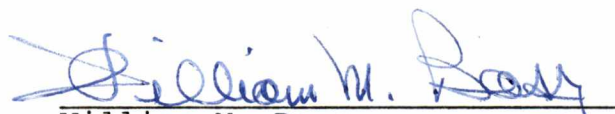
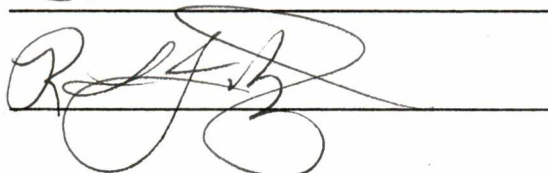


To the Graduate Council:

I am submitting herewith a thesis written by Dwight John Schmidt entitled "Histomorphological Age Changes Associated with the Growth and Development of the Femur in Saguinus fuscicollis and Saguinus oedipus (Callitrichidae, Primates)." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts, with a major in Anthropology.


William M. Bass
Major Professor

We have read this thesis and
recommend its acceptance:

Accepted for the Council:


The Graduate School

2

HISTOMORPHOLOGICAL AGE CHANGES ASSOCIATED WITH THE GROWTH
AND DEVELOPMENT OF THE FEMUR IN SAGUINUS FUSCICOLLIS
AND SAGUINUS OEDIPUS (CALLITRICHIDAE, PRIMATES)

A Thesis
Presented for the
Master of Arts
Degree
The University of Tennessee, Knoxville

Dwight John Schmidt

June 1984

This thesis is dedicated to
two very special people in my life.

My wife, Candace Lewis Rhyne,
whose love and patience enabled me to
complete this research,

and my sister, Bonnie Beth Schmidt,
whose generosity made
my graduate career possible.

It is also dedicated to all primates
who have become or are in danger of becoming extinct
due to their exploitation by man.

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ABSTRACT

Although skeletal histology has been investigated for a number of primate species, only for humans has research been in depth enough to adequately document specific histomorphological changes with age. In fact, our knowledge of comparative ageing in nonhuman primate skeletal histology is almost nonexistent.

This study documents histological age changes in the femoral midshaft of the South American tamarin, genus Saguinus. Examined were 86 Saguinus fuscicollis and 26 S. oedipus age and sex documented specimens. The entire approximate 13 year life span of these primates is represented within the sample. Two histomorphological aspects were investigated, the change in total cortical area and associated dimensions, as well as the pattern of osteonization or remodeling.

Results of this research indicate that although there are differences between the two species in cortical area expansion and patterning of osteonization, they conform to traditional growth curves for primate species. Cortical stability was evident for the posterior and medial quadrants of the bone due to posteriomedial muscular attachment, while cortical drift occurred anteriolaterally. The trends of intracortical remodeling are such that specific techniques of age determination from the quantification of

microstructures (i.e., osteons, osteon fragments) are not applicable. Due to continual cortical involution, most evidence of prior intracortical remodeling is lost in old age. Osteonization in older specimens was found to be limited to the posterior quadrant of the femur, indicating remodeling due to muscle stress rather than remodeling of compression.

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CHAPTER I

INTRODUCTION

In 1691, Clopton Havers published the manuscript Osteologica Nova, and in doing so introduced what it took the medical community nearly two centuries to recognize as the most dynamic aspect of skeletal tissue--the Haversian system or osteon. Nearly three centuries have passed and although the Haversian system and its role in the dynamics of skeletal tissue has been extensively investigated, major gaps remain in our understanding of just how the cells which affect these systems in bone are involved.

Havers' discovery (after whom Haversian systems are named) paved the way for biologists to recognize that bone is much more than merely nonliving mineral, but is indeed a living tissue. The biological processes which enable bone to grow from initial ossification into mature adult bone also perform essential homeostatic functions throughout an individual's life. In doing so, these dynamic functions play an important role in the maintenance of health in all vertebrate organisms. For this reason, it is of biological importance to investigate all aspects of the skeletal system as a living tissue, from the role it plays in the growth and development of an organism, to the contributions it effects in the maintenance of homeostasis after maturity.

Most research into the biology and pathology of bone, whether on Haversian systems or broader skeletal parameters, focuses almost entirely on conditions as they relate to humans. Investigations are concerned specifically with either human samples and data or with animal models as they apply to human conditions (see for example, Bourne, 1971; Dixon and Sarnat, 1982; Frost, 1964; Frost, 1964 (ed.); Hall, 1965; McLean and Urist, 1968; Rodahl et al., 1960; Trueta, 1968; and Urist, 1980, to list but a few). Comparatively few investigations have been concerned with comparative bone biology. Most of these studies have focused on growth and developmental changes as they relate to aging (Klevezal and Kleinenberg, 1967; Perrin and Myrick, 1980; Rhoads and Lutz, 1981; Sognaes, 1960). It is evident from the absence of literature on the subject that the bone biology of many vertebrate organisms has yet to be adequately investigated. In fact, our knowledge of comparative histoskeletal morphology is almost nonexistent. This thesis will conduct such an investigation, the documentation of the bone biology for a species which has not yet been described. Special attention will be given to histomorphological changes which are found to occur with growth and ageing.

Statement of Purpose

The purpose of this thesis is twofold: (1) to investigate and describe the histomorphological age changes in the cortex of the femoral midshaft of genus Saguinus (Callitrichidae, Primates), and (2) to assess the effects that functional morphology contributes to these changes. More specifically, it deals with the biological aspects of the growth and development of the femoral cortex as well as changes effected by senescence in one species, Saguinus fuscicollis. It also attempts to assess the value of estimating the chronological age of wild caught individuals of unknown age utilizing the histomorphological parameters investigated.

Defining the Research Problem

To investigate histoskeletal changes with age for any given species, one must begin with an acceptable sample size of age and species documented individuals. As this current study will analyze the histomorphological age changes beginning in infancy and continuing through old age, the sample must include individuals from the entire known life span of the species. In addition, all phases of skeletal growth, maturation and degeneration must be represented. The scarcity of such collections is one explanation for the lack of descriptive research of this type. Such a sample

of age, sex and species documented individuals for genus Saguinus is available, however, and is described in Chapter II.

Since this investigation will focus on the histomorphology of skeletal tissue, that aspect must be defined here. Histomorphology is the focus of bone biology on the cellular level or microscopic form and function of skeletal tissue. The microscopic structure of bone results from, and is a reflection of the two major functional aspects of bone, (1) mechanical (support and movement) and (2) physiological (mineral metabolism).

As a result of these two aspects, bone histology varies dramatically, not only between species, but between individuals of the same species as well. This creates considerable confusion among investigators concerning the identifiable histological elements seen in microscopic cross section of bone. What are so often succinctly described as distinct structural units of bone (non-Haversian canals, primary and secondary Haversian systems) are rarely so clear. Biological reality seldom conforms to textbook definitions. Histomorphological aspects of bone are, at best, often difficult to distinguish and interpret, a factor compounded by use of undecalcified sections 100 microns thick, a factor discussed in Chapter IV. Thus, it is of importance to describe and define (even if broadly) the histomorphology of skeletal

tissue to initiate the reader as to how the author interprets given structures. This topic is discussed in Chapters III and IV.

Microscopic age changes in cortical bone have been well documented for humans to the extent that their quantification is now one of the most reliable methods to estimate the age of unknown adults (Ahlquist and Damsten, 1969; Bouvier and Ubelaker, 1977; Jowsey, 1960; Kerley, 1965; Kerley and Ubelaker, 1978; and Ortner, 1975). However, little has been done concerning similar changes in other primate groups. This thesis attempts to close that gap.

Research on skeletal morphology has included, however, documented changes in the size and shape of cortical bone and the medullary cavity. These studies have focused primarily on that period when a significant loss of cortical bone occurs in old age due to osteoporosis (Carlson et al., 1976; Garn, 1970; Van Gerven, 1973; Van Gerven et al., 1969). They have not included changes in the size and shape of the cortex and medullary cavity throughout the entire life span of the species. In this study, however, changes in the cortex, medullary cavity and histomorphology will be correlated with age.

Research Assumptions

No scientific investigation is able to account for or control all variables which might influence the parameters

under analysis. There are, however, certain research assumptions which acknowledge these uncontrolled aspects and yet enable the research to proceed.

Two characteristics of the sample used in this study must be taken into account. First, the Saguinus specimens utilized in this study are all captive-born and colony reared individuals. Their captive conditions therefore, differ from those of their natural habitat. Second, all specimens included in the research sample are individuals which were not sacrificed but died as a result of disease, complications arising from captive breeding and rearing, or other natural causes. Excluded from the sample were any individuals with pathological conditions involving the skeleton.

Given these circumstances, the following assumptions have been made: (1) although the living conditions of the captive animals differ from those of their free ranging counterparts, the histomorphology and cortical parameters identified in the present study are applicable to both, and (2) although these individuals died prematurely, their histoskeletal development did not significantly diverge from normal growth and development.

Glassman (1982:7), utilizing a similar sample of Saguinus specimens, states:

Two considerations may be raised regarding the validity of the above assumptions. The first is

that since biological growth of animals is controlled by the complex interaction of genetic and environmental components, a change in one or both components may be expected to alter normal development. Since the colony-raised tamarins included in the study sample existed within a totally artificial environment wherein the diet, habitat, exposure to seasons, and almost all other behavioral patterns had been modified, a corresponding influence on development may be suspected. However, all attempts were made to provide the best possible environment for normal development of colony-raised animals.

Second, the immature specimens examined were not sacrificed, but died from disease or other natural causes. Since they had health-related problems significant enough to cause death, this may have altered their normal growth rate.

Although the validity of the assumptions can be questioned, the extent of influence in normal growth patterns by the effects of captivity and illness remains unknown. The position taken here is that the general acceptance of the assumptions is justified as long as recognition of their existence and possible implications are acknowledged.

Glassman later, however, investigated one aspect of these assumptions, that the functional morphology of colony-born individuals was comparable to those which lived the majority of their lives in their native habitat. Utilizing a sample of Saguinus specimens from the same colony as is used in this study (see Chapter II), Glassman (1983:293) states:

Approximately 37% of the sample was comprised of animals which were colony-born and 63% represent animals born in the wild and later integrated into the colony from their natural habitat. To test for possible effects of colony versus wild birth within the two species, data from the 14 morphologic variables used in the primary analysis for this study and which are defined below were subjected to Student's t-test procedures. . . . For S. fuscicollis, two variables were found to differ significantly

while only one was indicated for S. oedipus. Consequently, the degree of effect from colony birth on morphology has been assumed minimal and the pooling of colony and wild-born individuals justified for the purposes of this study.

Thus, the assumption that some types of data are comparable between colony-born and feral animals is justified. This research proceeds, therefore, on the acknowledgment of the presented assumptions.

Need for Nonhuman Primate Growth and Ageing Research

An appreciation of the growth and ageing process in living organisms is essential for understanding many aspects of their biology. Our current knowledge of normal or pathological ageing processes in nonhuman primates, however, is extremely limited, and for skeletal tissue is almost nonexistent. Klevezal and Kleinenberg (1967) discuss ageing in nine orders of the Class Mammalia, but do not include the Order Primates. In an extensive review of animal models being utilized in ageing research, Getty and Ellenport (1974) found information on nonhuman primates to be so inconsequential that they did not even discuss their role. More recently, Caminiti (1978) has presented a complete listing of nonhuman primate ageing research published since 1940. Of 148 references listed, only a few deal with the skeleton. Bowden (1979) has edited the most comprehensive work on the subject to date. Although the primary

focus is on soft tissue and biochemical age differences, one section does cover the skeletal system.

This lack of research on nonhuman primates is curious when compared to the extensive ageing data and research that has accumulated on other vertebrate organisms. Again, Klevezal and Kleinenberg (1967) discuss nine orders of the Class Mammalia. Perrin and Myrick (1980) have edited a comprehensive volume on whales and sirenians. Although much of this research has focused on age determination from dental and skeletal structures, it is directed at the biology of the individual organism rather than as a model for human research.

A number of other investigations on growth and ageing aspects of the skeleton have involved laboratory animals, including the rat (Kimmel and Jee, 1980), the rabbit (Stoker and Epker, 1971), and especially the dog (Anderson, 1970; Anderson and Floyd, 1963; Detenbeck and Jowsey, 1969). These, however, have focused not so much on the individual biology of the species studied, but have been used as animal models for skeletal age changes in humans. Although it may be assumed (as these authors have) that skeletal growth, remodeling and ageing in these animals is similar to man, and that parallels may be drawn, it is still a biological leap from laboratory rats or dogs to human beings.

Thus, the necessity for nonhuman primate ageing research is twofold. First, to investigate the growth and ageing of primates in order to gain a better understanding of their biology, and secondly, to assess their usefulness as a model for human ageing research.

One of the important applications of ageing research on nonhuman primates is the need for determining the age of wild-caught individuals. This need for accurately estimating the age of wild-caught individuals was expressed at the International Conference on Neotropical Primates sponsored by the National Academy of Sciences. Heltne and Thorington (1976: 118) state:

Age criteria for field evaluation of population structure are extremely important . . . more effort should be made to refine our techniques for estimating the age of wild primates.

A later publication by Thorington and Voreck (1976:1020-1021) restated this need:

It is obviously important that the age variation of Aotus be studied in the future in animals of known age . . . It would be useful to know the age structure of the sample that is being imported into the U.S. . . . to know the age structure of populations in different habitats throughout South America.

Currently, age estimation in biological, demographic or differential mortality studies is based on epiphyseal union, tooth eruption or longitudinal wear (Froehlich et al., 1981; Otis et al., 1981; Tappen and Seversen, 1971; Thorington and Voreck, 1976). Neyman (1977:49-51) has

estimated the age of wild caught Saguinus oedipus individuals based on their weight, head and body length, and dental age categories. While valuable, these techniques are unreliable for subadults and only grossly reliable for adults. Thus, the need for ageing research on, and an accurate age estimation technique for nonhuman primates has been well established.

It should be mentioned here that three sections of this thesis are given considerable attention: (1) the history of the ORAU marmoset colony (Chapter II), (2) bone biology (Chapter III), and (3) undecalcified bone microsectioning technique (Chapter IV). This was necessitated by several factors. First, it was felt necessary to document the historical background of the marmoset and tamarin cadaver collection on which this study was based. This had not been done prior to this thesis. Second, a knowledge of bone biology is essential to comprehend the results. And third, the thin section technique is described in detail in hopes that it will aid users of the Thin Section Laboratory, Department of Anthropology, The University of Tennessee.

CHAPTER II

THE SAMPLE: SAGUINUS FUSCICOLLIS
AND SAGUINUS OEDIPUSSaguinus: Model for Human Biomedical Research

Although many animals other than primates (i.e., the laboratory mouse, rat and dog) have a long history of involvement in medical research, nonhuman primates serve as the best animal models for research on human biology and disease due to their close phylogenetic relationship to humans. Thus, the importance of nonhuman primate utilization for such research has been well established (Austin, 1978; Bourne, 1973; Freiherr, 1983; Goldsmith and Moor-Jankowski, 1969, 1971; Heltne, 1976; Hill, 1969).

In the past, however, selection of an appropriate genus or species of primate as a research model often depended simply on their availability through importation. At one time, nonhuman primates could be obtained with relative ease. In the 1950s for example, more than 200,000 rhesus monkeys alone were imported into the United States. Although the number has decreased in subsequent years, as many as 77,000 primates were imported annually into the United States in the early 1970s (Committee on Conservation on Nonhuman Primates, 1975; Muckenhirn, 1975). As a result,

many species are now on the verge of extinction. Recent legislation has either limited or banned their importation or exportation, significantly reducing their availability and, fortunately, their exploitation.

The significance of this to the medical investigator is that if a new supply of primates is no longer available through importation, then the existing stocks within the United States must be maintained and expanded to the point where importation is no longer necessary. The only solution to this problem is the establishment of viable, self-sustaining breeding populations. Captive breeding of non-human primates has been successful for some species but not for others (Beveridge, 1972).

Successfully bred as early as the 1920s (Lucas et al., 1927), the small South American tamarins and marmosets, genera Saguinus and Callithrix (Callitrichidae), have since demonstrated their ability to reproduce in a laboratory environment (Epple, 1970; Fritzgerald, 1935; Gengozian et al., 1978a,b; Hampton, 1964; Hampton et al., 1966; Hampton and Hampton, 1967; Wolfe et al., 1975). The need for continued breeding of Callitrichids, however, has recently been formally restated. At the recent International Conference on the Biology and Conservation of the Callitrichidae sponsored by the National Zoological Park, Smithsonian Institution, a joint resolution by participants

recommended the following suggestions be urgently adopted (Kleiman, 1978:8):

- a. Development of breeding centers and research within the home countries.
- b. Development within the user countries of breeding centers that apply rigorous genetic management of stocks.

The reason for these steps, as stated at the symposia, was that several species were rapidly diminishing in number and on the verge of extinction. Fortunately, breeding programs have been successful and, as a result, at least one species of tamarin has recently been saved from extinction and is about to be reintroduced into its native habitat (Mitchell, 1983).

Marmosets and tamarins have become more increasingly utilized as models for a host of biomedical investigations (Deinhardt, 1971; Gengozian, 1969; Gengozian and Deinhardt, 1978; Levy and Artecona, 1964). They have proven to be adequate models for investigation of human disease, ranging from parasitic infection (Hershkovitz, 1977:368-394) to malignant carcinomas (Lushbaugh et al., 1978; Moreland and Woodward, 1968; Richter et al., 1980; Williamson and Hunt, 1970). Thus, marmosets have proven to be an invaluable research tool in pathology and medicine. This factor, coupled with their ability to reproduce outside their ecological habitat, make them an ideal candidate as a nonhuman primate which will enable medical research programs in the

United States to become self-sufficient and independent of importation markets.

The Marmoset Research and Breeding Colony, ORAU

History

In 1978, the Department of Anthropology, The University of Tennessee, Knoxville, was fortunate enough to be selected as the repository for an extensive, well documented collection of autopsied marmoset and tamarin cadavers. These cadavers, primarily representing several species of genera Saguinus and Callithrix, were a series of individuals who had been imported or bred by the Marmoset Research and Breeding Program, Medical and Health Sciences Division, Oak Ridge Associated Universities (ORAU), Oak Ridge, Tennessee.

Established in 1961, this colony was to provide non-human primates for studies in radiation biology (Gengozian, 1969:351-355; Gengozian et al., 1978b:71; ORAU, 1982:7). The colony began with the importation of a few individuals of a number of different species, including Saguinus fuscicollis ssp., S. oedipus ssp., S. mystax, S. nigricollis, Callimico goeldii, Cebuella pygmae and Callithrix jacchus. Continued importation and a successful breeding program of several of the species has resulted in the present colony of approximately 400 individuals comprised of three species: (1) Saguinus fuscicollis illigeri, the saddleback tamarin,

(2) Saguinus oedipus oedipus, the cotton-top tamarin, and
(3) Callithrix jacchus, the common marmoset (Tardif, 1984).
The colony has therefore grown from a few individuals of a
variety of species to a sizeable breeding population of
three representative species.

Gengozian (1969) presents a detailed description of the
ORAU colony, including laboratory care of marmosets, their
caging and colony environment, diet, early breeding observa-
tions, and their experimental potential in biological re-
search. Throughout its twenty three year history, the col-
ony has been utilized to approach an extended number of
research problems and has provided a wealth of biological
data. These include immunological, hematological (blood
chimerism and antigens), radiobiological, toxic, parasitic,
genetic, social organization, reproductive, pathological and
behavioral studies. The list of published data based on
the ORAU colony is simply too extensive to list here.
Thus, much of the experimental potential that Gengozian
(1969) first perceived has been realized.

One of the most important discoveries was made by
Dr. C. C. Lushbaugh who, in 1968, recognized the first spon-
taneously occurring colon adenocarcinoma in an imported
Saguinus oedipus oedipus individual (Clapp, 1983). The
importance of this is that no animal other than humans was
known to spontaneously develop this tumor with any

frequency. The occurrence was at first thought to have been an isolated event, however, neoplasms have now been observed in animals which were born and reared in captivity (Richter et al., 1980). Thus, S. oedipus oedipus is proving to be an invaluable experimental model for human colon cancer research.

Breeding efforts to improve the survival rate of colony born infants have been successful. Tardif (1984) reports that survival to one year of age for colony born, S. oedipus oedipus infants, increased from 23% in 1977 to 50% in 1981 and 69% in 1984. Gengozian et al. (1978a,b) state that first year survival rate of full term young for S. fuscicollis is 65%. An apparent reason for the higher infant mortality rate among S. oedipus is one of parental neglect. As a result of these findings, the ORAU colony has expanded their reproductive studies to include behavioral aspects of breeding in a laboratory environment.

As a result of twenty three years of importation and breeding, a sizeable collection of marmoset and tamarin cadavers was retained for future investigation. Dr. N. Gengozian, former senior research scientist of the marmoset colony, began the cadaver collection by preserving skulls only, but later expanded the gross collection by freezing the entire autopsied and eviscerated cadavers. All records were saved which included data on species, sex, time in

colony if imported, age if born in colony, genealogy, pathology and cause of death.

The cadaver collection was stored at the Medical and Health Sciences Division, ORAU, until, in the late 1970s, their freezers literally became too full. At that time, the need had arisen to estimate the age of imported adults in an effort to determine the onset and sequence of the spontaneous colon carcinoma which has been previously discussed. As a result, Dr. Conrad Richter, veterinary pathologist for the marmoset research facility at that time, suggested that the frozen cadaver collection be utilized to document skeletal age changes.

Thus, the Marmoset Use Committee, ORAU, selected the Department of Anthropology, The University of Tennessee, Knoxville, as the repository for the frozen cadaver collection. Here, the colony was to be housed and appropriately processed for skeletal age change studies.

The Sample

Table 1 presents a summary of the cadaver collection size by species and age group as of December 1983. Not all of those listed are repositied at the Department of Anthropology, UTK. A number of specimens were sent to the Department of Vertebrate Zoology, Smithsonian Institution, Washington, D.C., to become part of the primate collections at the National Museum of Natural History.

Table 1. ORAU Marmoset and Tamarin Cadaver Collection Size by Species and Age Group as of December 1983^a

Species	Unknown Age	Known Age			Species Total
		Subadults ^b	Adults		
			2 Years	3 Years	
<u>Callimico goeldii</u>	21	5	4		30
<u>Callithrix jacchus jacchus</u>	82	101	14		197
<u>Cebuella pygmaea</u>	4	0	1		5
<u>Saguinus fuscicollis illigeri</u>	343	485	210		1,038
<u>Saguinus fuscicollis lagonotus</u>	98	43	10		151
<u>Saguinus fuscicollis leucogenys</u>	29	11	8		48
<u>Saguinus fuscicollis nigrifrons</u>	114	31	9		154
<u>Saguinus mystax</u>	49	3	0		52
<u>Saguinus nigrifrons</u>	30	24	11		65
<u>Saguinus oedipus oedipus</u>	321	253	29		603
<u>Hybrids^c</u>	13	141	74		228
Unknown	664	6	0		670
				Collection Total	3,241

^aNot all specimens are deposited at the Department of Anthropology, The University of Tennessee, Knoxville. Approximately only 25% have been macerated, allowing soft tissue or anatomical study.

^bThe majority of the subadult specimens are less than two weeks of age.

^cThe majority of the hybrids are S. fuscicollis ssp.

Of the collection total, only approximately 25% have been macerated for skeletal study, thus allowing future soft tissue or anatomical studies. For those specimens macerated, each was first x-rayed, and a scalp hair sample and the palmer and plantar pads removed and preserved in formalin (Glassman and Webb, 1984). Since all cadavers had been eviscerated during autopsy at ORAU (with the exception of fetal specimens), the maceration process consisted of first skinning and then "fleshing out" the thawed cadavers. Each animal was then disarticulated at the neck, shoulder and pelvic girdles, and two caudal joints to facilitate their fit into the maceration jar. The disarticulated skeleton was then placed in a one quart glass jar and filled with tap water. Each jar was sealed and placed in a warm room to facilitate the maceration method, that of bacteriological decay.

After approximately two months, each jar was opened individually, the contents poured into a fine mesh filter screen, and the skeleton cleaned and rinsed with hot water. Although malodorous and time-consuming, this method is extremely cheap and sufficiently degreases small skeletons. The skeletal material was dried overnight, then numbered and placed in individual specimen boxes which were labeled and catalogued.

The sample utilized in this study included only macerated skeletons of known age, and is present in Table 2 by species, sex, and age in years. Only colony born individuals exhibiting no skeletal pathology were included. Initially, the entire life span of the S. fuscicollis and S. oedipus were to be compared. However, due to the limited sample of known age adult S. oedipus individuals (N = 5), the two species were compared for subadults alone. Only the S. fuscicollis sample was used to examine the cortical and histomorphological age changes of the femoral midshaft throughout the entire life span of the species.

Sexes were pooled for each species. This was based on Analysis of Covariance (ANOCOVA) tests (see Analytical Methods, Chapter IV), which indicated that the sexes were not significantly different. This agrees with sexual dimorphic data presented by Hershkovitz (1977:674,783). By pooling the sexes, sample sizes were maximized.

Saguinus fuscicollis and Saguinus oedipus

Although the study of Callitrichids began in the 18th century, no period has seen more research than the past decade. This observation may be explained by their usefulness in medical research, as described above, but it is also a result of a broad effort to understand the ecology and behavior of marmosets due to their diminishing population in their native habitat (Kleiman, 1978).

Table 2. Sample Size of Saguinus Specimens Included in Histomorphometric Analysis of the Femoral Midshaft by Species, Sex and Age

Age in Years	<u>Saguinus fuscicollis</u>		<u>Saguinus oedipus</u>	
	Male	Female	Male	Female

Due to the small sample size of S. oedipus adults (N = 5), these were excluded from the final analysis. Only S. oedipus subadults were used for comparison with S. fuscicollis subadults.

As a result, their taxonomy, habitat distribution, ecology, behavior and anatomy have been reexamined since earlier reports, and is briefly discussed here. The biology of the two species of Saguinus included in this study is emphasized.

Taxonomy

Within the infraorder Platyrrhini, Saguinus fuscicollis and Saguinus oedipus are members of the South American primate family Callitrichidae. The Callitrichidae includes four genera: Cebuella, the pygmy marmosets; Callithrix, the marmosets; Saguinus, the tamarins; and Leontopithecus, the golden lion-tamarins (Hershkovitz, 1977:9-10). Genus Saguinus consists of nine species (although not all authors agree) and of the two species investigated in this research, Saguinus fuscicollis includes thirteen subspecies while S. oedipus includes two subspecies (Hershkovitz, 1977:436-437). Of these, four subspecies of S. fuscicollis, S. f. illigeri, S. f. nigrifrons, S. f. lagonotus, and S. f. leucogenys; and one subspecies of S. oedipus, S. o. oedipus were included in this study.

Geographical Distribution

The distribution of genus Saguinus is generally limited to southern Central America and northern South America, approximately ten degrees latitude both north and south of

the equator (Hershkovitz, 1977:400). Saguinus fuscicollis inhabits the equatorial rain forest of the "Upper Amazonian region from west bank of the Rio Madeira south of the Rio Amazonas in Brazil, and the south (right) bank of the Japura-Rio Caqueta-Caguan north of the Amazonas in Brazil and Colombia, west to the eastern base of the Cordillera Oriental in Colombia, Ecuador, Peru, and Bolivia" (Hershkovitz, 1977:636). Although most subspecies of Saguinus occur entirely or partly in Brazil (Mittermeier et al., 1978:143), the four subspecies of S. fuscicollis used in this study, S. f. illigeri, S. f. nigrifrons, S. f. lagonotus, and S. f. leucogenys, inhabit eastern Ecuador and northeastern and central Peru.

Saguinus oedipus inhabits the "tropical forested zones of Colombia, Panama and Costa Rica" (Hershkovitz, 1977:753). However, the subspecies utilized in this study, S. o. oedipus, inhabits the northwestern region of Colombia bordering the Carribbean Sea.

The geographical distribution of the Saguinus subspecies included in this study is presented in Figure 1.

Morphology

Both body and tail of Saguinus are covered with hair with the exception of the face in some species. Most are recognizable by a variety of hair color patterns and a

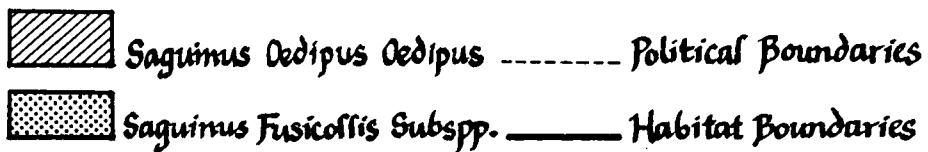
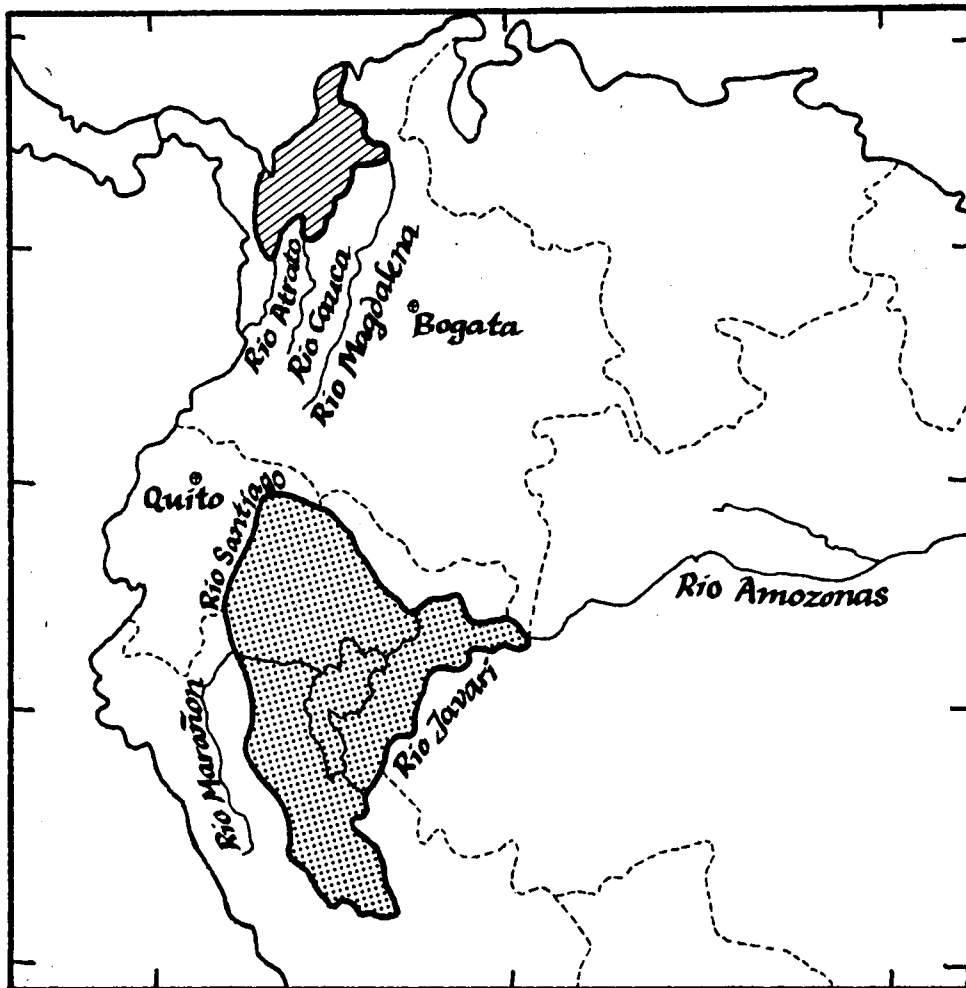


Figure 1. South American geographical distribution of the native habitats of *Saguinus fuscicollis* spp. and *Saguinus oedipus oedipus* included in this analysis. Adapted from Chiarelli (1972), Hershkovitz (1977), Hill (1957), and Neyman (1977).

distinguishing hairy crests, tufts or a mane (Herskovitz, 1977:414; Hill, 1957:193).

S. fuscicollis, shown in Figure 2, is recognizable by the black or dark brown hair on its crown and forehead with short white or gray hair surrounding the mouth. In addition, they exhibit a rather well defined gray and brown marbled or striated "saddle" on their back, hence, the common name, saddle-back tamarin. The four subspecies included in this study, possess dark brown to reddish-brown, or chestnut to reddish hair on their shoulders, rump and limbs (Hill, 1957:229).

S. oedipus oedipus, shown in Figure 3, is recognized by a "crest of long whitish hairs from forehead to nape flowing over shoulders and sharply defined from practically bare black" (Herskovitz, 1977:766) face. Hence, the common name, cotton-top tamarin. The body and tail are most often dark brown with a whitish chest, abdomen, arms and legs.

Overall body size of members of the family Callitrichidae are rather small, relative to other primates. Herskovitz (1977:123) reports that combined head and body length ranges between 100-350 mm for adults, making them one of the smallest living primates. Saguinus is the largest and most varied genus of the tamarins (Moynihan, 1970:1) within this family. Adult combined head and body length ranges between



Figure 2. Adult female Saguinus fuscicollis illigeri. (Courtesy of John Richards, Oak Ridge Associated Universities.)



Figure 3. Adult male Saguinus oedipus oedipus. (Courtesy of San Diego Zoo.)

170-310 mm. Tables 3 and 4 present available data on newborn S. oedipus (Hampton et al., 1971; Wolfe et al., 1975) and adult S. fuscicollis and S. oedipus body weight and size, and adult tail length (Hershkovitz, 1977:782,949; and Napier and Napier, 1967:301,303).

At birth, S. oedipus oedipus weighs an average 34-44 grams and measures 89-96 millimeters. Note in Table 3 that the larger the litter the smaller the newborn. Adult individuals of S. oedipus weigh an approximate mean of 405 grams with head and body length an average 237 millimeters. The overall body length of S. fuscicollis adults averages 222 millimeters, weighing approximately 315 grams. While some of the sample sizes on which these data are based are too small to be demographically reliable, they do indicate that although there is considerable size overlap, S. oedipus tends to be the larger of the two species.

Tails of Saguinus are considerably long, especially relative to body length. Hershkovitz (1977:945) presents tail length means of 322 millimeters for S. fuscicollis and 368 millimeters for S. oedipus as presented in Table 2, p. 22. Tail length, therefore, is nearly always greater than the combined head and body length. Tails of Saguinus are not prehensile, however, as they are in other groups of Platyrrhine monkeys. Although tail coiling has been observed, this is a "specialization unrelated to the power

Table 3. Average Head and Body Length and Body Weight for Newborn Saguinus oedipus oedipus of Different Litter Size. From Hampton et al. (1971) and Wolfe et al. (1975)

Litter Size	Newborn Head and Body Length in Millimeters			Newborn Body Weight in Grams		
	<u>N</u>	Mean	Range	<u>N</u>	Mean	Range
Singletons	4	96	94-97	4	37	34-40
	—	—	—	2	44	44
Twins	20	92	82-103	20	36	24-45
	—	—	—	32	43	35-52
Triplets	4	89	87-91	3	34	32-40
	—	—	—	8	36	30-43

Table 4. Head and Body Length, Tail Length and Body Weight Data for Adult Saguinus fuscicollis and Saguinus oedipus.
From Hershkovitz (1977:782,945) and Napier and Napier (1967:301,303).

Species	Sex	Adult Head and Body Length in Millimeters			Adult Tail Length in Millimeters			Adult Body Weight in Grams		
		N	Mean	Range	N	Mean	Range	N	Mean	Range
<u>S. fuscicollis</u>	Combined	96	222	175-270	95	322	250-383			
	Male	23	-	170-310	23	-	275-420	3	312	264-341
	Female	25	-	155-280	25	-	325-425	4	320	264-395
<u>S. oedipus</u>	Combined	149	237	200-287	147	368	307-423	-	-	-
	Male	5	-	219-245	5	-	362-382	28	417	300-510
	Female	1	-	250	1	-	370	30	394	337-567

grip coiling of prehensile tails" (Hershkovitz, 1977:447). Thus tails play no functional role in locomotor or grasping mechanisms, but do serve "as an accessory support in sitting or as a balancing organ" (Hershkovitz, 1977:447; Moynihan, 1970:4).

Locomotor Behavior and Functional Morphology of the Femur

As this study will document the age changes in the femoral cortex and cortical microstructure at midshaft, attention must be given to the locomotor pattern of S. fuscicollis and S. oedipus, and the functional morphology of the femur reflecting that pattern. This need is based on the phenomenon that "function dictates structure." Femoral morphology, therefore, reflects the mechanical demands (stress or lack of stress) placed upon it (Frost, 1964). The adult form of every skeletal element is accomplished only by extensive cellular (osteoblastic and osteoclastic) modeling and remodeling. Thus, the cortex size and shape and the cortical microstructure data analyzed in this study might be a reflection of function and not limited to the ageing process itself.

Saguinus, as all members of the primate family Callithricidae, are arboreal and diurnal (Epple, 1975:199). Predominantly inhabiting the forest canopy, Hill (1957:180) states that they "are among the most perfectly adapted of

all primates for arboreal existence." Moynihan (1970:3) reports that S. oedipus geoffroyi is "most frequently seen between 10 and 40 feet above ground," although they may go higher (60-75 feet) depending on the forestation.

Moynihan (1970:4) further classifies them as being:

. . . almost completely quadrupedal. They only stand up on their legs (hind limbs) very occasionally, during certain disputes or to get a better view of some distant animal or object. They usually run on the ground and on branches in much the same way as squirrels. But they tend to leap from branch to branch somewhat more frequently than do squirrels . . . and their leaps seem to be somewhat longer on the average.

Herskovitz (1977:45) describes them as:

. . . essentially quadrupedal branch runners and springers with hind limbs averaging approximately 25% longer than forelimbs. The long, sharp claws permit vertical clinging on rough tree trunks and branches and the long hind limbs provide the power for springing and, in rudimentary form, the type of vertical leaping

Thorington (1968:97), observing a number of troops of S. midas, noted that "tamarins frequently made long leaps between trees," the longest measuring 25 feet horizontally with a vertical drop of 25 feet. He continues that "short leaps were frequently made from small branches, but the longest leaps were made from vertical trunks, which provided better launching sites." One Saguinus troop was seen to "regularly move through a grove of . . . trees by leaping five to ten feet from one vertical trunk to another."

Neyman (1978:58) has also observed S. oedipus oedipus as moving "through trees using mainly a quadrupedal gait, at times jumping from one vertical support to another."

Napier and Napier (1967:302) also classify Saguinus locomotor behavior as quadrupedal with springing or jumping considerable distances a prominent feature in the wild. Garber (1980) reports that S. oedipus moves through the forest canopy by a series of long and acrobatic leaps. Castro and Soini (1978) also indicate that S. fuscicollis individuals walk and run along branches quadrupedally with horizontal and diagonal leaps from tree to tree.

Thus, genus Saguinus may be classified as quadrupedal branch runners with hind limbs adapted for powerful springing and leaping. Although this has been well documented for this genus, comparative intraspecies data is almost nonexistent. However Glassman (1983) reports that locomotor behavioral differences between the two sympatric species, S. fuscicollis illigeri and S. oedipus oedipus, have been noted in captive animals at the ORAU marmoset breeding colony.

Glassman (1983:292) states:

. . . . data were obtained from observations of captive animals. Richter and Davis (personal communication) suggest that observable differences can be identified between S. fuscicollis illigeri and S. oedipus oedipus in both the utilization of cage space and locomotor activity. S. fuscicollis sits and walks on the cage floor much more frequently than does S. oedipus which rarely comes down to this level. In terms of locomotion, they suggest

that both progress similarly when walking, yet in terms of rapid movement and leaping, S. oedipus is undoubtedly a more powerful and longer leaper than S. fuscicollis.

Thus, differences in locomotor behavior might be reflected in skeletal morphology and histomorphology.

Jamieson (1904), Beattie (1927), and Hill (1957) have published the major manuscripts on marmoset anatomy. Each discusses the femur and its myology, and while basically agreeing on the anatomy described, Hill (1957:118) informs us that Beattie, at least, is "far from complete and contains errors." Beattie (1927:595) does state that the differences between the genera Callithrix and Saguinus "are so few and so slight that it is doubtful if full generic rank ought to be given to the two divisions." His observations were not supported by later taxonomic revisions as distinctions were recognized and upheld (HersHKovitz, 1977).

Beattie (1927:636-637) states:

The great trochanter is large and possesses an extensive lateral surface which is prolonged downwards and posteriorly to form a postero-lateral flange on the shaft of the bone. Immediately below the lateral surface and half-way to the termination of the flange there is a well-marked third trochanter. The small trochanter is well developed. Posteriorly it is continued down by a thin ridge to meet the flange from the great trochanter. They thus form the linea aspera, which is really a small area lying between a lateral and a medial line, which lines are continuous below with the supracondylar ridges. The two lines never fuse to form the linea aspera which is seen in Man.

Beattie's general description of the femur was accurate, however, and agrees with observations made by this author during the maceration process and while obtaining the femoral measurements used in this study.

Additionally, Hill (1957:135) describes the femur as being "remarkable for its straight shaft There is, however, a slight axial concavity on its flexor aspect. The shaft is circular in section There is no true linea aspera, but its site is marked by a slight longitudinal sulcus The periosteum is thickened over the sulcus and if dried on the bone gives the impression of a linea aspera, and no doubt serves physiologically as such."

The morphology observed when isolating a cross section of the femoral midshaft supports the observations of the femur's gross anatomy. The shaft is generally "circular in section," and lacks a well defined linea aspera. Rather, the posterior surface is marked by two parallel nondescript ridges which create a slight longitudinal sulcus. These serve as the points of insertion for the adductor longus and adductor magnus, both of which lie adjacent to the posterior borders of the vastus intermedius as it extends both medially and laterally around the femur. A generalized muscle bone map is presented in Figure 4, and is derived from the reports of Beattie (1927:665-670), Hill (1957:152-155) and Jamieson (1904).

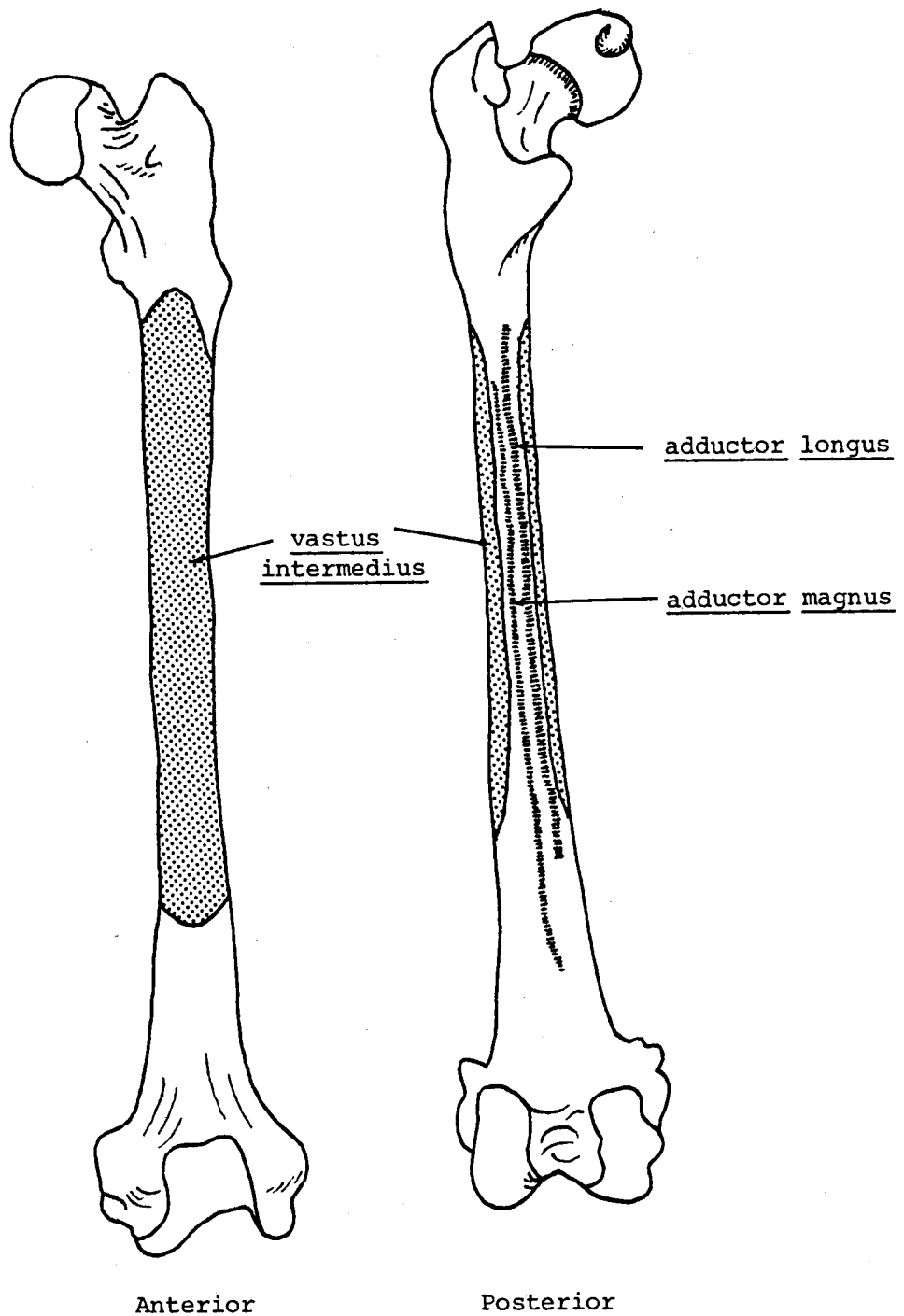


Figure 4. Generalized femoral muscle-bone map of Saguinus. The posterior musculature particularly may affect the cortical histomorphology. Adapted from Beattie (1927), Hill (1957) and Jamieson (1904).

Thus, the femoral anatomy described by these authors illustrates differences when compared to primates of other locomotor groups. These differences might also be reflected in the structure of the femoral cortex and its histomorphology which is under investigation in this thesis. If possible, correlations will be made between the functional morphology of the Saguinus femur and the observed histomorphology.

CHAPTER III

BONE BIOLOGY: THE SKELETAL SYSTEM
AS A LIVING TISSUEIntroduction

Bone is indeed a living tissue. The biological processes which enable bone to grow from the onset of ossification to mature adult bone also perform dynamic biological functions throughout an individual's life. These homeostatic functions force one to view the skeleton as more than inert tissue to be grossly measured; it is a dynamic tissue capable of dramatic changes even after growth has stopped at maturity.

Bone is a highly specialized form of connective tissue and is composed of two basic components. First, an organic or protein matrix onto which is deposited a second component, an inorganic or mineral substance, primarily calcium and phosphate. In addition to these two components, bone has specialized cells which form (osteoblasts), maintain (osteocytes), or rejuvenate, remodel and destroy (osteoclasts) bone. These characteristic bone cells, as do all cells of an organism, arise from one of three cellular germ layers of the embryo. It is with these embryological germ layers that this brief discussion of bone as a tissue, its

origin, growth, development, maturation and degeneration with ageing, must begin.

The genesis of any vertebrate organism commences of course, with fertilization, the union of the nuclei of both the sperm and the ovum. Almost immediately the fertilized ovum (zygote) undergoes rapid cell division. Cleavage occurs so quickly that in three days, the zygote has increased from one to sixteen cells (Arey, 1974). Under normal conditions, this group of cells continues its geometric division as it progresses down the uterine tube and into the uterus of the female.

Once in the uterus the group of cells (now termed a blastocyst) becomes implanted in the endometrium through which a vascular (nutrient) supply is established. The cells of the blastocyst continue to divide and begin to differentiate into the three primary germ layers of embryonic tissue: ectoderm, entoderm and mesoderm. It is from these three basic tissues or pluripotential cells that all specialized tissues and organs of an individual develop. As mesoderm gives rise to cartilage and bone, it is on this germ layer that the discussion will focus.

Mesoderm gives rise to a somewhat more specialized yet still general type of tissue, mesenchyme. Mesenchymal cells are extraordinarily versatile (pluripotential) and further differentiate or specialize depending on the vascular environment or conditions surrounding the cells as they

develop (Arey, 1974:22; Ham and Cormack, 1979:378-381; Ortner and Putschar (1981:8). These specialized cells form all of the connective tissues of the body, serving to support, protect and bind all body structures together.

Mesenchymal cells secrete between themselves a semi-fluid gel, or ground substance, which possesses characteristic protein fibers. The structural or supportive difference between connective tissues depends greatly upon the nature of this ground substance. In fibrous connective tissues the ground substance remains a gel, in cartilage it stiffens greatly, providing more support, while in bone it achieves rigidity by the deposition of calcium salts. Thus, the intercellular component of connective tissue, especially bone, performs a mechanical or supportive function.

The steps which lead to the differentiation of connective tissues further specialize in vertebrate organisms by forming skeletal tissue, a process termed ossification or osteogenesis. The discussion which follows is by no means complete, but is intended as an introduction to the histology of osteogenesis and skeletal remodeling, that of both growth remodeling and secondary remodeling. For a more detailed discussion see Johnson (1964, 1966).

Histomorphology of Osteogenesis and Growth Remodeling

Ossification is the deposition of calcium salts onto a preexisting network of supporting connective tissue. The

timing of the onset of ossification has not yet been established for many primate groups, of which Saguinus is one. Kraus and Hampton (1969) have investigated only the foot in Saguinus oedipus. Thus, there are several areas of research available for the ORAU marmoset colony.

As previously mentioned, mesenchymal cells differentiate into more specialized types of cells depending upon the environment surrounding them. Two basic types of cells arise, chondroblasts and osteoblasts, which are responsible for the formation of cartilage and bone, respectively. These cells initiate the two different sequences by which osteogenesis proceeds: intramembranous or endochondral. These two methods of bone formation do not lead to different types of adult bone; they refer, rather, to the two processes by which ossification begins (Ham and Cormack, 1979: 380).

Intramembranous ossification is the more direct of the two methods and forms the bones of the cranial vault and mandible. Here, osteoblasts differentiate directly from clusters of mesenchymal cells, such a cluster being termed an ossification center. Osteoblasts then form bone.

Endochondral or intracartilagenous ossification differs, however, as osteoblasts are not directly differentiated from mesenchymal cells. Rather, chondroblasts arise first, producing a cartilage preform (anlage) of the

developing bone. Most of the skeletal system is formed in this manner, including the face, base of the skull and the postcranial skeleton. As endochondral ossification gives rise to the femur, this discussion will focus on the osteogenesis and growth of the femoral cortex.

Initially, a model (anlage) of the future bone is formed in cartilage by a surrounding membrane, the perichondrium, which has differentiated from mesenchymal cells. The perichondrium, depending upon its vascular environment, eventually gives rise to osteoblasts instead of cartilage cells, and thus acquires osteogenic properties (Ham and Cormack, 1979: 424; Ortner and Putschar, 1981:8). A sleeve of bone is deposited by the newly formed osteoblasts around the middle portion of the cartilage model. In this manner, the cartilage model is destroyed and replaced with growing bone, the perichondrium being transformed into the bone forming membrane, the periosteum.

Once an ossified shaft (diaphysis) has been established, osteoblasts within the periosteal membrane form bone by first secreting an intercellular organic (protein) matrix which is primarily composed of collagen fibers. Following the formation of this tissue, termed osteoid, mineralization occurs by deposition of calcium salts into the supportive collagen network (calcification).

As the osteoblasts of the periosteum continue to lay down osteoid tissue, some become entrapped within the bone

matrix in microscopic spaces termed lacunae. These trapped osteoblasts become mature bone cells, osteocytes, which later mediate the final stages of mineralization of bone. Johnson (1964:570) states that "when mineralization advances into the osteoid, better than two-thirds of all the calcium is deposited in a matter of hours." Ortner and Putschar (1981:10) report that "full mineralization of bone matrix, particularly in the adult, may take months or years."

During this stage of primary ossification, the fibers are interwoven without the lamellar organization found in mature bone (McLean and Urist, 1968:32). Bone at this stage is thus termed fiber or woven bone. Due to an extensive capillary network at the site of forming osteoid, the cortex is seen in cross section to possess a great number of vascular canals (VC), with relatively evenly distributed osteocytes. Vascular canals occur in bone as capillaries are incorporated into the rapidly expanding cortex of growing bone. No Haversian type of inner circumferential lamellae are seen, thus, the structure is also termed a non-Haversian canal. A photomicrograph is presented in Figure 5 of a femoral midshaft cross section of a S. oedipus oedipus newborn exhibiting these features.

As the periosteum and the expanding cortex become established and more organized, further growth occurs by deposition of lamellar bone. This appears histologically as concentric layers of circumferential bone and is shown

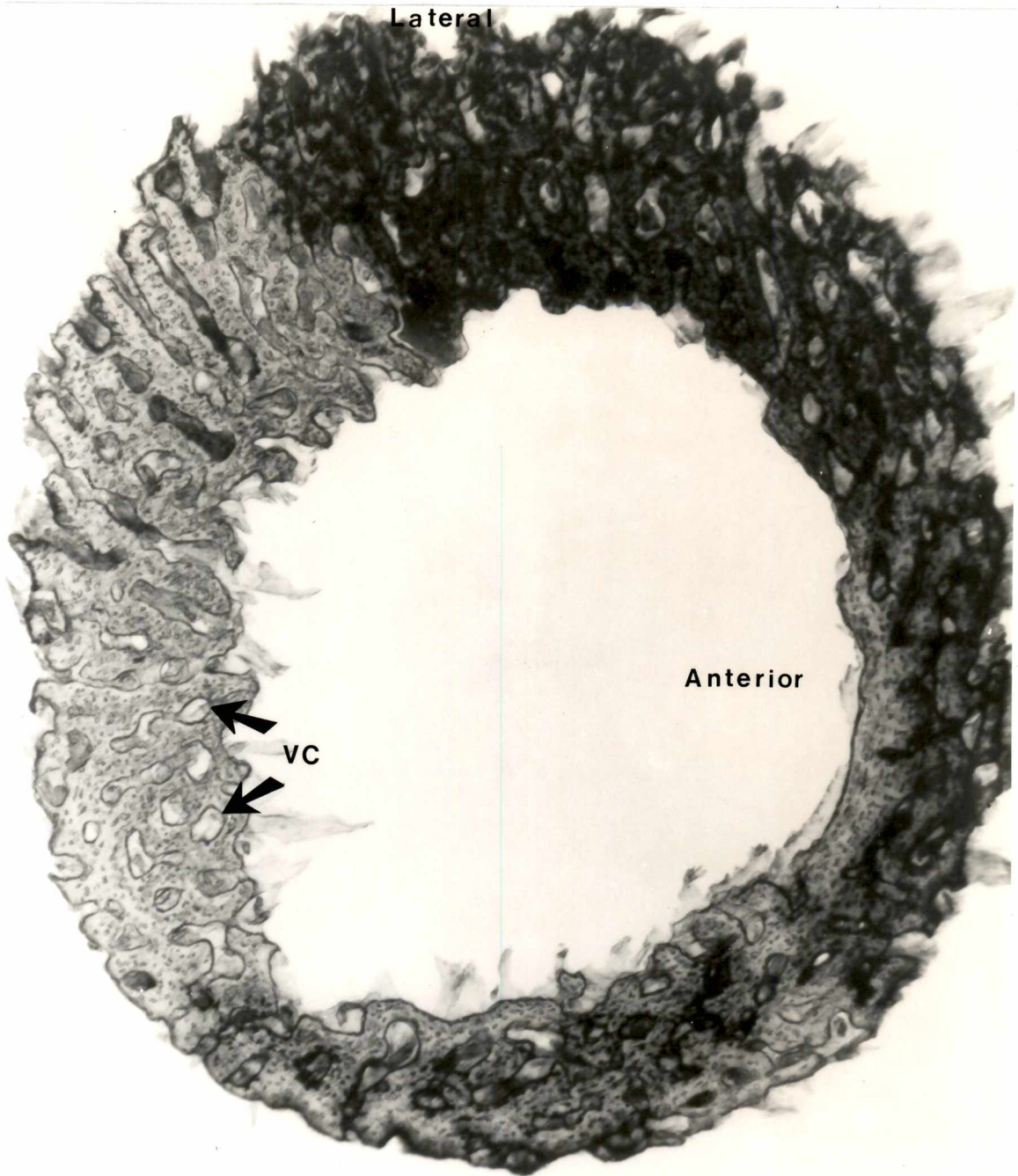


Figure 5. Photomicrograph of an undecalcified femoral midshaft cross section of a one day old male Saguinus oedipus oedipus. At this stage, the cortex is composed entirely of fiber or woven bone. Note the extensive network of vascular canals (VC) throughout the cortex and the absence of Haversian systems. The dark anteriolateral quadrants of the cortex are due to the orientation stain.

in Figure 6 in the femoral midshaft of a three month old female S. fuscicollis illigeri.

During this cortical expansion, there is continual demand for an extensive vascular network to supply proteins and minerals to the site of the forming osteoid tissue. Within the cortex, however, the vascular supply decreases as the nutrient demand decreases. As a result, these vessels constrict within their vascular (non-Haversian) canals, which is followed by the pervasion of the endosteum into the channel. The endosteum is a bone forming membrane and begins to fill the now partially empty canal by depositing, from the outside in, the typical concentric rings of bone seen in a Haversian system.

The resulting histoskeletal feature is termed a primary osteon (PO) and is shown in both normal transmitted and polarized light in Figure 7 in a nine month old male S. fuscicollis lagonotus. Primary osteons, therefore, are a result of blood vessels being incorporated into a rapidly expanding cortex. They have a separate origin and are distinguishable from secondary osteons which will be discussed under secondary remodeling.

For bone to reach its adult size and shape, its growth is dependent not only on the apposition of cortical bone, but internal resorption as well. While lamellar bone is being deposited periosteally, osteoclasts come in to play on

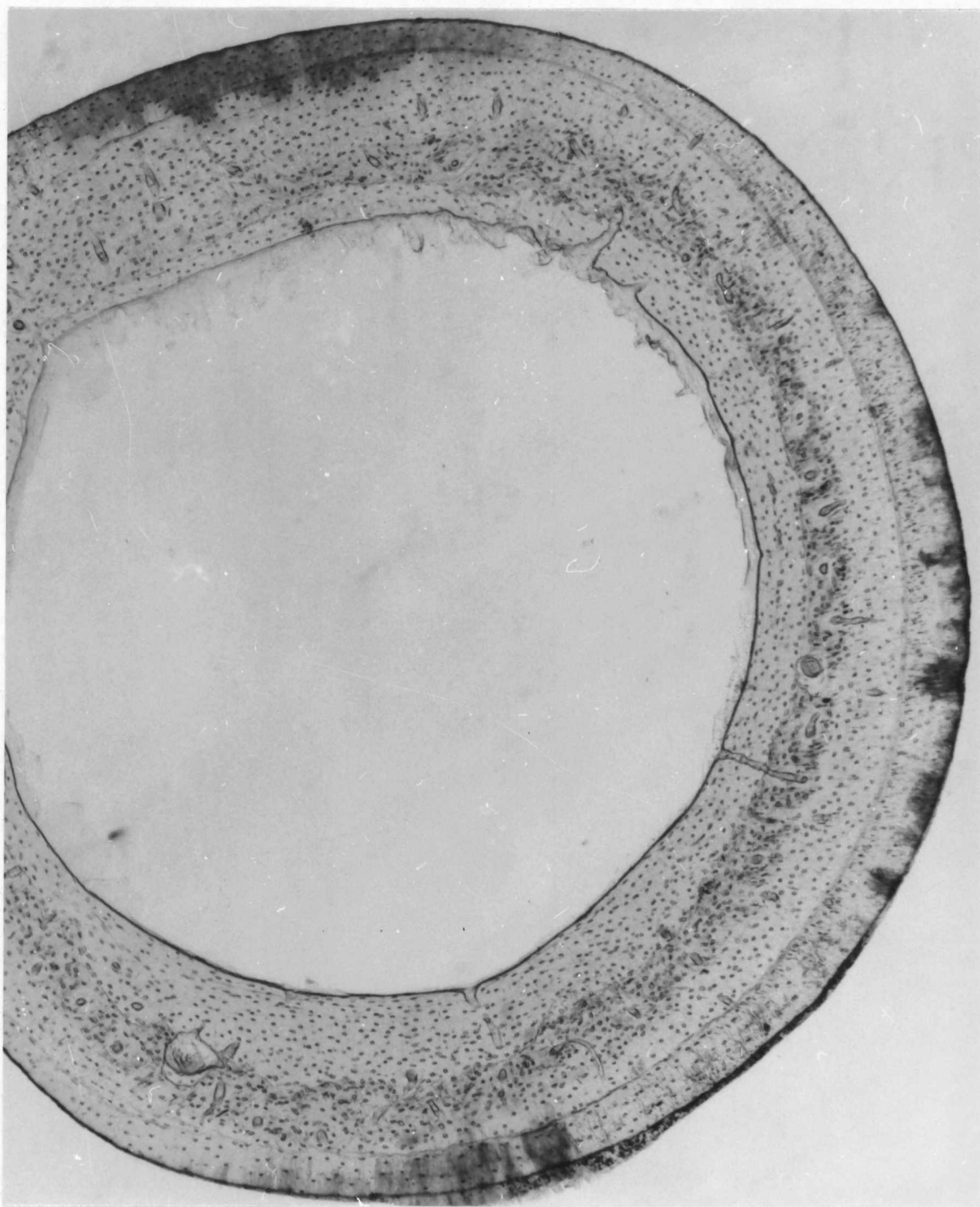


Figure 6. Photomicrograph of an undecalcified femoral midshaft cross section of a three month old female Saguinus fuscicollis illigeri. The cortex is composed of circumferential lamellar bone which can be seen in the organized pattern of osteocyte distribution.

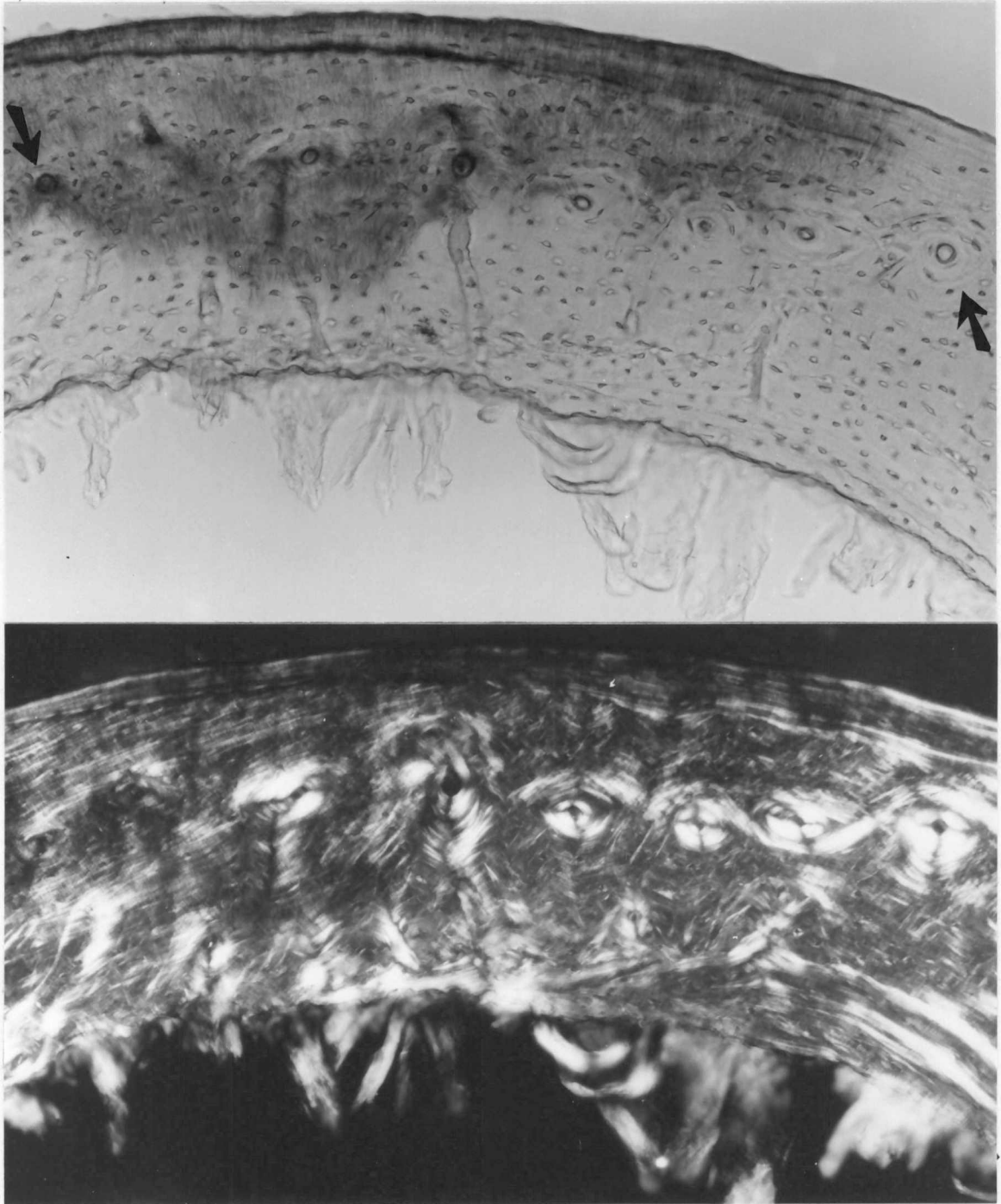


Figure 7. Photomicrograph of the medial quadrant of an undecalcified femoral midshaft cross section of a nine month old male *Saguinus fuscicollis lagonotus*. Both normal transmitted light (above) and polarized light (below) are presented. The arrows indicate a row of eight primary osteons in the center of the cortex.

the endosteal surface. These serve to remove bone, thereby expanding the medullary cavity and providing space for hemopoietic marrow tissue (Ortner and Putschar, 1981:16).

The result of this osteoclastic action is shown in Figure 8, in a femoral midshaft cross section of a six month old male S. fuscicollis illigeri. Here, the scalloping of osteoclastic resorption is seen along the entire endosteal surface. Massive resorption spaces are also seen in the anteriomedial portion of the section, with vascular canals and primary osteons scattered throughout the cortex. Thus, the original cortex, such as one seen in Figure 5, has been completely resorbed by the growth process.

This process, however, does not continue to occur equally on all surfaces of a growing bone. Enlow (1963, 1976) describes the phenomenon of cortical drift, where apposition and resorption occur on opposite sides of a localized region of the cortex. For example, the anterior cortex may exhibit periosteal apposition and endosteal resorption, while the posterior cortex undergoes endosteal apposition and periosteal resorption. The cortex will therefore "drift" anteriorly while the medial and lateral quadrants appear stable. This results in a change in the position and/or shape of the cortex.

While cortical growth continues until maturity, the process of drift may occur throughout adulthood as it is not

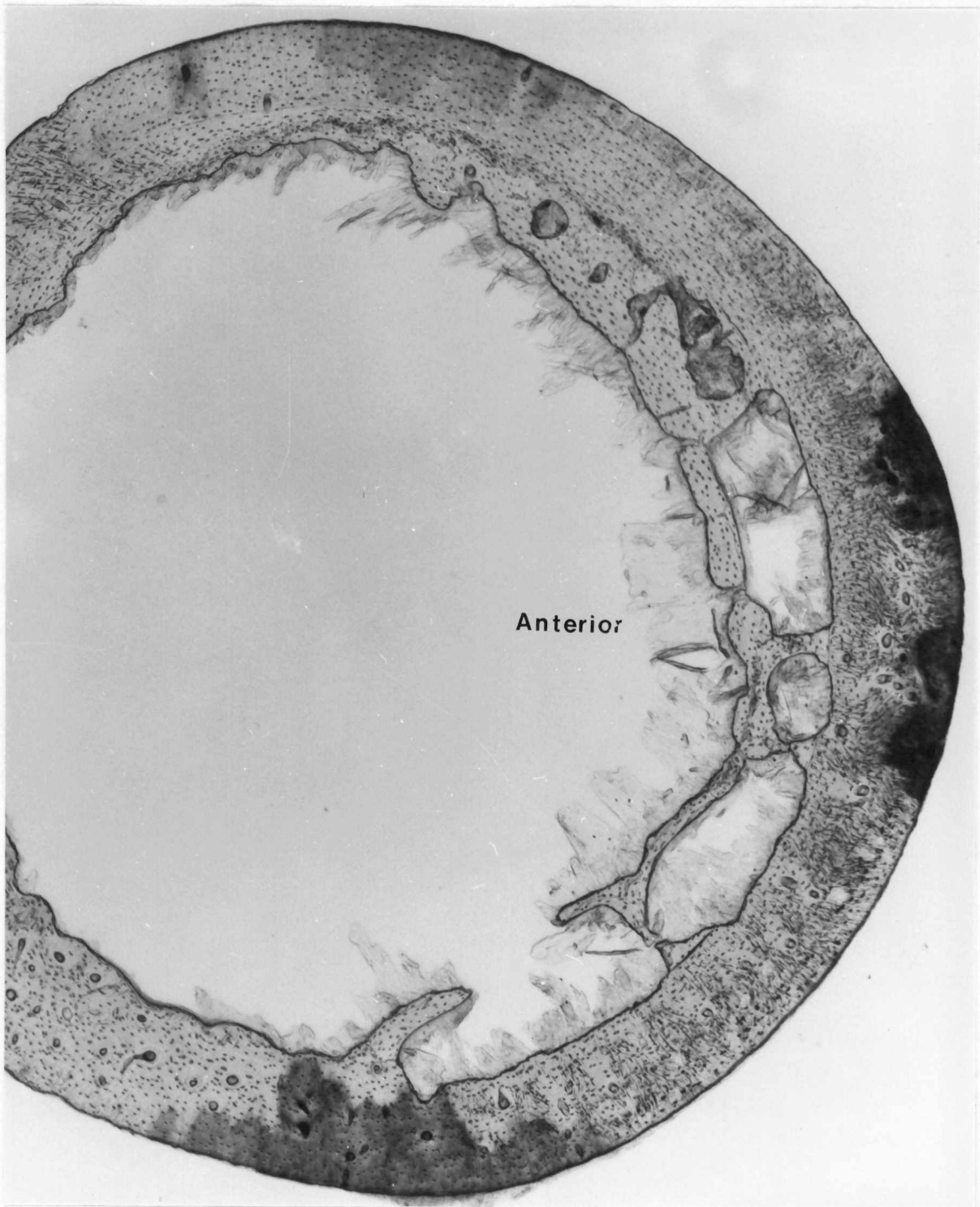


Figure 8. Photomicrograph of an undecalcified femoral midshaft cross section of a six month old male Saguinus fuscicollis illigeri. The scalloped appearance of the endosteal border and the large resorption spaces in the anterior half of the cortex is evidence of osteoclastic action enlarging the marrow cavity. Apposition of circumferential lamellae periosteally is evidence of cortical expansion or growth.

only under genetic control, but is also a response to mechanical changes in weight bearing bones such as the femur. The function of this growth remodeling is primarily that of structural or architectural modeling to transform bone from its cartilage preform to its adult form. This differs from secondary remodeling, however, which is described below.

Histomorphology of Secondary Remodeling

The secondary remodeling of skeletal tissue may also be termed internal, Haversian, or osteon remodeling. Although it appears first during growth and involves bone cells (osteoclasts and osteoblasts), secondary remodeling differs from growth remodeling as it serves to renew bone tissue (Ham and Cormack, 1979:441; Ortner and Putschar, 1981:19). Enlow (1976:30) refers to the process as Haversian reconstruction since it involves the replacement of primary bone.

The process itself involves the removal of cortical bone by osteoclastic activity which leaves a resorption space (RS) or, rather, a resorption tunnel through the cortex. Once osteoclastic activity has ceased, the space is pervaded by the endosteum and a vascular supply (capillary). As in a primary osteon, the osteoblasts of the endosteum deposit concentric lamellae within the cavity from the

periphery inward until only a small central canal remains through which the capillary passes.

During its fill-in stage, the structure is termed a forming osteon (FO), and once completed, is termed a Haversian system or secondary osteon (SO). Figure 9 presents each of these features in an adult female S. fuscicollis illigeri. A higher magnification of the posterior quadrant in Figure 9 is presented in Figure 10 utilizing polarized light. Evident is the sharp reversal line at the periphery of each secondary osteon. This reversal line is not seen in primary osteons (as is evident in Figure 7) which is one method of distinguishing the two.

Once secondary remodeling begins intracortically, it continues throughout the lifespan of the individual. Subsequent osteoclastic action will often tunnel through an existing secondary osteon. If the vascular canal is destroyed as a result, the osteon loses its capillary supply and becomes what is termed an osteon fragment (OF). (Several authors define this structure differently which will be discussed in Chapter IV). Figure 11 presents a femoral cross section of an adult female S. fuscicollis illigeri which possesses several osteon fragments. Near the endosteal border is seen a field of both osteons and osteon fragments sandwiched in between two layers of circumferential lamellae. Views under both normal transmitted and polarized light are presented.

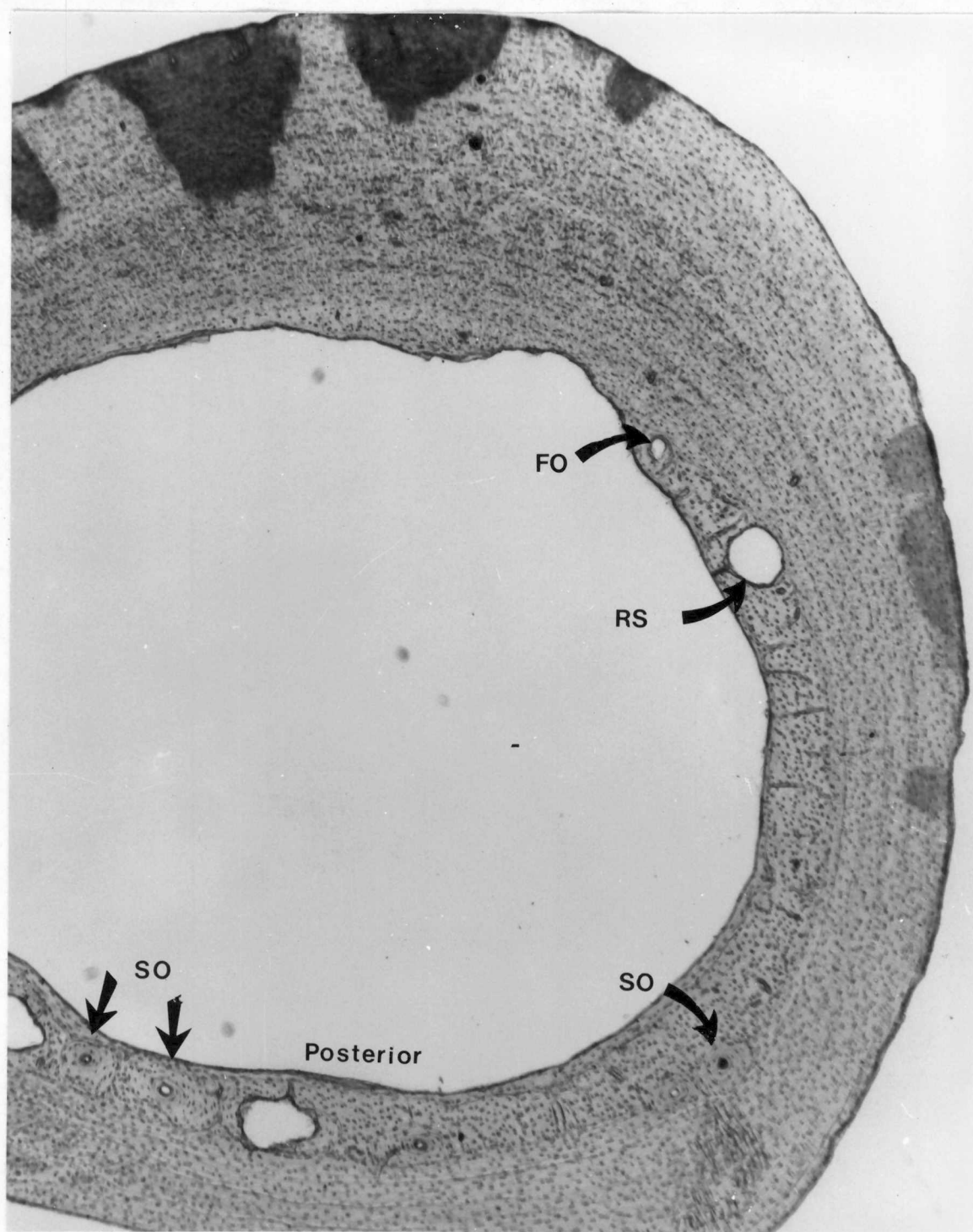


Figure 9. Photomicrograph of an undecalcified femoral midshaft cross section of a five and one half year old female Saguinus fuscicollis illigeri. Along the endosteal border are seen three resorption spaces (RS), a forming osteon (FO) and several secondary osteons (SO). An enlargement of the posterior quadrant is presented in Figure 10.

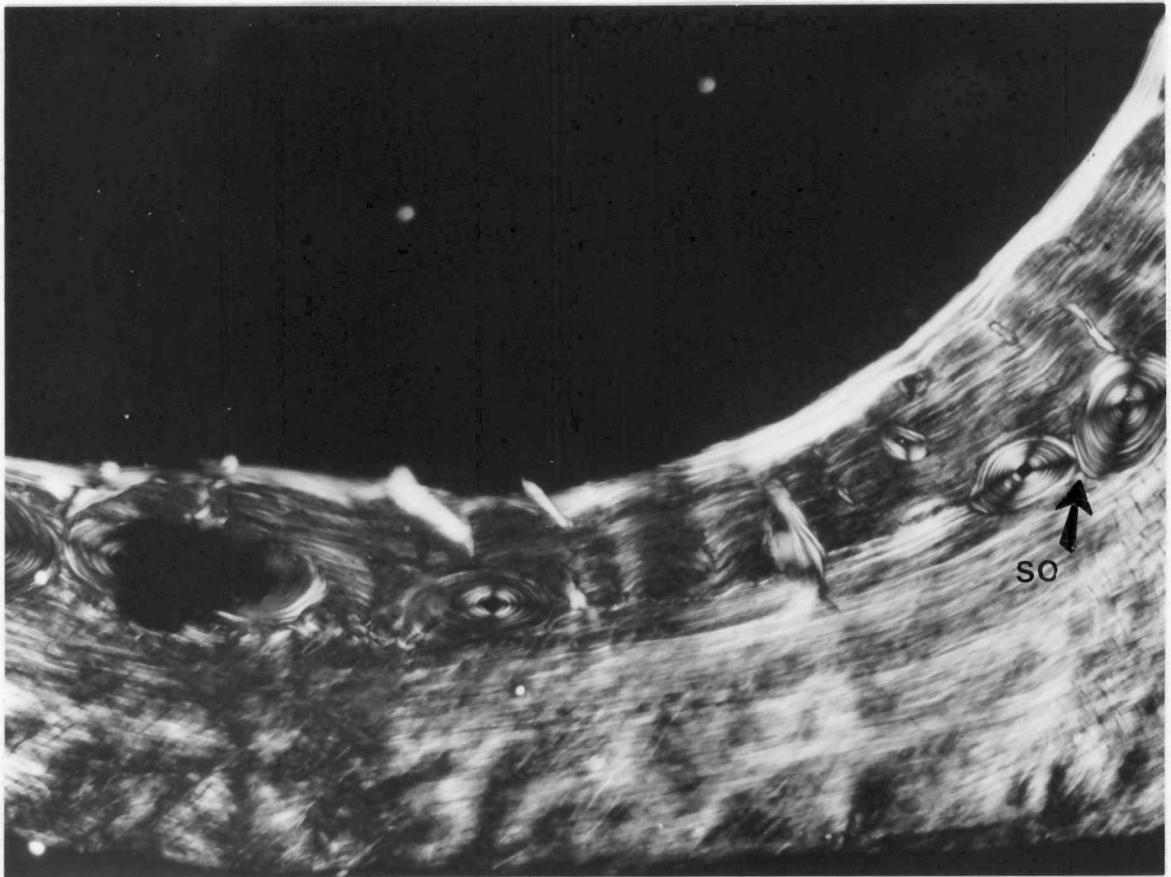


Figure 10. Polarized photomicrographic enlargement of the posterior quadrant of the undecalcified section presented in Figure 9. Under polarized light, secondary osteons (SO) are easily distinguishable from the surrounding cortex.

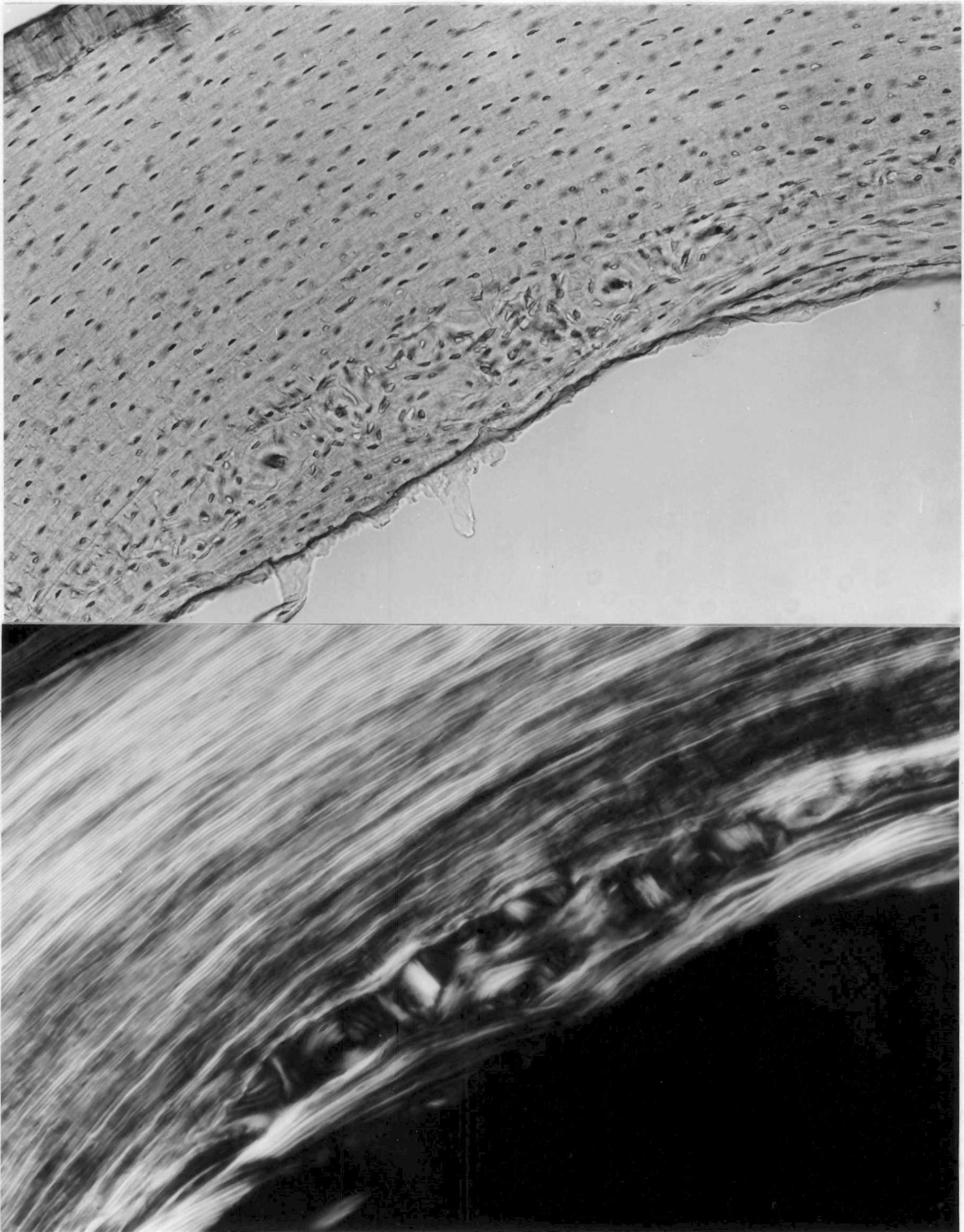


Figure 11. Photomicrograph of the medial quadrant of an undecalcified femoral midshaft cross section of a six and one half year old female *Saguinus fuscicollis illigeri*. Both normal transmitted light (above) and polarized light (below) are presented. The periosteal border is visible in the upper left corner. A field of secondary osteons and osteon fragments is seen near the endosteal border between layers of circumferential lamellae.

The process of secondary remodeling, as mentioned, continues throughout the lifespan of an individual. Once old age is reached, however, an overriding phenomenon commonly begins which has been described extensively for humans (Carlson et al., 1976; Epker, 1973; Garn, 1970; Van Gerven, 1973; Van Gerven et al., 1969) but for few other primates (Bowden et al., 1979). This is the process of cortical involution or loss of the cortex along its endosteal border due to osteoclastic activity. Although periosteal apposition may continue at a very slow rate after maturity (Epker and Frost, 1966; Garn, 1970), endosteal resorption exceeds apposition once senescence is reached, resulting in overall cortical thinning.

Cortical involution is evident in Figure 12 in a ten and one half year old male S. fuscicollis illigeri. Here, the cortex of the posterior quadrant is seen to be particularly thin. Note also that only a few secondary osteons are present within the cortex. All evidence of previous secondary remodeling activity has been lost as a result of cortical involution. Although this is not the case in humans and other large mammals, it is a significant factor in Saguinus, the implications of which will be discussed in Chapter V.

Thus, the stages of embryonic osteogenesis, subadult growth, secondary remodeling and the cortical involution of senescence all exhibit different histomorphological features. These aspects will be investigated in this thesis.

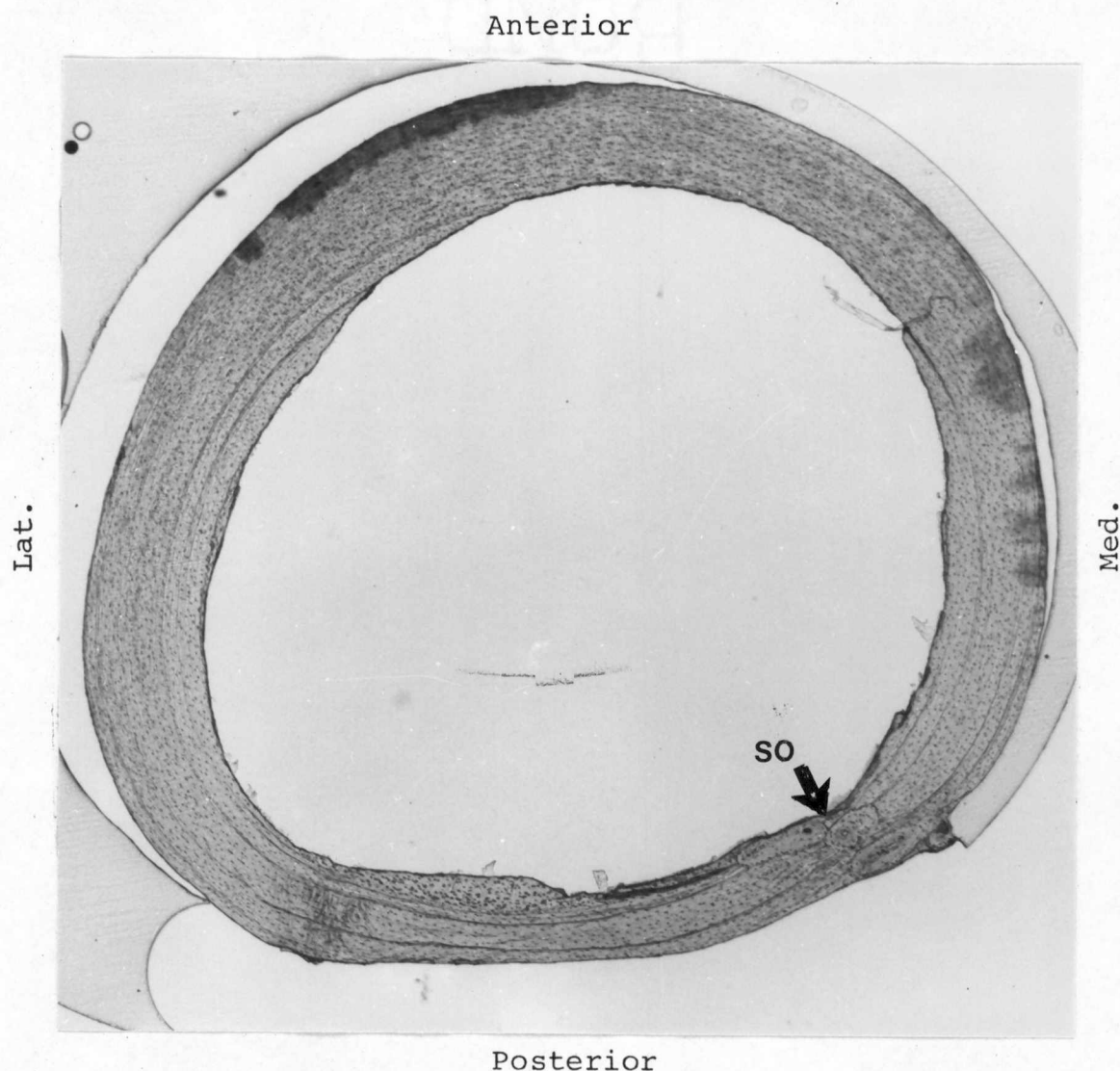


Figure 12. Photomicrograph of an undecalcified femoral midshaft cross section of a ten and one half year old male Saguinus fuscicollis illigeri. The cortex has become extremely thin as a result of cortical involution. The posterior quadrant has become particularly thin and several secondary osteons (SO) are seen at the site of muscle attachment.

Histoskeletal Age Change Studies: Historical Note

Physical Anthropology's role in the study of bone biology and the importance of Haversian system remodeling, has only recently come of age. Its importance was appreciated nearly seventy-five years ago, however, by Dr. Ales Hrdlicka, curator, Division of Physical Anthropology, Smithsonian Institution. Hrdlicka became interested in the pioneering work of Dr. J. S. Foote, of the Creighton Medical College, who had published initial findings on the comparative histology of the femur (Foote, 1911).

Hrdlicka recognized the need and potential for comparative histology studies and greatly supported them. Based on this support, Foote was able to extend his research well beyond the initial 1911 report utilizing a number of specimens which Hrdlicka provided from the Smithsonian's collections. Due to Hrdlicka's personal recommendation, Foote published, through the Smithsonian Institution, a follow-up report (Foote, 1913), and later an extensive manuscript in the Smithsonian Contributions to Knowledge (Foote, 1916).

One cannot help but reason that Hrdlicka, who was at that time conducting his own research into the normal variation of the shaft of long bones (Hrdlicka, 1934a; 1934b), expected Foote to discover histological differences between human races, just as species differences had been found.

Foote's (1916) contribution documented the histology of the femoral midshaft based on sections of 39 amphibians, 34 reptiles, 40 birds and 334 mammals, including 19 nonhuman primates and 146 humans. He also attempted to include individuals of different ages, and classified or "typed" each animal based on its histological features. These were assessed by three structural aspects: lamellae, laminae, and Haversian systems. His manuscript remains, to date, the only comprehensive published research on comparative skeletal histology.

The half century following Foote's manuscript saw little in the way of this type of research, especially that dealing with histological ageing. The work of Enlow (1957) and Jowsey (1960) began a period of renewed interest in histoskeletal changes with age (e.g., Atkinson, 1969; Atkinson et al., 1962; Chatterji and Jeffery, 1968; Currey, 1964; Epker and Frost, 1966; Sedlin et al., 1963). It was during this period that Physical Anthropology began to appreciate the potential of skeletal histomorphology first realized by Hrdlicka in the early 1900s.

In the early 1960s, Dr. Ellis Kerley, then at the Armed Forces Institute of Pathology, Washington, D.C., investigated one aspect of the Haversian system and its role in the remodeling of skeletal tissue. Using a documented human sample of undecalcified sections from the midshaft of the

femur, tibia and fibula, Kerley (1965) identified changes with age in the type and quantity of Haversian systems. Once these changes were quantified, he formulated a standardized technique whereby one is able to evaluate the cortical microstructure of unidentified individuals and arrive at an accurate estimation of skeletal age at death.

Since Kerley's study, numerous approaches have been applied to microscopic age changes in bone. Bouvier and Ubelaker (1977) and Stout and Gehlert (1980) both present comparisons between several of these methods in a test of their reliability. Both studies indicate that Kerley's method of "osteon counting" is the more reliable.

Histomorphological age changes in bone, however, have not been limited to osteon counting. Studies have included measures of bone mass and bone turnover rates, and have employed microradiography, autoradiography, tetracycline labeling and x-ray, to mention but a few. The volume of research compounded on these parameters is simply too extensive to list or discuss here. Several of the most important studies have been mentioned in the previous discussion.

The potential application of skeletal histology to anthropological analysis and interpretation, however, is largely unrealized. Ortner (1977) presented an introductory background to bone biology and demonstrated its applicability to understanding biological problems from an

anthropological view. It is the attempt of this thesis to contribute to our knowledge of bone biology from such an anthropological perspective.

CHAPTER IV

METHODS

Histological Technique: Introduction

Any study of skeletal histomorphology must begin with an appropriate method to section the material for microscopic study. As there are numerous techniques whereby sectioning may be accomplished, a brief description of the most common procedures is presented here. The importance of this is that selection of an appropriate method is essential, as different techniques yield different types of microscopic sections. The investigator must be aware that the sectioning procedure employed will produce sections applicable to the proposed research.

Standard histological preparation of osteological material usually commences with decalcification (Brain, 1966:3; Brown, 1978:39-51; Mancl, 1982; Sheehan and Hrapchak, 1980: 89-96). Decalcification is the complete removal of the calcium and other mineral salts from mineralized tissues. As with all histological techniques, the procedure has both advantages and disadvantages.

Decalcified bone, easily sectioned on a standard microtome, yields sections of desired thinness for light microscopy, usually ranging from four to twelve microns (Mancl, 1983). Sections of this size afford better resolution of

microstructures under a wide range of magnifications while enhancing the ability of skeletal tissue to accept histochemical stains. As most living cells are unpigmented, staining increases the contrast of tissue components enabling higher contrast of structural details (Bloom and Fawcett, 1968:10; Ham and Cormack, 1979:15). An added advantage is the fact that prior to decalcification, skeletal tissues are fixed (preserved) in formalin. This serves to "freeze" bone cells in action which then may be viewed after sectioning. Thus, decalcification of skeletal tissue followed by histochemical staining highlights desired tissue or cytological features, facilitating microscopic analysis and interpretation of both normal and pathological bone.

There are, however, several disadvantages to decalcification. First, there may be a loss or alteration of organic materials within bone since these compounds may be extracted along with the inorganic or mineral component of bone (Bonucci and Reurink, 1978:179; Schajowicz and Cabrini, 1955). Second, there is a structural change which occurs with the removal of mineral salts from the organic (protein) matrix of the bone. This structural change manifests itself in the form of tissue shrinkage (Frost, 1958:276-277; Frost, 1973a: 11; Cathey, 1963:216), an often ignored aspect of histoskeletal preparation. Recently, however, Lane and Ralis (1983) have fully documented the amount of shrinkage of

decalcified bone based on sectioning human femoral heads. They recorded up to 25-30% shrinkage after the completion of all steps of preparation and mounting. Although shrinkage for thicker cortical bone (i.e., femoral midshaft) is not likely to be nearly as much, at least a small percentage will undoubtedly occur.

If the underlying research problem involves the quantification of the area of bone and/or area of cortical remodeling, an undetermined amount of tissue shrinkage introduces an additional unknown into the study. Thus, decalcification was not an acceptable technique of microsectioning for use in this study. Instead, undecalcified thin sections of bone were obtained in order to eliminate any possibility of tissue shrinkage.

Several histological techniques to section undecalcified bone have been introduced that yield sections thin enough for light microscopy. Due to the mineralized nature of bone these histological techniques have been adapted from methods traditionally used to section petrographic (Moreland, 1968; Buehler, 1976; 1980), or metallographic substances (Buehler, 1973; Kosick, 1979). These techniques, whether borrowed or innovated, may be divided into three major groups: (1) the Frost or hand method, (2) the microtome, and (3) thin section machines. Each method is only briefly outlined here as all investigators adapt the general procedures described below to their specific needs.

First is the hand or Frost method, introduced by Frost (1958) and subsequently named after him. Slices of bone 1-2 mm thick are obtained with a fine-toothed hack saw. A sheet of abrasive carborundum paper (320-400 grit) is placed on plate glass, lubricated with water, and the bone is ground by hand with a circular motion. Once the section thins, it is held in place while grinding continues with another piece of carborundum paper covering a hand held glass slide. Frost (1958:273) claims that sections from one hundred to three microns are obtainable. Modifications of this procedure have been described and utilized by Anderson (1982:38-40), Bourque et al. (1978), Laws (1952), and Villanueva (1973:341-342; 1980:97-98). The technique has been widely applied as it is simple and inexpensive, although it requires steady hands, a great deal of practice and care to prevent tilting the section while grinding.

The second method employs a microtome which possesses a razor sharp knife to slice sections just a few microns thick. Microtomes are almost exclusively designed to cut soft tissues and decalcified bone, but have been adapted to section undecalcified specimens as well. These adaptations require special "heavy duty" microtomes which possess a glass or diamond knife. The most common are the Jung-K microtome (Anderson 1982:41), the Leitz Sledge microtome (Villanueva, 1980:97) and the Sorvall JB-4 microtome

(Sheehan and Hrapchak, 1980:85). A critical step is the embedment of the specimen in methyl methacrylate or some other type of durable yet sectionable plastic. This procedure yields sections of four to ten microns and is of special use in autoradiographic and microradiographic studies (Arnold, 1951; Axelrod, 1947; Low and Cowan, 1963; Woodruff and Norris, 1955).

The third group of undecalcified bone sectioning techniques are those that use thin sectioning machines. These are of two types, high speed saws which operate in excess of 2,200 rpm, and low speed saws which operate at less than 300 rpm. While high speed saws employ either "toothed" carbon steel or circular diamond bonded blades, the low speed saw utilizes exclusively the latter.

High speed saws were originally designed to section ceramic or petrographic materials (Moreland, 1968), but they have also been widely applied to undecalcified bone. One of the first high speed procedures applied to bone was the adaption of an old brain microtome to a war surplus rotary aircraft motor (Roofe et al., 1949). Mention is made of the fact that the circular blades were manufactured by stamping, and as a result were often off center. Since this resulted in "excessive vibration which interfered with the cutting of very thin sections," instructions were given to overcome this difficulty (Roofe et al., 1949:620). Later adaptations

of high speed saws have included "gang sawing" for simultaneously cutting serial sections (Kropp, 1956), or modified procedures for teeth (Fisher and Mackenzie, 1954; Perrin and Myrick, 1980, Sognnaes, 1947), as well as bone (Istock and Miller, 1958; Jowsey, 1955; Ortner, 1970:31-36; Schott, 1969; Ubelaker, 1974:54-56).

High speed sectioning of undecalcified bone, whether with carbon steel "toothed" or diamond bonded blade, follows essentially the same basic steps as presented by Moreland (1968) and Ortner (1970:31-36). Briefly, the specimen is first reduced to a workable piece (5-10 mm) on an Ingram thin section saw, cleaned (especially archaeological specimens), and then impregnated under a vacuum in either epoxy or soluble plastic. Next, the specimen is passed through a series of steps to first grind, polish and mount one side permanently with epoxy onto a glass microslide, and then cut, grind and polish the specimen down to a microscopically visible 80-100 microns. Note that grinding and polishing wheels are required in addition to the high speed saw. For a more detailed description, see Ortner (1970:30-36).

As all cutting and grinding steps are water cooled, problems may arise when the dry bone absorbs the water, which leads to expansion which in turn may cause the slide to crack (Ubelaker, 1974:55). Although the technique is

practical, additional problems are encountered in maintaining an even thickness and securely mounting the section onto the slide (Ortner, 1970:31-32). Also, much of the originally cut slice of bone is lost during the grinding process. The procedure is therefore time consuming as well as destructive and may utilize 3-5 mm of bone to produce a single section.

Additionally, high speed cutting occurs at no less than 2,200 rpm and in some cases is as high as 20,000 rpm (Istock and Miller, 1958:497). Thus, other problems such as cracking, burning or shattering of the specimen may occur (Istock and Miller, 1958:497, McQueen et al., 1972:23-24) making this not the most desirable method.

Sectioning by low speed thin section saw, on the other hand, has few if any of these problems. As mentioned, most saws of this type operate at speeds between 10-300 rpm, so there is little chance of the specimen cracking or burning due to the sectioning process itself. The debut of the low speed saw equipped with diamond bonded blade has introduced a much less destructive and more versatile microsectioning process for tooth and bone. Several models that have been introduced include the Macrotome II (Metals Research Ltd., Royston, England) described by McQueen et al. (1972), the Gillings-Hamco sectioning machine (Bronwill Scientific Division, Rochester, NY) described by Kwan (1970), Seliger

(1968) and Smith and Karagianes (1974), and more recently, the Buehler Isomet 11-1180 (A. Buehler, Ltd., Evanston, Ill.) described by Anderson (1982:36-39), Buehler (1978), and Clark (1980: 605). Of these, the Buehler Isomet is becoming the most widely used in histology and histopathology as well as archaeological and forensic investigation laboratories due to its portability, relative inexpensiveness, and great versatility. Sectioning procedures for all of these machines is essentially the same, so attention will focus on just one, the Buehler Isomet which was utilized in this study.

In the typical hospital or histopathology laboratory, the low speed thin sectioning machine is generally not used to microsection, but rather to cut a slab approximately 5 mm thick from a surgical or autopsy specimen. A specimen of this size greatly facilitates the decalcification of the tissue, reducing processing time by a matter of days (Sheehan and Hrapchak, 1980:36-37). Sectioning of archaeological, dental or osteological materials, differs however, as the purpose is to directly produce a microsection visible for light microscopy. Therefore an 80-100 micron section is cut directly from the parent sample or specimen. This was the procedure used in this research, so is described in detail here.

Microsectioning of Saguinus Femora

A femur from each of the one hundred twelve Saguinus individuals used in this research was microsectioned at midshaft. The left femur was used whenever possible, however, the right was substituted when necessary. Prior to this measurements were taken which would be unobtainable after sectioning was completed. As described by Bass (1971:168) and Wilder (1920:122), these measurements are: (1) maximum length, (2) physiological length, (3) maximum lateral length (trochanteric), (4) maximum medial length, (5) maximum diaphyseal length, and (6,7) maximum and minimum midshaft diameters. Measurements were taken with standard metric dial calipers to the nearest 0.1 millimeters. The midshaft was measured and then marked with a graphite pencil to facilitate later orientation.

Since the femoral shaft of marmosets is nondescript, there being no prominent linea aspera as in humans, it was necessary to stain the midshaft prior to sectioning to facilitate later anterior-posterior and medial-lateral orientation. Although staining of undecalcified bone has been widely utilized (Frost, 1959, 1964; Fullmer et al., 1964; Irving, 1958; Villaneuva, 1974, 1979; Villanueva et al., 1964), the method applied in the present study was not staining per se. Rather cardinal points at the midshaft of

each bone were color coded for orientation. This was accomplished by utilizing indelible Sharpie felt tip pens (red = anterior, green = posterior, blue = medial, purple or orange = lateral), which survived subsequent cleaning in organic solvents (xylene or acetone). Later, mounted thin sections were oriented with the remaining halves of the bone to insure correct orientation and color coding of cardinal points.

Next, specimens were embedded in or coated with standard epoxy. Since bones may crack during sectioning it is usually necessary to embed them. Kwan (1970), McQueen et al. (1972:24), Sognnaes (1947), and Villanueva (1973) have described the technique and need for specimen embedding. To minimize the likelihood of cortical breakage, only fresh bone with organic matrix intact may be sectioned without embedment. Even so, approximately 0.1 mm of the cortical periosteal border will fracture or "hinge" as the blade passes through the last 0.1 mm of the section just before completion of the cut. If this slight amount of cortex is sacrificeable, then embedment may not be required. With the current study, however, it was found that due to the extremely thin cortex of the Saguinus femur (0.07-1.14 mm), even the slightest loss of the periosteal border would result in a noticeable decrease in the total cortical area (TCA), hence, inaccurate results. Thus, the sectioning

technique described by Glassman (1982:63-65) was not acceptable. The only way to prevent breakage or "hinging" of the cortex was to first embed the specimen. Fragile and small specimens (less than four months of age) were completely embedded in standard epoxy. With larger specimens, it was found adequate to coat the midshaft with a thin (2 mm) layer of epoxy. This prevented any "hinging" effect and breakage or loss of the cortex. Once hardened, each specimen was ready for sectioning utilizing the Isomet low speed saw with a four inch circular, high concentration diamond wafering blade. The Isomet possesses a pivoting, counterbalanced arm for regulation of specimen pressure against the blade. The specimen is therefore gravity fed onto the blade, the counterbalanced arm allowing a specimen to literally float on the cutting edge if no additional weight is added. Attachable to this arm are a variety of specimen holders or chucks which allow specimens of almost any shape to be securely fastened and oriented in any direction with respect to the cutting edge of the blade. In addition, the Isomet possesses an automatic cut-off switch which stops action after completion of the cut, as well as a moveable, calibrated gauge at the base of the pivoting arm. This gauge allows movement of the arm (i.e., specimen) with respect to the blade, enabling the operator to select the precise location of the initial cut and later the desired thickness of the

section. As mentioned, there is variable speed control ranging from 0-300 rpm. During the cutting procedure, the specimen and blade are cooled as the diamond edge of the blade is submerged in a liquid bath. Although Buehler, Ltd., manufactures a special fluid for this purpose (Iso-cut), it was found that a water bath into which was placed a few milliliters of aerosol solution or dishwashing liquid was adequate. As the blade passes through the bath, it drags the cooling solution with it and onto the specimen.

After embedment, each femur was cushioned by being wrapped in a thin (4 mm) layer of cotton or styrofoam. The holding chuck is essentially a miniature clamp or vise, and when securely mounted, this padding prevented the specimen from cracking. The chuck with specimen properly secured was then attached to the pivoting arm and the gauge dialed to move the specimen into correct cutting position. When obtaining sections from a specific location, the thickness of the diamond wafering blade, 305 microns, must be taken into account as well as any slight deviation of the blade due to warpage. This is due to the fact that this portion of the section will be ground away. Thus, to obtain two 100 micron sections would require three cuts, totaling approximately 1.3 mm of the specimen expended. (This procedure has an advantage over high speed sectioning which may require up to 5 mm of a specimen to produce a single section.)

Therefore, the first cut was made approximately 0.75 mm off center.

The specimen arm was weighted with a 25 gram weight, the blade set in motion at 85 rpm, and the specimen, oriented perpendicular and at a right angle to the blade, was gently lowered onto the cutting edge. Care must be taken to insure that the blade is not deflected by any uneven epoxy embedding medium. Once cutting was established, blade speed was increased to approximately 165 rpm and was later reduced just prior to completion of the cut. The saw automatically shuts off as the arm drops onto the cut off switch.

With the initial cut, the section has been cut in half. Next the specimen must be moved approximately 0.4 millimeters in relation to the blade to first account for the 305 micron thickness of the blade and then the desired thickness of the section. After completion of the second cut, the thinness of the resulting section is measured with a micrometer caliper, optimal thinness being 80-90 microns. As sections were often 100-120 microns thick, it was necessary to fine grind and polish most of them.

Fine grinding was accomplished by a slight adaption of the Frost method, previously described. Carbi-met paper, 600 grit, was placed on plate glass and lubricated with water. The section was placed on the carbimet and using a finger

tip only, was gently ground in a circular motion. Often, the thinness of the specimen was checked as it is easy to go below 80 microns and begin to grind away the edges of the section. This was found to be a most difficult and therefore critical step.

When a thickness between 80-90 microns was achieved, the section was polished using Metadi 6 micron diamond polishing compound (Buehler, Ltd.), lubricated with a light oil, not water. Polishing removed most or all scratches which had resulted from the fine grinding process.

Next, the specimen was cleaned in a 25 ml beaker with 5 ml of xylene which was partially suspended in the water bath of an ultrasonic cleaner. This was done for one half minute and served to free the section, including its vascular channels, from the grit of the sectioning process.

Each section was then mounted and cover slipped on a standard 1 x 3 inch glass microslide with Permount or Protex medium. Due to the relative thickness of the sections, it was necessary to weight each cover slip for a twenty-four hour period as the mounting medium cured to prevent curling of the section.

Once completed, each slide was labeled with its identification number. No record of the individual's age was recorded on the slide label.

Analysis of Cortex and Histomorphology

Each of the 86 Saguinus fuscicollis and 26 Saguinus oedipus sections were analyzed for growth and development of the femur as reflected at midshaft. This analysis was based on two sets of parameters, (1) dimensions of the cortex, and (2) quantification of histomorphological structures. The entire cortex was analyzed. This analysis will establish the patterning of both macro and microstructural cortical changes with age.

Measurement of Cortical Dimensions

To determine the age related changes of the femoral cortex from birth, the following areas were calculated and distances measured directly from the cortex and are shown in Figures 13 and 14:

Cortical Area Calculations

1. Total Periosteal Area (TPA)--the total area of the section calculated by tracing the periosteal perimeter of the cortex.
2. Total Endosteal Area (TEA)--the total area of the marrow cavity calculated by tracing the endosteal border of the cortex.
3. Total Cortical Area (TCA)--the area of cortical bone based on the equation, $TPA - TEA = TCA$.

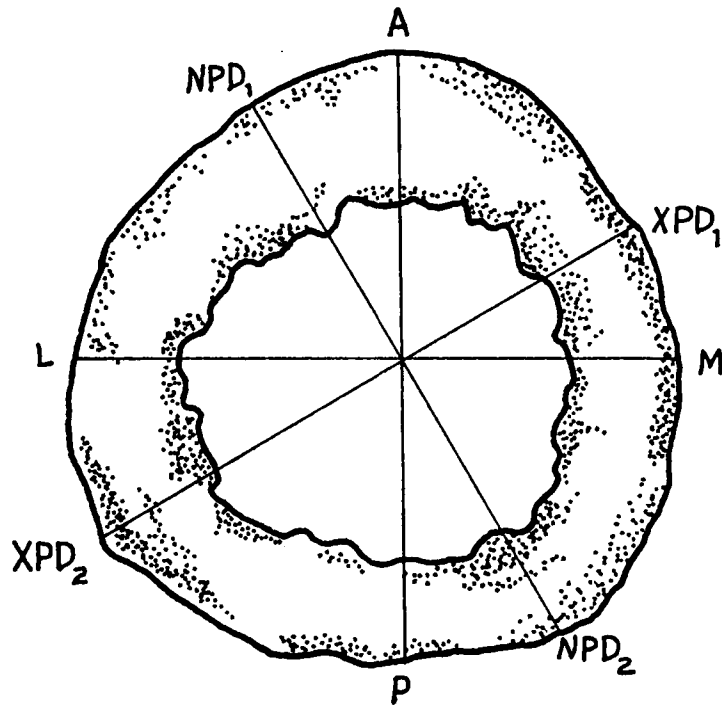


Figure 13. Cortex size and shape variables measured from the periosteal border.

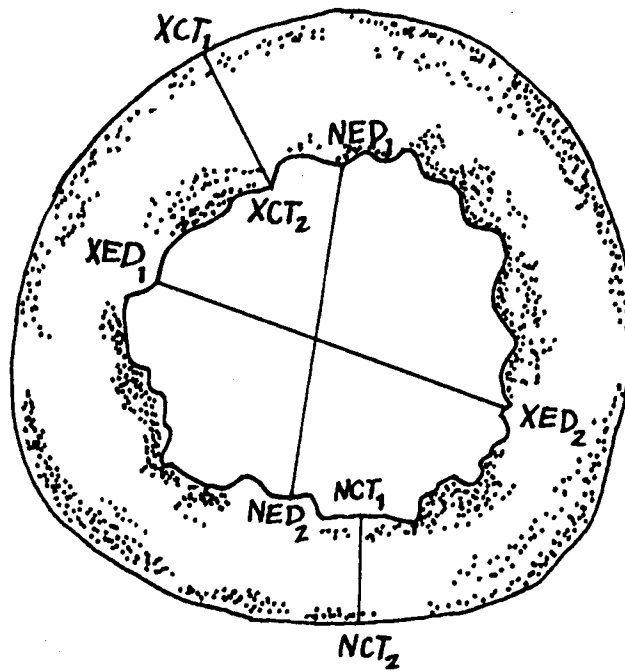


Figure 14. Cortical thickness variables and marrow cavity size and shape variables measured from the endosteal border.

Cortical Size and Shape Dimensions

4. Maximum Periosteal Diameter (XPD)--the maximum diameter of the cortex measured from the periosteal border.
5. Minimum Periosteal Diameter (NPD)--the minimum diameter of the cortex measured from the periosteal border.
6. Maximum Endosteal Diameter (XED)--the maximum diameter of the marrow cavity measured from the endosteal border.
7. Minimum Endosteal Diameter (NED)--the minimum diameter of the marrow cavity measured from the endosteal border.
8. Maximum Cortical Thickness (XCT)--the maximum thickness of the cortex.
9. Minimum Cortical Thickness (NCT)--the minimum thickness of the cortex.

In addition, the maximum anterior-posterior and medial-lateral diameters (XAPD, XMLD), as well as the total area of the resorption spaces within the cortex (TARS), were measured for future analysis.

Each of these cortical area and dimension parameters were measured and calculated utilizing a Zeiss Videoplan Imaging System (Zeiss, 1981) as shown in Figure 15. The Videoplan System is an electronic planimeter interfaced with



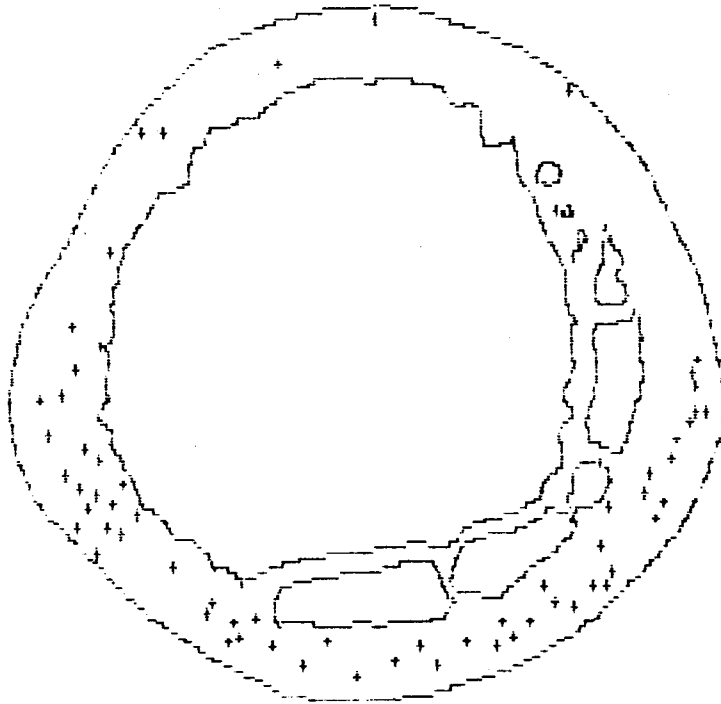
Figure 15. Zeiss Videoplan Imaging System with integrated Zeiss IVB stereomicroscope and Zeiss standard 16 microscope with polarizing analyzer. Note the periscopic tube (A) attached to the stereomicroscope and overlooking the electronic planimeter (B), on which rests the lighted cursor (C).

microcomputer and is designed to measure and analyze a variety of structural or morphological characteristics. The generated data may be statistically computed, graphically displayed, or transferred to interface devices by this system.

The electronic planimeter, or digitizer tablet possesses magnetic wires at regular intervals in the X and Y directions which provide a permanent magnetic field. When activated with the accompanying stylus or cursor, coordinate data is generated at the tablet surface whenever a structure is outlined or when distances or locations are defined. The generated data is immediately displayed on a video monitor and the sum, mean, and standard deviation are automatically calculated for each parameter as shown in Figure 16. A video display of the outlined cortex and resorption spaces is also obtainable as shown in Figure 16.

The Videoplan System is unique from the aspect that most types of image media may be analyzed, including photographs, projected images, drawings, printed material or directly from video transmission. Microscopic images may also be quantitated directly from a microslide in a binocular field of view.

This research employed the latter of the imaging systems and is pictured integrated with the Zeiss Videoplan Imaging System in Figure 15. Microslides were viewed



IDENT.NR 270196 07 01 CHANNEL 1

CHAN/COUNT		AREA	PERIMET.	DMAX	DELLIP-A	DELLIP-B
1	1	8.13	10.22	3.35	3.29	3.16
1	2	3.99	7.85	2.55	2.41	2.13
1	3	.01	.33	.11	.11	.10
1	4	.00	.18	.07	.06	.04
1	5	.00	.28	.12	.10	.04
1	6	.03	.88	.36	.36	.13
1	7	.12	1.62	.67	.70	.24
1	8	.03	.68	.24	.25	.17
1	9	.12	1.57	.66	.65	.26
1	10	.19	2.00	.83	.87	.28

Figure 16. Video image display of cortex traced on electronic planimeter with calculated area and distance measurements. Count number one is taken from the periosteal border (TPA, XPD, NPD), number two from the endosteal border (TEA, XED, NED), and counts three through ten are intracortical resorption spaces (RSA). The scattered dots are predominantly primary osteons. A photograph of this section is presented in Figure 8 (p. 50).

directly through a Zeiss IVB stereomicroscope with fiberoptic illuminator. A periscopic drawing tube was attached to the stereomicroscope, which, when placed next to the digitizer tablet, overlooks the tablet. This allows the specimen field (microslide section) and the drawing or measuring surface (digitizer tablet) to appear simultaneously in the field of view. Used in conjunction with this drawing tube is a digitizing cursor equipped with a light emitting diode. Thus, one may simultaneously view the microscopic section and the lighted cursor.

The data acquisition program of the Zeiss Videoplan System enables a number of geometric parameters to be measured simultaneously. Available parameters include any type of area calculation, linear measurement or a number of special arithmetic functions. Selected for this phase of the analysis were the (1) area, (2) perimeter, (3) maximum diameter, (4) major and minor axes, and (5) distance parameters of the Videoplan System (Zeiss, 1981:17-22). Of these, the first four were calculated simultaneously, while the individual distance parameters were measured separately in a second step.

For the Videoplan System to directly compute distance measurements or area calculations, one must first establish a known scale at the magnification at which the microsections will be viewed. This is determined by viewing a scale

of known length at the selected magnification and activating the end points of that scale with the lighted cursor. The known scale used here was a Leitz stage micrometer.

As the scale must be reestablished prior to each data capturing session, the magnification at which sections are viewed may be changed as long as the linear scale is redefined before measurements are taken. This necessity adapted well to this research as sections were viewed at three different magnifications--the highest magnification possible where the entire cortex was visible within the field of view. Thus, specimens with maximum diameters less than 3.5 mm were viewed at 62.5 power, specimens with diameters between 3.5 and 4.5 mm were viewed at 50 power and specimens with diameters greater than 4.5 mm were viewed at 40 magnifications.

Throughout the study, however, the same specimen was measured at the beginning of each data capturing session after the scale had been established. This served as a check against which the measurements at any magnification could be compared to those taken previously, thus ensuring accurate and replicable results.

Once the scale was established, data were obtained by viewing the entire cortex of each section with the Zeiss stereomicroscope with periscopic drawing tube attached and

overlooking the digitizer tablet. Sections were analyzed at random without their age, sex or species being known.

Cortical area calculations were obtained by tracing the periosteal, endosteal, or resorption space boundaries with the lighted cursor as viewed through the periscopic drawing tube. The Videoplan System automatically calculated area to the 0.01 mm^2 . Each area was measured six times and the average used for analysis. As the cortical area was quantifiable directly from each section, it was not necessary to photograph, cut, piece, and tape together a composite of each section as described by Glassman (1982:66).

To obtain linear distance measurements, the lighted cursor is activated when placed at the desired set of endpoints. The Videoplan automatically computes the distance between the two. In this study, the cortex for each individual was subjected to a series of up to fifteen pairs of endpoints to determine the maximum and minimum dimensions of the parameters described. Values for distance measurements are calculated to the nearest 0.01 mm by the Zeiss Videoplan System.

As an additional check, the maximum and minimum periosteal diameters (XPD, NPD) measured with the electronic planimeter were compared to the midshaft diameters obtained with the dial calipers prior to the sectioning process. Although the calipers measured to the nearest 0.1 mm and the

videoplan to the nearest 0.01 mm, all results were comparable within 0.5 mm.

In addition, the plane of orientation of each of these cortical dimensions was noted with respect to the longitudinal axis of the shaft. These provided a visual representation of the orientation of the maximum and minimum dimensions which aided in determining any patterns of shift (drift) in the maximum and minimum load axes of the femoral shaft.

Quantification of Histomorphology

To determine age related changes in the intracortical histomorphology of Saguinus, the cortex was analyzed for patterning of two phases of bone development: (1) primary vascularization of growing bone, and (2) secondary modeling or osteonization of mature bone. Both of these aspects reflect age changes in bone as their quantity is expected to increase or decrease with respect to age (Kerley, 1965).

These aspects, defined by the following criteria, are shown in the designated microphotographs and are described in detail in Chapter III. Each histomorphological feature was quantified individually by anterior, medial, posterior and lateral quadrants for the entire cortex. Described, these features were quantified as follows:

Primary Vascularization

1. Vascular Canals and Primary Osteons (by quadrant: VCPOA, VCPOM, VCPOP, VCPOL)--vascular canals (non-Haversian canals) and primary osteons were quantified for each quadrant of the cortex.

Secondary Remodeling

2. Resorption Spaces (by quadrant: RSA, RSM, RSP, RSL)--resorption spaces were quantified for each quadrant of the cortex.
3. Forming Osteons (by quadrant: FOA, FOM, FOP, FOL)--forming osteons were quantified for each quadrant of the cortex.
4. Secondary Osteons (by quadrant: SOA, SOM, SOP, SOL)--secondary osteons were quantified for each quadrant of the cortex.
5. Osteon Fragments (by quadrant: OFA, OFM, OFP, OFL)--osteon fragments were quantified for each quadrant of the cortex.

For the analysis, the following sets of variables were correlated with age: (1) the quadrant count of each individual histological feature as listed above, (2) the secondary remodeling total within each quadrant (SAT, SMT, SPT, SLT), and (3) the total secondary remodeling count within the entire cortex (TSR).

Although the formation of each of these histological features is described in Chapter III, their use in this study must be defined here, as not all authors use these terms synonymously.

Initially, vascular canals (VC) and primary osteons (PO) were quantified separately. However, as Saguinus possesses such an extremely thin femoral cortex (0107-1.14 mm), microstructures within that cortex are relatively small and difficult to distinguish. This was especially true of VC and PO. It was often not possible to distinguish if a vascular canal possessed none, just one, or a few circumferential lamellae. Thus, vascular canals and primary osteons were quantified together.

The histoskeletal features used to establish the pattern of osteonization or secondary remodeling with age, involve "the formation of a resorption cavity and its refill with concentric lamellar bone" (Johnson, 1964:585). Those used in this study generally correspond to Ortner's (1970: 21-26) descriptive phases of the life cycle of an osteon. Here, resorption spaces correspond to Ortner's Phase I in the life cycle of an osteon, forming osteons to Phase II, mature, whole, secondary osteons to Phase III, and osteon fragments to the completion of the osteon life cycle. Each is defined in greater detail below.

Jaworski et al. (1972), Ortner (1975:29), Richman et al. (1979), and Tappen (1977) have described criteria for recognizing different types of resorption spaces (RS), based primarily on size and the presence of Howship's (absorption) lacunae, the result of osteoclastic activity. In this study, however, a resorption space is simply defined as one within the cortex that has not yet begun to fill with circumferential lamellae (Ortner, 1970:22).

Forming osteons (FO) are secondary osteons where deposition of inner circumferential lamellae had begun but was not yet complete. Thus they possess large Haversian canals relative to all other osteons within the cortex (Ortner, 1970:23; 1975:29). These may also be recognized when viewed under polarized light as the innermost lamellae will appear yellow, a characteristic of incompletely mineralized osteoid tissue (Ortner, 1983).

Secondary osteons (SO) are those which possess "a vascular canal surrounded by concentric lamellae" (Kerley, 1965:151), where formation of the central canal is complete. Kerley (1965:162) later clarifies this definition and states that a recognizable osteon is "one that is easily distinguishable over 80% or more of its area and has the canal intact." However, less than 80% of an Haversian system might be present while its vascular function is maintained. Thus, secondary osteons were considered whole (as opposed

to fragmentary) if the central Haversian canal remained intact, even when the outer lamellae of the osteon had been partially destroyed by later osteoclastic action.

An osteon fragment (OF) is the remnant of an osteon which has been partially destroyed by subsequent osteoclastic reaction. A fragment was considered as such if the Haversian canal had been invaded, thus terminating the vascular function of the osteon, that of supplying nutrients through the Haversian capillary to the surrounding osteocytes. This corresponds to Ortner's (1970:26) definition of an osteon fragment, yet differs considerably from Kerley's (1965:151,162) description.

Although Kerley (1965:162) defines fragments as "any osteones that have discernible encroachment by subsequent generations of osteones and are exemplified by arcs of concentric lamellae between newer osteons," he never clarifies just how little of an osteon may be destroyed before it is classifiable as a fragment. Thus, he considers a whole osteon to possess over 80% of its area, which must indicate that a fragment is any osteon with less than 80% of its original area. Since an osteon may lose 20% of its area and maintain its vascular supply, osteon fragments were considered to be those where the vascular canal had been invaded.

Before histomorphological analysis began, a bisecting cross hatch was drawn on the top of the cover slip with a

felt tip pen. This served to divide the section into equal anterior, medial, posterior and lateral quadrants.

For this aspect of the analysis, a Zeiss Standard 16 microscope (Figure 15, p. 80) with polarizing analyzer was utilized. The entire cortex was first viewed for each section under low power (40x). This was to insure proper orientation of the section and equal division of the quadrants by the bisecting cross hatch. It also allowed initial scanning of the entire section to note the general histomorphological pattern within the cortex.

As the entire cortex was analyzed in this analysis and not just given microscopic fields, each quadrant was viewed at both 100 and 200 power to identify the features within that quadrant alone. Whether primary vascularization or secondary remodeling, all structures within a quadrant were first counted to establish the total number of features within that quadrant. Next, each of the five histomorphological features were quantified individually. These were recorded on a data sheet along with any other observations pertinent to the case.

After the individual features in each quadrant were totaled, these figures were compared to the original total for that quadrant. This prevented any discrepancy where structures which were difficult to distinguish might be omitted or counted twice as different features.

Quantification of histological structures were tedious and difficult. Regardless of how well defined and described these features are by any author, they are often indistinguishable from the surrounding lamellae or are cluttered in a field of osteonization. This is especially true of Saguinus due to their thin cortex and relatively small vascular canal and osteon size. Thus, it must be assumed that quantification of the defined histological features will incorporate a certain degree of error because of the inability to distinguish some features. However, the author collected all the data, thus eliminating any interobserver error.

Microphotographs were taken of desired sections with a Reichart Univar photomicroscope utilizing both normal transmitted and polarized light. Kodak Tungsten 50 ASA color and Kodak Ektapan 100 ASA black and white films were used.

Analytical Methods

Following quantification of all variables, data were entered onto the DEC-10 terminal of The University of Tennessee Computer Center.

Descriptive statistics. Descriptive statistics for each variable were calculated by the MEANS procedure of SAS82 (SAS Institute, 1982). Sample size, means, standard deviations and ranges for Saguinus fuscicollis adults are

presented in Table 5 for cortical dimension variables and in Table 6 for microstructural variables.

Next it was necessary to determine whether age changes between the sexes were significantly different and would therefore necessitate separate analysis or could they be pooled. This analysis was performed using the most critical variable, Total Cortical Area (TCA), and was tested by an analysis of covariance (ANOCOVA) by the General Linear Model Procedure (PROC GLM) of SAS 82 (SAS Institute, 1982). Total cortical area was taken as the dependent variable, with sex as the treatment and age as the covariate.

There are four tests of significance calculated by this procedure: (1) a test for the overall model, (2) a test for the heterogeneity of the slopes, (3) a test to determine if the slopes are equal to zero, and (4) a test to determine if the adjusted means differ among populations. These tests are F ratios calculated with various sums of squares produced by the analysis. Table 7 summarizes the ANOCOVA results for Saguinus fuscicollis.

The results indicate that the overall model is significant (Test 1); the slopes are equal (Test 2); the slopes do not equal zero (Test 3); and the adjusted means do not differ from the population means (Test 4). The adjusted means for total cortical area are 4.679 for males and 4.667 for females. Thus, pooling the sexes for this analysis is justified.

Table 5. Descriptive Statistics of Cortical Dimensions for Adult Saguinus fuscicollis

Variable	Code	Mean	SD	Range
Age in Days	AGE	2608.42	1058.6	770.00-5445.00
Total Cortical Area	TCA	5.57	0.9	3.99-7.61
Total Periosteal Area	TPA	12.10	1.7	9.28-17.84
Total Endosteal Area	TEA	6.53	1.5	4.18-10.45
Maximum Periosteal Diameter	XPD	4.21	0.3	3.61-4.98
Minimum Periosteal Diameter	NPD	3.66	0.3	3.19-4.32
Maximum Endosteal Diameter	XED	3.17	0.4	2.41-3.96
Minimum Endosteal Diameter	NED	2.61	0.3	1.98-3.32
Maximum Cortical Thickness	XCT	0.67	0.1	0.49-1.10
Minimum Cortical Thickness	NCT	0.38	0.1	0.19-0.56

Table 6. Descriptive Statistics of Cortical Microstructure for Adult *Saguinus fuscicollis*

Variable	Code	Mean	SD	Range
Vasc Canal Prim Osteon--Anterior	VCPOA	7.31	10.8	0.0-49.0
Vasc Canal Prim Osteon--Medial	VCPOM	2.37	4.9	0.0-24.0
Vasc Canal Prim Osteon--Posterior	VCPOP	1.70	3.5	0.0-16.0
Vasc Canal Prim Osteon--Lateral	VCPOL	2.80	5.1	0.0-25.0
Vasc Canal Prim Osteon--Cortex Total	VC POT	14.30	19.3	0.0-83.0
Resorption Space--Anterior	RSA	0.02	0.1	0.0-1.0
Resorption Space--Medial	RSM	0.03	0.2	0.0-1.0
Resorption Space--Posterior	RSP	0.15	0.5	0.0-2.0
Resorption Space--Lateral	RSL	0.02	0.1	0.0-1.0
Resorption Space--Cortex Total	RST	0.22	0.6	0.0-3.0
Forming Osteon--Anterior	FOA	0.07	0.3	0.0-2.0
Forming Osteon--Medial	FOM	0.04	0.2	0.0-1.0
Forming Osteon--Posterior	FOP	0.07	0.3	0.0-1.0
Forming Osteon--Lateral	FOL	0.02	0.1	0.0-1.0
Forming Osteon--Cortex Total	FOT	0.20	0.5	0.0-3.0
Secondary Osteon--Anterior	SOA	0.48	1.3	0.0-7.0
Secondary Osteon--Medial	SOM	1.83	2.8	0.0-11.0
Secondary Osteon--Posterior	SOP	3.28	4.2	0.0-19.0
Secondary Osteon--Lateral	SOL	1.11	2.2	0.9-9.0
Secondary Osteon--Cortex Total	SOT	6.70	8.1	0.0-38.0
Osteon Fragment--Anterior	OFA	0.43	1.6	0.0-10.0
Osteon Fragment--Medial	OFM	1.44	2.6	0.0-11.0
Osteon Fragment--Posterior	OFF	1.54	2.1	0.0-10.0
Osteon Fragment--Lateral	OFL	0.94	1.7	0.0-7.0
Osteon Fragment--Cortex Total	OFT	4.35	4.4	0.0-16.0
Secondary Remodeling--Anterior Total	SAT	1.00	2.5	0.0-12.0
Secondary Remodeling--Medial Total	SMT	3.35	4.7	0.0-17.0
Secondary Remodeling--Posterior Total	SPT	5.03	5.2	0.0-22.0
Secondary Remodeling--Lateral Total	SLT	2.09	3.2	0.0-11.0
Secondary Remodeling--Cortex Total	TSR	11.48	10.5	0.0-42.0

Table 7. Summary of the ANCOVA Results for Total Cortical Area with Age between Male and Female Saguinus fuscicollis

Test	Source	Sum of Squares	Mean Squares	df	F
1 MSA/MS1	Among Error	146.73 106.66 (S ₁)	29.35 1.33	5 80	22.01*
2 MS4/MS1	AGE*SEX (AGE) ^a *SEX TOTAL Error	0.384 ^a 2.092 ^a <u>2.476 (S₄)^a</u> 106.66	1.238 1.33	2 80	0.9288
3 MSO/MS2	AGE (AGE) ^b TOTAL Error	67.988 ^a 75.936 ^a <u>143.924 (S₀)^a</u> 109.134 (S ₂)	71.971 1.331	2 82	54.009*
4 MS3/MS2	SEX Error	0.002 (S ₃) ^b 109.134 (S ₂)	0.002 1.331	1 82	0.001

* $p > .0001$

^aType I Sum of Squares

^bType II Sum of Squares

As this study attempts to determine the changes of the described variables with age, the initial procedure to accomplish this was to subject the pooled data with the variable age, to a Pearson product-moment correlation analysis (SAS Institute, 1982). This analysis provided a matrix of the correlation coefficients between all variables. These values are presented in the correlation matrix in the Appendix.

Each variable determined to be significantly correlated with chronological age was first subjected to bivariate plots by the PLOT procedure of SAS82 (SAS Institute, 1982). These plots are presented in Chapter V, where the variable count is plotted on the vertical (Y) axis and age on the horizontal (X) axis. Significant growth curve trends are evident in these bivariate plots and are discussed in Chapter V.

To further test the relationships between the significant cortical variables and their changes with age, a general linear regression analysis from the General Linear Models (GLM) procedure of SAS82 (SAS Institute, 1982) was utilized. The object of the linear regression analysis is to predict the value of the dependent variable with the value of the independent variable. The formula for such a regression is:

$$Y = b_0 + b_1(X_1) + e$$

where Y is the dependent variable, b_0 is the intercept, b_1 is the regression coefficient for the given variable, x_1 is the known independent variable, and e is the error, as age does not account for all of the variation in Y .

A summary of the regression analysis is presented in Chapter V for subadult and adult Saguinus fuscicollis cortical dimensions and cortical microstructure.

A regression plot is obtained for each test by plotting the predicted value for a given variable along the Y axis, with age along the X axis. Once plotted, a line will be produced conforming to the regression model, indicating the relationship between each variable with age. This relationship helps to interpret the age change events being studied, the results of which are also discussed in Chapter V.

CHAPTER V

RESULTS AND DISCUSSION

The results of the Pearson product-moment correlations between each variable are presented in the correlation matrix in the Appendix. Results of the regression analysis are presented in Tables 8 and 9. This analysis focuses on one species, Saguinus fuscicollis, as it was the only species of the two samples with an adequate sample size for the entire lifespan of the species. However, S. oedipus is compared with S. fuscicollis for subadults as there were adequate sample sizes for both species.

The cortical dimension and microstructural variables found to be significantly correlated with age at the 0.05 level of probability or better are listed here with their r value. The r value measures the strength of association and is not just a test of significance. For subadults, they include:

1. Total cortical area (TCA) - 0.66
2. Total periosteal area (TPA) - 0.86
3. Total endosteal area (TEA) - 0.80
4. Maximum periosteal diameter (XPD) - 0.88
5. Minimum periosteal diameter (NPD) - 0.79
6. Maximum endosteal diameter (XED) - 0.84
7. Minimum endosteal diameter (NED) - 0.75

Table 8. Linear Regression Analysis Summary of Cortical Dimensions for Saguinus fuscicollis

Dependent Variable	Age Group	N	Age Mean Square	Error Mean Square	F Value	R
Total Cortical Area	Subadult	32	61.21	1.04	59.09***	0.66
	Adult	54	4.57	0.75	6.12*	0.11
Total Periosteal Area	Subadult	32	327.53	1.81	180.89***	0.86
	Adult	54	1.25	3.08	0.41	0.01
Total Endosteal Area	Subadult	32	105.56	0.87	120.70***	0.80
	Adult	54	1.04	2.34	0.44	0.01
Maximum Posterior Diameter	Subadult	32	17.36	0.08	228.97***	0.88
	Adult	54	0.01	0.11	0.09	0.00
Minimum Posterior Diameter	Subadult	32	12.13	0.11	113.24***	0.79
	Adult	54	0.14	0.07	2.16	0.04
Maximum Endosteal Diameter	Subadult	32	9.45	0.06	153.79***	0.84
	Adult	54	0.09	0.14	0.67	0.13
Minimum Endosteal Diameter	Subadult	32	6.39	0.07	91.32***	0.75
	Adult	54	0.02	0.09	0.24	0.00
Maximum Cortical Thickness	Subadult	32	0.50	0.02	21.17***	0.41
	Adult	54	0.06	0.01	4.30*	0.08
Minimum Cortical Thickness	Subadult	32	0.17	0.01	18.08***	0.38
	Adult	54	0.09	0.01	17.73***	0.25

*Probability > 0.05

**p > 0.01

***p > 0.001

Table 9. Linear Regression Analysis Summary of Cortical Microstructure for Saguinus fuscicollis

Dependent Variable	Age Group	N	Age Mean Square	Error Mean Square	F Value	R
VCPOA	Subadult	32	1.22	274.27	0.00	0.00
	Adult	54	10.41	118.75	0.09	0.00
VCPOM	Subadult	32	702.54	65.08	10.80**	0.26
	Adult	54	6.24	24.89	0.25	0.00
VCPOP	Subadult	32	662.17	42.33	15.64***	0.34
	Adult	54	4.53	12.54	0.36	0.01
VCPOL	Subadult	32	220.69	41.63	5.30*	0.15
	Adult	54	0.76	26.89	0.03	0.00
VCPOT	Subadult	32	4651.21	784.12	5.93*	0.16
	Adult	54	48.70	379.88	0.13	0.00
RSA	Subadult	32	0.68	2.03	0.33	0.01
	Adult	54	0.06	0.02	3.20	0.58
RSM	Subadult	32	0.00	0.69	0.01	0.00
	Adult	54	0.03	0.04	0.85	0.02
RSP	Subadult	32	0.00	0.16	0.61	0.00
	Adult	54	0.03	0.25	0.13	0.00
RSL	Subadult	32	0.08	0.50	0.15	0.01
	Adult	54	0.01	0.02	0.29	0.01
RST	Subadult	32	0.27	7.05	0.04	0.00
	Adult	54	0.45	0.45	1.11	0.02
FOA	Subadult	32	0.47	0.45	1.04	0.03
	Adult	54	1.12	0.09	12.67***	0.19
FOM	Subadult	32	0.01	0.21	0.03	0.00
	Adult	54	0.01	0.04	0.23	0.00
FOP	Subadult	32	0.00	0.36	0.00	0.00
	Adult	54	0.03	0.07	0.37	0.01
FOL	Subadult	32	0.26	0.57	0.45	0.01
	Adult	54	0.01	0.02	0.29	0.01
FOT	Subadult	32	1.17	3.39	0.34	0.01
	Adult	54	1.53	0.29	5.22*	0.09
SOA	Subadult	32	0.82	1.91	0.43	0.01
	Adult	54	5.86	1.65	3.56	0.06
SOM	Subadult	32	33.69	2.22	15.19***	0.34
	Adult	54	16.33	7.83	2.09	0.04
SOP	Subadult	32	27.85	2.69	10.33**	0.26
	Adult	54	10.35	17.51	0.59	0.01
SOL	Subadult	32	0.87	1.37	0.63	0.02
	Adult	54	2.89	4.74	0.61	0.01

Table 9 (continued)

Dependent Variable	Age Group	<u>N</u>	Age Mean Square	Error Mean Square	<u>F</u> Value	<u>R</u>
SOT	Subadult	32	123.41	14.12	8.74**	0.23
	Adult	54	129.55	64.30	2.01	0.04
OFA	Subadult	32	0.37	0.32	1.15	0.04
	Adult	54	0.73	2.59	0.28	0.01
OFM	Subadult	32	18.92	0.83	22.75***	0.43
	Adult	54	14.22	6.52	2.18	0.04
OFF	Subadult	32	1.27	0.21	6.12*	0.17
	Adult	54	0.36	4.33	0.08	0.00
OFL	Subadult	32	0.92	0.18	5.19*	0.15
	Adult	54	15.27	2.49	6.13*	0.11
OFT	Subadult	32	49.55	2.54	19.48***	0.39
	Adult	54	2.53	20.23	0.12	0.00
SAT	Subadult	32	3.27	6.75	0.48	0.02
	Adult	54	0.56	6.29	0.09	0.00
SMT	Subadult	32	106.15	5.39	19.67***	0.39
	Adult	54	62.36	20.92	2.98	0.05
SPT	Subadult	32	40.09	5.17	7.91***	0.21
	Adult	54	6.95	27.71	0.25	0.00
SLT	Subadult	32	2.76	4.94	0.56	0.02
	Adult	54	4.23	10.12	0.42	0.01
TSR	Subadult	32	273.94	49.67	5.52*	0.16
	Adult	54	85.07	109.74	0.78	0.01

*Probability > 0.05

**p > 0.01

***p > 0.001

8. Maximum cortical thickness (XCT) - 0.41
9. Minimum cortical thickness (NCT) - 0.38
10. Vascular canal and primary osteon counts, medial quadrant (VCPOM) - 0.26
11. Vascular canal and primary osteon counts, posterior quadrant (VCPOP) - 0.34
12. Vascular canal and primary osteon counts, lateral quadrant (VCPOL) - 0.15
13. Vascular canal and primary osteon counts, cortex total (VCPOT) - 0.16
14. Secondary osteon counts, medial quadrant (SOM) - 0.34
15. Secondary osteon counts, posterior quadrant (SOP) - 0.26
16. Secondary osteon counts, cortex total (SOT) - 0.23
17. Osteon fragment counts, medial quadrant (OFM) - 0.43
18. Osteon fragment counts, posterior quadrant (OFP) - 0.17
19. Osteon fragment counts, lateral quadrant (OFL) - 0.15
20. Osteon fragment counts, cortex total (OFT) - 0.39
21. Secondary remodeling, total count, medial cortex (SMT) - 0.39
22. Secondary remodeling, total count, posterior cortex - (SPT) - 0.21
23. Secondary remodeling, total count, cortex total (TSR) - 0.16

For adults, the significant variables and their r values include:

1. Total cortical area (TCA) - 0.11
2. Maximum cortical thickness (XCT) - 0.08
3. Minimum cortical thickness (NCT) - 0.25
4. Forming osteon count, anterior quadrant (FOA) - 0.19
5. Forming osteon count, cortex total (FOT) - 0.09
6. Osteon fragment count, lateral quadrant (OFL) - 0.11

Cortical Dimensions and Histomorphological
Changes with Growth

The high number of variables significantly correlated with age for subadults is evident when one examines the bi-variate plots presented in Figures 17 through 21. Age is represented in days, cortical dimensions by actual measurement or area calculation, and histomorphological features by actual count. Although substantial variation is represented within these plots, general linear relationships with age are evident. This linear relationship is presented as a predicted regression growth line for several of the most significant variables. Each generally involves an increase in area or dimension from birth to maturity as the result of growth.

The total area of cortical bone, and the maximum and minimum dimensions of the cortex, whose growth is plotted in Figures 17 through 19, all linearly increase from birth

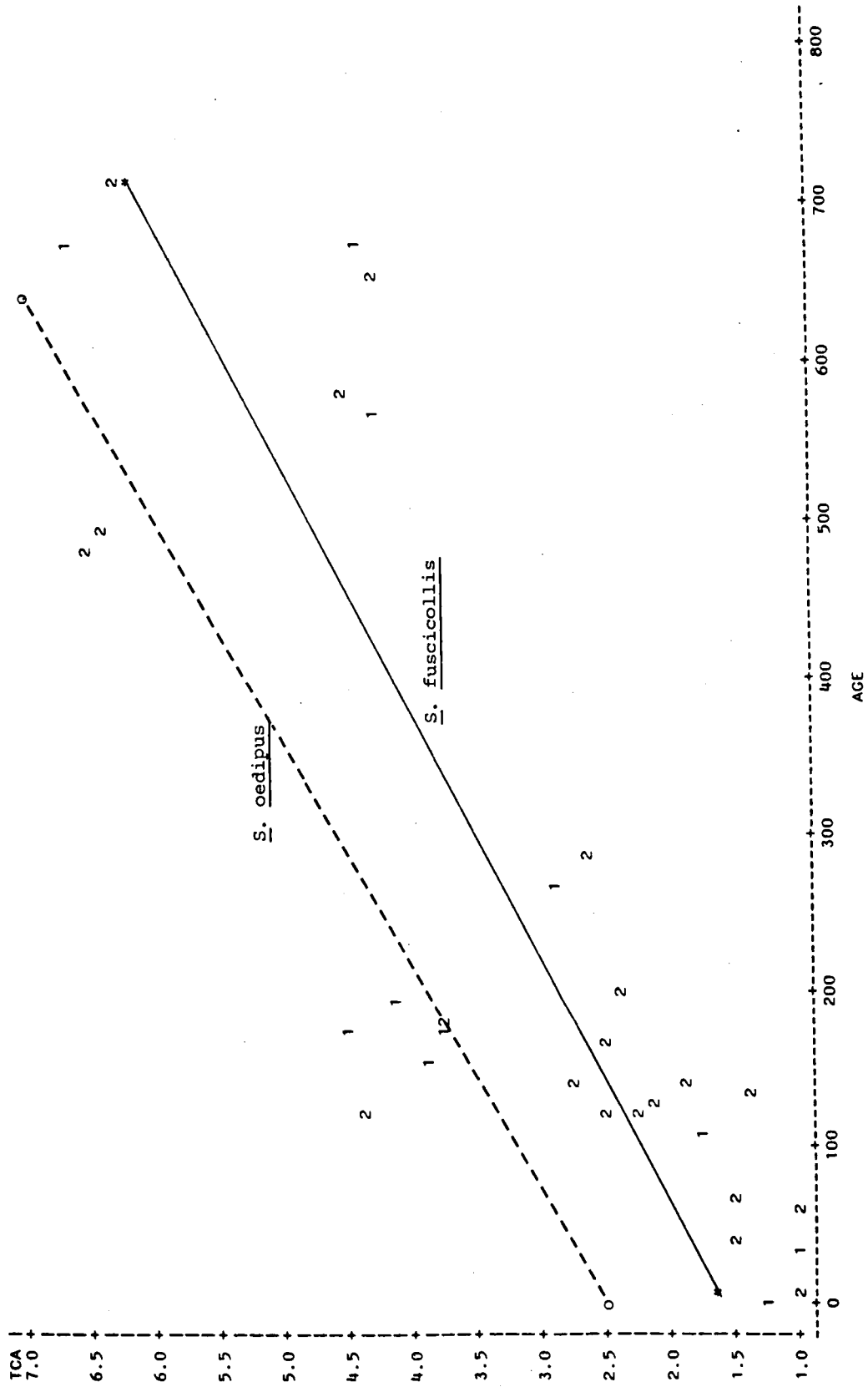


Figure 17. Plot of actual and predicted values of total cortical area on age for Saguinus subadults.

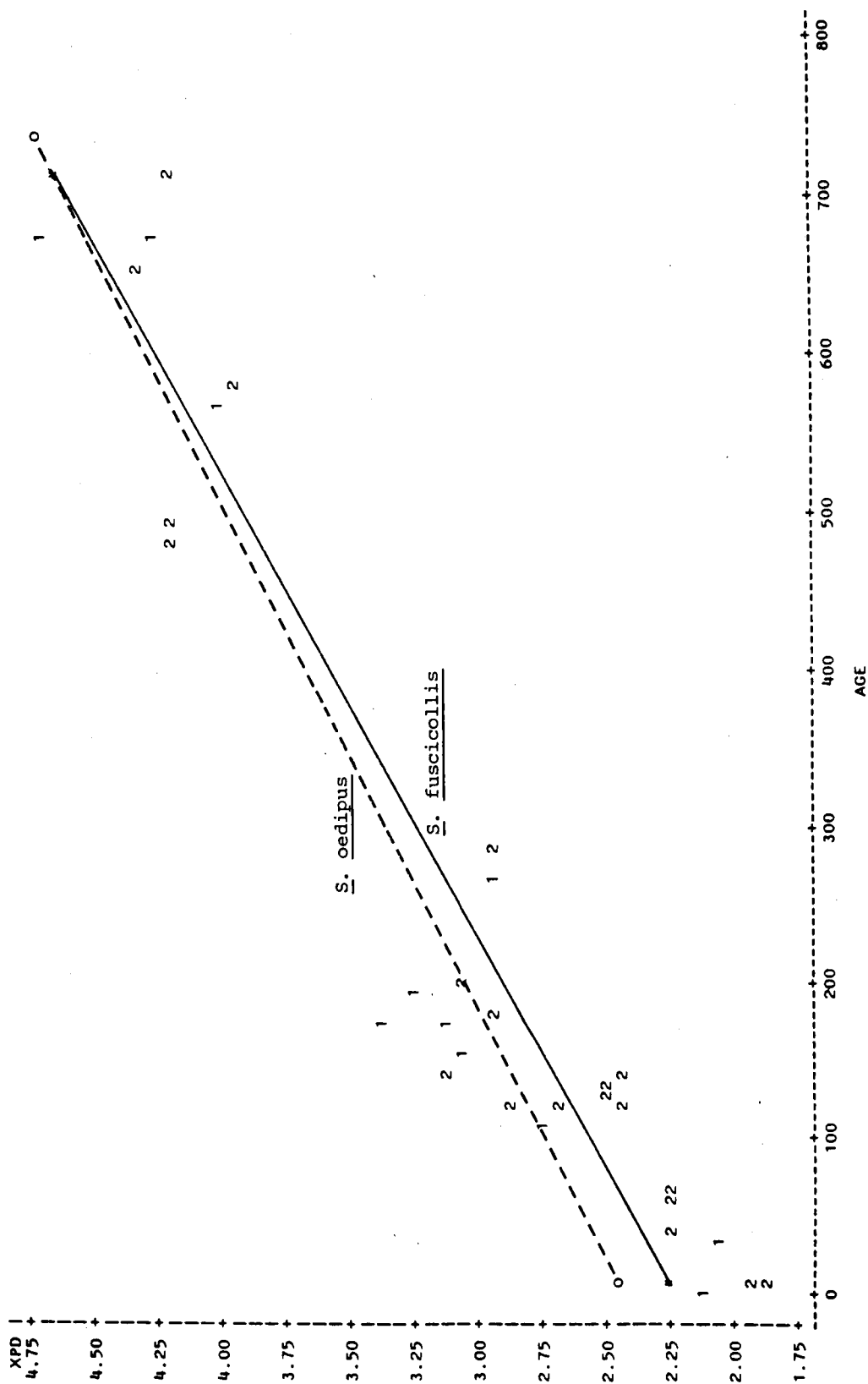


Figure 18. Plot of actual and predicted values of maximum periosteal diameter on age for Saguinus subadults.

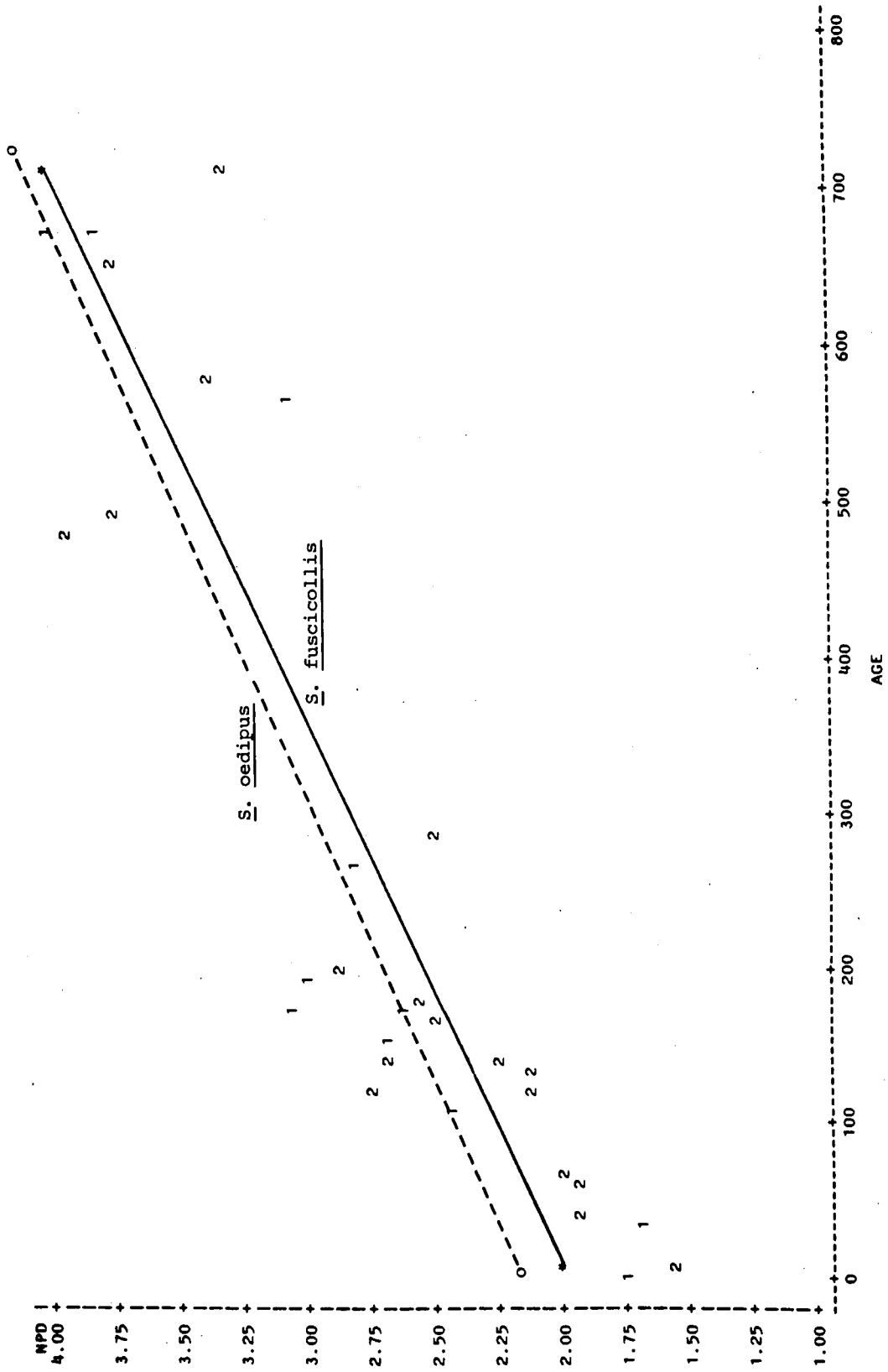


Figure 19. Plot of actual and predicted values of minimum periosteal diameter on age for *Saguinus* subadults.

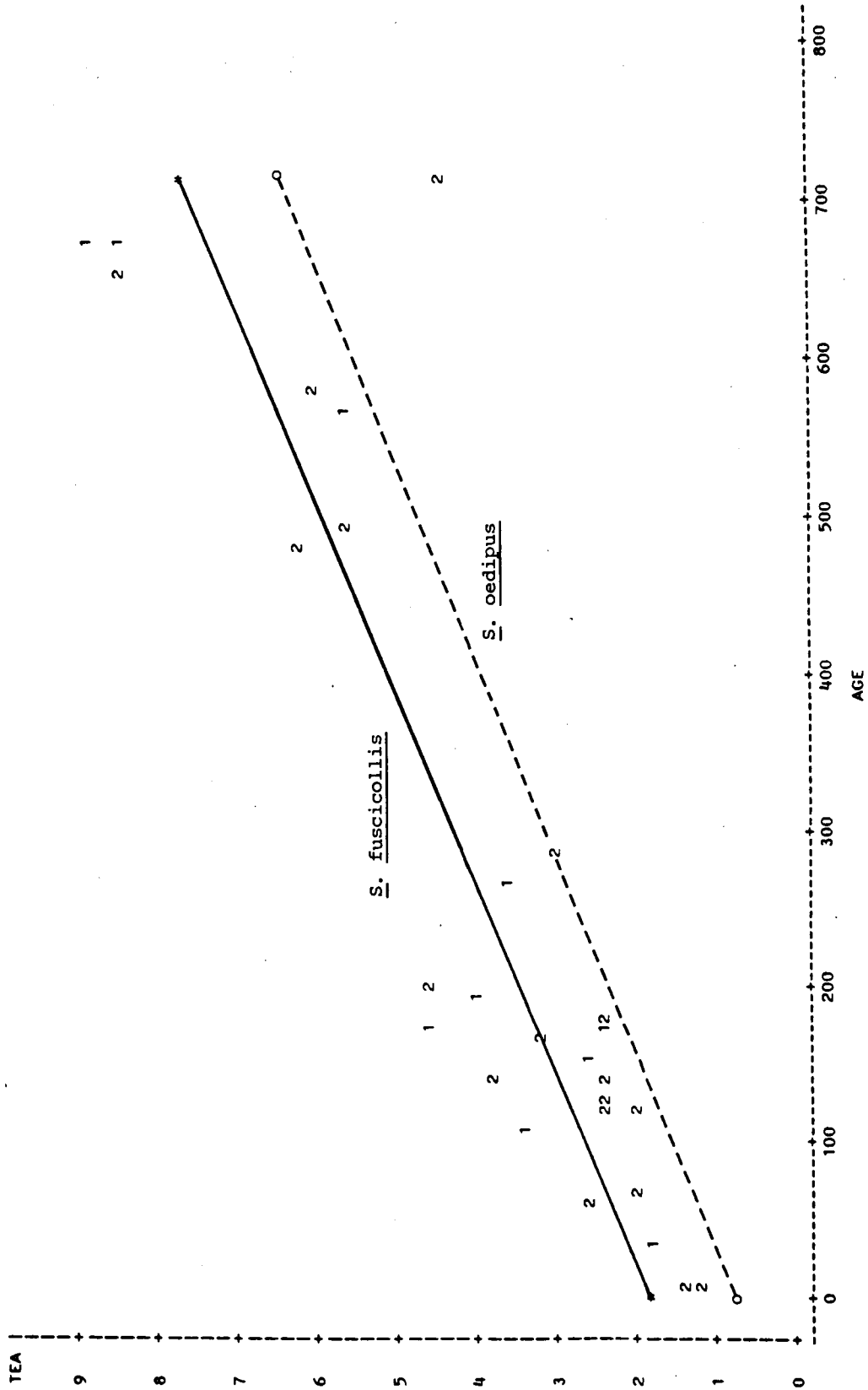


Figure 20. Plot of actual and predicted values of total endosteal area (marrow cavity) on age for *Saguinus* subadults.

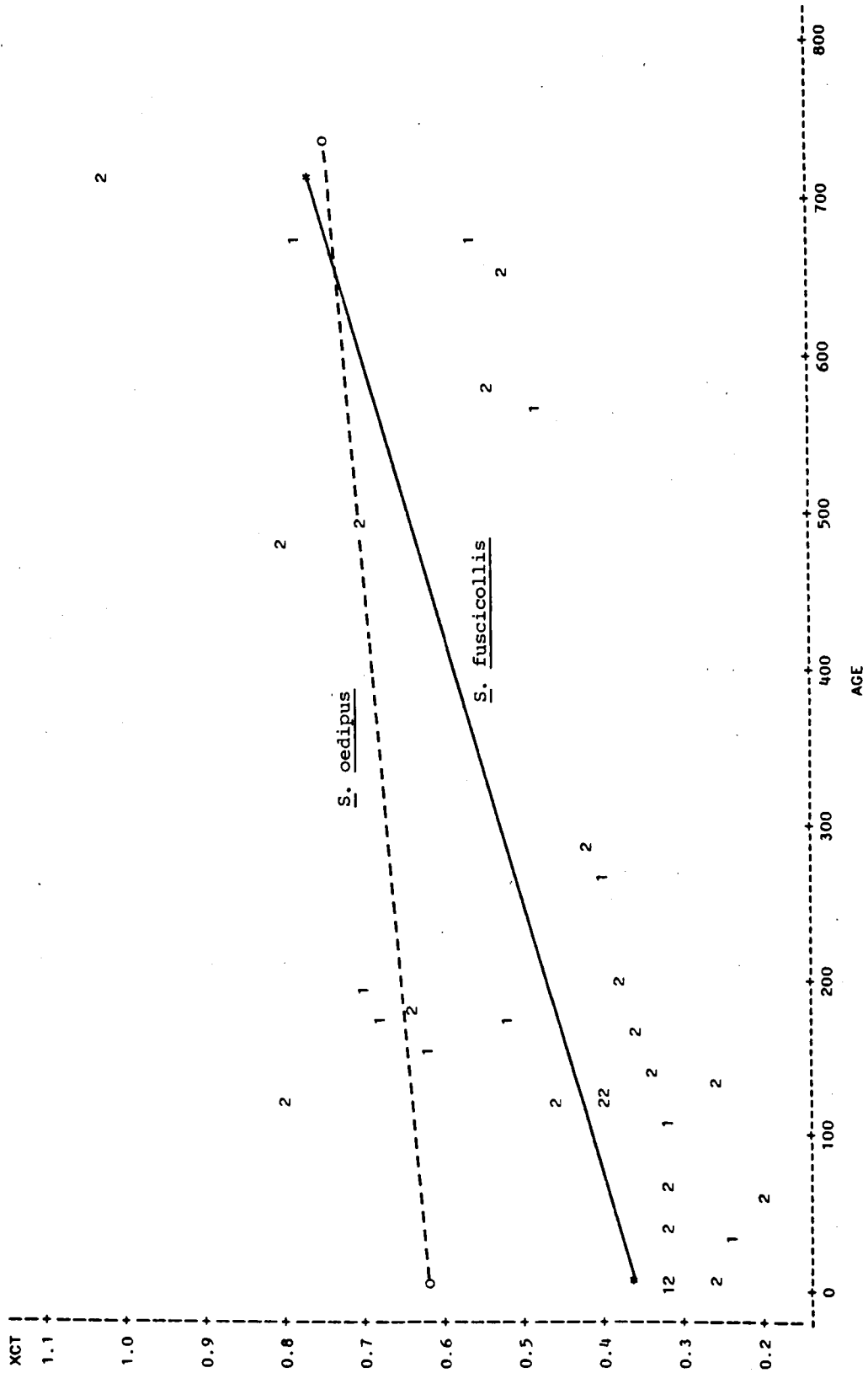


Figure 21. Plot of actual and predicted values of maximum cortical thickness on age for *Saguinus* subadults.

to skeletal maturity (two years of age) as is expected. The minimum total cortical area at birth, based on those surviving birth and into their first week of infancy, ranges from 0.95 mm² to 1.31 mm² for S. fuscicollis and 1.44-2.15 mm² for S. oedipus. From birth, the mean total cortical area for S. fuscicollis doubles within the first four months and then doubles again by one year.

Cortical area, therefore, progressively increases as the cortex is reconstructed and periosteal apposition enlarges the cortex to an approximate 6.5 mm² for mature S. fuscicollis and 5.5-8.5 mm² for S. oedipus. The plot of the subadult total cortical area comparing both S. fuscicollis and S. oedipus, Figure 17, indicates this linear trend and the gradual increase in cortical area. It is also evident that while both species demonstrate increases in cortical area at approximately the same rate, S. oedipus is, from birth, the larger of the two species. This supports data previously presented for S. fuscicollis and S. oedipus in Chapter II and data reported by Glassman (1982).

Essentially the same linear growth trends are seen in total periosteal area (TPA or total section area), total endosteal area (TEA or total marrow cavity area), and the maximum and minimum diameters of the cortex and marrow cavity. They differ from the total cortical area regression plot, however, as there appears to be less species dimorphism

between S. fuscicollis and S. oedipus. This species similarity is evident in the maximum and minimum cortical dimensions as shown in Figures 18 and 19. The maximum diameters of the cortex is just under 2.0 mm at birth for both species, while minimum cortex diameter measures an approximate 1.6 mm for both species.

While these cortical dimensions are similar, the greater cortical area for S. oedipus may be accounted for by the size of the marrow cavity. Here, a smaller marrow cavity area (TEA) is seen for S. oedipus than S. fuscicollis, as shown in Figure 20. The maximum diameter of the marrow cavity at birth is approximately 1.2 mm, while the minimum diameter is roughly 1.0 mm for S. oedipus. S. fuscicollis, however, possesses a larger marrow cavity at birth, roughly 1.5 mm by 1.2 mm. This trend continues throughout subadult growth until several months prior to skeletal maturity (approximately 1.7 years), when marrow cavity size stabilizes.

Greater total cortical area seen in S. oedipus is also due to a thicker cortex. This is apparent when examining the maximum cortical thickness data, approximately 0.8 mm at birth for S. oedipus while only 0.3 mm for S. fuscicollis, as shown in Figure 21. Minimum thickness of the cortex measures 0.17 mm for S. oedipus and 0.10 mm for S. fuscicollis.

Thus, while growth velocity is essentially the same for both species, S. oedipus exhibits a larger femoral cortical area which reflects the trend of their larger body size and possibly their greater locomotor activity.

The histomorphological features analyzed in this study also indicate linear trends with growth, as most, but not all, follow expected growth patterns to maturity. Here, histological parameters are divided into two broad categories, (1) those features associated with the growth and development or modeling (reconstruction) of the cortex, and (2) those associated with internal remodeling of the cortex. For two of the variables most significantly correlated with age, plots of the actual and predicted values are presented in Figures 22 and 23.

The first category involves vascular canals and primary osteons, both described and pictured in Chapter III, whose use in this study was defined in Chapter IV. Both are the result of a rapidly expanding cortex where bone is increasing to its adult size. One would expect to find a high vascular canal and primary osteon count (combined in this study) during infancy, with a steadily decreasing amount with growth as the more slowly expanding cortex of middle age subadult (one year) for Saguinus gives way to internal or secondary osteon remodeling.

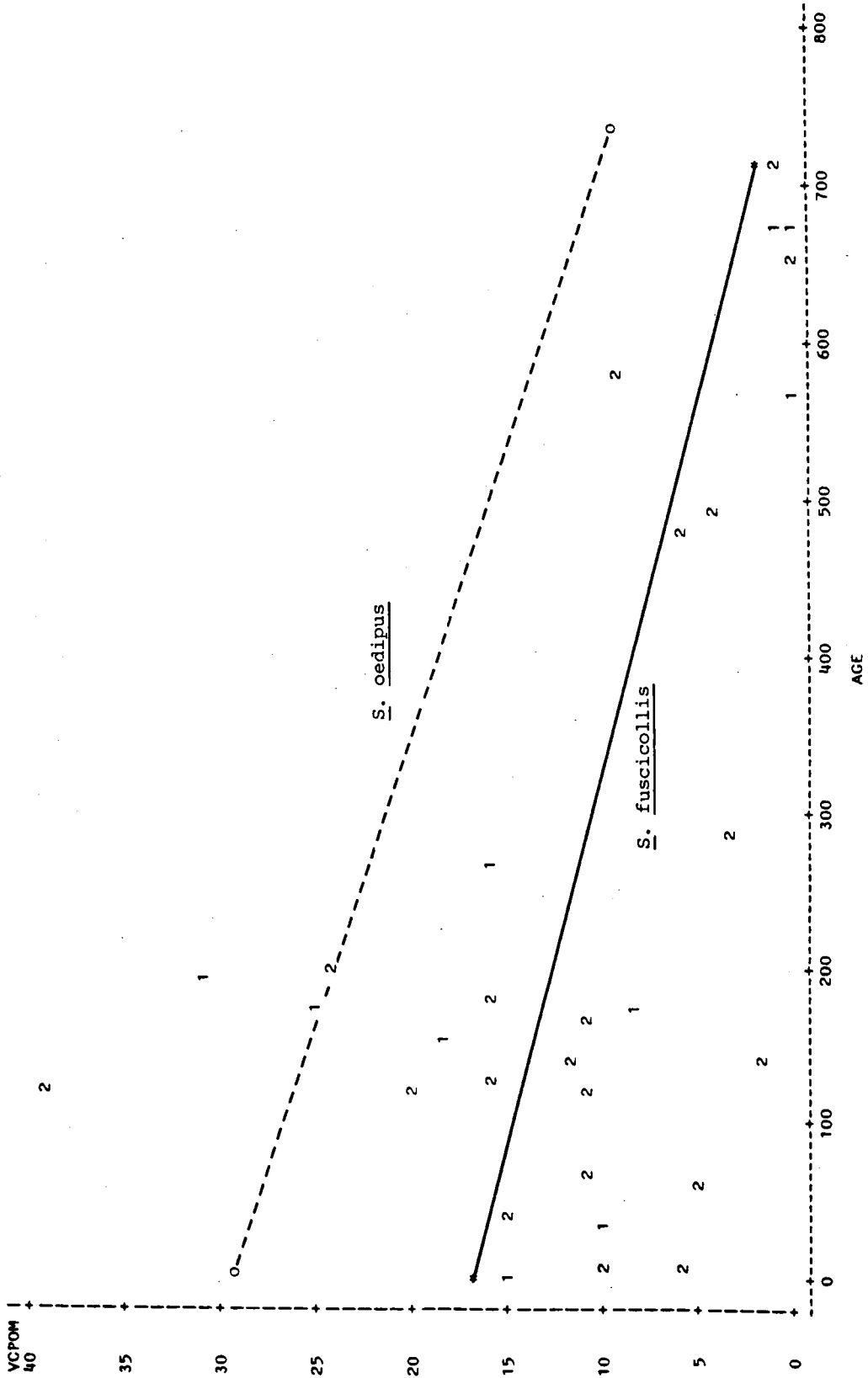


Figure 22. Plot of actual and predicted values of vascular canal and primary osteon count on age for Saguinus subadults.

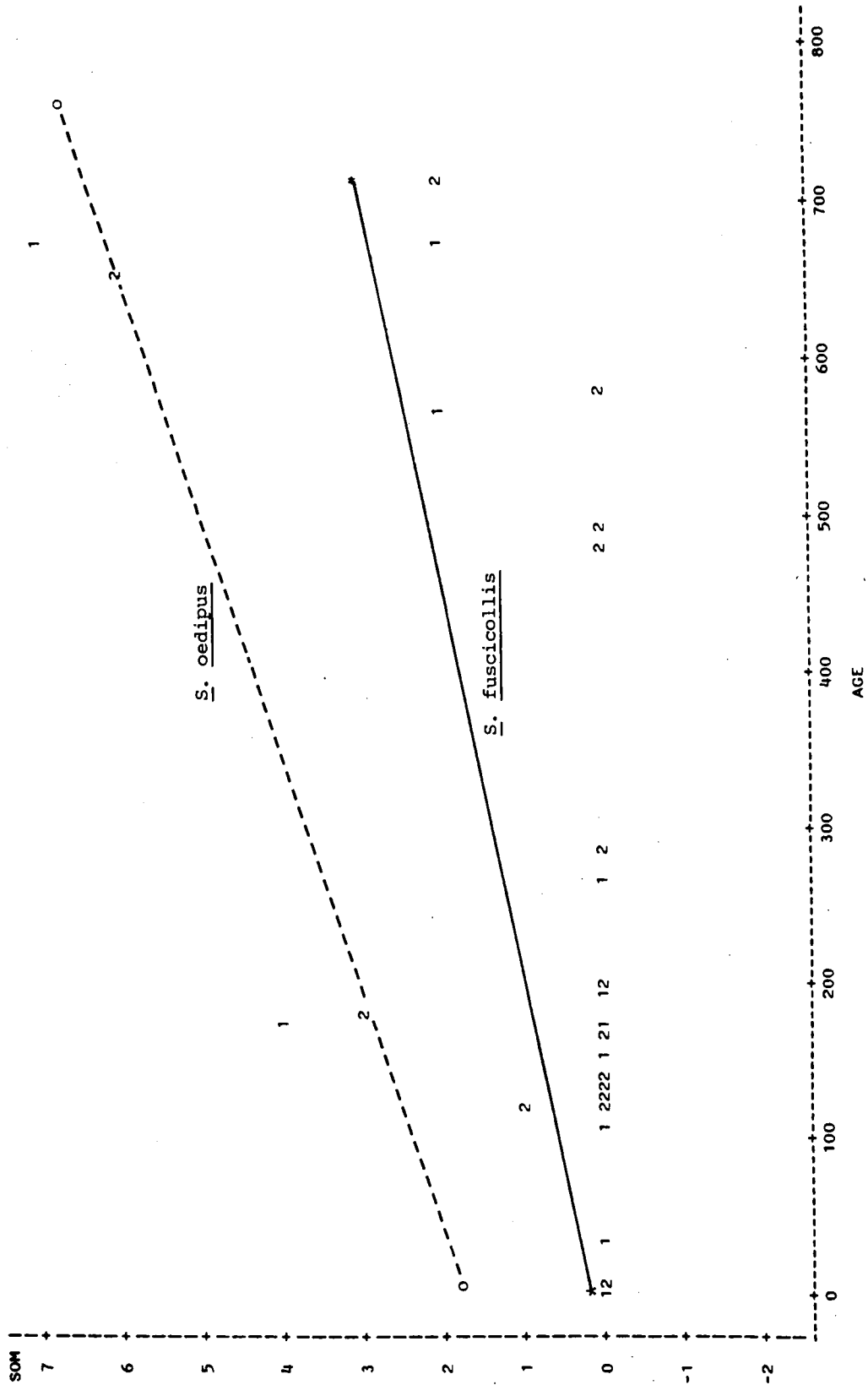


Figure 23. Plot of actual and predicted values of secondary osteon count, medial quadrant, on age for Saguinus subadults.

Such is the case for all counts of VCPO, with the exception of the anterior quadrant. VCPO counts range from 6-22 per quadrant at birth for S. fuscicollis, and from 35-42 per quadrant at birth for S. oedipus. VCPO counts per quadrant decrease with growth (and age) to range from 0-2 for S. fuscicollis and 4-33 for S. oedipus, as they reach maturity. A plot of actual and predicted values is presented for the medial quadrant only in Figure 22.

As mentioned, and as indicated in the correlation matrix and regression analysis summary, the VCPO count for the anterior quadrant was not correlated with age, but displayed a random scattering of counts when plotted against age. This is possibly the result of a cortex that is drifting anteriorly. This cortical drift, described by Enlow (1963:51-59), is influenced by the size and activity of the individual and is therefore less likely to show a linear trend with growth, as is the case. Here, appositional growth occurs on the anterior periosteal surface while osteoclastic resorption follows on the endosteal border. Thus, the cortex drifts anteriorly, does not necessarily increase in thickness, and incorporates capillaries at an irregular rate.

Cortical microstructure, indicative of internal remodeling and the result of maintenance of both the mechanical and physiological (mineral metabolism) aspects of skeletal tissue, also displays expected growth trends.

For secondary osteon counts, both the medial (SOM) and posterior (SOP) quadrants, and the cortex total (SOT) are significantly correlated with age. A plot of the actual and predicted values is presented for the medial quadrant only in Figure 23. Osteon fragment totals in the medial, posterior and lateral quadrants and the cortex total count (OFM, OFP, OFL, OFT) are also significant. In addition (most likely due to the secondary osteon counts, SOM and SOP, and osteon fragment counts, OFM and OFP), the secondary remodeling total counts for the medial and posterior quadrants (SMT, SPT) is significantly correlated with age, as well as the secondary remodeling total (TSR) for the entire cortex.

All of these individual variables exhibit a general linear regression with age where variable counts roughly increase with age. However, the scattered nature of each plot for all secondary remodeling totals is evidence of the individual diversity between the two species, S. fuscicollis and S. oedipus, as well as for individuals within each species.

It must be noted that an aspect of those cortical quadrant counts which are significantly correlated with age indicate a biological phenomenon not apparent when interpreting the cortical area and dimension data. Here we find that for the VCPO, SO and OF microstructural variables, both the medial and posterior quadrants consistently correlate with

age while the anterior and lateral quadrants do not. This phenomenon is most likely due to factors described by Enlow (1963, 1976) and Anderson (1970). Anderson (1970:165), describing the skeletal growth of the beagle, states:

Some areas of this bone persist for long periods of time following its formation and others are rapidly removed. This is partly necessitated by the realignment of the cortex with respect to the curvature of the shaft, known as "drift." For example, the primary bone tissue about the region of the linea aspera is an area which is retained for a relatively long period of time. However, bone tissue opposite the linea aspera is quickly removed because this is the site of maximum expansion. Therefore, drift is accomplished by addition of bone in certain sites and removal of bone from others.

Thus, it is apparent that the posterior quadrant of the cortex remains stable during growth due to posterior muscle attachments and relative to the drift occurring in the other quadrants. Thus we would expect to find a higher correlation with age for histomorphological features seen in the posterior quadrant, which is the case.

Saguinus, however, possesses strong correlations with age for the medial quadrant as well. This is understandable when one examines the femoral functional morphology and muscle-bone map discussed in Chapter II. As a quadruped and powerful springer, Saguinus possess no true linea aspera. Several of the major muscle origins and insertions along the posterior half of the femur, are displaced medially. This would stabilize the medial quadrant of the cortex as well as

the posterior, and account for the high correlation with age of both quadrants.

Cortical Dimensions and Histomorphological
Changes with Maturity and Senescence

Of the three cortical dimensions and three histomorphological features significantly correlated with age for adult S. fuscicollis, four are the same variables described for subadults. These are not as highly correlated with age for adults, however, and only one, minimum cortical thickness (NCT) is significant to the 0.0001 level.

As described by Duncan (1973), Garn et al. (1969), Garn (1970), Van Gerven (1973), and Van Gerven et al. (1969) the total cortical area (TCA) and maximum and minimum cortical thickness parameters (SCT, NCT) exhibit significant linear regression trends from the time skeletal maturity is reached, throughout adulthood, and into senescence. Plots of actual and predicted values for these three parameters are presented in Figures 24, 25 and 26, respectively. Here, area and thickness of the cortex have a negative correlation with age and are seen to significantly decrease with advancing senescence. This is the result of cortical involution. The slope of maximum cortical diameter is near zero, indicating that periosteal apposition throughout life does not occur to a significant degree, as has been described for humans (Epker and Frost, 1966).

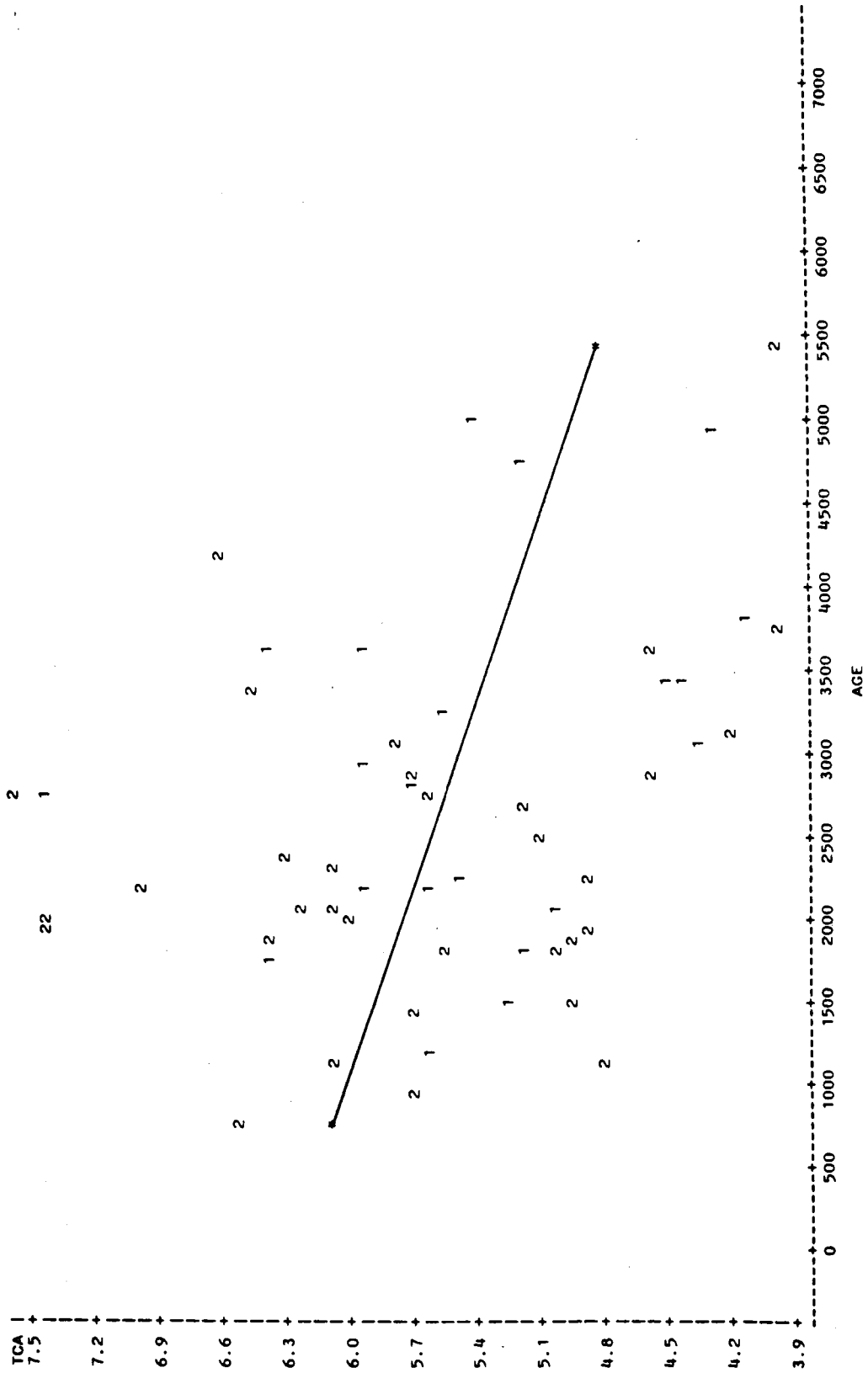


Figure 24. Plot of actual and predicted values of total cortical area on age for *Saguinus fuscicollis* adults.

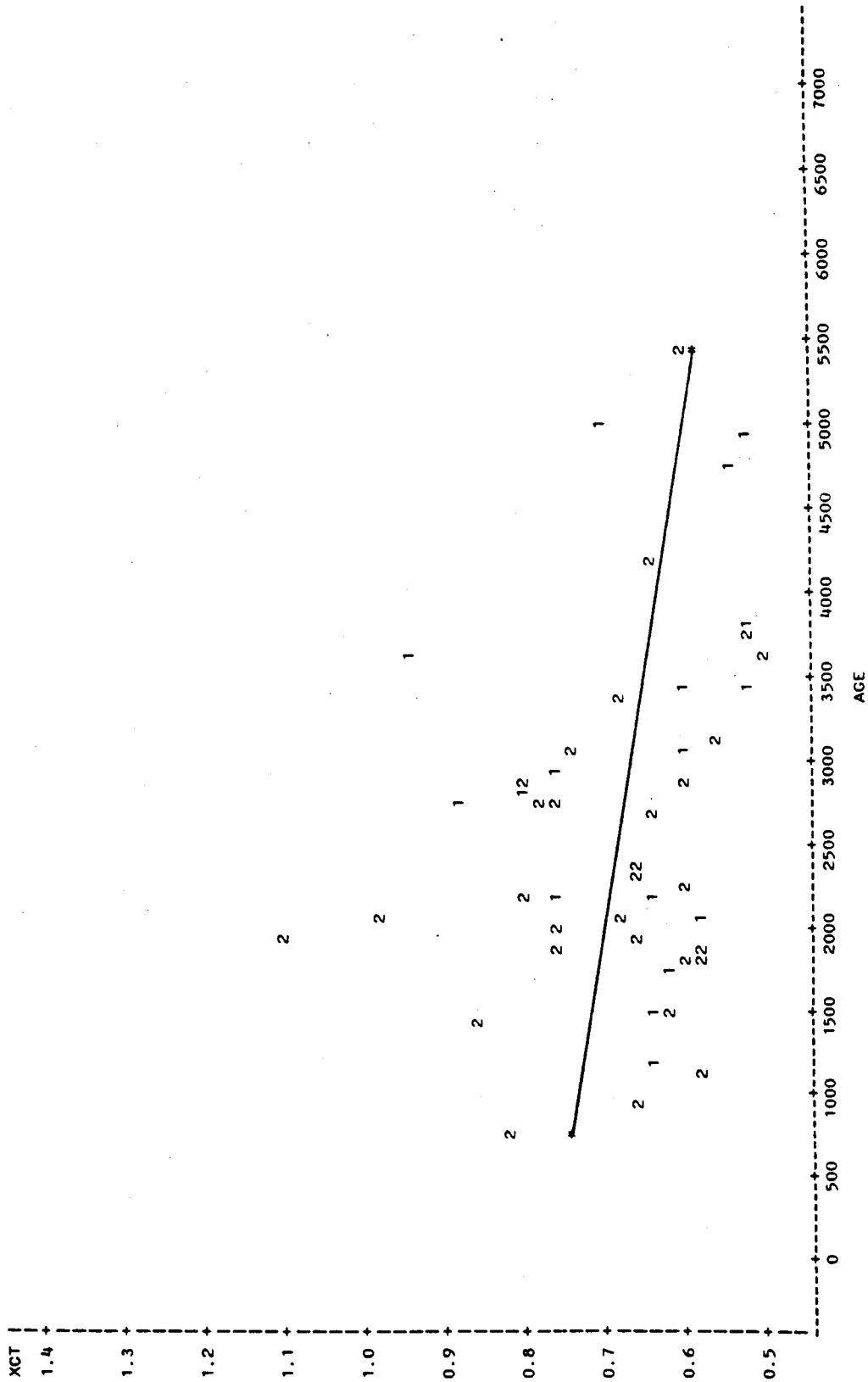


Figure 25. Plot of actual and predicted values of maximum cortical thickness on age for Saguinus fuscicollis adults.

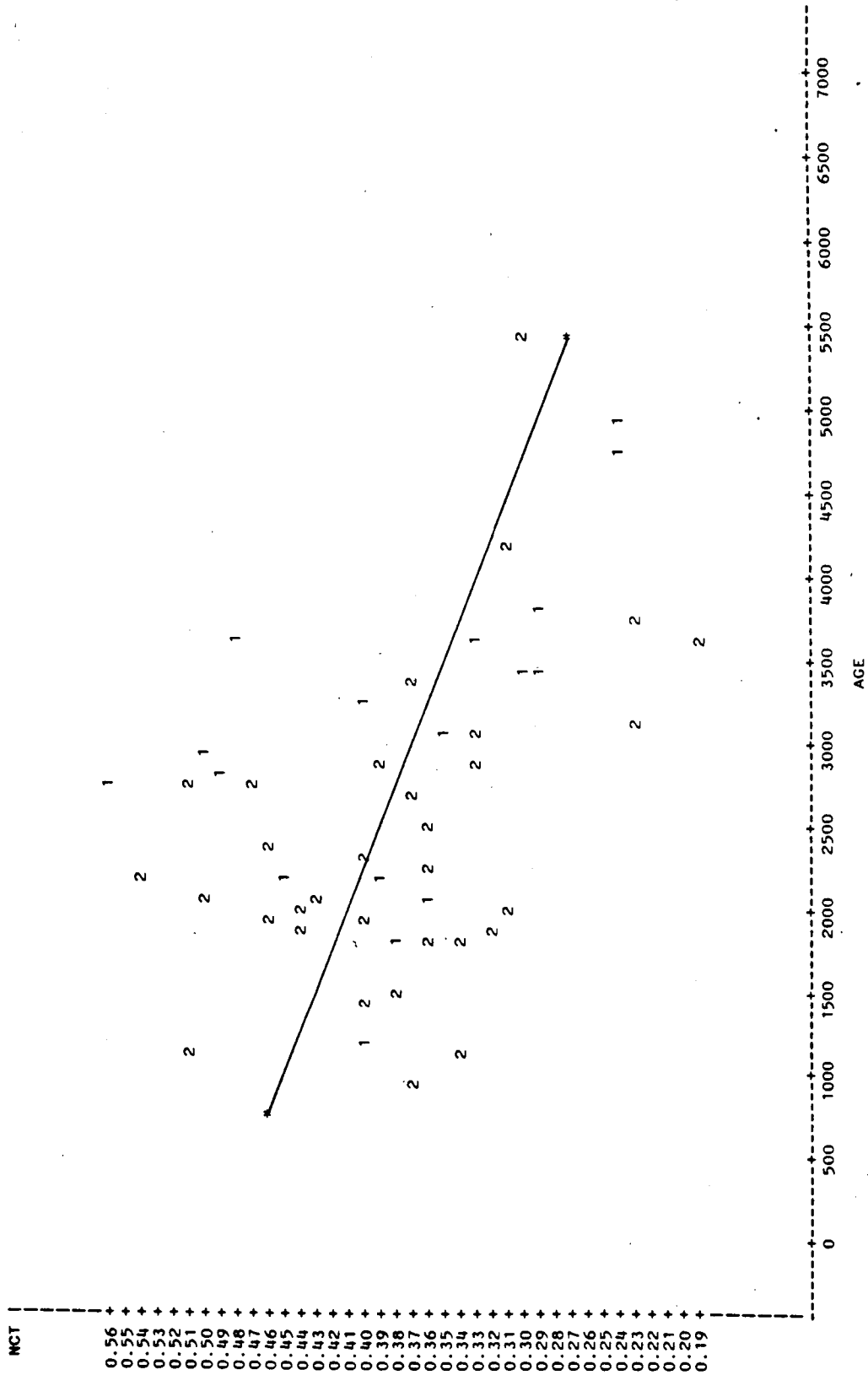


Figure 26. Plot of actual and predicted values of minimum cortical thickness on age for Saguinus fuscicollis adults.

For the cortical microstructural variables significantly correlated with age for adult S. fuscicollis, the meaning of the forming osteon count for the anterior quadrant and cortex total is unclear. Of the adults included in this analysis (N = 54), only three individuals had an anterior quadrant forming osteon count (FOA) greater than zero, and seven individuals for the forming osteon cortex total (FOT) count. Thus, the correlation and predictability with age might be due to the fact that most values are zero.

Although the skeletal histomorphology of Saguinus is that characteristic of all primates, microscopic ageing techniques such as those established for humans are only of limited applicability. However, broad qualitative ageing trends were recognized and are summarized here. These trends are similar to those previously reported by this author (Glassman and Schmidt, 1980).

At birth through approximately three months, the cortex incorporates a highly vascular network of non-Haversian canals, without exhibiting well defined periosteal and endosteal borders.

By three months the cortex becomes more structurally organized with rapid deposition of circumferential lamellae coupled with the endosteal resorption characteristic of growth remodeling. Secondary osteon remodeling initially appears at three months of age, and soon thereafter, secondary osteon fragments appear. Secondary remodeling is

located endosteally due to a rapidly expanding marrow cavity and is found to be most stable in the posterior and medial quadrants of subadults. Although there is no true linea aspera, the posteriomедial age correlation with osteon remodeling suggests a stabilization of those quadrants by the attachments of the major femoral locomotor muscles.

From nine to approximately 15 months, the rapid cortical expansion continues with more variable internal remodeling seen in all quadrants. By one year a pronounced anteriorly directed cortical drift begins which continues into skeletal maturity at age two years. From two to five and one half years, as cortical drift continues, the posterior zone of osteon remodeling shifts from the inner border of the cortex to midcortex as endosteal deposition proceeds. Between six and nine years, the degree of variability within this sample was such that it was difficult to isolate any characteristic age trend.

Specimens older than nine years, however, were distinguishable due to medullary cavity expansion. Cortical involution resulted in an endosteal thinning of the cortex to such an extent that all evidence of internal remodeling were lost, with the exception of the posteriomедial region of the cortex at a site of muscle attachment. Involution proceeded until the cortex was approximately one half to one third the average thickness of a middle aged adult.

Thus, the present analysis of histomorphologic age-changes does yield useful though limited age criteria for estimation in Saguinus. It is suggested that specimens may be assigned to general age categories. For example, infants are characterized by rapid cortical expansion and posteriorly, localized endosteal remodeling, adolescents by an onset of anterior cortical drift and increased endosteal remodeling, young adults by an increased degree of anterior cortical drift and posterior endosteal deposition, and finally old adults by extreme endosteal resorption resulting in cortical thinning.

Future study, directed at specific quantification of the histomorphology may better isolate the changes between these categories and subsequently increase the reliability of estimating age in unknown specimens.

CHAPTER VI

CONCLUSIONS

This thesis has attempted to investigate and document the normal histomorphological age changes in the femoral cortex at midshaft of two species of tamarin, Saguinus fuscicollis and Saguinus oedipus. These changes were investigated on two sets of cortical data: (1) area and dimensions of the cortex itself, and (2) histomorphological features within the cortex. These data were analyzed separately for subadults and adults, as the changes which occur with growth are different from those which occur with ageing. All conclusions presented below reflect the results of this study.

At birth, Saguinus fuscicollis possesses a mean total cortical area of 1.1 mm^2 , with a maximum and minimum diameter of 1.9 mm and 1.6 mm respectively. From birth, the mean total cortical area doubles within the first four months and doubles again by one year of age.

For subadults, cortical areas and dimensions are all significantly correlated with age. Each exhibits a significant linear increase with age as a result of the growth process. Data indicate a significant difference in size between S. fuscicollis and S. oedipus, although their rate of growth is essentially the same. Additionally, S. oedipus exhibits a thicker cortex than does S. fuscicollis, a

reflection of larger body size and possibly greater locomotor activity.

Cortical microstructure also exhibit significantly correlated linear trends with growth. Vascular canals and primary osteons, both associated with the rapid expansion of a growing cortex, decrease significantly with age as growth slows nearing maturity. It seems that much of this cortical expansion occurs on the anterior and lateral quadrants, reflecting a more stable posteriomedial half of the cortex.

The histomorphology of secondary remodeling, however, exhibits positive correlations with age and increases in count with growth. Here, the medial and posterior quadrants for secondary osteon and osteon fragment counts, as well as the total secondary remodeling counts, are seen to be significantly correlated with age. This is most likely a reflection of the stabilization at the posterior and medial quadrants due to the origin of the adductor longus muscle or other posterior musculature.

Adults, however, show fewer cortical features correlated with age. Those dimensions which do exhibit a significant relationship are all negative. These include the total cortical area, as well as the maximum and minimum cortical thicknesses. These progressively decrease with age as a result of cortical involution.

The histomorphological features associated with secondary remodeling of the cortex normally seen in the adult do not exhibit trends found in larger primates. Here, one would expect to find increasing secondary osteon and osteon fragment counts, a factor seen only for osteon fragments in the lateral quadrant. It is suspected that the lack of additional correlations is a result of the femoral cortical involution previously described for the decrease in cortical area and thickness. Evidence of secondary remodeling is apparently lost to cortical involution.

Although growth and age related patterns are evident in the cortical dimensions and histomorphology analyzed in this study, none were found to significantly correlate with age throughout the entire lifespan of the species. Therefore, due to the extensive variation evident for all variables at any age, estimation of chronological age for Saguinus individuals of unknown birth date based on these femoral parameters is suggested to be unreliable. It is recommended, however, that subsequent analysis subject the data to multiple linear regression analysis to further test their relationships.

It is also suggested that subsequent investigation employ microradiographs of the undecalcified sections to better distinguish histomorphological features and document mineralization phases of growing and remodeling bone.

Additionally, osteon size and area, as well as osteon area for the total cortex, will help to further explain the patterns of histomorphology documented by this thesis.

However, the documentation of the bone biology of genus Saguinus with respect to growth and age changes will remain incomplete until x-rays of the entire bone (femur) are correlated with the cortical drift and histomorphological events described here. Only with such x-rays may the statistical events investigated be fully interpreted biologically.

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APPENDIX

Table A-1. Correlation Matrix for the Pooled Sexes of the Subadult Saguinus fuscicollis,
Section 1

Variable	AGE	TPA	TEA	XPD	NPD	XED	NED	XCT	NCT	XAP
AGE	1.0									
TPA	0.93	1.0								
TEA	0.89	0.94	1.0							
XPD	0.94	0.99	0.92	1.0						
NPD	0.89	0.98	0.91	0.97	1.0					
XED	0.91	0.95	0.98	0.95	0.92	1.0				
NED	0.87	0.81	0.97	0.89	0.90	0.95	1.0			
XCT	0.64	0.73	0.47	0.74	0.74	0.53	0.43	1.0		
NCT	0.61	0.70	0.45	0.73	0.74	0.48	0.43	0.88	1.0	
XAP	0.89	0.98	0.93	0.97	0.99	0.93	0.81	0.70	0.70	1.0
XML	0.93	0.97	0.88	0.99	0.97	0.93	0.86	0.76	0.73	0.94
TARS	0.28	0.27	0.11	0.29	0.26	0.19	0.14	0.61	0.30	0.23
VCPOA	-0.01	0.22	0.09	0.16	0.27	0.10	0.05	0.37	0.32	0.26
VCPOM	-0.51	-0.32	-0.40	-0.32	-0.22	-0.39	-0.34	0.02	-0.03	-0.23
VCPOP	-0.59	-0.46	-0.54	-0.47	-0.39	-0.57	-0.52	-0.04	-0.09	-0.41
VCPOL	-0.39	-0.30	-0.34	-0.33	-0.29	-0.32	-0.39	-0.09	-0.17	-0.28
SOA	-0.12	-0.03	-0.19	0.01	-0.02	-0.17	-0.19	0.28	0.34	0.02
SOM	0.58	0.59	0.58	0.57	0.50	0.52	0.48	0.43	0.43	0.58
SOP	0.51	0.53	0.57	0.51	0.47	0.51	0.55	0.24	0.28	0.53
SOL	0.14	0.18	0.02	0.22	0.19	0.10	0.07	0.47	0.35	0.19
FOA	-0.18	-0.16	-0.24	-0.16	-0.13	-0.25	-0.25	0.06	0.12	-0.11
FOM	0.03	0.09	-0.02	0.12	0.09	-0.01	-0.06	0.26	0.39	0.12
FOP	0.01	0.09	-0.05	0.09	0.08	-0.01	-0.07	0.35	0.42	0.15
FOL	-0.12	-0.01	-0.18	0.01	0.03	-0.16	-0.21	0.38	0.36	0.01
OFA	0.19	0.12	0.06	0.14	0.17	0.09	0.10	0.25	0.23	0.14
OFM	0.67	0.47	0.40	0.52	0.42	0.46	0.37	0.56	0.37	0.40
OFF	0.41	0.37	0.32	0.41	0.35	0.36	0.34	0.35	0.33	0.34
OFL	0.38	0.33	0.20	0.34	0.30	0.25	0.17	0.53	0.37	0.29
RSA	-0.10	-0.04	-0.16	-0.02	-0.01	-0.13	-0.15	0.31	0.19	-0.02

Table A-1 (continued)

Variable	AGE	TPA	TEA	XPD	NPD	XED	NED	XCT	NCT	XAP
RSM	0.01	0.01	-0.16	0.04	0.02	-0.11	-0.15	0.43	0.28	0.01
RSP	-0.02	0.01	-0.03	-0.01	-0.04	-0.05	-0.06	0.07	0.06	0.04
RSL	0.07	0.13	-0.04	0.12	0.10	-0.07	-0.10	0.48	0.46	0.14
MDL	0.89	0.93	0.85	0.95	0.96	0.87	0.85	0.73	0.78	0.95
TCA	0.81	-0.91	0.72	0.92	0.91	0.76	0.68	0.92	0.89	0.89
TCMD	0.54	-0.68	0.42	0.69	0.67	0.47	0.37	0.91	0.84	0.64
VCPOT	-0.41	-0.17	-0.29	-0.21	-0.09	-0.29	-0.03	0.17	0.10	-0.10
SOT	0.48	-0.53	0.45	0.53	0.47	0.42	0.41	0.51	0.52	0.54
FOT	-0.11	-0.01	-0.19	0.01	0.01	-0.19	-0.22	0.36	0.43	0.04
OFT	0.63	-0.47	0.38	0.52	0.45	0.44	0.37	0.61	0.44	0.43
RST	-0.04	0.02	-0.15	-0.03	0.02	-0.13	-0.16	0.44	0.32	0.04
SAT	-0.13	-0.05	-0.24	-0.02	-0.01	-0.21	-0.23	0.39	0.36	0.01
SMT	0.63	0.56	0.47	0.59	0.49	0.47	0.39	0.65	0.55	0.54
SPT	0.46	-0.49	0.47	0.48	0.43	0.42	0.45	0.34	0.38	0.51
SLT	0.14	-0.20	-0.02	0.22	0.20	0.02	-0.04	0.65	0.53	0.21
TSR	0.39	-0.43	0.25	0.45	0.39	0.26	0.22	0.69	0.62	0.44

Table A-2. Correlation matrix for the pooled sexes of the subadult Saguinus fuscicollis,
Section 2

Variable	XML	TARS	VCPOA	VCPOM	VCPOP	VCPOL	SOA	SOM	SOP	SOL
AGE										
TPA										
TEA										
XPD										
NPD										
XED										
NED										
XCT										
NCT										
XAP										
XML	1.0									
TARS	0.31	1.0								
VCPOA	0.18	0.06	1.0							
VCPOM	-0.31	0.23	0.36	1.0						
VCPOP	-0.46	0.12	0.25	0.83	1.0					
VCPOL	-0.31	-0.21	0.36	0.28	0.33	1.0				
SOA	-0.01	0.08	-0.01	0.17	0.09	0.01	1.0			
SOM	0.49	0.14	-0.12	-0.42	-0.35	-0.31	0.35	1.0		
SOP	0.45	0.05	0.04	-0.29	-0.35	-0.37	0.07	0.58	1.0	
SOL	0.23	0.44	0.19	0.18	-0.02	-0.13	0.34	0.10	0.05	1.0
FOA	-0.17	0.02	0.12	0.32	0.40	0.17	0.32	0.03	-0.11	0.05
FOM	0.11	-0.06	-0.17	-0.07	-0.07	-0.03	0.77	0.51	0.02	0.10
FOP	0.03	0.06	0.05	0.15	0.15	0.16	0.76	0.45	0.22	0.15
FOL	0.04	0.28	0.21	0.42	0.41	-0.19	0.59	0.15	-0.01	0.25
OFA	0.18	0.22	0.11	0.36	0.22	0.02	-0.07	-0.15	-0.07	0.11
OFM	0.52	0.55	-0.15	-0.34	-0.31	-0.30	-0.11	0.41	0.19	0.43
OFF	0.44	0.11	-0.13	-0.21	-0.29	-0.26	0.18	0.22	0.41	0.44
OFL	0.34	0.60	0.08	0.06	0.02	-0.09	-0.02	0.27	0.17	0.41
RSA	0.01	0.52	0.17	0.62	0.56	-0.09	0.09	-0.18	-0.21	0.08

Table A-2 (continued)

Variable	XML	TARS	VCPOA	VCPOM	VCPOP	VCPOL	SOA	SOM	SOP	SOL
RSM	0.04	0.55	0.15	0.46	0.47	-0.08	0.21	-0.03	-0.17	0.28
RSP	-0.07	0.03	-0.03	-0.06	-0.01	0.15	0.49	0.40	0.42	0.01
RSL	0.08	0.33	0.28	0.28	0.36	-0.09	0.33	0.38	0.22	0.19
MDL	0.94	0.26	0.19	-0.21	-0.38	-0.33	0.07	0.52	0.45	0.20
TCA	0.92	0.41	0.33	-0.18	-0.29	-0.20	0.17	0.51	0.40	0.33
TCMD	0.70	0.47	0.36	-0.02	-0.05	-0.04	0.31	0.39	0.26	0.42
VCPOP	-0.19	0.09	0.80	-0.79	0.73	0.60	0.08	-0.36	-0.24	0.12
SOT	0.47	0.23	0.02	-0.20	-0.28	-0.33	0.60	0.83	0.73	0.45
FOT	-0.01	0.13	0.14	0.32	0.35	0.03	0.80	0.35	0.03	0.21
OFT	0.54	0.56	-0.08	-0.14	-0.19	-0.26	-0.06	0.31	0.23	0.48
RST	0.02	0.55	0.21	0.55	0.54	-0.07	0.27	0.06	-0.04	0.18
SAT	-0.01	0.40	0.14	0.59	0.51	0.01	0.65	0.06	-0.12	0.26
SMT	0.54	0.45	-0.12	-0.28	-0.22	-0.34	0.34	0.85	0.39	0.38
SPT	0.42	0.07	0.04	-0.23	-0.28	-0.27	0.34	0.64	0.97	0.16
SLT	0.23	0.55	0.28	0.37	0.25	-0.18	0.47	0.28	0.13	0.76
TSR	0.41	0.50	0.10	0.12	0.07	-0.27	0.61	0.65	0.46	0.50

Table A-3. Correlation Matrix for the Pooled Sexes of the Subadult Saguinus fuscicollis,
Section 3

Variable	FOA	FOM	FOP	FOL	OFA	OFM	OFF	OFL	RSA	RSM
AGE										
TPA										
TEA										
XPD										
NPD										
XED										
NED										
XCT										
NCT										
XAP										
XML										
TARS										
VCPOA										
VCPOM										
VCPOP										
VCPOL										
SOA										
SOM										
SOP										
SOL										
FOA	1.0									
FOM	0.19	1.0								
FOP	0.36	0.49	1.0							
FOL	0.46	0.47	0.34	1.0						
OFA	0.42	-0.16	0.05	0.32	1.0					
OFM	-0.06	-0.01	-0.03	-0.01	0.21	1.0				
OFF	-0.07	0.28	-0.03	0.13	-0.09	0.49	1.0			
OFL	0.08	-0.13	0.13	0.18	0.48	0.72	0.13	1.0		
RSA	0.18	-0.05	0.06	0.45	0.33	0.05	-0.05	0.21	1.0	

Table A-3 (continued)

Variable	FOA	FOM	FOP	FOL	OFA	OFM	OFF	OFL	RSA	RSM
RSM	0.76	-0.05	0.27	0.41	0.38	0.35	-0.02	0.48	0.84	1.0
RSP	0.42	0.37	0.53	0.18	0.07	-0.11	-0.05	0.01	-0.21	-0.09
RSL	0.43	0.13	0.54	0.59	0.34	0.24	-0.04	0.54	0.50	0.65
MDL	-0.05	0.17	0.16	0.07	0.23	0.46	0.39	0.29	0.04	0.95
TCA	-0.04	0.21	0.25	0.21	0.18	0.47	0.35	0.43	0.12	0.19
TCMD	-0.01	0.29	0.30	0.37	0.11	0.36	0.29	0.42	0.20	0.27
VCPOT	0.31	-0.14	0.21	0.31	0.23	-0.34	-0.27	0.05	0.41	0.33
SOT	0.09	0.52	0.58	0.32	-0.09	0.34	0.56	0.28	-0.12	0.06
FOT	0.32	0.67	0.72	0.81	0.27	-0.04	0.08	0.12	0.26	0.34
OFT	0.08	-0.01	0.02	0.16	0.49	0.93	0.54	0.81	0.16	0.42
RST	0.36	0.05	0.34	0.56	0.40	0.19	-0.05	0.41	0.91	0.93
SAT	0.62	0.40	0.54	0.75	0.47	0.01	0.03	0.20	0.71	0.72
SMT	0.10	0.45	0.52	0.27	0.08	0.75	0.37	0.57	0.14	0.39
SPT	0.06	0.24	0.48	0.13	-0.05	0.22	0.48	0.19	-0.19	-0.08
SLT	0.34	0.26	0.39	0.70	0.37	0.45	0.29	0.65	0.40	0.59
TSR	0.37	0.47	0.62	0.61	0.29	0.50	0.40	0.54	0.35	0.55

Table A-4. Correlation Matrix for the Pooled Sexes of the Subadult Saguinus fuscicollis,
Section 4

Variable	RSP	RSL	MDL	TCA	TCMD	VCPOT	SOT	FOT	OFT	RST
FOP										
FOL										
OFA										
OFM										
OFP										
OFL										
RSA										
RSM										
RSP	1.0									
RSL	0.22	1.0								
MDL	-0.03	0.15	1.0							
TCA	0.06	0.32	0.88	1.0						
TCMD	0.09	0.39	0.60	0.89	1.0					
VCPOT	0.01	0.31	-0.14	0.01	0.16	1.0				
SOT	0.52	0.42	0.50	0.54	0.50	-0.20	1.0			
FOT	0.50	0.61	0.10	0.20	0.32	0.27	0.48	1.0		
OFT	-0.06	0.35	0.49	0.51	0.41	-0.19	0.35	0.10	1.0	
RST	0.07	0.78	0.09	0.22	0.31	0.41	0.15	0.49	0.30	1.0
SAT	0.27	0.64	0.09	0.18	0.30	0.40	0.26	0.81	0.19	0.82
SMT	0.24	0.53	0.55	0.59	0.50	-0.28	0.74	0.39	0.68	0.38
SPT	0.58	0.32	0.57	0.43	0.34	-0.18	0.85	0.29	0.27	0.05
SLT	0.14	0.73	0.23	0.43	0.55	0.28	0.54	0.60	0.59	0.62
TSR	0.42	0.75	0.47	0.56	0.57	0.04	0.82	0.70	0.59	0.62

Table A-5. Correlation Matrix for the Pooled Sexes of the Subadult Saguinus fuscicollis,
Section 5

Variable	SAT	SMT	SPT	SLT	TSR
FOP					
FOL					
OFA					
OFM					
OFF					
OFL					
RSA					
RSM					
RSP					
RSL					
MDL					
TCA					
TCMD					
VCPOT					
SOT					
FOT					
OFT					
RST					
SAT	1.0				
SMT	0.30	1.0			
SPT	0.08	0.50	1.0		
SLT	0.64	0.55	0.27	1.0	
TSR	0.67	0.82	0.63	0.82	1.0

Table A-6. Correlation Matrix for the Pooled Sexes of the Adult *Saguinus fuscicollis*, Section 1

Variable	AGE	TPA	TEA	XPD	NPD	XED	NED	XCT	NCT	XAP
AGE	1.0									
TPA	-0.09	1.0								
TEA	0.09	0.86	1.0							
XPD	-0.04	0.92	0.85	1.0						
NPD	-0.19	0.91	0.69	0.74	1.0					
XED	0.11	0.75	0.95	0.84	0.53	1.0				
NED	0.07	0.83	0.94	0.76	0.77	0.83	1.0			
XCT	-0.28	-0.01	-0.46	-0.09	0.11	-0.54	-0.43	1.0		
NCT	-0.50	-0.07	-0.49	-0.12	0.14	-0.53	-0.40	0.72	1.0	
XAP	-0.15	0.91	0.71	0.73	0.96	0.54	0.77	0.06	0.09	1.0
XML	-0.01	0.87	0.85	0.94	0.66	0.86	0.74	-0.19	-0.19	0.65
TARS	-0.19	0.08	-0.08	0.13	0.07	-0.09	-0.10	0.56	0.19	0.02
VCPOA	0.04	-0.02	-0.25	-0.14	0.07	-0.31	-0.19	0.31	0.32	0.10
VCPOM	0.07	-0.08	-0.26	-0.15	-0.02	-0.34	-0.20	0.18	0.27	0.03
VCPOP	0.08	0.07	-0.09	0.01	0.09	-0.10	-0.02	0.19	0.23	0.05
VCPOL	-0.02	0.12	-0.08	0.11	0.07	-0.11	-0.04	0.29	0.22	0.06
SOA	-0.25	-0.14	-0.23	-0.19	-0.11	-0.24	-0.21	0.07	0.19	-0.08
SOM	-0.19	-0.05	-0.14	-0.04	-0.03	-0.13	-0.15	0.19	0.19	-0.01
SOP	-0.11	-0.34	-0.35	-0.29	-0.25	-0.33	-0.35	0.10	0.20	-0.30
SOL	-0.11	-0.35	-0.40	-0.34	-0.22	-0.40	-0.38	0.11	0.20	-0.24
FOA	0.44	-0.14	-0.06	-0.10	-0.17	-0.07	-0.07	-0.06	-0.09	-0.14
FOM	0.07	0.09	0.19	0.09	0.05	0.19	0.19	-0.14	-0.18	0.03
FOP	0.08	-0.02	0.08	-0.07	0.06	-0.01	0.12	-0.01	-0.06	0.02
FOL	-0.08	-0.13	-0.21	-0.18	-0.10	-0.21	-0.28	0.33	0.19	-0.15
OFA	0.07	-0.06	-0.07	-0.06	-0.09	-0.10	-0.08	0.06	0.02	-0.05
OFM	-0.22	0.36	0.32	0.27	0.34	0.21	0.31	0.13	-0.05	0.35
OFF	0.04	-0.13	-0.18	-0.12	-0.07	-0.21	-0.13	0.10	0.20	-0.09
OFL	0.32	-0.15	-0.09	-0.14	-0.10	-0.09	-0.11	-0.04	-0.13	-0.08
RSA	-0.24	0.13	0.06	0.13	0.17	0.08	-0.01	0.16	0.13	0.15

Table A-6 (continued)

Variable	AGE	TPA	TEA	XPD	NPD	XED	NED	XCT	NCT	XAP
RSM	-0.13	-0.10	-0.18	-0.11	-0.07	-0.23	-0.16	0.33	0.11	-0.06
RSP	-0.05	-0.11	-0.08	-0.15	-0.08	-0.10	-0.12	0.13	0.13	-0.11
RSL	-0.08	-0.13	-0.21	-0.18	-0.10	-0.21	-0.28	0.33	0.19	-0.15
MDL	-0.22	0.52	0.27	0.44	0.54	0.20	0.34	0.19	0.32	0.58
TCA	-0.32	0.49	-0.03	0.34	0.58	-0.16	0.02	0.74	0.70	0.56
TCMD	-0.31	0.37	-0.14	0.23	0.47	-0.26	-0.10	0.79	0.70	0.43
VCPOT	0.05	0.01	-0.24	-0.08	0.07	-0.31	-0.17	0.36	0.35	0.09
SOT	-0.19	-0.31	-0.37	-0.28	-0.22	-0.36	-0.37	0.16	0.26	-0.24
FOT	0.30	-0.09	0.01	-0.11	-0.08	-0.03	0.01	-0.01	-0.09	-0.09
OFT	0.05	0.07	0.04	0.03	0.09	-0.04	0.05	0.13	0.02	0.11
RST	-0.14	-0.11	-0.15	-0.16	-0.07	-0.18	-0.20	0.30	0.21	-0.11
SAT	-0.04	-0.12	-0.17	-0.14	-0.13	-0.19	-0.17	0.08	0.11	-0.08
SMT	-0.23	0.17	0.09	0.12	0.17	0.03	0.08	0.20	0.08	0.18
SPT	-0.07	-0.33	-0.35	-0.29	-0.23	-0.35	-0.33	0.13	0.25	-0.28
SLT	0.09	-0.33	-0.34	-0.32	-0.21	-0.34	-0.34	0.08	0.08	-0.22
TSR	-0.12	-0.22	-0.28	-0.22	-0.14	-0.31	-0.27	0.20	0.22	-0.15

Table A-7. Correlation Matrix for the Pooled Sexes of the Adult Saguinus fuscicollis, Section 2

Variable	XML	TARS	VCPOA	VCPOM	VCPOP	VCPOL	SOA	SOM	SOP	SOL
AGE										
TPA										
TEA										
XPD										
NPD										
XED										
NED										
XCT										
NCT										
XAP										
XML	1.0									
TARS	0.06	1.0								
VCPOA	-0.17	0.08	1.0							
VCPOM	-0.24	-0.07	0.60	1.0						
VCPOP	0.01	0.01	0.49	0.23	1.0					
VCPOL	0.03	0.29	0.42	0.41	0.47	1.0				
SOA	-0.15	-0.06	0.07	0.17	-0.08	-0.10	1.0			
SOM	-0.06	0.18	-0.02	-0.06	-0.11	0.03	0.46	1.0		
SOP	-0.27	0.14	0.01	0.06	-0.12	-0.04	0.47	0.09	1.0	
SOL	-0.37	-0.04	-0.01	0.13	-0.14	-0.16	0.22	-0.07	0.21	1.0
FOA	-0.09	-0.04	0.20	0.18	0.05	0.01	0.30	0.01	0.03	0.07
FOM	0.13	-0.03	-0.05	-0.07	-0.04	0.06	-0.10	-0.03	0.07	-0.10
FOP	-0.03	-0.05	-0.05	0.01	-0.06	0.01	-0.05	-0.04	0.06	0.08
FOL	-0.14	0.09	-0.04	-0.04	-0.03	-0.08	-0.05	-0.04	-0.01	-0.01
OFA	0.01	-0.04	-0.11	-0.09	0.05	-0.13	0.27	0.04	-0.01	-0.01
OFM	0.23	0.18	-0.19	-0.02	-0.02	-0.03	-0.07	0.46	0.12	-0.12
OFF	-0.07	0.06	0.02	-0.08	-0.07	-0.04	0.08	0.12	0.29	0.16
OFL	-0.13	-0.07	0.11	0.20	-0.08	-0.10	-0.05	-0.09	0.13	0.36
RSA	0.16	0.09	-0.04	0.02	-0.07	-0.08	-0.05	0.08	-0.11	-0.01

Table A-7 (continued)

Variable	XML	TARS	VCPOA	VCPOM	VCPOP	VCPOL	SOA	SOM	SOP	SOL
RSM	-0.13	0.63	0.10	-0.07	-0.09	0.20	-0.07	0.08	0.03	-0.01
RSP	-0.09	0.14	-0.14	-0.04	-0.11	-0.13	-0.05	-0.09	0.06	0.14
RSL	-0.14	0.09	-0.04	-0.04	-0.03	-0.08	-0.05	-0.04	0.06	-0.01
MDL	0.35	-0.07	0.08	0.03	0.13	0.04	-0.13	0.13	-0.31	-0.38
TCA	0.24	0.29	0.39	0.28	0.29	0.36	0.11	0.15	-0.06	-0.01
TCMD	0.14	0.36	0.41	0.31	0.28	0.40	0.17	0.13	0.05	0.13
VCPOP	-0.15	0.10	0.91	0.74	0.64	0.69	0.04	0.01	-0.01	-0.04
SOT	-0.28	0.11	0.01	0.07	-0.15	-0.02	0.53	0.65	0.96	0.72
FOT	-0.06	-0.04	0.06	0.07	-0.02	0.01	0.17	-0.06	0.17	0.04
OFT	0.05	0.09	-0.05	-0.11	-0.05	-0.12	0.07	0.30	0.26	0.13
RST	-0.11	0.33	-0.10	-0.06	-0.13	-0.07	-0.09	-0.04	0.05	0.11
SAT	-0.07	-0.06	-0.01	0.06	-0.01	-0.14	0.73	0.03	0.26	0.24
SMT	0.09	0.23	-0.07	-0.15	-0.08	0.11	-0.02	0.86	0.40	-0.02
SPT	-0.25	0.15	-0.01	0.01	-0.14	-0.06	0.38	0.46	0.92	0.62
SLT	-0.33	-0.06	0.04	0.19	-0.14	-0.17	0.29	0.01	0.53	0.87
TSR	-0.20	0.15	-0.02	0.01	-0.15	-0.07	0.45	0.63	0.86	0.62

Table A-8. Correlation Matrix for the Pooled Sexes of the Adult Saguinus fuscicollis, Section 3

Variable	FOA	FOM	FOP	FOL	OFA	OFM	OFF	OFL	RSA	RSM
AGE										
TPA										
TEA										
XPD										
NPD										
XED										
NED										
XCT										
NCT										
XAP										
XML										
TARS										
VCPOA										
VCPOM										
VCPOP										
VCPOL										
SOA										
SOM										
SOP										
SOL										
FOA	1.0									
FOM	0.26	1.0								
FOP	0.15	0.32	1.0							
FOL	-0.03	-0.03	-0.04	1.0						
OFA	0.41	0.01	-0.31	-0.04	1.0					
OFM	0.03	0.16	0.28	-0.78	0.20	1.0				
OFF	0.41	-0.09	-0.01	-0.04	0.19	0.09	1.0			
OFL	0.22	0.31	0.31	-0.08	-0.06	0.01	0.01	1.0		
RSA	-0.03	-0.03	-0.04	-0.02	0.13	-0.08	-0.04	0.01	1.0	

Table A-8 (continued)

Variable	FOA	FOM	FOP	FOL	OFA	OFM	OFF	OFL	RSA	RSM
RSM	-0.04	-0.04	0.32	-0.03	-0.05	0.04	0.04	0.01	-0.03	1.0
RSP	-0.07	-0.06	0.20	0.52	-0.08	-0.14	-0.04	0.01	-0.04	0.14
RSL	-0.03	-0.03	-0.04	-0.07	-0.04	-0.08	-0.04	-0.08	-0.02	-0.03
MDL	-0.18	-0.08	-0.32	-0.07	0.13	0.23	-0.12	-0.23	0.11	-0.18
TCA	-0.17	-0.13	-0.16	0.11	0.01	0.15	0.04	-0.14	0.14	0.12
TCMD	-0.15	-0.12	-0.06	0.15	-0.04	0.09	0.08	-0.08	0.12	0.21
VC POT	0.17	-0.04	-0.03	-0.06	-0.11	-0.12	-0.03	0.07	-0.05	0.07
SOT	0.14	0.04	0.03	0.01	0.05	0.18	0.25	0.13	-0.05	0.03
FOT	0.73	0.63	0.66	0.20	0.22	0.18	0.19	0.36	-0.05	0.10
OFT	0.43	0.16	0.27	-0.10	0.54	0.70	0.59	0.35	-0.01	0.03
RST	-0.08	-0.07	0.24	0.61	-0.06	-0.13	-0.04	-0.01	0.17	0.40
SAT	0.51	0.20	-0.06	-0.06	0.85	0.09	0.22	-0.04	0.11	-0.08
SMT	-0.02	0.13	0.16	-0.07	0.14	0.84	0.12	-0.04	-0.01	0.11
SPT	0.33	-0.01	0.12	0.08	0.06	0.14	0.62	0.12	-0.11	0.07
SLT	0.16	0.09	0.21	0.04	-0.05	-0.09	0.11	0.76	-0.04	-0.01
TSR	0.33	0.13	0.18	0.01	0.28	0.44	0.45	0.27	-0.03	0.07

Table A-9. Correlation Matrix for the Pooled Sexes of the Adult Saguinus fuscicollis, Section 4

Variable	RSP	RSL	MDL	TCA	TCMD	VCPOT	SOT	FOT	OFT	RST
FOP										
FOL										
OFA										
OFM										
OFF										
OFL										
RSA										
RSM										
RSP	1.0									
RSL	0.52	1.0								
MDL	-0.26	-0.07	1.0							
TCA	-0.06	0.11	0.55	1.0						
TCMD	0.03	0.15	0.28	0.95	1.0					
VCPOT	-0.14	-0.06	0.09	0.44	0.46	1.0				
SOT	0.03	0.01	-0.24	0.04	0.13	-0.01	1.0			
FOT	0.16	0.20	0.30	-0.19	-0.12	0.05	0.11	1.0		
OFT	-0.13	-0.10	0.04	0.06	0.04	-0.10	0.28	0.41	1.0	
RST	0.92	0.61	-0.24	0.04	0.14	-0.11	0.02	0.19	-0.11	1.0
SAT	-0.09	-0.06	-0.01	0.05	0.05	-0.03	0.33	0.32	0.44	-0.08
SMT	-0.13	-0.07	0.20	0.17	0.13	-0.06	0.50	0.09	0.58	-0.08
SPT	0.14	0.08	-0.34	-0.04	0.07	-0.04	0.86	0.27	0.44	0.12
SLT	0.15	0.04	-0.39	-0.07	0.06	0.01	0.56	0.23	0.26	0.12
TSR	0.03	0.01	-0.20	0.05	0.12	-0.05	0.90	0.32	0.66	0.04

Table A-10. Correlation Matrix for the Pooled Sexes of the Sdult Saguinus fuscicollis, Section 5

Variable	SAT	SMT	SPT	SLT	TSR
FOP					
FOL					
OFA					
OFM					
OFF					
OFL					
RSA					
RSM					
RSP					
RSL					
MDL					
TCA					
TCMD					
VCPOP					
SOT					
FOT					
OFT					
RST					
SAT	1.0				
SMT	0.08	1.0			
SPT	0.28	0.36	1.0		
SLT	0.14	-0.04	0.49	1.0	
TSR	0.45	0.63	0.88	0.57	1.0

VITA

Dwight John Schmidt was born in Jacksonville, Florida, on November 10, 1952. At age two he moved to Okinawa where he began elementary school. Moving to Pennsylvania at age eight he was graduated from Elizabethtown Area Highschool in June 1970. After working for one year as an operating room assistant at the Milton S. Hershey Medical Center, he entered Catawba College, Salisbury, North Carolina, and in August 1975 received a Bachelor of Arts degree in anthropology.

He worked for one year at the Department of Anthropology, Smithsonian Institution, Washington, D.C., and in the fall of 1977 entered the Graduate School of The University of Tennessee, Knoxville. Upon completion of his course work he returned to the Smithsonian Institution and continued his thesis research. He received a Master of Arts degree in anthropology in June 1984.

Since 1982 he has been employed as a museum specialist, Anatomical Collections, by the Armed Forces Institute of Pathology, Washington, D.C. He is married to Candace Lewis Rhyne of Gastonia, North Carolina!