QUANTITATIVE HISTOLOGICAL STUDIES ON THE ESTIMATION OF CHRONOLOGICAL AGE FROM SKELETAL MATERIAL

by

Inder Jit Singh, B.D.S.

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611 S. W. Campus Drive
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APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

Everything one does enough of eventually generates its own interest and one then begins to believe in it.

Alan Dunn In Is There Intelligent Life on Earth? Cited by N.R.F. Maier In Psychology In The Wry (Editor, R.A. Baker). Princeton, Van Nostrand, 1963

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I. INTRODUCTION

A. General Considerations

In the literature on skeletal tissue, a wide variety of research and opinion exists pertaining to the use of skeletons for the identification of such items as race, sex and age. An important shortcoming of the skeletal tissues for such purposes has been the understanding that bone is far from inert, but is throughout life, highly susceptible to a host of systemic and local influences, both prenatal and postnatal in origin. Nevertheless, unlike the soft tissues, the osseous tissues maintain their structural and morphologic integrity for a longer period after death, even under relatively adverse conditions. Thus, skeletal and dental tissues have become more useful and widely depended upon for purposes of identification.

Over the years, many attempts have been made toward using skeletal material for the identification of sex, race or age. Sex identification has been extensively discussed by Stewart (1954) and Krogman (1962); whereas, Giles and Elliot (1963) and Howells (1967) have recently attempted race determination by discriminant function analyses of multiple skull measurements on adult crania. Also, in recent years, a number of authors have compiled authoritative reviews on age estimation from skeletal material (Stewart and Trotter, 1954; Krogman, 1955, 1962; McKern and Stewart, 1957). This dissertation deals primarily with the use of skeletal material for the estimation of age at death.

For a long time the major sources of information on age estimation were textbooks of anatomy and too often they contained partial-

ly substantiated and oversimplified statements ascribing definite dates to various skeletal maturational events. Even if one were to acknowledge that these conclusions were based upon adequate data, the almost complete absence of any information on normal variation would seriously impair their usefulness. This unfortunate tendency exists in anatomy textbooks even today, and in them central tendency is the rule and variability rates only an occasional mention. Such data are obviously of limited value in individual identification, and it was not until the 1920's that anatomists and anthropologists began reappraising skeletal maturational events with emphasis on sampling techniques and variability.

The earliest studies devoted explicitly to the estimation of age at death from skeletons of known background were conducted from 1920 to 1942 by Todd and his associates at Western Reserve University where they had access to possibly the largest and best preserved selection of skeletons in this country. Later there will be more occasion to discuss their work. It will be seen that while these investigations have been heuristic in the development of the problem, their deficiencies, both methodological and statistical, are so severe by present-day standards that it becomes difficult to assess their substantive contribution.

Individual identification in anthropology and in forensic medicine, especially regarding age, race or sex is even now often based upon fragmentary evidence from skeletal and dental material (Krogman, 1962). It is surprizing, therefore, to note the paucity of

quantitative evidence existing in this area, although to an extent, this state of affairs reflects the fact that much of the available evidence was not initially collected for that purpose.

In order that age dependent parameters of bone may be used to provide estimates of age at death, it is also necessary to evaluate the variability and errors introduced in such estimation by the administration of some metabolic agents which are known to effect skeletal tissue. To a large extent, the dependability of such estimates, and thus their usefulness, depends upon understanding the effects of such environmental influences to which skeletal tissues may at times be exposed.

In this dissertation, the review of literature is presented in two parts. The major part presents the current methods and concepts of estimating age at death from skeletal material. Also discussed in this part are some other morphologic (gross and histologic) studies of age-changes in cortical bone although many of them were neither conducted nor analyzed for the specific purpose of age estimation. The second part of the review briefly considers the effects of a metabolic agent (estrogen) on skeletal tissue.

B. Age Estimation from Skeletal Material

For the younger age groups, timing and sequence of appearance of ossification centers have provided the most useful determinants of age at death (among others Stevenson, 1924; Greulich & Pyle, 1950; Pyle & Hoerr, 1955; Kraus, 1961). After 25 years of age, however, estimation of age at death is still based upon strictly subjectively determined morphologic criteria; as for example, the rate of cranial suture closure, presence of lipping on bony margins, and symphyseal changes in the pubic bone.

It has been claimed that bony lipping on the margins of lumbar vertebral bodies and on the inner border of ischial tuberosities initiates at about 35 to 40 years of age and, in almost all cases, is well advanced by 45 years of age (Stewart, 1954; Krogman, 1962). However, such morphologic age-changes have not yet been critically and extensively investigated.

That the closure of cranial sutures is related to age was known to Vesalius (Montagu, 1938) and perhaps even earlier to Hippocrates (McKern and Stewart, 1957). In more recent years, Dwight (1890), Parsons and Box (1905) and Frederic (1906) have demonstrated age, race and sex differences in the timing of closure of the cranial sutures. For the most part, however, these studies were based on inadequate samples, frequently of poorly known background. Todd and Lyon (1924, 1925a, 1925b, 1925c) were the first investigators who had a statistically adequate sample, carefully documented as to age, race and sex. Using Broca's (1861) and Frederic's (1906) system of

classification, they assigned numerical values to the degree of cranial suture closure, ectocranially and endocranially. Unfortunately, however, they deliberately excluded all cases where suture closure was "abnormal" or when evidence of "growth deviations was seen in the postcranial skeleton". The authors recognized the limitations of their work; nevertheless, their standards have been generally accepted and are even now frequently utilized.

Criticism of the above methods has been relatively recent. From his studies on the identification of 225 North American soldiers, 18 to 38 years of age who were killed in Europe during World War II, Vandervael (1952) pointed out the inadequacy of the standards proposed by Todd and Lyon (1924, 1925a, 1925b, 1925c).

The unreliability of age estimates based on suture closure has also been demonstrated by Singer (1953) on crania of 100 Cape Coloureds, 190 Bantu, 20 White German, 60 North American Indians and 30 Eskimos. He concluded that age of the individual at death cannot be estimated from the degree of closure of the various cranial sutures, whether taken individually or collectively or whether observed exocranially or endocranially.

More recently, investigations on White, Negro and American Indian skeletons (including those used by Todd and Lyon) have demonstrated that the variability in the chronology of cranial suture closure is so large that is seriously impairs the usefulness of the method (Brooks, 1955; Cobb, 1955). It appears that Hrdlicka (1920) was being too optimistic when he stated that by considering all the sutures together, one could estimate age to within ten years.

In looking at another widely used parameter for estimating age at death, the morphology of the innominate bone, one is impressed by the antiquity of the idea and disappointed by the almost total reliance on it even today in spite of ample evidence documenting its lack of validity and reliability. Gross morphological age-changes in the pubic bone have long been recognized (among others Hunter, 1761; Cleland, 1889; and an excellent historical review by Todd, 1920); but a systematic study, from skeletons of known ages, had to await the attentions of Todd (1920, 1921, 1942). Notwithstanding the subjective nature of the approach and its inherent limitations, Todd's description remains even today the most widely depended upon for age identification. He divided the pattern of observed pubic symphyseal differentiation into ten age ranges (Table 1). A perusal of his description clearly suggests that in practice such subjective criteria would be far from easy to identify.

Hanihara (1952) has applied Todd's standards to a study of age changes in pubic symphysis of Japanese male skeletons and found large errors in age estimation; he was unable to determine, however, whether the errors were due to racial variation or to the large difference of opinion between examiners which would be inherent in such an approach.

More recently, the validity of age estimation based on Todd's standards of pubic symphyseal development has been questioned mainly on two grounds: first, that the inherently subjective approach does not lend itself to easy interpretation, and second, that these

TABLE I.

MORPHOLOGICAL AGE CHANGES IN THE PUBIC SYMPHYSIS BASED ON TODD (1920, 1921, 1942).

Stage	Age Range (Years)	Morphology
1	18-19	Typical adolescent ridge and furrow formation with no sign of beveling on margins
2	20-21	Slight indication of a) dorsal margin and b) ventral beveling.
3	22-24	Progressive obliteration of ridge and furrow system. Increased definition of dorsal margin and ventral beveling.
4	25-26	Complete dorsal margin. Increase of ventral beveling. Commence delimitation of lower extremity.
5	27-30	Commence formation of upper extremity. Slight formation of ventral rampart.
6	30-35	Development and completion of ventral rampart. Increasing definition of extremities.
7	35-39	Changes in symphyseal face and ventral aspect of pubis. Bony outgrowths into pelvic attachments of tendons and ligaments.
8	39-44	Smoothness and inactivity of symphyseal face and ventral aspect of pubis. No rim formation or lipping.
9	45-50	Development of rim on symphyseal face. Lipping of dorsal and ventral margins.
10	50 +	Breaking down of ventral margins. Erosion on symphyseal face.

standards show so much inter-examiner variability that large errors in age estimation result even when applied to the same skeletons from which the standards were originally derived (Brooks, 1955).

The most recent morphologic study of age changes in bone was conducted by McKern and Stewart (1957). The material consisted of 450 skeletons of American war dead, 17 to 50 years of age, which were recovered from North Korea under "Operation Glory." Many parameters were investigated including cranial suture closure, epiphyseal union in several bones, differentiation in the pubic symphysis and the presence of bony lipping. As expected in such an age stratified group, sample size was largest in the early twenties and dwindled to three by 37 years of age. These sampling problems, when added to the fact that race was not always known, suggest that the conclusions be interpreted conservatively. They utilized subjectively determined criteria and, by numerically ranking developmental stages, were able to provide regression equations for the estimation of chronological age from composite scores derived from measures taken on many bones. However, no measure of the error of estimation was provided.

The problem remains that such subjective criteria would necessarily have large variability between examiners. Moreover, it must be pointed out that morphologic criteria in the pubic symphysis are of little use beyond fifty years of age - an age limit that falls far short of the human life span. One must conclude that, although currently advocated for age estimation (Krogman, 1962), such methods can only be regarded as having limited practical merit.

Turning now toward the more objective studies, one finds that

research reports dealing explicitly with age determination from skeletal material are few and far between. Many of the early attempts in this area were focused on estimating age at death from the size of long bones. Studies on fetuses have demonstrated a primarily linear relationship between Crown-Rump (C-R) length and length of femur (Scammon, 1937). However, variability in femur length was considerable and, consequently, estimates of C-R length were highly imprecise.

A similar approach has also been used to estimate age, from birth to 18 years, from human femur length (Stewart, 1954). The data, however, are of dubious practical merit because no information on measurement variability was provided and also because part of the curves were based upon Eskimo material "adjusted for known Eskimo-White differences." This conclusion is further reinforced by the fact that the error of prediction at later ages may be expected to be considerably larger than in Scammon's fetal data because variability of every human dimension is known to increase with age (Shuttleworth, 1937).

Stewart (1954) also recognized that a complete femur may not always be available; therefore, he provided separate estimates of femur size based upon length of tibia, fibula, humerus and ulna. Then even in cases where the femur was incomplete or missing and any one of the mentioned long bones was available, the method could still be used. Presumably these relationships were derived from adult material. There seems to be an additional untested a priori assumption here, that interrelations between measurements of long bones are very consistent and remain unchanged throughout life. In

view of some recent evidence, such assumptions do not seem justified (Meredith, 1962; Singh, Savara and Miller, 1967).

In a cross-sectional study, Smith and Walker (1964) examined radiographs from 2030 women ranging in age from 45 to 90 years. They showed an increase in the periosteal and endosteal diameters and a slight decrease in cortical thickness at femoral midshaft as a function of age. Such an increase has also been noticed in the diameter of the second metacarpal on roentgenograms (Garn, Wagner, Rohmann and Ascoli, 1967, 1968); men and women were measured 24 and 15 years apart, respectively, and the second measurement for both sexes was made in the sixth decade. Trotter and Peterson (1967) confirmed Smith and Walker's earlier findings on femora from skeletons but reported significantly lower values; the discrepancy was probably due to radiographic enlargement in the latter work. However, in a more recent study on femora from skeletons, Trotter, Peterson and Wette (1968) concluded that the hypothesis of continued bone expansion with age was not tenable. Frances (1956) reported a tendency towards a decrease with age in the height of cervical vertebral bodies; the change is probably not significant.

It is well known that with age there is a decrease in the amount of red bone marrow and a concomitant increase in yellow bone marrow. An index expressing the proportion of red to yellow bone marrow from humeri and femora in 50 adults, 20 to 90 years of age, was calculated by Jaaskelainen (1968), and from this he was able to estimate age to the order of accuracy of two decades. To the obvious shortcoming of a large error in estimation, one must add another, the difficulty of

measuring red and yellow bone marrow especially where death had occurred a long time prior to autopsy.

An increase with age in the amount of light seen through the scapula by transillumination has been suggested by Graves (1922). Presumably this is due to osteoporosis and the reduced level of cortical thickness in old age. In the earlier ages, however, no consistent pattern was seen either in his work or in the later report of Krogman (1949) along similar lines. Incidentally, from Graves' and Krogman's photographs, it appears that, except between the extreme ages, an age change in the scapula is difficult to demonstrate by transillumination alone.

The development of ideas and techniques in this area, though not necessarily chronological in their exposition here, have evolved from the gross morphological observations which are strongly subjective toward the more quantitative and objective measurements of microstructure. The next logical step in the study of skeletal ageing, therefore, would be the application of histological techniques.

A slight increase in rib diameter with age has been suggested (Sedlin, Frost and Villanueva, 1963); this evidence, however, is not unequivocal because the location of the removed rib varied among patients (5th, 6th, 7th rib) and no attempt was made to standardize the site of sectioning within a rib. Recently tetracycline label has been observed in the subperiosteal circumferential lamellae in rib cross sections from subjects 70 years of age (Epker and Frost, 1966), and some even as old as 88 years of age

(Barer, 1966; Barer and Jowsey, 1967). This lends further credence to the view that some periosteal turnover does occur in old age.

Microradiographic studies have demonstrated an increase in the number of osteons and in cortical porosity as a function of age (Jowsey, 1960). Epker, Klein and Frost (1965) and Barer (1966) have noted a slight decrease with age in the size of the osteons and in cortical thickness but a slight increase in the Haversian canal perimeter. On the other hand, some investigators (Arnold, Bartley, Tont and Jenkins, 1966) suggest an age associated decrease in cortical thickness of vertebrae only in females; their data do not appear to support such a hypothesis for males. The amount of cortical bone is said to increase with age until about 20 years of age and decrease slightly thereafter (Takahashi and Frost, 1966).

After the fourth decade, as compared to periosteal and endosteal surfaces, more remodeling occurs in Haversian bone (Epker and Frost, 1965a). Also, with age there is a slight tendency for osteons to drift in a periosteal direction in the rib (Epker and Frost, 1965b). By means of tetracycline labeling, it has been suggested that an age associated decrease in Haversian canal perimeter continues into the eighth decade and averages about 0.086μ per day between the ages of 52 and 84 years (Epker, Hattner and Frost, 1964; Landeros and Frost, 1964a). There is also some evidence to suggest that the rate of remodeling is slowest from 35 to 40 years of age (Bromley, Dockum, Arnold and Jee, 1966; Villanueva, Sedlin and Frost, 1963).

The surface area of Howship's lacunae in a given amount of cortical bone has been found to be highest at infancy and least in adult life (Sedlin, Villanueva and Frost, 1963). They found no adolescent spurt and, therefore, postulated that the internal remodeling of bone is probably independent of influences that govern skeletal growth and maturation.

As long ago as 1911, Balthazard and Lebrun claimed that, after 10 years of age, it was possible to estimate age quite accurately from the diameter of the Haversian canal, regardless of the choice of bone. Implied in their work was the assumption that differences in Haversian canal diameter are completely age dependent and variability between that of different bones of an individual is negligible. However, it has been recently demonstrated that even within a given bone, Haversian canal diameter shows so much variability that it may have to be considered undependable for the purpose of estimating age at death (Deslypere and Baert, 1958). Variability in Haversian canal diameter between different bones has not been investigated.

From studies of undecalcified sections of femora from 19 subjects, 23 to 89 years of age, it has been suggested that with age there is an increase in the Haversian canal diameter and a concomitant decrease in the osteon diameter (Currey, 1964). The latter finding agrees with that of Barer (1966) and Landeros and Frost (1964b) but the former finding is inconsistent with those of others (Epker, Hattner and Frost, 1964; Landeros and Frost, 1964a).

These contradictions are probably due to the small sample size and the lack of any definition of Haversian canal diameter in Currey's report.

Recently a quantitative histological approach towards age estimation from cross sections of femur, tibia and fibula at mid-shaft has also been attempted (Kerley, 1965). However, the standard error of estimate is so large that the technique becomes of doubtful value. His study does demonstrate that the number of Haversian systems (osteons) in a given area of bone is positively and highly correlated to age. Lacroix and Dhem (1967) have also suggested that the number of osteons per unit area of bone may be a useful estimator of age. Their recommendations, however, were based on a study of ground sections of human tibias from only two subjects, 24 and 89 years of age.

Age changes in several additional parameters of bone have also been reported. As has been the case in several of the investigations reviewed so far, much of the data were neither collected nor analyzed for the purpose of estimation of age at death, and from this point of view, are of little value.

Garn and Schwager (1967), in a radiographic study, have demonstrated the persistence of high density transverse lines in tibia in 14% of women and 8% of men between 51 and 85 years of age. Density of bone has been reported to decrease with age by some investigators (Broman, Trotter and Peterson, 1958) and to show no relationship to age by others (Williams and Samson, 1960). Refractive index of bone has been reported to be constant from birth to three years

(1.560 ± 0.002), decrease to 1.549 by middle age and increase thereafter to 1.564 ± 0.002 (Antonio, 1949). Diameter of the collagen fibers has been claimed to increase with age (Hall, 1957) as also the proportion of fibrous elements to ground substance (Sobel and Marmorston, 1956). A number of investigators (among others Trotter and Peterson, 1962; Woodard, 1962, 1964; Mellors, 1964; Menczel, Posner and Harper, 1965; Arnold and Tont, 1967) have analyzed the relationship to age of various parameters, for example, content of calcium, phosphorus, water, ash and configuration of apatite crystals. These studies are not considered further here because they do not contribute much towards elucidating the problem of age estimation from skeletal material.

In summary, estimates of age at death from epiphyseal closure are quite dependable until the age of 25 years. Thereafter, until about fifty years of age, estimates of chronological age depend upon subjective criteria derived from differentiation in the pubic symphysis which are rather difficult to interpret and apply. Beyond age fifty, the odds against accurate age determination from skeletal morphology may best be termed as overwhelming. The accuracy of such estimates depends largely on the experience of the examiner and the completeness of the available material. However, not often does one find complete skeletons in well preserved condition. Needless to say, such methods are not easily replicated or quantitated. Histological studies have demonstrated age-associated changes in some parameters of cortical bone. In most of these studies, however, estimation of age at death was not the primary objective and from

them data for such purposes are not available.

Thus it is seen that over the years many different measurements of bone have been correlated with chronological age, but the results have been equivocal perhaps as a function of the experimental design. Gross bone observations, whether qualitative or quantitative, are obviously of limited value. Quantitative histology is a relatively unexplored tool, but it can reveal information at the microscopic level which is obviously not available from examination of gross specimens. It also lends a measure of reliability and objectivity to the observations and, therefore, offers a new promise. Quantitative histological techniques assume added importance when one realizes how small a sample of tissue is required for analysis.

In view of the above literature, it becomes of interest to define histological bone parameters which are age dependent and then, by superimpsing a known disturbance of bone metabolism, to be able to determine the constancy of such parameters in estimating chronological age. Later there will be a further consideration of the rationale for age determination from bone; but let us, for a moment, concede that histological measurements of bone can provide accurate estimates of age and very briefly consider a known disturbance of bone metabolism relevant to this study.

C. Effect of Estrogens on Skeletal Tissue

Many investigators have suggested a strong and positive correlation between maturity ratings (skeletal age, age at menarche, age at peak height, velocity, etc.) and measures of physique such as fat, muscle and bone size. Early maturing girls are known to show more subcutaneous fat (Reynolds, 1946; Garn and Haskell, 1960), and smaller relative breadth of bone and muscle (Reynolds, 1946), than late maturing girls. Girls who before puberty are one standard deviation above the average in normalized fat scores reach menarche earlier, are advanced in skeletal maturation and are taller at all ages from one and one-half to eleven and one-half years of age (Garn and Haskell, 1960).

Sex differences in components of body build are well known (Tanner, 1962; Singh, Savara and Newman, 1967). While early and late maturing subjects show differences in physique before, during and after adolescence, the time and duration of adolescence is related to linearity of body build (Tanner, 1962). Since early maturing subjects have completed a larger percentage of their mature size by a given age, the late maturing subjects, by virtue of their longer adolescent period, are larger at the end of the growth period (Tanner, 1965). It is also well known that high levels of circulating estrogens lead to an earlier cessation of epiphyseal growth resulting in shorter long bones at maturity (Lloyd, 1963). It follows then that in subjects who exhibit high levels of circulating estrogens, differences in body build should exist when

compared to the norm.

In some animals, estrogens are known to effect both soft and skeletal tissues. Prolonged administration of estradiol benzoate to rabbits results in a significantly lower weight gain, narrowing of the epiphyseal cartilage plate, a decrease in cartilage and bone weight and a reduction in metaphyseal height (Bernsten, 1968). A reduction of metaphyseal height in estrogen treated rabbits had also been reported earlier (Urist, Budy and McLean, 1948).

A similar decrease in weight gain following estrogen treatment in rats also has been demonstrated (Day and Follis, 1941; Meyer, 1961; Tjan and Gunberg, 1967). By controlling food intake, it has been suggested that the difference in weight gain is due to a catabolic effect of estrogens rather than any difference in food ingestion (Day and Follis, 1941). In the rat, Tjan and Gunberg (1967), among others, have demonstrated that, as determined by organ weight, different organs manifest different responses to estrogen treatment.

It is also known that during the ovulatory phase, female pigeons produce a secondary system of endosteal spongy bone; these calcium deposits are later used during mineralization of the egg shell (Kyes and Potter, 1934). Intramedullary bone is normally produced during ovulation in all avian females and can be produced in the males by the administration of estrogens.

Such changes can also be produced in mice by estrogen administration, but not in the growing rat (McLean and Urist, 1968). In mice, estrogen administration inhibits proliferation and hypertrophy of cartilage cells in the epiphyseal plate (Silberberg and Silberberg,

1946), produces endosteal ossification of long bones (Urist, 1950), and retards linear skeletal growth (Suzuki, 1958, 1959). In guinea pigs, prolonged estrogen administration produces a decrease in epiphyseal cartilage height (Silberberg and Silberberg, 1939).

Conflicting evidence exists on the effect of estrogen treatment in the growing rat; an earlier appearance of ossification centers only in females has been claimed by some investigators (Talbot, 1939), whereas others (Noback, Barnett and Kupperman, 1949; Urist, Budy and McLean, 1948) have suggested that estrogen administration in the rat results only in an inhibition of resorption of the spongy bone of metaphysis.

The reason for the differential response of species to estrogen treatment is unknown (Weinmann and Sicher, 1955). In any event, even in the rat, estrogen does appear to have some influence on osseous tissue. Whether or not any changes in bone cortex of the rat may be expected in response to such treatment is not known.

Several investigators (among others Priest and Koplitz, 1962; and Bernsten, 1968) have studied biochemical alterations in bone resulting from estrogen treatment. Beyond this passing reference, such studies are not discussed here as they are not pertinent to this dissertation.

It is also worth noting that, as determined by tetracycline labeling techniques, the rate of decrease in size of Haversian canals appears to be slower in diabetics (Landeros and Frost, 1964b), as well as in subjects treated with adrenal cortical steroids (Klein, Villanueva and Frost, 1965). Microradiographic studies have suggested

that in osteoporotic individuals the marrow cavity is probably larger (Santoro and Frost, 1968), and the resorption rate may be slightly increased but with a normal formation rate (Jowsey, Kelly, Riggs, Bianco, Scholz, and Gershon-Cohen, 1965). Other investigators, however, have reported a reduced rate of Haversian bone formation in such subjects (Villanueva, Ilnicki, Duncan and Frost, 1966; Jett, Wu and Frost, 1967). In recent years several authors, but particularly Frost (1963a, 1963b; 1964), have developed and emphasized the view that histological parameters of bone cortex constitute a sensitive measure of the metabolic state of the organism.

In view of this literature, therefore, the argument has been substantially strengthened that the effects of some exogenous agent on bone cortex should be included in an investigation of morphologic age-changes in bone.

D. Statement of the Problem

Broadly speaking, this investigation aims to elucidate some facets of the ageing process in cortical bone of both humans and rats. As proposed here, the problem has two natural subdivisions: first, the specific objectives and second, a brief consideration of the rationale governing the experimental model.

1. Objectives

The investigation reported here consists of the following two parts:

Part I. Animal Study

The objectives here are to determine in an animal population, less variable than the human:

1) the possibility of devising a model for estimating chronological age from histological measurements of bone cortex and to assess the usefulness as well as the errors inherent in such a model.

2) the variability in age estimation and the errors introduced in the predictive model when animals are subjected, during a period of rapid skeletal development, to an exogeous agent suspected of eliciting an effect on the osseous tissues.

Part II. Human Study

The objective of this study is to derive a practical and useful model for the estimation of age from quantitative histology of bone cortex.

2. Rationale

Before any alterations in bone age may be attributed to administration of an exogenous agent, it is necessary to define a set of skeletal parameters which can estimate chronological age and which can thus be shown to be age dependent. Theoretically speaking, if it were possible to measure all of the age dependent, histological parameters in bone, then estimation of age from them should be precise. However, the set of all age dependent variables is never available and, therefore, is not measureable. On the other hand, any one given parameter would probably manifest considerable variation and alone would be a very poor estimator of age.

The fallacy of relying upon a single criterion for estimating age at death was dramatically illustrated in the case of the "Tepexpan Man." Based on suture closure alone, the skull was assigned an age of 55-65 years (Romero, 1949), but on reevaluation with dental radiographs, an age of no more than 25-30 years could be assigned (Genoves, 1960; Moss, 1960).

It follows then that a judiciously chosen subset of age dependent variables could provide practically useful estimates of chronological age within a certain range of permissible error. In other words, a multivariate approach might succeed where an univariate model is obviously inadequate.

Ageing of skeletal tissue is a complex phenomenon and, in the present state of knowledge, it is possible that the independent

estimates of the selected predicting (control or independent) variables have a very limited functional relationship to ageing. Thus, the ability to select control variables with high cause and effect relationships to ageing is limited. Nevertheless, for this problem, a predictive multivariate model may be postulated. It must be emphasized, however, that such a model does not imply the existence of a functional relationship between the response variable (age in this study) and the predicting variables (histological measurements in this study). Such an assumption is neither implied nor required for the statistical model proposed here (Draper and Smith, 1968). In one sense then the model is unrealistic, but as long as it is capable of reproducing the main features of the response variable (age), it is useful.

For purposes of age estimation from histological bone measurements, a multiple linear regression model may be conceived as follows:

$$Y_{ij} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots\ldots + \beta_p X_p + \xi_{ij}$$

where Y_{ij} is the real age, β_0 is a regression constant, and β_1 to β_p are regression coefficients (weights) for the respective estimating variables, X_1 to X_p, and where ξ_{ij} is random unmeasured error which is reflected as the standard error of the estimate. This concept is further discussed in the section, "Material and Methods."

A reasonably simple and accurate model for estimating age at death from incomplete skeletal remains should prove useful in forensic medicine and anthropology. The method proposed here should be

equally useful for such diverse applications as relative ageing of a species of animal or the use of bone as an indicator of the metabolic state of the organism. As discussed earlier, attempts at such morphometric analyses of bone indicated that the effects on the measurement system of some experimental paradigm, such as one to which skeletal system may be exposed during life, should be included.

II. MATERIAL AND METHODS

In the preceding sections the literature was explored, and both theoretical and practical considerations relevant to this dissertation were examined. In accord with the objectives, three different experiments were undertaken and in this summary each experiment is considered in relation to its objectives. This very brief summary will be followed by a fuller description of each experimental procedure in the same order.

The first experiment investigated the possibility of deriving a model for estimation of age at death from some histological parameters of bone cortex. The experiment was conducted on rats and, from these data, mean values and range of histological parameters were obtained and nomographs were constructed for age estimation in the rat from soon after birth to maturity.

The second experiment, by exposing skeletal tissues to an exogenous metabolic agent, investigated the variability and errors introduced in the system for age estimation in the rat derived in the first experiment. Thus the individual and collective responses of a number of age dependent variables of bone to an experimental paradigm were assessed.

The third experiment was conducted on skeletal material from human subjects and it provided normative data for estimation of age at death in an older human population. In this problem, the choice of the experimental design was guided by the results of the first two experiments.

Some specialized statistical techniques were necessary for analyses, and the fourth and fifth parts of this section present respectively:

1) Experimental design for analysis of measurement errors in the histological variables and

2) Design of problems for data analysis.

A. Experiment 1: Untreated Animal Group

1. Material

Thirty-five female Sprague-Dawley rats procured locally* were sacrificed in groups of five at ages 2, 10, 20, 30, 60, 90 and 120 days.

2. Histologic Technique

The left femur and tibia and the left half of each mandible were dissected out from each animal, and divested of soft tissues. The bones were simultaneously fixed and decalcified in formalin-formic acid mixture, dehydrated, and cleared in toluene. They were embedded in paraffin and oriented so as to provide cross sections of femur and tibia at the midshaft and of the mandible at the gonial angle (Figure 1).

Over the age period investigated, 35 bone specimens each of mandible, femur and tibia were available. Ten micra thick sections were cut with a rotary microtome and mounted on albuminized slides. Four such slides were prepared from each bone specimen and each slide contained a ribbon of two to four adjacent sections. One slide for each bone specimen was stained with thionin and examined. Relevant details of decalcification and staining are provided in Section A of the Appendix.

3. Histologic Measurements

Sections were examined under high power (40X) with 10X wide-field oculars. One ocular contained a micrometer standardized against a stage micrometer and was used to measure the shortest

* Rush Laboratories, Beaverton, Oregon

diameter of the Haversian canals. This combination of objective and ocular lenses covered a microscopic field that was 0.5 mm in diameter.

Measurements were made on the 105 thionin-stained slides, 35 for each of the three bones. On each slide, two sections were randomly chosen and on each such section, one microscopic field in the periosteal third was randomly selected. The following data were obtained from these two fields (Figure 2A):

a) X_1 - the total number of osteons (complete Haversian systems). In cross sections, these appear as concentric lamellae arranged around a central vascular canal. Histologically, each osteon is clearly demarcated from the neighbouring ones as well as from the interstitial systems by prominent cement lines; lamellae are seen as alternating dark and light rings, each ring containing a number of lacunae filled with osteocytes. The total number of osteons in two fields was counted. Osteons that were partially obscured by the periphery of the field, or those cut obliquely or seen only as fragments, were included only if the complete Haversian canal could be seen. Hereafter, X_1 refers to the total number of osteons in two fields as defined here and not an average.

b) X_2 - the number of lamellae per osteon. In two fields, lamellae were counted on each osteon and averaged to provide the mean number of lamellae per osteon for every bone specimen.

c) X_3 - Haversian canal diameter. An ocular micrometer containing a 1cm scale divided into 100 equal parts was used to obtain

the shortest diameter of each Haversian canal. Diameter was obtained only when the complete Haversian canal was visible. In obliquely cut osteons this measurement was not made when the canal was three times or more longer than it was wide. Measurements were made on all Haversian canals meeting these criteria and in two fields. An average value for each specimen was computed.

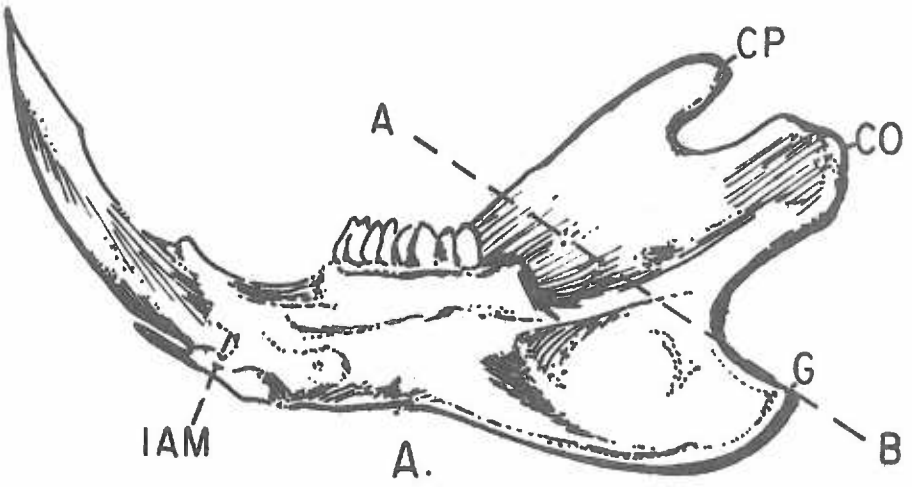
d) X_4 - the number of primitive or non-Haversian longitudinal canals. Histologically these bear a superficial resemblance to the definitive or secondary Haversian system (X_1) but are distinguished by the lack of any cement lines or any other sharply defined border. The total number seen in two microscopic fields was ascertained.

Hereafter, this material is referred to as the untreated rat material in this dissertation, and from this material were derived the regression relationships for age estimation in the rat.

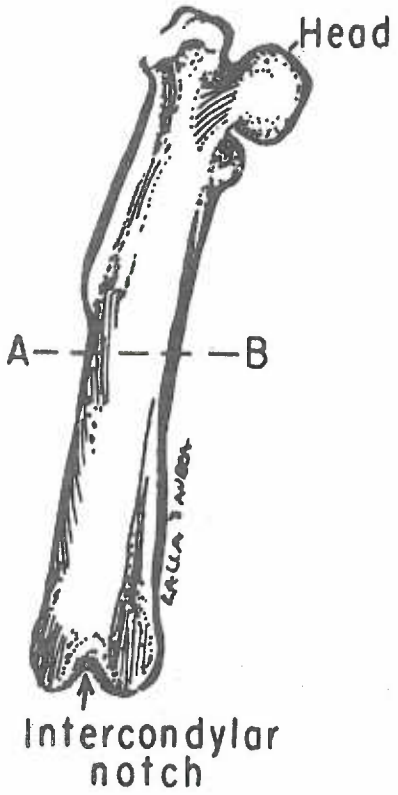
FIGURE 1.

Mandible, Femur and Tibia of the rat indicating landmarks used and level of sectioning (broken line)

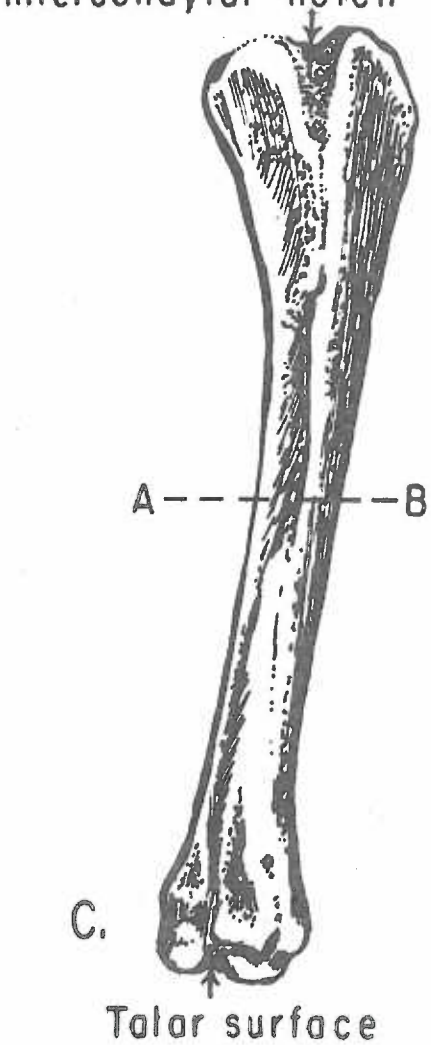
A. Mandible, medial aspect
B. Femur, Anterior aspect
C. Tibia, Anterior aspect


CP
A
CO
G
B
IAM

A.


Head
A
B
Intercondylar
notch

B.

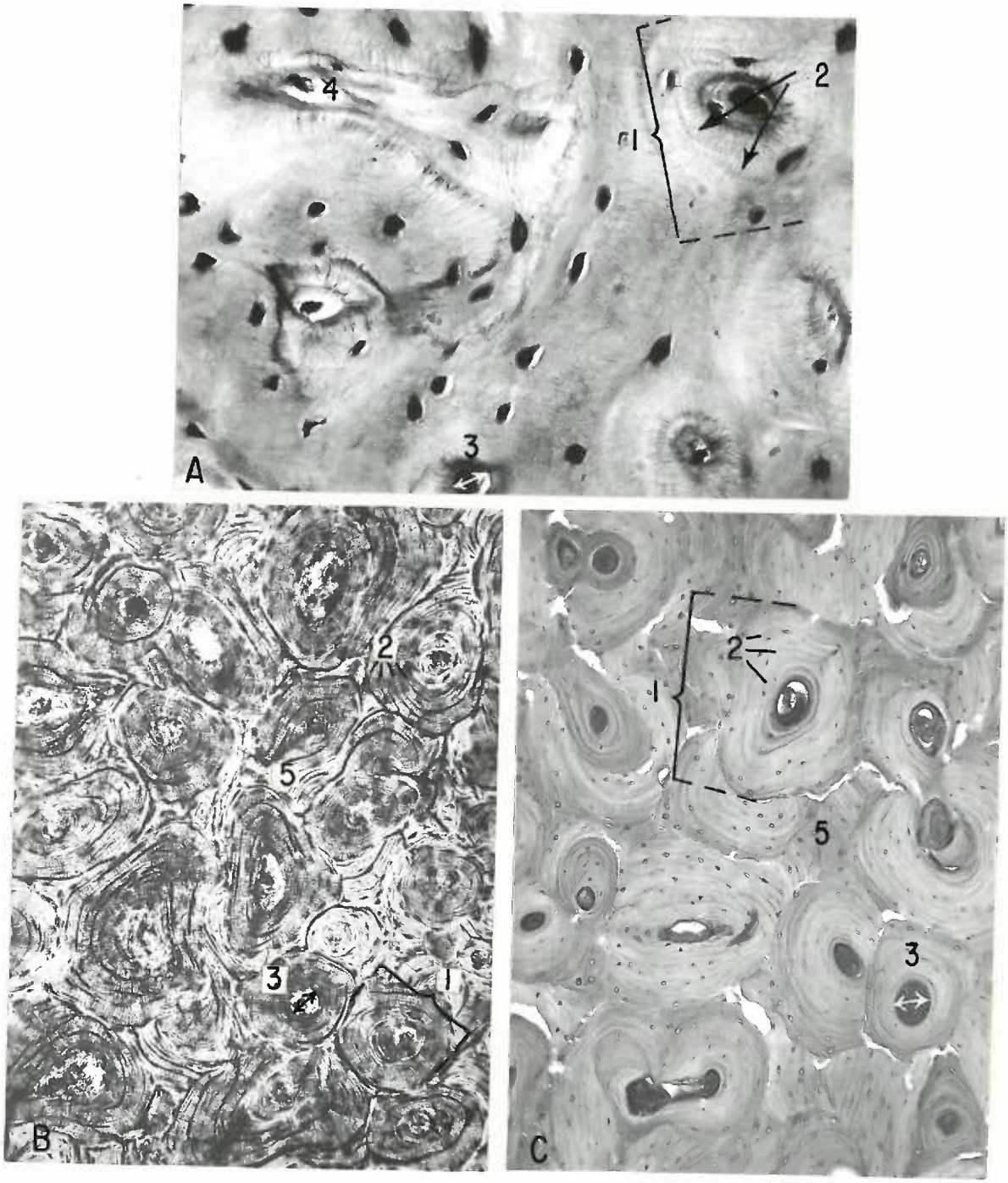

Intercondylar notch
A
B
Talar surface

C.

FIGURE 2.

Histological Measurements on rat and human skeletal material.
A. Decalcified section of 60 day old rat tibia (480X)
B. Ground section of 60 year old human mandible (90X)
C. Decalcified section of 60 year old human femur (90X)

1. Complete osteon
2. Lamellae
3. Haversian canal diameter
4. Non-Haversian longitudinal canal
5. Interstitial bone.


4
2
1
3
A
2
5
3
1
B
2
1
5
3
C

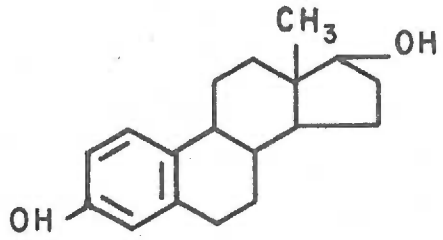
B. Experiment 2: Treatment with 17 Beta Estradiol

1. Material

Forty female Sprague-Dawley rats, 30 days old, were procured locally*. For the duration of the experiment, they were housed in a reverse light cycle environment of 12 hours duration and maintained on Purina laboratory chow and tap water ad libitum. The rats were divided into two groups of twenty animals each.

2. Treatment

One group was designated as the treated group and received 0.2 ug of 17 Beta Estradiol** daily. This was administered subcutaneously in the dorsal region of the neck from 30 to 60 days of age inclusive, each injection consisting of 0.4 ml of an aqueous suspension of the steroid. This dose is equivalent to 2.4 Rat Units or 24 International Units. This steroid has the following configuration:



The remaining twenty animals served as sham controls and received the same daily volume of diluent during a comparable age period.

*Rush Laboratories, Beaverton, Oregon.

**Progynon manufactured by Schering Corporation. Aqueous suspension containing, per ml, 0.25 mg of 3, 17 Beta Dihydroxyoestra - 1,3,5 (10)-triene with 0.015 mg polysorbate 80 and 0.5 percent phenol, suspended in isotonic saline.

3. Histologic Technique and Measurement

Five animals from each group were sacrificed at ages 45, 60 90 and 120 days. From each animal, the left femur and tibia, and the left half of the mandible were removed, cleaned of soft tissues, and prepared for histological examination. This processing of tissues, definition of landmarks, and measurements made (X_1 to X_4) were as in Experiment 1 (Figure 2A).

4. Non-Histologic Measurements

In addition, the following non-histological data were also obtained on rats of both sham and treated groups:

I. Body weight measured every seventh day

II. Age in days at which, by visual examination, vaginal plates were seen to be open.

III. Some gross bone measurements were obtained at the time of sacrifice of each animal. Landmarks and linear measurements recorded were as follows (Figure 1):

Mandible

1) Weight
2) Tip of the coronoid process (CP) to maximum curvature on the mandibular condyle (CO).
3) CO to tip of the gonial angle (G)
4) CO to the maximum curvature on the incisal alveolar margin of the central incisor on the medial surface of the mandible (IAM).
5) G to IAM

Femur

1) Weight
2) Length from femoral head to the notch of the intercondylar fossa.
3) Maximum diameter at midshaft

Tibia

1) Weight
2) Length from intercondylar fossa to talar surface
3) Maximum diameter at midshaft

Body weight was measured to the nearest gram and bones were weighed wet on a Roller-Smith torsion balance to the nearest 0.2 mg. Linear measurements of bones were obtained by graduated vernier calipers to the nearest 0.1 mm.

C. Experiment 3: Human Studies

1. Material

Human material was obtained from 59 cadavers from the dissection rooms of the University of Oregon Medical and Dental Schools. The sample was well documented as to sex, pathological history, and age at death; and in no case was there any gross or microscopic pathology associated with any mandible, femur or tibia in the sample.

Samples of all three bones were available from 40 subjects (33 males), but in the remaining 19 males only mandibular samples could be collected. There were only seven females in the sample; therefore, derivation of models for estimation of age at death was based exclusively on the males. The female sample was used to test the model for age estimation and for some very preliminary observations on sex differences.

2. Histologic Technique

Approximately 1 cm x 1 cm fragments of bone were removed from the midshaft of anterior surface of femur and tibia and from the posterior border of the mandibular ramus opposite the lingula (Figures 3, 4, and 5). The diagrams also show that although bone fragments did not extend through the complete circumference of any bone, each bone sample was complete from the periosteal to the endosteal border of the anterior surface in the tibia and femur and posterior border of the mandibular ramus.

Bone fragments from 40 subjects were decalcified and processed

as in the earlier two experiments. From each of these 120 bone specimens, ten micra thick decalcified sections were cut and mounted on albuminized slides. As in the earlier experiments, four such slides were prepared for each bone specimen, each slide contained from one to three adjacent sections. One slide for each specimen was stained with thionin and examined. Procedural details are provided in Section A of the Appendix.

In the 19 males, where only mandibular samples were available, bone fragments were cleaned of soft tissues, dehydrated in absolute alcohol, and embedded in Caroplastic*. Thick sections, 150 to 200 micra, were cut with a saw and hand ground to approximately 30 to 50 micra thickness. One ground section per bone specimen was mounted and examined. Details relevant to making ground sections are provided in Section B of the Appendix.

3. Histologic Measurements

Sections were examined under low power with 10X objective and 10X widefield ocular lenses. This combination of objective and ocular lenses permitted examination of a microscopic field that was 2.0 mm in diameter. One ocular contained the standardized micrometer described in Experiment 1.

Measurements were made on the 120 thionin-stained and 19 unstained ground sectioned bone specimens. Two microscopic fields were selected at random from the periosteal third of bone sections. Wherever possible, each field was from a different section. On most, but not all, decalcified sections this could be done; on ground

*A methacrylate embedding material manufactured by Carolina Biological Supply.

sections selection of microscopic fields on different sections of the same specimen was not possible since only one section per specimen was made. However, even within the same section, selection of the two fields was randomized. Landmarks were defined as in Experiment 1 and measurements were made as follows (Figures 2B and 2C):

a) X_1 - the total number of osteons in two microscopic fields

b) X_2 - average number of lamellae per osteon calculated from all osteons in two fields

c) X_3 - average shortest diameter of the Haversian canal measured as defined in Experiment 1.

FIGURE 3.

Human mandible indicating the level of sectioning and the fragment removed for histologic study.

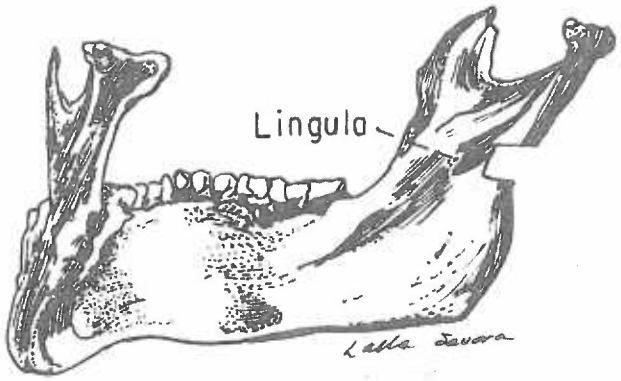

Lingula

FIGURE 4.

Human femur indicating the level of sectioning and the fragment removed for histologic study.

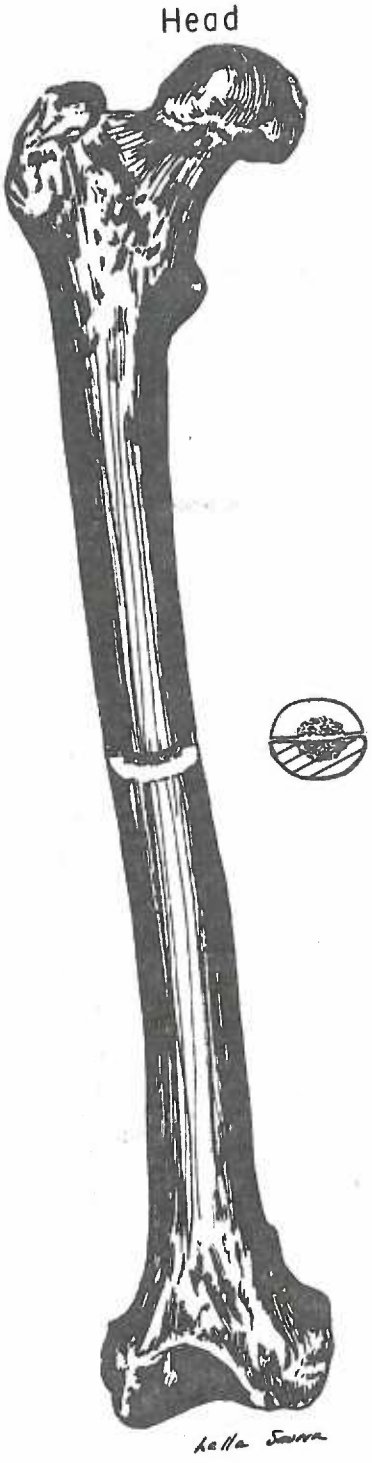
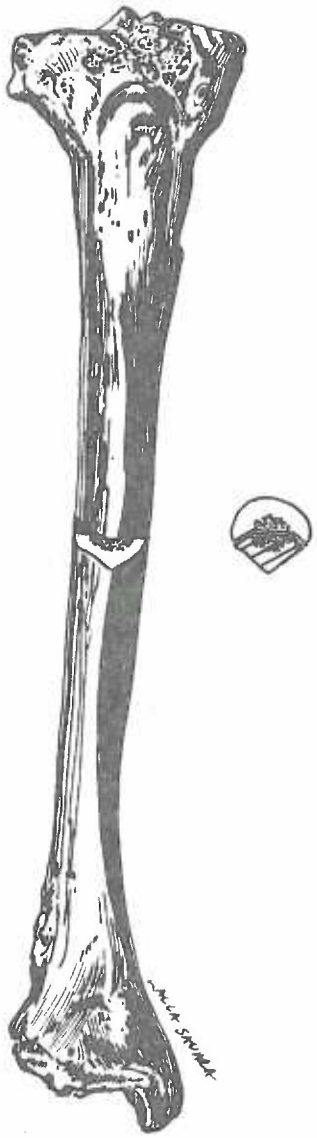

Head
Lella Sarra

FIGURE 5.

Human tibia indicating the level of sectioning and the fragment removed for histologic study.



Talar surface

D. Analysis of Measurement Errors

Reliability of measurements was tested on untreated rat material collected in Experiment 1. There were 35 specimens each, of the mandible, femur and tibia, between the ages of two to 120 days. From among these, five slides of each bone were randomly selected for measurements by another examiner. The second examiner (R.G.W. Anderson) obtained his set of measurements of the variables, X_1 to X_4, with no prior knowledge of the measurements obtained earlier by this investigator.

Consistency between examiners was evaluated for inter-examiner differences, pooled over all ages and all bones investigated in this study. Each of the 15 sections was examined for each variable (X_1 to X_4) by each examiner and data were analyzed by a two-way, repeated measures, analysis of variance (Winer, 1962).

By this technique, it was possible to partition the total variation in the measurements into various contributing factors, namely, the variability due to:

a) pooling of sections over all ages and bones

b) inter-examiner differences

c) interaction between examiners and measurements, some measurements being easier to measure than others. For example, one examiner may find it easier to count the number of lamellae than to measure Haversian canal diameter.

d) various other interaction terms represented the argument

that some measurements may be more easily measured in a certain bone, in a specific age period and by a particular examiner.

An assumption necessary to this analysis, that of a fixed model, was made. The experimental model, expected values of mean squares, and appropriate F-rations are presented in Table 2. The two examiners are represented by the levels of factor A (a_1, a_2) and the measurements obtained from each bone specimen (X_1, X_2, X_3, X_4) are respectively represented by the four levels of factor B (b_1, b_2, b_3, b_4).

LEGEND FOR TABLE 2

X_{ijkm}, the measurement on section from the $k^{\underline{th}}$ subject, by the $i^{\underline{th}}$ examiner and for the $j^{\underline{th}}$ variable.

Factor A. represents levels of examiners: two levels in this design because there are two examiners
α represents the effect due to differences between examiners a_1 and a_2.

Factor B represents levels of measurements: four levels in this design because there are four variables, X_1, X_2, X_3, and X_4.
β represents the effect due to differences between measurements b_1, b_2, b_3, and b_4.

Variability among the 15 sections here is represented by π, therefore π_k is variability within the $k^{\underline{th}}$ subject.
Sampling here is pooled over all seven age groups and all three bones.

The various interaction terms are self-explanatory and are represented by $\alpha\pi$, $\beta\pi$, $\alpha\beta$, and $\alpha\beta\pi$.

$\epsilon_{m(ijk)}$ is randomly distributed error. $\epsilon \sim N(0, \sigma^2)$

The model means that any measured value X_{ijk} in fact represents the true mean ($\mu..$) plus the effects of differences between examiners, measurements, sections, plus the various interaction terms of these main effects, and plus some random experimental error.

TABLE 2. EXPERIMENTAL DESIGN FOR ANALYSIS OF MEASUREMENT ERRORS

Model: $X_{ijkm} = \mu.. + \pi_k + \alpha_i + \alpha\pi_{ik} + \beta_j + \beta\pi_{jk} + \alpha\beta_{ij} + \alpha\beta\pi_{ijk} + \epsilon_{m(ijk)}$

Factors A and B fixed

Analysis of Variance

Source	df	E(MS)	F
Between Subjects (Sections)			
Subjects Within Group	n-1		
Within Subjects (Sections)	n(pq-1)		
A	p-1	$\sigma^2_\epsilon + \sigma^2_{\alpha\pi} + \sigma^2_\alpha$	$MS_A/MS_{A \times Subj\ W\ Grp}$
A X Subj W Grp	(p-1)(n-1)	$\sigma^2_\epsilon + \sigma^2_{\alpha\pi}$	
B	q-1	$\sigma^2_\epsilon + \sigma^2_{\beta\pi} + \sigma^2_\beta$	$MS_B/MS_{B \times Subj\ W\ Grp}$
B X Subj W Grp	(q-1)(n-1)	$\sigma^2_\epsilon + \sigma^2_{\beta\pi}$	
AB	(p-1)(q-1)	$\sigma^2_\epsilon + \sigma^2_{\alpha\beta\pi} + \sigma^2_{\alpha\beta}$	$MS_{AB}/MS_{AB \times Subj\ W\ Grp}$
AB X Subj W Grp	(p-1)(q-1)(n-1)	$\sigma^2_\epsilon + \sigma^2_{\alpha\beta\pi}$	
TOTAL	npq-1		

Ho: $\sigma^2_\alpha = \sigma^2_\beta = \sigma^2_{\alpha\beta} = 0$

H_1: Not H_0

$\alpha = .05$

E. Data Analysis

1. Experiments 1 & 3: Estimation of Age in Rats and Humans

Data were analyzed by multiple linear regression techniques. The program for this technique computes first a simple correlation matrix for each of the predicting variables with the dependent variable (age in this study). Then a linear multiple regression equation is computed from a preselected set of independent variables.

Thus regression equations are of the form:

$$Y_{ij} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots\ldots + \beta_p X_p + \epsilon_{ij}$$

and the value of p is determined a priori. To determine whether or not the regression relationship is significant, the following hypothesis was tested:

$$\text{Ho: } \beta_1 = \beta_2 = \ldots\ldots = \beta_p$$

The 5 percent level of significance based on analysis of variance was selected as the criterion for the regression equation (Draper and Smith, 1968).

From measurements on each bone, separate series of prediction relationships for age were derived. Tables 3 and 4 respectively present experimental designs for regression relationships for the rat and the human material.

From these regression models, it was possible to construct several nomographs from which, given the two best correlated variables with age, one could with a certain error estimate chronological age in both rats and in humans. Methods for constructing such

nomographs are well explained by Levens (1948).

2. Experiment 2: Effect of 17 Beta Estradiol

The general effects of 17 Beta Estradiol were assessed by differences, between treated and sham groups, in:

a) age at which vaginal plates were first seen to be open

b) body weight tested by Student's t statistic, at age of maximum difference between the two groups (Li, 1964).

Effects on skeletal growth were investigated by calculating the ratio of bone weight/body weight at ages 45, 60 90 and 120 days, and also from growth patterns of linear bone measurements.

Changes in skeletal microstructure resulting from the experimental treatment were analyzed by computing differences between real age and age estimated from the reference standards derived in Experiment 1.

TABLE 3.

DESIGN OF PROBLEMS FOR REGRESSION ANALYSES ON UNTREATED RAT MATERIAL

Dependent Variable:

Y

Selection of Independent Variables:

I	X_1,	X_2,	X_3,	X_4,	X_5,	X_6,	X_7,	X_8
II	X_1,	X_2,	X_3,	X_4				
III	X_1,	X_2						
IV	X_1,	X_3						
V	X_1,	X_4						
VI	X_2,	X_3						
VII	X_2,	X_4						
VIII	X_3,	X_4						

List of Measurements:

Y = Log Age in Days

X_1 = Total number of osteons in two fields — $X_5 = X_1^2$

X_2 = Ave. no. of lamellae per osteon — $X_6 = X_2^2$

X_3 = Ave. diameter of Haversian canal (μm) — $X_7 = X_3^2$

X_4 = Total number of non-Haversian longitudinal canals in two fields — $X_8 = X_4^2$

This design was performed thrice, once each for the mandible, femur and tibia.

TABLE 4.

DESIGN OF PROBLEMS FOR REGRESSION ANALYSES ON HUMAN MATERIAL

Dependent Variable:

Y

Selection of Independent Variables:

I	X_1, X_2, X_3
II	X_1, X_2
III	X_1, X_3
IV	X_2, X_3
V	X_1
VI	X_2
VII	X_3

List of Measurements:

Y = Age in years

X_1 = Total number of osteons in two fields

X_2 = Ave. No. of lamellae per osteon

X_3 = Ave. diameter of Haversian canal (μm)

This design was performed thrice; once each for the mandible, femur and tibia.

III. RESULTS

In this section analysis of measurement errors is presented first and is followed by the results of each experiment in order. Some specific and general correlative aspects of these findings will be further considered in the section: Discussion.

A. Measurement Errors

Table 5 presents analysis of variance for evaluating errors inherent in these histological measurements. Variability due to examiners (levels of factor A) or examiner-measurement interaction (factor AB) is not significantly different from zero. Quite expectedly, significant variability exists among sections (levels of factor B).

Thus measurements as made by one examiner are not judged to be significantly different from those made by the second examiner for any of the four variables (X_1 to X_4) at the 5 percent level of significance.

TABLE 5.

ANALYSIS OF VARIANCE FOR MEASUREMENT ERRORS

Source	d.f.	M.S.	F
Between subjects (sections)	14		
Subjects within group			
Within subjects (sections)	105		
A (Examiners)	1	0.1390	< 1.0
AX Subj. W Grp.	14	0.9663	
B (Variables)	3	601.3975	42.1005*
BX Subj W Grp.	42	14.2848	
AB	3	0.4185	< 1.0
ABX Subj W Grp.	42	0.7121	
Total	119		

* P < .05

B. Experiment 1: Untreated Rat Material

1. Age Changes in Histological Measurements

Means and standard deviations of histological measurements are presented in Table 6, and to illustrate these changes, age trends for number of osteons (X_1) and number of lamellae (X_2) in the mandible, and Haversian canal diameter (X_3) and number of non-Haversian longitudinal canals (X_4) in the femur are also shown (Figures 6 and 7).

Age associated increase in X_1 and X_2, and decrease in X_3 and X_4 are seen. In trends, major points of inflection are seen at age 10 to 20 days for all four measurement. In addition, X_1, X_3 and X_4, but not X_2, show an inflection at approximately 60 days of age. With age, growth in every measurement occurs at a declining rate.

These comments apply equally well to measurements derived from any of the three bones investigated.

2. Regression Equations for the Estimation of Age in Rats

Regression equations for the estimation of age in rats were based only on untreated rat material and were derived separately for each of the three bones: mandible, femur and tibia.

Table 7 presents a simple correlation matrix between each of the four histological measurements and age for each bone. Between the ages of two to 120 days, the number of non-Haversian longitudinal canals are best correlated to age; this is followed closely by Haversian canal diameter, number of osteons, and number of lamellae

per osteon.

The various predicting equations along with their multiple correlation (R), and the standard error of estimate are provided in Table 8. Significant multiple R exist for every prediction equation and from every combination of independent variables investigated. The highest multiple R and the least standard error of estimate are obtained with a full model containing all first order and their quadratic components, however, almost equally good results are obtained with other regression models containing fewer terms.

Residuals (errors in prediction) resulting from the full models, as well as those containing only the first order terms, are provided in Section G of Appendix. Their perusal suggests that there is no systematic bias in the distribution of errors of prediction; this is also true of all other regression functions investigated.

When a combination of any two independent variables is regressed on age, significant estimating equations result; however, the best estimating equation for any bone is based on the number of osteons (X_1) and the number of non-Haversian longitudinal canals (X_4). Although the femoral regressions based on X_3 and X_4 are slightly better than the ones found on X_1 and X_2, the difference is not significant. Comparisons of regression functions based on different bones show that the coefficients of multiple correlation or the standard errors of estimate are not significantly different.

3. Nomographs for the Estimation of Age in Rats

Three nomographs, one each based on the mandible, femur and

tibia, were prepared from the number of osteons and the number of non-Haversian longitudinal canals (Figures 8, 9 and 10).

These regression relationships provide multiple correlations (R) ranging from 0.967 for the mandible to 0.952 for the femur. The standard error of estimates ranges from 0.137 to 0.184 log days which is approximately one and one-half days in age. Slightly better estimates result when regressions are based on the mandible.

Thus between the ages of two and 120 days, age may be estimated from these nomographs to the order of accuracy of $\pm$ 1.5 days in 67 percent, and to within $\pm$ 3 days in 95 percent of the cases, of the true value.

TABLE 6.

HISTOLOGICAL MEASUREMENTS ON UNTREATED NORMAL RATS

		AGE IN DAYS						
		2	10	20	30	60	90	120
MANDIBLE								
Osteons	$\bar{X}$	9.20	11.20	13.20	14.20	15.40	17.20	18.60
	S.D.	0.74	0.76	0.73	0.74	1.01	1.16	1.10
Lamellae	$\bar{X}$	1.49	1.91	2.14	2.40	2.90	3.45	4.09
	S.D.	0.14	0.22	0.17	0.14	0.26	0.34	0.65
Hav. Can. Dia.	$\bar{X}$	10.97	9.12	6.55	6.07	5.25	4.20	3.40
	S.D.	1.01	0.57	1.19	0.65	0.70	0.56	1.02
Non-Hav. Can.	$\bar{X}$	14.60	11.60	9.20	7.60	5.80	5.00	4.60
	S.D.	0.48	1.01	0.74	1.01	0.74	0.60	1.20
FEMUR								
Osteons	$\bar{X}$	9.80	11.00	12.60	13.80	16.20	17.00	18.00
	S.D.	1.16	1.09	0.80	0.74	0.74	1.09	1.41
Lamellae	$\bar{X}$	1.53	2.10	2.54	2.79	3.18	3.50	4.31
	S.D.	0.31	0.16	0.28	0.24	0.17	0.38	0.84
Hav. Can. Dia.	$\bar{X}$	10.16	9.65	8.27	6.48	5.43	4.69	4.10
	S.D.	0.43	0.20	0.30	0.59	0.38	0.47	0.44
Non-Hav. Can.	$\bar{X}$	16.80	12.20	10.40	8.80	7.80	6.40	5.20
	S.D.	0.74	1.32	1.01	1.16	0.97	1.01	1.16
TIBIA								
Osteons	$\bar{X}$	7.60	9.40	11.00	12.40	14.60	15.60	16.20
	S.D.	1.01	1.01	0.89	1.01	1.01	0.80	1.16
Lamellae	$\bar{X}$	1.60	1.88	2.35	2.42	2.74	3.57	4.15
	S.D.	0.22	0.19	0.20	0.14	0.16	0.22	0.40
Hav. Can. Dia.	$\bar{X}$	10.05	8.05	7.09	6.71	5.74	4.28	3.91
	S.D.	0.60	0.62	0.47	0.40	0.46	0.48	0.34
Non-Hav. Can.	$\bar{X}$	17.40	13.60	11.80	10.60	7.80	6.80	5.80
	S.D.	1.01	1.01	1.16	1.06	0.74	0.76	0.74

Sample size in each cell equals five.
Measurements of Haversian canal diameter in μm

FIGURE 6.

Age changes in the total number of osteons in two microscopic fields (X_1) and the average number of lamellae per osteon (X_2) in the rat mandible.

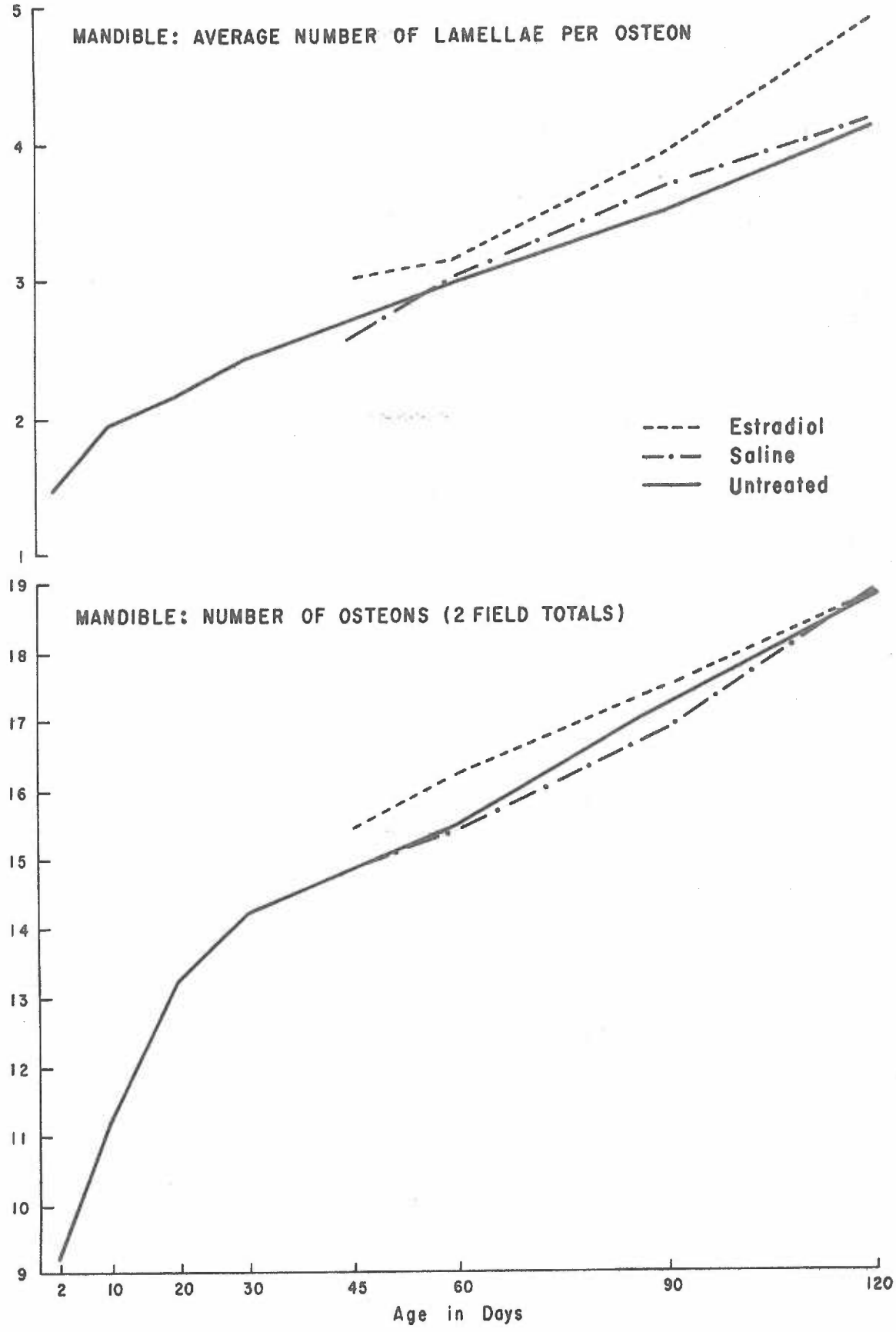

MANDIBLE: AVERAGE NUMBER OF LAMELLAE PER OSTEON
5
4
3
2
1
Estradiol
Saline
Untreated
MANDIBLE: NUMBER OF OSTEONS (2 FIELD TOTALS)
19
18
17
16
15
14
13
12
11
10
9
2
10
20
30
45
60
90
120
Age in Days

FIGURE 7.

Age changes in the average Haversian canal diameter (X_3) and the total number of non-Haversian longitudinal canals in two microscopic fields (X_4) in the rat femur.

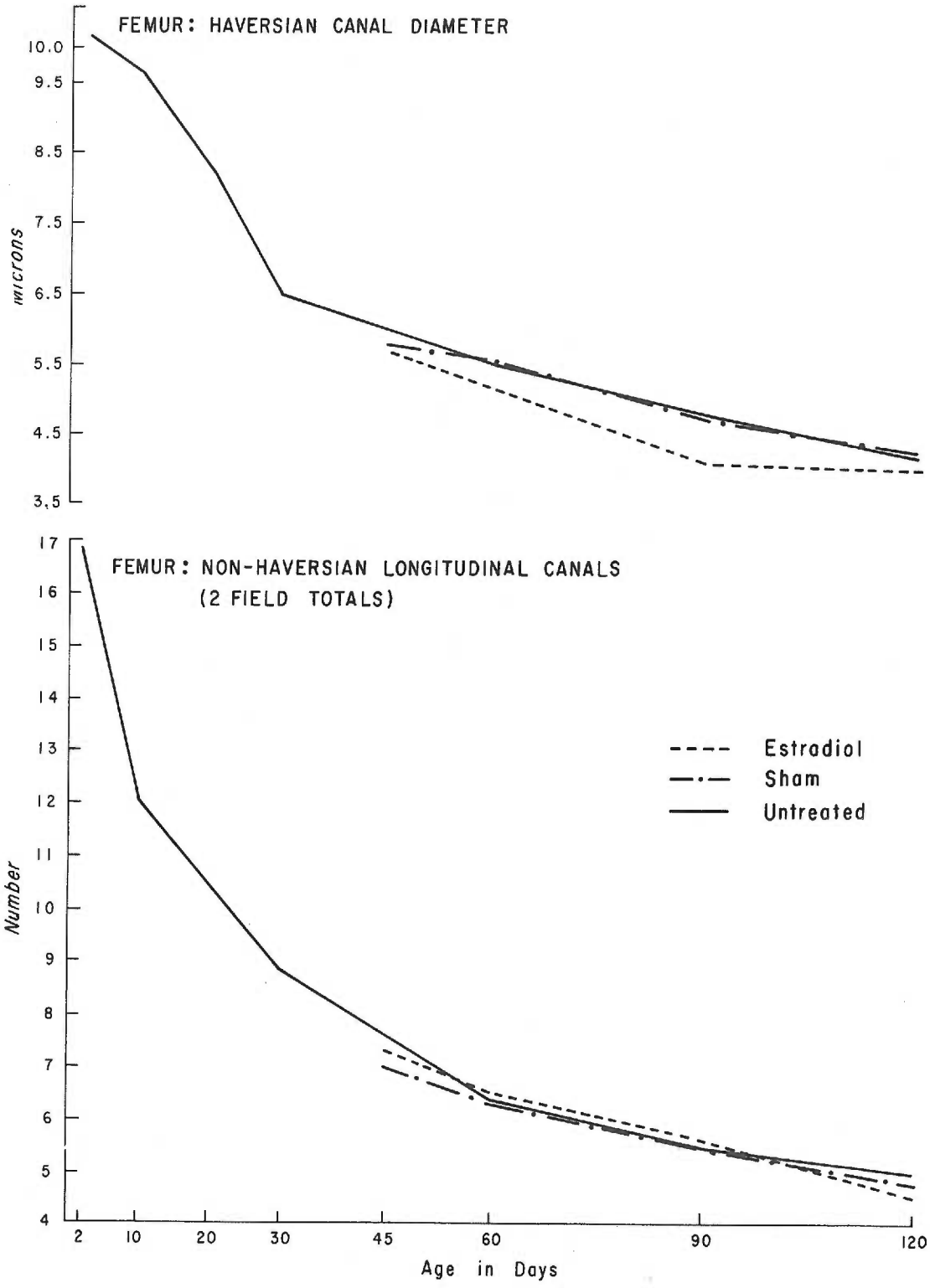

FEMUR: HAVERSIAN CANAL DIAMETER
microns
10.0
9.5
8.5
7.5
6.5
5.5
4.5
3.5
FEMUR: NON-HAVERSIAN LONGITUDINAL CANALS
(2 FIELD TOTALS)
Number
17
16
15
14
13
12
11
10
9
8
7
6
5
4
Estradiol
Sham
Untreated
2
10
20
30
45
60
90
120
Age in Days

TABLE 7.

SIMPLE CORRELATIONS OF UNTREATED RAT MEASUREMENTS WITH LOG AGE IN DAYS

	Bone		
Variable	Mandible	Femur	Tibia
Osteons	.936	.906	.928
Lamellae	.859	.858	.866
Hav. Can. Dia.	-.933	-.935	-.957
Non-Hav. Long.Can.	-.963	-.940	-.966

All correlations are significantly different from zero, $P < .05$

TABLE 8.

REGRESSION EQUATIONS FOR ESTIMATING AGE OF RATS FROM HISTOLOGICAL MEASUREMENTS

Dependent Variable: Log age in days

Selection	Regression Equations	Multiple R*	Std. Error of Estimate
	MANDIBLE		
I	$.55 - .002 X_1 + .48 X_2 + .05 X_3 + .001 X_4 + .001 X_5 - .05 X_6 - .003 X_7 - .005 X_8$	.982	.125
II	$1.51 + .07 X_1 - .07 X_2 - .02 X_3 - .10 X_4$	.974	.139
III	$-1.17 + .21 X_1 - .17 X_2$	.940	.204
IV	$.735 + .09 X_1 - .10 X_3$	.947	.192
V	$1.40 + .06 X_1 - .10 X_4$	.973	.137
VI	$2.25 + .10 X_2 - .17 X_3$	.936	.210
VII	$2.22 + .11 X_2 - .13 X_4$	.967	.151
VIII	$2.76 - .06 X_3 - .11 X_4$	.970	.145
	FEMUR		
I	$3.0 - .13 X_1 + .65 X_2 - .34 X_3 + .10 X_4 + .003 X_5 - .07 X_6 + .01 X_7 - .007 X_8$	.984	.117
II	$3.79 - .04 X_1 + .02 X_2 - .16 X_3 - .08 X_4$	.965	.163
III	$-.94 + .16 X_1 + .01 X_2$	.906	.253
IV	$2.73 + .01 X_1 - .22 X_3$	.935	.212
V	$1.50 + .06 X_1 - .10 X_4$	.952	.184
VI	$2.75 + .05 X_2 - .21 X_3$	.936	.211
VII	$2.17 + .12 X_2 - .11 X_4$	.946	.194
VIII	$2.99 - .12 X_3 - .08 X_4$	.963	.160
	TIBIA		
I	$4.13 - .11 X_1 + .16 X_2 - .36 X_3 + .05 X_4 - .002 X_5 - .02 X_6 + .02 X_7 - .01 X_8$	.976	.146
II	$4.55 - .06 X_1 - .06 X_2 - .14 X_3 - .12 X_4$	.973	.142
III	$-.65 + .16 X_1 + .03 X_2$	.928	.223
IV	$2.85 + .01 X_1 - .25 X_3$	.958	.172
V	$3.69 - .04 X_1 - .17 X_4$	.967	.152
VI	$3.58 - .08 X_2 - .30 X_3$	.958	.171
VII	$3.04 - .03 X_2 - .15 X_4$	.966	.155
VIII	$3.01 - .10 X_3 - .09 X_4$	.969	.147

* Significant as assessed by F-test for regression at 5% level of significance.

FIGURE 8.

Nomograph for the estimation of age at death in the rat from histologic measurement of the mandible.

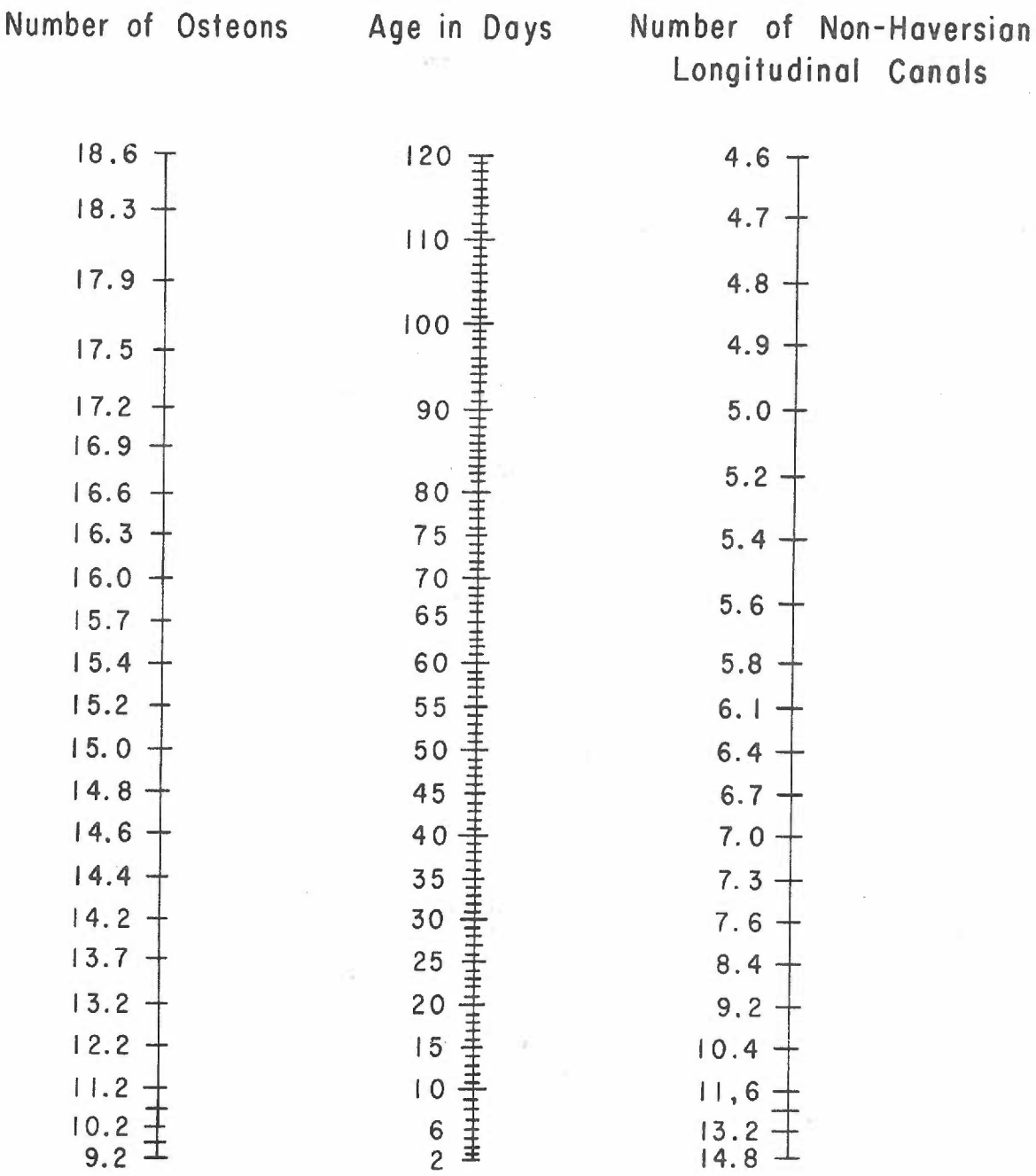

Number of Osteons
18.6
18.3
17.9
17.5
17.2
16.9
16.6
16.3
16.0
15.7
15.4
15.2
15.0
14.8
14.6
14.4
14.2
13.7
13.2
12.2
11.2
10.2
9.2
Age in Days
120
110
100
90
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
6
2
Number of Non-Haversian Longitudinal Canals
4.6
4.7
4.8
4.9
5.0
5.2
5.4
5.6
5.8
6.1
6.4
6.7
7.0
7.3
7.6
8.4
9.2
10.4
11,6
13.2
14.8

FIGURE 9.

Nomograph for the estimation of age at death in the rat from histologic measurements of the femur.

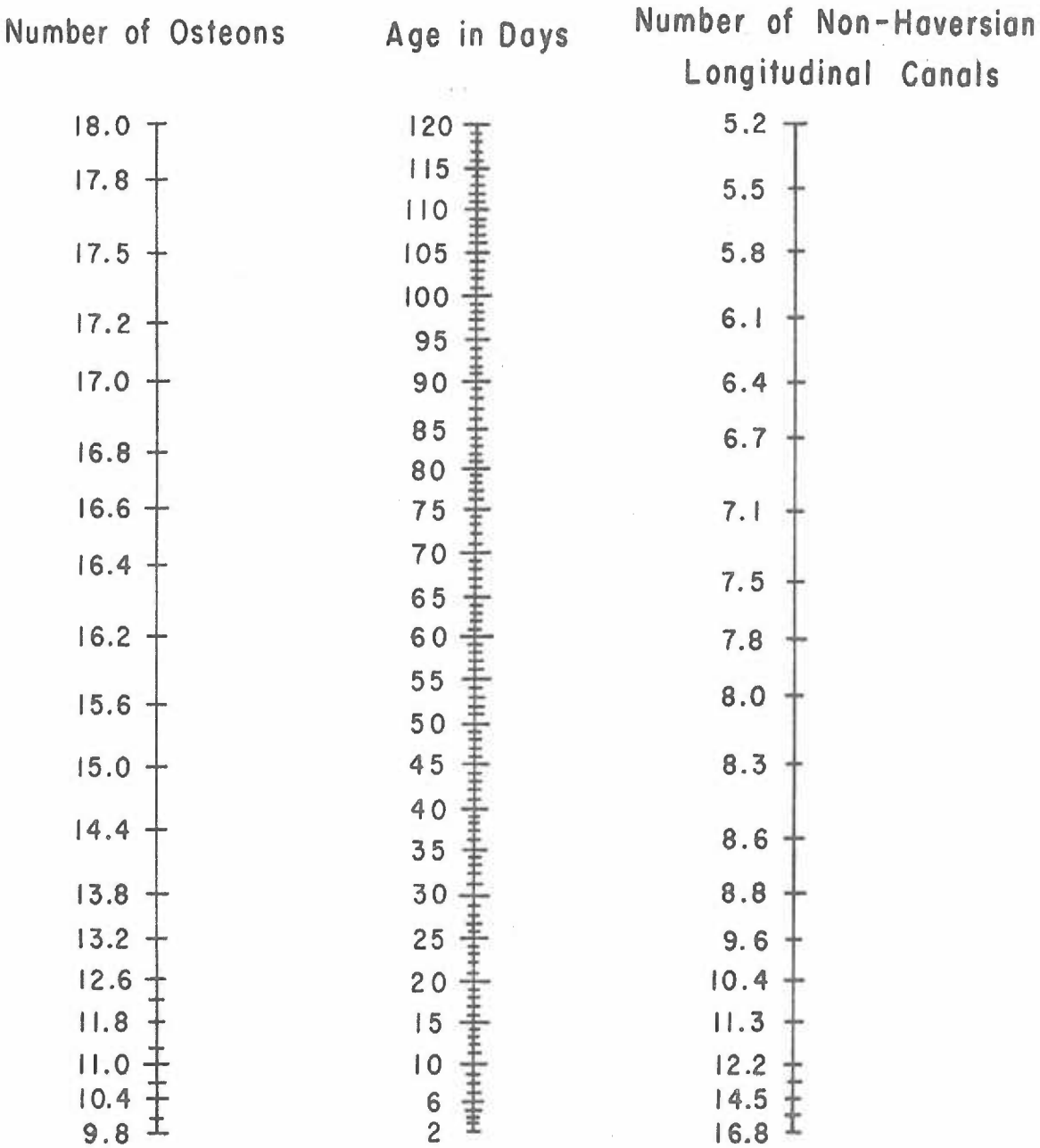
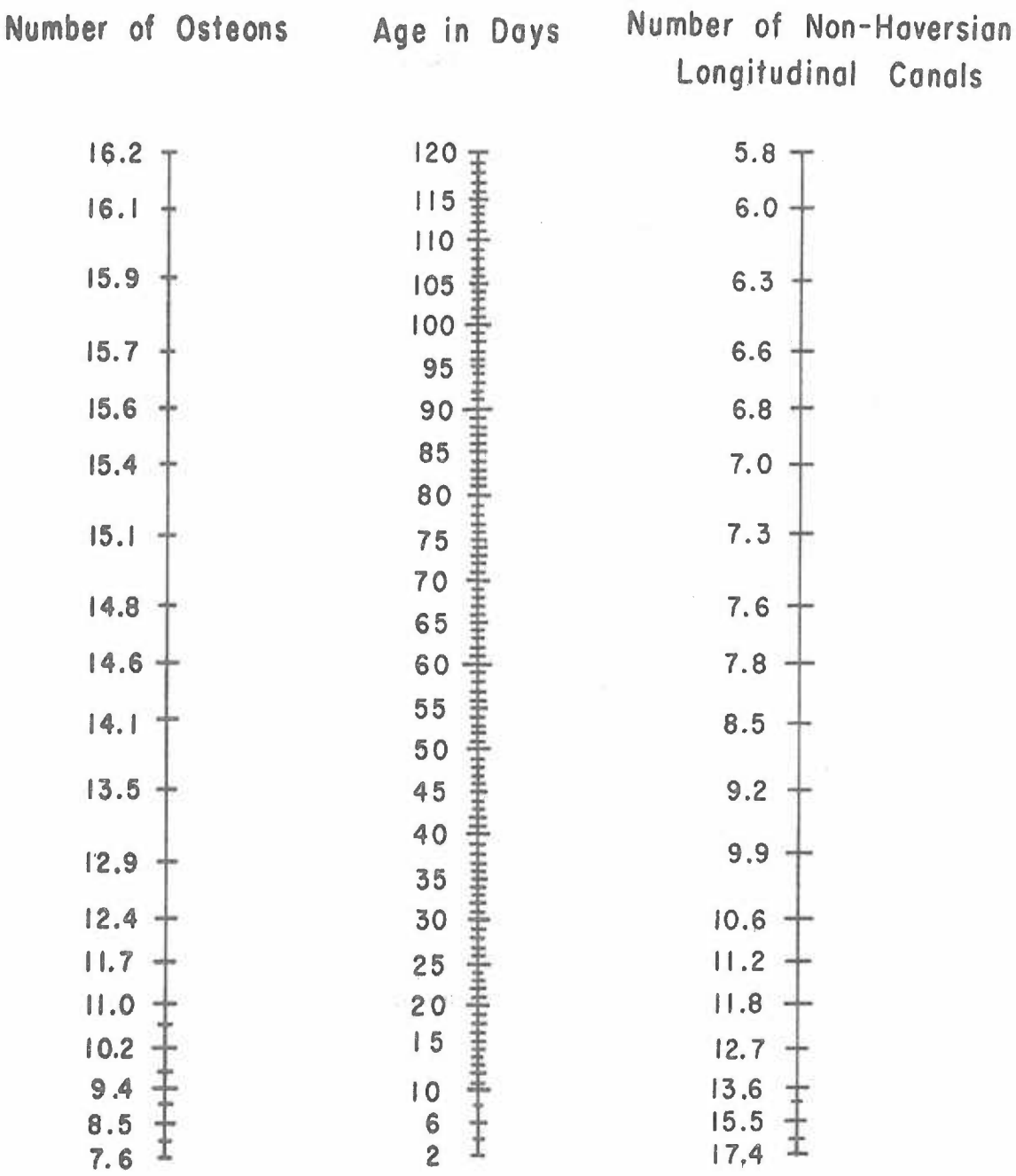

Number of Osteons
18.0
17.8
17.5
17.2
17.0
16.8
16.6
16.4
16.2
15.6
15.0
14.4
13.8
13.2
12.6
11.8
11.0
10.4
9.8
Age in Days
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
6
2
Number of Non-Haversian Longitudinal Canals
5.2
5.5
5.8
6.1
6.4
6.7
7.1
7.5
7.8
8.0
8.3
8.6
8.8
9.6
10.4
11.3
12.2
14.5
16.8

FIGURE 10.

Nomograph for the estimation of age at death in the rat from histologic measurements of the tibia.


Number of Osteons
16.2
16.1
15.9
15.7
15.6
15.4
15.1
14.8
14.6
14.1
13.5
12.9
12.4
11.7
11.0
10.2
9.4
8.5
7.6
Age in Days
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
6
2
Number of Non-Haversian Longitudinal Canals
5.8
6.0
6.3
6.6
6.8
7.0
7.3
7.6
7.8
8.5
9.2
9.9
10.6
11.2
11.8
12.7
13.6
15.5
17.4

C. Experiment 2: Effects of 17 Beta Estradiol

1. General Effects

Increase in body weight, in the sham and estradiol-treated animals, is presented in Figure 11 and Table 9. A significant difference between the two by the Student's t statistic is only noted at age 65 days (α = .10). By 90 days of age, the trend for the two groups come together and no discernable difference exists.

Vaginal plates open at a mean age of 70 days in the sham and 37.6 days in the estradiol-treated animals respectively (Table 10). This age distribution in the two groups is completely different and has no points of overlap (Figure 12); therefore, a test for significant differences is not necessary. The sham group shows a slightly higher variability for this measure.

2. Gross Skeletal Measurements

Mean weight of either bone does not appear to be altered as a result of estradiol administration. However, when it is considered as a proportion of body weight, remarkable differences in bone weight of the treated and sham groups are seen (Figure 13). These differences are highest at 90 days of age and are least at 45 and 120 days of age.

Measurements of tibial and femoral diameters do not show any differences between the two groups.

Linear measurements of all three bones show alterations as a result of estradiol treatment (Figures 14 and 15). Differences are maximum at 60 to 90 days of age and are less pronounced for the mandibular measurements, condylion to coronoid process and condylion to gonion. Tibial length at age 90 days shows the maximum difference between the two groups.

3. Histological Measurements

Measurements from both sham and estradiol-treated groups are respectively presented in Tables 11 and 12. Although the comments made here apply equally well to measurements from other bones, age trends are presented only for the number of osteons and lamellae in the mandible and for Haversian canal diameter and number of non-Haversian systems only in the femur (Figures 6 and 7). Patterns of age changes are similar to the ones described in Experiment 1.

Measurements from the sham animals follow closely those of untreated animals but the estradiol-treated group exhibits significant points of departure. This group is maximally different from the other two at 60 days of age for the number of osteons and at 90 days of age for Haversian canal diameter. The number of lamellae, however, remain markedly higher in the estradiol group at 120 days of age when the highest difference in this measurement is seen. The pattern for non-Haversian longitudinal canals is very similar in the three groups and no significant differences at any age are seen. With the exception of lamellar counts differences between the two

groups are higher at 45 days of age than at age 120 days.

At a probable error of less than five percent, significant differences between the sham and estradiol-treated group exist at age 60 days for the number of osteons; at age 90 days for number of lamellae and Haversian canal diameter; and at age 120 days for the number of lamellae only.

4. Age Estimation

For both treated groups, histological measurements for femur, tibia and mandible were available at 45, 60, 90 and 120 days of age. From nomographs based on untreated rat material in Experiment 1 (Figures 8, 9, 10), a new set of estimated ages were derived from each bone. Differences between real and estimated ages for the experimental and control groups are shown in Table 13.

Age estimates of sham animals are within four days of the real age but those of estradiol-treated animals show marked departures especially when such estimates are based on the long bones. Data from estradiol-treated animals consistently provide age estimates that are higher than true values.

Differences between estradiol and control groups range from a minimum of zero days to a maximum of 28 days which is well outside the third deviation of the standard error of the estimate. Also, such differences are highest at 60 and 90 days of age and are consistently higher for the femur.

TABLE 9.

AVERAGE BODY WEIGHT (GMS.) OF SHAM AND ESTRADIOL-TREATED RATS

Age in Days		Sham Group	Estradiol-Treated Group
30	$\bar{X}$	85.63	87.00
	S.D.	9.00	10.63
	N	20	20
37	$\bar{X}$	121.20	122.05
	S.D.	11.01	11.29
	N	20	20
44	$\bar{X}$	144.84	143.15
	S.D.	12.50	12.42
	N	20	20
51	$\bar{X}$	168.78	163.53
	S.D.	11.45	11.72
	N	15	15
58	$\bar{X}$	184.21	178.07
	S.D.	11.72	11.96
	N	15	15
65*	$\bar{X}$	199.10	190.40
	S.D.	12.12	11.88
	N	10	10
72	$\bar{X}$	207.90	202.50
	S.D.	11.05	10.64
	N	10	10
79	$\bar{X}$	219.40	214.40
	S.D.	9.19	10.08
	N	10	10
86	$\bar{X}$	224.00	221.20
	S.D.	7.16	7.44
	N	10	10
93	$\bar{X}$	233.80	232.40
	S.D.	7.85	8.57
	N	5	5
100	$\bar{X}$	236.00	235.40
	S.D.	8.32	8.53
	N	5	5
107	$\bar{X}$	241.00	240.00
	S.D.	10.02	9.97
	N	5	5
114	$\bar{X}$	244.60	244.40
	S.D.	9.75	9.95
	N	5	5
120	$\bar{X}$	246.60	246.00
	S.D.	11.06	11.79
	N	5	5

* P $<.10$

FIGURE 11.

Age changes in average body weight of sham and estradiol-treated rats.

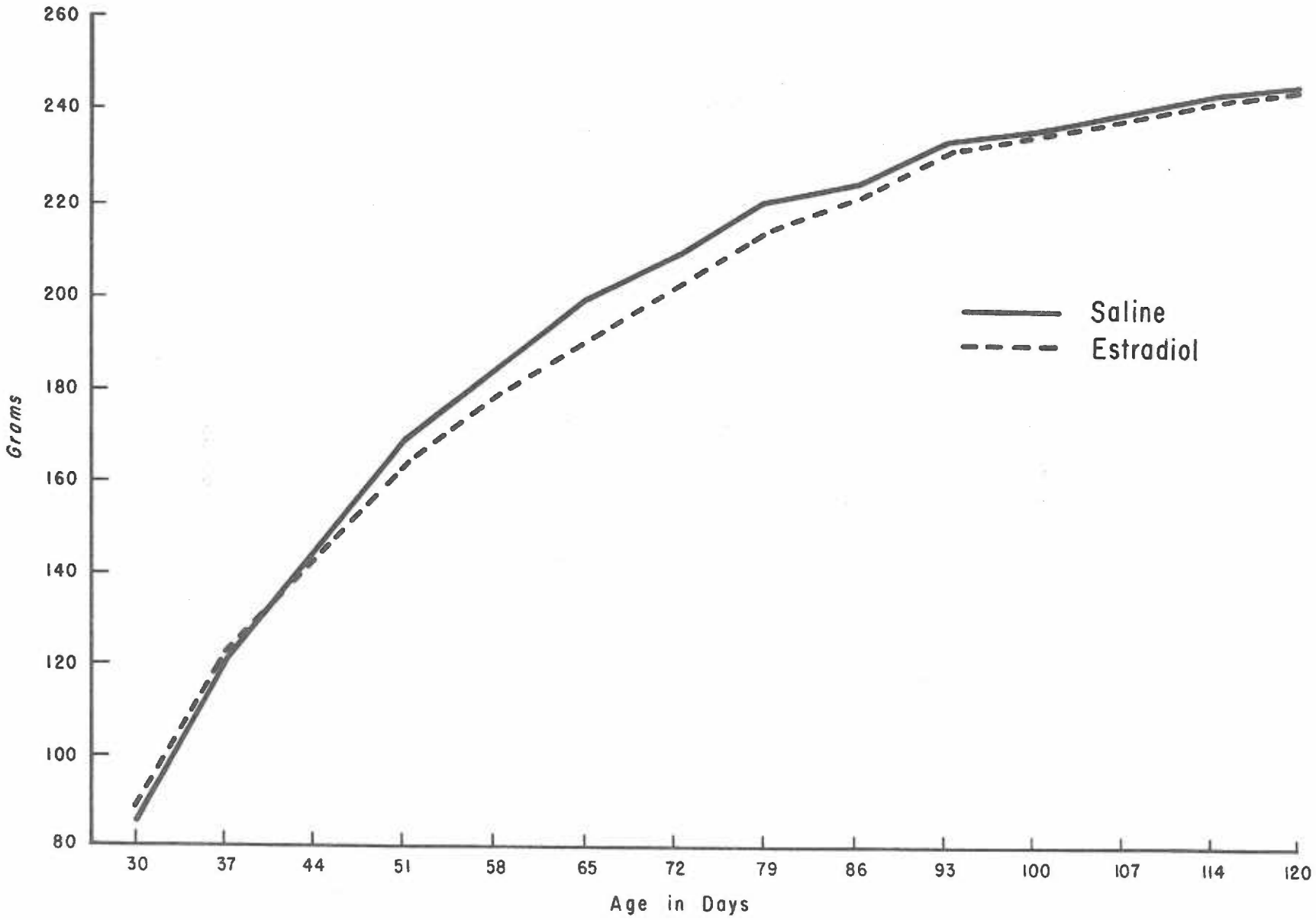

260
240
220
200
180
160
140
120
100
80
Grams
30
37
44
51
58
65
72
79
86
93
100
107
114
120
Age in Days
Saline
Estradiol

TABLE 10.

AGE OF OPENING OF VAGINAL PLATES IN RATS

Estradiol-Injected Animals		Saline-Injected Animals	
Age in Days	Number with Open Plates	Age in Days	Number with Open Plates
33	0	63	0
34	2	64	1
35	3	65	1
36	7	66	2
37	11	67	3
38	14	68	3
39	15	69	4
40	17	70	6
41	18	71	6
42	20	72	7
		73	7
		74	9
		75	10

		Age (days) of opening of plates			
	N	Mean	±	Std. Dev.	Median
Estradiol Group	20	37.65	±	2.38	37.0
Saline Group	10	70.01	±	3.69	69.5

FIGURE 12.

Effect of estradiol on cumulative percent distribution of rats with open vaginal plates.

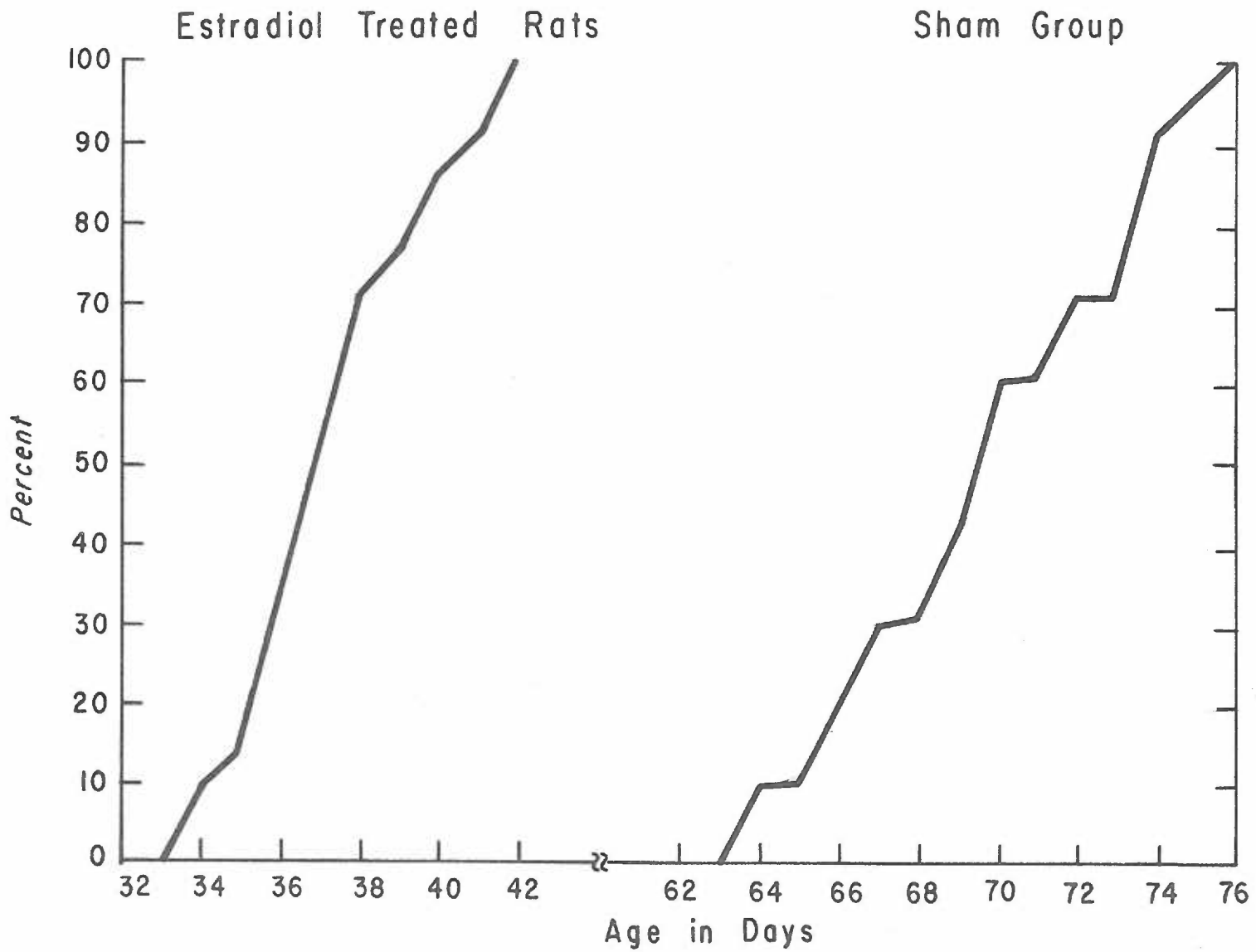

Estradiol Treated Rats
Sham Group
Percent
100
90
80
70
60
50
40
30
20
10
0
32
34
36
38
40
42
62
64
66
68
70
72
74
76
Age in Days

FIGURE 13.

Effect of estradiol on bone weight/body weight

FIGURE 14.

Effect of estradiol on length measurements of femur and tibia

FIGURE 15.

Effect of estradiol on linear measurements of the mandible

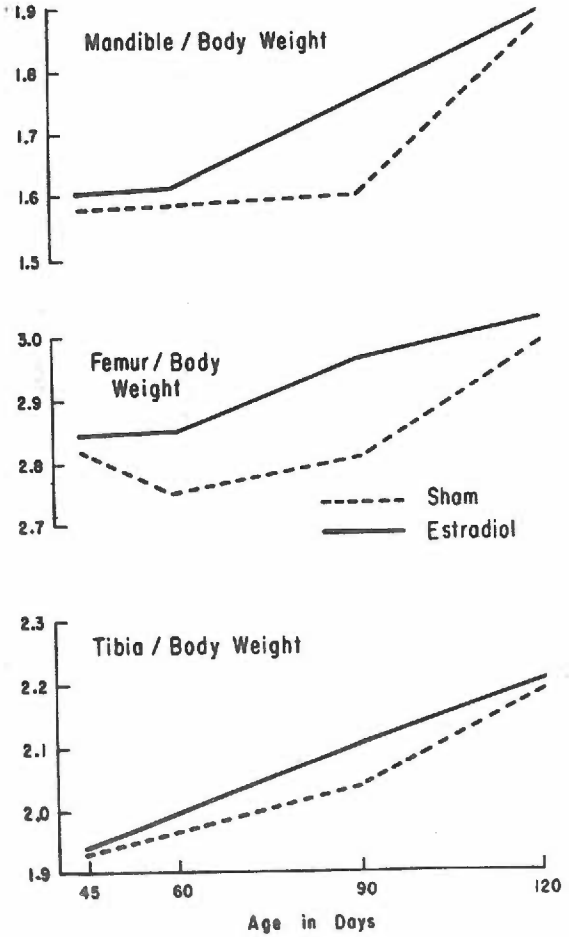


Figure 13

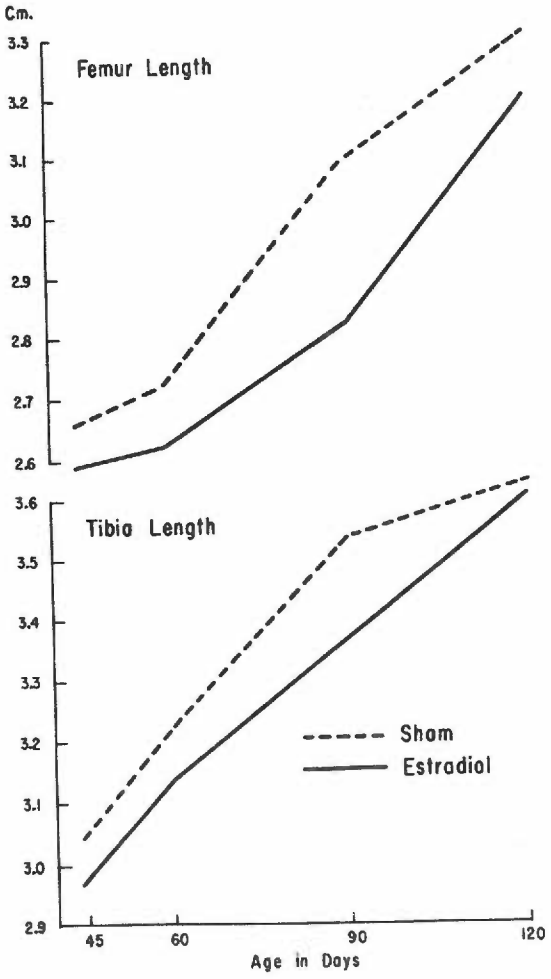


Figure 14

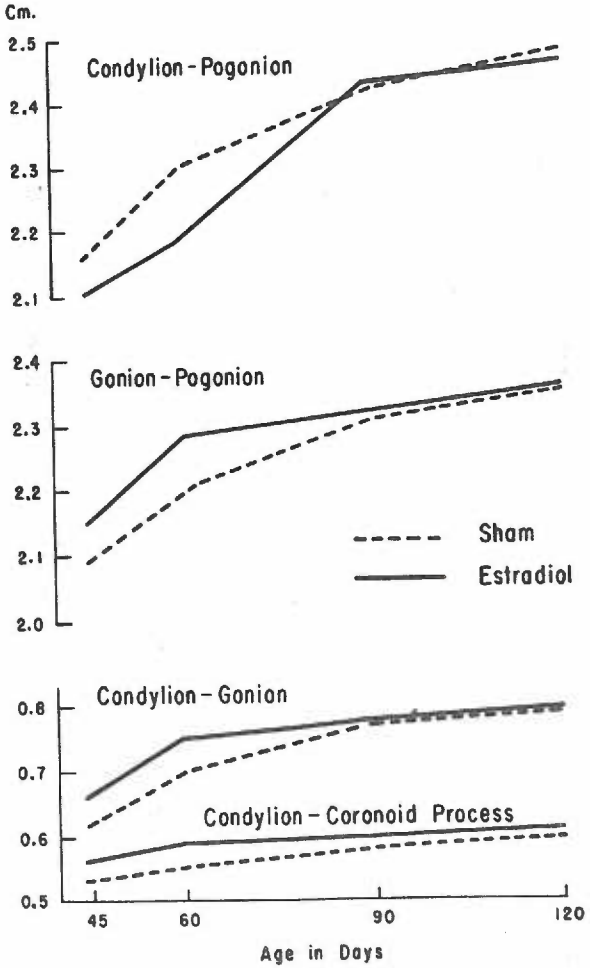


Figure 15

TABLE 11.

HISTOLOGICAL MEASUREMENTS ON SALINE-INJECTED RATS

Dimension		Age In Days 45	60	90	120
MANDIBLE					
Osteons	$\bar{X}$	14.80	15.40	16.80	18.80
	S.D.	1.16	1.01	1.16	1.18
Lamellae	$\bar{X}$	2.58	3.00	3.55	3.96
	S.D.	0.22	0.52	0.33	0.70
Hav. Can. Dia.	$\bar{X}$	5.65	5.24	4.20	3.16
	S.D.	0.59	0.53	0.44	0.84
Non-Hav. Can.	$\bar{X}$	7.00	6.20	5.00	4.80
	S.D.	0.89	1.16	0.89	1.16
FEMUR					
Osteons	$\bar{X}$	15.40	16.20	17.40	18.20
	S.D.	1.01	1.16	1.01	1.83
Lamellae	$\bar{X}$	3.00	3.16	3.75	4.35
	S.D.	0.14	0.30	0.30	0.22
Hav. Can. Dia.	$\bar{X}$	5.76	5.50	4.70	4.20
	S.D.	0.46	0.56	0.85	0.82
Non-Hav. Can.	$\bar{X}$	7.00	6.40	5.40	4.80
	S.D.	0.89	1.01	1.02	1.30
TIBIA					
Osteons	$\bar{X}$	13.20	14.60	15.40	16.40
	S.D.	1.16	1.49	1.01	1.01
Lamellae	$\bar{X}$	2.60	2.83	3.60	4.20
	S.D.	0.53	0.14	0.43	0.84
Hav. Can. Dia.	$\bar{X}$	6.51	5.69	4.30	3.90
	S.D.	0.40	0.55	0.47	0.55
Non-Hav. Can.	$\bar{X}$	9.80	8.20	7.20	5.60
	S.D.	1.16	1.16	0.74	1.01

Sample size in each cell equals five. Measurements of Haversian canal diameter in μm

TABLE 12.

HISTOLOGICAL MEASUREMENTS ON ESTRADIOL-INJECTED RATS

Dimension		Age in Days 45	60	90	120
MANDIBLE					
Osteons	$\bar{X}$	15.40	16.20	17.40	18.80
	S.D.	1.20	1.60	1.01	1.16
Lamellae	$\bar{X}$	3.00	3.25	3.89	4.85
	S.D.	0.34	0.40	0.44	0.50
Hav. Can. Dia.	$\bar{X}$	5.54	5.10	4.20	3.30
	S.D.	0.54	0.61	0.36	0.50
Non-Hav. Can.	$\bar{X}$	7.20	5.60	4.80	4.60
	S.D.	1.16	1.01	0.74	0.80
FEMUR					
Osteons	$\bar{X}$	15.40	17.00	18.00	18.60
	S.D.	1.35	1.41	0.89	1.85
Lamellae	$\bar{X}$	3.27	3.60	4.00	4.69
	S.D.	0.60	0.45	0.59	0.26
Hav. Can. Dia.	$\bar{X}$	5.70	5.20	4.10	3.90
	S.D.	0.88	0.64	0.37	0.56
Non-Hav. Can.	$\bar{X}$	7.40	6.60	5.60	4.60
	S.D.	1.01	1.01	1.35	1.03
TIBIA					
Osteons	$\bar{X}$	14.20	15.40	16.20	17.20
	S.D.	0.74	1.01	1.46	1.40
Lamellae	$\bar{X}$	2.99	3.36	4.16	4.45
	S.D.	0.50	0.33	0.38	0.63
Hav. Can. Dia.	$\bar{X}$	6.00	5.90	4.10	3.30
	S.D.	0.55	0.60	0.28	0.70
Non-Hav. Can.	$\bar{X}$	9.60	8.40	7.00	5.40
	S.D.	1.85	1.01	0.89	1.20

Sample size in each cell equals five.

Measurements of Haversian canal diameter in μm

TABLE 13.

EFFECT OF ESTRADIOL TREATMENT

ON ESTIMATION OF AGE AT DEATH

Bone from which age is estimated	True age in days	Mean age in days estimated from nomographs	
		Saline-injected group	Estradiol-injected group
Mandible	45	42	48
	60	57	71
	90	89	101
	120	122	122
Femur	45	46	59
	60	62	88
	90	92	116
	120	119	125
Tibia	45	41	48
	60	58	68
	90	88	107
	120	119	130

D. Experiment 3: Human Studies

1. Age Changes in Histological Measurements

Characteristics of the various histological measurements of the mandible, femur and tibia are presented in Table 14; individual observations for each subject are in Section F of the Appendix.

Sample size for the mandible is considerably larger than that for the other two bones and is more skewed toward the later ages, consequently, mean age is slightly higher than that for femur and tibia.

Although all three variables show age associated changes, considerable variability is present. As determined by the coefficient of variation, variability is least for number of osteons (X_1) and higher for the other two. However, not much difference exists between bones, except in mean diameter of Haversian canal which is significantly higher for the mandible ($\alpha = .05$).

Age changes are illustrated by a scatter-diagram for the number of lamellae per osteon (X_2) in the femur (Figure 16). As a function of age, an increase in X_1 and X_2 and a concomitant decrease in X_3 is seen. In all three measurements, a slightly non-linear component, especially at later ages, seems to be present. For further analysis, however, a linear model was assumed and any non-linearity ignored for reasons which shall be discussed later.

2. Regressions Equations for the Estimation of Age in Humans

Simple correlation matrix for X_1, X_2 and X_3 with age shows that

the number of osteons and the Haversian canal diameter are best correlated with age, the average number of lamellae per osteon rank last (Table 15). Also, slightly higher correlations with age are obtained from mandibular measurements than from femoral or tibial data.

From each of the three bones, multiple regression equations were computed with age as the dependent variable and either three, two, or one independent variable(s): the predicting equations are provided in Table 16. In some of the equations a negative intercept is seen which is to be expected in view of the fact that the age distribution of the sample is skewed toward the later ages and every subject is above 38 years of age. Significant regressions result from every combination of independent variables investigated in this experiment and the residuals resulting therefrom do not show any systematic bias.

Mandibular measurements provide the best regressions with age and with the least standard errors of estimate for every combination of independent variables; femoral and tibial regressions on age follow close behind. In every bone, the error in estimation is least when all three predicting variables are regressed on age and is maximum when prediction of age is based only on the average number of lamellae per osteon.

3. Nomograph for the Estimation of Age in Humans

Because of sample limitations, a nomograph was constructed only from mandibular measurements, X_1 and X_3 (Figure 17). This

regression relationshp provides a multiple correlation (R) of 0.978 with a standard error of estimate of 2.58 years.

Thus between the ages of 40 to 80 years, age may be estimated to the order of accuracy of $\pm$ 2.58 years in 67 percent, and within $\pm$ 5.16 years in 95 percent of the subjects, of the true value from these nomographs.

A sample size of 33 for the femur and tibia measurements was not considered adequate for the construction of nomographs.

The real age of the seven females in the sample was compared with the male standards, but the errors in estimation of age are judged to be within experimental and measurement error, and therefore, are not considered to be significant.

FIGURE 16.

Age changes in the average number of lamellae per osteon in the human femur.

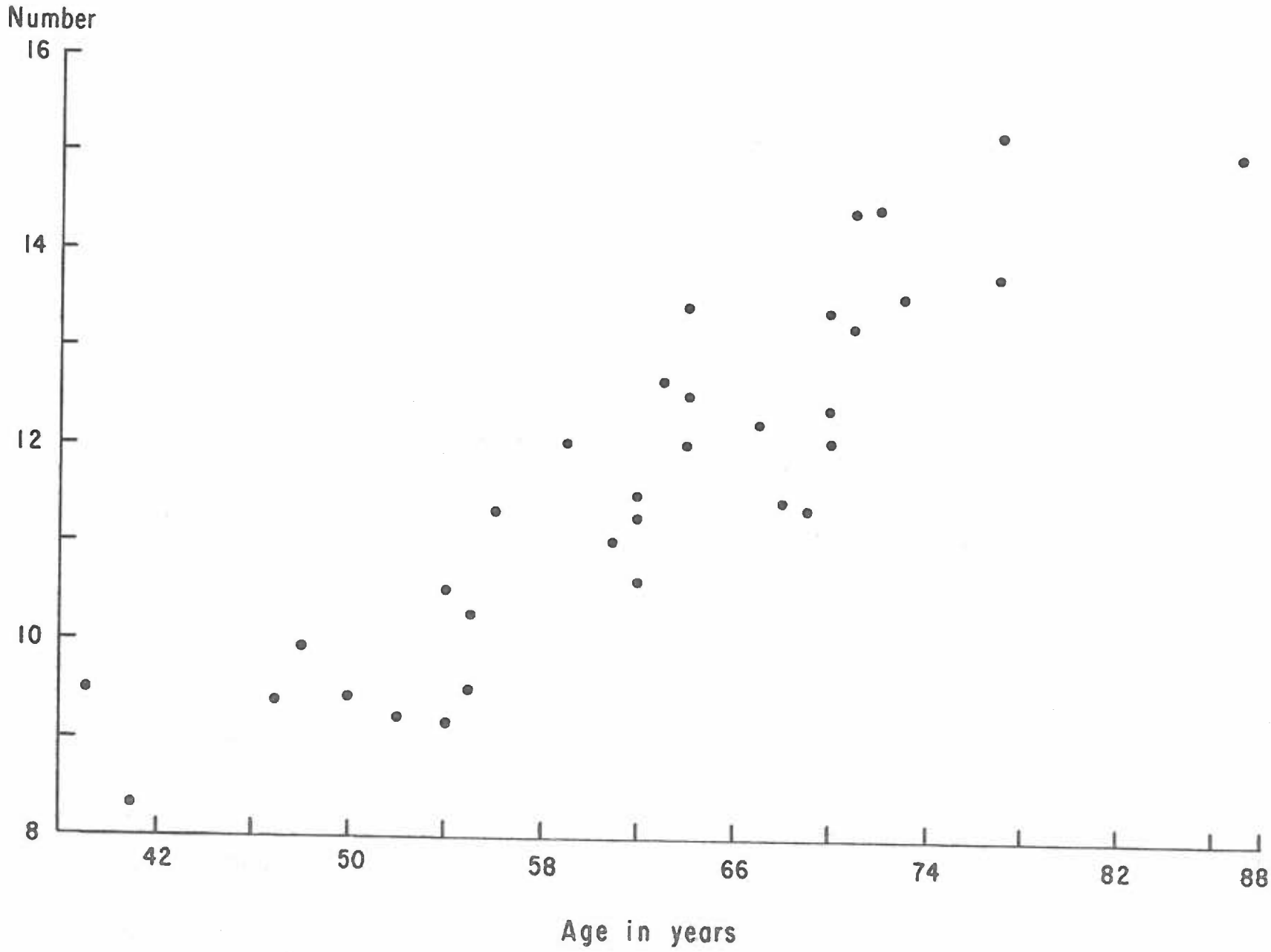

Number
16
14
12
10
8
42
50
58
66
74
82
88
Age in years

TABLE 14.

CHARACTERISTICS OF HUMAN DATA

Variable		BONE Mandible	Femur	Tibia
Age (Yrs.)	$\bar{X}$	64.25	62.333	62.333
	S.D.	12.14	10.80	10.80
	N	52	33	33
Osteons	$\bar{X}$	55.02	56.85	58.48
	S.D.	7.00	7.39	8.65
	N	52	33	33
Lamellae	$\bar{X}$	11.78	11.68	11.74
	S.D.	2.30	1.86	2.01
	N	52	33	33
Hav.Can. Dia. (μm)	$\bar{X}$	63.44	43.24	45.54
	S.D.	18.72	16.42	15.87
	N	52	33	33

TABLE 15.

SIMPLE CORRELATIONS OF HUMAN HISTOLOGICAL MEASUREMENTS WITH AGE IN YEARS

Variable	BONE Mandible	Femur	Tibia
Osteons	.969	.945	.919
Lamellae	.950	.890	.908
Hav. Can. Dia.	-.966	-.937	-.935

All correlations are significantly different from zero, $P < .05$

TABLE 16.

REGRESSION EQUATIONS FOR ESTIMATING AGE IN HUMANS FROM HISTOLOGICAL MEASUREMENTS

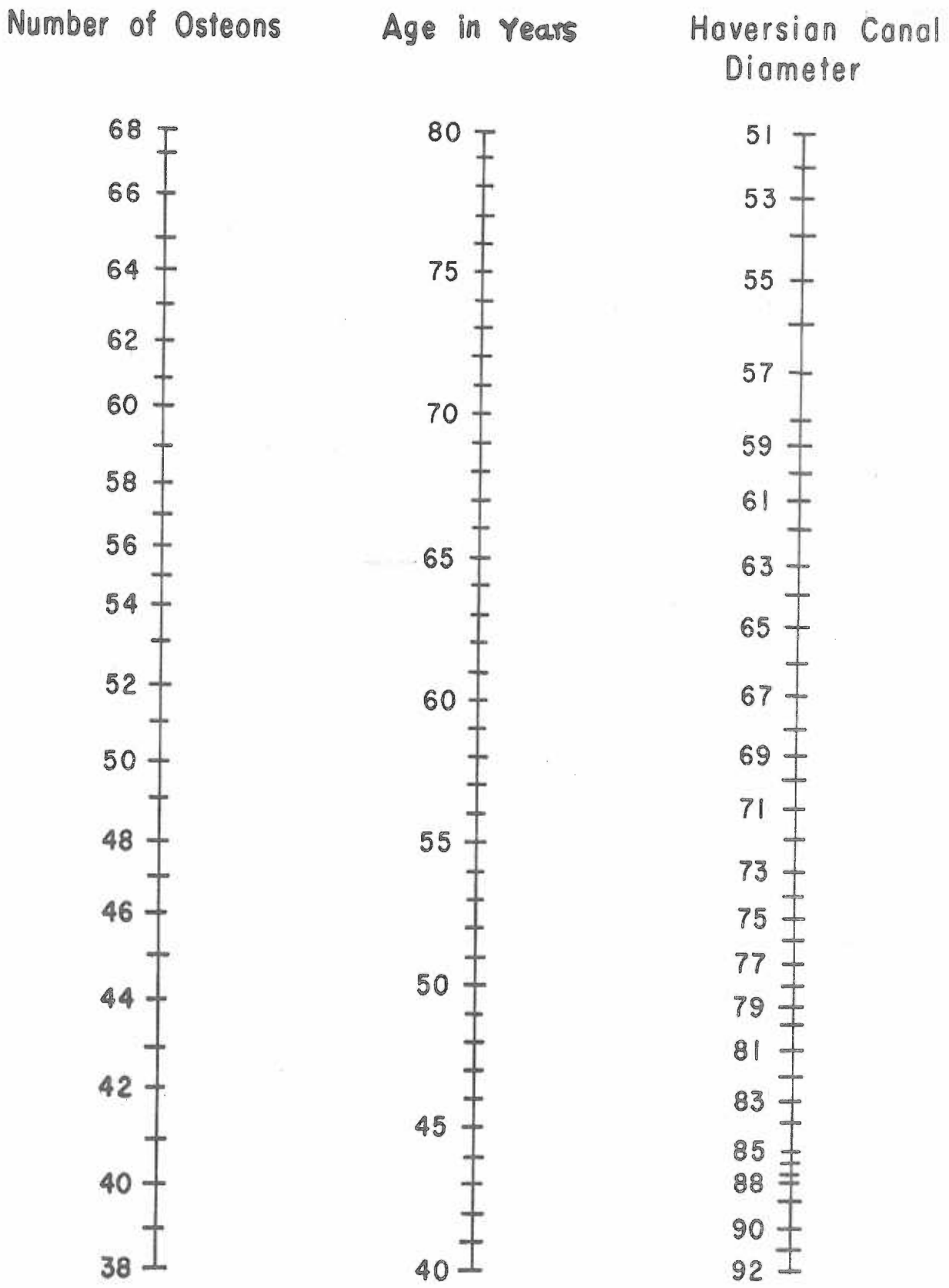
Dependent Variable: Age in years

Selection	Regression Equations	Multiple R*	Std. Error of Estimate
	MANDIBLE		
I	$20.82 + .85 X_1 + .87 X_2 - .22 X_3$	.979	2.55
II	$-18.99 + 1.13 X_1 + 1.76 X_2$	.976	2.69
III	$32.23 + .92 X_1 - .30 X_3$	.978	2.58
IV	$74.73 + 1.52 X_2 - .45 X_3$	.969	3.04
V	$-28.24 + 1.68 X_1$	.969	3.02
VI	$5.31 + 5.00 X_2$	.950	3.83
VII	$103.99 - .63 X_3$	.966	3.16
	FEMUR		
I	$27.65 + .65 X_1 + .78 X_2 - .26 X_3$	.958	3.24
II	$-14.69 + 1.13 X_1 + 1.11 X_2$	.948	3.55
III	$29.59 + .79 X_1 - .28 X_3$	.957	3.25
IV	$61.25 + 1.74 X_2 - .44 X_3$	.949	3.52
V	$16.10 + 1.38 X_1$	.045	3.60
VI	$2.00 + 5.16 X_2$	.889	5.01
VII	$89.01 - .62 X_3$	.937	3.82
	TIBIA		
I	$43.52 + .291 X_1 + 1.47 X_2 - .34 X_3$	.964	3.02
II	$-3.40 + .67 X_1 + 2.27 X_2$	.936	3.93
III	$48.61 + .53 X_1 - .38 X_3$	.957	3.22
IV	$54.79 + 2.19 X_2 - .4 X_3$	.960	3.12
V	$-4.76 + 1.15 X_1$	.919	4.33
VI	$5.10 + 4.88 X_2$	.908	4.59
VII	$91.32 - .64 X_3$	.935	3.88

* Significant as assessed by F-test for regression at 5% level of significance.

FIGURE 17.

Nomograph for the estimation of age at death in human males from histologic measurements of the mandible.


Number of Osteons
68
66
64
62
60
58
56
54
52
50
48
46
44
42
40
38
Age in Years
80
75
70
65
60
55
50
45
40
Haversian Canal Diameter
51
53
55
57
59
61
63
65
67
69
71
73
75
77
79
81
83
85
88
90
92

VI. DISCUSSION

In a broad sense, this dissertation was concerned with ageing phenomena in cortical bone and had three major investigative aspects. This brief summary presents their integrative concepts; a fuller discussion will follow.

The major emphasis was on demonstrating that there were age-dependent histological parameters in bone cortex which could be quantitated to arrive at good estimates of age at death. In this part, therefore, measurement systems and models for estimation of age at death were explored in the rat.

In order that such a system be practically useful, one needs to ask what are the nature of errors in age estimation that result when the skeletal system is subjected to some exogenous influence. It follows that the choice of such influences, at least initially, be one to which skeletal tissues may be ordinarily exposed during life. The second part of this thesis investigated the effect of such an influence on the histologic parameters used for estimating age at death in the rat.

Such an experimental approach on rat skeletal tissues, as in the first two experiments, was undertaken because existing information on histology of aging in bone cortex was considered to be inadequate for purposes of age estimation. Based on the results of these experiments, the proposed models for estimating age at death

were applied to some age-stratified human skeletal material in the third experiment.

An important aspect of any measurement system is whether or not another investigator, using the same techniques and criteria, could arrive at comparable results. A small experiment was, therefore, designed to investigate measurement errors in the proposed system and to establish its reliability.

In the following discussion, the analysis of measurement errors is considered first and is followed by results from various other facets of these investigations. These separate areas and the rationale governing the investigative approach are then integrated in the last two sections, Correlative Discussion and General Comments.

A. Measurement Errors

The contribution of any measurement system depends upon the sources and magnitude of its inherent errors. An important aspect of this study, therefore, was an evaluation of measurement errors of the proposed system. Theoretically speaking, a study of errors should distinguish between validity and reliability and examine the argument that a certain measurement variable may be more easily measured in a particular bone at a specific age as opposed to other bones and other ages. In practice, however, as in this investigation, the important consideration was whether or not the reliability of the total measurement system was such that it would be possible to estimate age within a certain degree of permissible error. Thus when measurement errors were analyzed, it was considered desirable to pool material over all ages and bones investigated.

An analysis of the type conducted here, analysis of variance, was considered to be superior to multiple t tests on the four variables, because such t tests are known to result in large errors of the type where the null hypothesis of no differences is rejected even when true (Petrinovich and Hardyck, 1969).

Analysis indicated that measurement errors were negligible, that no systematic bias in measurement was present, and that the proposed model, when utilized by other investigators could provide useful estimates of age.

B. Experiment 1: Age Estimation in the Rat

The first experiment was designed to investigate the possibility of deriving a model for estimation of age from rat skeletal material. This purpose was adequately fulfilled. In this experiment, age was estimated as the logarithm of age in days. A standard error of 0.15, for example, implies that based upon that particular regression function, age could be estimated to within the antilogarithm of 0.15 or approximately one and one-half days on either side of the true value in two-thirds of the subjects. In 95 percent of the cases, however, age estimates would be within three days of the true age. This implies a high degree of accuracy in age estimation from two to 120 days of age when based upon these regression formulae.

It was seen that depending upon which histological parameters are available, or are most easily measured in a particular case, appropriate regression equations could be used to arrive a fairly accurate and useful estimates of age. However, nomographs were prepared only from the number of osteons (X_1) and of the number of non-Haversian longitudinal canals (X_4), because these were considered to be the most easily identified variables in histological sections. The number of lamellae are at times difficult to count especially in the older osteons, and the measurement of Haversian canal diameter requires ocular reticules or other measuring devices

thus rendering such measurements inconvenient to make.

These methods for estimation of age at death in female rats of this strain are accurate and simple to use. They are an addition to the traditional standards based on appearance of ossification centers (Walker and Wirtschafter, 1957) and compared to them, also cover a longer age span.

It must be pointed out that these nomographs, and all other regressions of rat material, apply within the range examined and to females of this particular strain only. Extrapolations beyond the limits of this investigation, whether regarding age, sex or strain, may not be justified. Extrapolations even regarding other bones may not be safely made, although the resulting errors in estimation would probably not be too large. This assumption is being made in view of the fact that no marked differences were seen among the three bones investigated, two of which were long bones.

Although individual parameters differed between bones, age estimates from the three bones were comparable, and mandibular regressions were only slightly better. This and other aspects of the results are more fully discussed later. Growth patterns of histological measures are in keeping with those generally accepted for most somatic measurements; this aspect is discussed further under the section, Correlative Discussion.

C. Experiment 2: Effects of 17 Beta Estradiol

1. General Effects

The severity of the effects of this dose of 17 Beta Estradiol on bone cortex could not be anticipated a priori because of conflicting evidence in the literature on susceptibility of rat skeletal material to this steroid (review by Weinmann and Sicher, 1955). Therefore, observations were made of some other well known general effects of estrogens and the results were in accord with those reported in the literature.

Estrogens are well known to induce skeletal and reproductive maturity, but the opening of vaginal plates is rather a gross criterion for determining the effectiveness of the steroid. Perhaps this is why even the estradiol-treated animals exhibited considerable variability. Tjan and Gunberg (1967), among others, noted that following estrogen treatment in the immature rat, vaginal plates open within a few days. Similar results were obtained in the present investigation; in fact, the sham and estradiol-treated groups were completely different with no points of overlap. Therefore, no test for statistical significance of difference was deemed necessary.

It is well known that female reproductive organs respond to estrogens dramatically in other ways also. There is ample evidence that vaginal and uterine weights constitute a sensitive measure of the effects of estrogens (among others, Talalay, Dobson, Ebersol, and Huggins, 1952; Pliske, Baker and Johnson, 1953). In rats, such

responses have been reported as the result of dosages as small as 0.05 ug. of 17 Beta Estradiol administered every 24 hours for three days (Meyer, 1961; Tjan and Gunberg, 1967). Proliferation and cornification with an increased deposition of mucopolysaccharides in vaginal epithelium have been noted by many investigators and Lloyd (1963) reviews such experiments. Such observations were not available here.

Fluctuations in body weight corresponding to phases of the menstrual cycle were observed in young women and were probably related to the pattern of circulating levels of estrogens (Thomas, 1953). Following estrogen administration, a lower weight gain has been reported by several investigators in many different species; for example, Silberberg and Silberberg (1939) in the guinea pig; Zondek (1936) in the fowl; Gardner and Pfieffer (1943) in the mouse; Day and Follis (1941) and Tjan and Gunberg (1967) in the rat; and Bernsten (1968) in the rabbit. This is confirmed here, but the differences from sham animals are maximum five days after cessation of treatment and even then are of no more than borderline significance. One month after suspension of estradiol treatment and thereafter, no difference in the control and experimental group exists. Such "catch up" growth although not surprizing, occurs in a very short period of time and had not been demonstrated before.

Estrogen administration in large doses also results in hyperproteinemia in turtles (Urist and Schjeide, 1961), snakes (Dessauer and Fox, 1959), lizards (Suzuki and Prosser, 1968) and crocodiles

(Prosser and Suzuki, 1968). Such general effects and more sensitive measures of the effectiveness of estrogens exist but were not utilized.

2. Effects on Skeletal Tissues

In different experimental animals, a variety of effects on calcified tissues have been attributed to estrogens. The literature covers a multitude of species and is voluminous. Hyperphosphatemia and hypercalcemia have been noted in fish, mice, rats, chickens, dogs and man (review by Gardner and Pfieffer, 1943). Similar results have also been obtained in lizards (Suzuki and Prosser, 1968) but only a hypercalcemic response has been seen in crocodiles (Prosser and Suzuki, 1968). It is also well to point out that most of these experimental studies have used doses ranging from 100 ug. to 2.0 mg. per week for several weeks, whereas in the present study, the dose employed was a total of 6 ug. administered over a 30 day period.

Some puzzling results following estrogen treatment have been noted by Manunta, Saroff and Turner (1957). Ca^{45} was injected into adult rats and after sacrificing animals, radioactivity in bone and serum was determined. The estradiol-treated group had significantly higher radioactivity in serum, but bone activities were similar. Parathyroidectomy altered bone activity but not serum activity. One would suspect that urinary or fecal excretion of Ca^{45} had been altered, but such measurements were not available. Suzuki and Prosser (1968), however, believe that the increase in serum calcium is brought about by a removal of calcium from the bones, but no substantiating evidence has been brought forth. Other studies, however,

indicated that removal of calcium from bones does not occur but, in fact, in animals that had not been parathyroidectomized, there was a concomitant increase in bone density. Such an increase is apparent from radiographs of epiphyseal zones from estradiol-treated rats (Budy, Urist and McLean, 1952) and also forms the basis for treatment of osteoporosis of old age by estrogens (review by Tonna, 1965).

In this study, effects of estradiol on skeletal tissues were seen even in gross bone measurements. Effects on bone weight were easily demonstrated when it was considered as a proportion of body weight but not otherwise; similar observations have been reported in rabbits by Bernsten (1968). The probable reason for this was the large body weight variability present in the sample.

Inhibition of linear bone growth because of higher circulating levels of estrogens have been reported in a variety of animal species including man (Suzuki, 1958, 1959; reviews by Gardner and Pfieffer, 1943; and Lloyd, 1963); such findings are confirmed here in the rat. In this study, however, treatment was suspended at 60 days of age and observations continued until 120 days of age by which time estrogen levels had presumably returned to normal. Therefore, the inhibitory effect on linear measurements was transitory and, by the end of the experimental period, bone size was normal. Had treatment been continued to maturity, such inhibitory effects would probably have persisted throughout the animal's life span.

Histologically the number of osteons and of lamellae were maximally altered at the time of cessation of treatment. This pattern was expected but not found in the other two variables. Haversian canal diameter exhibited little difference whereas the number of lamellae were significantly higher as late as two months after cessation of treatment. It seems that in estradiol-treated animals, Haversian canals remain relatively patent while permitting at the same time a faster deposition of lamellae on existing osteons. In other words, the interrelation between X_2 and X_3 would necessarily fall.

It is well to recall that the rate of decrease in size of Haversian canals is slower in diabetic humans (Landeros and Frost, 1964b) as well as in those treated with adrenal cortical steroids (Klein, Villanueva and Frost, 1965). Whether or not this is a specific effect of such steroids cannot be known from such studies.

Although individual measurements do not generally show marked differences, nevertheless, they do interact so that significant errors in age estimation result. Errors in age estimation were consistently higher when based on the femur, less so for the tibia and least of all for the mandibular measurements. Over the age period investigated, mandibular measurements provided better estimates than the two long bones. Pertinent to these observations are the findings of inhibition of linear growth in this study and similar reports of Suzuki (1958, 1959) and others cited earlier.

The mandible ossifies from one center, but the other two bones have multiple ossification centers. Although the ossification of all three bones starts 17 days after conception in the rat, the appearance of other centers occurs later in the femur than in the tibia (Walker and Wirtschafter, 1957). In this study, estrogen administration was too late to have delayed the appearance of ossification centers, but ossification at time of treatment could have been less complete in the femur than in the tibia. Therefore, the femur probably was more susceptible to treatment. This may explain why femoral estimates were consistently higher and erroneous.

Interesting species differences in response to estrogen administration are known to exist (Gardner and Pfeiffer, 1943; Weinmann and Sicher, 1955). Estrogens are known to stimulate endosteal bone and inhibit proliferation of cartilage cells in birds and mice (review by McLean and Urist, 1968), but no histological changes have been noted in lizards and crocodiles (Prosser and Suzuki, 1968; Suzuki and Prosser, 1968). Other skeletal and non-skeletal effects of estrogens have already been considered. Prior to this investigation, the only reported effect of estrogens on skeletal tissues of the growing rat had been an inhibition of resorption of the spongy bone of metaphysis (Urist, Budy and McLean, 1948; Noback, Barnett and Kupperman, 1949; Budy, Urist and McLean, 1952). An earlier appearance of ossification centers, at least in the female rat, had been claimed (Talbot, 1939) but not confirmed. Effects of estrogens

on bone cortex in the rat are reported here for the first time.

Other aspects of the effects of this estrogen on the histological parameters used in estimation of age are discussed later in the section, Correlative Discussion.

D. Human Studies

The human data were limited to a small sample with a highly skewed age distribution; also, sample size was considerably larger for the mandible than for the femur and tibia. As fairly reliable methods are available for the estimation of age below 25 years (review by Krogman, 1962) the human part of this dissertation concentrated on estimating age for an older population.

The estimating equations for age based on mandibular measurements demonstrated that fairly good regression relationships could be proposed and from them age estimates could be made to within three years of the true age in two-thirds of all subjects. Again depending upon the parameters available, one may use different regressions to estimate age. In 95 percent of the subjects, estimates of age at death would be accurate to within 6 years of the true age.

Sample size for the femur and tibia was even more limited and conclusions, therefrom, have been presented only as preliminary findings. The results, however, justify more extensive investigations on long bones and on other bones of the body. The same limitations to interpretation and extrapolation apply here as were discussed earlier for rat skeletal material. Results from human material, especially on the long bones, should be interpreted even more conservatively because of sample limitations.

For the limited material available, significant sex differences were not noted; this is in accord with the finding of Kerley (1965).

Racial differences could not be analyzed because data on race distribution in the sample were not available. Further studies along these lines should take into account the possible variations due to sex or race. Such variability should be expected in view of the voluminous literature on sexual and racial variation in developmental age and size; Tanner (1962) provides an excellent review of this area.

Most somatic measurements are known to show a decreasing growth rate and a nonlinear relationship to age, especially during the older ages. This may also be true for the histological measurements of this study; however, any nonlinear component in the human material was ignored, because measurements on rats had indicated that accurate estimation of age was possible from strictly first order models.

Kerley (1965) attempted to estimate age from the number of osteons, Haversian fragments, non-Haversian systems, and the amount of lamellar bone. In the present study, non-Haversian systems were not counted because the sample was composed of individuals almost all above 40 years of age, and Kerley's work had demonstrated that after about 50 years of age, these systems are rarely observed. Haversian fragments were not included for two reasons: first, all interstitial bone in reality consists of fragments which would be impossible to count, and second, Haversian systems do not always travel parallel to the long axis of a bone (Cohen and Harris, 1958), and therefore, some of them are bound to be cut obliquely. In cross

sections these would appear as fragments, and errors in counting would result. Instead, the number of lamellae per osteon and the Haversian canal diameter were included.

For the variables utilized in this investigation, ground sections were easier to measure and interpret than thionin stained decalcified sections. Facilities for making ground sections however, were not available, and therefore, decalcified sections were utilized for most of the preparations. It must be pointed out that decalcified sections provided equally valid and highly useful data although they required slightly longer to measure and interpret.

In view of the differences in parameters and methods of analyses reported in the literature and those utilized in this study, it is not possible to compare in detail the findings of other investigators with data reported here, only general comparisons are possible.

The findings of this investigation are in opposition to those of Currey (1964) and Barer (1966) who concluded that there is either a slight age-associated increase or no change in size of the Haversian canal. However, this contradiction may be due to the fact that whereas they measured perimeter, in this investigation diameter was measured. The findings, however, are in agreement with those of others (Landeros and Frost, 1964a; Epker, Hattner and Frost, 1964) that closure of Haversian canal continues into the eighth decade.

This investigation demonstrates that contrary to some opinions (Deslypere and Baert, 1958; Krogman, 1962), it is possible to estimate age quite accurately from histological measurements of bone cortex.

E. Correlative Discussion

The growth pattern of the different histological variables in this investigation were similar in the rat and human material. Human growth in most body measurements, such as stature, weight, size of long bones, etc., is known to occur at a declining rate and the superimposition of an adolescent spurt results in the typical "S" shaped age-size curve. An exception to this rule is the lymphatic tissue which attains a value equal to 200 percent of its adult size at puberty and regresses thereafter. A childhood spurt and later an adolescent spurt in most body measurements have been noted, and Tanner (1962) provides a recent review on growth curves with emphasis on adolescent spurts.

Results of the present study indicated that, at least in the rat, histologic measurements exhibit similar growth patterns. This is contrary to other observations in human ribs that bone remodeling is probably independent of influences that govern skeletal growth and maturation (Sedlin, Villanueva, and Frost, 1963). Perhaps the large variability in their human sample was responsible. This would not be wholly unexpected, and for a long time now human studies have demonstrated adolescent spurts only by superimposing individual growth curves, not on chronological age, but on other measures of physiologic maturity such as age at menarche or age at peak height velocity (Shuttleworth, 1937; Tanner, 1965). Perhaps such treatment of human histological data would demonstrate adolescent spurts in

internal remodeling of bone; they certainly appear to be present in histological data from rats. The human sample in this study was limited to an older age group and such treatement of data was not applicable.

The number of osteons and lamellae were positively correlated to age, while Haversian canal diameter and non-Haversian systems were inversely related. This was to be expected in view of the fact that deposition of new lamellae occurs centripetally and continues even as late as the eighth decade; this would necessarily reduce Haversian canal diameter. As a certain minimum size of Haversian canal is reached, formation of new lamellae probably slows down to near cessation. If this is true, then given the Haversian canal diameter and the thickness of lamellae at an early age, one should be able to predict the number of lamellae that a given osteon might have at the end point of its development. Because it is not known if the variability in lamellar thickness refers to variability among lamellae of the same osteon or among lamellae of different osteons in the same bone, the above hypothesis may be postulated in spite of the fact that lamellae do vary in thickness (Bloom and Fawcett, 1968).

Indirect evidence of variability in lamellar thickness within the same osteon can, however, be provided from these investigations. In estradiol-treated animals, the number of lamellae was significantly increased but the Haversian canal diameter did not show significant and comparable decrease. Therefore, the newer forming lamellae must have been reduced in thickness.

Evidence for the above hypothesis could be obtained from measurements of osteon size (area or diameter). Because with age, or as a result of estradiol-treatment, there are more osteons per unit area of bone, there should be a comparable decrease in their size. In estradiol-treated animals, therefore, osteon size should be reduced but only to the extent that osteons are increased in number which in these animals is not as accentuated as the increase in lamellae. Ergo, osteon size may be less susceptible to exogenous influences like estrogens than the number and/or size of lamellae. Indirect evidence for this lies in the contradictory reports in the literature; Currey (1963) and Barer (1966) observed an age-associated decrease in osteon diameter, but Takahashi, Epker and Frost (1965) noted no such change.

In both rats and humans, at any given age, one can find both young and old osteons. A young osteon is defined as one that has a large Haversian canal and few lamellae. In fact, any bone section will generally show many different sizes and ages of osteons and this has in the past contributed to the opinion that histologic observations of bone are not useful for age estimation (Deslypere and Baert, 1958; Krogman, 1962). By relying upon traditionally subjective histologic approach, it would be impossible to discuss ageing in bone cortex except in terms of porosity and a general impression of more osteons in older specimens. Even the above conclusions can be safely made only between extreme ages, a finer discrimination of age or anything else, would be difficult.

From data presented in this dissertation, it appears that with age, there is a shift in the relative distribution of number of osteons and their relative age; such changes are reflected in the figures calculated from many osteons in a given microscopic field.

It has been claimed that the average formation time for osteons in humans is 4 to 5 weeks, sexual dimorphism exists in this parameter and different values have been reported in pathologic states and old age (Bloom and Fawcett, 1968). That the latter is probably true is indicated in this study by the fact that some histological variables were effected by estradiol which speeded up formation of new osteons. Consequently, more osteons with a larger number of lamellae per unit area of bone were found. However, Haversian canal diameter was only slightly altered and, two months after cessation of treatment, every histological variable except the number of lamellae had returned to normal. This indicated that the animal had returned to a normal metabolic state as had the rate of bone formation, but the osteons formed during the experimental period had a higher number of lamellae and two months later such osteons were still apparent.

It was also noted that bones from estradiol-treated animals needed as much as a day longer for decalcification than the controls. This was a strictly subjective observation and no attempt was made to rigorously examine this, but it does lend some support to the school which believes that estrogen treatment results in increased

bone density and not a loss of calcium from the bones.

The experimental work on rats also provided support for the multivariate approach utilized here as well as some bases for human studies. If age were to be estimated from one variable only, then errors due to exogenous influences such as the estradiol used in the experimental portion of this investigation would result. These errors would be serious if say the number of lamellae were the variable utilized. Errors resulting from the use of number of osteons should be less and should be least when Haversian canal diameter is used for age estimation. Errors would also be more serious if estimates were based on lamellae and osteons jointly than if based on Haversian canal diameter and any one of the other two variables. It is better, therefore, to have in a nomograph a set of predicting variables, one of which is positively correlated and the other negatively correlated to age, provided that the two are themselves not highly correlated.

Effects of estrogen treatment as an example of an exogenous influence was tested only on rats. It was reasoned that in them both, genetic and environmental variability could be better controlled than in humans. Thus, rats could be used as a model for evaluating the influence of an exogenous agent to which humans might be exposed during life, and the systems of age estimation proposed here could be tested.

Experiments on rats extended to animals four months of age, therefore, estradiol treatment for one month constituted a chronic

factor. Even then, 15 days after initiating treatment, mandibular and tibial measurements provided adequate age estimation; femoral estimates were, on the average, higher by 14 days. Throughout the study, mandibular measurements provided adequate age estimates; tibial estimates were unsuitable at 90 days of age whereas femoral estimates were tolerable only at 120 days of age, 60 days after cessation of treatment. This indicated that age estimates based on long bones are more susceptible to metabolic agents such as estrogens, than are bones like the mandible. It must be pointed out that the investigation was conducted on female rats who are more susceptible to estrogens than males and that a 0.2 ug. of 17 Beta Estradiol per day for 30 days is, for the rat, a fairly high dose of the strongest known estrogen.

Based on the experimental work, it was expected that in the human sample, large variability and errors in age estimation would be seen but these were not found. It is possible that the dissection room population is not a random cross section of the human male population. Although slightly larger variability in age estimation did exist for the long bones, these findings cannot be termed as conclusive because sample size could best be called modest and there were too few females to look for sexual dimorphism. Nevertheless, the experimental study on rats indicated that the conclusions of the human study be cautiously applied to the ageing of skeletal remains. Errors in age estimation however, would still be considerably less than the errors resulting from existing criteria, as for example,

morphodifferentiation in the pubic symphysis. The experimental approach also suggests that more human data be analyzed along the lines indicated in this dissertation and by other approaches such as tetracycline labeling studies which have indicated that the age associated decrease in size of Haversian canals is slower in diabetics (Landeros and Frost, 1964b), as well as in subjects treated with adrenal cortical steroids (Klein, Villanueva and Frost, 1965).

Thus the influence of various abnormal metabolic states on the practical problem of age estimation could be further evaluated. The total variability in the measurement system derived in this manner would be more useful in practice where unknown skeletal remains are to be identified and upper and lower limits on age estimation are hazarded. The second experiment investigated such variability in the proposed system for estimating age at death in rats.

F. General Comments

Age estimation from skeletal material is hardly a new idea, but as pointed out in the literature, its usage and popularity bears an inverse relationship to its validity and reliability. This investigation was conceived because often only small fragments of bone are available for individual identification, and an important aspect of such identification is the estimation of age. Traditional methods of age estimation based upon subjective observations, as for example, closure of cranial sutures, or gross morphological changes as in the pubic symphysis, have been useful but are obviously limited in application, reliability and validity. Also a prerequisite for the application of such methods has been the availability, not of skeletal fragments, but of substantial portions of a skeleton. Given even the best skeletal material, however, such methods are low in reliability and high in inter-examiner variability. Consequently, age estimation from skeletal material has not, heretofore, been dependable. This is especially true for individuals over 25 years of age.

Quantitative histology, on the other hand, lends objectivity and a measure of reliability to such estimates. Such an approach assumes added importance when one realizes how small a piece of tissue is necessary.

It is not surprising, therefore, that quantitative histological techniques are now being accorded their well deserved recognition

and are increasingly supplanting the traditional qualitative descriptive approach in areas as diverse as diagnostic pathology (Rosenberg, Ledeen and Kline, 1969) and cellular ageing (Strehler, 1962). Although few techniques have been utilized, the general area of quantitative histology has remained relatively unexplored (Elias and Pauly, 1966).

During consideration of the rationale of this investigation, a case was made for the use of multivariate methods in biological research. This has been amply justified by the results reported in this dissertation. The superiority of the multivariate approach over the univariate one has long been recognized. In fact, it is practiced, perhaps in an intangible fashion, by almost everyone in a diagnostic or decision making situation. A subjective multivariate approach is inherent in Todd's (1920, 1921, 1942) standards of morphological changes in the pubic bone, in evaluating ossification in various bones of the hand and wrist (Greulich and Pyle, 1959) or in profile analysis for age determination from various bones as advocated by Kerley (1965).

Multiple regression techniques are relatively unexplored and have been infrequently used in biology but they can be a powerful tool for simultaneously analyzing interrelations among a number of variables. In biological research, where an array of variables, or a series of measurements, may be more important in their interrelations rather than any one single variable alone, such statistical techniques become of great importance. As stated earlier, the

choice of the predicting variables does not imply that a cause and effect relationship exists between them and age. The model only suggests that if it is possible to estimate the response variable, age in this study, from a preselected set of variables, then the predicting relationship could be a useful estimator whose limits are defined by the standard error of the estimate.

To consider an example, skeletal ageing may be regarded as an n-dimensional phenomenon. Of course, if all n-dimensions were available, age estimation would be precise; but there is another aspect which asks whether or not this n-dimensional phenomenon can be described either equally well or perhaps slightly less so by p-dimensions where

$$n > p \geq 1$$

As the value of p decreases, error in estimation increases and its proportional complement R^2 decreases. In the simplest case, the regression model when p = 1 cannot be significantly improved upon by the model when p = n. Examination of R^2 and the standard error of estimate at each step, provide criteria for the cutoff point for the value of p. This concept is well discussed by Beale, Kendall and Mann (1967), and also by Draper and Smith (1968). This description would apply to regression analyses based on all possible combinations of all independent variables, individually and collectively. In this dissertation, the number of independent variables was limited and the value of p was preselected.

Measurements were made only in the periosteal third of the anterior quadrant of the femur and tibia and posterior border of mandibular ramus. A review of the literature indicated that the microscopic structure of the periosteal third is different from the middle and endosteal third but, at least in the femur, the structure of different quadrants in cross section is similar (Jowsey, 1960).

Mandibular specimens were selected in relation to an easily defined landmark, the lingula; but femoral and tibial specimens were taken from the general area of midshaft. Hence, considerably more variability in the site of sectioning could exist for the long bones than for the mandible. Although Kerley (1965) stated that a block almost three inches long approximately in the middle third of adult human long bones is histologically the same throughout its length, evidence for this assumption was not presented by him. In this dissertation, such a hypothesis was not tested, therefore, an *a priori* assumption was implied that the one or two sections used are representative of the histology of an easily recognized part of a bone. In order to keep this approach to the estimation of age simple and as easily replicable as possible, nomographs were prepared from the variables judged to be the easiest to measure. This investigation also emphasizes the importance of applying quantitative criteria to histological measurements.

It was concluded that the experimental approach utilized in this investigation can be of use not only in age estimation for anthropology and forensic medicine but also in studies of ageing and in the use of skeletal tissues as indicators of the metabolic state of the organism.

V. SUMMARY

Studies were conducted to quantitate histological age changes in bone cortex of both rats and humans. From decalcified sections of mandible, femur and tibia of female rats, two to 120 days of age measurements were made of: 1) number of osteons in two microscopic fields, 2) number of non-Haversian longitudinal canals in two microscopic fields, 3) average Haversian canal diameter, and 4) average number of lamellae per osteon. Multiple regression techniques were then utilized to estimate age from several combinations of the above measurements of Haversian and non-Haversian bone.

In another experiment, 30 day old female rats were injected with 24 I.U. of 17 Beta Estradiol per day for the next 30 days. Animals were sacrificed at varying intervals and histological measurements as in the first experiment were made. The effect of estrogen on skeletal ageing was thus assessed. Additional non-histological observations made in this group were; a) date of opening of vaginal plates, b) periodic body weight, c) bone weight, and d) linear bone measurements.

In the third part of this thesis, ground and decalcified sections of human mandible, femur and tibia were measured for; a) number of osteons in two fields, 2) average number of lamellae per osteon, and c) average Haversian canal diameter. These data were used to estimate age at death in a male sample of 52 subjects ranging in age from 39 to 87 years.

These investigations indicated that:

1) With age there is an increase in the a) number of osteons per unit area of bone, and b) number of lamellae per osteon; but a concomitant decrease occurs in Haversian canal diameter. In rats the number of non-Haversian canals per unit area of bone also shows a strong decline with age; in the human sample this measurement could not be made. The number of lamellae per osteon show the least correlation with age, but it is still significant.

2) Multiple regressions analyses indicated that in the age group investigated, estimates of age at death could be made to within 3 days of the true value in 95 percent of rats and to within 6 years of the true age in 95 percent of human males. Mandibular measurements provided estimates that were consistently more accurate than those based on the two long bones.

For the rat material, three nomographs for estimating age at death were prepared from the number of osteons and of non-Haversian canals; one each was from the mandible, femur and tibia. In the human material, because of sample limitations, a nomograph was constructed only from mandibular measurements of number of osteons and Haversian canal diameter. These nomographs can provide accurate estimates of age for the age span of two to 120 days in the Sprague-Dawley female rat and between 40 to 80 years of age in the male human population.

3) Prolonged administration of estradiol to female rats during the second postnatal month resulted in a) an earlier opening

of vaginal plates, b) reduction in rate of gain in body weight until estradiol administration was stopped, c) inhibition of linear skeletal growth but not of bone thickness, d) decrease in bone weight which was demonstrable only when considered as a proportion of body weight, and e) increase in aforementioned histological measurements of bone. The number of lamellae demonstrated the maximum increase which was apparent even two months after suspension of treatment, but by that time no differences could be detected in any of the other measurements.

Variability in age estimation resulting from estradiol treatment was maximum when estimates were based on long bones and least when mandibular measurements were utilized. Such estimates were consistently higher for the femur than for the other two bones.

APPENDIX

A. Decalcified Sections

1. Decalcification of Tissues

Decalcifying Solution

End Point of Decalcification

2. Staining

Stains and Reagents

Procedure

B. Ground Sections

C. Histological Measurements

1. Mandibular Measurements: Untreated Rats

2. Femoral Measurements: Untreated Rats

3. Tibial Measurements: Untreated Rats

D. Histological Measurements

1. Mandibular Measurements: Saline-Injected Rats

2. Femoral Measurements: Saline-Injected Rats

3. Tibial Measurements: Saline-Injected Rats

E. Histological Measurements

1. Mandibular Measurements: Estradiol-Injected Rats

2. Femoral Measurements: Estradiol-Injected Rats

3. Tibial Measurements: Estradiol-Injected Rats

F. Histological Measurements

1. Mandibular Measurements: Human Males

2. Femoral Measurements: Human Males

3. Tibial Measurements: Human Males

G. Residuals from Regressions on Age in Untreated Rats

Model 1.

Model 2.

A. Decalcified Sections

1. Decalcification of Tissues

Decalcifying Solution

Formic acid	20 ml
Formalin	10 ml
Distilled water to make	100 ml

End Point of Decalcification

Adequacy of decalcification was determined by one of two methods. In all specimens from humans and estradiol-treated rats, this was subjectively ascertained by the ease with which tissues could be sliced with a sharp razor blade. In all untreated rat material, determination of decalcification was done by the following procedure recommended by Humason (1962): to 5 ml of solution containing the tissue, 1 ml of 5 percent ammonium oxalate was added. After five minutes of standing, a precipitate indicated incomplete decalcification; clear solution was indicative of complete decalcification.

The usual decalcification time for rat skeletal material was 48 hours except in very young material (2 or 10 days old) when decalcification was judged to be complete in 12 to 36 hours. Human skeletal material required approximately one week.

Decalcification was carried out at room temperature for all rat skeletal material, but human bone fragments were warmed to approximately 58-60^{o}C for 4-6 hours per day.

2. Staining

Stains and Reagents

Ammoniated Carbol-Thionin (Schmorl, 1900)

Phenol, 1% in distilled water	45 ml
Thionin*, saturated solution in 50% ethyl alcohol	10 ml
Ammonium hydroxide, specific gravity 0.88, add just before use	4 drops

Carbol-Xylol (Humason, 1962)

Phenol, melted	1 part
Xylene	3 parts

Procedure

The procedure was modified after Schmorl's (1900) technique for picro-thionin staining. A step of absolute isopropyl alcohol was added but counterstaining with picric acid was not done. Sections were hydrated following standard histological procedures and their further treatment was as follows:

Wash in three changes of distilled water	
Stain in ammoniated carbol-thionin	3 minutes
Wash in distilled water	30 seconds
Differentiate in 70% ethyl alcohol	30 seconds
Dehydrate in 96% ethyl alcohol	30 seconds
Dehydrate in absolute isopropyl alcohol	1 minute
Clear in carbol-xylol	1 minute

* Color Index 52000, manufactured by Allied Chemicals

B. Ground Sections

Bone fragments were embedded in methacrylate by the following procedure:

Dehydrate in absolute ethyl alcohol	24 hours
Coat with amyl acetate	
Place in unpolymerized Caroplastic*	24 hours
Cover with mixture of 15 ml unpolymerized Caroplastic* to 28 drops of catalyst (benzoyl peroxide)	
Let stand at room temperature	24 hours
Cure at 140° F	3-4 hours
Allow to cool at room temperature	

A milling machine with a rotary metal saw was used to cut sections at 150 to 200 micra thickness. These were hand ground between ground glass with progressively finer grain size of pumice until a thickness of 30 to 50 micra was attained.

* A methacrylate manufactured by Carolina Biological Supply

Sections C through F of the Appendix present histologic data of both rat and human samples. Definition of measurements is as follows:

X_1 - total number of osteons (Haversian Systems) in two 40X microscopic fields in the rat, but in two 10X fields in the human sample

X_2 - average number of lamellae per osteon

X_3 - average Haversian canal diameter (μm)

X_4 - total number of non-Haversian longitudinal (primary) canals in two 40X microscopic fields in the rat.

In rats all four measurements were obtained but in humans only the first three were available and in them the number of non-Haversian canals was not counted.

C. Histologic Measurements

1. Mandibular Measurements: Untreated Rats

No.	Age in Days	X_1	X_2	X_3	X_4
1	2	8	1.25	9.92	14
2	2	10	1.50	12.33	15
3	2	9	1.48	12.10	15
4	2	10	1.73	10.15	14
5	2	9	1.50	10.39	15
6	10	11	1.70	9.27	10
7	10	12	2.01	8.29	12
8	10	10	1.72	10.02	13
9	10	12	2.35	9.30	11
10	10	11	1.81	8.75	12
11	20	12	1.91	8.20	10
12	20	13	2.15	7.56	9
13	20	13	1.95	6.50	8
14	20	14	2.34	5.25	10
15	20	14	2.39	5.25	9
16	30	14	2.35	7.01	6
17	30	15	2.60	6.15	7
18	30	15	2.52	5.20	8
19	30	13	2.17	6.50	8
20	30	14	2.37	5.50	9
21	60	14	2.60	6.25	6
22	60	15	2.55	5.52	5
23	60	15	3.15	5.13	5
24	60	16	3.04	5.31	6
25	60	17	3.20	4.05	7
26	90	16	2.98	5.20	5
27	90	17	3.15	4.12	4
28	90	16	3.52	4.34	5
29	90	18	3.91	3.86	6
30	90	19	3.73	3.48	5
31	120	18	3.50	3.25	4
32	120	19	4.35	4.05	6
33	120	20	4.40	2.50	4
34	120	17	3.20	5.02	6
35	120	19	5.02	2.21	3

See page 113 for definition of measurements.

2. Femoral Measurements: Untreated Rats

No.	Age in Days	X_1	X_2	X_3	X_4
1	2	10	1.58	10.11	17
2	2	9	1.15	10.52	16
3	2	8	1.17	10.72	16
4	2	11	1.88	10.02	17
5	2	11	1.91	9.43	18
6	10	10	1.98	9.48	14
7	10	11	2.21	9.52	12
8	10	11	2.05	9.50	12
9	10	13	2.25	9.72	10
10	10	10	2.02	10.03	13
11	20	12	2.25	8.75	12
12	20	13	2.45	8.12	10
13	20	12	2.35	8.03	10
14	20	14	3.08	7.95	9
15	20	12	2.57	8.50	11
16	30	14	2.80	6.13	7
17	30	13	2.45	7.50	10
18	30	15	3.18	5.77	8
19	30	14	2.95	6.25	9
20	30	13	2.57	6.75	10
21	60	16	3.25	5.75	6
22	60	15	2.98	6.02	8
23	60	16	3.00	5.20	8
24	60	17	3.50	5.20	9
25	60	17	3.20	4.98	8
26	90	17	3.45	5.13	6
27	90	18	4.25	4.43	8
28	90	17	3.20	4.47	6
29	90	15	3.12	5.37	5
30	90	18	3.50	4.05	7
31	120	17	3.40	4.60	7
32	120	20	5.52	3.35	4
33	120	16	3.28	4.45	5
34	120	19	4.75	3.85	4
35	120	18	4.63	4.25	6

See page 113 for definition of measurements.

3. Tibial Measurements: Untreated Rats

No.	Age in Days	X_1	X_2	X_3	X_4
1	2	9	2.02	9.09	16
2	2	6	1.33	10.07	18
3	2	8	1.71	10.00	17
4	2	8	1.45	10.07	17
5	2	7	1.52	11.02	19
6	10	10	1.94	8.03	14
7	10	9	1.75	8.08	13
8	10	9	1.90	8.07	14
9	10	11	2.12	7.04	12
10	10	8	1.72	9.03	15
11	20	11	2.43	6.75	12
12	20	10	2.28	7.95	13
13	20	12	2.48	6.60	11
14	20	10	1.98	7.25	13
15	20	12	2.62	6.90	10
16	30	12	2.24	6.52	11
17	30	13	2.57	6.42	10
18	30	12	2.32	7.02	11
19	30	14	2.68	6.24	9
20	30	11	2.30	7.35	12
21	60	14	2.70	6.20	8
22	60	13	2.58	6.35	8
23	60	15	2.72	5.15	9
24	60	16	3.02	5.29	7
25	60	15	2.69	5.71	7
26	90	17	3.92	3.76	6
27	90	15	3.60	5.02	7
28	90	16	3.62	3.92	6
29	90	15	3.18	3.98	8
30	90	15	3.53	4.72	7
31	120	15	3.78	4.11	6
32	120	16	4.01	3.93	5
33	120	17	4.13	3.72	6
34	120	15	3.92	4.10	7
35	120	18	4.95	3.69	5

See page 113 for definition of measurements.

D. Histologic Measurements

1. Mandibular Measurements: Saline-Injected Rats

No.	Age in Days	X_1	X_2	X_3	X_4
1	45	13	2.25	6.52	8
2	90	17	4.01	4.50	4
3	90	15	3.05	4.30	6
4	45	16	2.45	5.21	8
5	90	16	3.75	4.83	6
6	60	14	2.25	6.15	8
7	90	18	3.28	3.57	4
8	45	15	2.72	5.10	6
9	45	14	2.93	6.23	7
10	120	20	4.20	2.52	6
11	120	18	3.25	3.18	3
12	90	18	3.66	3.80	5
13	60	15	2.50	5.10	7
14	60	16	3.50	4.60	6
15	45	16	2.58	5.19	6
16	60	17	3.60	4.85	5
17	120	20	5.20	2.80	4
18	120	19	3.80	2.51	6
19	120	17	3.35	4.79	5
20	60	15	3.15	5.50	5

See page 113 for definition of measurements.

2. Femoral Measurements: Saline-Injected Rats

No.	Age in Days	X_1	X_2	X_3	X_4
1	45	15	2.75	6.42	8
2	90	17	3.73	5.23	6
3	90	18	3.92	4.58	5
4	45	17	3.23	5.18	6
5	90	17	4.21	4.19	5
6	60	15	2.93	5.34	8
7	90	19	3.61	3.50	4
8	45	14	2.89	6.20	7
9	45	16	3.15	5.33	6
10	120	20	4.78	3.20	5
11	120	16	4.27	3.72	4
12	90	16	3.28	6.00	7
13	60	16	2.82	5.88	5
14	60	17	3.15	5.08	7
15	45	15	2.98	5.67	8
16	60	15	3.20	6.40	6
17	120	20	4.19	4.11	5
18	120	19	4.42	4.29	3
19	120	16	4.09	5.68	7
20	60	18	3.70	4.80	6

See page 113 for definition of measurements.

3. Tibial Measurements: Saline-Injected Rats

No.	Age in Days	X_1	X_2	X_3	X_4
1	45	14	2. 15	6. 18	10
2	90	17	4. 25	3. 72	6
3	90	16	3. 75	4. 10	7
4	45	12	3. 10	6. 79	11
5	90	15	3. 77	4. 32	8
6	60	15	2. 70	6. 10	9
7	90	14	2. 98	5. 20	7
8	45	13	2. 30	7. 12	9
9	45	15	3. 40	6. 00	8
10	120	16	5. 68	3. 81	7
11	120	18	4. 32	3. 05	4
12	90	15	3. 25	4. 18	8
13	60	16	2. 92	5. 71	8
14	60	14	2. 77	5. 31	7
15	45	12	2. 05	6. 50	11
16	60	16	3. 08	4. 89	7
17	120	17	3. 73	3. 67	6
18	120	15	3. 10	4. 69	5
19	120	16	4. 17	4. 30	6
20	60	12	2. 68	6. 48	10

See page 113 for definition of measurements.

E. Histologic Measurements

1. Mandibular Measurements: Estradiol-Injected Rats

No.	Age in Days	X_1	X_2	X_3	X_4
1	45	16	3.22	5.30	6
2	90	19	4.53	3.62	4
3	90	18	3.87	4.21	4
4	90	17	4.22	4.08	5
5	60	18	3.96	4.33	5
6	45	14	2.58	6.07	9
7	60	16	2.78	5.68	6
8	45	16	2.90	5.49	6
9	45	17	3.58	4.67	7
10	60	15	3.22	5.09	6
11	120	18	4.78	4.03	4
12	120	20	4.92	3.17	6
13	120	19	5.12	3.19	4
14	120	20	5.48	2.50	4
15	60	18	3.34	4.49	4
16	60	14	2.95	5.91	7
17	45	14	2.72	6.20	8
18	90	16	3.21	4.79	6
19	120	17	3.95	3.61	5
20	90	17	3.62	4.30	5

See page 113 for definition of measurements.

2. Femoral Measurements: Estradiol-Injected Rats

No.	Age in Days	X_1	X_2	X_3	X_4
1	45	17	4. 25	4. 42	6
2	90	17	3. 36	4. 43	8
3	90	18	3. 60	3. 91	5
4	90	17	3. 75	4. 58	6
5	60	16	3. 25	5. 82	8
6	45	17	3. 64	5. 28	7
7	60	19	4. 02	4. 72	7
8	45	14	2. 75	7. 08	8
9	45	14	2. 56	6. 21	9
10	60	15	3. 03	6. 03	7
11	120	20	5. 05	3. 52	3
12	120	19	4. 78	3. 71	5
13	120	21	4. 87	3. 19	6
14	120	17	4. 52	4. 78	4
15	60	18	4. 25	4. 29	5
16	60	17	3. 45	5. 18	6
17	45	15	3. 15	5. 51	7
18	90	19	4. 24	4. 10	5
19	120	16	4. 23	4. 31	5
20	90	19	5. 05	3. 49	4

See page 113 for definition of measurements.

3. Tibial Measurements: Estradiol-Injected Rats

No.	Age in Days	X_1	X_2	X_3	X_4
1	45	15	2.52	5.52	8
2	90	16	4.35	3.81	6
3	90	15	3.97	4.49	8
4	90	16	4.05	4.32	8
5	60	17	3.90	5.13	7
6	45	14	3.42	6.21	10
7	60	16	3.56	5.31	10
8	45	13	3.13	6.69	11
9	45	14	2.27	6.41	12
10	60	15	3.19	6.22	8
11	120	20	5.05	2.62	4
12	120	17	3.98	4.02	4
13	120	16	4.83	2.89	6
14	120	17	4.97	2.70	7
15	60	14	2.87	6.06	8
16	60	15	3.28	6.80	9
17	45	15	3.61	5.18	7
18	90	15	3.63	3.71	7
19	120	16	3.42	4.31	6
20	90	19	4.80	4.19	6

See page 113 for definition of measurements.

F. Histologic Measurements

1. Mandibular Measurements: Human Males

No.	Age in Years	X_1	X_2	X_3
1	64	55	11.25	65
2	72	61	14.72	46
3	47	45	8.59	94
4	55	50	9.00	78
5	63	54	11.20	67
6	73	59	14.61	44
7	68	60	12.95	62
8	70	59	13.32	57
9	69	57	13.17	60
10	62	54	10.32	74
11	64	57	11.74	69
12	71	62	13.29	48
13	59	53	10.43	72
14	52	48	8.93	78
15	64	55	12.18	66
16	67	59	12.87	63
17	87	62	14.95	35
18	54	48	8.92	77
19	50	49	8.80	80
20	73	60	14.59	42
21	56	52	10.05	76
22	55	49	8.91	76
23	77	62	14.29	39
24	61	53	10.55	73
25	62	55	10.43	70
26	41	42	8.61	93
27	71	60	13.57	45
28	70	60	13.40	47
29	62	53	11.00	68
30	54	47	8.85	79
31	77	63	14.63	36
32	39	40	8.75	97
33	48	44	8.62	90
34	40	39	8.50	96
35	46	43	8.51	95
36	55	52	10.21	75
37	59	51	10.21	73
38	62	53	11.29	69

F. Histologic Measurements

1. Mandibular Measurements: Human Males (Cont'd)

No.	Age in Years	X_1	X_2	X_3
39	63	56	11. 17	67
40	64	56	12. 35	64
41	68	60	12. 50	52
42	69	58	13. 20	58
43	76	61	14. 70	41
44	76	62	14. 77	38
45	80	64	14. 23	37
46	81	63	14. 75	40
47	81	65	14. 53	41
48	84	65	14. 80	43
49	85	64	14. 77	42
50	85	66	14. 73	33
51	49	47	8. 74	92
52	61	49	11. 20	77

See page 113 for definition of measurements.

2. Femoral Measurements: Human Males

No.	Age in Years	X_1	X_2	X_3
1	64	60	13. 39	38
2	72	65	14. 42	25
3	47	50	9. 38	70
4	55	48	9. 45	65
5	63	57	12. 65	41
6	73	63	13. 52	28
7	68	58	11. 43	29
8	70	64	12. 34	32
9	69	60	11. 26	32
10	62	55	10. 62	40
11	64	58	12. 50	35
12	71	64	13. 20	26
13	59	57	12. 25	42
14	52	49	9. 21	62
15	64	62	12. 00	38
16	67	63	12. 24	35
17	87	69	15. 05	29
18	54	51	10. 50	59
19	50	48	9. 37	61
20	73	66	12. 00	26
21	56	51	11. 29	48
22	55	50	10. 25	57
23	77	66	15. 20	28
24	61	58	11. 00	43
25	62	56	11. 25	42
26	41	45	8. 28	80
27	71	67	14. 42	25
28	70	61	13. 35	27
29	62	58	11. 50	39
30	54	46	9. 15	56
31	77	63	13. 75	26
32	39	42	9. 50	75
33	48	46	9. 83	68

See page 113 for definition of measurements

3. Tibial Measurements: Human Males

No.	Age in Years	X_1	X_2	X_3
1	64	62	12.00	41
2	72	62	14.28	30
3	47	48	9.15	74
4	55	46	9.50	62
5	63	59	12.25	41
6	73	68	13.25	30
7	68	56	11.25	33
8	70	61	12.50	29
9	69	60	11.50	36
10	62	57	11.95	45
11	64	61	12.29	40
12	71	68	14.00	28
13	59	59	12.57	47
14	52	51	9.20	59
15	64	65	12.50	37
16	67	62	12.00	39
17	87	75	16.20	33
18	54	48	10.00	58
19	50	51	9.25	63
20	73	72	12.25	30
21	56	53	11.00	50
22	55	48	10.00	60
23	77	73	16.00	31
24	61	60	12.00	47
25	62	60	11.10	43
26	41	43	8.29	82
27	71	69	14.00	29
28	70	59	13.75	28
29	62	61	11.20	41
30	54	49	8.95	57
31	77	69	13.90	31
32	39	45	9.47	79
33	48	50	9.73	70

See page 113 for definition of measurements

G. Residuals from Regressions on Age in Untreated Rats

Errors in estimation of age at death in untreated rats are provided in the following tables. Since measurements of age in days were converted to logarithmic functions, the resulting errors should be interpreted accordingly. Two tables of residual errors are provided; these were based on the mandible, femur and tibia and result from two different regression functions. In these regression models measurements are defined as follows:

Y = log age in days

X_1 = total number of osteons in two 40X microscopic fields

X_2 = average number of lamellae per osteon

X_3 = average Haversian canal diameter (μm)

X_4 = total number of non-Haversian longitudinal (primary) canals in two 40X microscopic fields

$X_5 = X_1^2$

$X_6 = X_2^2$

$X_7 = X_3^2$

$X_8 = X_4^2$

Model 1: $Y_{ij} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + \varepsilon_{ij}$

Bone On Which Regression Estimates Of Age Were Based

		Mandible		Femur		Tibia	
Case No.	Y Value	Y Estimate	Residual	Y Estimate	Residual	Y Estimate	Residual
1	0. 30102	0. 36408	-0. 06305	0. 31548	-0. 01445	0. 58239	-0. 28136
2	0. 30102	0. 29121	0. 00981	0. 31047	-0. 00944	0. 31695	-0. 01592
3	0. 30102	0. 27001	0. 03101	0. 39171	-0. 09068	0. 44181	-0. 14078
4	0. 30102	0. 55568	-0. 25465	0. 36618	-0. 06515	0. 42421	-0. 12318
5	0. 30102	0. 31607	-0. 01504	0. 27745	0. 02357	0. 06519	0. 23583
6	0. 99999	1. 05528	-0. 05528	0. 88282	0. 11716	0. 92440	0. 07559
7	0. 99999	0. 95898	0. 04100	1. 05527	-0. 05527	1. 17028	-0. 17028
8	0. 99999	0. 68789	0. 31209	1. 00460	-0. 00460	0. 99268	0. 00731
9	0. 99999	1. 15028	-0. 15028	1. 03838	-0. 03838	1. 26236	-0. 26236
10	0. 99999	0. 87486	0. 12513	0. 94380	0. 05619	0. 85848	0. 14151
11	1. 30102	1. 14882	0. 15220	1. 07793	0. 22308	1. 29779	0. 00323
12	1. 30102	1. 33497	-0. 03394	1. 26595	0. 03507	1. 13691	0. 16411
13	1. 30102	1. 36003	-0. 05900	1. 30954	-0. 00851	1. 40148	-0. 10045
14	1. 30102	1. 29590	0. 00512	1. 41604	-0. 11501	1. 13830	0. 16271
15	1. 30102	1. 40080	-0. 09978	1. 26251	0. 03851	1. 52406	-0. 22303
16	1. 47711	1. 63355	-0. 15643	1. 67085	-0. 19373	1. 39438	0. 08273
17	1. 47711	1. 65077	-0. 17365	1. 34937	0. 12774	1. 49681	-0. 01969
18	1. 47711	1. 54622	-0. 06910	1. 75484	-0. 27772	1. 36628	0. 11083
19	1. 47711	1. 41743	0. 05968	1. 65111	-0. 17399	1. 58052	-0. 10341
20	1. 47711	1. 40054	0. 07656	1. 49632	-0. 01920	1. 25872	0. 21839
21	1. 77814	1. 68129	0. 09684	1. 72940	0. 04874	1. 68536	0. 09277
22	1. 77814	1. 74911	0. 02903	1. 67146	0. 10668	1. 72171	0. 05643
23	1. 77814	1. 84850	-0. 07035	1. 79242	-0. 01427	1. 65399	0. 12415
24	1. 77814	1. 81825	-0. 04010	1. 81503	-0. 03688	1. 78245	-0. 00430
25	1. 77814	1. 79009	-0. 01194	1. 84215	-0. 06401	1. 76685	0. 01129
26	1. 95423	1. 86043	0. 09379	1. 84273	0. 11150	2. 06039	-0. 10615
27	1. 95423	1. 94606	0. 00817	2. 01432	-0. 06008	1. 87266	0. 08157
28	1. 95423	1. 91478	0. 03945	1. 94372	0. 01051	2. 07315	-0. 11891
29	1. 95423	1. 96309	-0. 00885	1. 79919	0. 15504	1. 95595	-0. 00171
30	1. 95423	2. 03703	-0. 08279	2. 05101	-0. 09677	1. 91678	0. 03745
31	2. 07917	2. 00728	0. 07188	1. 95403	0. 12513	2. 08341	-0. 00423
32	2. 07917	2. 03070	0. 04847	2. 06635	0. 01282	2. 11517	-0. 03599
33	2. 07917	2. 12715	-0. 04797	1. 98196	0. 09720	2. 06460	0. 01457
34	2. 07917	1. 87597	0. 20319	2. 05793	0. 02124	2. 01891	0. 06026
35	2. 07917	2. 09033	-0. 01116	2. 05102	0. 02815	2. 04792	0. 03125

Model 2: $Y_{ij} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \epsilon_{ij}$

Bone On Which Regression Estimates Of Age Were Based

Case No.	Mandible Y Value	Mandible Y Estimate	Mandible Residual	Femur Y Estimate	Femur Residual	Tibia Y Estimate	Tibia Residual
1	0. 30102	0. 44406	-0. 14303	0. 45243	-0. 15140	0. 59867	-0. 29764
2	0. 30102	0. 42380	-0. 12277	0. 50159	-0. 20056	0. 42116	-0. 12013
3	0. 30102	0. 35764	-0. 05662	0. 51074	-0. 20971	0. 41811	-0. 11708
4	0. 30102	0. 55120	-0. 25017	0. 43306	-0. 13203	0. 42381	-0. 12278
5	0. 30102	0. 39295	-0. 09192	0. 44318	-0. 14215	0. 08968	0. 21134
6	0. 99999	1. 03010	-0. 03010	0. 80657	0. 19342	0. 94939	0. 05060
7	0. 99999	0. 90961	0. 09038	0. 93032	0. 06966	1. 13558	-0. 13558
8	0. 99999	0. 65105	0. 34894	0. 92998	0. 07001	1. 00331	-0. 00332
9	0. 99999	0. 96106	0. 03893	0. 98517	0. 01482	1. 27407	-0. 27407
10	0. 99999	0. 84097	0. 15902	0. 80420	0. 19579	0. 80755	0. 19244
11	1. 30102	1. 11114	0. 18988	1. 01126	0. 28976	1. 29729	0. 00373
12	1. 30102	1. 27728	0. 02374	1. 23874	0. 06228	1. 06482	0. 23619
13	1. 30102	1. 41008	-0. 10905	1. 29048	0. 01054	1. 38330	-0. 08227
14	1. 30102	1. 28990	0. 01111	1. 32142	-0. 02039	1. 18484	0. 11618
15	1. 30102	1. 38286	-0. 08183	1. 13957	0. 16145	1. 45573	-0. 15470
16	1. 47711	1. 63698	-0. 15987	1. 76377	-0. 28666	1. 40957	0. 06754
17	1. 47711	1. 61440	-0. 13729	1. 33532	0. 14178	1. 47122	0. 00588
18	1. 47711	1. 54388	-0. 06676	1. 70575	-0. 22863	1. 33204	0. 14507
19	1. 47711	1. 39502	0. 08208	1. 58343	-0. 10631	1. 55793	-0. 08081
20	1. 47711	1. 37886	0. 09824	1. 45475	0. 02235	1. 21805	0. 25906
21	1. 77814	1. 63618	0. 14196	1. 83539	-0. 05724	1. 68708	0. 09105
22	1. 77814	1. 82409	-0. 04595	1. 66248	0. 11566	1. 72991	0. 04823
23	1. 77814	1. 79140	-0. 01326	1. 91566	-0. 13752	1. 65656	0. 12158
24	1. 77814	1. 77115	0. 00699	1. 96905	-0. 19090	1. 80974	-0. 03159
25	1. 77814	1. 76331	0. 01483	1. 83194	-0. 05379	1. 82615	-0. 04801
26	1. 95423	1. 87399	0. 08023	1. 89642	0. 05781	2. 04437	-0. 09013
27	1. 95423	2. 05436	-0. 10012	1. 81800	0. 13623	1. 87086	0. 08336
28	1. 95423	1. 85549	0. 09874	1. 82894	0. 12529	2. 09673	-0. 14249
29	1. 95423	1. 88764	0. 06659	1. 68432	0. 26991	1. 92304	0. 03119
30	1. 95423	2. 07694	-0. 12271	1. 86097	0. 09325	1. 91872	0. 03551
31	2. 07917	2. 12153	-0. 04236	1. 89546	0. 18371	2. 11665	-0. 03747
32	2. 07917	1. 92592	0. 15325	2. 26374	-0. 18456	2. 19603	-0. 11686
33	2. 07917	2. 22095	-0. 14178	2. 12100	-0. 04182	2. 03737	0. 04180
34	2. 07917	1. 83888	0. 24028	2. 20907	-0. 12990	1. 98500	0. 09417
35	2. 07917	2. 20867	-0. 12949	2. 01915	0. 06001	2. 05895	0. 02022

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