QUANTITATIVE MORPHOMETRY OF THE VERTEBRAE AND FEMUR OF THE BEAGLE AS A FUNCTION OF AGE AND SEX

by

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ABSTRACT

New methodology was developed to study bone microradiographs on a television image analyzing system. The optimum exposure time for producing microradiographs of 100 micron ground bone sections with sufficient contrast for the quantitative television microscope was determined. The microradiographs were standardized by using a step wedge made from aluminum foil.

The microradiographs produced from a bone section (cross-section of lumbar vertebra) ground in several steps from 120 to 70 microns were found to give the same morphometric measurements.

With the use of the image editor light pen of the image analyzer (QTM-720) to separate cortical and trabecular bone, complete information of the bone was obtained from the serial cross-sections. The variations of the measurements were detected from one end of the bone to the other leading to the reconstruction of the bone and to a determination of the accuracy of selecting samples for a specific morphometric study.

The first lumbar vertebra and the proximal femur from each of 14 beagles were chosen to study changes in morphometric parameters as a function of age and sex. In the cross-sections of the ventral vertebra the maxima of trabecular bone area, percent bone and surface area per volume of tissue were detected at the end portions; these values decreased gradually and had a minimum at the middle part of the main vertebral body. The specific bone surface was more or less uniform within the main vertebral body but dropped abruptly as it reached the

end portions where the epiphysis was located. In contrast there were no significant changes in the measurements among sections in the dorsal vertebra; greater trabecular width and higher percent bone were characteristics of this portion. In the cross-sections of the proximal femur the head region had greater trabecular width and higher percent bone than the non-head portion; percent bone was nearly uniform within the head portion but decreased gradually from the proximal end of the non-head portion down into the shaft. The morphometric parameters and dimensions of the bones were presumed to be a function in response to stresses; the variations were observed in beagles of the same age and sex and to a higher degree in the proximal femur than in the vertebra.

Bone is rapidly developing in the three month old beagles and could not be compared with the adults. No specific differences in measurements were detected in beagles from 17 months to 11 years, male and female. There was no indication of osteoporosis.

Cross-sections of the vertebra and proximal femur showed isotropic orientation of the trabeculae while in the longitudinal sections anisotropic trabeculae were detected in all portions of the vertebra and the non-head portion of the proximal femur. The isotropic structures were preferred for study since the three dimensional measurements could be calculated from a universal k value of $\frac{4}{\pi}$, whereas in anisotropic structures the k value could not be given directly, since the anisotropy varied from section to section and within different regions of the same section.

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1. INTRODUCTION

1.1 Background

The Radiobiology Laboratory at the University of Utah is currently investigating, among other things, the toxicity of Radium-226 and Plutonium-239 in beagles with the objective of determining the toxicity of the two radioisotopes at selected ages of the dogs. One of the major investigative efforts of the project is centered on bone because of the bone-seeking characteristics of the nuclides and because osteosarcomas arising in the bone provide the single, most useful indicator of radionuclide toxicity. The use of osteosarcomas and other bone pathology as indicators of toxicity makes it desirable to have an estimate of the microdoses of radiation received at various locations. The microdosimetry in turn requires a knowledge of the quantitative micromorphometry of the bone.

1.2 Objectives of this study

Until now no studies have been made in which quantitative micromorphometry has been studied in detail for a whole bone and the detailed quantitative structures reported and overall summaries given. Such a study is highly desirable from the standpoint of both the increase of knowledge of bone anatomy and the needs of bone microdosimetry. Until comparatively recently such a study was not feasible because of the great amount of work required to measure completely all serial sections of a bone by point counting techniques. It was not until the development of reliable, high-speed computers and their application to the data handling and calculation problems of already existent density scanning devices that devices become available that could measure such things as the area and perimeter of trabecular bone microradiographs. The earlier models of these were quite primitive but over the years development has taken place until nowadays it is possible to purchase highly complex 'Image Analyzers' on the commercial market from several companies. One of these analyzers, the Quantimet-720, is in the possession of the Radiobiology Laboratory and it was desired to use this instrument to obtain quantitative micromorphometric information on bones of the beagle as the basis for studies in microdosimetry. It was soon found that a great number of problems would have to be solved before accurate information could be obtained.

Since there was considerable need for information on quantitative micromorphometry and an instrument was available to carry the actual load of measurement, it was decided to undertake this project with the following principal aims:

1. To develop a methodology for the quantitative micromorphometry of bones which in the final stage would allow for semi-automatic data acquisition.

2. To test the methodology using beagles and selecting one bone from the axial skeleton (first lumbar vertebra) and one from the appendicular skeleton (proximal femur).

3. To use as many beagles of various ages and sexes as were available (14) for the tests of the methodologies and to use the data thus obtained for developing methods of analysis.

4. To present the results of analysis as a preliminary report and as an illustration of the information that can be obtained from quantitative micromorphometry.

While some of the aims may sound more or less standard for biological studies, number of new contributions have emerged from the work and are reported herein. Not the least of the novel contributions has been the development of a methodology that would produce quantitative information on the microscopic details of the anatomy of an entire bone and the presentation of information, hitherto not known on changes of trabecular distributions within a whole bone.

1.3 Outline of methodology

1.3.1 Background

Although details of some of the component parts of the methodology are given in the section on Materials and Methods and others are covered in the appendix, it seems desirable to give a general discussion here of the methodology as finally worked out unencumbered by numerous details.

The sequence employed in going from the fresh bone removed at autopsy to a printout of the parameters used to express the quantitative micromorphometry is given below. Some of the elements are standard in bone investigations and will be considered only briefly; novel constituents are discussed in more detail.

1.3.2 Preparation of bone slices ready for X-ray radiographs

This is standard procedure in bone investigatory work and does not need much discussion here. The bones removed at autopsy are measured, fixed, defatted, embedded in plastic, sawed into thin sections and then ground to a thickness of about 100 micrometers. During the process of sawing and grinding records are kept of the bone loss so that it will be known how much bone the finished section represents.

1.3.3 Radiographs of bone section

Using a low voltage (12 kilovolts) X-ray source a radiograph is made of the bone section on high contrast film. From this point on all measurements are made on projections or on magnified images of the negative film.

The making of the radiograph is one of the most critical steps in the entire procedure. It is necessary for all films to be of the same density to ensure comparability of measurements. Constant monitoring of the development procedures and adjustments for different batches of film by means of step wedge calibration is necessary. In addition it is highly desirable that the films be exposed to give the optimum density for purposes of measurement. Considerable investigative work was necessary to determine the optical density and its limits of variation which were optimal for measurement purposes. Details are given in the Appendix B.

1.3.4 Measurement of micromorphological details from the radiograph

The radiograph of the bone section is magnified by a conventional optical microscope and projected onto the camera tube of a television type image analyzer - the Quantimet 720. In this device the image is scanned and broken down into a series of discrete points known as picture points or pixels. Using as a guide a threshold entered into the machine by the operator, the device determines the number of picture points above the threshold and passes this information to a small programmed computer which converts the number of picture points, greater than the threshold, to the area of bone in square millimeters and prints this information out. Further sequential instructions programmed into the image analyzer cause it to measure the marrow area, the total area of the tissue and the perimeter of the trabecular bone-marrow interface. The results of these are also printed out in absolute units. The results were also punched onto paper tape for later processing on a larger computer.

A special light pen editor circuit in the image analyzer allows specific areas, e.g. trabecular bone only, to be outlined on the monitor image and measured independently of cortical bone.

Types of measurement other than bone area and perimeter can be made with the image analyzer. Examples are: orientation of trabecular bone, mean path length through bone marrow and trabecular bone, average area and frequency distribution of size of marrow spaces. Investigation of such measurements as these were not undertaken because of limitations of time.

The great advantage of the image analyzer in this type of work is the semi-automatic acquisition of data and the immense increase of speed with which measurements can be made.

1.3.5 Quantification of microanatomical structures of the trabecular bone and marrow

The image analyzer produced measurements of the trabecular bone and marrow in the areas specified by the investigator and printed the measurements in square millimeters for the areas and linear millimeters for the perimeter. The specific measurements printed out were:

Trabecular bone cross-section

Bone marrow cross-section

Total tissue cross-section (bone marrow + bone)

Perimeter of interface between bone and bone marrow

Note that these measurements could be made on separate sections or different parts of the same section so that comparisons were available from one location to another. From these four measurements parameters which describe some aspects of microscopic anatomy of the bone can be computed and used for analysis. The parameters are not all independent of each other but they do measure different things. Some discussion of the parameters is desirable at this point; further discussion will be found in the section on Materials and Methods.

Bone density, proportion bone, percent bone:

This parameter is derived by dividing the measured area of the bone by the total tissue area. This is the proportion bone. It may be multiplied by 100, for the sake of convenience, to give the percent bone. Bone density is perhaps a more descriptive term of what this parameter measures. This particular parameter is one of the few derived from measurements on thin sections which does not require a correction factor to compensate for difference in orientation of the elements being measured.

Trabecular width and three-dimensional trabecular thickness:

When a group of objects, such as trabeculae, are sliced and the width of the section measured at a large number of positions then the average of these measured widths will not be the true width; it will generally be larger than the true width by a factor that depends upon the shape of the sectioned objects and the degree of orientation. The true width could of course be obtained by measuring the width or diameter of the three-dimensional objects at a number of locations and averaging the results. Unfortunately the measurement of the thickness of three-dimensional trabeculae is seldom possible and it is necessary to arrive at the conversion factor from widths taken on sections by means of some theoretical considerations. The conversion factor for the bone width problem appears in the equation:

$$\frac{\text{True width of trabeculae}}{\text{(Three-dimensional thickness)}} = \frac{\text{Measured width of trabeculae}}{k}$$

Where k, or the reciprocal of λ, is a dimensionless constant. The value of k is $\frac{4}{\pi}$ where orientation of the trabeculae is either absent or of a comparatively minor degree, i.e. isotropic. The derivation of this expression is out of place here but a discussion of it and references to where it is derived can be found in Schenk (11) and others (3,8,14,15). This correction factor $\frac{4}{\pi}$, is numerically equal to 1.273..., and also appears in the equations for the surface to volume ratios which follow. In principle, if the structures are oriented or anisotropic, k can have any values between one and infinity and there is no simple general method for finding k. It is for this reason that most of the work in this study was done on cross-sections; the isotropic nature of these allows the use of simpler constants.

Surface of bone to volume of bone ratio, specific bone surface (S_{vb}):

This parameter relates the surface of the bone to the volume of the bone and in a way is a measure of the shape. For a bone like trabeculae the thinner the width, the larger the ratio for the same volume of bone. It is useful in dosimetry of surface bone seeker elements as it measures the relative area of bone on which the element can be deposited.

The surface to volume ratio of bone is reciprocally related to the thickness of the trabeculae; the biological interpretation is different, however.

Surface of bone to volume of tissue (S_{vt}):

This relates the area of the bone surface to the volume of the tissue and is a parameter of potential usefulness in microdosimetry.

1.4 Composition of sample of beagles used for testing methodology

The sample of dogs used to validate the methodology of this study and to provide numerical results is shown in Table 1.4.

It is highly desirable in a study such as this to have a wide range of ages and also to have a balanced age-sex composition in the experimental animals used for the study. The beagle, however, is a fairly long-lived animal and one cannot afford to wait 10 to 15 years for the oldest animals. For this particular study and some other allied experiments it was necessary to take the dogs which were available in the beagle colony. For the young ages such as three months and for young adults at 17 months of age, the desired numbers could be obtained; for older dogs the choice was limited to those available in the colony and not needed for other purposes.

1.5 Beagle skeletal data

There were few data reported on bone morphometry of beagle. Sampling sections were used and quantitated by various methods. Bartley et al (1) worked on lumbar vertebral bodies; undecalcified sections were stained by Von Kossa and analyzed by three different methods, tracing paper-cut out-weighing, point counting, and the Quantimet. Jee et al (6,7) made the sagittal cut of lumbar vertebral bodies, undecalcified and Von Kossa stained sections were determined by the Quantimet. An automatic scanning device was used by Lloyd and Hodges (8) to analyze microradiographs which had been prepared from 100

Table 1.4 Age-sex composition of experimental beagles. Parentheses contain the weights of animals in kilograms. The other figures are the identification numbers of the animals.

Age \ Sex	Male	Female	Total
3 Months	MAC165 (4.30 kg) MAC171 (3.90 kg)	FAC173 (4.30 kg) FAC174 (5.25 kg)	4
17 Months	MAC168 (13.15 kg) MAC169 (12.60 kg)	FAC172 (11.20 kg) FAC175 (10.85 kg)	4
3 Years	MAC178 (11.50 kg) MAC181 (12.50 kg)	Not available	2
9-11 Years	MAC177 (8.45 kg) MAC179 (10.80 kg)	FAC180 (8.80 kg) FAC107 (8.85 kg)	4
Total	8	6	14

micron thick sections in the sagittal cut of the vertebra. Woodbury et al (21) investigated the microradiographs on the Quantimet-720 in cross- and longitudinal sections of the vertebra. Beddoe (2) worked on mid-sagittal plane of lumbar vertebra and oblique sagittal plane of proximal femur; 30 micron thick sections were radiographed, and the radiographs and photographic positives were analyzed on a bone scanning microscope.

The relevant results of the above work are included in Tables 4.5.1 and 4.5.2 of the Discussion section.

2. MATERIALS AND METHODS

2.1 Bone and bone preparation

Beagles used in this experiment have been tabulated in Table 1.4.

2.1.1 Preautopsy and autopsy

Before dogs were sacrificed, tetracycline and tritiated thymidine had been given as routine laboratory procedure for other purposes. At sacrifice bones were removed, trimmed and sawed into appropriate pieces. The first lumbar vertebra was sawed into anterior and posterior halves, and the right proximal femur was used for approximately a two to three centimeter length; they were used in cross-section. The second lumbar vertebra was sawed along the median sagittal plane into two halves and the left half was used, and the left proximal femur was sawed into medial half and lateral half; they were used in longitudinal section.

Bones were then fixed in acetone and measurements of length and width were made. In the process of fixation and defatting bones were placed in five to six changes of acetone followed by twelve changes of ether for at least three hours in each solution. Bones were dried at room temperature overnight and embedded in bioplastic casting resin.

2.1.2 Cutting section

Embedded bones were sawed to a thickness of approximately 0.5 mm each. All sections were numbered consecutively and the measurement of thickness of each section was made at three different places to get the average thickness. Sections were then ground equally on both sides to 100 micron thickness in preparation for X-ray. Identification numbers of beagles used for serial cross sections and longitudinal sections are shown in Table 2.1.2. For the other dogs cross-sections were selected uniformly throughout the bone and these are referred to as sampling cross-sections.

2.1.3 Division of the lumbar vertebra

In the three month old beagles the component primitive bones have not yet fused together to produce the final vertebral bone, so measurements were made of each of the distinguishable segments as illustrated in Figure 2.1.3 (a, b, and c). In cross-sections where the spinal canal is complete, a horizontal line was drawn across the canal and surrounding bone about one-third of the diameter of the canal in the dorso-ventral direction as illustrated in Figure 2.1.3 b. Thus the lower or ventral portion and the upper or dorsal portion were designated. Where the spinal canal was open at the side as in Figure 2.1.3 c the lower and upper segments were measured separately.

For the 17 month old and older beagles the measurements were made only on the ventral and dorsal portions; no attempt was made to distinguish the centrum from the adjacent bones as was done in the three month old beagle.

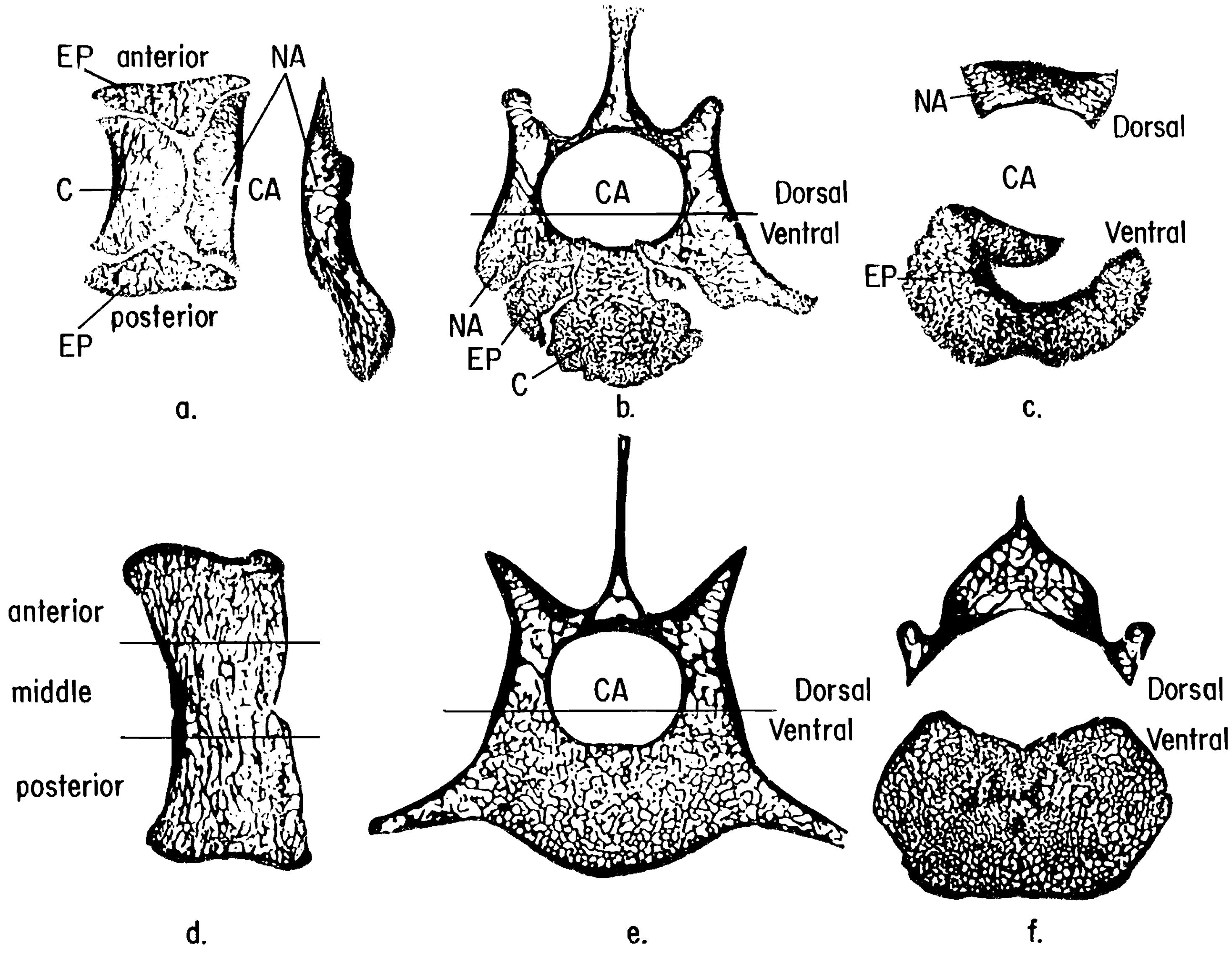
2.1.4 Division of the proximal femur

In cross-section the proximal part of the femur of the three month old beagle is composed of three separate parts which can be clearly seen in section. These may be designated as the head, the middle and the lateral parts and they are illustrated in Figure 2.1.4.1. In older beagles, however, the three component parts visible in the young dogs have fused together and only epiphyseal line separating the head of the

Table 2.1.2 Beagles selected for serial cross-sections and longitudinal sections of the lumbar vertebra and the proximal femur.

	Serial cross-sections		Longitudinal sections	
Age	Lumbar vertebra	Proximal femur	Lumbar vertebra	Proximal femur
3 months	MAC165 MAC171 FAC173 FAC174	FAC173	FAC174	FAC173
17 months	FAC175	FAC175	FAC175	FAC175
11 years	FAC107	FAC107		

Figure 2.1.3 Cross- and longitudinal sections of lumbar vertebra of three and 17 month old beagles illustrating the areas which were measured separately: a = longitudinal section of three months; b, c = cross-section of 3 months; d = longitudinal section of 17 months; e, f = cross-section of 17 months; C = centrum, CA = canal area of spinal cord, EP = epiphysis, NA = neural arch.


EP
anterior
NA
C
CA
posterior
EP
a.
CA
Dorsal
Ventral
NA
EP
C
b.
NA
Dorsal
CA
Ventral
EP
c.
anterior
middle
posterior
d.
CA
Dorsal
Ventral
e.
Dorsal
Ventral
f.

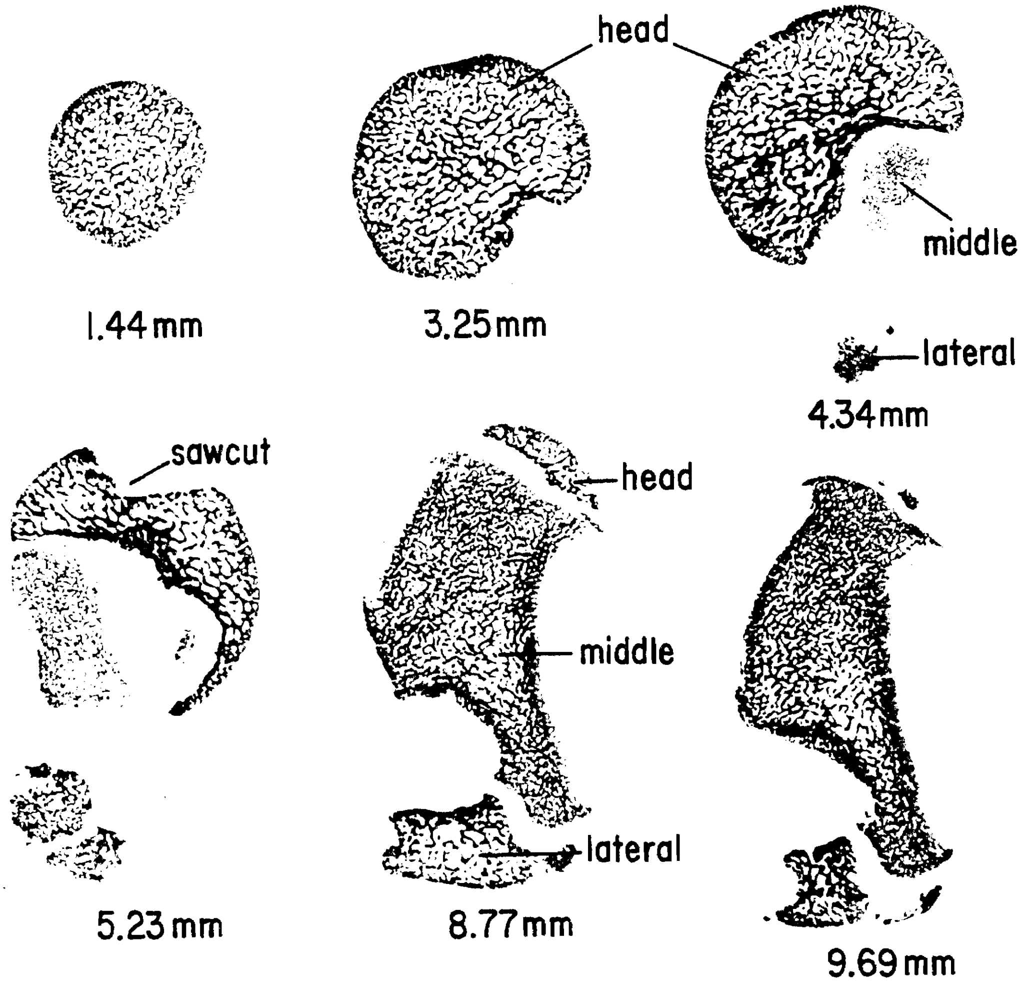


Figure 2.1.4.1 Representative cross-sections of the proximal femur of a three month old beagle. Three portions (the head, the middle, and the lateral) are clearly separated. The figures under the sections are distance in millimeters from the proximal end (2.8X).

femur from the other bones can be distinguished. Measurements therefore were perforce limited to the head and to the non-head bones combined. This is illustrated in Figure 2.1.4.2.

In longitudinal (sagittal) section of the three month old beagle, the head of the femur is clearly distinguished from the other bone as illustrated in Figure 2.1.4.3. In older beagles where component bones are fused together, the epiphyseal line is used to separate the head from the non-head portions as illustrated in 2.1.4.4.

2.2 X-ray of bone

The X-ray or microradiograph unit (Radiobiology Laboratory, University of Utah) consists of a high voltage power supply, a line voltage regulator, an X-ray tube with a tungsten filament and a camera to position the film. The unit operates at 12 kilovolts and 25 milliamperes. A rotary camera is required to obtain uniformity of the X-ray beam.

Exposure time and the optical density of the film are discussed later in section 2.4.1.

2.3 Morphometric analysis

2.3.1 Quantitative television microscope (Q.T.M.)

The Imanco Quantimet-720 is an automatic image analyzing computer. The section to be investigated is placed under a microscope where the field of view is then projected on to a television screen with higher magnification. With sufficient contrast of the projected image of the specimen, the Quantimet defines a visible boundary between areas of low and high contrast, permitting several kinds of measurements to be made.

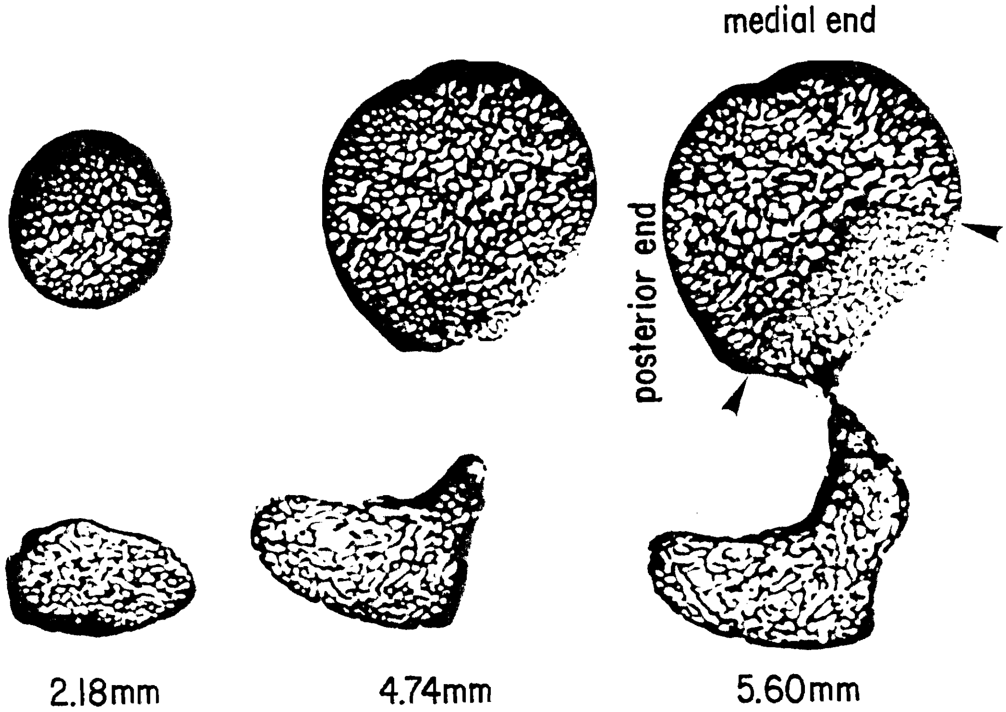
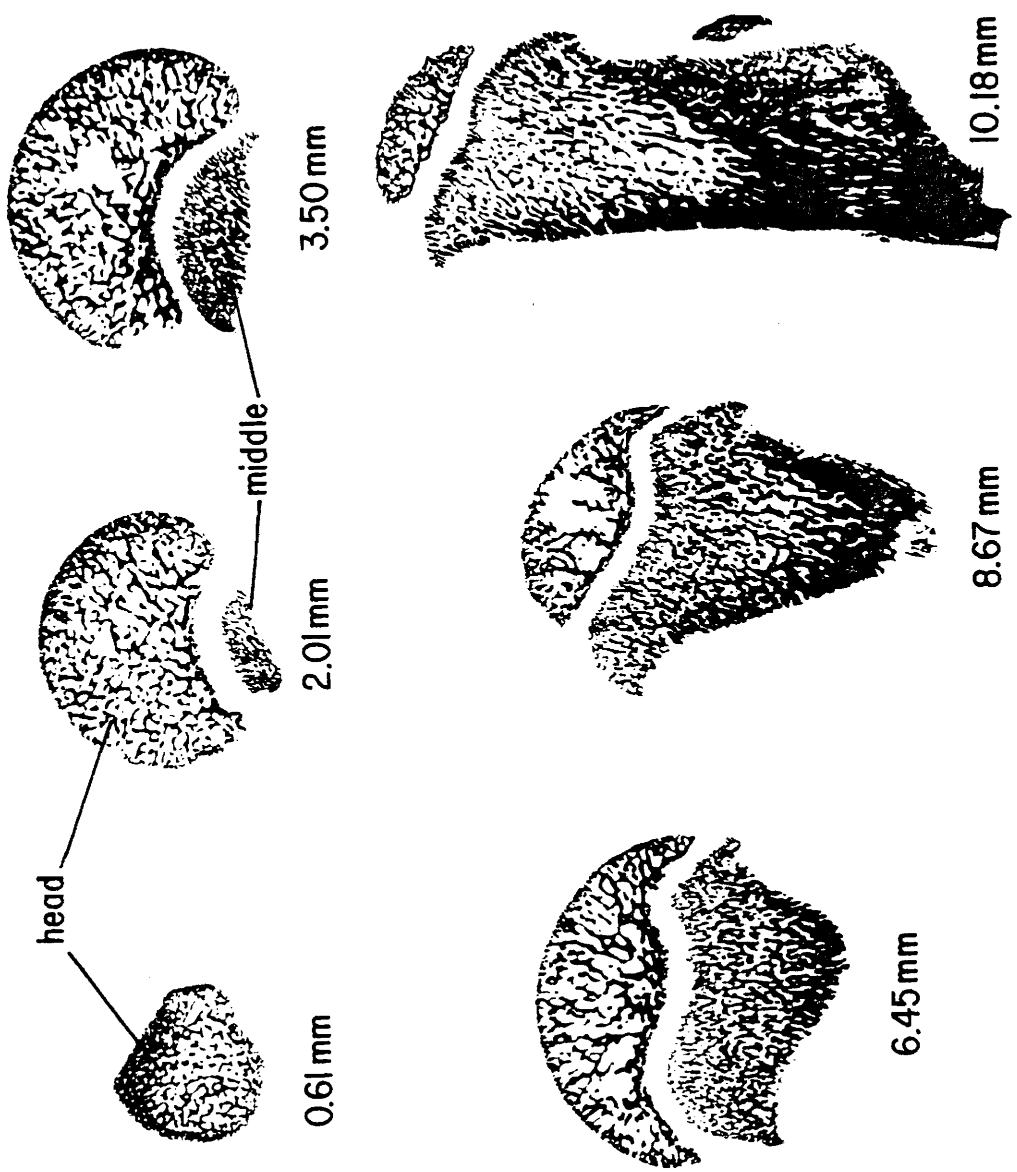


Figure 2.1.4.2 Representative cross-sections of the proximal end of the femur of a 17 month old beagle. Sections are taken in a plane oriented at right angle to the shaft of femur. The head of the femur is at the top of the photograph and the greater trochanter is at the bottom. The arrow indicates the epiphyseal line to distinguish the head and non-head portions. The figures under the sections are distances in millimeters from the proximal end (2.8X).

Figure 2.1.4.3 Longitudinal (sagittal) section of the proximal femur in three month old beagle. The figures under the sections are distance in millimeters from the medial end of bone (3.5X).


head
middle
0.61mm
2.01mm
3.50mm
6.45mm
8.67mm
10.18mm

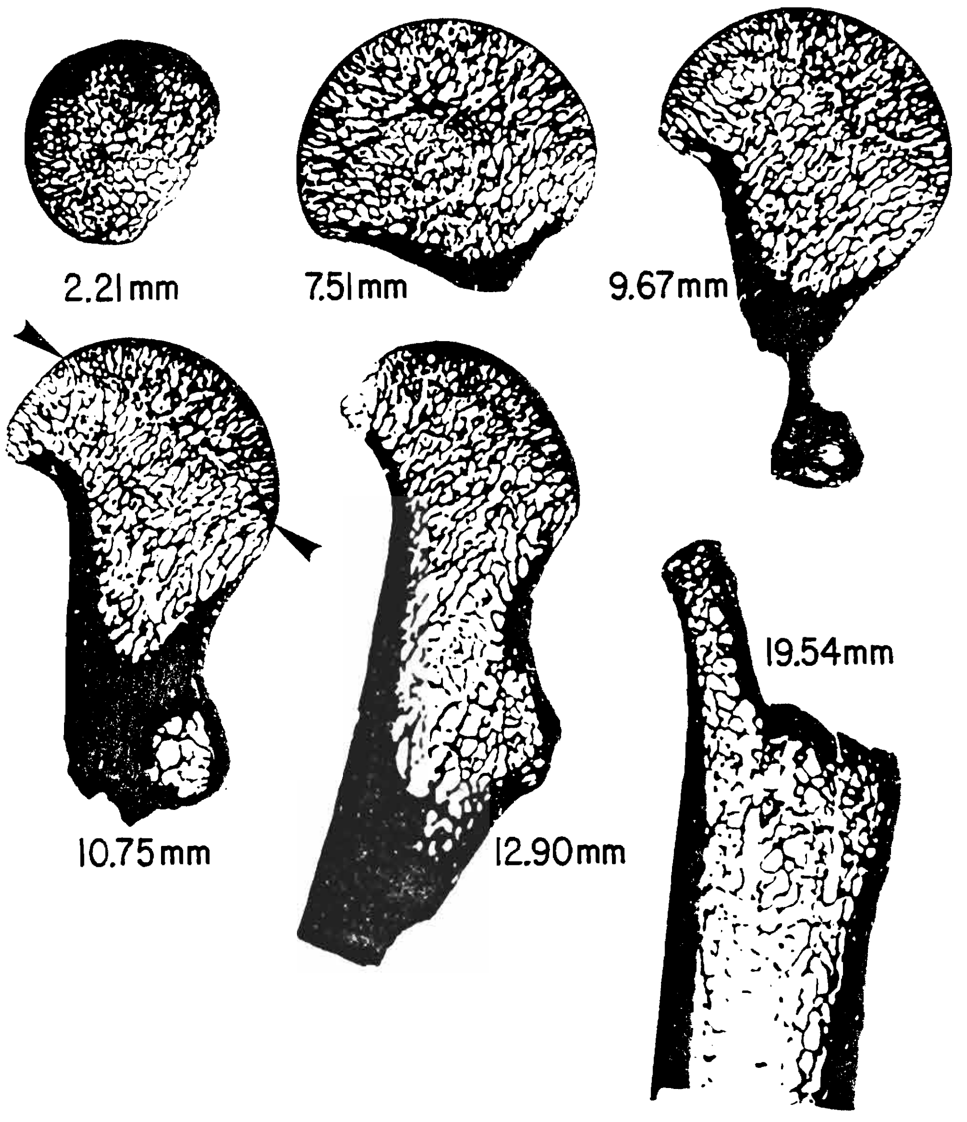


Figure 2.1.4.4 Longitudinal (sagittal) section of the proximal femur in a 17 month old beagle. Note that the epiphyseal line (arrows) separating the head portion from the rest of the structure (non-head) can be clearly seen in most sections. The figures are distances in millimeters from the medial end of bone (2.5X).

Specific details for using the Quantimet-720 in order to quantify bone micromorphometry are presented in Appendix A.

2.3.2 Types of measurements

Terminology and formulae used in bone morphometry were given in several papers (4,7,8,9,11,12,17,18,20,21). They are combined and used in this text in the following sections.

2.3.2.1 Direct measurements

Direct measurements on trabeculae are made on bone area in mm^2 (A_b), bone marrow area in mm^2 (A_m), tissue area in mm^2 (A_t, which is bone area plus marrow area) and bone perimeter in mm (P).

2.3.2.2 Derived measurements

Derived measurements are calculated from direct measurements with a number of formulae as follows.

Mean trabecular width in mm (W_b):

The mean width of trabeculae in two dimensions is estimated by the equation.

$$W_b = \frac{2\ A_b}{P}$$

Mean trabecular thickness in mm (T_b):

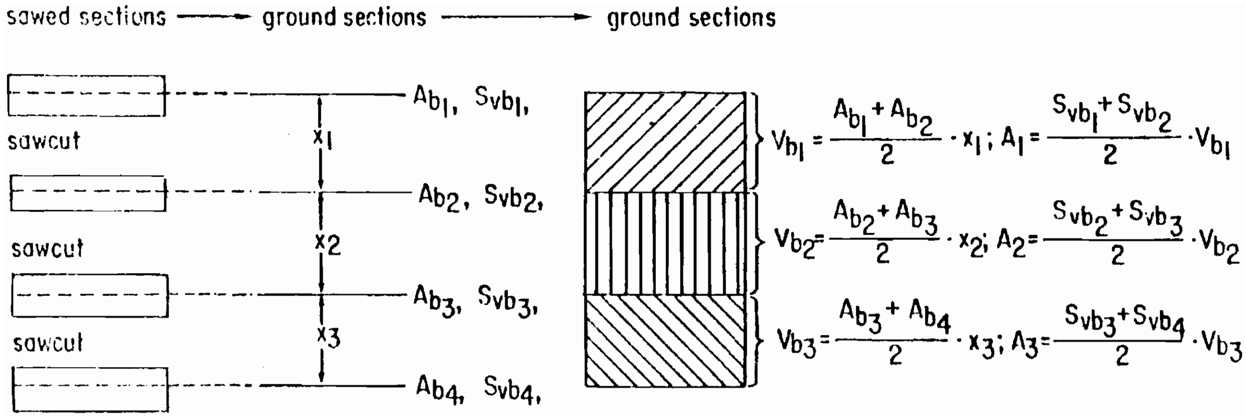
The mean thickness of trabeculae in three dimensions is estimated by the equation.

$$T_b = \frac{W_b}{k}$$

Where k is $\frac{4}{\pi}$ or 1.273... for isotropic structures.

Percent bone (A_{ab}):

The ratio of bone area to tissue area is called bone proportion. The percentage is used here and referred to as percent bone.



If: L = length of bone before cutting
n = number of cuts
T = accumulative thickness of all sections
t = average thickness of slots left by the saw

thus $t = \frac{L - T}{n - 1}$

x = distance between the middle of two consecutive sections
A_b = trabecular bone area
S_{vb} = specific bone surface
V_b = trabecular bone volume
A = trabecular surface area

Figure 2.3.2.2 Calculation of bone volume (V_b) and surface area (A).

Percent marrow (A_{am}):

This is the ratio (in percent) of bone marrow area to tissue area.

Specific bone surface in mm^2/mm^3 (S_{vb}):

Specific bone surface is a three dimensional value of the surface of bone to bone volume ratio. It is given by the formula.

$$S_{vb} = \frac{P}{A_b} \cdot k$$

Surface area of bone per volume of tissue in mm^2/mm^3 (S_{vt}):

This parameter is calculated by the equation.

$$S_{vb} = \frac{P}{A_t} \cdot k$$

Volume in mm^3 and surface area in mm^2:

In two consecutive sections bone volume (V_b) and trabecular surface area (A) can be calculated as illustrated in Figure 2.3.2.2. By addition the total or accumulative values of bone volume (ΣV_b) and surface area (ΣA) will be given. The total volumes of bone marrow (ΣV_m) and tissue (ΣV_t) are estimated in the same manner.

2.4 Factors affecting bone measurements

2.4.1. Microradiographs: exposure time and density

Different exposure times of the microradiographs give different values of direct measurements made on the Quantimet. The optical density of the microradiographs should be constant and be in the range of small variation with exposure time so that the Quantimet measurements are consistent. For the purposes of calibration a step wedge of aluminum foil is used as a control and is X-rayed along with the bone section. For this experiment three layers of aluminum foil were used, giving a microradiograph with an optical density of 0.80 ± as a standard of reference. Details are shown in Appendix B.

The minor potential problems with development of the microradiographs are given in the final protocol for X-ray in Appendix B.

2.4.2 Thickness of the ground bone section

It was thought that the thickness of the ground bone section may cause some error on Q.T.M. measurements. To check this point the same section was ground from 120 to 70 microns with X-rays being taken serially at the optimum exposure time. It was found that there was no significant difference in the measurements as a function of thickness. The quantitative results are shown in Appendix C.

2.5 Statistical analysis

Simple one-way Analysis of Variance (ANOVA) is used to compare differences of two groups or more. This is treated in any general reference work on statistical analysis. The tables are made for the comparisons of various ages in male (or female) to be read horizontally; the comparisons between sexes are given vertically. It is also used to compare parameters among different portions of a specific bone in one animal such as in a longitudinal section of the vertebra of a 17-month old beagle.

Significance of comparison is determined by p-value, using stars to indicate the degree of statistical significance. For example, one, two and three stars represent low ($p < 0.05$), high ($p < 0.01$) and very high ($p < 0.001$) statistical differences respectively; and N.S. is an abbreviation for not statistically significant. Arithmetic mean (M), standard deviation (S) and the number of sections under test (N) are used in the table of the analysis.

3. RESULTS

3.1 Introduction

Although the primary aim of this study was the development of a methodology for the quantitative micromorphometry of bones, a secondary aim was the analysis of the data obtained in testing out the methodology. The results of the analysis will also be useful in judging the success of the methodology that was developed.

The raw data resulting from the methodology were in the form of readouts from the Quantimet-720 and these were converted to numerical values of the desired parameters in the proper system of units by a small computer and it is these values that are to be analyzed.

The pattern of analysis to be used with the data needs some discussion since a number of factors must be considered. Four major micromorphometric parameters are being considered, namely: trabecular width in microns (W_b), percent bone (A_{ab}), specific bone surface in mm^2/mm^3 (S_{vb}), and surface of bone to volume of tissue (surface to volume ratio) in mm^2/mm^3 (S_{vt}). The values of these parameters are to be evaluated for the first lumbar vertebra and the right proximal femur. Average values for the two bones and for portions of the bones are to be derived as well as changes in the structure of the bone along its length as indicated by changes in the various parameters. The effects of the age and sex of the dog upon the parameters and their changes are also to be evaluated.

Each parameter is to be considered in turn and an assessment made of the effects of the various factors upon the values of the parameters and their changes. Changes of the numerical value of the parameter from one end of the bone toward the other end is probably the most fundamental factor in the analysis and will be considered first. The first lumbar vertebra will be considered followed by the proximal femur.

3.2 Cross-sections of the ventral vertebra in adult beagles

3.2.1 Area of trabecular bone (A_b), marrow (A_m), and tissue (A_t)

The cross-sectional areas of trabeculae and bone marrow and their sum, the total tissue, are not part of the group of parameters discussed in the previous section, but since they are fundamental to those parameters they are discussed here as background data.

Measurement of the three areas on consecutive cross-sections through the first lumbar vertebral body are plotted in Figure 3.2.1. Technically, the material covered is the ventral part of the lumbar vertebra as defined in Materials and Methods and this includes the vertebral body as well as some additional material.

Inspection of Figure 3.2.1 shows that the parameters have an abrubt rise from no trabecular bone at the ends of the vertebral body and a pronounced minimum near the center. The abrupt rise at the two ends is largely due to intrusion of trabecular bone into the epiphysis which constitutes most of the end sections. The minimum towards the center of the vertebral body can be largely attributed to the narrowing or "necking in" of the vertebral body.

The total values, in square millimeters, of trabecular bone, marrow and tissue are of little direct use in comparisons. The areas are highly correlated with the size of the animal and thus the bone area of

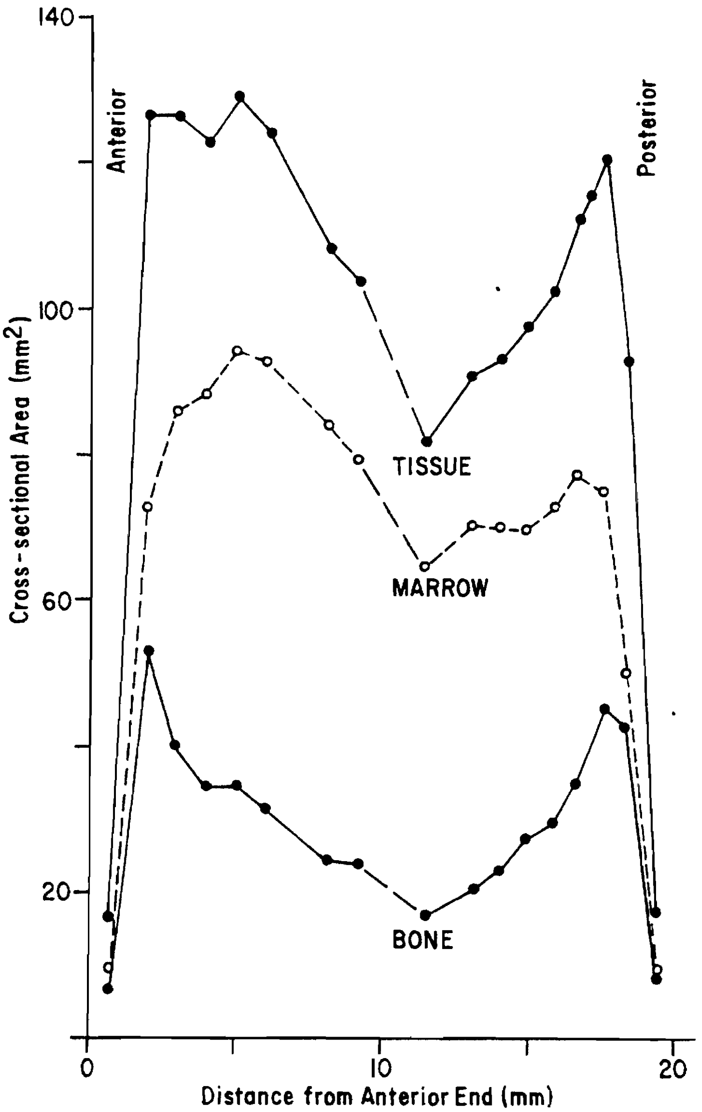


Figure 3.2.1 Area in square millimeters of trabecular bone, bone marrow, and tissue measured in consecutive cross-sections of the ventral part of the vertebra starting at the anterior end. The curves given here are from a 17-month old beagle (FAC 175).

a small dog and a large dog of the same age would be quite different. In general the variability of the direct area measurements is too large for them to be of much use for comparisons. Relative measurements in which the effect of size has been largely eliminated are generally more useful.

3.2.2 Percent bone - Relative area of bone (A_{ab})

The cross-sectional area of trabecular bone expressed as a percentage of the total tissue area (trabecular bone area + marrow area) is a composite parameter which is largely independent of the absolute size of the animal and thus allows comparisons to be made between dogs of different sizes, ages and sexes. The relative bone area is high at both ends of the vertebral body and drops abruptly to about half of the end value at the middle of the body. A typical curve of the relative bone area is shown for a 17 month old beagle in Figure 3.2.2.

The percentage of bone marrow (relative area of bone marrow) is not shown on the graph as it is a simple inverse of the percent bone obtained by subtracting percent bone from 100.

Average value of percent bone:

Because of the U-shaped curve of percent bone as a function of distance from the anterior end of the vertebral body, it is not a simple matter to define the average percent bone. In the case of the measurements illustrated in Figure 3.2.2, all of the consecutive cross-sections were measured and plotted and so it is a fairly simple matter to average all of the individual points. Measurement of all sections, however, was not feasible for all dogs and in general only a representative sample of about 6 or 7 sections was measured. Extreme end sections

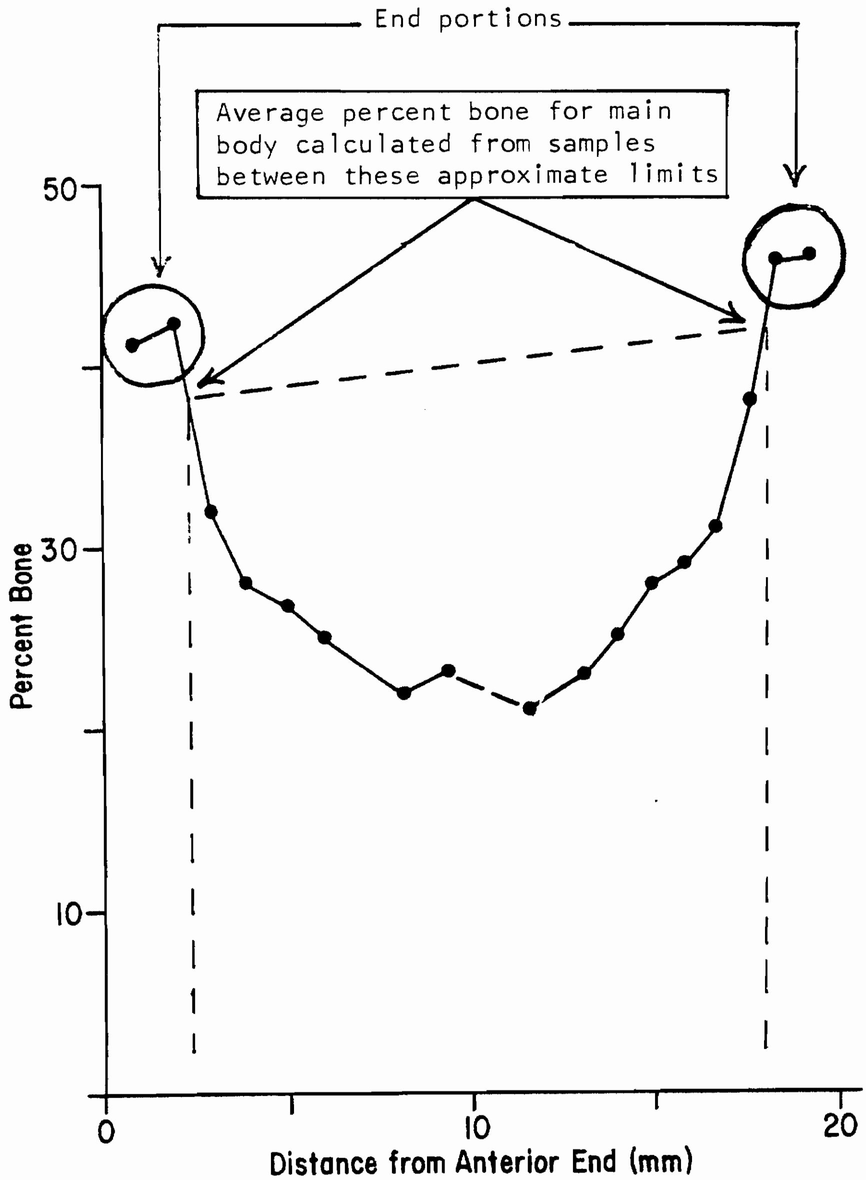


Figure 3.2.2 Percent bone in the ventral vertebra as a function of the distance from the anterior end of the vertebra (FAC 175). See text for discussion.

were excluded from the sampling because these were largely epiphyseal bone with epiphyseal type trabeculae of greater width. The sample sections were scattered evenly along the shaft of the vertebral body. The average percent bone for a specific vertebra was obtained by averaging the individual measurements of percent bone. The approximate boundaries for the end and the main body regions are shown in Figure 3.2.2.

Effects of age and sex:

To determine whether percent bone varied with the age or sex of the dog, a preliminary analysis was made using the percent bone for each section as the variable and checking to determine if the average value varied significantly from one dog to another. Simple one-way Analysis of Variance (ANOVA) was used to check for differences among dogs, ages and sexes and no significant difference ($P > 0.05$) was found. This is not to say that such differences do not exist, but that if they do they could not be found with the number of dogs available for study. Since no statistical significant differences were found, it was judged permissible to combine the averages for percent bone for all dogs in the study. This was done and yielded a value of 27.5. This may be rephrased for emphasis and clarity:

> The average value of the percent bone of the ventral portion of the first lumbar vertebra and with the end sections excluded is 27.53 and the standard deviation is 4.45. Ages covered were from 17 months through 11 years and the values are based on 10 dogs.
>
> The average value for the percent bone for the end sections which were excluded from the above average is 45.4 for the

same dogs. Since the excluded sections were largely epiphysis, the included trabeculae are largely epiphyseal in nature and the high percentage of bone may be a reflection of this.

3.2.3 Mean trabecular width (W_b)

Values of mean trabecular width are approximate uniform within the main vertebral body. Where trabeculae reach the small end portions of the vertebra, the width changes abruptly, having a pronounced maximum and then declining slightly at the very end sections. The maximal mean width is found within the epiphysis which has the greatest bone area. The end sections, one at each end of the bone, are incomplete and always have the smallest bone volume which may contain part of the cortex covering both ends of the vertebra. These observations, together with the high density of trabeculae as mentioned in the previous section, are probably in response to stresses around the epiphysis.

Curves of mean trabecular width (not shown) are identical in the serial cross-sections of one 17-month old and one 11 year old female beagle, and the comparison is shown in Appendix D.

Effect of age and sex:

Certain degrees of variations in mean trabecular width were detected with dog. Marked differences of the width were found in dogs of the same age and sex, and no specific relation was detected among beagles of different age and sex.

To avoid misinterpretation, all dogs of the same sex are combined and the grand mean for the width along with other parameters within the ventral portion are shown in Table 3.2.3.1. The results for the end portions are summarized in Table 3.2.3.2.

3.2.4 Specific bone surface (S_{vb})

Specific bone surface is reciprocally related to mean trabecular width. Changes within the ventral portion of lumbar vertebra are plotted in Figure 3.2.4.

Inspection of Figure 3.2.4 shows that specific bone surface is more or less uniform within the main vertebral body. Although the value of the middle section has slightly decreased, this is judged not to be significant and almost the straight curve is found in the serial cross-section of an 11-year old dog (see values in Appendix D). Specific bone surface decreases in the end portions with the pronounced minimum found in the largest section of the epiphysis. Values of specific bone surface are shown in Tables 3.2.3.1 and 3.2.3.2.

3.2.5 Surface area per volume of tissue (S_{vt})

As shown in Figure 3.2.4, surface area per volume of tissue has the highest value at the very end section of both ends of the vertebra. The value decreases gradually and reaches the minimum around the middle section of the bone. The curve is somewhat similar to percent bone because surface area per tissue volume is directly proportional to percent bone but indirectly proportional to bone area. The values are summarized in Tables 3.2.3.1 and 3.2.3.2.

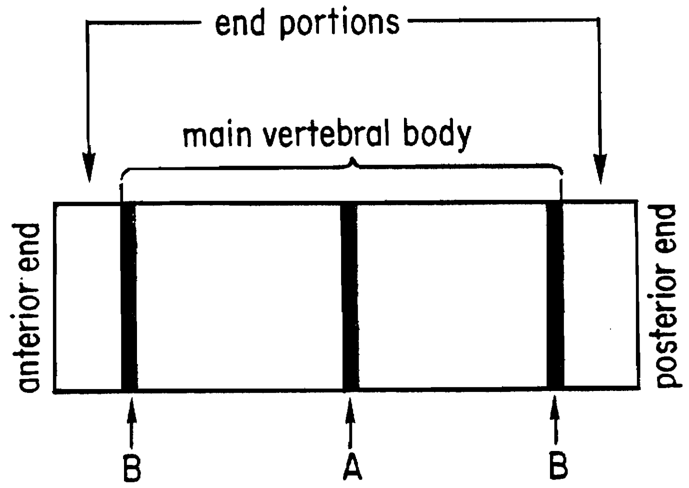
3.2.6 Total volume and total trabecular surface area

Total volumes of trabecular bone (ΣV_b), bone marrow (ΣV_m) and tissue (ΣV_t) and total trabecular surface area (ΣA) of individual beagles

Table 3.2.3.1 Summary of results of measurements of cross-sections within ventral and dorsal portions of first lumbar vertebra in combined ages from 17 months to 11 years.

		W_b (microns)	A_{ab} (percent)	S_{vb} (mm^2/mm^3)	S_{vt} (mm^2/mm^3)
Male	ventral	110	32	24	7.3
	dorsal	134	35	20	6.8
Female	ventral	100	32	26	8.0
	dorsal	120	34	22	7.3

Table 3.2.3.2 Summary of results of measurements of the cross-sections within the ventral portion of the first lumbar vertebra in combined ages from 17 months to 11 years. Subdivisions of the main vertebral body (approximately middle six-eighth) and end portions (anterior one-eighth and posterior one-eighth) are illustrated.



	End portions	Main vertebral body A	Main vertebral body B
W_b (microns)	130	98	99
A_{ab} (percent)	45	23	33
S_{vb} (mm^2/mm^3)	21	27	26
S_{vt} (mm^2/mm^3)	9.1	6.1	8.4

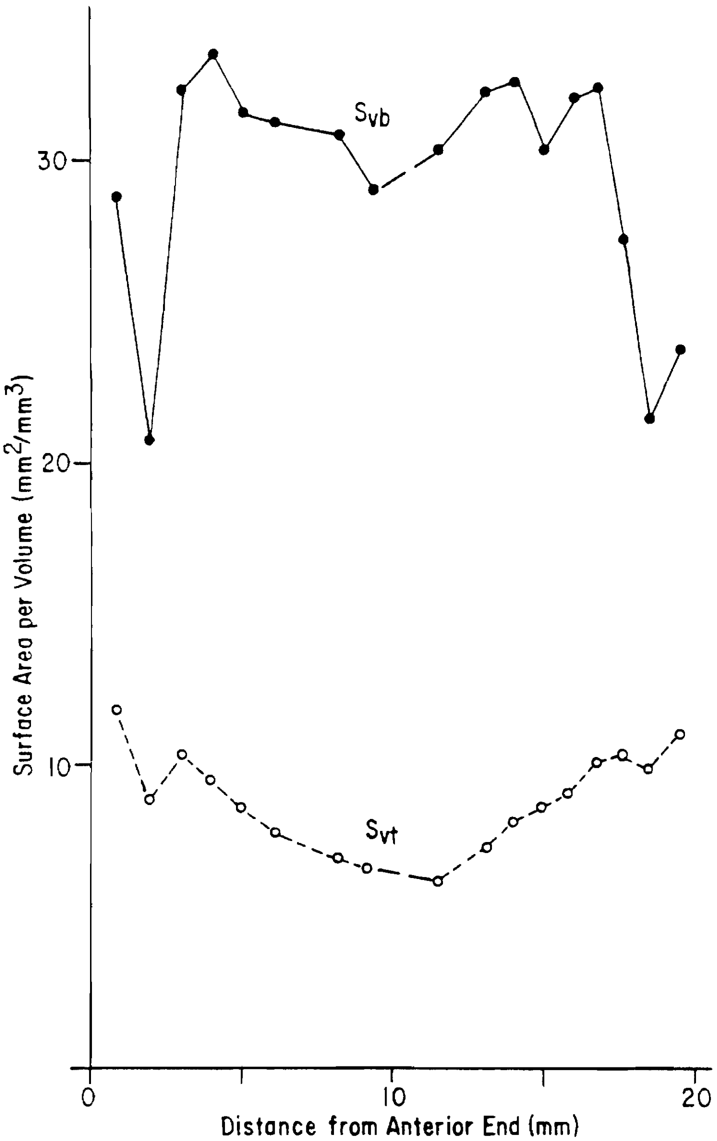


Figure 3.2.4 Specific bone surface (S_{vb}) and surface area per tissue volume (S_{vt}) in the ventral vertebra as a function of the distance from the anterior end of the vertebra (FAC175). See text for discussions.

are given in Table 3.2.6. These values depend mainly on the dimensions of the bone. Their relative values are already determined in the form of percent bone, specific bone surface, and surface area per volume of tissue.

3.3 Cross-sections of the dorsal vertebra in adult beagles

All sections used to investigate the dorsal vertebra are the same as those of the ventral vertebra. The results are quite different from the vertebral body. All parameters (W_b, A_{ab}, S_{vb}, S_{vt}) are found to be more or less uniform in all sections from the anterior end to the posterior end of the vertebra in all animals. No specific relation is detected among beagles of different ages and sexes. For similar reasons as mentioned in section 3.2.3, all parameters of combined ages from 17 months to 11 years are summarized in Table 3.2.3.1.

3.4 Longitudinal sections of the ventral vertebra

The left half of the second lumbar vertebra from one beagle (FAC175) was chosen for this study. The anisotropic structure is present in the sagittal section where the trabeculae of all consecutive sections are parallel to one another in the antero-posterior direction of the vertebra. The three dimensional parameters cannot be estimated directly. However, the two dimensional mean trabecular width and dimensionless percent bone can be determined.

As shown in Table 3.4.1, mean trabecular width is very uniform throughout the entire section. The same bone density is found in the anterior and the posterior portions but it decreases significantly in the middle portion.

Table 3.2.6 Comparison of total volumes and total trabecular surface area in the ventral vertebra of individual beagles.

			Vertebral length (mm)	ΣV_b (mm^3)	ΣV_m (mm^3)	ΣV_t (mm^3)	ΣA (mm^2)
17 months	Male	MAC168	20.5	863	1980	2843	19393
		MAC169	19.2	636	1474	2110	15030
	Female	FAC172	19.8	760	1592	2352	17494
		FAC175	19.4	547	1365	1912	15877
3 years	Male	MAC178	20.3	818	1613	2431	18196
		MAC181	21.9	1028	2252	3280	24666
9-11 years	Male	MAC177	18.6	687	1507	2194	18092
		MAC179	19.1	645	1264	1909	13622
	Female	FAC180	18.1	617	1322	1939	14910
		FAC107	18.0	473	1098	1571	11443

Table 3.4.1 Comparisons of mean trabecular width (W_b) and percent bone (A_{ab}) between complete cross-section (X) and longitudinal section (L) in the same female beagle (FAC175, ventral portion). The vertebra is subdivided into three equidistant portions, the anterior, the middle, and the posterior.

	Anterior		Middle		Posterior		Total	
	X	L	X	L	X	L	X	L
Wb (microns)	79	96	84	95	80	98	81	96
A_{ab} (percent)	28	28	22	19	28	29	26	25

To compare the two different sectional planes in the same dog, the serial cross-section of the vertebra is subdivided into three portions as found in the longitudinal section. However, sections in the small epiphyseal regions are excluded because of the occurrence of marked changes of mean trabecular width and percent bone. It is found that, except for the middle portion where percent bone in the longitudinal section is slightly lower ($p < 0.05$) than the value in the cross-section, bone density is equal in the other corresponding portions of these two sectional planes. Thus on the assumption that the three dimensional trabecular width (T_b) of the same bone is the true width for any direction of sectioning:

$$T_b = W_b . k = W_{bl} . k_l \qquad (1)$$

where T_b = mean trabecular thickness (three dimensions)

W_b = average mean trabecular width (two dimensions) of total sections in cross-section

$k = \frac{4}{\pi}$ (correction factor for isotropic trabecular in cross-section)

W_{bl} = average mean trabecular width of total sections in longitudinal section

k_l = correction factor in longitudinal section

From the equation (1), the value of k_l is 1.5157. Thus specific bone surface and surface area per volume of tissue can be calculated and all parameters in both the cross- and longitudinal sections are compared and summarized in Table 3.4.2. It is noted that in the longitudinal section, no variation is detected among consecutive sections for all parameters.

Table 3.4.2 Summary of results for complete cross-sections and longitudinal sections in the same beagle (FAC175, ventral portion).

		T_b (microns)	A_{ab} (percent)	S_{vb} (mm^2/mm^3)	S_{vt} (mm^3/mm^3)
Cross-section	Anterior	62	28	32	9.0
	Middle	66	22	31	6.8
	Posterior	63	28	32	9.0
	Mean of total portions	64	26	32	8.3
Longitudinal section*	Anterior	63	28	32	8.7
	Middle	63	19	32	6.1
	Posterior	65	29	31	8.8
	Total	64	25	31	7.9

* k = 1.5157

3.5 Cross- and longitudinal sections of lumbar vertebra in young beagles

3.5.1 Serial cross-sections in three month beagle

It is not the purpose of this study to determine all details in young beagle bones and the results will be mentioned only briefly.

One male and one female were chosen to study the ventral portion of the first lumbar vertebra. The bone is small, approximately 10 mm in length. The trabeculae are isotropic, small trabeculae are found in the periphery of all portions, and some are scattered in the middle part of the portion where large trabeculae are located. The comparisons in male and female beagles are illustrated in Table 3.5.1. The table includes the results of the dorsal vertebra in which only large trabeculae are observed.

3.5.2 Longitudinal sections in three month beagle

Consecutive longitudinal sections were taken in the left half of the second lumbar vertebra in one dog. Portions are clearly distinguished and the trabeculae appear to be isotropic. The results are summarized in Table 3.5.2.

Further information for the three month old dog may be seen in the Appendix E.

3.6 Cross-sections of the proximal femur

3.6.1 Trabecula bone area (A_b)

As previously mentioned in Materials and Methods, the proximal femur is divided into the head and non-head portions. Measurements of

Table 3.5.1 Summary of results for complete cross-sections of first lumbar vertebra in a three month old beagle. Within ventral portion (MAC171, FAC174) 'L' and 'S' stand for large and small trabeculae, respectively, and within dorsal portion (MAC165, MAC171, FAC173, FAC174) only large trabeculae are present.

Parameter (Portion)			Ventral Portion: Anterior plate	Ventral Portion: Centrum	Ventral Portion: Neural arch	Ventral Portion: Posterior plate	Ventral Portion: Portions combined	Dorsal portion
W_b (microns)	Male	L	78	67	68	86	72	81
		S	50	33	36	48	39	
	Female	L	75	76	67	81	73	79
		S	42	31	32	54	37	
A_{ab} (percent)	Male	L	31	23	21	40	26	28
		S	39	28	24	38	29	
	Female	L	30	23	20	32	24	29
		S	38	24	24	43	29	
S_{vb} (mm^2/mm^3)	Male	L	33	39	38	30	36	32
		S	52	76	71	53	68	
	Female	L	34	34	38	32	35	32
		S	64	84	81	48	74	
S_{vt} (mm^2/mm^3)	Male	L	10.1	8.8	7.7	11.9	9.0	8.8
		S	19.4	21.0	16.6	20.3	19.0	
	Female	L	10.1	7.7	7.8	10.2	8.5	9.2
		S	22.7	19.4	18.9	19.9	19.8	

Table 3.5.2 Summary of results for longitudinal sections within ventral portion of second lumbar vertebra in a three month old female beagle (FAC174). 'L' and 'S' stand for large and small trabeculae, respectively.

Parameter \ Portion		Anterior plate	Centrum	Posterior plate	Portions Combined
W_b (microns)	T	77	72	79	76
	S	47	32	47	42
A_{ab} (percent)	T	28	19	28	25
	S	40	20	40	33
S_{vb} (mm^2/mm^3)	T	33	36	32	34
	S	55	79	55	63
S_{vt} (mm^2/mm^3)	T	9.3	6.7	8.9	8.3
	S	21.8	15.6	21.7	19.7

the trabecular area on serial cross-sections are plotted in Figure 3.6.1.

In all portions bone area starts from zero at the proximal end of the bone and then increases slowly for the first few sections. The first section can be the head or non-head portion depending on the variability of the bone which portion is higher when the shaft of the femur is perpendicular to the ground. Area of bone increases abruptly and has the pronounced maximum about the end of the first half of the head portion where the proximal femur has the greatest width. The bone area then decreases sharply, however in the head portion of bone of the last two or three sections decreases gradually and disappears whereas bone in non-head portion after this point slows down losing trabeculae and this continues into the shaft region.

In comparison of the head and non-head portions, the ending of the head portion is used as a landmark. Approximately five sampling sections are selected: two sections which have the smallest bone area from both ends, one (or two if uncertain) which has the largest bone area from the middle part, and the other two equidistant between the middle and end sections. From these samples total volume and total surface area can be determined.

3.6.2 Percent bone (A_{ab})

Typical curves of relative bone area in the head and non-head portions of the proximal femur (17-month female) are shown in Figure 3.6.2.

From the figure, percent bone in the head region does not have marked change from end to end, whereas in the non-head portion percent

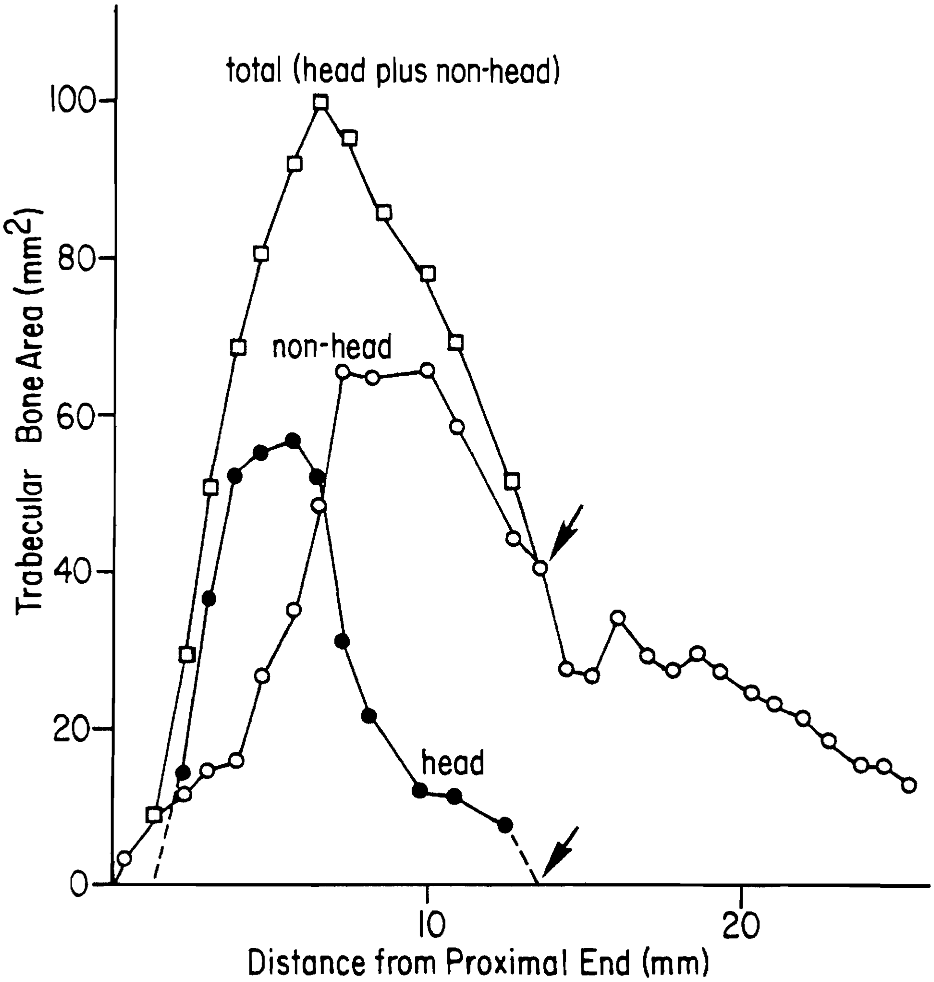


Figure 3.6.1 Area in square millimeters of trabecular bone in consecutive cross-sections of the proximal femur starting from the proximal end. The curves given here are from a 17-month old beagle (FAC175). Arrows indicate the end of the head portion.

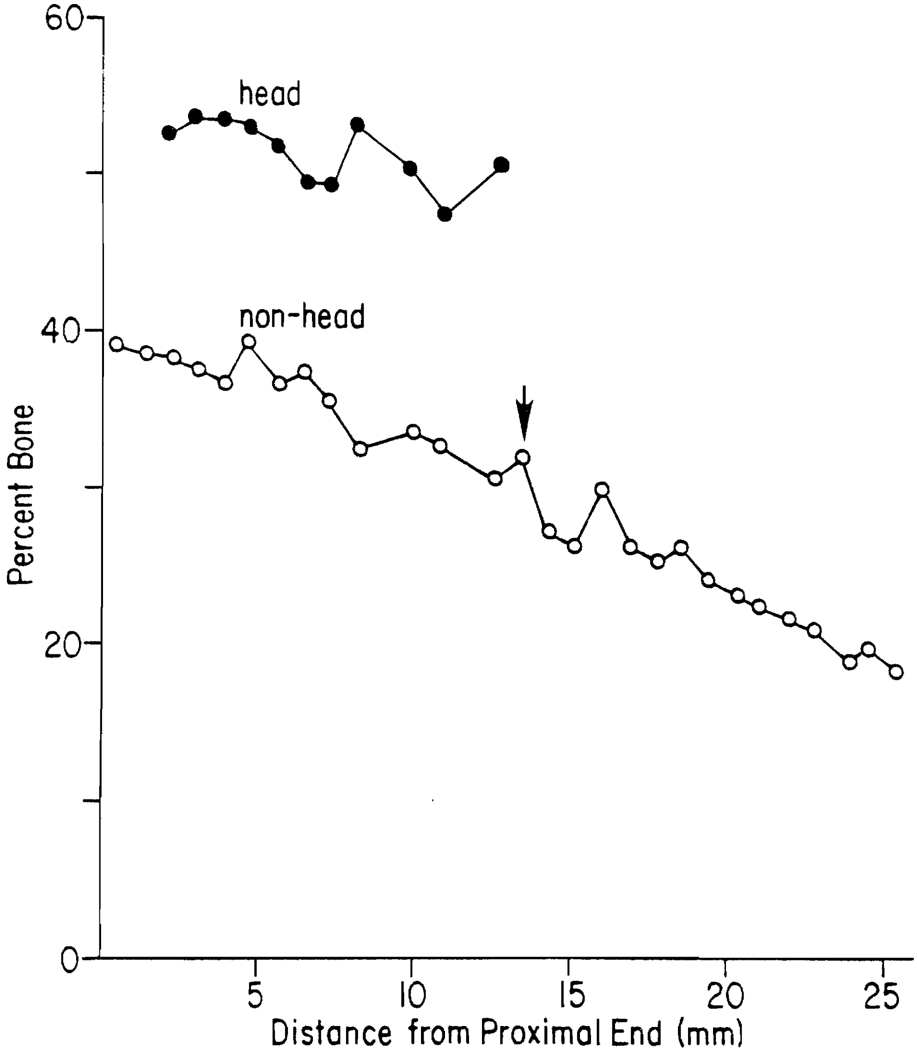


Figure 3.6.2 Variation of percent bone (A_{ab}) in head and non-head portions on different sections (cross-section of proximal femur, FAC175). Arrow indicates the end of the head portion.

bone decreases gradually from the proximal end down into the shaft. In all dogs investigated percent bone is higher in the head portion than in the non-head portion.

3.6.3 Mean trabecular width (W_b)

Mean trabecular width is greater in the head portion than in the non-head portion. In the head region the proximal half has greater width than in the distal half, whereas uniformity of the width is generally found in the non-head portion. It is noted that beyond the point where the head portion ends, trabecular width decreases gradually down into the shaft.

3.6.4 Specific bone surface (S_{vb}) and surface area per tissue volume (S_{vt})

Specific bone surface is proportional to the reciprocal of mean trabecular width, thus it has lower values in the head portion than in the non-head portion. In the head portion it has lower values in the proximal half than in the distal half, while in the non-head portion no marked change of specific bone surface is observed. Curves illustrating specific bone surface and surface area per tissue volume are shown in Figure 3.6.4.

Values of bone surface per tissue volume are about the same in both portions of the proximal femur. However the features are different. In the head region surface area per tissue volume is slightly higher at both ends while it gradually decreases from the proximal end of the non-head portion down into the shaft of bone.

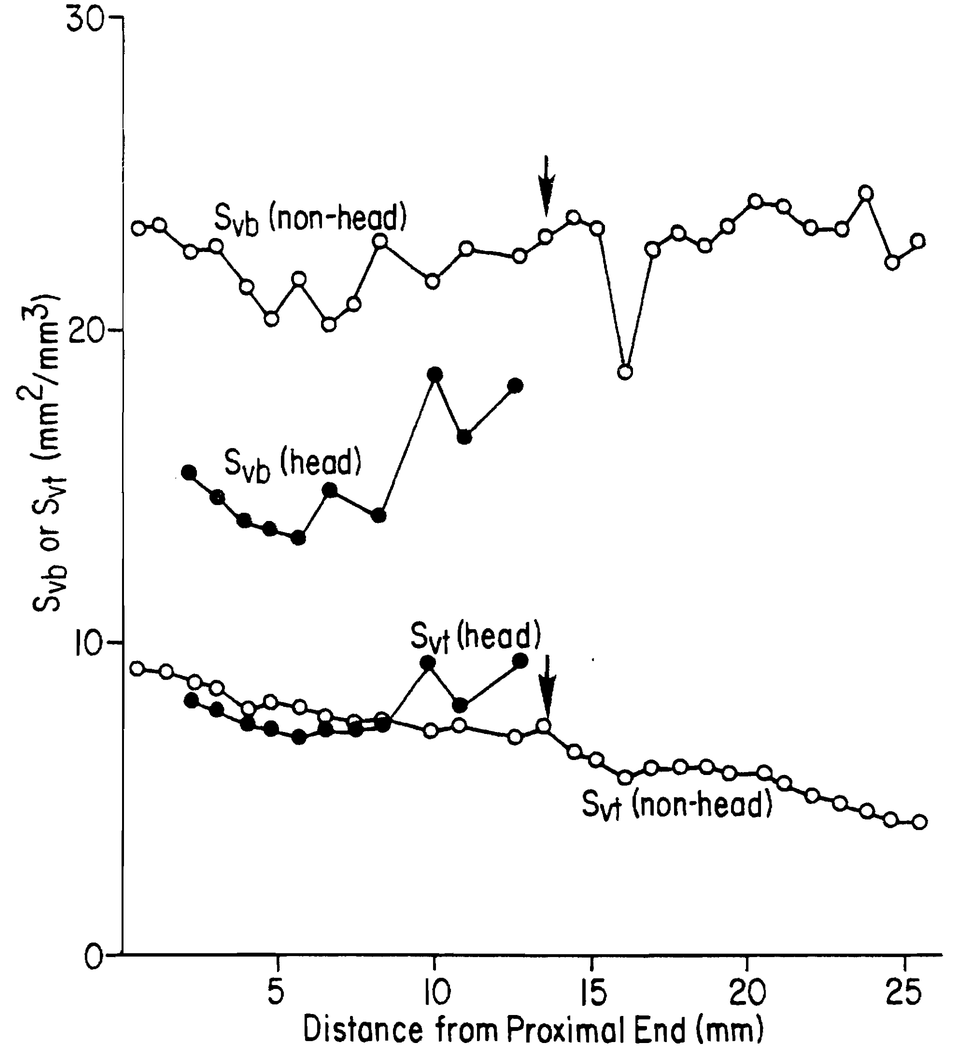


Figure 3.6.4 Variation of specific bone surface (S_{vb}) and surface area per tissue volume (S_{vt}) in head and non-head portions on different sections (cross-section of proximal femur, FAC175). Arrows indicate the end of head portion.

3.6.5 Effect of age and sex

All parameters of the serial cross-sections in 17-month beagle as mentioned above have the same features as found in the serial cross-sections of one 11-year old female and the sampling sections of the others. However a large number of variations were found in the proximal femur. For example, percent bone, mean trabecular width, specific bone surface, and surface area per tissue volume have marked differences between dogs of the same age and sex. This is critical because it is probably less meaningful to combine dogs of one age group and compare them with combined dogs in the other ages. The dimensions of the head portion are not the same in the same age beagle, especially in one dog (MAC169) where the length is about half of that of the other dog (MAC168). In one beagle (MAC177), it was found that the epiphyseal line which indicates the boundary between the head and non-head portions cannot be detected and this dog was excluded from the test. Although there are statistically significant differences among ages and sexes, for the reasons indicated it is probably more meaningful to combine all ages for comparison between males and females and these are shown in Table 3.6.5.

3.6.6 Total volume and total trabecular surface area

Total volumes of trabecular bone (ΣV_b), marrow (ΣV_m), and tissue (ΣV_t) and total trabecular surface area (ΣA) of individual beagles are given in Table 3.6.6.

3.7 Measurements of longitudinal sections of the proximal femur

One 17-month female beagle was chosen. The head portion of the proximal femur, where the portion ended is clearly seen, and the

Table 3.6.5 Summary of results for measurements of cross-sections of the proximal femur in combined ages from 17 months to 11 years.

		W_b (microns)	A_{ab} (percent)	S_{vb} (mm^2/mm^3)	S_{vt} (mm^2/mm^3)
Male	head	170	54	15	8.1
	non-head	128	37	20	7.6
Female	head	179	55	15	7.9
	non-head	129	37	20	7.4

Table 3.6.6 Comparisons of total volume and total trabecular surface area in the proximal femur of individual beagles. The first value is the head portion followed by the non-head portion in parentheses. Length is from the proximal end to the point where the head portion ends.

			Length (mm)	ΣV_b (mm^3)	ΣV_m (mm^3)	ΣV_t (mm^3)	ΣA (mm^3)
17 months	Male	MAC168	18.2	613(868)	493(1643)	1106(2511)	8050(17922)
		MAC169	10.9	324(470)	278(811)	602(1281)	3880(9200)
	Female	FAC172	15.2	376(550)	445(1092)	821(1642)	5642(11510)
		FAC175	12.6	325(484)	313(920)	638(1404)	4768(10583)
3 years	Male	MAC178	14.6	456(601)	470(1011)	926(1612)	7882(11939)
		MAC181	14.5	380(678)	344(1383)	724(2061)	5683(14834)
9-11 years	Male	Mac179	13.0	272(529)	161(717)	433(1246)	2808(8458)
	Female	FAC180	12.6	241(537)	139(788)	380(1325)	2572(9514)
		FAC107	13.2	329(481)	211(846)	540(1327)	4090(8899)

trabeculae are more or less isotropic, is to be considered. It is found that no marked variations among consecutive sections are detected in all parameters (W_b, A_{ab}, S_{vb}, S_{vt}). The values are shown in comparison with the serial cross-sections of the same dogs in Table 3.7.

No statistically significant difference is found between the two sectional planes for trabecular width, specific bone surface, and surface area per tissue volume. A statistically significant difference however is detected ($p < 0.05$) in percent bone, which is slightly higher in cross-section (52) than in longitudinal section (49).

3.8 Cross-sectional measurements of the proximal femur in three month beagles

One male and one female were chosen and the trabeculae in both dogs are isotropic. The larger trabeculae are located in the main part of the portions, whereas the smaller trabeculae are found in the periphery. In the middle portion of the male beagle only small trabeculae are detected. Comparisons of portions are shown in Table 3.8.

3.9 Longitudinal sections of the proximal femur in a three month old beagle

The head portion of one beagle was used. Isotropic trabeculae are present in all consecutive sections. The results compared with the cross-section of the same dog are summarized in Table 3.9.

Table 3.7 Summary of results for complete cross-section (right) and longitudinal section (left) in the head portion of proximal femur of the same 17-month old female beagle (FAC175).

	W_b (microns)	A_{ab} (percent)	S_{vb} (mm^2/mm^3)	S_{vt} (mm^2/mm^3)
Cross-section	169	52	15	7.9
Longitudinal section	159	49	16	7.9

Table 3.8 Summary of the results of measurements for cross-sections of the proximal femur in three month old beagles (MAC171 sampling sections, FAC173 complete sections). 'L' and 'S' stand for large and small trabeculae, respectively.

			Head portion	Middle portion	Lateral portion	Portions combined
W_b (microns)	Male	L	72	--	77	74
		S	37		37	42
	Female	L	85	62	63	73
		S	41	33	30	36
A_{ab} (percent)	Male	L	25	--	42	33
		S	30	36	36	33
	Female	L	35	39	35	36
		S	38	33	30	35
S_{vb} (mm^2/mm^3)	Male	L	36	--	34	35
		S	68	60	70	65
	Female	L	30	42	41	36
		S	65	79	86	74
S_{vt} (mm^2/mm^3)	Male	L	9.0	--	14.3	11.6
		S	20.9	20.1	23.2	21.1
	Female	L	10.5	16.4	14.6	13.2
		S	23.9	26.0	25.7	25.0

Table 3.9 Summary of the results of measurements of cross- and longitudinal sections in the head portion of proximal femur in the same three month old beagle (FAC173).

		W_b (microns)	A_{ab} (percent)	S_{vb} (mm^2/mm^3)	S_{vt} (mm^2/mm^3)
Cross-section	L	85	35	30	10.0
	S	41	38	65	23.9
Longitudinal section	L	81	28	32	9.0
	S	40	35	65	21.8

4. DISCUSSION

4.1 New techniques

4.1.1 Control of X-ray by step wedge

It is clearly seen that a number of variations can cause errors in making direct measurements on microradiographs using an image analyzing computer. A step wedge works as a control to check for variations during X-raying and/or the film developing process. For example, if the optical density of the step wedge is lower than the standard value (0.80 ± 0.05), values of the direct measurements will be higher than normal. Actually, any number of layers of aluminum foil from one to five may be used as long as the optical density is standardized.

4.1.2 Thickness of ground bone section

The thickness of the ground section from 120 to 70 microns gives no significant change in measurements as expected by the Holmes effect (5,19). This may be explained by the following reasons. First, the lumbar vertebra under investigation is from the cross-section where the parallel trabeculae are detected in the antero-posterior direction, trabecular surfaces are then more or less perpendicular to the sectional plane. Secondly, the mean trabecular width is fairly big when compared with the section thickness thus the angle between trabecular surface and the plane of cutting has a little effect on this. For future work it is suggested that the cross-section within the head region of the femur should be investigated in comparison with the current study. It is interesting to determine the upper and lower limits of the ground bone sections which can be used for morphometric measurements of microradiographs.

4.1.3 Serial sections

The serial sections apparently give full details of bone and the variation of measurements can be detected from one end to the other end. The accuracy of the sampling sections is then given and will be discussed in the later section.

4.2 New approach of selecting samples

4.2.1 Lumbar vertebra

In cross-section of lumbar vertebrae the complete information may be given by selecting two or three sections. The first section should be the first complete section in the epiphysis where the lowest specific bone surface, the highest percent bone, and the greatest trabecular area are detected. The second section should be from the middle section in the 'A' region (see Table 3.2.3.2) where the lowest percent bone and trabecular area are observed. It is recommended to have a third section equidistance between the 'A' and 'B' regions.

With longitudinal sectioning any sample may represent the whole bone in most parameters (W_b, A_{ab}, S_{vb}, S_{vt}) because no significant changes are detected among all consecutive sections. However, it requires a few more sections near the lateral end of bone to construct total volume and trabecular surface area. The drawback in longitudinal sectioning will be discussed later.

4.2.2 Proximal femur

In cross-section three to four samples may be adequate to have complete information about the proximal femur down to the end of the head region. Inspection of Figrues 3.6.1, 3.6.2 and 3.6.4 shows that the first sample is from the first section from the proximal end where the small bone areas are detected in both the head and non-head portions, and the highest percent bone is found in the non-head portion. The second sample is the last section where the smallest bone area and highest specific bone surface are detected in the head region. The third and/or fourth samples are from the middle where the greatest bone areas and probably the lowest specific bone surface are observed in both the head and non-head portions.

In contrast, no significant changes of parameters (W_b, A_{ab}, S_{vb}, S_{vt}) are found among all consecutive longitudinal sections within the head region of the femur, thus one sample from any part may represent the whole portion. However a few more sections from different parts are required to construct the total volume and total trabecular surface area.

4.3 Cross-sections vs. longitudinal sections

In most cases the reconstruction of the whole portion of bone, where total volume and trabecular surface area are calculated, is probably not needed. In lumbar vertebrae, for example, one longitudinal section from any part has enough information to observe changes in parameters from the anterior to the posterior ends of bone. However, the critical determination is based on the three dimensional values which cannot directly be given due to the anisotropic structures of trabeculae. Values of the correction factor (k) are expected to change from one section to the other, and furthermore the variation of k is anticipated among different portions or parts within the same section. Woodbury et al (21) calculated k value of 1.073 whereas k is 1.5157 in the current study. In the present study mean trabecular width is larger in longitudinal sections indicating either that an oblique cut was made or that the second lumbar vertebra is different from the first lumbar vertebra.

In the cross-sections of the proximal femur the isotropic structures are found in the head and non-head portions, thus the universal value of k (1.273...) can be applied to calculate the three dimensional parameters. However, in the longitudinal sections anisotropic trabeculae are detected in the non-head portion and k value is unknown.

With several questions remaining to be answered for the longitudinal section study, it is probably best to work on cross-sections where isotropic structures are observed.

4.4 Changes of parameters

4.4.1 Changes of parameters within bone portions

In the ventral portion of lumbar vertebra the end portions have greater trabecular and tissue areas, bigger trabecular width and higher percent bone and these values decrease toward the middle part of the main vertebral body where they have a minimum. This might be the function of bone in response to stresses at the junction with the other vertebra, stresses which are not effective at the middle. In the dorsal vertebra where larger mean trabecular width and higher percent bone are detected, the greater strength and better protection are presumed; the thicker cortex and greater number of muscle attachments in this portion help to support this idea.

In the proximal femur the head portion has greater trabecular width and higher percent bone than the non-head portion, and therefore the greater strength in response to stresses is presumed within the head region where contact between bone and acetabulum of the pelvic girdle is present.

4.4.2 Changes of parameters on age and sex

Bone is rapidly developing in the three month old beagles and could not be compared with the adults. No specific differences in the measurements were detected in adult beagles from 17 months to 11 years. No osteoporosis is observed and thus the dog age equivalent to human cannot be justified in bone measurements (see Table 4.4.2). Significant bone loss was found in 16 year old beagles (7).

4.5 Comparison with others

Results from the current study are compared with the previous work as shown in Table 4.5.1 for the lumbar vertebra and Table 4.5.2 for the proximal femur.

Table 4.4.2 Equivalent age of dog and human by Lebeau (13)

Dog age	Human age
17 months	18 years
3 years	28 years
9 years	51 years
11 years	60 years
16 years	80 years

Table 4.5.1 Comparison of the lumbar vertebra.

	Longitudinal (sagittal) sections					Cross-sections	
	Current study 17 months Female	Jee et al (7) 17 months Male	Jee et al (7) 17 months Female	Beddoe (2)	Lloyd and Hodges (8) Male	Current study* Combined ages Combined sexes	Woodbury et al (21) 17 months
W_b (microns)	96	94	96	--	--	98	96
A_{ab} (percent)	25	29	30	37	35	28	29
S_{vb} (mm^2/mm^3)	31**	28** 20***	30** 22***	19	23	26	27

*Values are from the main vertebral body.

**k = 1.5157

***k = 1.073

Table 4.5.2 Comparison of the proximal femur.

	Current study Sagittal section within the head portion (17 months, female)	Beddoe (2) Oblique sagittal section within head and neck
W_b (microns)	159	--
A_{ab} (percent)	49	54
S_{vb} (mm^2/mm^3)	16	13

5. APPENDIX A

USE OF THE QUANTIMET-720 FOR BONE MORPHOMETRIC MEASUREMENTS

5.1 Optical magnification

Bone sections are restricted to microradiographs of comparatively thick sections (ca. 100 microns). The specimens are much larger than the field of view of the microscope. The optical magnification is small, a 1.0 X objective is used with a 1.25 X ocular for a total effective magnification of 1.25 X. There is, however, 20 X of electronic magnification so that the bone section appears on the television screen with 25 X magnification.

5.2 Plumbicon system control

Plumbicon light integration is set at X1. In the manual mode, the light sensitivity is adjusted to 0.3 when the sensitivity is 'on' and to 1.0 when white level is 'on'.

5.3 Shade correction

Shade correction is required to obtain uniform detection of the specimen in the field of view. A transparent slide is used at the same distance as the microradiograph to the microscope objective. A distance of two millimeters is used to obtain a sharp image on the screen.

5.4 Display unit

Scale and figures, guard, and computer and amended are 'on' and nearly full clockwise. Image detected and brightness are 'on' and set at the proper position to get both a sharp image and good contrast.

5.5 Variable frame and scale

Frame position and frame size are adjusted as required. Position of the picture is also adjusted with the image rotation and X-Y stage micrometer.

5.6 1D Auto detector

The Auto detector is set to detect 'white' on field standard where it is found that the measurements are consistent and faster than the manual one.

5.7 MS3 computer

The MS3 computer can be set manually or automatically to detect area, perimeter, and intercept. It is important that the sizer is 'off'.

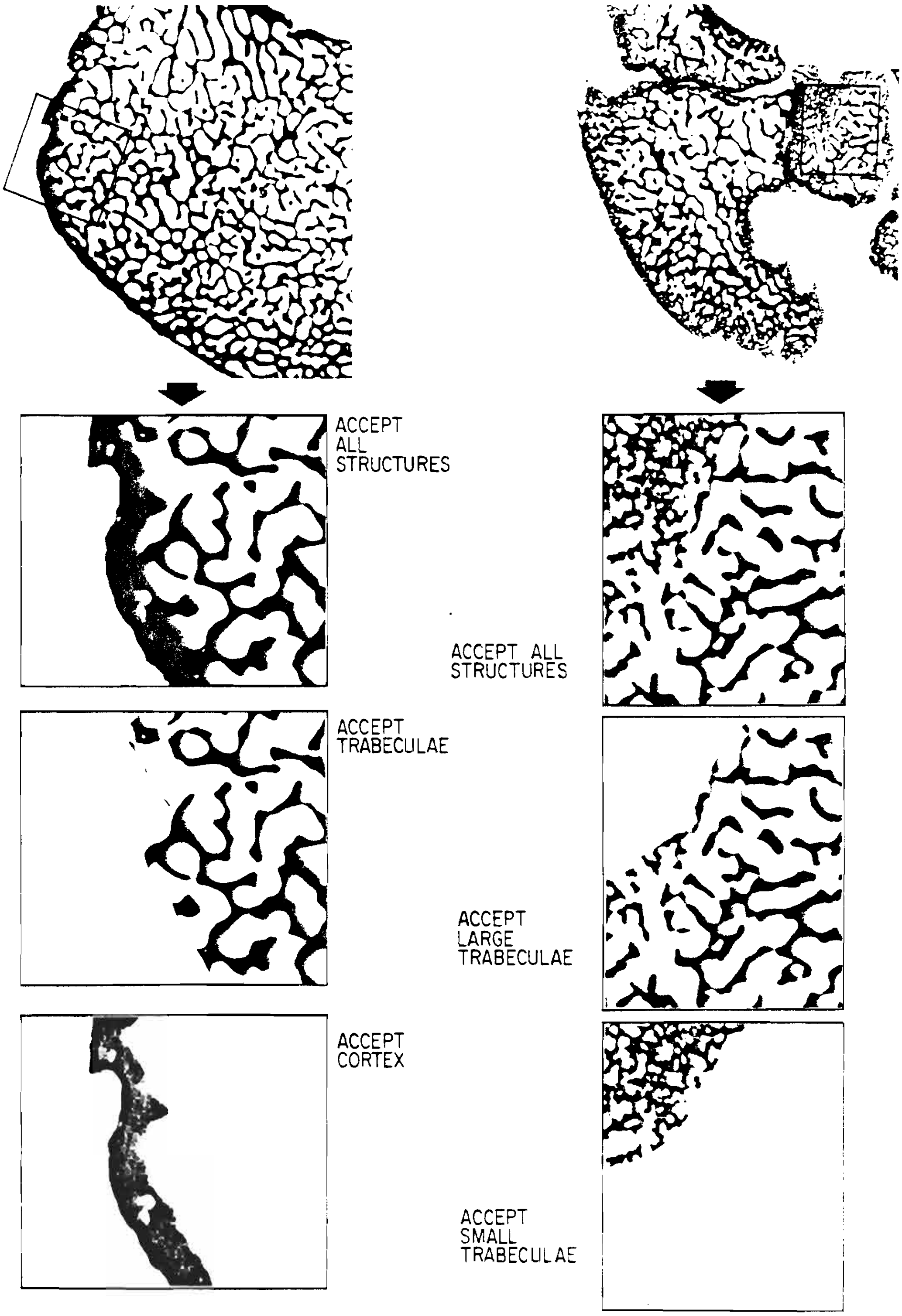
5.8 Programmer and Field Data Interface

The specific program is set on the auto programmer board to make direct measurements. Readings of direct measurements are automatically accepted by the field data interface and passed to the Hewlett Packard Model 10 calculator where they are converted to mm units and printed out.

5.9 Image editor pen

Image editor pen is a useful tool to accept or reject any part of the picture on the television screen for measurements. Two different structures can be separated from each other; for example, trabeculae from cortex, small trabeculae from large trabeculae, and the absolute parameters can be determined. The examples are shown in Figure 5.9.

Figure 5.9 Examples to illustrate how the image editor pen can accept or reject certain structures. The bottom six pictures (20X) are the magnification from the inset (7X) of the corresponding top pictures. The left column is the adult ventral vertebra, and the right column is the three month ventral vertebra.


ACCEPT ALL STRUCTURES
ACCEPT TRABECULAE
ACCEPT CORTEX
ACCEPT ALL STRUCTURES
ACCEPT LARGE TRABECULAE
ACCEPT SMALL TRABECULAE

6. APPENDIX B

MICRORADIOGRAPHS: EXPOSURE TIME AND DENSITY

6.1 Exposure time

To obtain the optimum exposure time, the same 100 micron ground section from a cross-section of a lumbar vertebra was X-rayed at different exposure times of 1/2,1,2,3,6,9,12,15 and 18 mintues. However, for exposure times of 1/2 minute and 18 minutes, the microradiographs produced picutres on the Quantimet television screen which could not be detected because they were under or over exposed. Trabecular bone area, perimeter and intercept on the same frame size, location and orientation were measured in picture points (pixels). Curves for these parameters are shown in Figures 6.1.1, 6.1.2 and 6.1.3.

From inspection of the figures the highest and the lowest readable values are averaged to get reasonable exposure times of 5.5, 5.0 and 4.9 minutes for area, perimeter and intercept, respectively. For convenience of X-raying it is preferable to use six minutes as an appropriate exposure time and this gives microradiographs of good contrast on the Quantimet screen.

6.2 Density

A step wedge for calibration purpose was made from standard aluminum foil (Reynolds Wrap) of approximately 100 microns thick. It was made by overlapping five layers to form a series of steps. A step wedge was glued at the center of the mylar of the X-ray holder and X-rayed at an exposure time of six minutes. The measurements of optical

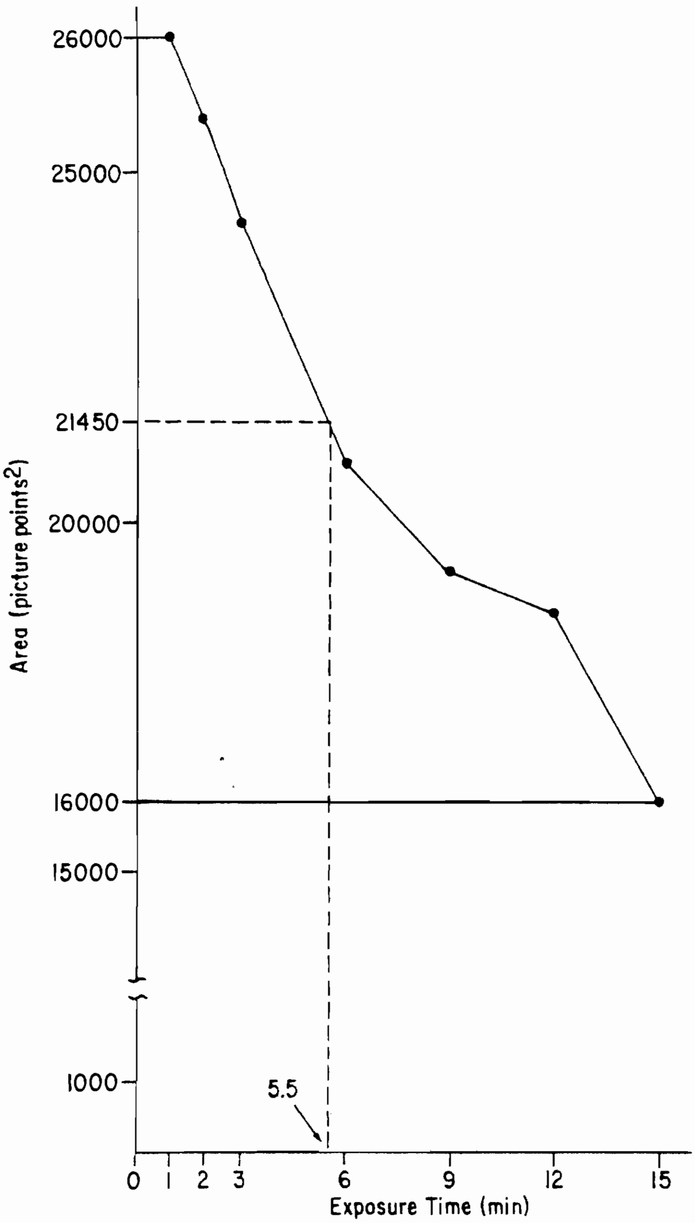


Figure 6.1.1 Variations of trabecular bone area for different exposure times.

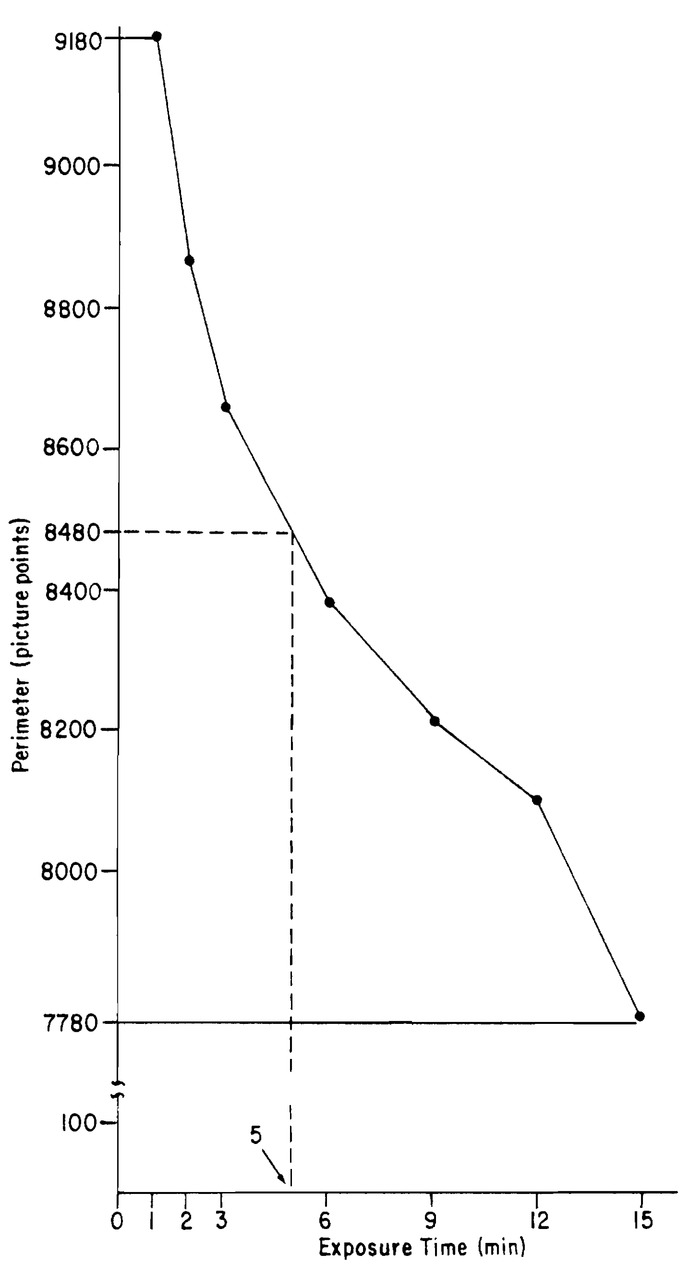


Figure 6.1.2 Variations of trabecular bone perimeter for different exposure time.

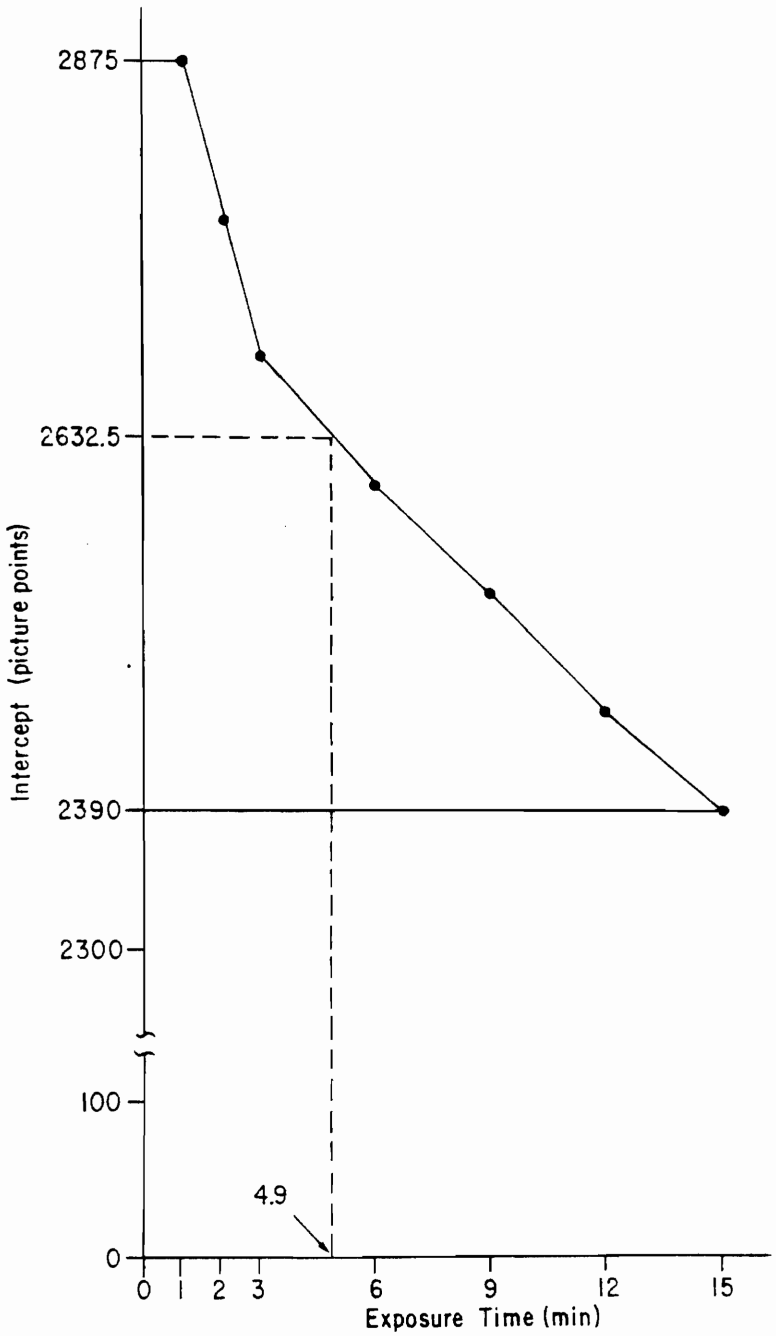


Figure 6.1.3 Variations of trabecular bone intercept for different exposure time.

density were made on one through five layers of the step wedge by a McBeth densitometer (set at the low beam light). Three layers of aluminum foil had an average density of 0.80 and this was used as a control. Variations of optical density are shown in Figure 6.2.

6.3 Final protocol for X-rays of 100 micron bone section*

6.3.1 Spectroscopic plates

Plates should be at room temperature before use. After they are taken from the freezer, leave them in the dark overnight or at least six hours before use. After this initial use, the remaining plates can be stored in the refrigerator provided they are all used within a few days. In this case, leave the box of plates in the darkroom for only two hours before X-raying. This is adequate time for the plates to reach room temperature. If no more X-raying is to be done for a week or longer, the plates should be placed in the freezer.

6.3.2 X-ray unit

The X-ray unit should be turned on approximately 10 to 15 minutes or longer before operating. During operation, if there is fluctuation of current and/or voltage and this does not stabilize within 30 seconds, the plate should be discarded. Meters should be checked frequently during exposure since the fluctuation may occur at any time.

6.3.3. Reagents

After mixing Kodak Developer D19, it should be used between 3.5

*Based on KODAK spectroscopic plate size 3 1/4 x 3 3/4 in.

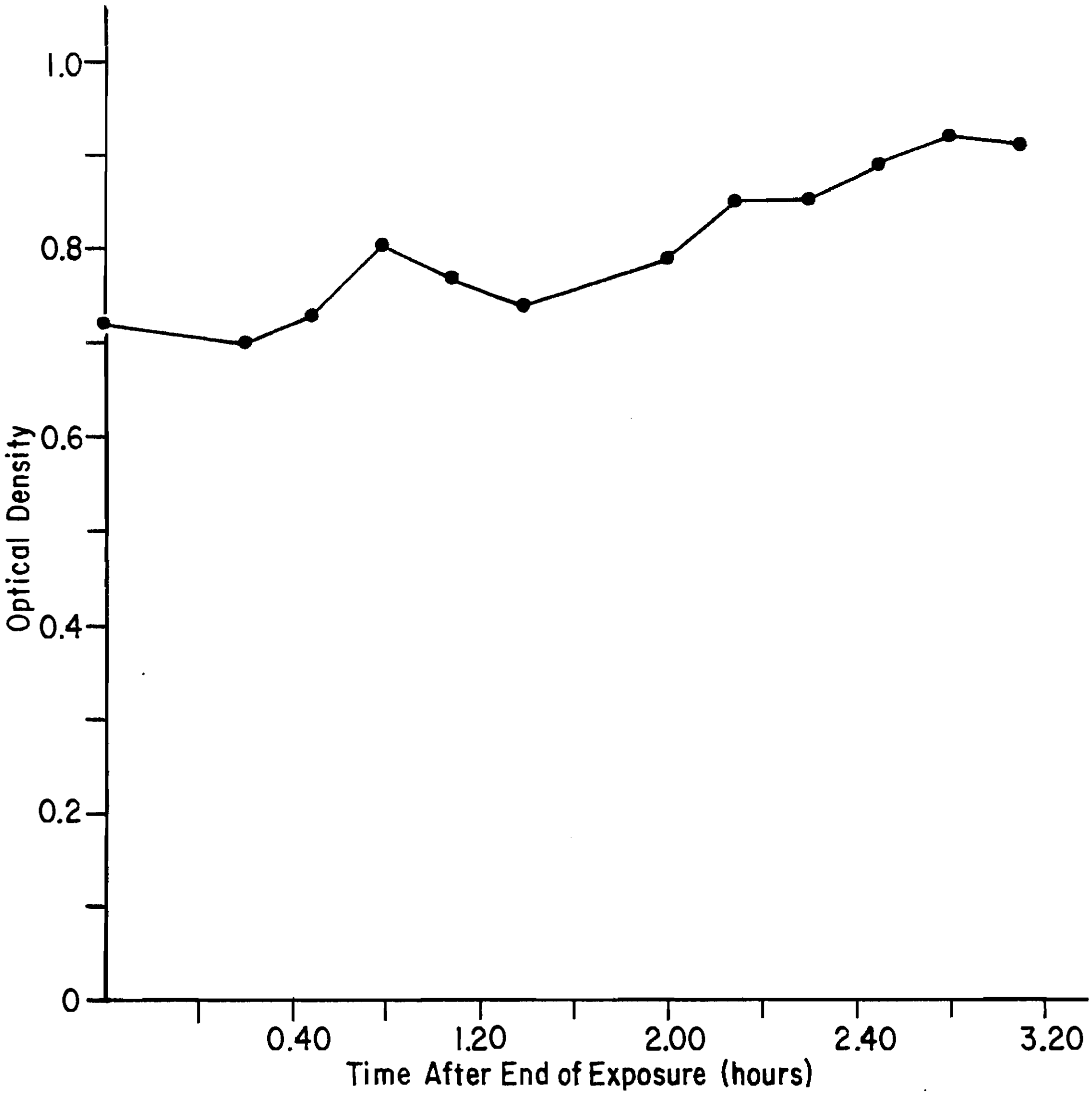


Figure 6.2 Variations of optical density of a three layer step wedge at six minutes exposure. On the x-axis is the time after the microradiograph of the first slide was developed. Fresh developer and fresh fixer were used and slides were developed at 24 minutes after the last slide was taken.

to 8 hours and may be used not more than three times. The Fixer can be stored for weeks after mixing and may be used up to four times.

6.3.4 Developing and fixing

Temperature is critical for D19 Developer, i.e., 20°C must be maintained during developing time. Solution should be put in a water-bath which is already set at 20°C. Measure the temperature of the D19, then start developing within 1 to 4 minutes (time needed for putting slides into tray). After the clock starts, not more than 5 seconds should elapse before slides are placed in D19.

Agitation is required for optimum contact between grains and D19 and it is necessary to agitate every 45 seconds, i.e., 4 times for a 4 minute developing time. Agitation frequency and intensity is critical. There should be a moderate speed of movement of the slide tray starting from the center of the container to right side-to left side-to right side-to left side-then back to the center.

When the development time has elapsed, place the slide tray into the waterbath (do not agitate) and then into the fixer. It will take about 6 seconds to complete this transfer.

Immediately move the tray from side to side as mentioned above for one cycle only, then cover the fixer with aluminum foil. Total time in the fixer is 10 minutes and the temperature should be 20 ± 0.5°C. The slides are rinsed in running tap water for at least two hours, then dried.

After processing, the temperature of the D19 should be checked as soon as possible to confirm that it remained constant at 20°C.

6.3.5 Miscellaneous

All slides should be developed between 10 to 30 minutes after the last slide is X-rayed. The total time from the first one taken to the last one is important and is recommended to be completed in approximately two hours, thus the total time prior to developing is approximately 2.5 hours and it should not be longer than three hours.

It is recommended to develop the full tray (14 slides) for fresh D19 and any number for used D19.

Before temperature is measured, solution should be stirred moderately. Always use the same thermometer to check temperature.

The bone section should be placed within an approximately 1.5 cm radius from the center of the mylar support, which is within a uniform X-ray beam exposure.

At least one step wedge should be used in every tray and every new slide box to confirm the uniformity of the X-ray exposure and the processing technique. For example, three layers of aluminum foil at six minutes of exposure should produce a microradiograph with an optical density of approximately 0.80 ± 0.05 (McBeth Comp. set at low light).

It is recommended to use the same step wedge for the entire experiment because different step wedges may have different optical densities unless they are pre-tested and determined to be the same quality.

If the X-ray tube is replaced, it must be calibrated to get the new appropriate exposure time to maintain the optical density of the control step wedge at 0.80 ± 0.05.

7. APPENDIX C

EFFECT OF THICKNESS OF THE GROUND BONE SECTION ON MEASUREMENTS

One cross-section for the epiphyseal region of a lumbar vertebra of FAC107 was ground serially and X-rayed. The microradiographs were measured on the Quantimet using the same frame size, location, and orientation. The comparisons of trabecular bone area, perimeter and intercept for different thickness are shown below:

	Thickness of ground sections in microns (All measurement units are in picture points*)					
	120	105	100	90	80	70
Area	83950	82915	83964	83883	85225	83545
Perimeter	17056	17114	17366	17336	17349	18042
Intercept	4930	4938	5014	5027	4997	5180

*One picture point is 0.0143 millimeter.

8. APPENDIX D

MEASUREMENTS AND ANALYSES OF THE FIRST LUMBAR VERTEBRA IN 17 MONTH TO 11 YEAR OLD BEAGLES

8.1 Serial cross-sections

The measurements in the serial cross-sections of one 17 month and one 11 year old female beagle are compared in Table 8.1.

8.2 Cross-sections within the main vertebral body

Summary of data and results of the analysis by age and sex of all measured cross-sections within the main vertebral body is shown in Tables 8.2.1 and 8.2.2 for mean trabecular width and specific bone surface, respectively. Percent bone and surface area per volume of tissue are not shown because no statistical differences are detected by age and sex.

8.3 Cross-sections of the ventral vertebral cortex

The cortex in the ventral portion of the vertebra is fairly thin. The comparative results of percent cortex are summarized in Table 8.3.

8.4 Cross-sections of the dorsal vertebra

Summary of data and results of the analysis of mean trabecular width, percent bone, specific bone surface, and surface area per volume of tissue are shown in Tables 8.4.1 through 8.4.4.

The cortex in the dorsal vertebra is thicker than in the ventral portion. Percent bone cortex of the dorsal vertebra is combined in Table 8.4.5.

8.5 Longitudinal sections of the ventral vertebra

One second lumbar vertebra of the 17-month female beagle is selected. Three equidistant portions (anterior, middle, posterior) are measured separately and the results are shown in Table 8.5.

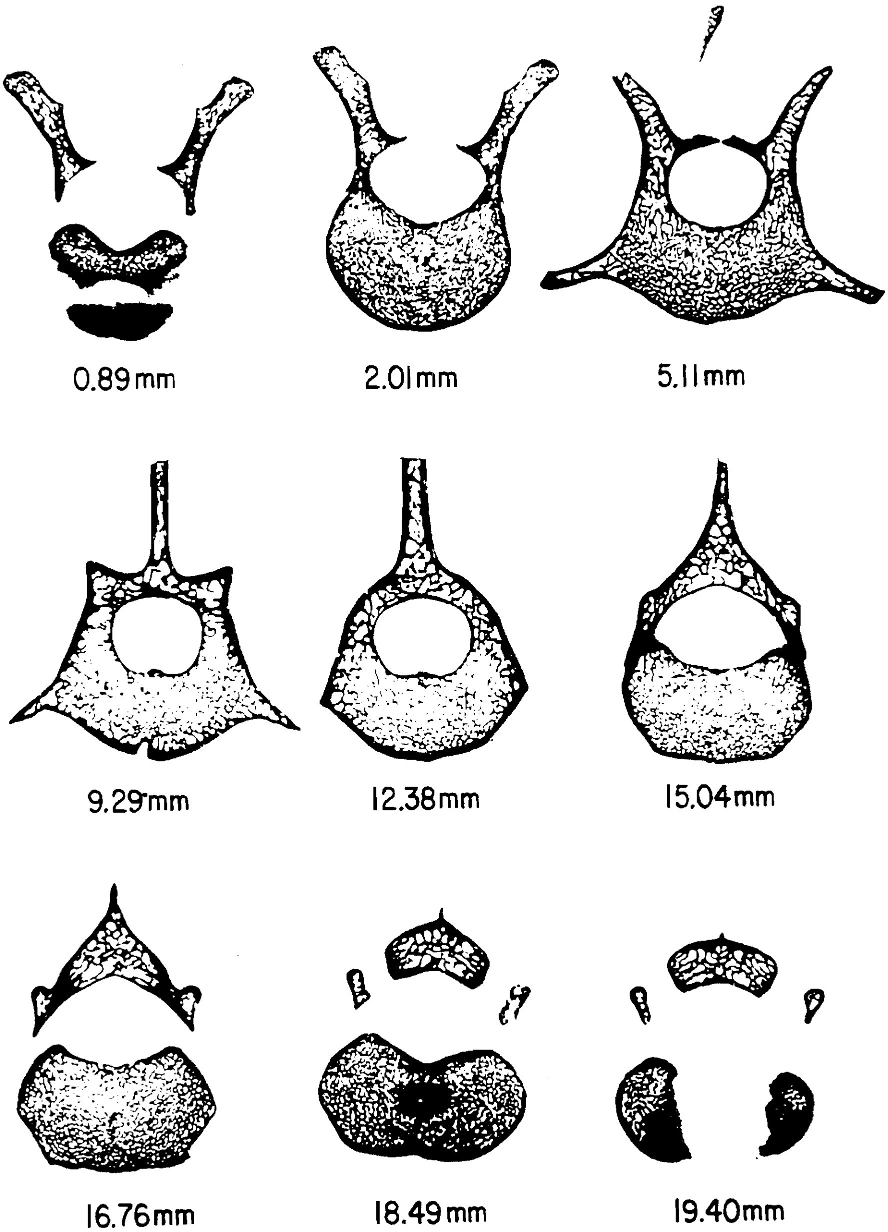


Figure 8.1 Representative cross-sections of the first lumbar vertebra (FAC175). The figures under the sections are distance in millimeters from the anterior end (1.6X).

Table 8.1 Comparison of the serial cross-sections within the ventral portion of the first lumbar vertebra in 17 month (FAC175) and 11 year (FAC107) old female beagles. Distance starts from the anterior end to the posterior end of the vertebra.

Distance (mm)	A_b (mm^2)		W_b (microns)		A_{ab} (percent)		S_{vb} (mm^2/mm^3)		S_{vt} (mm^2/mm^3)	
	17 mos.	11 yrs.	17 mos.	11 yrs.	17 mos.	11 yrs.	17 mos.	11 yrs.	17 mos.	11 yrs.
0-0.99	7		88		41		29		11.9	
1-1.99	--	12	--	122	--	55	--	21	----	11.5
2-2.99	53	34	122	138	42	47	21	18	8.8	8.7
3-3.99	40	41	79	111	32	36	32	23	10.3	8.2
4-4.99	35	35	76	99	28	29	34	26	9.4	7.5
5-5.99	35	31	81	94	27	25	32	27	8.5	6.9
6-6.99	32	28	81	95	25	24	31	27	7.9	6.4
7-7.99	--	25	--	93	--	23	--	27	----	6.2
8-8.99	24	23	83	98	22	23	31	26	6.9	5.9
9.9.99	24	--	88	--	23	--	29	--	6.7	----
10-10.99	17	19	84	97	21	24	30	26	6.3	6.2
11-11.99	--	--	--	--	--	--	--	--	----	----
12-12.99	21	19	79	98	23	23	32	26	7.3	6.1
13-13.99	23	24	78	97	25	27	33	26	8.1	7.1
14-14.99	27	31	84	99	28	30	30	26	8.6	7.8
15-15.99	29	39	79	103	29	35	32	25	9.2	8.6
16-16.99	35	48	79	124	31	43	32	21	10.0	8.7
17-17.99	45	25	93	168	38	58	27	15	10.3	8.7
18-18.99	43		119		46		21		9.8	
19-20.99	8		107		46		24		11.0	

Table 8.2.1 Mean trabecular width in microns (W_b).

Sex \ Age		17 Months	3 Years	9-11 Years	Ages Combined	Significance
Male	M	107.6	104.4	95.0	102.9	**
	S	5.61	4.27	14.62		
	N	14	10	10	34	
Female	M	87.2		99.4	92.9	***
	S	10.89		6.68		
	N	17		15	32	
Sexes Combined	M	96.4		97.6		
	N	31		25		
Significance		***		N.S.		

N.S. = Not statistically significant
* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$
M = Arithmetic average or mean
S = Standard deviation
N = Number of sections measured

Table 8.2.2 Specific bone surface in mm^2/mm^3 (S_{vb}).

Sex \ Age		17 Months	3 Years	9-11 Years	Ages Combined	Significance
Male	M	23.67	24.44	27.39	24.99	**
	S	1.21	0.99	4.14		
	N	14	10	10	34	
Female	M	29.65		25.72	27.81	***
	S	3.35		1.64		
	N	17		15	32	
Sexes Combined	M	26.94		26.40		
	N	31		25		
Significance		***		N.S.		

Table 8.3 Percent bone cortex (A_{ab}).

Sex \ Age		17 Months	3 Years	9-11 Years	Ages Combined	Significance
Male	M	86.29	89.02	89.27	88.1	**
	S	2.71	1.99	3.05		
	N	15	12	14	41	
Female	M	85.16		81.49	83.45	**
	S	2.77		5.14		
	N	23		20	43	
Sexes Combined	M	85.61		84.69		
	N	38		34		
Significance		N.S.		***		

Table 8.4.1 Mean trabecular width in microns (W_b).

Sex \ Age		17 Months	3 Years	9-11 Years	Ages Combined	Significance
Male	M	142.9	137.5	119.8	133.8	**
	S	20.14	15.34	17.61		
	N	15	12	13	40	
Female	M	114.1		126.9	119.9	*
	S	19.09		14.87		
	N	23		19	42	
Sexes Combined	M	125.5		124.0		
	N	38		32		
Significance		***		N.S.		

Table 8.4.2 Percent bone (A_{ab}).

Sex \ Age		17 Months	3 Years	9-11 Years	Ages Combined	Significance
Male	M	35.31	37.16	33.90	35.40	N.S.
	S	3.84	3.26	4.91		
	N	15	12	13	40	
Female	M	32.29		36.02	33.98	*
	S	4.39		5.45		
	N	23		19	42	
Sexes Combined	M	33.48		35.16		
	N	38		32		
Significance		*		N.S.		

Table 8.4.3 Specific bone surface in mm^2/mm^3 (S_{vb}).

Sex \ Age		17 Months	3 Years	9-11 Years	Ages Combined	Significance
Male	M	18.61	18.73	21.72	19.66	*
	S	2.97	2.08	3.24		
	N	15	12	13	40	
Female	M	22.97		20.31	21.77	*
	S	4.00		2.26		
	N	23		19	42	
Sexes Combined	M	21.25		20.87		
	N	38		32		
Significance		***		N.S.		

Table 8.4.4 Surface area per volume of tissue in mm^2/mm^3 (S_{vt}).

Sex \ Age		17 Months	3 Years	9-11 Years	Ages Combined	Significance
Male	M	6.37	6.94	7.23	6.82	*
	S	0.907	0.787	0.639		
	N	15	12	13	40	
Female	M	7.33		7.25	7.29	N.S.
	S	0.957		0.840		
	N	23		19	42	
Sexes Combined	M	6.95		7.24		
	N	38		32		
Significance		**		N.S.		

Table 8.4.5 Percent bone cortex (A_{ab}).

Sex \ Age		17 Months	3 Years	9-11 Years	Ages Combined	Significance
Male	M	89.78	91.39	89.97	90.33	N.S.
	S	2.31	1.69	4.21		
	N	15	12	13	40	
Female	M	87.49		85.93	86.78	N.S.
	S	3.03		3.98		
	N	23		19	42	
Sexes Combined	M	88.39		87.57		
	N	38		32		
Significance		*		***		

Table 8.5 Longitudinal sections of the ventral vertebra in 17 months old beagle (FAC175).

Parameter \ Portion		Anterior	Middle	Posterior	Portions combined	Significance
W_b (microns)	M	95.9	94.8	97.7	96.1	
	S	2.1	5.1	3.3	--	N.S.
	N	7	7	7	21	
A_{ab} (percent)	M	27.54	19.05	28.52	25.04	
	S	0.05	1.58	1.35	--	***
	N	7	7	7	21	

9. APPENDIX E

MEASUREMENTS AND ANALYSES OF THE FIRST LUMBAR VERTEBRA IN THREE MONTH OLD BEAGLES

9.1 Serial cross-sections within the ventral vertebra

Large and small trabeculae are measured separately. The derived measurements of mean trabecular width, percent bone, specific bone surface, and surface area per volume of tissue are summarized in Tables 9.1.1 through 9.1.8.

9.2 Serial cross-section within the dorsal vertebra

Only one type of trabeculae is found in the dorsal vertebra and the measurements are summarized in Table 9.2.

9.3 Longitudinal sections of the ventral vertebra

Large and small trabeculae are separated. The derived measurements in one beagle (FAC173) are shown in Tables 9.3.1 and 9.3.2.

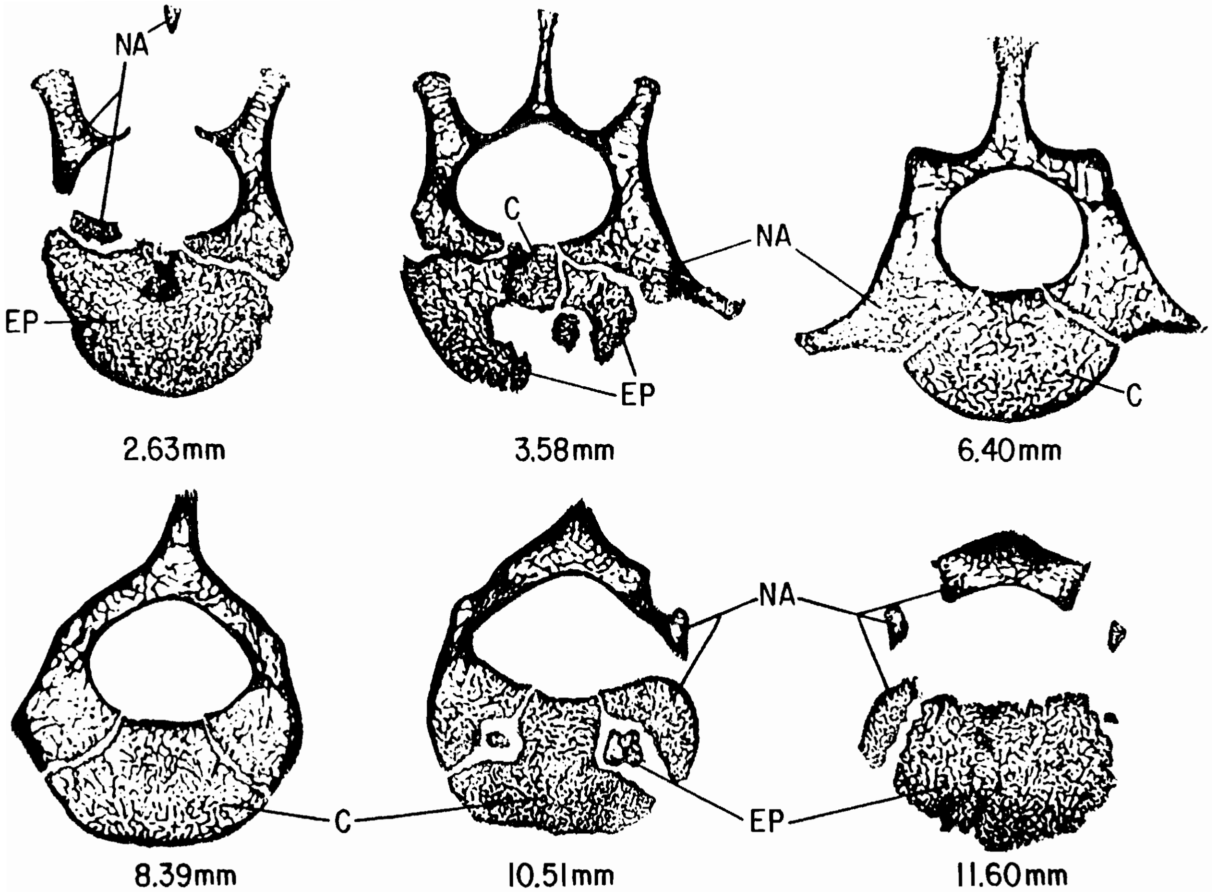


Figure 9.1 Representative cross-sections of the first lumbar vertebra. The figures under the sections are distances in millimeters from the anterior end (2.3X). See Figure 2.1.3 for abbreviations.

Table 9.1.1 Mean trabecular width (W_b) in microns of large trabeculae.

Sex \ Portion		Anterior plate	Centrum	Neural arch	Posterior plate	Portions combined	Significance
Male	M	78.40	66.80	67.56	86.00	71.58	**
	S	11.89	7.71	4.34	9.00		
	N	2	6	7	3	18	
Female	M	74.83	76.32	66.90	80.77	72.70	
	S	9.29	5.53	4.24	6.77		
	N	4	7	10	3	24	
Sexes Combined	M	76.02	71.93	67.17	83.38	72.22	
	N	6	13	17	6	42	
Significance		N.S.	*	N.S.	N.S.		

Table 9.1.2 Mean trabecular width (W_b) in microns of small trabeculae.

Sex \ Portion		Anterior plate	Centrum	Neural arch	Posterior plate	Portions combined	Significance
Male	M	49.63	33.46	36.33	48.20	38.62	***
	S	8.50	4.96	3.36	5.80		
	N	3	8	9	3	23	
Female	M	42.48	30.68	32.21	54.08	36.68	***
	S	12.03	2.88	5.31	10.39		
	N	4	8	10	4	26	
Sexes Combined	M	45.54	32.07	34.16	51.56	37.59	
	N	7	16	19	7	49	
Significance		N.S.	N.S.	N.S.	N.S.		

Table 9.1.3 Percent bone (A_{ab}) of large trabeculae.

Sex \ Portion		Anterior plate	Centrum	Neural arch	Posterior plate	Portions combined	Significance
Male	M	31.37	22.63	20.50	39.96	25.66	***
	S	7.69	2.38	3.27	4.17		
	N	2	6	7	3	18	
Female	M	29.55	23.20	20.47	32.20	24.25	*
	S	2.99	4.71	6.96	3.61		
	N	4	7	10	3	24	
Sexes Combined	M	30.16	22.93	20.49	36.08	24.85	
	N	6	13	17	6	42	
Significance		N.S.	N.S.	N.S.	N.S.		

Table 9.1.4 Percent bone (A_{ab}) of small trabeculae.

Sex \ Portion		Anterior plate	Centrum	Neural arch	Posterior plate	Portions combined	Significance
Male	M	37.86	27.55	23.64	38.19	28.75	**
	S	6.62	9.34	5.47	3.42		
	N	3	8	9	3	23	
Female	M	38.08	23.54	24.00	42.55	28.88	**
	S	11.90	6.76	7.51	10.30		
	N	4	8	10	4	26	
Sexes Combined	M	37.98	25.55	23.83	40.68	28.82	
	N	7	16	19	7	49	
Significance		N.S.	N.S.	N.S.	N.S.		

Table 9.1.5 Specific bone surface (S_{vb}) in mm^2/mm^3 of large trabeculae.

Sex \ Portion		Anterior plate	Centrum	Neural arch	Posterior plate	Portions combined	Significance
Male	M	32.84	38.59	37.81	29.83	36.19	*
	S	4.99	4.89	2.50	3.22		
	N	2	6	7	3	18	
Female	M	34.48	33.51	38.23	31.66	35.40	**
	S	4.76	2.40	2.39	2.57		
	N	4	7	10	3	24	
Sexes Combined	M	33.93	35.85	38.06	30.75	35.74	
	N	6	13	17	6	42	
Significance		N.S.	*	N.S.	N.S.		

Table 9.1.6 Specific bone surface (S_{vb}) in mm^2/mm^3 of small trabeculae.

Sex \ Portion		Anterior plate	Centrum	Neural arch	Posterior plate	Portions combined	Significance
Male	M	52.34	77.50	70.57	53.30	68.35	***
	S	9.06	11.13	6.15	5.99		
	N	3	8	9	3	23	
Female	M	63.69	83.61	80.88	48.37	74.08	***
	S	17.83	7.16	12.26	9.02		
	N	4	8	10	4	26	
Sexes Combined	M	58.83	80.56	75.99	50.48	71.39	
	N	7	16	19	7	49	
Significance		N.S.	N.S.	*	N.S.		

Table 9.1.7 Surface area per volume of tissue (S_{vt}) in mm^2/mm^3 of large trabeculae.

Sex \ Portion		Anterior plate	Centrum	Neural arch	Posterior plate	Portions combined	Significance
Male	M	10.11	8.75	7.74	11.94	9.04	*
	S	0.96	1.57	1.27	2.02		
	N	2	6	7	3	18	
Female	M	10.09	7.74	7.79	10.16	8.45	N.S.
	S	0.33	1.46	2.58	0.92		
	N	4	7	10	3	24	
Sexes Combined	M	10.09	8.20	7.77	11.05	8.70	
	N	6	13	17	6	42	
Significance		N.S.	N.S.	N.S.	N.S.		

Table 9.1.8 Surface area per volume of tissue (S_{vt}) in mm^2/mm^3 of small trabeculae.

Sex \ Portion		Anterior plate	Centrum	Neural arch	Posterior plate	Portions combined	Significance
Male	M	19.43	21.04	16.60	20.27	18.99	N.S.
	S	1.27	7.67	3.81	2.05		
	N	3	8	9	3	23	
Female	M	22.68	19.40	18.87	19.91	19.79	N.S.
	S	0.84	4.72	4.81	1.36		
	N	4	8	10	4	26	
Sexes Combined	M	21.29	20.22	17.79	20.06	19.40	
	N	7	16	19	7	49	
Significance		**	N.S.	N.S.	N.S.		

Table 9.2 The measurements of the serial cross-sections within the dorsal vertebra of the three month old beagle.

Parameter \ Sex		Male MAC165, MAC171	Female FAC173, FAC174	Sexes combined	Significance
W_b (microns)	M	80.61	79.44	79.99	
	S	11.08	9.19	-	N.S.
	N	23	26	49	
A_{ab} (percent)	M	27.60	28.57	28.12	
	S	5.47	6.52	---	N.S.
	N	23	26	49	
S_{vb} (mm^2/mm^3)	M	32.16	32.48	32.33	
	S	4.36	3.84	---	N.S.
	N	23	26	49	
S_{vt} (mm^2/mm^3)	M	8.77	9.16	8.98	
	S	1.64	1.87	---	N.S.
	N	23	26	49	

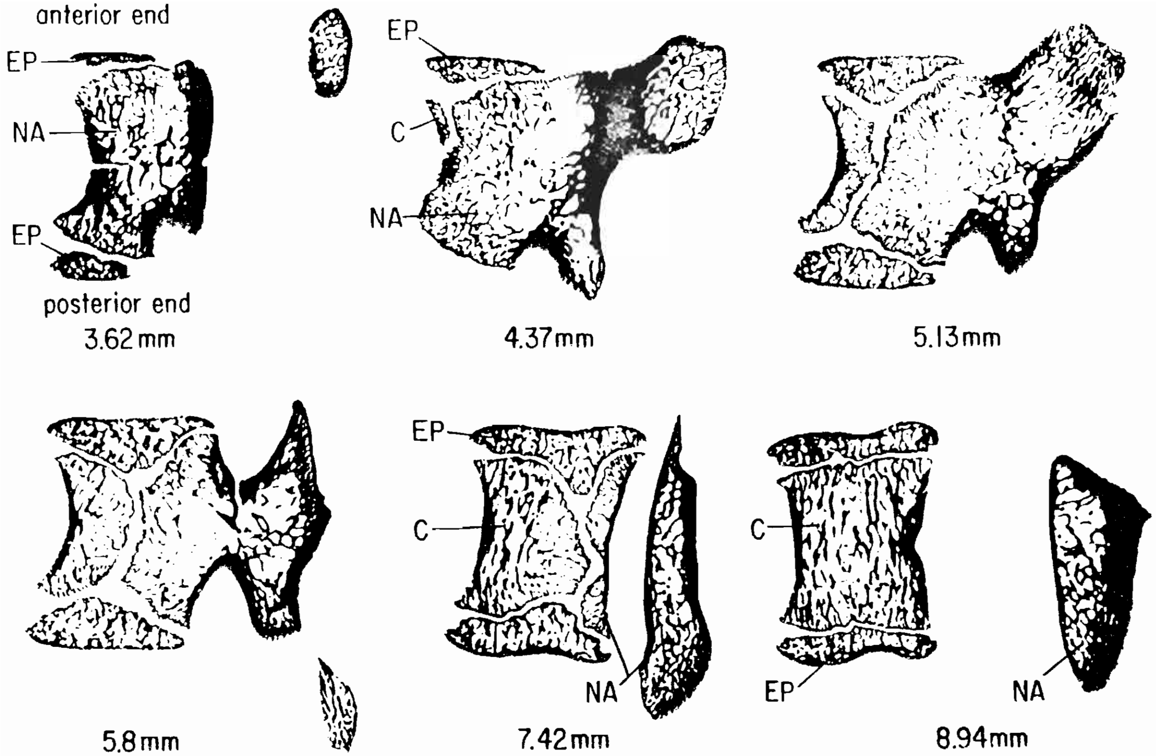


Figure 9.3 Longitudinal sections of the second lumbar vertebra (FAC175). The figures under the sections are distances in millimeters from the lateral end (2.3X). See Figure 2.1.3 for abbreviations.

Table 9.3.1 The measurements of the large trabeculae in a three month old beagle (FAC173).

Parameters \ Portion		Anterior plate	Centrum	Posterior plate	Portions combined	Significance
W_b (microns)	M	76.84	71.62	78.82	75.76	N.S.
	S	6.30	9.87	3.34		
	N	5	5	5	15	
$\dot{A}_{ab}$ (percent)	M	27.90	18.66	27.54	24.7	***
	S	2.87	1.86	1.29		
	N	5	5	5	15	
S_{vb} (mm^2/mm^3)	M	33.32	36.1	32.36	33.92	N.S.
	S	2.77	4.97	1.31		
	N	5	5	5	15	
S_{vt} (mm^2/mm^3)	M	9.26	6.72	8.91	8.30	***
	S	0.64	1.07	0.39		
	N	5	5	5	15	

Table 9.3.2 The measurements of the small trabeculae in a three month old beagle (FAC173).

Parameters \ Portion		Anterior plate	Centrum	Posterior plate	Portions combined	Significance
W_b (microns	M	46.58	32.26	46.82	41.89	***
	S	5.53	1.86	4.61		
	N	5	5	5	15	
A_{ab} (percent)	M	39.68	19.78	39.68	33.05	***
	S	3.78	2.80	3.21		
	N	5	5	5	15	
S_{vb} (mm^2/mm^3)	M	55.22	79.18	54.81	63.07	***
	S	5.82	4.47	5.25		
	N	5	5	5	15	
S_{vt} mm^2/mm^3)	M	21.80	15.57	21.65	19.67	***
	S	1.98	1.38	1.56		
	N	5	5	5	15	

10. APPENDIX F

MEASUREMENTS AND ANALYSES OF THE PROXIMAL FEMUR IN 17 MONTH TO 11 YEAR OLD BEAGLES

10.1 Cross-sections

The head and non-head portions of the proximal femur are used from the proximal end to the region where the head portion ends. The derived measurements of mean trabecular width, percent bone, specific bone surface, and surface area per volume of tissue are shown in Tables 10.1.1 through 10.1.8.

10.2 Longitudinal sections

All consecutive sections from the medial to lateral ends of the head portion of the proximal femur are used. The analyses of mean trabecular width and percent bone are shown in Table 10.2.

Table 10.1.1 Mean trabecular width in microns (W_b) of the head portion.

Sex \ Age		17 Months	3 Years	9-11 Years	Ages Combined	Significance
Male	M	178.8	150.1	204.4	170.2	***
	S	12.58	19.43	15.71		
	N	10	11	4	25	
Female	M	162.7		195.7	179.2	***
	S	19.24		23.08		
	N	16		16	32	
Sexes Combined	M	168.9		197.4		
	N	26		20		
Significance		*		N.S.		

Table 10.1.2 Mean trabecular width in microns (W_b) of the non-head portion.

Age / Sex		17 Months	3 Years	9-11 Years	Ages combined	Significance
Male	M	121.8	120.8	159.5	128.3	***
	S	12.68	9.76	12.25		
	N	10	12	5	27	
Female	M	117.3		140.3	128.8	***
	S	6.20		9.00		
	N	18		18	36	
Sexes Combined	M	118.9		144.5		
	N	28		23		
Significance		N.S.		***		

Table 10.1.3 Percent bone (A_{ab}) of the head portion.

Sex \ Age		17 Months	3 Years	9-11 Years	Ages combined	Significance
Male	M	55.22	50.46	61.23	54.09	***
	S	2.33	4.61	3.43		
	N	10	11	4	25	
Female	M	49.43		60.32	54.88	***
	S	3.75		3.35		
	N	16		16	32	
Sexes Combined	M	51.66		60.50		
	N	26		20		
Significance		***		N.S.		

Table 10.1.4 Percent bone (A_{ab}) of the non-head portion.

Sex \ Age		17 Months	3 Years	9-11 Years	Ages combined	Significance
Male	M	34.62	35.68	43.60	36.76	***
	S	2.35	2.31	3.22		
	N	10	12	5	27	
Female	M	35.36		38.90	37.13	**
	S	2.71		4.30		
	N	18		18	36	
Sexes Combined	M	35.10		39.92		
	N	28		23		
Significance		N.S.		*		

Table 10.1.5 Specific bone surface in mm^2/mm^3 (S_{vb}) of the head portion.

Sex \ Age		17 Months	3 Years	9-11 Years	Ages combined	Significance
Male	M	14.31	17.21	12.51	15.29	***
	S	0.98	2.06	0.93		
	N	10	11	4	25	
Female	M	15.86		13.20	14.53	***
	S	1.93		1.67		
	N	16		16	32	
Sexes Combined	M	15.26		13.06		
	N	26		20		
Significance		*		N.S.		

Table 10.1.6 Specific bone surface in mm^2/mm^3 (S_{vb}) of the non-head portion.

Sex \ Age		17 Months	3 Years	9-11 Years	Ages combined	Significance
Male	M	21.11	21.20	16.04	20.21	***
	S	2.28	1.68	1.21		
	N	10	12	5	27	
Female	M	21.76		18.22	20.27	***
	S	1.13		1.16		
	N	18		18	36	
Sexes Combined	M	21.53		17.74		
	N	28		23		
Significance		N.S.		**		

Table 10.1.7 Surface area per volume of tissue in mm^2/mm^3 (S_{vt}) of the head portion.

Sex \ Age		17 Months	3 Years	9-11 Years	Ages combined	Significance
Male	M	7.89	8.64	6.96	8.07	**
	S	0.524	0.941	0.519		
	N	10	11	4	25	
Female	M	7.80		7.92	7.86	N.S.
	S	0.727		0.726		
	N	16		16	32	
Sexes Combined	M	7.83		7.73		
	N	26		20		
Significance		N.S.		*		

Table 10.1.8 Surface area per volume of tissue in mm^2/mm^3 (S_{vt}) of the non-head portion.

Sex \ Age		17 Months	3 Years	9-11 Years	Ages combined	Significance
Male	M	7.27	7.64	8.03	7.57	N.S.
	S	0.543	0.711	0.758		
	N	10	11	5	26	
Female	M	7.69		7.07	7.38	*
	S	0.735		0.780		
	N	18		18	36	
Sexes Combined	M	7.54		7.28		
	N	28		23		
Significance		N.S.		*		

Table 10.2 Mean trabecular width (W_b) and percent bone (A_{ab}) in the head portion of the proximal femur (FAC175).

W_b (microns)	M	158.5
	S	13.2
	N	13
A_{ab} (percent)	M	48.95
	S	2.78
	N	13

11. APPENDIX G

MEASUREMENTS AND ANALYSES OF THE PROXIMAL FEMUR IN THREE MONTH OLD BEAGLES

11.1 Cross-sections

The head, middle and lateral portions of the proximal femur in young beagles are separated by the growth plates. Large and small trabeculae are measured separately. The measurements are made from the proximal end to the region where the head portion ends. The results of the analysis of mean trabecular width, percent bone, specific bone surface, and surface area per volume of tissue are shown in Tables 11.1.1 through 11.1.4.

11.2 Longitudinal sections

Large and small trabeculae in the head portion are measured separately. All consecutive sections are used and the results are shown in Table 11.2.

Table 11.1.1 Mean trabecular width (W_b) and percent bone (A_{ab}) in male beagles (MAC 165, MAC171).

Portion \ Trabeculae		W_b (Micrometers) Large	Small	A_{ab} (Percent) Large	Small
Head	M	72.2	37.32	24.88	30.26
	S	8.90	2.39	2.73	4.95
	N	4	5	4	5
Middle	M		49.14		36.49
	S		17.26		16.17
	N		5		5
Lateral	M	76.6	36.8	41.69	33.53
	S	12.41	3.60	6.32	3.69
	N	4	3	4	3
Portions combined	M	74.4	41.75	33.28	33.41
	N	8	13	8	13
Significance		N.S.	N.S.	**	N.S.

Table 11.1.2 Specific bone surface (S_{vb}) and surface area per volume of tissue (S_{vt}) in male beagles (MAC165, MAC171).

		S_{vb} (mm^2/mm^3)		S_{vt} (mm^2/mm^3)	
Portion \ Trabeculae		Large	Small	Large	Small
Head	M	35.68	68.44	8.97	20.85
	S	4.60	4.70	2.13	4.77
	N	4	5	4	5
Middle	M		59.76		20.07
	S		29.21		7.67
	N		5		5
Lateral	M	33.89	69.64	14.25	23.20
	S	5.32	6.81	3.76	0.98
	N	4	3	4	3
Portions combined	M	34.78	65.38	11.61	21.09
	N	8	13	8	13
Significance		N.S.	N.S.	N.S.	N.S.

Table 11.1.3 Mean trabecular width (W_b) and percent bone (A_{ab}) in female beagles (FAC 173, FAC174).

Portion \ Trabeculae		W_b (Micrometers) Large	W_b (Micrometers) Small	A_{ab} (Percent) Large	A_{ab} (Percent) Small
Head	M	84.93	40.79	34.72	37.97
	S	8.29	7.76	3.98	6.40
	N	8	10	8	10
Middle	M	61.66	32.54	39.40	32.95
	S	6.93	4.12	3.20	2.93
	N	5	7	5	7
Lateral	M	63.00	29.92	35.18	29.92
	S	9.64	3.76	4.66	2.86
	N	4	5	4	5
Portions combined	M	72.92	35.70	36.20	34.54
	N	17	22	17	22
Significance		**	*	N.S.	*

Table 11.1.4 Specific bone surface (S_{vb}) and surface area per volume of tissue (S_{vt}) in female beagles (FAC173,FAC174).

Trabeculae / Portion		S_{vb} (mm^2/mm^3)		S_{vt} (mm^2/mm^3)	
		Large	Small	Large	Small
Head	M	30.23	64.88	10.47	23.94
	S	2.83	14.40	1.35	2.74
	N	8	10	8	10
Middle	M	41.74	79.21	16.35	26.04
	S	4.83	9.22	1.27	3.24
	N	5	7	5	7
Lateral	M	41.21	86.20	14.64	25.65
	S	6.69	10.69	4.09	2.67
	N	4	5	4	5
Portions combined	M	36.20	74.28	13.18	25.00
	N	17	22	17	22
Significance		**	*	**	N.S.

Table 11.1.5 Comparison of total volumes and total trabecular surface area within the ventral vertebra of a three month old male beagle (MAC171).

Portion / Parameter	Components of Ventral Portion								
	Anterior plate		Centrum		Neural arch		Posterior plate		
	Large trabec.	Small trabec.	Large trabec.	Small trabec.	Large trabec.	Small trabec.	Large trabec.	Small trabec.	Dorsal portion
ΣV_b (mm^3)	25	15	73	19	53	21	37	14	114
ΣV_m (mm^3)	53	23	256	46	209	60	62	22	282
ΣV_t (mm^3)	78	38	329	65	262	81	99	36	396
ΣA (mm^2)	821	753	2566	1267	1891	1294	1061	744	3631

Table 11.2 Longitudinal sections in the head portion of the proximal femur (FAC173).

		Large trabeculae	Small trabeculae
W_b (microns)	M	80.68	40.44
	S	6.04	2.47
	N	11	11
A_{ab} (percent)	M	28.11	34.97
	S	2.45	2.84
	N	11	11
S_{vb} (mm^2/mm^3)	M	31.67	62.49
	S	2.57	2.84
	N	11	11
S_{vt} (mm^2/mm^3)	M	8.98	21.76
	S	1.43	1.35
	N	11	11

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