the force generated by the growth of the crystals is sufficient to mobilize the unstable protein matrix gel. However, neither explains why there is an apparently

sudden loss of organic matrix that is not paralleled by a matching increase in mineral uptake, nor the active cyclical changes in the organization of ameloblasts seen in the maturation zone, which strongly suggest active cellular participation. A different theory proposes that ameloblasts secrete proteases into the enamel matrix; however, no protease has yet been isolated (Boyde, 1989).

III.3.3. Teeth Developmental Chronology

Deciduous enamel formation begins *in uterus* and continues for several months after birth, although is largely an intrauterine process (Giro, 1947; Kelley and Larsen, 1991). Crowns are partially complete at birth, and total deciduous tooth takes only 2-3 years from initial mineralization to root completion. The order of initial mineralization in the deciduous teeth is: central incisor, lateral incisor, first molar, canine and second molar. Mineralization of the central incisor and first molar appear earlier in the maxilla but subsequent development in the mandible preceded the one in the maxilla (Sunderland et al., 1987).

First molar and anterior teeth are the first group of permanent teeth to form. A second group, premolars and second molars, begin their formation at about 1.5-3.0 years of age. After a pause, the third molars begin their formation at about 7 to 10 years of age. The upper teeth, as a rule, begin their formation slightly earlier than the lower ones do, although the lower teeth generally erupt just before the corresponding upper teeth (Schour and Massler, 1940a; Risnes, 1986; Kelley and Larsen, 1991).

The time required for the formation of permanent tooth crowns, between initial calcification and the crown-completed stage is 2.1 years for first molar and 2.8 years for second and third molars. The formation period of premolar crowns ranges between 3.1-

3.4 years, while in the canine it takes 3.5 years. At twelve years of age, the mature root length has been attained for all permanent teeth, with the exception of the third molar. Therefore, the calcification of permanent dentition is, usually, entirely postnatal and the formation of each tooth takes 8 to 12 years (Schour and Massler, 1940a; Risnes, 1986; Kelley and Larsen, 1991). Thus, the apposition of dental enamel is slower in permanent than deciduous teeth (Risnes, 1986).

The length of time required for crown development is practically the same in males and females, with differences occurring within a narrow range from 0.02-0.1 years (Schour and Massler, 1940a; Risnes, 1986). In fact, the variance in attainment of developmental stages is the same for males and females (Moorrees et al., 1963).

Teeth then, have the longest developmental chronology compared with any other organ in the body and unlike other hard tissues they are not subject of remodeling. In fact, once a layer is formed it cannot be altered structurally by systemic factors, due to the fact that formative cells have receded and lost their connection with the matrix (Massler et al., 1941; Sunderland et al., 1987). Thus, defects in dental formation due to environmental influences remain throughout the life of the tooth, and have a clinical value in timing disturbances. However, this timing must be related to accurate tabulations of dental development. Dental development, indeed, is much less affected than other tissues by endocrinopathies and other developmental insults. This is shown by studies of children with major abnormalities in sexual maturation, stature and bone age, who show small deviations in timing of dental development (Sunderland et al., 1987). Moreover, tooth formation timing also appears to be comparatively resistant to nutritional effects, as

shown by the low correlation (r=0.1-0.2) found between tooth formation and relative weight, fatness, stature, and bone age (Kelley and Larsen, 1991).

III.4. Enamel Hypoplasia

As teeth form by continuous incremental growth, any metabolic disturbance to which the ameloblasts are sensitive will cause a layer of abnormal tissue. If the disturbance is transitory, normal dental tissue growth is resumed and the evidence of the disturbance is an enhanced incremental growth line, pit or band (Newton et al., 1984). Developmental enamel defects, then, are a putative marker of childhood morbidity that can be analyzed by age of occurrence using a standard calcification chart (Corrucini et al., 1985).

Because some teeth start to form before birth, others do shortly afterwards, and others much later during the growth period there is a record of variation in growth from before birth until dental maturation (Dean, 1987).

There are different types of developmental enamel defects. In this particular study one type will be considered: enamel hypoplasia, which is by far the most commonly studied one (Blakeley and Armelagos, 1985).

III.4.1. Definition

Dental enamel hypoplasia is a quantitative class of enamel defect. In general, enamel hypoplasia (EH) refers to a deficiency in the amount or thickness of the enamel, which results from a deficiency in the enamel matrix composition. The presence of enamel hypoplasia, therefore, indicates differences in hardness or quality of the enamel.

There are different types of enamel hypoplasia. The defect can present the form of a pit, a line of pits, an irregular group of pits, a line or a band. The most severe form of

hypoplasia is a total deficiency of the enamel, known as enamel aplasia; this type of hypoplasia results from the death of the ameloblasts prior to, or at the time of, differentiation (Goodman and Rose, 1990). The defect is more easily seen in the labial face of the teeth, especially in incisors and canines (Clarkson, 1989; Lukacs, 1989; Goodman and Rose, 1990; Skinner and Goodman, 1992; Roberts and Manchester, 1995; Lewis, 2000).

III.4.2. Mechanism of Formation

Mature dental enamel is a non-cellular tissue constituted by an internal epithelium formed by the ameloblasts. Mature enamel is fully acellular and almost completely composed of inorganic salt (>97%), with small traces of remaining protein and water.

All developmental defects of the enamel (DDE) result from disruptions in the process of amelogenesis. This occurs when ameloblasts at one particular longitudinal address, on the developing tooth circumference, are unable to form enamel or the correct thickness of enamel (Boyde, 1989).

If nutritional insults are imposed early in the formation of the organic matrix, the clinical expression will be enamel hypoplasia. If the stress is imposed later, during the maturation process, the result will be a hypocalcification manifested by opaque or chalky areas (Giro, 1947; Alvarez and Navia, 1989; Goodman and Rose, 1990). These defects will be preserved, because there is no remodeling process. Consequently, enamel defects are a permanent and retrospective record of the stressful events experienced during childhood (Nikiforuk and Fraser, 1981; Rose et al., 1985; Lukacs, 1989; Goodman and Rose, 1990; Skinner and Goodman, 1992; Roberts and Manchester, 1995; Hillson, 1996; Lewis, 2000).

In the case of EH, as a result of the disturbance, the ameloblasts undergo retrogressive changes. Their height is shortened and instead of being cylindrical, they turn into cuboidal an even squamosal shape. These changes bring about a complete disruption of enamel formation. Later, when the disturbing agent ceases, the ameloblasts adjacent to the affected ones resume the enamel-building process (Giro, 1947).

Hence, any systemic disturbance that occurs during the enamel formation can disrupt ameloblast's metabolism. If a particular ameloblast does not recover from the metabolic insult, no further enamel will be formed by the affected ameloblast. This deficiency in enamel thickness due to premature degeneration of the ameloblasts is defined as enamel hypoplasia, which is observed macroscopically as an area of depressed enamel (Rose et al., 1985). The presence of multiple enamel defects in a tooth is the result of periodical stress events that may be the result of seasonal scarcity (Cohen and Armelagos, 1984; Hillson, 1996).

The location of the lesion depends on the period of calcification in which the disturbing agent acted, while the extension of the lesion will depend mainly on the span of action of the causative agent. Accordingly, the alterations quite accurately tell the onset and duration of the force that compelled the formation of the enamel defect. Consequently, the difference in the period of maturation and calcification of various teeth explains the different location of the lesion in several teeth in the same dentition (Giro, 1947).

At a population level, enamel defects register developmental alterations occurred between 5th month *in uterus* to 7 years of age (approximately), with a hiatus between the ages of 8 and 9 years (usually there is no enamel formation at this age), followed by a

deposition of the enamel between 10 and 13-16 years old -enamel deposited in the formation of the 3rd molar (Rose et al. 1985; Skinner and Goodman, 1992)-. Enamel hypoplasia, thus, can be present in deciduous and permanent teeth. In fact, damage to enamel epithelium and formation of abnormal enamel had been detected in the majority of perinatal deaths, indicating adverse prenatal influences (Gruenwald, 1973). Chronic fetal distress, as manifested by a significant subnormal birth weight, is nearly always associated with severe damage to the enamel organ. This hypoplasia then, is not the result of an event, which is usually how hypoplasia is interpreted, but a process; in fact, the presence of abnormal masses of enamel, attest for the long duration of the disturbance (Gruenwald, 1973). Gruenwald's study demonstrated that enamel epithelium, specifically the portion that is forming enamel at the time of injury, is highly susceptible to damage. In contrast, the epithelium closer to the apex, which has not yet begun to function, is structurally unaffected. This structurally unaffected epithelium remains functionally normal and will later be able to lay down normal enamel.

III.4.3. Etiology

Enamel hypoplasia may be the result of developmental disturbances of the enamel, and as such it records the interacting stresses of nutrition and illness during the period of tooth formation (Wright, 1997), but it can also have its origin in some form of genetic abnormality. In general terms, the presence of hypoplasia is an indicator that the causative agent affected the ameloblast at a definitive stage of development, hindering or completely nullifying enamel formation for a more or less extended period, but allowing the ameloblast to continue their work later (Giro, 1947).

During dental development, a variety of systemic stressors such as moderate malnutrition (Goodman et al., 1991) and infectious diseases can produce abnormal enamel growth pattern (Suckling, 1989). Many factors seem to be relevant in the formation of developmental enamel defects, but they can be categorized, broadly, in four major groups:

1) Hereditary anomalies;

2) Localized Trauma;

3) Nutritional deficiencies; and,

4) Childhood diseases (e.g. measles).

Defects resulting from hereditary causes generally affect all of the teeth in a set and are extremely severe. However, individuals with hereditary defects are rare (<1%) in most contemporary populations, and they are likely to be even less frequent in prehistoric populations, because they would have been eliminated through natural selection (Goodman and Rose, 1990).

Hereditary enamel defects are collectively known under the title of amelogenesis imperfecta (Winter and Brook, 1975; Hals, 1962). The main disorder in this group of conditions seems to be that of maturation, so that the tissue, although formed, remains with a high organic matrix and a low mineral content. It is, therefore, very soft and easily abraded, flaking off from the surface of what maybe a relatively normal dentine (Boyde, 1989). Amelogenesis imperfecta is associated with autosomal dominant, autosomal recessive, sex-linked dominant and sex-linked recessive conditions. Independent of which condition the defect is related with, amelogenesis imperfecta is rare (1:14,000) and the severity of these types of defects clearly differentiate them from the ones that are the

result of environmental disturbances (Winter and Brook, 1975). In fact, genetically determined enamel defects usually affect all areas of enamel in contrast to the environmentally induced hypoplastic defects (Pindborg, 1982).

Defects due to local trauma, local inflammation, and other non-systemic factors may also be relatively severe, but will influence only one tooth or few adjacent teeth (Suckling, 1989). Its causes include neonatal mechanical ventilation, fall and injuries, gunshot, ritual mutilation, electric burn, irradiation and local infection (e.g. periapical osteitis; Pindborg, 1982). Defects due to local trauma are likely to be rare in prehistoric populations, as most of the EH identified in paleopathological studies fit a chronological pattern and appear to be due to systemic metabolic stress (Goodman and Rose, 1990).

The vast majority of EH found in archaeological material fit a pattern of systemic metabolic stress (Goodman and Rose, 1990). This type of defect is likely to be found on most, or all, teeth developing at the time of the stress, and the location of the defects reflect the relative completeness of enamel crowns at the time of the disruption. Current research has demonstrated that the frequency of developmental disturbances such as EH is greatly influenced by the diet quality and quantity (Cook and Buikstra 1979; Corrucini et al., 1985; Rose et al., 1985).

Diet, may be one of the major causes of dental enamel hypoplasia, or it may be only one factor contributing to its appearance (Roberts and Manchester, 1995; Hillson, 1996). In fact, numerous conditions have been shown to cause these defects; diseases that interfere with calcium metabolism like rickets and other conditions like starvation have been proposed as one of them. Infectious diseases like measles, rubella, bacterial syphilis, tetanus, whooping cough, pneumonia, severe gastrointestinal disturbances, fever,

starvation, congenital infection, tuberculosis, hypoparathiroidism, fluorosis and low birth weight have been identified as some of it causes also (Giro, 1947; Pindborg, 1982; Goodman et al., 1988). In addition, other conditions like haemolitic anemia (usually in conjunction with erithroblastosis fetalis), endochrinopaties (like diabetes mellitus) and nephropathies can be the cause of EH (Pindborg, 1982).

It has been suggested that diseases can cause enamel hypoplasia because they create new nutritional demands, especially when severe deficiencies in vitamins D or A act in conjunction (Giro, 1947). There is, nevertheless, no conclusive evidence that diseases and/or specific nutritional deficiencies (vitamin A deficiency and scurvy) are the etiologic causes of enamel defects (Newton et al., 1984; Goodman et al., 1988). There is no one-to-one relationship between stress events and defect formation; EH appear to have no specific etiology, it is merely the reaction of a particular organ to some general disease process or processes: "The tooth appears to be a very sensitive indicator of environmental disturbance, but quite non-specifically so in that even febrile illness may cause a line to appear" (Newton et al., 1984). Therefore, any condition that is of sufficient constitutional severity to affect the growing cells of the body will likewise affect the enamel forming cells (Sarnat and Schour, 1942).

Formation of the defect depends not only upon the nature of the insult but also on the stage of maturation of the tooth crown; there are certain periods more vulnerable than others (Buikstra and Cook, 1980; Goodman, 1996). In some occasions, stresses occurred during the last five months of gestation, at birth, and the first year after gestation can be recorded by the presence of enamel defects in deciduous teeth. The presence/absence of this indicator on the deciduous teeth are a reflection of the state of health of both mother

and child. Prenatal hypoplasia, though, is uncommon; *in uterus* calcification normally takes place homogeneously and completely. It is, apparently, not markedly influenced by fluctuations in the condition of the mother, except in cases of severe maternal illnesses. However, once out of this environment, enamel is influenced by the state of the infant health (Kronfeld and Schour, 1939). Birth, in fact, can be registered in dental enamel through the presence of an accentuated Striae of Retzius (incremental lines; Schour, 1936) that is usually visible at the microscopic level. Studies considering children with normal births, with difficult birth and children born with cesarean procedure, show that there is a high correlation ($p(\alpha)<0.001$) between an increased width of the neonatal line (NNL) and pathological conditions (children with difficult birth). The data also shows that individuals born with cesarean procedure show a significant decrease in the NNL width. The results show that trauma to the ameloblasts is partially due to the birth process itself, partially to the change in environment, and it is more commonly found among socio-economic groups that suffer from protein-calorie malnutrition (Sweeney et al., 1971; Eli et al., 1986; 1989). However, there is no agreement as whether or not the dental changes indicate a pre or post-natal metabolic disturbance (Levine and Keen, 1974).

II.4.4. Alternative Methods of Analysis

As the chronology of tooth formation is known, measuring the distance between the defect and the cemento-enamel junction (CEJ) in the tooth helps to reconstruct when the defect was formed in the person's life. In order to do that is necessary to assume that the rate at which the permanent teeth developed was the same in the past, and that teeth are universally susceptible to enamel defects (Goodman et al., 1988; Goodman and Rose, 1990; Roberts and Manchester, 1995).

Table 4 Regression Equations for the Estimation of the Age in Years at the Time of the Defect Formation

Tooth	Equation
Maxilla	
Central Incisor (CI)	-(0.454 x Ht) +4.5
Lateral Incisor (LI)	-(0.402 x Ht) +4.5
Canine (C)	-(0.625 x Ht) +6.0
1st Premolar (Pm1)	-(0.494 x Ht) +6.0
2nd Premolar (Pm2)	-(0.467 x Ht) +6.0
1st Molar (M1)	-(0.448 x Ht) +3.5
2nd Molar (M2)	-(0.625 x Ht) +7.5
Mandible	
Central Incisor (CI)	-(0.460 x Ht) + 4.0
Lateral Incisor (LI)	-(0.417 x Ht) +4.0
Canine (C)	-(0.588 x Ht) +6.5
1st Premolar (Pm1)	-(0.641 x Ht) +6.0
2nd Premolar (Pm2)	-(0.641 x Ht) +7.0
1st Molar (M1)	-(0.449 x Ht) +3.5
2nd Molar (M2)	-(0.580 x Ht) +7.0

(After Goodman and Rose, 1990).

Goodman and Rose's (1990) method is one of the most popular ones. In this method the following steps have to be developed:

1) Determine the distance between the defect and the CEJ:

2) This value is processed by regression equations (see table 4) that estimate the age of the individual at the time of the defect formation; and,

3) These estimations are based in the division of the enamel development into half-year or yearly development periods.

The level of detail in the estimation of the age of the individual, at the time of the stress event, is due to the fact that the deposition of the enamel in humans is extremely slow in permanent teeth. This estimation, however, has an error of unknown magnitude originated in the assumption that smaller teeth take as long to develop as larger teeth (Goodman and Rose, 1990).

Hodges and Wilkinson (1990) have questioned the first assumption. These researchers examined the lower canines and maxillary central incisors in 34 individuals of an archaeological sample and 28 individuals of a modern collection. In their method, for every defect:

1) The distance between it and the CEJ was measured in millimeters;

2) Large enamel defects were measured considering the superior and inferior edge of the defect; and,

3) Crown height and the maximum distance from the incisal surface to the CEJ on the labial surface were measured also. This distance was converted into half-year age intervals, representing the individual's age-at-stress.

The comparison between their results with the ones obtained using Goodman and Rose's (1990) chart showed significant statistical differences between the two methods: most of the differences between age estimates were on the order of one half-year interval (older or younger). Therefore it seems that the first assumption exposed for Goodman and Rose's (1990) method can be questioned.

Reid and Dean (2000), however, evaluated 115 unworn anterior teeth through histological examination, and divided the cusp into 10^{th} percentiles. Using this data they calculated, in days, the time of initial mineralization, the time taken to form cuspal enamel, and the time taken to form each 10^{th} percentile of crown height. According to their results there is no evidence that longer crowns take longer to form than shorter ones. Thus, the discussion about whether or not crown height affects the results is not closed.

Ensor and Irish (1995) offered a completely different analysis of EH. Instead of trying to estimate the age of formation they tried to account for the range of variability in the duration of stress. Their method considered:

1) A categorization of lines and pits as acute events of stress, while bands were considered chronic defects. A differentiation based on the assumption that acute episodes of stress result in localized hypoplasia, whereas continuous hypoplasia represents a record of stress without immediate recovery;

2) An acute hypoplastic defect is of 0.5 mm or less in width, any defect over 0.5 mm in width was treated as continuous;

3) In order to avoid any error, no tooth with more than one third of the crown worn away was considered;

4) The hypoplastic defects were recorded by measuring the distance, to the nearest 0.1mm, from the CEJ to the center of each acute hypoplasia, or to the beginning and ending of each continuous defect; and,

5) Each acute hypoplasia received a value of 0.10. For each chronic hypoplasia, the percentage of crown disturbance was calculated by dividing the width of the insult by two-thirds crown of the estimated height. The sum of these values on a crown is called tooth hypoplastic area (THA) with which they established the level of stress present in the individual.

This method, although interesting, it is not useful for the purpose of this study, because it does not estimate the age of the individual at the time of the defect formation. However, most of the methods that do estimate age at the time of defect formation are not applicable to the deciduous dentition. The problem is that as they are formed largely in

the prenatal period and considering that the tibia starts to develop *in uterus*, it is impossible to ignore deciduous teeth in a study whose aim is to compare Harris lines with enamel hypoplasia (Blakely and Armelagos, 1985; Storey, 1992).

There is only one method available in the current literature to establish the age of the individual at the time of the stress event, when the event is register as EH in a deciduous tooth (Blakely and Armelagos, 1985). This method was developed using a sample of 50 individuals from the Dickson Mound site, in which ages fluctuated between 1 and 12 years. The sample was selected according the following criteria:

1) Complete crown enamel development and preservation was required on all the teeth studied; and,

2) Each individual had to have at least four teeth, if no defect was present.

The method requires determining:

1)The crown height for each specific tooth type;

2)The vertical width of the transverse segment of enamel that develops during a single month. This is established by dividing the crown height by the number of months that it takes to develop the crown. This same procedure must be repeated with every type of tooth considering its specific time of development (see table 5); and,

3)The timing of the defective enamel segment. The distance measured from CEJ to the defect is divided by the width of a monthly segment for that tooth. This becomes an expression of the number of months between the occurrence of the defect and the last month of enamel development. Finally, the number of months is subtracted from the standard developmental month, during which enamel is completed for that tooth, giving the month of occurrence. By the same process, the distance to superior and inferior

aspects of wide defects gives the age at onset and conclusion of enamel disruption in a given tooth. All teeth must be included, because studies have shown that all deciduous teeth are susceptible to hypoplasia (Storey, 1992; see table 6).

Table 5 Period of Enamel development in Deciduous Maxillary Teeth[1]

Tooth	Beginning of enamel development (prenatal months)	Completion of enamel development (postnatal months)	Duration of enamel development (months)
Central Incisors (CI)	5^{th}	4^{th}	9^{th}
Lateral Incisors (LI)	5^{th}	5^{th}	10^{th}
Canines (C)	6^{th}	9^{th}	13^{th}
1^{st} Molar	5^{th}	6^{th}	11^{th}
2^{nd} Molar	6^{th}	10^{th}-12^{th} (11^{th})	14^{th} -16^{th} (15^{th})

[1] This sequence applies approximately to lower dentition with only slightly variations. After, Blakely and Armelagos, 1985.

Table 6 Deciduous Teeth Susceptibility to Hypoplastic Defects

Tooth	% Hypoplastic Lesions	% Tooth affected by Tooth type
Mandible Central Incisor	3%	55%
Lateral Mandible Incisor	4%	---
Mandible Canine	38%	---
Mandible Second Molar	---	38%
Maxillary Incisors	27%	68-71%
Maxillary Canine	28%	68-71%
Maxillary 1^{st} molar	---	43%
Deciduous Molars	10-15%	---
All others	<20%	---

The major problem to be considered in the development of this technique is whether timings should be based upon 1-month enamel segments derived from crown height averages for each type of tooth in the sample, or whether timings for each individual should be based on the crown heights of the individual teeth. It is known that, there is variability in crown height and time of development: e.g. male dentition is larger than female dentition because male crown development begins earlier and ends later than in females. The timings, nevertheless, can also be estimated considering average crown

heights for the population, but only at the expense of objectivity in the assessment of age of defect occurrence. Considering, however, that individual crown height and average height methods are only slightly different in their results, it seems that for the study of the present population, in order to maximize the sample, the average method is more appropriate.

III.4. 5. Problems in the Interpretation

Theoretically all teeth whose enamel crowns are developing at the time of a systemic insult should bear a corresponding enamel defect. Thus, studies analyzing different teeth should yield comparable results as long as the tooth crowns examined developed over the same period of time. There is a variation, however, in enamel hypoplasia formation that may occur between different tooth types and between different regions of the enamel crown. Therefore, not all teeth and not all segments of a tooth are equally sensitive to the environment, therefore sensitivity is differential at inter and intra tooth level. In fact, susceptibility to enamel defect formation varies with the stage of crown development, which appears to be related with the rate of enamel deposition. The slower the rate the greater the susceptibility: enamel tends to be more stable or rigid (and thus less susceptible to defect formation) when it is expanding at its greatest rate, and correspondingly less stable at lower rates. Enamel deposition rates are greater cuspally, and least cervically, as it has been indicated by perykimata studies. Hypoplastic defects, then, are most abundant in the middle third of the enamel of each tooth (Dean, 1987; Goodman and Rose, 1990; Condon and Rose, 1992; Wright, 1997; Santos and Coimbra, 1999).

In general, the teeth most affected by hypoplasia are the anterior ones. Among these, the most sensitive ones are the lateral incisors and canines of the mandible. In the maxilla, central incisors and canines appear to be the most susceptible. A tentative explanation is that teeth in which the genetic control is strongest may be more likely to be hypoplastic because they are less able to alter developmental timing (Goodman and Armelagos, 1985; Goodman and Rose, 1990; Condon and Rose, 1992).

Considering the inter-tooth and intra-tooth segment differential sensitivity, the interpretation of the age of peak morbidity is complicated. In fact, at different ages the speed of calcification and therefore the susceptibility of the enamel to environmental disturbances varies (Massler et al, 1941). Moreover, when crowns of different teeth from one individual are matched, it is clear that one growth disruption causes defects in a different place in each of the different tooth crowns being formed at the time, with a difference in size and prominence (Hillson and Bond, 1997). These differences have usually been interpreted as the result of differential susceptibility between tooth classes. But, it is also possible that the geometry of the crown growth may be a major factor because each appositional zone of a crown hides a considerable proportion of enamel layers. These layers do not appear in the surface, and therefore unless a growth disruption generates a defect on the surface, no evidence of hypoplasia is visible for that part of the crown (Hillson and Bond, 1997).

Comparative studies, then, must take into account the type of tooth analyzed, while the position of the defects must be interpreted taking into account that some areas of the tooth are more sensitive, before any conclusion about social behavior (e.g. weaning) can be made. If hypoplastic defects are biased towards the middle period of amelogenesis for

each tooth (Wright, 1997), the peak occurrence of hypoplasia does not directly mirror the timing of the peak stress experienced.

III.5. Studies About Correlation Between Harris Lines And Enamel Hypoplasia

As indicators of stress, enamel hypoplasia and Harris lines have partially overlapping etiologies and a somewhat analogous mechanism of formation, because both involve the cessation or alteration of normal growth (McHenry and Schultz, 1976; Clarke, 1982).

McHenry and Schultz (1976) found Harris lines without an equivalent enamel defect. These authors have suggested that Harris lines are formed by insults that are not as severe or as long lasting as those that cause enamel hypoplasia. In fact, in their study, the association between radiopaque transverse lines and enamel defects was statistically non-significant, and the results did not change even when only the moderate or severe hypoplastic defects were considered.

In a study executed by Clarke (1982) the ordinary four-cell tables of contingency failed to show association between Harris lines and enamel hypoplasia. Nevertheless, when Chi-square was applied to a two-cell concordance-discordance contingency table, a significant association between the two indicators was revealed for lines and hypoplastic defects of all grades. Therefore, according to these results there is a co-occurrence of transverse lines and enamel hypoplasia. However, there was no one-to-one correlation between the two and their co-occurrence was interpreted as individual's dual susceptibility when they are in a high state of stress.

According to Maat (1984), the problem in the study of correlation between enamel hypoplasia and Harris lines is due to the fact that Harris lines are subject to resorption while hypoplastic defects are not. Therefore, in his conception, it is necessary to check Harris lines against enamel hypoplasia, and not vice versa. His study showed that there is a correlation between type III lines (moderate lines located in the diaphysis, plus well-marked lines located in the metaphyses) with enamel hypoplasia. Therefore, type III lines represent events disturbing both developmental systems (bone and teeth) within an individual.

As a consequence Maat (1984) considers that an emphasis on type III lines minimizes the risk of misinterpretations of lines as markers of serious health insults. However, he recognizes that the less-well marked lines may also represent originally well-marked cases partially obliterated by resorption.

Mays (1995) did not find a chronological association between these two indicators of stress. His study showed that enamel defects may occur when no corresponding Harris lines are visible on the bones, while in some cases Harris lines had been found without a correspondingly enamel defect although they were formed during the period in which the crowns of the anterior teeth were growing. Considering that these stress markers are non-specific and the result of the same biological process (halted bone/enamel deposition) there should be, at least in theory, a correlation between the two of them. However, as presented above, there is no agreement about the presence/absence of correlation between these two stress indicators. Thus, it is not only possible but also necessary to develop a study as the one proposed here.

CHAPTER IV

MATERIALS AND METHODS

VI.1.Populations Under Study

Two populations were considered for the purpose of this study, Az-71 (juvenile segment) and Az-140. These populations inhabited the Azapa valley, during the Early Intermediate period (B.C.1700-A.D.500) and the Middle Horizon (A.D.400-1000; see Figure 4, map 1 and map 2).

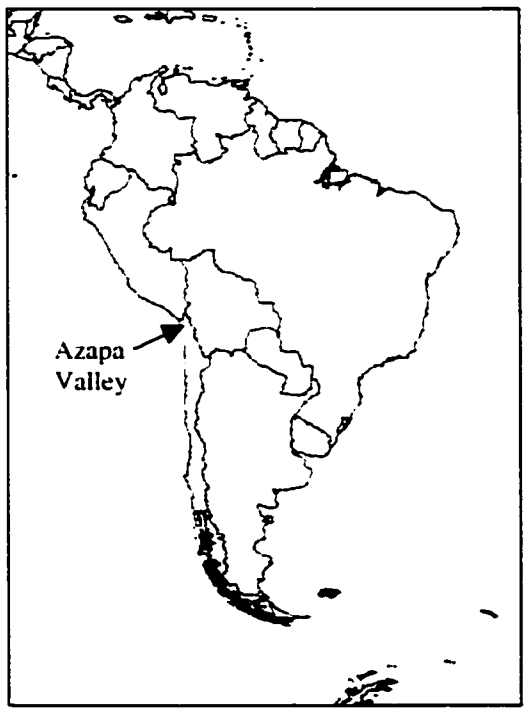
VI.1.1. The Azapa Valley

The Azapa valley is located in the south central Andean region, and is one of the occidental valleys (Lumbreras, 1981). These occidental valleys are found in southern Peru and northern Chile, and are formed as the result of the action of rivers and streams, which originate in the western slope of the Andes. It is, in fact, the presence of these rivers what allows the settlement of human groups in this area.

The Azapa valley (17°30'-19°10') is part of the Atacama Desert where rain is scarce, daily temperature oscillation is extreme and the vegetation is poor (Sepúlveda, 1962; Villagrán et al. 1982). The environmental aridity is the result of a set of variables; over the waters of the Pacific Ocean, due to the Humboldt Current, the air is cold and humid, but when the wind blows over the hot land, the humidity evaporates before any cloud can be formed. In addition, the "Cordillera de la Costa", a massive range of mountains at the coast, does not allow the humid winds from the ocean to reach the valley. Moreover, the

Andes stop the entrance of the humid winds from the tropics; as a result the scarcest resource is water (Nuño and Barros, 1984; Toledo and Zapater, 1991).

Map 1



Its cold water, rich in salts and nutrients, characterizes the ocean at the coast of the Azapa valley. A variety of fishes (*anchoveta, jurel*, tuna, *albacora*, etc.), crustaceans, sea mammals (e.g. sea lions -*Otaria florecens*- and *chungungos* -*Lutrafelina peruvensis*), rats, *chillas* (*Seudalopex griseus*), insects (e.g. spiders and scorpions) and birds (e.g pelican –*Pelecanus thagus*-, and *piqueros* –*Sula dactylatra*), are some of the resources that can be utilized in this area (Quintanilla, 1983).

The San José River originally formed the Azapa valley, through erosion. This river does not reach the ocean, most of its waters are subterranean (Urzúa, 1969), and had been used in agricultural practices since prehistoric times. Some of the cultivated products are

yuca, pumpkin, chili, tomato, cotton, avocado and corn –*Zea mays*- (Keller, 1946; Toledo and Zapater, 1991). Some of the native threes in the valley are *chañar* (*Geofrea decorticans*), *algarrobo* (*Prosopis atacamensis*) and *pimiento* (*Schinus molle*; Quintanilla, 1983). Snakes (e.g., *Dromicus angustilineatus*), lizards (*Phyllodactylus inaequaliscope and P. heterurus*), batrachians (*Bufo atacamensis* and *B. kalinowskii*), and birds (e.g., *Zontrichia capensis antofagastae*) are some of the faunal resources in the valley (Quintanilla, 1983).

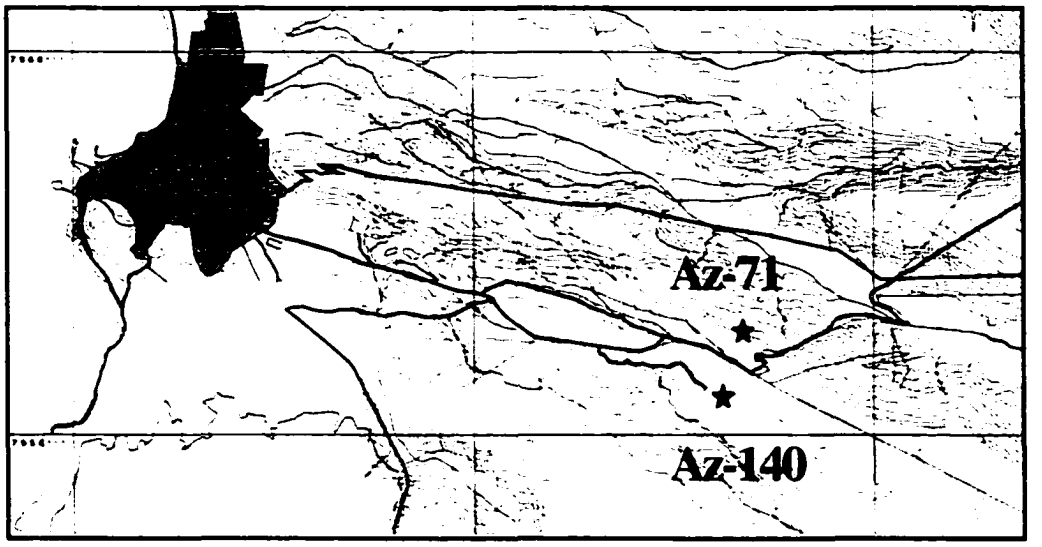
Towards the west, the altitude of the valley increases, to the point that it disappears in the slopes of the Andes (Keller, 1946), where the environmental conditions change, as do the fauna and flora.

VI.1.2. Formative Period (1700B.C.-500A.D.)

Az-71's population inhabited the Azapa Valley (see map 1 and 2) during the Formative period (1700B.C.-500A.D.). The Formative period can be characterized by the appearance of agricultural practices, which were fully adopted by 1300B.C. The presence of irrigation channels shows the existence of social coordination and organization. The houses present more permanent characteristics that indicate a lower degree of mobility among these populations, in comparison with the previous, Archaic, period (Nuñez, 1989).

Two cultural phases are recognized in the Azapa valley for this period: a) Azapa phase (1300B.C.-560B.C.), and b) Alto Ramirez (410B.C.-760A.D.; see figure 4). The first cultural phase is Azapa. The Azapa groups were mainly hunter-gatherers, but they also cultivated products such as chili, *achira*, gourds, and sweet potato. The habitational sites are located close to springs, and the cemeteries are found near to the habitational sites (Santoro 1980a; 1980b).

Map 2



Decorated and undecorated baskets, and gourds, mortars associated with pigments, combs, brushes, wood bags and the hallucinogenic kit are part of the material remains. Other remains are fishhooks, harpoons, projectile points, bows, leather bags, and ceramic pots (Santoro 1980a; 1980b).

Individuals covered with plant mats compose the mortuary pattern. Some of them present turbans and in some cases the faces were painted with red ocher and covered with thin wood textiles. The Azapa phase's populations represent a transition in which hunting and gathering were still practiced, but among whom the first manifestations of agriculture were also present (Santoro 1980a; 1980b). Although part of Az-71 material belongs to this period, this sample does not include them.

The following cultural phase, Alto Ramirez (410B.C.-760A.D.; see figure 4), which apparently extends into what has been classified as Middle Horizon, is mainly characterized by a more intense occupation of the Azapa valley. The villages are

associated with burial mounds. The burials were usually sealed with plant mats, under which the funerary bundles were deposited. The bodies were usually flexed, on their side or seated. Some of them present hatbands, hats or turbans, and loincloths. Grave goods are scarce, and the bodies do not present a direct association with them; in some cases the grave goods are intrusive in the archaeological record. Necklaces (made of *malaquita* or shells), spindles, baskets, shells, fishes, pottery, mortars, pestles, camelid bones, and some trophy skulls are part of the material remains (Rivera et al., 1987; Muñoz, 1983a; 1883b; 1987, 1989; Nuñez, 1989).

Alto Ramirez's populations had a mixed economy in which *mandioca*, *quínoa*, beans, sweet potatoes, corn, *yuca*, and chili were cultivated. The use of tools for agricultural activities became more common in comparison with the previous period, and *chañar* and *algarrobo* flours were part of the diet. The technology used in the elaboration of ceramics was more sophisticated in comparison with the Azapa phase (Rivera et al., 1973; Muñoz, 1983a; 1989).

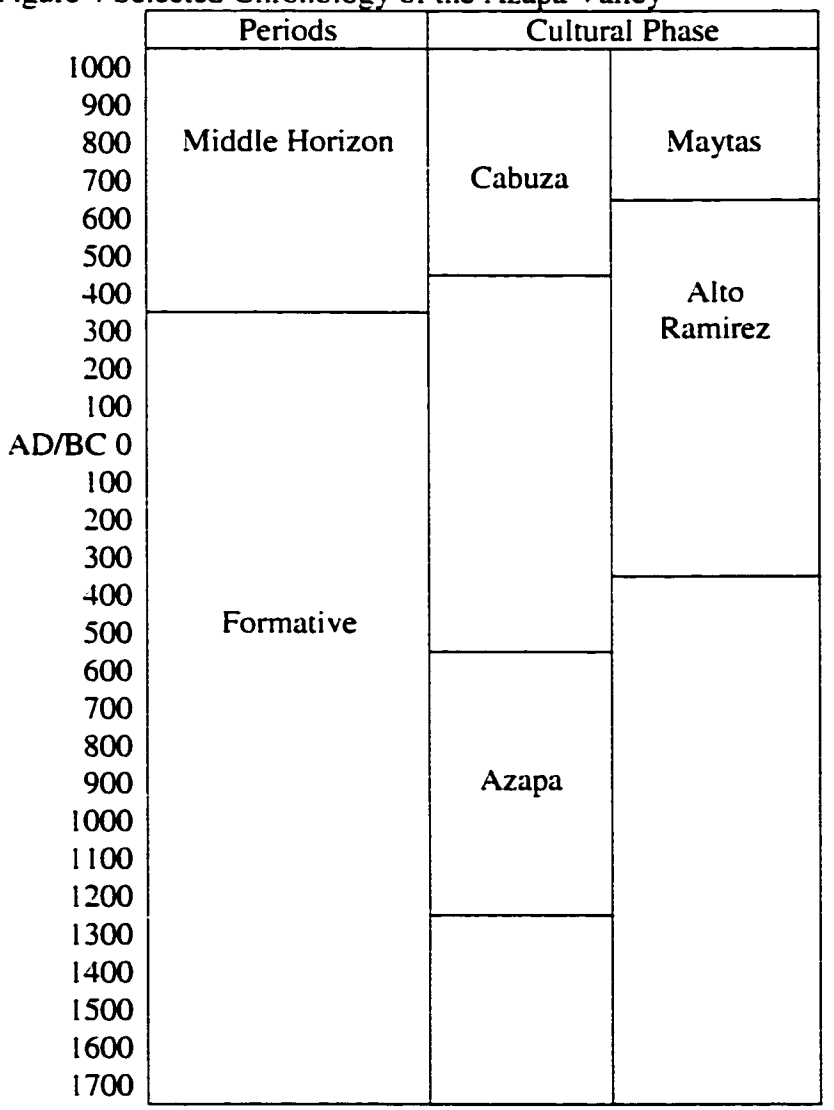
Intensive cultivation of corn, gourd, pumpkin, sweet potato, *achira* and *quínoa*, demonstrate that at this time the adoption of agriculture was complete, and it was accompanied by the domestication of camelids (Nuñez, 1989).

The latest moments of the Alto Ramirez phase can be characterized by the absence of burial mounds and grave goods. The bodies were generally deposited in large baskets, and they show fractures and alterations of their limbs (Muñoz, 1983a).

It has been suggested that altiplanic populations migrated to the Azapa valley during the Formative period. The archaeological evidence, however, shows that the importance of the foreign products is limited, the objects that suggest the present of an altiplanic population are scarcely represented in the archaeological record, and up to this moment,

there is not one altiplanic site recognized in this area. The bioanthropological evidence has not identified foreign individuals among these groups either (Sutter, 1997; 2000).

Figure 4 Selected Chronology of the Azapa Valley



VI.1.3. The Middle Horizon (400-1000A.D.)

The population of the site Az-140 inhabited the Azapa valley (see map 1 and 2) during the archaeological period known as the Middle Horizon (400-1000A.D.). This

period can be characterized by the presence of populations that practiced farming and herding, while complex social organization is evidenced through the appearance of elite groups. Two archaeological phases are recognized for this period: Cabuza (400-700 A.D.) and Maytas (700-1000A.D.; Muñoz, 1983b; Focacci, 1990; Uribe, 1999).

The populations inhabited small villages, located in the high slopes of the hills, characterized by the presence of rectangular houses. The burials were located next to the villages, far away from the farm fields. The bodies were flexed, dressed with decorated wool shirts and accompanied with varied funerary goods. The tombs were directly excavated on the earth and present a cylindrical shape (Muñoz, 1983b; Berenguer and Dauelsberg, 1989; Focacci, 1990; Uribe, 1999). During the Middle Horizon, in general, the archaeological remains show a high intake of corn, potato, sweet potato, gourd, beans, and a minor intake of *llama*, *guanaco*, guinea pigs and seafood (Berenguer and Daulesberg, 1989; Dauelsberg, 1992-1993).

Two archaeological phases are recognized for this period: a) Cabuza (400-1000A.D.) and b) Maytas (700-1000A.D.). Ceramic types differentiate one phase from the other; Cabuza presents ceramic pots with right angles, plane bases, painted (black over red), and modeled decoration. There is certain similarity between this ceramic type and the one produced in the altiplanic site of Tiwanaku (Muñoz, 1983b; Focacci, 1990; Uribe, 1999). Maytas ceramic type is three-colored, and is currently understand as a transformation of the Cabuza style, that has been interpreted as a reaction to the influence of Tiwanaku (Uribe, 1999).

It was originally postulated that during this period altiplanic colonies (Tiwanaku) were settled in the valley (Murra, 1972; Berenguer, 1975; Focacci, 1982; Nuñez and Dillehay, 1995) as some archaeological remains in the Azapa valley (sites Az-143, Az-

144, and Atoca-1) suggest the influence of Tiwanaku culture in this area. The evidence, however, does not demonstrate the presence of altiplanic colonies in the valley, and bioanthropological studies do not confirm the presence of these colonies (Goldstein 1995-1996; Sutter, 1997, 2000).

IV.2. Material

The osteological collections that are the subject of this analysis come from the archaeological sites of Az-71 and AZ-140. These collections are curated at the "Museo San Miguel de Azapa" (MASMA) in the Azapa Valley, northern Chile. The composition of the collections is described in table 7.

Az-71's and Az-140's populations inhabited the Azapa valley (see map 2) during two archaeological periods: Formative period (1700 B.C.-400 A.D.) and the Middle Horizon (400-1000 A.D.) respectively. The sample from Az-71 includes only the juvenile individuals in order to increase the sample size for this age segment. This was considered necessary in order to identify a possible correlation between the two indicators (HL and EH), since it may disappear in older individuals due to the process of resorption that affects Harris lines.

From these two populations only 136 individuals were analyzed (see table 7 and graph 2) because of two restrictions:

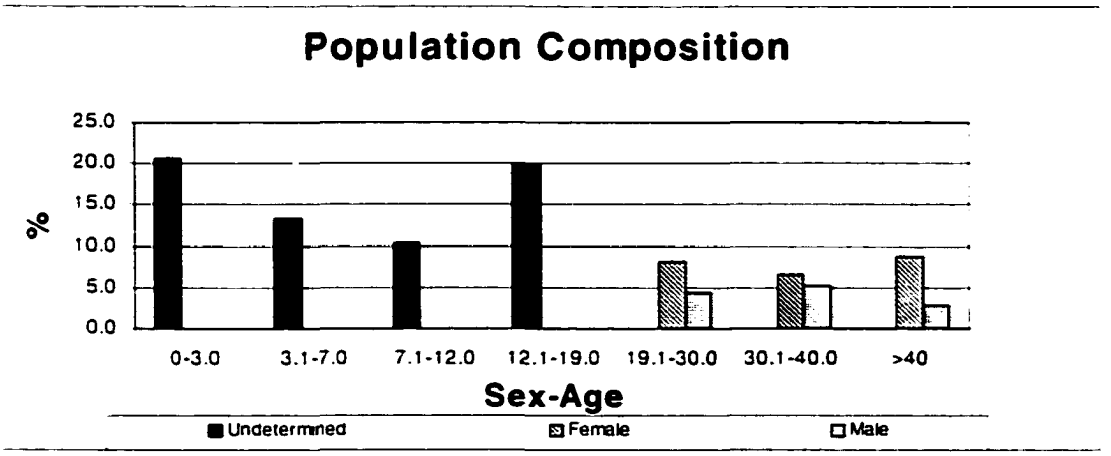
1) Only the individuals that had at least one observable tooth were considered; and,

2) Of the above sample, only the individuals with at least one available tibia were considered.

Table 7 Sample Composition

Age/Sex	Undetermined	Female	Male	Total
0-3.0	28			28
3.1-7.0	18			18
7.1-12.0	14			14
12.1-19.0	27			27
19.1-30.0		11	6	17
30.1-40.0		9	7	16
>40		12	4	16
Total	87	32	17	136

Graph 2



In the case of tibial length determination the sample included a larger number of individuals (see table 8) from these two sites. The size of the sample was increased in order to construct a table of growth, necessary for the application of Hummert and Van Gerven's (1985) method.

Over 83% of the radiographs taken correspond to the left tibia, while the remaining 16% were taken from right tibias (see table 9) and correspond to the case in which the left tibia was not available.

Table 8 Population Composition for Radiographs

Age/Sex	Undetermined	Female	Male	Total
Unborn	3			3
0-3.0	44			44
3.1-7.0	17			17
7.1-12.0	14			14
12.1-19.0	29			29
19.1-30.0		11	6	17
30.1-40.0		9	7	16
>40		12	4	16
Total	107	32	17	156

Table 9 Radiographs Taken

Radiographs	N	%
Left	131	83.97
Right	25	16.03
Total	156	100

IV.2. Methods

IV.2.1. Sex and Age Determination

Sex, in adult individuals was determined considering the following aspects of the Os Coxae morphology: 1) Ventral arch, 2) Subpubic concavity, 3) Ischiopubic Ramus Ridge, and 4) Greater Sciatic Notch (Phenice, 1969; Buikstra and Mielke, 1985). In addition, the size or robusticity of five cranial morphological traits were considered: 1) Nuchal crest, 2) Mastoid process, 3) Supra-orbital margin, 4) Glabella, and 5) Mental eminence (Acsádi and Neméskeri, 1970; Buikstra and Ubelaker, 1994).

Although it is desirable to determine the sex of the juveniles in the sample, there is, so far, no method that can be applied to this entire age segment. Loth and Hennenberg (2001) have developed a method to determine sex in juvenile individuals considering the mandible morphology. This method, however, is only accurate (μ=81%) for individuals between ages 0-7. Above the age 7, the predictive accuracy of this method is only of

69%. Schutkowski (1993) offered a second sex determination method for juveniles. In this method mandible and ilium were considered. The analysis showed six traits that were sexually dimorphic in juveniles: 1) chin 2) anterior dental arcade, 3) gonion, 4) angle of the greater schiatic notch, 5) depth of the greater schiatic notch, and 6) curvature of the iliac crest. The method is highly accurate to determine sex in males (95%) but not as accurate in females (71.4%). In addition, the few individuals of more than five years of age that were analyzed in this study did not show the typical sex-distribution of these traits. Therefore, this method is not recommended for individuals of five or more years of age. La Velle (1995) compared the breadth of the ischium and the acetabular regions among 8 years olds and found significant sex differences. Application of this method in the population under study, however, has shown low accuracy (Sutter, 2002 pers. com.).

Among the adults, age was determined considering age-associated changes in the pubic symphisis, according to Todd's (1921a; 1921b) and Brooks and Suchey's (1990) methods. In addition, age associated changes were evaluated in the auricular surface, and in the lateral-anterior cranial sutures (Meindl and Lovejoy, 1985; 1989).

The subadult age was determined considering: 1) Dental maturation (Ubelaker, 1989); 2) union of the primary ossification centers (Steele and Bramblett, 1988); and, 3) union of epiphyses (McKern and Steward, 1957; Suchey et al., 1984; Krogman and Iscan, 1989; Ubelaker, 1989).

Once age and sex of the individuals was determined, the populations was divided into the following age-sex groups:

- 0-3.0
- 3.1-7.0
- 7.1-12.0

- 12.1-19.0
- 19.1-30.0 Female/ 19.1-30.0 Male
- 30.1-40.0 Female/ 19.1-30.0 Male
- >40 Female/ >40 Male

IV.2.2. Harris Lines

Harris lines were studied using radiographs, instead of cutting the bones cross sectional, for two reasons: 1) Harris lines are more visible in radiographs (Machiarelli et al., 1994) and 2) radiographs are non-destructive. The radiographs were obtained from the laboratory in the "Museo San Miguel de Azapa".

The radiograph equipment utilized in this study was: Portable ProfexRay, model A, serial 2228 XRAY. The radiographs were obtained using 85-90volt. mA 2-3. In adults the time of exposition was 3-4 seconds, while in juveniles the time varied between 2-3 seconds.

The tibia was selected for the identification of Harris lines because:

1) It is the most commonly used bone, so this study can be compared with others; and

2) It presents nearly horizontal non-convoluted epiphyses whose shape will not significantly distort the geometry of a transverse line in relation to the plane of the X-ray.

The study of Harris lines was done according to the following process:

1) If the two tibias were present, only the left one was considered (because it has been shown that it presents a greater number of lines, in comparison with the right one);

2) In the cases where the left tibia was absent the right one was considered, and its use was recorded;

3) Determining the presence of the line: the presence of a radiopaque line in the radiograph was counted as a Harris line if it covered at least 30% of the shaft width, and only if the angle was higher than 45° and lower than 135° (Clark and Mack, 1988); and,

4) The lines for both the proximal and distal segments were considered.

Although Hummert and Van Gerven (1985) recommend researchers to count only the lines in the distal shaft, the analysis of the radiographs showed that some individuals only presented lines in the proximal end of the diaphysis. Then, if the analysis were restricted only to the distal end, the results would have been biased.

The age of the individual at the time of the line formation was calculated according to the specifications of the two methods (Hummert and Van Gerven, 1985; Byers, 1991) selected in this study.

Several methods have been proposed for the estimation of the age of the individual at the time of the line formation (Allison et al., 1974; Hunt and Hatch, 1981; Matt, 1984;). Byers' (1991) method was chosen in this particular study because it offers a series of advantages like:

1) A table of percentage of growth per year (see table 2);

2) It does not assume an average tibia's length at birth (*contra* Allison et al., 1974);

3) It differentiates males and females;

4) It considers the different rates of growth for proximal (57%) and distal (43%) sections of the tibia; and,

5) It is case specific because it considers each individual tibia's length in the calculation.

A second method (Hummert and Van Gerven, 1985) will also be applied. This one has been selected because:

1) It allows the analysis of immature remains, although it can also be applied in the adult segment of the population;

2) It is population specific, because it demands the construction of a table for the tibial growth;

3) It does not assume an average length of the tibia at birth (*contra* Allison et al., 1974); and,

4) It considers the different rates of growth for the proximal (57%) and distal (43%) sections of the tibia.

Although this method has several advantages in common with the one offered by Byers (1991), its major contribution is its applicability to immature remains. Since Harris lines are subject to resorption, if only the adult segment was analyzed, the number of lines recognized would be lower due to resorption. Therefore the absence of correlation between Harris lines and enamel hypoplasia in the adults would be misleading, and it is through the analysis of immature' remains that this erroneous conclusion can be avoided. Hummert and Van Gerven (1985) applied their method to the distal end of the tibia only. The analysis of the radiographs, however, showed that some individuals, especially juveniles, have HL only, or mostly, on the proximal end. A total of 87 tibia's radiographs from individuals aged Unborn-19 years of age were considered in the construction of proximal and distal tibial growth tables (see Table 19 and Table 20). For both methods, the tibia's length was measured from the radiograph.

IV.2.3 Enamel Hypoplasia

The following steps were used in the analysis of Enamel Hypoplasia:

1) Identify the presence of an enamel defect;

2) Identify the type of defect: a) line, b) pit, c) line of pits, d) group of pits or e) band;

3) Measure the distance between the defect and the cemento-enamel junction (CEJ). A dental caliper was utilized to ensure accuracy; and,

4) Calculate the age of the individual at the time of defect formation.

Several methods have been proposed for the estimation of the age of the individual at the time of the formation of the defect (e.g. Hodges and Wilkinson, 1990). In this specific case, Goodman and Rose' (1990) regression equations were applied (1990; see table 4). The Goodman and Rose (1990) method was selected over others because:

1) It is simple to apply;

2) It does not require a control population (not available for the current study); and,

3) It is the most popular method used in the study of enamel hypoplasia.

Limitations of this specific method include the assumption that crown height and mean age at enamelization onset and completition are the same for the population under study, and the one studied by Goodman and Rose (1990; Berti and Mahaney, 1992). The method was also adjusted for the average crown height per tooth-type in the sample; this method will be referred as "adjusted Goodman and Rose method". Mean crown height per tooth-type was calculated using teeth of the left maxilla and mandible that were unworn or that presented a minimum degree of wear (Rogers, 1984; Smith, 1984). Thus, two methods, Goodman and Rose (1990) and adjusted Goodman and Rose, were applied in the determination of the age of the individuals at the time of the EH formation in permanent teeth.

In deciduous teeth, Blakeley and Armelagos' (1985) method was applied. In order to do that the following steps were completed:

1) Teeth that present less than two degrees of abrasion (determined using the Smith, 1984 method) were measured following Rogers' indications (1984);

2) According to the previous results, a mean crown height for each tooth type was established and then it was divided into the numbers of months that if takes the crown to develop; and

3) Then, the distance between the enamel defect and the CEJ was converted in years, according to the previous calculations.

In all the methods, for permanent and deciduous teeth, the following assumptions were accepted:

1) The assumed date of initial calcification is valid, although geographical variations due occur;

2) 4μm/day is the regular mean of enamel deposition, although incremental growth rates in excess of this may occur; and

3) Continual growth of the tooth crown during its developments occurs, for if the retreating cell layers rest at any time, then the time scale of the record is distorted (Levine et al., 1979).

IV.2.4. Statistical Analysis

After the data was collected, statistical analyses (descriptive, parametric and non-parametric) were performed in order to establish whether these two indicators:

1) Result from the same environmental conditions;

2) Are equally sensitive to the same environmental conditions;

3) Are equally reliable as indicators of stressful conditions experienced by past populations; and,

4) Show a coincidence in the time of formation.

Descriptive statistic was obtained using Excel 97, while parametric and non-parametric statistic tests were applied using SPSS 10.1.

The incidence of HL and EH was first calculated considering the number of individuals that presented them. The number of HL in the distal and proximal end of the tibia was also calculated. In the case of EH the numbers of teeth affected in the sample were calculated along with the average number of defects formed in deciduous and permanent teeth per tooth affected. In the cases were differences between age-sex groups, in terms of the frequency of EH, were detected Z tests were applied in order to determine if these differences were significant. The difference in average number of EH defects between deciduous and permanent teeth were evaluated with the student t-test (Steel and Torrie, 1995).

The age of the individuals at the time of EH and HL formation was calculated with the methods already described. The results are expressed considering the age-sex groups and by one-year range in terms of the age at the time at their formation, except for the segment <1 which groups in HL and EH formed *in uterus* and during the 11 months before the year of age.

The age at the time of HL and EH formation was compared in terms of general distribution and also specific distribution for the adult segment. The results for HL and EH were then compared. For EH in the deciduous teeth, or the results obtained with Blakeley and Armelagos' (1985) method were grouped with the Goodman and Armelagos (1990) and the Goodman and Armelagos Adjusted method, in order to have a complete description of the distribution.

The results obtained with the Byers' (1991) method and the Hummert and Van Gerven's (1985) method (considering only the same individuals) were compared using the t-test for paired samples. The same test was applied to compare the results obtained

with the Goodman and Rose's (1990) and Goodman and Rose adjusted method (Steel and Torrie, 1995).

Between these two markers (HL and EH), at the individual level (presence/absence), and in order to establish the presence/absence of correlation a general Chi^2 test and Chi^2 for each sex-age group were obtained. Between these two markers and at the individual level the distribution of age of the individuals at the time of EH and HL formation was compared.

Finally, and in order to remove the noise, the data concerning age of the individual at the time of HL and EH formation, was smoothed by subtracting the average number of HL and EH formed at each specific year. The remaining HL were matched with the remaining EH. In order to avoid the danger of having subtracted the average number of HL formed due to stress and not to growth, three correction factors were added to the residual HL (0.1; 0.2; 0.3). The remaining HL once the correction factor was added, were matched each time with the remaining EH. Remaining HL were compared with remaining EH and not the other way around in order to avoid the possibility that the absence of Hl was due to the resorption process. The proportion of matches and disagreements were then compared.

CHAPTER V

RESULTS

This chapter presents the results of the analyses of the radiographed tibias and the dental enamel of the sample. First, the Harris lines results will be presented, in terms of the percentage of individuals affected and the frequency of lines. This section will be followed by the results obtained for age of the individual at the time of Harris lines formation. Next, the enamel hypoplasia results will be presented, and they include percentage of individuals affected and frequency of the defects. In addition, the results obtained by percentage of teeth affected will be exposed.

Next, the results of the analysis of the correlation between these two indicators will be presented, in terms of their correspondence at the individual level and in terms of age of the individuals at the time HL and EH formation. All the comparisons were carried out considering the age and sex of the individuals in the sample.

V.1. Harris Lines

V.1.1. Harris Line's Incidence at the Individual Level

The incidence of Harris lines at the individual level was assessed to determine the percentage of individuals that exhibited this particular indicator. The analysis showed that

67.82% of the juveniles, 62.25 % of the females and 58.82% of the males presented HL (see table 10). In total, 63.23% of the population examined presented HL (see Table 11).

Among the juvenile population (0-19 years; see table 10) the percentage of individuals that have HL appear to follow a bell-shaped curve, with an increase and then decrease of HL through the 0-19 years of age range. The incidence is lower in the age range 0-3 years (64.29%) in comparison with that of 3.1-7.0 years (88.89%) and 7.1-12.0 years (71.43%). These differences, though, are not statistically significant (see Table 12), using a Z test. The frequency continues to diminish among the 12.1-19.0 years old (55.56%), this difference, also, is not statistically significant (see Table 12). Among the adults, both males and females, the frequency of individuals that present HL tend to diminish with increasing age, this is especially evident among the females and males of 19.1-30.0 years compared with those of ages 30.1-40.0 years (see table 10). These differences, however, are not statistically significant, except for the 19.1-30.0/30.1-40.0 age range for males (see Table 12).

Table 10 Frequency of HL at the Individual Level

Harris Lines									
Age/Sex	Undetermined			Female			Male		
	n	N	%	N	N	%	N	N	%
0-3.0	18	28	64.29						
3.1-7.0	16	18	88.89						
7.1-12.0	10	14	71.43						
12.1-19.0	15	27	55.56						
19.1-30.0				8	11	72.73	5	6	83.33
30.1-40.0				5	9	55.56	3	7	42.86
>40				7	12	58.33	2	4	50.00
Total	59	87	67.82	20	32	62.50	10	17	58.82

Table 11 Harris Lines and Enamel Hypoplasia General Frequency

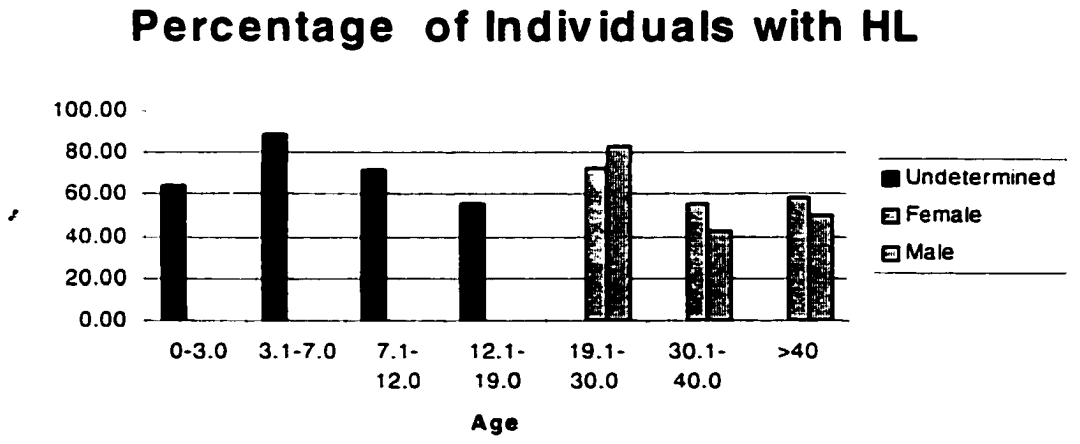
Condition	n	N	%
Harris Lines	87	136	63.23
Enamel Hyp.	108	136	79.41

Table 12 Harris Lines: Z test

Pop. 1	Pop. 2	p1	p2	n1	n2	Z	P(a)
0-3.0	3.1-7.0	0.6429	0.8947	28	19	1.21	0.1137
3.1-7.0	7.1-12.0	0.8889	0.7143	18	14		
7.1-12.0	12.1-19.0	0.7143	0.5526	14	27	1.05	0.1469
19.1-30.0F	30.1-40.0F	0.7273	0.5556	11	9	0.698	0.2451
19.1-30.0M	30.1-40.0M	0.8333	0.4286	6	7	2.82	0.0024*

Pop: population; p1: frequency in population 1; p2: frequency in population 2; n1: number of individuals in population; n2: number of individuals in population 2; *= significant.

Graph 3



Of the entire population, a total of 86 (63.23%) of them presented HL. Among the individuals affected by HL, a total of 263 lines (see Table 13) were identified in the radiographs; 31.94% of them are located in the proximal end of the tibia, while the remaining 68.06% are present towards the distal end (see Table 14).

Table 13 Number of HL by Age-Sex Groups

	Undetermined		Female		Male	
	N	%	N	%	N	%
0-3.0	52	25.62				
3.1-7.0	67	33.00				
7.1-12.0	40	19.70				
12.1-19.0	44	21.67				
19.1-30.0			19	44.19	4	23.53
30.1-40.0			16	37.21	7	41.18
>40			8	18.60	6	35.29
Total	203		43	100	17	100

Table 14 Number, Percentage and Location of HL.

Proximal		Distal		Total	
n	%	n	%	N	%
84	31.94	179	68.06	263	100

These results are in agreement with other studies, which had identified a higher incidence of HL towards the distal end of the tibia (Garn et al., 1968). When this distribution is analyzed considering both sex and age, it is possible to see that there is a different distribution of the lines that appears at an early age (age range 0-3.0 years; see Table 15).

The 0-3.0 years old age range, however, is the only one that presents an equal distribution of lines at both distal and proximal ends. At more advanced ages, the distribution shows a clear preference for HL to be formed in the distal end of the tibia (see Table 15).

The majority of HL are present in immature individuals (74.90%), followed by the females (16.35%), while males are the ones who present the lowest number of lines (7.22%). This difference between sexes does not reveal any significant statistical

association between HL and the sex of the individual (Chi^2=0.000, df=1 p(α)=1.00) and it might be the result of the limited sample size.

Table 15 Number, Percentage and Location of HL by Age-Sex Category

	Undetermined			Female			Male		
		Proximal	Distal		Proxima	Distal		Proximal	Distal
	N	%	%	N	%	%	N	%	%
0-3.0	54	50.00	50.00						
3.1-7.0	67	44.78	55.22						
7.1-12.0	40	15.00	85.00						
12.1-19.0	40	22.50	77.50						
19.1-30.0				19	15.79	84.21	8	12.50	87.50
30.1-40.0				16	37.50	62.50	5	20.00	80.00
>40				8	12.50	87.50	6	0.00	100.00

V.1.2. Harris Lines Frequency According to Age at Time of Line Formation

Two methods were utilized in order to estimate the age of the individuals at the time of HL formation: 1) Byers (1991); and, 2) Hummert and Van Gerven (1985). The first method is only applicable in individuals that have fused epiphyses, while the second one can be applied in both adults and juveniles.

Age at the time of HL formation will subsequently be compared with the age of the individuals at the time of EH formation in order to determine if any correlation between the two indicators exist.

V.1.2.1. Harris Lines Results:

Byers's (1991) Method

The total sample analyzed with this method includes males, females, and some juveniles of the 12.1-19.0 years old group who presented their epiphyses fused. Thirty-seven individuals, then, were analyzed with this method to determine when the HL formed. These individuals presented a total of 81 lines, distal and proximal. Distance to the closest epiphysis was calculated and then converted into an age of the individual at the time of line formation (see Chapter II for an explanation of the method). Fifty six (69.14%) of these lines correspond to female individuals, while 25 (30.86%) correspond to males.

When all the lines under analysis are considered together, the results show that (see Table 16) for males and females, the age at the time of line formation presents its highest peak before the first year (19.75%) and during adolescence (14.81%; see graph 4). While at other ages the incidence of HL seems more equally dispersed (see graph 3). Before age 1, the individuals, both males (28.00%) and females (16.07%), form the greatest number of lines. This trend tends to diminish towards the age of 3, and the formation of the lines presents a more even, and low, frequency until the age of 11. At this point female individuals present their second peak, while males exhibit this second peak two years later at the age of 13 (12.00%; see graph 4).

It is extremely interesting that 37.04% (see table 16) of the lines were formed before the age of three. A number of studies have shown that there is a common peak age of occurrence between the one-to-three years of age interval in the formation of HL among different groups (Clarke and Gindhart, 1981).

When considered by age-sex categories (see Table 17) it is evident that most of the groups show a clear peak of HL formation before the 1st year, except for the males over 40 years, although this difference may be the result of the few number of lines identified in this group. Most of the age-sex groups show a second peak between the ages 10 and 14, with some exceptions (like the 30.1-40.0 males) that are possibly the result of the reduced size of the sample.

Table 16 Age at the Time of HL Formation, Byers' (1991) Method

	Female		Male		Total	
Age at Occurrence	N	%	N	%	N	%
<1	9	16.07	7	28.00	16	19.75
1	5	8.93	1	4.00	4	4.94
2	4	7.14	0	0.00	4	4.94
3	4	7.14	4	16.0	6	7.41
4	3	5.36	1	4.00	4	4.94
5	2	3.57	0	0.00	2	2.47
6	3	5.36	2	8.00	4	4.94
7	2	3.57	1	4.00	3	3.70
8	3	5.36	1	4.00	4	4.94
9	5	8.93	2	8.00	7	8.64
10	4	7.14	1	4.00	8	9.88
11	8	14.29	0	0.00	12	14.81
12	1	1.79	1	4.00	3	3.70
13	2	3.57	3	12.0	3	3.70
14	1	1.79	1	4.00	1	1.23
15	0	0.00	0	0.00	0	0.00
16	0	0.00	0	0.00	0	0.00
17	0	0.00	0	0.00	0	0.00
Total	56	100.00	25	100.00	81	100.00

Thus when using Byer's method (1991), considered either in general or by sex-age categories, HL formation presents two clear peaks one during infancy, and a second one during adolescence.

Graph 4 Age at Time of HL Formation: Byers' (1991) Method

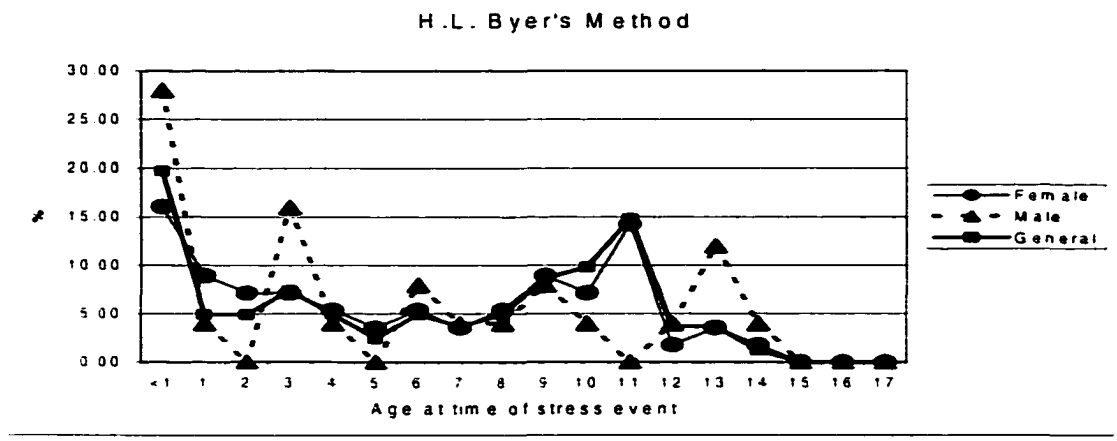


Table 17 Age at the Time of HL Formation by Age and Sex Group, Byers' (1991) Method.

	12.1-19.0		19.1-30.0 Female		19.1-30.0 Male		30.1-40.0 Female		30.1-40.0 Male		>40 Female		>40 Male	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
<1	5	23.81	4	21.05	3	75.00	6	19.35	2	28.57	1	12.50	0	0.00
1	2	9.52	1	5.26	0	0.00	1	3.23	0	0	0	0.00	0	0.00
2	1	4.76	1	5.26	0	0.00	2	6.45	0	0	1	12.50	0	0.00
3	3	14.29	0	0.00	0	0.00	1	3.23	1	14.29	0	0.00	1	16.67
4	2	9.52	0	0.00	0	0.00	1	3.23	1	14.29	0	0.00	0	0.00
5	0	0.00	1	5.26	0	0.00	0	0	0	0	1	12.50	0	0.00
6	0	0.00	0	0.00	0	0.00	2	6.452	1	14.29	1	12.50	1	16.67
7	1	4.76	1	5.26	0	0.00	0	0	0	0	0	0.00	1	16.67
8	0	0.00	1	5.26	0	0.00	2	6.45	1	14.29	1	12.50	0	0.00
9	1	4.76	2	10.53	0	0.00	3	9.68	0	0	1	12.50	1	16.67
10	2	9.52	3	15.79	0	0.00	3	9.68	1	14.29	0	0.00	0	0.00
11	2	9.52	3	15.79	0	0.00	10	32.26	0	0	2	25.00	0	0.00
12	1	4.76	2	10.53	0	0.00	0	0	0	0	0	0.00	0	0.00
13	1	4.76	0	0.00	0	0.00	0	0	0	0	0	0.00	2	33.33
14	0	0.00	0	0.00	1	25.00	0	0	0	0	0	0.00	0	0.00
15	0	0.00	0	0.00	0	0.00	0	0	0	0	0	0.00	0	0.00
16	0	0.00	0	0.00	0	0.00	0	0	0	0	0	0.00	0	0.00
17	0	0.00	0	0.00	0	0.00	0	0	0	0	0	0.00	0	0.00
Total	21	100.00	19	100.00	4	100.00	31	100.00	7	100.00	8	100.00	6	100.00

V.1.2.2. Harris Lines Results: Hummert and Van Gerven's (1985) Method

Hummert and Van Gerven's (1985) method was used to obtain HL data from the younger aged individuals who did not have fused epiphyses. This method was also used on older individuals, and the results obtained among individuals who presented fused epiphyses were compared with the results obtained with the Byers' (1991) method.

In order to apply Hummert and Van Gerven's (1985) method, two tables of tibial growth had to be constructed. These tables of growth differentiate distal from proximal growth and, therefore, allow one to estimate the age of the individual at the time of line formation for both distal and proximal HL. Eighty-seven tibias, corresponding to 87 different individuals, ranging between unborn to 19 years of age were considered (see Table 18). The diaphyseal length was measured directly from the radiographs.

Following Hummert and Van Gerven's (1985) recommendation, the two tables for proximal and distal diaphyseal length were constructed (see Table 19 and Table 20). The sample did not contain any individuals of 7, 11 or 13 years of age: those ages were omitted from the tables 19 and 20.

Because Hummert and Van Gerven's (1985) method is applicable to both juveniles and adults, all the individuals (N=136; see Table 7) present in the sample, and all the lines (N=263; see Table 13) identified in them, where analyzed. Most of the transverse lines are present among the juveniles (undetermined sex), followed by females, while the ones with fewest lines are the males (seeTable 21). This difference among males and females, as already mentioned, is not statistically significant, and might be the result of the small size of the sample.

Table 18 Tibial Length

		Total Diaphyseal Length		Proximal		Distal	
Age	N	μ	%	μ	%	μ	%
U.B.	3	62.33	21.16	35.53	21.16	26.80	21.15
Birth	6	68.67	23.31	39.14	23.30	29.53	23.30
6m	2	75.50	25.62	43.04	25.62	32.47	25.62
9m	1	83.00	28.17	47.31	28.17	35.69	28.17
1	3	94.67	32.13	53.96	32.13	40.71	32.13
1.5	11	100.55	34.12	57.31	34.12	43.23	34.12
2	7	122.29	41.50	69.70	41.50	52.58	41.50
3	6	148.67	50.46	84.74	50.46	63.93	50.46
4	9	156.00	52.95	88.92	52.94	67.08	52.94
5	3	172.67	58.60	98.42	58.60	74.25	58.60
6	3	195.33	66.30	111.34	66.29	83.99	66.29
8	3	208.67	70.82	118.94	70.82	89.73	70.82
9	3	227.67	77.27	129.77	77.27	97.90	77.27
10	5	250.60	85.05	142.84	85.05	107.76	85.05
12	4	251.50	85.36	143.36	85.36	108.15	85.36
14	1	252.00	85.53	143.64	85.53	108.36	85.52
15	5	284.00	96.39	161.88	96.39	122.12	96.39
16-19	12	294.64	100.00	167.95	100.00	126.70	100.00

Table 19 Chronology of radiopaque transverse lines for the proximal tibia as measured by the percentage of proximal growth complete by age Group

	UB	Birth	6m	9m	1	1.5	2	3	4	5	6	8	9	10	12	14	15	16-19
UB	100	90.8	82.6	75.1	65.8	62.0	51.0	41.9	40.0	36.1	31.9	29.9	27.4	24.9	24.8	24.7	21.9	21.2
Birth		100	90.9	82.7	72.5	68.3	56.2	46.2	44.0	39.8	35.2	32.9	30.2	27.4	27.3	27.2	24.2	23.3
6m			100	91.0	79.8	75.1	61.8	50.8	48.4	43.7	38.7	36.2	33.2	30.1	30.0	30.0	26.6	25.6
9m				100	87.7	82.6	67.9	55.8	53.2	48.1	42.5	39.8	36.5	33.1	33.0	32.9	29.2	28.2
1					100	94.2	77.4	63.7	60.7	54.8	48.5	45.4	41.6	37.8	37.6	37.6	33.3	32.1
1.5						100	82.2	67.6	64.5	58.2	51.5	48.2	44.2	40.1	40.0	39.9	35.4	34.1
2							100	82.3	78.4	70.8	62.6	58.6	53.7	48.8	48.6	48.5	43.1	41.5
3								100	95.3	86.1	76.1	71.2	65.3	59.3	59.1	59.0	52.3	50.5
4									100	90.3	79.9	74.8	68.5	62.3	62.0	61.9	54.9	52.9
5										100	88.4	82.7	75.8	68.9	68.7	68.5	60.8	58.6
6											100	93.6	85.8	77.9	77.7	77.5	68.8	66.3
8												100	91.7	83.3	83.0	82.8	73.5	70.8
9													100	90.8	90.5	90.3	80.2	77.3
10														100	99.6	99.4	88.2	85.0
12															100	99.8	88.6	85.4
14																100	88.7	85.5
15																	100	96.4
16-19																		100

Table 20 Chronology of radiopaque transverse lines for the distal tibia as measured by the percentage of distal growth complete by Age Group

	UB	Birth	6m	9m	1	1.5	2	3	4	5	6	8	9	10	12	14	15	16-19
UB	100	90.8	82.5	75.1	65.8	62.0	51.0	41.9	40.0	36.1	31.9	29.9	27.4	24.9	24.8	24.7	21.9	21.2
Birth		100	90.9	82.7	72.5	68.3	56.2	46.2	44.0	39.8	35.2	32.9	30.2	27.4	27.3	27.3	24.2	23.3
6m			100	91.0	79.8	75.1	61.8	50.8	48.4	43.7	38.7	36.2	33.2	30.1	30.0	30.0	26.6	25.6
9m				100	87.7	82.6	67.9	55.8	53.2	48.1	42.5	39.8	36.5	33.1	33.0	32.9	29.2	28.2
1					100	94.2	77.4	63.7	60.7	54.8	48.5	45.4	41.6	37.8	37.6	37.6	33.3	32.1
1.5						100	82.2	67.6	64.4	58.2	51.5	48.2	44.2	40.1	40.0	39.9	35.4	34.1
2							100	82.2	78.4	70.8	62.6	58.6	53.7	48.8	48.6	48.5	43.1	41.5
3								100	95.3	86.1	76.1	71.2	65.3	59.3	59.1	59.0	52.4	50.5
4									100	90.3	79.9	74.8	68.5	62.2	62.0	61.9	54.9	52.9
5										100	88.4	82.7	75.8	68.9	68.7	68.5	60.8	58.6
6											100	93.6	85.8	77.9	77.7	77.5	68.8	66.3
8												100	91.7	83.3	83.0	82.8	73.5	70.8
9													100	90.9	90.5	90.3	80.2	77.3
10														100	99.6	99.4	88.2	85.1
12															100	99.8	88.6	85.4
14																100	88.7	85.5
15																	100	96.4
16-19																		100

Using Hummert and Van Gerven's (1985) method, the analysis of age at time of line formation shows that, among the juveniles (age ranges 0-3.0 and 3.1-7.0) there is an important concentration of line formation at the age of 2 years. This trend is not as clearly present among the 7.1-12.0 years old, who peak at age 6 and at the age of 7 (see Table 22).

Table 21 Number of HL by Sex-Age Groups: Hummert and Van Gerven's (1985)

Undetermined		Female		Male		Total	
N	%	N	%	N	%	N	%
203	77.19	43	16.35	17	6.46	263	100

Among the adults, and because they lived longer than the juveniles, it is possible to distinguish two peaks, one between the ages <1-3 years, and a second one around 14-15 years (see Table 23). These two peaks are not clear among the females and males of 30.1-

40.0 years or among the females of >40years of age. This may be the result, however, of the small number of lines present in these segments (see Table 23).

Table 22 Age at the Time of HL Formation Among Juveniles, Hummert and Van Gerven's (1985) Method.

Age/Age group	0-3.0		3.1-7.0		7.1-12.0		12.1-19.0	
	N	%	N	%	N	%	N	%
<1	10	19.23	4	5.97	2	5.00	8	18.18
1	12	23.08	1	1.49	2	5.00	5	11.36
2	25	48.08	35	52.24	6	15.00	9	20.45
3	5	9.62	16	23.88	1	2.50	1	2.27
4			4	5.97	4	10.00	1	2.27
5			5	7.46	5	12.50	5	11.36
6			2	2.99	10	25.00	1	2.27
7			0	0.00	0	0.00	0	0.00
8					7	17.50	0	0.00
9					3	7.50	4	9.09
10					0	0.00	3	6.82
11					0	0.00	0	0.00
12					0	0.00	0	0.00
13							0	0.00
14							5	11.36
15							2	4.55
16							0	0.00
17							0	0.00
Total	52	100.00	67	100.00	40	100.00	44	100.00

When all the lines present in the population are considered together, the greatest incidence of HL occur at the age of 2 years (30.80%; see Table 24). This distribution, however, is skewed due to the high number of juveniles in the population. As these individuals died prematurely, their remains only account for the earliest years of development.

Table 23 Age at the Time of HL Formation Among Adults, Hummert and Van Gerven's (1985) Method.

Age/Age group	19.1-30.0 Female		19.1-30.0 Male		30.1-40.0 Female		30.1-40.0 Male		>40 Female		>40 Male	
	N	%	N	%	N	%	N	%	N	%	N	%
<1	5	26.32	2	50.00	1	6.25	0	0.00	1	12.50	0	0.00
1	0	0.00	1	25.00	1	6.25	2	28.57	0	0.00	0	0.00
2	1	5.26	0	0.00	1	6.25	2	28.57	1	12.50	1	16.67
3	0	0.00	0	0.00	2	12.50	0	0.00	0	0.00	0	0.00
4	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
5	0	0.00	0	0.00	1	6.25	1	14.29	1	12.50	1	16.67
6	1	5.26	0	0.00	0	0.00	1	14.29	1	12.50	1	16.67
7	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
8	2	10.53	0	0.00	1	6.25	0	0.00	0	0.00	1	16.67
9	2	10.53	1	25.00	3	18.75	1	14.29	2	25.00	0	0.00
10	2	10.53	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
11	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
12	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
14	2	10.53	0	0.00	1	6.25	0	0.00	0	0.00	1	16.67
15	4	21.05	0	0.00	5	31.25	0	0.00	2	25.00	1	16.67
16	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
17	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Total	19	100.00	4	100.00	16	100.00	7	100.00	8	100.00	6	100.00

When all the lines present in the individuals with fused epiphyses are grouped (the same ones as considered with the Byers' method), it is clear that there are two main peaks (see Table 25). The fist peak occurs before the 1st year of age and a second one at the age of 15 (see Graph 5 and Table 25).

In general, the defects formed during early years tend to concentrate between the ages 0-3. There is a mild peak at 9 years of age and a second important one at the age of 15 (see Graph 5 and Table 25). Recall that the absence of lines at ages 7, 11 and 13 is due to the absence of individual of those ages in the sample used to construct the tibial growth tables. Thus, even if some of these lines were formed at those ages, we will only get an approximated age at time of formation. There is no difference in the peaks present among

males and females due to the fact that this method does not apply different tabulation for each sex.

Table 24 Age at the Time of HL Formation All lines Considered, Hummert and Van Gerven's Method (1985).

Age at Occurrence	N	%
<1	33	12.55
1	24	9.13
2	81	30.80
3	25	9.51
4	9	3.42
5	19	7.22
6	17	6.46
8	11	4.18
9	16	6.08
10	5	1.90
12	0	0.00
14	9	3.42
15	14	5.32
Total	263	100.00

Graph 5

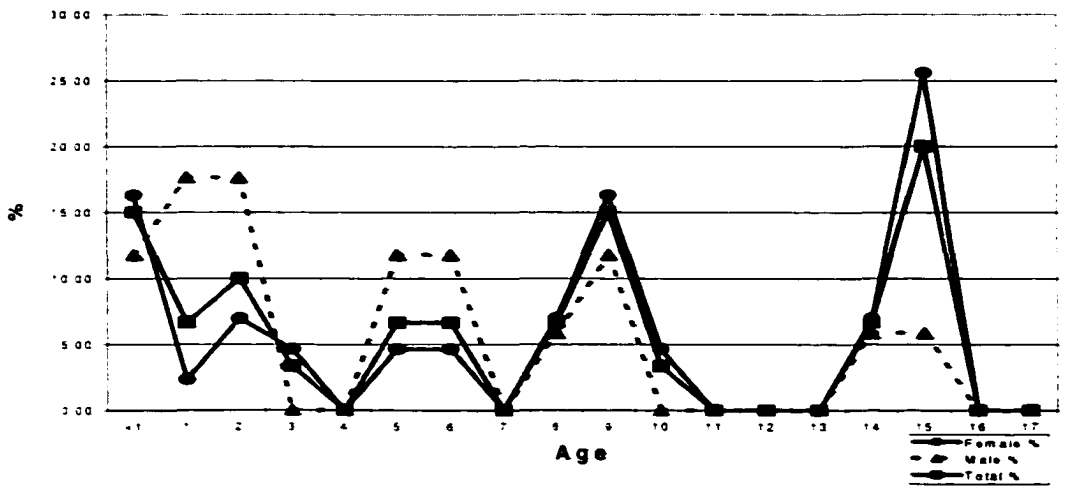


Table 25 Age at the Time of HL Formation among Individuals with Fused epiphyses: Hummert and Van Gerven's (1985) Method.

Age at Occurrence	Female		Male		Total	
	n	%	n	%	N	%
<1	7	16.28	2	11.76	9	15.00
1	1	2.33	3	17.65	4	6.67
2	3	6.98	3	17.65	6	10.00
3	2	4.65	0	0.00	2	3.33
4	0	0.00	0	0.00	0	0.00
5	2	4.65	2	11.76	4	6.67
6	2	4.65	2	11.76	4	6.67
7	0	0.00	0	0.00	0	0.00
8	3	6.98	1	5.88	4	6.67
9	7	16.28	2	11.76	9	15.00
10	2	4.65	0	0.00	2	3.33
11	0	0.00	0	0.00	0	0.00
12	0	0.00	0	0.00	0	0.00
13	0	0.00	0	0.00	0	0.00
14	3	6.98	1	5.88	4	6.67
15	11	25.58	1	5.88	12	20.00
16	0	0.00	0	0.00	0	0.00
17	0	0.00	0	0.00	0	0.00
Total	43	100.00	17	100.00	60	100.00

V.1.2.3. Comparing the Results Obtained with the Two HL Methods: Byers' (1991) v/s Hummert and Van Gerven's (1985)

Thus, the results obtained with Hummert and Van Gerven's (1985) method shows a trend that resembles the one detected with the Byers (1991) method. When comparing the results using a t-test for paired samples, considering only the lines present in individuals with fused epiphyses, it turns out that the differences between the two methods are statistically significant ($t=-2.52$, $df=80$, $p(\alpha)=0.014$). This means that when studying Harris lines, more than one method should be applied, considering that their application result in statistically different out comes. Moreover, considering the discrepancy showed

by the two methods the results may conduct to a different interpretation, and thus the researcher should be aware of the existing differences between the two methods.

V.2. Enamel Hypoplasia

Recall that HL are only one indicator of possible stress. The second indicator of stress to be considered is EH. Thus, the next step of the analysis was to consider the percentage of individuals affected, as well as the number of teeth that presented EH. In addition the age at the time of EH formation was calculated. The methods used for assessing the presence of EH were outlined in Chapter 2 and 4.

V.2.1. Enamel Hypoplasia Incidence at the Individual Level

The analysis showed that 79.41% of the populations under analysis present at least one tooth with a hypoplastic defect (see Table 11). When analyzed by sex (undetermined, female and male) and age category (see Table 26), the results show a common trend, to present a higher frequency of EH with increasing age. These frequencies, however, are not very informative, as studies have shown that it is the number of teeth affected, which more accurately indicates specific trends among the age-sex groups (Lukacs, 1989).

V.2.2. Enamel Hypoplasia: Incidence in Teeth

Each tooth in each individual's dentition was examined to determine the presence of incidents of EH. A total of 2,467 teeth were analyzed, of these 650 correspond to deciduous while 1,817 are permanent (see Table 27).

Table 26 Frequency of EH at the Individual Level

Enamel Hypoplasia									
Age/Sex	Undetermined			Female			Male		
	n	N	%	n	N	%	n	N	%
0-3.0	12	28	42.86						
3.1-7.0	12	18	66.67						
7.1-12.0	13	14	92.86						
12.1-19.0	26	27	96.30						
19.1-30.0				10	11	90.91	5	6	83.33
30.1-40.0				9	9	100.00	7	7	100.00
>40				10	12	83.33	4	4	100.00
Total	63	87	72.41	29	32	90.63	16	17	94.12

Table 27 Teeth Sample: Deciduous and Permanent

	n	N	%	Σ EH	μ
Deciduous	32	650	4.92	37	1.16
Permanent	633	1817	34.84	989	1.56
Total	665	2467	26.96	1026	1.54

Σ EH: Sumatory of hypoplastic defects; μ: average number of defects per affected tooth.

A low percentage of deciduous teeth exhibit EH (4.92%), while 34.84% of permanent teeth show some type of enamel hypoplasia; a difference that is statistically significant ($Z=5.09$; $p(\alpha)= 0.0000$). Among deciduous teeth the average number of defects per affected tooth is 1.16, while among permanent teeth the average is slightly higher (1.56). Although small, this difference is statistically significant (see Table 28).

Table 28 τ test for Average Number of EH Defects: Deciduous v/s Permanent Teeth

S.1	S. 2	μ1	μ2	S1 K-S	P2 K-S	σ^2	Fç	σ_2^2	n1	n2	τ	Df	p (α)
Decid	Perman	1.16	1.58	2.89	8.93	0.30	1.46	No	32	633	5.9	18	<0.05

S.1: sample 1; S.2: sample 2; μ1: average of EH defects per tooth affected in sample 1; μ2: average of EH defects per tooth affected in sample 2. K-S: Kolmogorov-Smirnov; σ: variance; t= Student's t-test; Df: degrees of freedom.

Individuals between 0-7.0 years of age (see Table 29) present a low percentage of teeth with EH, mainly because these individuals have, primarily, deciduous teeth. Adults, in general, have around 30% of their teeth affected, and the average number of defects per tooth affected is relatively similar among all the different age and sex categories. The average number of defects does not reach 2.0 and the results do not show any consistent pattern of higher or lower percentage or average between the sexes.

Table 29 EH Incidence by Teeth counting in each Age-Sex Category

	n	N	%	Σ EH	μ
0-3.0	23	365	6.30	28	1.22
3.1-7.0	24	263	9.13	30	1.25
7.1-12.0	100	270	37.04	191	1.91
12.1-19.0	225	598	37.63	355	1.58
19.1-30.0F	73	241	30.29	113	1.55
19.1-30.0M	46	155	29.68	86	1.87
30.1-40.0F	67	174	38.51	86	1.28
30.1-40.0M	30	142	21.13	39	1.30
>40F	46	133	34.59	65	1.41
>40M	26	59	44.07	33	1.27

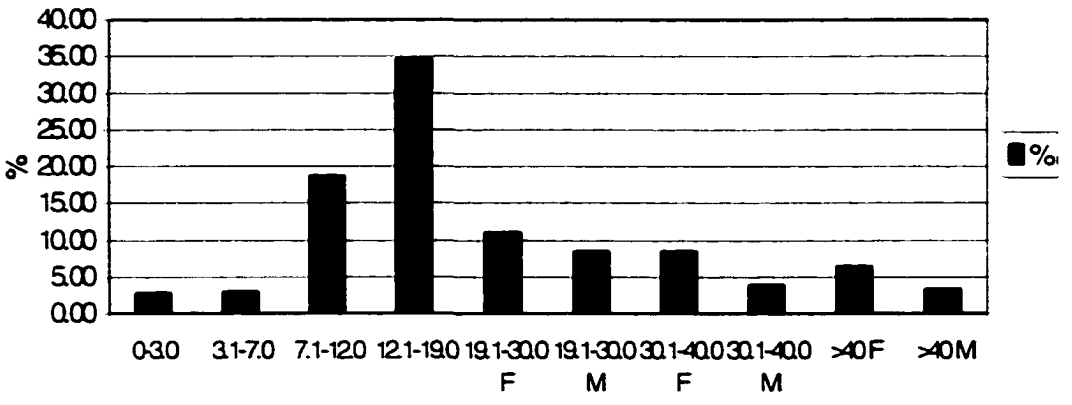
Σ EH: Addition of hypoplastic defects; μ: average number of defects per affected tooth.

Table 30 Number of EH Defects by Age-Sex Groups

	N	%
0-3.0	28	2.73
3.1-7.0	30	2.92
7.1-12.0	191	18.62
12.1-19.0	355	34.60
19.1-30.0 Female	113	11.01
19.1-30.0 Male	86	8.38
30.1-40.0 Female	86	8.38
30.1-40.0 Male	39	3.80
>40 Female	65	6.34
>40 Male	33	3.22
Total	1026	100.00

In the 665 teeth that presented EH, a total of 1026 defects were identified (see Table 30 and Graph 6). The majority of the defects were formed in individuals of 12.1-19.0 years (34.60%).

Graph 6



Of the 1026 EH defects, 37 correspond to defects in deciduous teeth while the remaining 989 are located in permanent teeth (see Table 31).

Table 31 Number of EH Defects in Deciduous and Permanent Teeth

	N	%
Deciduous	37	3.61
Permanent	989	96.39
Total	1026	100.00

Deciduous teeth are not only less affected, they also present a considerably lower average number of defects per affected tooth (see Table 27).

V.2.3. Enamel Hypoplasia Frequency According to Age at Time of Formation

In total, three different methods were applied to estimate the age of the individual at the time of EH formation. Enamel hypoplasia in deciduous teeth was analyzed according to Blakeley and Armelagos' (1985) method. Those defects present in the permanent teeth were analyzed with both the Goodman and Armelagos' (1990) method, and an adjusted version of this method.

Considering that there are only 37 defects in the deciduous teeth in total, and that all of them were formed before the first year of age, the results will be presented as follow: the results of Blakeley and Armelagos's (1985) method are combined with the ones obtained with the Goodman and Rose's (1990) method. A second analysis considers the Blakeley and Armelagos' (1995) method results along with the ones obtained using the adjusted version of the Goodman and Rose's method. Thus the results for the deciduous teeth are included in the comparison between the Goodman and Rose *versus* the "adjusted method" results.

Blakeley and Armelagos' (1985) method required the estimation of mean crown height by tooth type in deciduous dentition. Left maxilla and mandible deciduous teeth that presented minimum (2^{nd} degree according to Smith, 1984) or no wear were considered. The results for mean crown height are presented in tables 32 and 33.

Table 32 Upper Deciduous Teeth Crown Heights

	i1	i2	C	m1	m2
Mean	6.63	5.83	6.66	5.84	6.40
N	14	14	15	31	24
Standard Deviation	1.89	0.39	0.60	0.36	0.50
Sample Variance	3.57	0.15	0.35	0.13	0.25
Minimum	5.26	4.85	5.72	5.1	5.64
Maximum	13	6.52	7.8	6.44	7.3

Table 33 Lower Deciduous Teeth Crown Heights

	i1	i2	C	m1	m2
Mean	5.62	5.94	6.86	6.73	6.58
Count	17	18	22	30	19
Standard Deviation	1.05	0.33	0.56	0.42	0.59
Sample Variance	1.10	0.11	0.31	0.18	0.35
Minimum	4.61	5.19	6	5.57	5.29
Maximum	9.3	6.59	7.76	7.4	7.55

Table 34 Blakeley and Armelagos (1985): Deciduous Dentition Adjusted

	Crown Height			Developmental Age (in months)			Regression Equation	
	Mean	s.d.	N	At cusp (prenatal)	At CEJ (postnatal)	Duration	Average	Equation
Maxilla								
i1	6.630	1.889	14	5	-4	9	0.737	-(Ht/0.737)+4
i2	5.828	0.392	14	5	5	10	0.583	-(Ht/0.583)+5
C	6.661	0.595	15	6	9	13	0.512	-(Ht/0.512)+9
m1	5.837	0.361	31	5	6	11	0.531	-(Ht/0.531)+6
m2	6.404	0.500	24	6	12	15	0.427	-(Ht/0.427)+12
Mandible								
i1	5.624	1.052	17	5	-4	9	0.625	-(Ht/0.625)+4
i2	5.941	0.332	18	5	5	10	0.594	-(Ht/0.594)+5
C	6.862	0.558	22	6	9	13	0.528	-(Ht/0.528)+9
m1	6.732	0.422	30	5	6	11	0.612	-(Ht/0.612)+6
m2	6.578	0.591	19	6	12	15	0.439	-(Ht/0.439)+12

Ht: distance EH-CEJ

Blakeley and Armelagos's (1985) regression equations were then adjusted to the mean deciduous crown height in this sample (see Table 34). Utilizing the mean crown

height for each type of deciduous teeth and considering its time of development the Blakeley and Armelagos' (1985) formulas were applied to estimate the age of each individual at the time of the defect formation.

In order to adjust the Goodman and Rose's (1990) formulas to this specific sample, teeth of the left maxilla and mandible that presented none or minimum degree of wear (Smith, 1984) were considered (see tables 35 and 36).

Table 35 Permanent Upper Teeth Crown Heights

	I1	I2	C	PM1	PM2	M1	M2	M3
Mean	11.21	9.73	10.97	8.20	7.17	7.44	7.45	6.26
Count	18	18	17	33	34	21	32	21
Standard Deviation	0.67	0.76	1.06	0.86	0.82	0.58	0.57	0.55
Sample Variance	0.46	0.58	1.13	0.74	0.67	0.34	0.33	0.30
Minimum	10.29	8.3	9.13	6.49	5.39	6.02	6.23	4.94
Maximum	12.44	11.56	12.92	9.99	8.67	8.64	8.62	7.12

Table 36 Permanent Lower Teeth Crown Heights

	I1	I2	C	PM1	PM2	M1	M2	M3
Mean	9.42	9.66	11.37	8.33	7.29	7.83	7.47	6.45
Count	16.00	20.00	16.00	31.00	28.00	14.00	18.00	15.00
Standard Deviation	0.57	0.53	1.31	0.98	0.79	0.52	0.70	0.75
Sample Variance	0.32	0.28	1.71	0.96	0.63	0.27	0.49	0.57
Minimum	7.95	8.55	8.99	6.17	5.33	6.96	5.79	4.57
Maximum	10.15	10.74	14.12	10.36	8.72	8.71	8.78	7.66

Then, considering the time needed for the crown to be formed (assuming that this is standard), the regression equations of Goodman and Rose (1990) for permanent teeth were adjusted (see Table 37). Since the defects present in third molars were not

considered in these calculations the number of defects that were converted into age of the individual at the time of the defect formation is slightly smaller than the total number of defects identified (N=1016).

Table 37 Goodman and Rose (1990): Permanent Dentition Adjusted

	Crown Height			Developmental Age (in years)			Regression Equation	
	Mean	s.d.	N	At cusp	At CEJ	Duration	Average	Equation
Maxilla								
I1	11.209	0.675	18	1.0	4.5	3.5	0.312	=-(Ht*0.312)+4.5
I2	9.729	0.764	18	2.0	4.5	2.5	0.257	=-(Ht*0.257)+4.5
C	10.972	1.062	17	1.0	6.5	5.5	0.501	=-(Ht*0.501)+6.5
PM1	8.197	0.863	33	3.0	6.0	3.0	0.366	=-(Ht*0.366)+6.0
PM2	7.174	0.819	34	3.5	6.0	2.5	0.348	=-(Ht*0.348)+6.0
M1	7.439	0.579	21	1.0	3.5	2.5	0.336	=-(Ht*0.336)+3.5
M2	7.452	0.570	32	4.0	7.5	3.5	0.470	=-(Ht*0.470)+7.5
M3	6.258	0.549	21					
Mandible								
I1	9.417	0.568	16	1.0	4.0	3.0	0.319	=-(Ht*0.319)+4.0
I2	9.660	0.527	20	1.0	4.0	3.0	0.311	=-(Ht*0.311)+4.0
C	11.367	1.307	16	1.5	4.5	3.0	0.264	=-(Ht*0.2.64)+4.5
PM1	8.326	0.979	31	2.0	6.0	4.0	0.480	=-(Ht*0.480)+6.0
PM2	7.290	0.791	28	3.0	7.0	4.0	0.549	=-(Ht*0.549)+7.0
M1	7.826	0.516	14	1.0	3.5	2.5	0.319	=-(Ht*0.319)+3.5
M2	7.472	0.700	18	4.0	7.0	3.0	0.401	=-(Ht*0.401)+7.0
M3	6.451	0.755	15					

Ht: distance EH-CEJ

Table 38 Age at Time of EH Formation Juveniles: Blakeley and Armelagos (1985), Goodman and Rose (1990)

Age	0-3.0		3.1-7.0		7.1-12.0		12.1-19.0	
	N	%	N	%	N	%	N	%
<1	28	100.00	9	30.00	5	2.62	2	0.56
1	0	0.00	1	3.33	7	3.66	16	4.52
2	0	0.00	9	30.00	20	10.47	78	22.03
3	0	0.00	10	33.33	79	41.36	103	29.10
4	0	0.00	1	3.33	34	17.80	74	20.90
5	0	0.00	0	0.00	29	15.18	53	14.97
6	0	0.00	0	0.00	15	7.85	22	6.21
7	0	0.00	0	0.00	2	1.05	6	1.69
Total	28	100.00	30	100.00	191	100.00	354	100.00

Table 39 Age at Time of EH Formation Adults: Blakeley and Armelagos (1985), Goodman and Rose (1990)

Age	19.1-30.0 Female		19.1-30.0 Male		30.1-40.0 Female		30.1-40.0 Male		>40 Female		>40 Male	
	N	%	N	%	N	%	N	%	N	%	N	%
<1	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
1	3	2.70	1	1.16	1	1.18	1	2.70	0	0.00	0	0.00
2	25	22.52	7	8.14	8	9.41	1	2.70	5	7.69	4	12.50
3	39	35.14	24	27.91	21	24.71	9	24.32	16	24.62	4	12.50
4	22	19.82	24	27.91	17	20.00	12	32.43	13	20.00	5	15.63
5	17	15.32	24	27.91	22	25.88	10	27.03	17	26.15	9	28.13
6	5	4.50	6	6.98	14	16.47	4	10.81	12	18.46	6	18.75
7	0	0.00	0	0.00	2	2.35	0	0.00	2	3.08	1	3.13
Total	111	100.00	86	100.00	85	100.00	37	100.00	65	100.00	29	90.63

In the permanent teeth, for the age range of 7.1-30.0 (see Table 39), all the age-sex groups (except for the 19.1-30.0 males), present a peak of EH formation at the age of 3. From individuals of 30.1 years and older, the peak moves to the age of 5.

V.2.3.2. Results Obtained with the Blakeley and Armelagos' (1985) Method and Adjusted Goodman and Rose's Method.

The adjusted formulas for the Goodman and Rose's (1990) method, shows a similar distribution of the frequencies compared with the results obtained with the original method (see tables 40 and 41). In a comparison of juveniles, similar results were obtained and the peak age of EH formation was 3.0 years of age (seeTable 40); the same peak was obtained with the Goodman and Rose's (1990) and Blakeley and Armelagos' (1985) method.

Among the adults, however, the results obtained with the adjusted regression equations show a slightly earlier peak, at age 4 (see Table 41). Nevertheless, the difference, between the individuals that died earlier and the ones that died after adulthood was achieved, is still present.

Table 40 Age at the Time of EH Formation by Sex-Age Group Among Juveniles: Blakeley and Armelagos (1985), Adjusted Goodman and Rose.

Age	0-3.0		3.1-7.0		7.1-12.0		12.1-19.0	
	N	%	N	%	N	%	N	%
<1	28	100.00	9	30.00	0	0.00	0	0.00
1	0	0.00	0	0.00	1	0.52	0	0.00
2	0	0.00	2	6.67	14	7.33	41	11.58
3	0	0.00	17	56.67	81	42.41	139	39.27
4	0	0.00	2	6.67	57	29.84	85	24.01
5	0	0.00	0	0.00	21	10.99	58	16.38
6	0	0.00	0	0.00	15	7.85	21	5.93
7	0	0.00	0	0.00	2	1.05	10	2.82
Total	28	100.00	30	100.00	191	100.00	354	100.00

Table 41 Age at the Time of EH Formation by Sex-Age Group Among Adults: Blakeley and Armelagos (1985), Adjusted Goodman and Rose.

Age	19.1-30.0 Female		19.1-30.0 Male		30.1-40.0 Female		30.1-40.0 Male		>40 Female		>40 Male	
	N	%	N	%	N	%	N	%	N	%	N	%
<1	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
1	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
2	11	9.91	3	3.49	4	4.71	2	5.41	2	3.08	2	6.25
3	38	34.23	21	24.42	21	24.71	6	16.22	20	30.77	6	18.75
4	36	32.43	27	31.40	28	32.94	17	45.95	25	38.46	3	9.38
5	20	18.02	26	30.23	14	16.47	9	24.32	7	10.77	7	21.88
6	6	5.41	9	10.47	15	17.65	2	5.41	9	13.85	9	28.13
7	0	0.00	0	0.00	3	3.53	1	2.70	2	3.08	2	6.25
Total	111	100.00	86	100.00	85	100.00	37	100.00	65	100.00	29	90.63

V.2.3.3. Comparison between Goodman and Rose's (1990) Regression Equations and Goodman and Rose (1990) adjusted Regression Equations.

A paired t-test was used to establish if the original regression equations and the ones adjusted to the mean crown height alter the results (see Table 42). The results show not only a significant difference in the general results obtained with both systems of equations, but also that the results are different when analyzed by tooth type (see Table 42).

Usually, in EH studies, the results are rounded to the closest whole number (e.g. Goodman and Rose, 1990; Wright, 1997; Santos and Coimbra, 1999). In this case, even when the results are rounded, the difference is clearly reflected in the distribution (see tables 38, 39, 40 and 41), and thus the discrepancy between the two methods may result in a different interpretation, depending upon which method is applied to a particular sample of individuals. Moreover, when grouped by age category, the distribution is also dissimilar. While the unadjusted formula shows a higher number of defects formed between the ages 0-3.0 years of age, the adjusted one shows a higher frequency in the segment 3.1-7.0 years of age (see Table 43).

Table 42 Paired t-test Goodman and Rose (1990) / Goodman and Rose Adjusted

	Mean	SD	SE of Mean	Mean	SD	SE of Mean	t-value	Df	p(α)
Maxilla									
I1	2.72	0.698	0.829	3.28	0.48	0.057	-21.423	70	0.000
I2	3.11	0.716	0.101	3.61	0.458	0.647	-13.761	49	0.000
C	3.6	1.017	0.988	4.58	0.815	0.079	-49.845	105	0.000
PM1	4.13	0.702	0.108	4.61	0.521	0.803	-17.306	41	0.000
PM2	4.53	0.632	0.117	4.91	0.471	0.874	-12.437	28	0.000
M1	2.22	0.431	0.073	2.53	0.322	0.544	-17.347	34	0.000
M2	5.75	0.786	0.126	6.03	0.592	0.095	-9.11	38	0.000
Mandible									
I1	2.39	0.749	0.838	2.89	0.52	0.58	-19.19	79	0.000
I2	2.71	0.543	0.527	3.04	0.406	0.039	-24.681	105	0.000
C	4.22	1.16	0.867	3.48	0.521	0.039	15.59	178	0.000
PM1	3.81	0.934	0.105	4.36	0.67	0.787	-20.874	78	0.000
PM2	5.39	0.848	0.126	5.62	0.726	0.108	-12.716	44	0.000
M1	2.18	0.578	0.099	2.56	0.411	0.07	-13.352	33	0.000
M2	5.72	0.704	0.131	6.12	0.487	0.09	-9.795	28	0.000
General	3.63	1.331	0.044	3.89	1.145	0.038	-12.69	923	0.000

The difference is equally statistically significant for both age-sex groups (0-3.0; 3.1-7.0; $Z=119.66$; $p(\alpha)=0.000$). Therefore, if these methods are applied to a population, the results will show differences that are significant in two important ways; a) analysis of the

peak age at the time of EH formation. b) analysis of the distribution for the age of the individuals at the time of EH formation.

Table 43 Age at the Time of EH formation by Age-Group Goodman and Armelagos (1990)/ Goodman and Armelagos Adjusted.

Age at time of Stress event	Goodman and Rose (1990)		Goodman and Rose Adjusted	
	N	%	N	%
0-3.0	578	56.89	471	46.36
3.1-7.0	438	43.11	545	53.64
Total	1016	100.00	1016	100.00

V.3. Correlation between Harris Lines and Enamel Hypoplasia

The aim of this thesis was to determine if a correlation existed between EH and HL. In order to examine the correlation between these two non-specific indicators two aspects needed to be considered: a) correlation at the individual level, and b) assessment of the age of each individual at the time of HL and EH formation.

V.3.1. Correlation of Harris Lines and Enamel Hypoplasia at the individual Level

The analysis of the correlation at the individual level considered only the presence/absence of these two skeletal markers in each individual. The results were obtained considering the entire sample (juvenile and adults), and also dividing it by the age-sex categories (see Table 44).

As seen in Table 44, when the whole sample is considered there is no identifiable correlation between these two indicators of stress using a chi^2 test (chi^2: 0.21024; p(α):

0.64658). Moreover, when the sample is divided according to the age-sex categories none showed any significant correlation between EH and HL.

Table 44 Chi^2 for Enamel Hypoplasia and Harris Lines Presence/Absence at the Individual Level.

	EH-/HL-	EH+/HL-	EH-/HL+	EH+/HL+	Chi^2	Df	p(α)
General	12	37	17	70	0.21	1	0. 64658
0-3.0	8	2	8	10	2.025	1	0.15468
3.1-7.0	0	2	7	10	0.14	1	0.71357
7.1-12.0	1	3	0	10	0.24	1	0.62254
12.1-19.0	0	12	1	14	0.00	1	1
19.1-30.0 F	1	2	0	7	0.21	1	0.64548
19.1-30.0 M	0	1	1	4	0.00	1	1
30.1-40.0 F	0	4	0	5	na	na	na
30.1-40.0 M	0	4	0	3	na	na	na
>40 F	2	5	0	5	0.27	1	0.06047
>40 M	0	2	0	2	na	na	na

EH: enamel hypoplasia; HL: Harris Lines; +: positive; -: Negative; Df: degrees of freedom; p(α): type one error probability; na: not available.

V.3.2. Correlation of Harris Lines and Enamel Hypoplasia for the Age at the Time of Formation

When time of formation is taken into account, the two indicators show a very different distribution. While HL (see Graph 4 and Graph 5) show a high percentage of lines before the 1st year of age and during adolescence (11 years in females and 13 years in males; see Graph 4) EH shows a peak between ages 3 and 5 (see tables 38, 39, 40 and 41). The difference, though, may be due to the fact that EH can only be formed between >1-7 years of age while HL can be formed between >1-16 years of age, therefore if any HL formed, after the age of 7, "noise" is introduced into the data.

In order to avoid this bias, only the distribution observed in adults will be considered, as the bias is due to the fact that individuals who died at an early age did not live long enough to continue forming HL (see Wood et al., 1992). Thus, the evaluation of HL, by age at the time of formation, is skewed towards younger ages. This distribution needs to be compared, then, with EH distribution in the adult segment of the sample.

One problem is, however, that if only adults are considered, HL formed <1 year of age should be omitted, because even if a EH defect was formed at that time, it might have been registered in deciduous, but not in permanent teeth. If that were the case, the defect would not be observable if the permanent teeth had already replaced the deciduous ones. By omitting data from the <1 year of age, this bias is avoided.

One must consider that, if the population, in general, was forming a high number of EH before the 1st year of age, deciduous dentition will show a large percentage of teeth affected. Not only should the percentage of deciduous teeth be large, but also the number of defects, by deciduous tooth affected, should show a greater average. This, however, is not the case (see Table 27). These potential problems then, are not an issue in this sample: EH was not formed at the same rate as HL before the 1st year of age. Thus, it is possible to say that HL defects were formed more frequently than EH *in uterus* and during the first year of age. After the first year of age, though, the pattern is reversed and EH was formed at a faster rate than HL.

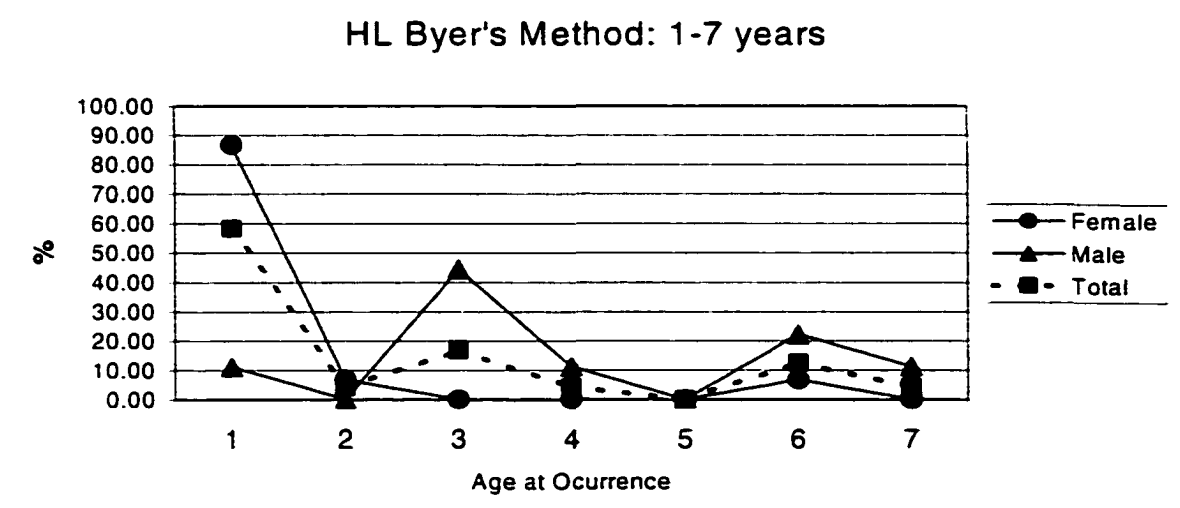
Next, the HL formed between the ages 1-7 will be considered. When the HL data is "cleaned" (only the lines present in adult and formed between 1-7 years of age are considered) and analyzed with Byer's (1990) method, the pattern of distribution shows

that most of the lines (58.33% of the total) are formed at the age of 1 (see Table 45 and Graph 7). After that their formation decreases notably.

Table 45 HL 1-7 years Byer's (1990) Method

	Female		Male		Total	
Age	N	%	N	%	N	%
1	13	86.67	1	11.11	14	58.33
2	1	6.67	0	0.00	1	4.17
3	0	0.00	4	44.44	4	16.67
4	0	0.00	1	11.11	1	4.17
5	0	0.00	0	0.00	0	0.00
6	1	6.67	2	22.22	3	12.50
7	0	0.00	1	11.11	1	4.17
Total	15	100.00	9	100.00	24	100.00

Graph 7



When the transverse HL lines, formed between the ages of 1-7 years calculated with the Hummert and Van Gerven's (1985) method, are considered it is clear that most of the HL (40%) are formed at the age of 2. A total of 60% is formed between the ages 1-2: after the age of 2. HL formation decreases noticeably (see Table 46 and Graph 8).

When EH defects are analyzed for the adult population only, the results show a contrastingly different distribution in comparison with HL. When the Goodman and Rose's (1990) formulas are considered, the majority of EH defects (72.6% of the total) are concentrated between the ages 3-5 years (see tables 47 and 48).

Table 46. HL 1-7 years Hummert and Van Gerven's (1985) Method

Age/Sex	Female		Male		Total	
Age	n	%	n	%	n	%
1	2	11.76	4	30.77	6	20.00
2	7	41.18	5	38.46	12	40.00
3	2	11.76	0	0.00	2	6.67
4	1	5.88	0	0.00	1	3.33
5	3	17.65	2	15.38	5	16.67
6	2	11.76	2	15.38	4	13.33
7	**	**	**	**	**	**
Total	17	100.00	13	100.00	30	100.00

Graph 8

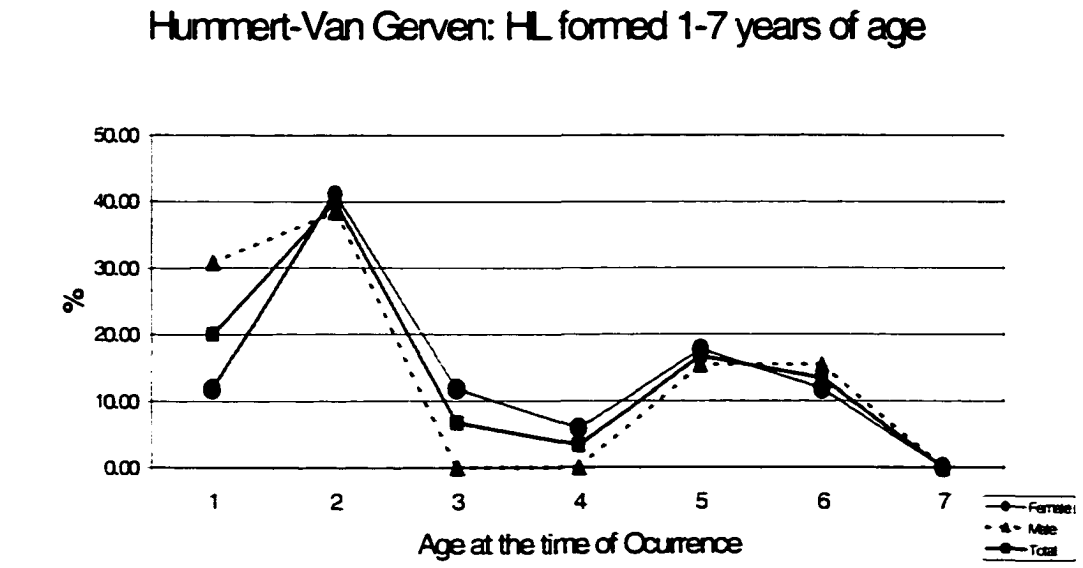


Table 47. EH in Adults by Age at the Time of Formation: Goodman and Rose (1990)

Age/Sex	Female		Male		Total	
Age	N	%	N	%	N	%
1	5	2.15	0	0.00	5	1.41
2	36	15.45	7	5.79	43	12.15
3	55	23.61	33	27.27	88	24.86
4	61	26.18	19	15.70	80	22.60
5	53	22.75	36	29.75	89	25.14
6	22	9.44	22	18.18	44	12.43
7	1	0.43	4	3.31	5	1.41
Total	233	100.00	121	100.00	354	100.00

Table 48 EH in Adults by Age at the Time of Formation: Goodman and Rose Adjusted

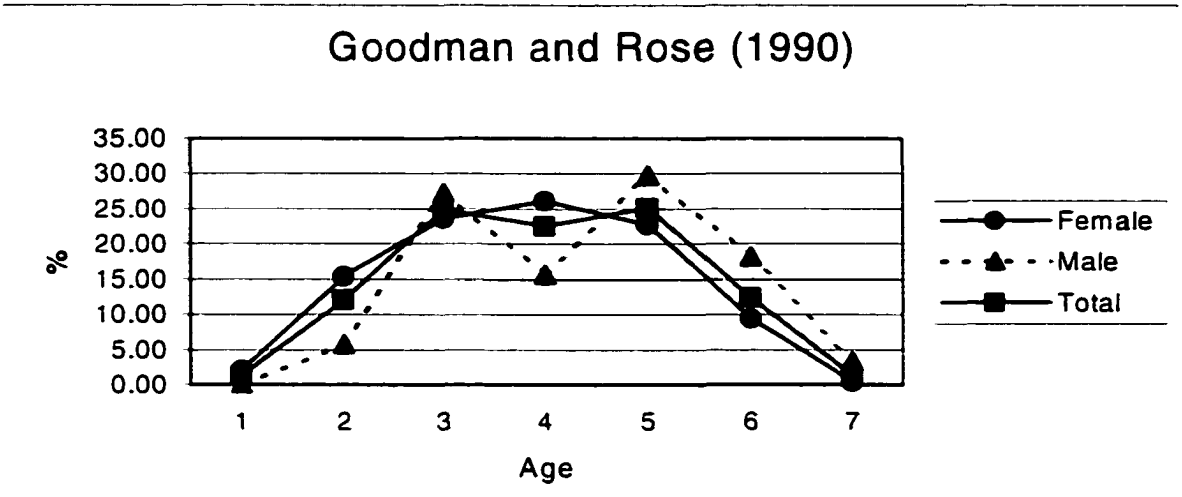
Age/Sex	Female		Male		Total	
Age	N	%	N	%	N	%
1	0	0.00	0	0.00	0	0.00
2	17	6.51	7	4.61	24	5.81
3	79	30.27	33	21.71	112	27.12
4	89	34.10	47	30.92	136	32.93
5	41	15.71	42	27.63	83	20.10
6	30	11.49	20	13.16	50	12.11
7	5	1.92	3	1.97	8	1.94
Total	261	100.00	152	100.00	413	100.00

When EH in adults is assessed according to the age at the time of formation, with the adjusted equations, the distribution is similar to the one discussed previously. A very high percentage (80.15%) of the defects formed between the ages 3-5 years (see Table 48 and Graph 10).

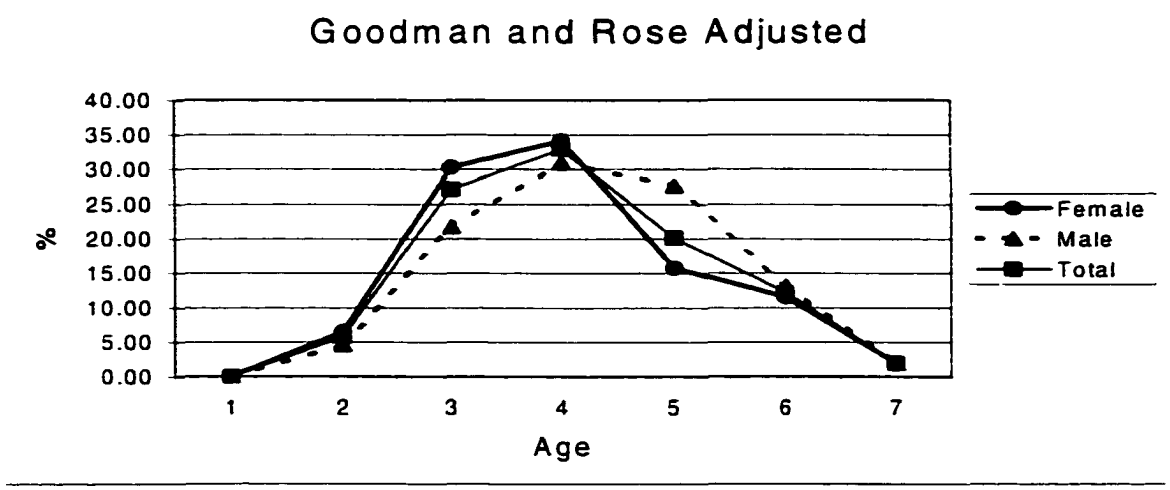
Therefore, even if the sample is reduced to avoid the biases already identified, EH and HL show a completely different distribution. At this point, then, it is possible to say that there is no correlation between EH and HL. In fact, the distribution of HL (see tables 17,22 and 23) is actually more similar to the tibial grown pattern (see graph 1).

Considering this, it is possible to question HL's status solely as stress indicator. It is not conceivable, however, to discard the possibility that some HL are the result of stress.

Graph 9



Graph 10



V.4. Filtering the Noise

If HL is the result of growth, but there are some HL that are formed as the result of stress, it becomes necessary to remove those lines that are the result of growth, and match the residual ones with EH defects to see if they coincide. At the same time, EH defects may be the result of a higher sensitivity and not the product of stress solely and that "noise" in the data must also be removed.

In order to avoid these two factors, and considering that sensitivity to stress and growth are specific to each year of life, it is assumed that an average number of EH and HL are formed each year of life due to this differential sensitivity (EH) and due to growth (HL). The data available for these two indicators then, was smoothed, by subtracting the specific average number of HL (see Tables 49 and 50) and EH formed each year by the individuals (see Tables 51,52 and 53). The remaining HL, were then matched with the remaining EH, to see if there was any coincidence. HL were match with EH and not EH with HL because the number of EH is larger than the number of HL, and thus, by comparing HL with EH the probability of finding a match is increased. The number of remaining HL at any specific age in any specific individual was not considered; only its presence/absence was analyzed in the matching process with EH.

These smoothing factors were subtracted in all the individuals that presented both HL and EH. These individuals are the only ones where it is possible to find a correlation between these two indicators. Harris lines formed between the ages <1-7 were considered in this analysis. Harris lines formed before the 1st year of age were considered in order to increase the possibility to find a match.

Table 49 HL Smoothing Factor for the Byers' (1990) Method

Age	N HL	N individuals	F(HL-By)
<1	16	14	1.14
1	4	4	1.00
2	4	4	1.00
3	6	5	1.50
4	4	3	1.00
5	2	2	0.50
6	4	4	1.00
7	3	3	0.75

F(HL-By): smoothing factor for HL calculations obtained with the Byers' (1990) method.

Table 50 HL Smoothing Factor for the Hummert and Van Gerven's (1985) Method

Age	N HL	N individuals	F(HL-HVg)
<1	33	26	1.27
1	24	21	1.14
2	78	39	2.00
3	75	17	4.41
4	9	6	1.50
5	19	12	1.58
6	16	10	1.60
7	**	**	**

F(HL-HVg): smoothing factor for HL calculations obtained with the Hummert and Van Gerven's (1985) method.

Table 51 EH Smoothing Factor for the Goodman and Rose's (1990) Method

Age	N EH	N individuals	F(EH-GR)
<1	5	3	1.67
1	31	23	1.35
2	157	49	3.20
3	306	68	4.50
4	203	63	3.22
5	182	62	2.94
6	84	38	2.21
7	13	8	1.63

F(EH-GR): smoothing factor for EH calculated with the Goodman and Rose's (1990) method.

Although 70 individuals present both HL and EH, the year-by-year analysis showed that 7 of them did not present HL formed in this age range. Thus, only 63 individuals were suitable for this analysis.

Table 52 EH Smoothing Factor for the Goodman and Rose Adjusted Method

Age	N HL	N individuals	F(HL-GRadj)
<1	0	0	0
1	1	1	1.00
2	81	36	2.25
3	351	73	4.81
4	281	75	3.75
5	162	55	2.95
6	86	34	2.53
7	20	13	1.54

F(HL-GRadj): smoothing factor for EH calculated with the Goodman and Rose Adjusted Method

Table 53 EH Smoothing Factor for the Blakeley and Armelagos's (1985) Method

Age	N EH	N Individuals	F(EH-BA)
>1	37	19	1.95

F(EH-BA): smoothing factor for EH calculated with the Blakeley and Armelagos' (1985) method

Considering that the smoothing factor of HL may contain the average number of HL formed each year due to stress, several correction factors (0.1-0.3) were added to the remaining HL, and then the result was rounded, in order to increase the chance to find a match. These correction factors are arbitrary because it is impossible to determine, with the available data how many HL, if any, were the result of stress.

Once the smoothing factors were subtracted, the results showed that with or without the correction factors the majority of HL disappeared (89.74%-76.86%; see Table 54).

Table 54 Remaining HL after Subtraction of the Smoothing Factors

μ_s	HL	HL-F(HL-By)	% of Lines Eliminated	HL	HL-F(HL-HVg)	% of Lines Eliminated
0.0	39	4	89.74	121	26	78.51
0.1	39	4	89.74	121	27	77.69
0.2	39	4	89.74	121	27	77.69
0.3	39	7	82.05	121	28	76.86

μ_s: supposed number of HL formed each year due to stress and added to the remaining HL.

The remaining lines were then matched with the remaining EH. The comparison did not consider the cases in which HL was absent and EH was present because the absence of HL might be the result of resorption. Remaining EH defects were grouped by method as follow: a) Blakeley and Armelagos (1985) and Goodman and Rose (1990); and b) Blakeley and Armelagos (1985) and Adjusted Goodman and Rose. If there are HL that resulted from stress and not from growth we should expect to find a greater proportion of matches than miss-matches. The different methods were then tested, and the number of possible matches, and the actual number of matches and matches-matches found were assessed (see Table 55).

The results, however, show that the number of matches is never greater than the number of matches-matches (see Table 55), no matter what method is considered or what correction factor is added. Thus, the few occasions in which a match is found is, most probably, due to chance and not to stress as a common etiological factor. With this in mind, the status of HL as stress marker can be further questioned. Thus, the lack of correlation between the two markers, HL and EH, provides important information of significance to studies of stress in past and present populations.

Table 55 Matches Obtained with the different Smoothing Factors and the different Correction Factors

	By-GRBA			By-GradjBA			HVg-GRBA			HVg-GRadjBA		
μ_s	N possible matches	% Mat	% Dis	N possible matches	% Mat	%Dis	N possible matches	%Mat	% Dis	N possible matches	% Mat	% Dis
0.0	4	0	1	4	25.00	75.00	14	0.14	0.86	14	0.21	0.79
0.1	4	0	1	4	25.00	75.00	15	0.13	0.87	15	0.19	0.81
0.2	4	0	1	4	25.00	75.00	15	0.13	0.87	15	0.2	0.8
0.3	7	0	1	7	14.00	86.00	15	0.13	0.87	15	0.2	0.8

μ_s: supposed number of HL formed each year due to stress and added to the remaining HL: By: Byers; GRBA: Goodman and Rose (1990) and Blakeley and Armelagos (1985); GRadjBA: Adjusted Goodman and Rose and Blakeley and Armelagos (1985); % Mat: % of matches; % Dis: % of disagreements.

CHAPTER VI

CONCLUSIONS

In order to survive, an organism must be able to respond to any changing environmental conditions. If the change in the environment occurs while the individual is growing and developing, certain phenotypic changes might occur in response to the stimuli: a phenomenon called developmental adaptation. Developmental plasticity, then, especially in humans, carries a high level of adaptation compared to the one attainable during adulthood (Little, 1995).

Among these phenotypic changes, due to stressful environmental conditions, HL and EH are recognized in the corpus of the literature as stress markers (Elliot, 1927; Harris, 1932; Suckling, 1989; Goodman and Rose, 1990; Goodman et al., 1997; Wright, 1997; Lewis, 2000). According to the literature, although these indicators show certain similarities in their etiology they also present differences:

1) Harris Lines can be formed over a longer span of years (*in uterus*-16 years), than hypoplasia (*in uterus*-13 years; Martin et al., 1985); and,

2) Harris lines are affected by resorption and remodeling, a process that does not alter enamel and thus has no effect on EH (Martin et al., 1985).

The aim of this study was to determine the existence of any correlation between these two markers. In order to identify if the two markers were correlated, their incidence was

analyzed at the individual level (presence/absence) and the distribution of the age of the individuals at the time of EH and HL formation. The analyses were carried on each marker separately, to determine if any patterns emerged. Then, the two markers were compared in several analyses to determine if any correlation existed between them.

VI. 1. Harris Lines

The results obtained in the analysis of HL considered its presence/absence by individual and the age of the individual at the time of HL formation. At the individual level, the results showed that 63.23% of the sample has HL. A higher percentage of juvenile individuals (67.82%) present HL in comparison with the adult segment of the sample (62.25% in females and 58.82% in males). There are two possible explanations for such phenomena: a) it is possible that individuals who lived longer had more time to resorb the lines and that is why they present a smaller number of HL (Wood et al., 1992) or; b) it is possible that, if HL is the result of stress, individuals who formed less lines and thus were exposed to less stressful events during childhood had a better chance to survive. The second possibility can be eliminated if HL is not a stress marker.

In general, the distribution of the transverse Harris lines show that they are more numerous towards the distal end: this result is in accordance with the findings of other studies (Garn et al., 1968). This phenomenon is hard to explain; however, it is possible that although the tibia grows faster towards proximal (57%), and thus enhances its ability to form lines, this high rate of growth may also result in a higher rate of resorption and remodeling. The resorption and remodeling process, then, may result in an increased resorption of HL towards the proximal end. This interpretation can also explain why

among the segment of 0-3 years of age, HL presents an even distribution between distal and proximal (see Table 15). In this specific age range, the large number of lines formed before the first year, and the accelerated growth of the proximal end (57%) in comparison with the distal end, would result in the same numbers of lines in the distal and the proximal end. Later, the remodeling and resorption would eliminate a higher number of lines in the proximal end, as the individuals aged. As a result then, a higher number of HL are identifiable at the distal end among older individuals.

This study did not identify any special correlation between HL and sex of the individuals. In this sample, however, the number of male individuals is small (n=17) and, thus, the results in this study cannot be considered conclusive. In contrast, other studies have identified a clear association between this skeletal marker and male individuals (Gindhart, 1969).

When the results for age of the individuals at the time of HL formation were considered, the analysis of the results obtained with the Byers' (1991) and the Hummert and Van Gerven's method (1985) were shown to be significantly different ($p(\alpha)$: 0.014).

The analysis of HL with the Byers' (1991) and the Hummert and Van Gerven's (1985) method, showed two peaks for the age of the individuals at the time of HL formation. The first peak correspond to <1 year, and the second one to adolescence; 11 years for females and 13 for males with the Byers' method, and a general peak of 15 years with the Hummert and Van Gerven's method. This curve of occurrence is interesting because it clearly resembles the tibial growth curve (see graph 1), which in turn resembles the general velocity growth curve for humans (see figure 1). It is interesting to note that in the womb (through the placenta; Daunter, et al 1992; Telemo

and Hanson, 1996), and during the first year of life (through lactation; Telemo and Hanson, 1996; Hanson 2000; Kelleher and Lonnerdal, 2001) humans are usually protected from environmental stresses. Thus, if Harris lines are the consequence of stressful conditions, it is quite surprising that the highest peak of formation occurs during this period of development, when the most protection is available.

In humans, during *in uterus* development, and also during the 1st year of life, growth velocity is highly accelerated. Although it is true that, especially during the first year of life, an important part of the growth correspond to the brain, the analysis of the tibial growth graph (see graph 1) shows that tibiae undergo an accelerated growth during this period too; thus, the highest rate of HL formation coincides with a period of high growth rate.

It is necessary to emphasize that with the Hummert and Van Gerven (1985) method, a mild peak is revealed at the age of 9. This peak coincides with the mid-childhood growth spurt (Bogin, 1999). The mid-childhood growth spurt is associated with adrenarche, an endocrine event. Adrenarche, which is mainly an increase in the secretion of adrenal androgen hormones (Bogin, 1999), results in a transient acceleration of skeletal growth and maturation that might be related with an accelerated formation of HL in the bones.

The results are even more striking using the Byer's (1990) method, which offers different regression equations according to the sex of the individuals. The results indicate two different peaks for males and females during adolescence, where the peak in females occurs two years earlier than in males. This difference is significant because the adolescence growth spurt occurs earlier in girls than in boys (Bogin, 1999). The fact that this difference does not show up with the Hummert and Van Gerven's (1985) method is

not surprising considering that this method does not differentiate between males and females. Adolescence is defined by an overall skeletal growth spurt (independent of the gonads; Bogin, 1999), and so it is obvious that the two most important peaks, and the third mild one recognized here, coincide with periods of accelerated growth.

If Harris lines were the consequence of stress events, we would expect to find them with a higher frequency when growth is slowed down, and not when growth is accelerated. It is necessary to consider, however, that it is possible that during accelerated growth the osteoblasts were more sensitive to deleterious environmental conditions. If that was the case, we should be able to find a correlation between Harris Lines and other stress markers.

VI.2. Enamel Hypoplasia

A large percentage of individuals in the sample (79.41%) present EH. This frequency, although high, is not uncommon for populations that had already adopted agriculture (Goodman, 1989). There is no clear association between EH and sex or age of the individuals. Although it has been proposed that enamel hypoplasia is correlated with diminished life expectancy (Goodman, 1989; Goodman, 1996), this sample does not show that pattern when the number of teeth affected was considered.

When the age of the individuals at the time of the defect formation was estimated, however, two different peaks appeared for juveniles and adults. With the Goodman and Rose's (1990) method, juveniles and young adults (19.1-30.0) show a peak at age 3, while adults (30.1-40.0) and advance adults (>40) show a peak at age 5. With the "adjusted method" a similar pattern is observed, where juveniles show a peak at the age

of 3, and adults show it at the age of 4. This pattern suggests that it is possible that individuals exposed to stressful conditions at an earlier age have a lower life expectancy in comparison with the ones that were exposed to these events later in life. The small size of the adult segment in this sample, limits this interpretation, which should be further explored in future studies of this kind.

Enamel hypoplasia does show a clear difference in its incidence between deciduous and permanent teeth. Deciduous teeth present not only a significantly lower number of teeth affected by EH, but also a significantly lower average number of defects per tooth affected in comparison with permanent teeth. This pattern is not uncommon; other studies have shown a low frequency of EH in deciduous teeth (Goodman et al, 1984; Larsen, 1987). Enamel hypoplasia then, is more common after the first year of life, and not before. Although this pattern is the one usually observed, it is interesting to note that this distribution is exactly the opposite to the one observed in HL, which were more frequently formed before the first year of age.

The results obtained for age at the time of EH formation, with the Goodman and Rose's (1990) method and the "adjusted method" were compared. A paired t-test analysis showed that the results obtained using these two methods were statistically significantly different. These results suggest that in order to obtain a realistic result, it is necessary to adjust the method to the average crown height per tooth type in the population under study; if not, the results will be biased. This adjustment is especially necessary in a study like this in which the presence or absence of correlation between EH and HL may erroneous, and attributable to the bias introduced by the unadjusted method.

VI.3. Correlation between Two Non-Specific Indicators of Stress: Harris Lines and Enamel Hypoplasia

No correlation between these two indicators was identifiable at the individual level. The analysis of the presence/absence of these indicators showed no association when the sample was considered as a whole or when each age-sex group was considered individually. Although Chi^2 was not applicable to the 30.1-40.0 years female and male segments and the >40 male group, this deficiency in the data is not as significant if we consider that the results of this test showed no significant association between HL and EH among juveniles. It was juveniles where a positive correlation was expected, since HL were exposed to resorption for a shorter duration.

When the age of the individuals at the time of HL and EH formation was analyzed, the two indicators show a very different behavior. While HL showed a high rate of formation before the first year of age, and a second peak towards adolescence, EH shows a peak between the ages 3 and 5. In HL and EH these trends are valid for the sample as a whole and most of the age-sex groups. These differences, however, might have been the result of the differential range of years in which EH (<1-7) and HL (<1-16) can be formed. Thus, when the HL formed after the age of 7 are considered, "noise" is introduced into the comparison. Another source of "noise" is the data that came from the juvenile individuals, especially considering that they constitute 63.97% of the sample, which skewed the distribution of HL toward younger ages.

Although the use of HL formed before the first year of age might be questioned, because EH formed at this time rarely appears in permanent teeth, we cannot omit the fact that there is a very low percentage of deciduous teeth with EH (4.92%). Moreover,

not only the percentage, but also the average number of defects per affected deciduous tooth (1.16) is low. Thus, if EH was forming at the same time HL was, that is, at a fast rate *in uterus* and during the first year of life, we would expect a large percentage of deciduous teeth affected with EH and a high average number of defects per affected deciduous tooth, but this does not occur in the sample analyzed.

In order to solve these problems only the HL and EH present in adults, and formed between the ages 1-7, were considered. Again, the distribution of the two indicators showed a completely different behavior. Harris lines showed a high rate of formation at age 1 using Byer's (1991) method, and at the age of 2 with Hummert and Van Gerven's (1985) method; after the age of two a very low frequency of HL is detected. The distribution of EH is, in contrast, completely different. When EH defects present in the adult population are analyzed with the Goodman and Rose's (1990) method, and then again with the "adjusted" one, the distribution shows that the majority of the defects are formed between the ages of 3 and 5 years.

We can conclude then that these two indicators show a completely different distribution by age of formation. It is extremely interesting that no matter how the data are "cleaned", HL keeps showing a high peak before or at the first year of age. Moreover, Harris Lines are very common before the first year of age and not as common at older ages. In contrast, Enamel Hypoplasia shows the opposite pattern; it is rarely formed before the first year of age and form more commonly after the age of 2. This distribution, at least for EH, has been attributed to the protection that the uterine environment and, later, maternal milk provides (Kronfeld and Schour, 1939; Sarnat and Schour, 1942; Gruenwald, 1973; Levine and Keen, 1974).

It is widely known that intra uterine environment and lactation provides the fetus and the neonate with protection (Daunter et al., 1992; Telemo and Hanson, 1996; Hanson 2000; Kelleher and Lonnerdal, 2001), after this period the individual has to develop its own immune system. Considering that, we would expect to find a low incidence of stress markers before the first year of age, as in the case of EH. The high incidence of HL at this time, however, does not fit this model. If HL were an indicator of stress we would expect to find a low frequency of HL before the first year and a high incidence after this period. Moreover, if HL is the consequence of stress that results in slowed or stopped growth, we should expect to find it not only correlated with other stress markers (as EH), but also at times when growth speed is decelerated.

The results obtained here do not fit with any of these expectations. Harris lines are not associated with EH; they do not only show a completely opposite distribution, but also HL shows a pattern of distribution that is similar to the growth curve of the tibia. These results are interesting because HL is associated not with stopped, or slowed growth, but with accelerated growth.

It is possible that HL are the result of growth and not the result of stress. Nevertheless, it still remains possible that some of the HL were the result of stress. In order to assess that possibility the data was "smoothed" by subtracting the average number of HL formed each year between the ages <1-7. Enamel hypoplasia was also smoothed considering that there is inter and intra-tooth differential sensitivity to EH. Thus a greater amount of EH is formed at specific years during the development of an individual. Thus, an average number of EH formed each year was also subtracted.

The data were smoothed for individuals that presented both EH and HL. Once the data were smoothed the residual HL were matched against the residual occurrences of EH. A larger number of disagreements than matches were identified; where HL occurred and no EH formed at the same age in the same individual. In order to omit the possibility that in the average number of HL subtracted, the average number of HL formed due to stress was included three different correction factors were added to the remaining HL (0-1-0.3). With each correction factor added, the new remaining HL were matched with the remaining occurrences of EH. The three analyses showed a considerably higher frequency of disagreements than matches. The few number of matches found are, most probably, the result of chance, rather than the result of these two skeletal markers having stress as a common etiological factor in common.

VI.4. Harris Lines Reevaluated

Magennis (1990) postulated that HL were the result of an imbalance between GH and IGF-I, and that this imbalance might have been the result of normal growth, diseases or protein deficiency that decreased the production of IGF-I and with it the hypertrophy of chondrocytes. Thus, according to Magennis (1990) it is possible that some HL are the result of stressful conditions.

The results obtained in this study do not support the possibility that HL are the result of stressful conditions. Moreover, although Magennis (1990) considered the possibility that a deficiency in IGF-I, due to protein deficiency, might result in the formation of HL, studies in Rhesus monkeys have shown that a larger mean number, and a greater

percentage of HL occurred in controls, rather than in protein-restricted infants (Murchison et al., 1984).

It is possible, then, that HL are not pathological; they might instead be the normal consequence of growth. Thus they are not a stress indicator and should not be used to reconstruct or evaluate the health status of past and present populations.

There are a number of hormones and growth factors that are involved in bone growth. In fact, the hormonal influence on bone formation is understood as the interaction of many factors. In the interaction systemic hormones, such as glucocorticoids (GC), insuline, thyroxine (T^3), sex hormones, growth hormone (GH) and growth factors (IGF-I and IGF-β), play a decisive role (Stracke et al., 1984).

Growth hormone (GH) expands the pool of progenitor chondrocytes (Lindahl et al., 1987; Ohlsson et al., 1992; Siebler et al., 2001); it stimulates growth, directly, through the Growth Hormone Receptor (GHR) and indirectly by stimulating IGF-I expression (Sims et al., 2000). It also promotes bone formation (Maor et al., 1993), and collagen production (Sims et al., 2000).

Insuline-like growth factor-I (IGF-I) mediates most of the effects of GH. It is a mitogen that stimulates the clonal expansion, and later the hyperthropy, of the chondrocytes (Bikle et al., 1994). All chondrocytes layers produce IGF-I, and the one produced by the lower layer of the growth plate targets osteoblasts, and thus it is involved in bone deposition (Reinecke et al., 2000). IGF-I is especially important for growth *in uterus*, when the fetus lacks GH (Maor et al., 1993). Moreover, a deficiency in IGF-I results in hypoplasia of different organs (Siebler et al., 2001). Thus, although the importance of IGF-I for uterine growth coincides with the high number of HL formed *in*

uterus that were identified in this study, and therefore with Magennis's (1990) theory, the problem is that if IGF-I deficiency results in hypoplasia we should find EH associated with HL.

Thyroid hormone (T^3) and IGF-β also influence bone growth. Thyroid hormone (T^3) stimulates osteoblast activity, chondrocyte differentiation, and it is indispensable for chondrocyte hypertrophy and vascular invasion of the growth plate (Siebler et al., 2001). IGF-β enhances bone formation by intra-membranous ossification in vivo (Seino, 1994).

None of these factors or hormones, per se, can explain the increased bone deposition and vascular invasion, and the decreased mitosis of the progenitor chondrocytes, associated with the appearance of HL. Glucocorticoids (GC), however, stimulate osteoblast activity and, therefore, bone production. Although it is apparent that IGF-I and GH might mediate some of its actions, GC stimulate insensitivity to GH and IGF-I, and with that it reduces their expression (Siebler et al, 2001). Secretion of GC may increase with stress but studies in rats have shown no differences between the rats exposed to stress and the control ones (Bikle et al., 1994). Thus by stimulating osteoblast activity and reducing GH sensitivity, and thus mitosis in the reserve layer, GC might be involved in the appearance of transverse lines. This does not mean that this is the only hormone involved in the HL formation, and as long as bone growth at the endocrinological level is not fully understood it is not possible to give a definite answer to this question. Further studies and analysis of these hormones and growth factors are needed as well as other studies in HL that may or may not agree with the results obtained here.

The purpose of this study was to investigate the possible correlation between two recognized stress indicators: Harris lines and enamel hypoplasia. The results indicate that

there is no correlation. Instead, while EH corresponds to possible periods of environmental stress, and so it can be considered an indicator of pathology, Harris lines do not. Instead they correspond to periods of growth. These results have important implications for clinical studies and studies in past populations. Future studies will not be able to use HL as an indicator of stress and therefore a proxy for health in past populations. Since HL are most probably influenced by rate of growth in an individual, their formation at a hormonal and developmental level warrant further investigation.

APPENDIX I

SAMPLE ANALYZED

Site	Individual	Sex	Age Group
AZ71	49	Undetermined	0-3.0
AZ71	300	Undetermined	0-3.0
AZ71	Y	Undetermined	0-3.0
AZ71	246	Undetermined	0-3.0
AZ71	285	Undetermined	0-3.0
AZ71	116 A	Undetermined	0-3.0
AZ71	156 B	Undetermined	0-3.0
AZ71	277	Undetermined	0-3.0
AZ71	N1	Undetermined	0-3.0
AZ71	165	Undetermined	0-3.0
AZ71	470	Undetermined	0-3.0
AZ71	504	Undetermined	0-3.0
AZ71	201	Undetermined	3.1-7.0
AZ71	315	Undetermined	3.1-7.0
AZ71	150 A	Undetermined	3.1-7.0
AZ71	17 A	Undetermined	3.1-7.0
AZ71	280	Undetermined	3.1-7.0
AZ71	69 B	Undetermined	3.1-7.0
AZ71	256 B	Undetermined	7.1-12.0
AZ71	169	Undetermined	7.1-12.0
AZ71	224	Undetermined	7.1-12.0
AZ71	222	Undetermined	7.1-12.0
AZ71	230	Undetermined	7.1-12.0
AZ71	336	Undetermined	7.1-12.0
AZ71	AA	Undetermined	7.1-12.0
AZ71	232	Undetermined	12.1-19.0
AZ71	105	Undetermined	12.1-19.0
AZ71	258	Undetermined	12.1-19.0
AZ71	235 A	Undetermined	12.1-19.0
AZ71	NMT3	Undetermined	12.1-19.0
AZ71	328	Undetermined	12.1-19.0
AZ71	206	Undetermined	12.1-19.0
AZ71	288	Undetermined	12.1-19.0
AZ140	15	Undetermined	12.1-19.0
AZ140	26	Undetermined	0-3.0
AZ140	95	Undetermined	0-3.0
AZ140	31	Undetermined	0-3.0
AZ140	131B	Undetermined	0-3.0
AZ140	24A	Undetermined	0-3.0
AZ140	SUP.2	Undetermined	0-3.0

AZ140	88	Undetermined	0-3.0
AZ140	115	Undetermined	0-3.0
AZ140	1	Undetermined	3.1-7.0
AZ140	60	Undetermined	3.1-7.0
AZ140	94	Undetermined	3.1-7.0
AZ140	65B	Undetermined	3.1-7.0
AZ140	127B	Undetermined	3.1-7.0
AZ140	46A	Undetermined	3.1-7.0
AZ140	101	Undetermined	3.1-7.0
AZ140	114	Undetermined	3.1-7.0
AZ140	72	Undetermined	3.1-7.0
AZ140	90	Undetermined	3.1-7.0
AZ140	20	Undetermined	7.1-12.0
AZ140	96	Undetermined	7.1-12.0
AZ140	109	Undetermined	7.1-12.0
AZ140	102	Undetermined	7.1-12.0
AZ140	24B	Undetermined	7.1-12.0
AZ140	53	Undetermined	7.1-12.0
AZ140	127	Undetermined	7.1-12.0
AZ140	SUP.1	Undetermined	7.1-12.0
AZ140	76	Undetermined	12.1-19.0
AZ140	74	Undetermined	12.1-19.0
AZ140	14	Undetermined	12.1-19.0
AZ140	66	Undetermined	12.1-19.0
AZ140	XPB3	Undetermined	12.1-19.0
AZ140	68	Undetermined	12.1-19.0
AZ140	28	Undetermined	12.1-19.0
AZ140	29	Undetermined	12.1-19.0
AZ140	36	Undetermined	12.1-19.0
AZ140	140	Undetermined	12.1-19.0
AZ140	XPB	Undetermined	12.1-19.0
AZ140	35	Undetermined	12.1-19.0
AZ140	79	Undetermined	12.1-19.0
AZ140	10	Undetermined	19.1-30.0
AZ140	113	Undetermined	19.1-30.0
AZ140	45B	Undetermined	19.1-30.0
AZ140	50	Undetermined	19.1-30.0
AZ140	38	Undetermined	30.1-40.0
AZ140	58	Undetermined	>40
AZ140	92	Undetermined	>40
AZ140	124	Undetermined	>40
AZ140	128	Undetermined	>40
AZ140	40	Undetermined	0-3.0
AZ140	87	Undetermined	0-3.0
AZ140	121	Undetermined	0-3.0
AZ140	140A	Undetermined	0-3.0
AZ140	44	Undetermined	12.1-19.0
AZ140	39	Undetermined	12.1-19.0
AZ140	21	Undetermined	12.1-19.0
AZ140	67	Undetermined	12.1-19.0
AZ140	51	Undetermined	12.1-19.0
AZ140	XPB2A	Undetermined	12.1-19.0
AZ140	106	Undetermined	12.1-19.0
AZ140	4	Female	19.1-30.0
AZ140	18	Female	19.1-30.0
AZ140	111	Female	19.1-30.0

AZ140	104	Male	19.1-30.0
AZ140	78	Female	19.1-30.0
AZ140	116	Female	19.1-30.0
AZ140	107	Female	19.1-30.0
AZ140	118	Female	19.1-30.0
AZ140	80	Male	19.1-30.0
AZ140	91	Male	19.1-30.0
AZ140	48	Female	19.1-30.0
AZ140	73	Female	30.1-40.0
AZ140	XPB4	Female	30.1-40.0
AZ140	23	Male	30.1-40.0
AZ140	82	Male	30.1-40.0
AZ140	122	Male	30.1-40.0
AZ140	108A	Male	30.1-40.0
AZ140	117	Female	30.1-40.0
AZ140	8	Female	30.1-40.0
AZ140	41	Female	30.1-40.0
AZ140	100	Female	30.1-40.0
AZ140	110	Female	30.1-40.0
AZ140	120	Female	30.1-40.0
AZ140	75	Male	30.1-40.0
AZ140	97	Male	30.1-40.0
AZ140	64A	Male	30.1-40.0
AZ140	47	Female	30.1-40.0
AZ140	7	Male	>40
AZ140	55	Male	>40
AZ140	105	Male	>40
AZ140	5	Female	>40
AZ140	32	Female	>40
AZ140	56	Female	>40
AZ140	63	Female	>40
AZ140	125	Female	>40
AZ140	126	Female	>40
AZ140	112A	Female	>40
AZ140	27	Female	>40
AZ140	MMB	Female	>40
AZ140	2	Undetermined	3.1-7.0
AZ140	42B	Undetermined	0-3.0
AZ71	271	Undetermined	0-3.0
AZ71	604	Undetermined	0-3.0

APPENDIX 2

RADIOGRAPHED INDIVIDUALS

Site	Individual	Sex	Age	Age Group	Radiograph Left/Right
AZ140	1	Undetermined	4	3.1-7.0	Left
AZ140	2	Undetermined	6	3.1-7.0	Left
AZ140	4	Female	19-24	19.1-30.0	Left
AZ140	5	Female	45-49	>40	Left
AZ140	7	Male	40-49	>40	Left
AZ140	8	Female	35-39	30.1-40.0	Left
AZ140	10	Female	20	19.1-30.0	Right
AZ140	14	Undetermined	15	12.1-19.0	Left
AZ140	15	Undetermined	1.5	0-3.0	Left
AZ140	18	Female	20-22	19.1-30.0	Left
AZ140	20	Undetermined	8	7.1-12.0	Left
AZ140	21	Undetermined	17-18	12.1-19.0	Left
AZ140	23	Male	30-35	30.1-40.0	Left
AZ140	24A	Undetermined	2	0-3.0	Left
AZ140	24B	Undetermined	10	7.1-12.0	Left
AZ140	26	Undetermined	1.5	0-3.0	Left
AZ140	27	Female	45-50	>40	Left
AZ140	28	Undetermined	17	12.1-19.0	Right
AZ140	29	Undetermined	17	12.1-19.0	Right
AZ140	31	Undetermined	2	0-3.0	Left
AZ140	32	Female	45-49	>40	Left
AZ140	35	Undetermined	18	12.1-19.0	Left
AZ140	36	Undetermined	18	12.1-19.0	Right
AZ140	38	Male	30	19.1-30.0	Left
AZ140	39	Undetermined	17-18	12.1-19.0	Left
AZ140	40	Undetermined	1.5	0-3.0	Right
AZ140	41	Female	35-39	30.1-40.0	Right
AZ140	42B	Undetermined	9m	0-3.0	Left
AZ140	44	Undetermined	16-17	12.1-19.0	Left
AZ140	45B	Female	25	19.1-30.0	Left
AZ140	46A	Undetermined	4	3.1-7.0	Left
AZ140	47	Female	35-40	30.1-40.0	Right
AZ140	48	Female	25-30	19.1-30.0	Left
AZ140	50	Female	30	19.1-30.0	Left
AZ140	51	Undetermined	18-19	12.1-19.0	Left
AZ140	53	Undetermined	12	7.1-12.0	Right

AZ140	55	Male	40-49	>40	Right
AZ140	56	Female	45-49	>40	Right
AZ140	58	Female	(+50)	>40	Left
AZ140	60	Undetermined	4	3.1-7.0	Right
AZ140	63	Female	45-49	>40	Left
AZ140	64A	Male	35-39	30.1-40.0	Left
AZ140	65A	Undetermined	0-6m	0-3.0	Left
AZ140	65B	Undetermined	4	0-3.0	Left
AZ140	66	Undetermined	15	12.1-19.0	Left
AZ140	67	Undetermined	17-18	12.1-19.0	Left
AZ140	68	Undetermined	16	12.1-19.0	Left
AZ140	69	Undetermined	14-15	12.1-19.0	Left
AZ140	70	Undetermined	17-19	12.1-19.0	Left
AZ140	72	Undetermined	6	3.1-7.0	Left
AZ140	73	Female	30-35	30.1-40.0	Right
AZ140	74	Undetermined	15	12.1-19.0	Left
AZ140	75	Male	35-39	30.1-40.0	Left
AZ140	76	Undetermined	14	12.1-19.0	Right
AZ140	78	Female	20-25	19.1-30.0	Right
AZ140	79	Undetermined	19	12.1-19.0	Right
AZ140	80	Female	25-29	19.1-30.0	Right
AZ140	82	Female	30-35	30.1-40.0	Left
AZ140	87	Undetermined	1.5	0-3.0	Left
AZ140	88	Undetermined	3	0-3.0	Left
AZ140	90	Undetermined	7	3.1-7.0	Left
AZ140	91	Male	25-29	19.1-30.0	Left
AZ140	92	Female	(+50)	>40	Left
AZ140	94	Undetermined	4	3.1-7.0	Left
AZ140	95	Undetermined	1.5	0-3.0	Left
AZ140	96	Undetermined	8	7.1-12.0	Right
AZ140	97	Male	35-39	30.1-40.0	Left
AZ140	100	Female	35-39	30.1-40.0	Left
AZ140	101	Undetermined	5	3.1-7.0	Left
AZ140	102	Undetermined	10	7.1-12.0	Left
AZ140	103	Undetermined	6m	0-3.0	Left
AZ140	104	Male	20-24	19.1-30.0	Left
AZ140	105	Male	40-50	>40	Left
AZ140	106	Male	18-20	19.1-30.0	Left
AZ140	107	Female	25-29	19.1-30.0	Left
AZ140	108A	Male	30-35	30.1-40.0	Left
AZ140	109	Undetermined	9	7.1-12.0	Left
AZ140	110	Female	35-39	30.1-40.0	Right
AZ140	111	Female	20-24	19.1-30.0	Left
AZ140	112A	Female	45-49	>40	Right
AZ140	112B	Undetermined	Birth	0-3.0	Left
AZ140	113	Male	20	19.1-30.0	Left
AZ140	114	Undetermined	5	3.1-7.0	Left
AZ140	115	Undetermined	3	0-3.0	Left
AZ140	116	Female	20-25	19.1-30.0	Left
AZ140	117	Female	30-40	30.1-40.0	Left
AZ140	118	Female	25-29	19.1-30.0	Right

AZ140	119	Undetermined	4-6m	0-3.0	Left
AZ140	120	Female	35-39	30.1-40.0	Left
AZ140	121	Undetermined	1.5	0-3.0	Left
AZ140	122	Male	30-35	30.1-40.0	Left
AZ140	123	Undetermined	Birth	0-3.0	Right
AZ140	124	Female	(+50)	>40	Left
AZ140	125	Female	45-49	>40	Left
AZ140	126	Female	45-49	>40	Left
AZ140	127	Undetermined	12	7.1-12.0	Right
AZ140	127B	Undetermined	4	3.1-7.0	Left
AZ140	128	Male	(+50)	>40	Left
AZ140	131	Undetermined	6m	0-3.0	Left
AZ140	131A	Undetermined	Birth	0-3.0	Left
AZ140	131B	Undetermined	2	0-3.0	Left
AZ140	140	Undetermined	18	12.1-19.0	Left
AZ140	MMB	Female	45-50	>40	Left
AZ140	SUP.1	Undetermined	12	7.1-12.0	Left
AZ140	SUP.2	Undetermined	2	0-3.0	Left
AZ140	XPB	Undetermined	18	12.1-19.0	Right
AZ140	XPB2A	Undetermined	18-19	12.1-19.0	Left
AZ140	XPB2B	Undetermined	Birth	0-3.0	Left
AZ140	XPB3	Undetermined	15	12.1-19.0	Left
AZ140	XPB4	Female	30-35	30.1-40.0	Left
AZ140	140A	Undetermined	1.5	0-3.0	Right
AZ71	8	Undetermined	0-6m	0-3.0	Left
AZ71	17 A	Undetermined	5	3.1-7.0	Left
AZ71	36	Undetermined	Unborn	Unborn	Left
AZ71	48	Undetermined	Birth	0-3.0	Left
AZ71	49	Undetermined	2	0-3.0	Left
AZ71	69 B	Undetermined	7	3.1-7.0	Left
AZ71	105	Undetermined	16	12.1-19.0	Left
AZ71	116 A	Undetermined	1.5	0-3.0	Left
AZ71	150 A	Undetermined	4	3.1-7.0	Left
AZ71	156 B	Undetermined	1.5	0-3.0	Left
AZ71	165	Undetermined	3	0-3.0	Left
AZ71	168	Undetermined	0-6m	0-3.0	Left
AZ71	169	Undetermined	9	7.1-12.0	Left
AZ71	188	Undetermined	Unborn	Unborn	Left
AZ71	201	Undetermined	4	3.1-7.0	Left
AZ71	206	Undetermined	16-18	12.1-19.0	Left
AZ71	219	Undetermined	6-12m	0-3.0	Right
AZ71	222	Undetermined	10	7.1-12.0	Left
AZ71	224	Undetermined	9	7.1-12.0	Left
AZ71	230	Undetermined	10	7.1-12.0	Left
AZ71	232	Undetermined	15	12.1-19.0	Left
AZ71	235 A	Undetermined	18	12.1-19.0	Left
AZ71	241	Undetermined	0-6m	0-3.0	Left
AZ71	246	Undetermined	1.5	0-3.0	Left
AZ71	256 B	Undetermined	8	3.1-7.0	Left
AZ71	258	Undetermined	17	12.1-19.0	Left
AZ71	271	Undetermined	1	0-3.0	Left

AZ71	275	Undetermined	1.5	0-3.0	Left
AZ71	277	Undetermined	2	0-3.0	Left
AZ71	280	Undetermined	6	3.1-7.0	Left
AZ71	285	Undetermined	1.5	0-3.0	Left
AZ71	288	Undetermined	17-18	12.1-19.0	Left
AZ71	300	Undetermined	1	0-3.0	Left
AZ71	315	Undetermined	4	3.1-7.0	Left
AZ71	328	Undetermined	16-17	12.1-19.0	Left
AZ71	336	Undetermined	10	7.1-12.0	Left
AZ71	369	Undetermined	Unborn	Unborn	Left
AZ71	470	Undetermined	3	0-3.0	Left
AZ71	504	Undetermined	3	0-3.0	Left
AZ71	604	Undetermined	3	0-3.0	Left
AZ71	AA	Undetermined	12	7.1-12.0	Left
AZ71	N1	Undetermined	2	0-3.0	Left
AZ71	NMT3	Undetermined	19	12.1-19.0	Left
AZ71	W	Undetermined	Birth	0-3.0	Left
AZ71	Y	Undetermined	1	0-3.0	Left

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