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Radiogrammetric Study of Width, Area of Cortex and Medullary Cavity of Long Bones of *Macaca Mulatta* From Juvenile to Adult Stages

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INTRODUCTION

With the invention of more accurate and improved techniques the studies of variation and development changes in composition of animal bone are rapidly multiplying. One such technique is the measurement of cortical thickness in radiographs. The amount of cortex in a bone at any given time is determined by two interrelated processes the deposition of bone on the periosteal surface and resorption of bone from endosteal surface (Ham, 1965). As changes occur in the amount of cortical bone there are corresponding changes that occur in the size of the medullary cavity as well.

There is also a clear sexual dimorphism in both, amount and percentage of cortical bone (Baker et al., 1958; Garn et al., 1963; Johnston and Malina, 1966). Careful study of this parameter is necessary so that these changes may be documented and sexual dimorphism may be evaluated more accurately.

The present study, is aimed at quantifying the width, cross–sectional area of cortex and medulary cavity of some long bones of Macaca mulata at several growth stages within and between sexes. The data presented could be a useful tool in the hands of biologists more so of Paleoanthropologists/Paleoprimatologists in ageing and sexing excavated unknown bone specimens. The data could also of help in studying the effects of ecological imbalances caused by man on wild life by examining the bones especially the cortical thickness in wild animals. Owing to the diagnostic value in certain metabolic bone disorders such as osteoporosis and osteomalacia the study of cortical thickness could be of interest to orthopaedists. Also there is increasing evidence that measurement of compact bone may prove to be of clinical value independent of bone growth and development. In Human Auxology the similar type of study is useful in highlighting the nature of disease in diagnosing the reason for growth retardation/deviation. In testing the effects of newly discovered medicines/vaccines etc. in the bones of experimental animals can pave way for eliminating the harmful factors of the same.

MATERIAL AND METHODS

The basic data presented in this study consist of measurements of subperiosteal and endosteal widths of long bones of *Macaca mulatta*. The investigation was carried out on 699 bones (376F, 323M). The specimens of the long bones were procured from the skeletal collection available in the Primatological laboratory of the Department of Anthropology, Panjab University, Chandigarh. In this collection complete individual skeletons with cranium, mandible, vertebral column, the bones of the extremities, hip bones etc. are available. Each bone was assigned a reference number.

Age Groups: The following age categories were defined on the basis of criteria of dental eruption and the degree of dental wear (Chopra, 1957). These are briefly described below.

Newborn: Specimens with no eruption of deciduous teeth.

Infant: Specimens with incomplete to complete deciduous dentition.

Juvenile 1: Specimens with partial deciduous dentition and with eruption of first permanent molars and first permanent incisors.

Juvenile II: Specimens with partial deciduous dentition and with further eruption of the second molars and the second incisors.

Juvenile III: Specimens with more advanced stages in dental eruption than in juvenile II stage but without eruption of third permanent molars (M3).

Adult I: Specimens with complete permanent dentition and pratically no dental wear (=+0).

Adult II: Specimens with complete permanent dentition and medium dental wear (=++).

Senile: Specimens with complete permanent dentition and extreme dental wear (=+++).

Sexing: The cadaveric sex was known for most of the specimens. The same was reconfirmed for the bony specimens employing the following criteria.

The shape and size of the permanent canine was the main information in this regard. In *M. mulatta*, the canine is comparatively larger, dagger shaped and incurved in adult males than in females (Zuckerman, 1928). In females the canine is smaller, less curved and little tappering.

Whenever the canine tooth was missing the shape and size of its socket was taken as an indirect evidence of sex. Due to the larger canine the socket is also larger in males and so shaped as to accommodate the incurved canine. In younger specimens where permanent canine had not erupted yet, the general criteria of comparatively smooth, light and smaller size of the female skull was used to distinguish female specimens from those more rugged skulls of the males. The sample size in each age group and sex in given in table 1.

Choice of Suitable View: Each bone was radiographed in three different views – anterior posterior and lateral views. The radiographs were examined carefully to select which particular view was the best exposure of the periosteal and endosteal margins of the bones. The anterior view was to be the best. All the bones were then fixed in their medio-lateral (anterior) view, with the help of plastocine and were thus radiographed.

Radiological Technique: A focus film distance of 170cm. was used throughout and the typical exposure time was 0.6-0.4 secs. At 10-15 mA and 60 Kv. Screen type "INDU" fast films with intensifying screens were used for all these radiographs.

Development of the films was done by hand. The developing time of the dark room was 2-5 mins. The films took 2-3 minutes in the developer and 10 mins in the fixer. The films were then washed for 15-20 minutes in running water.

Measurements and Techniques: The dried radiographs were analysed by means of a light box and a vernier caliper having least count of .02mm. Measurements on the medio-lateral bone radiographs included the total subperiosteal width and medullary cavity width. From these two measurements four other parameters were derived and these included Cortical thickness. Cross-sectional Cortical area, Medullary area and Percentage cortex. The areas were calculated by assuming a cylindrical model as described in Garn (1970) and Garn et al. (1971).

Definitions of the Measurements

Sub periosteal Width: This measures the straight distance between the two periosteal surface of the bone. The measurement is taken at mid shaft and perpendicular to the long axis.

Medullary Width: This measures the straight distance between the two endosteal surfaces of the bone. This measurement is taken at right angles to the long axis and at the same level as the previous measurement.

Cortical Thickness: This measurement was found indirectly by subtracting the second measurement above from the first and then dividing by two.

$$\text{Cortical thickness} = \frac{\text{Subperiosteal width} - \text{Medullary width}}{2}$$

The area of a circle is generally calculated as πr^2 where 'r' is the radius of the circle. This formula can also be written as $\pi (D/2)^2$, Where 'D' is the Diameter of the circle. This can be further simplified as $\pi 4(D)^2$ or $0.785 (D)^2$.

Table 1: Number of long bones studied in various age groups

	Female				Male				
Age Gp.	*Femur*	*Tibia*	*Humerus*	*Radius*	*Femur*	*Tibia*	*Humerus*	*Radius*	*Total*
J. I	26	23	25	18	16	15	14	19	156
J. II	27	22	24	26	44	42	42	38	265
A. I	34	32	32	31	18	19	18	17	201
A. II	17	11	16	12	02	05	02	04	69
Senile	-	-	-	-	02	02	02	02	08
Total	104	88	97	87	82	83	78	80	699

Cross-sectional Cortical Area: Cortical area= 0.785 (T^2-M^2) where 'T' is the Total subperiosteal width and 'M' is the Medullary cavity width.

Medullary area: Medullary area = 0.785 $(M)^2$.

Percentage Cortex:

$$= \frac{\text{Medullary width}}{\text{Total width}} \times 100$$

This data thus collected were then subjected to computational analysis for descriptive statistics.

Reliability of Measurement: The measurer's reliability was tested by selecting eighty five radiographs randomly as given in Fisher and Yates statistical tables. The subperiosteal and medullary width of these bones were taken a second time. The first readings and the second readings were compared by finding Correlation coefficients and t-test values between them.

The correlation between these double measurements were not less than 0.9998, indicating a high degree of reliability. The t-value results were also statistically insignificant at 5 percent level of probability. These results are displayed in table 2.

Table 2: The mean values, correlations and t-test values

Statistical analysis	*Subperiosteal width (mm)*		*Medullary width (mm)*	
	First obs.	*Second obs.*	*First obs.*	*Second obs.*
Mean	9.21	9.21	5.04	5.04
Standard error	1.96	1.96	1.47	1.45
Correlation (r)	0.99	N.S.	0.99	N.S.
t-value	0.01	N.S.	0.01	N.S.

N.S. indicated that the value is statistically not significant at 5% level.

RESULTS

Sample size, Means and Standard deviations of all the parameters studied in the respective bones are displayed in tables 3-4 by age and sex.

Superiosteal Width: The results indicate that in all the bones studied of both the sexes there is a gradual increase in the mean subperiosteal width with age. It is noted that bone deposition is high in the juveniles but this is slowed down as adulthood is approached. Attained mean width in males is more than in females at every successive age group. However, statistically significant sex differences ($P<.05$) were observed at JII and All in Humerus and Radius and in AII in Femur and JII in Tibia.

Medullary Width: Mean Medullary width also increases with age in both the sexes. However, the difference between the successive stages is less showing that the amount added at each successive age is comparatively very little. Infact a tendency towards reduction in medullary width is apparent in female hind limb bones. No statistically significant sex differences ($P> .05$) was observed at any age group. This indicates that that marrow cavity is almost equal in the two sexes at each age. SD tends to remain more or less constant across age groups.

Cortical Thickness: It is noticed that the increase in the mean Cortical thickness with age in females is comparatively less than in males. Sex differences ($P< .05$) are significant at Jll, Al, All in all the bones except Tibia whose value is significant at JII age group only.

For the bone to retain its approximate form it is necessary that remodelling of bone at the outer surface takes place inspite of the fact that increase in length occurs by addition of bone at the ends of the diaphysis. The reconstruction of bone is effected by process of absorption and produces an enlargement of the marrow cavity, the diaphysis of the bone is thus continually reconstructed during growth. The process of bone remodelling can be assessed by studying area changes at the midshaft of the bone.

Cross-sectional Cortical Area: At midshaft of these bones, medullary and cortical areas reflect remodelling process. Cortical area changes reflect same pattern as depicted by cortical thickness. The mean area increases with age in both the sexes. The increase is however, high in males than in females at each successive age group. It is noted that the difference between the mean Cortical areas at successive age group reduces drastically with the onset of adulthood. Sex differences ($P<.05$) are significant at JII and All in all the bones except Tibia where the value is significant at JII only.

Medullary Area: Mean medullary area changes reflect same pattern as depicted by medullary width changes in both sexes. There is a gradual increase in the means of this parameter in both sexes across ages. The values are higher in

Table 3: Mean (M) and standard Deviation (SD) for subperiosteal width. medullary width and cortical thickness of humerus and radius by age and sex

		Males							
		Humerus				*Radius*			
Age		*JI*	*JII*	*AI*	*AII*	*JI*	*JII*	*AI*	*AII*
N**		14	42	18	2	18	26	17	4
Subperiosteal width (mm)	M	7.42	9.78*	10.39	12.57*	6.07	8.02*	8.27	9.54*
	SD	0.81	1.45	1.89	0.27	0.50	1.78	1.78	0.87
Medullary width (mm)	M	4.00	5.28	5.69	6.10	2.93	3.69	3.69	3.96
	SD	0.51	1.21	1.25	0.14	0.77	1.03	1.03	0.26
Cortical thickness (mm)	M	1.71	2.26	2.35	3.24*	1.54	2.21*	2.29*	2.79*
	SD	0.26	0.39	0.57	0.06	0.39	0.52	0.52	0.47
Cortical area (mm^2)	M	31.02	53.82*	60.82	94.93*	21.74	41.46*	44.59	59.62*
	SD	6.93	15.12	21.56	3.95	5.58	16.57	17.04	12.68
Medullary area (mm^2)	M	12.73	23.02	26.59	29.24	7.43	10.65	11.46	12.33
	SD	3.40	10.52	11.60	1.36	4.53	4.68	6.43	1.64
Percentage Cortex (%)	M	46.08	46.42	45.16	51.47*	50.76	55.00	55.58*	58.24*
	SD	4.24	7.18	6.71	0.09	11.51	6.43	6.25	5.21
		Females							
N**		25	24	32	16	25	24	32	16
Subperiosteal width (mm)	M	7.27	8.92	10.14	10.57	5.77	7.27	7.69	8.31
	SD	0.67	0.83	1.45	0.79	0.47	0.82	1.73	0.74
Medullary width (mm)	M	4.02	5.06	5.95	5.98	2.68	3.40	3.80	4.21
	SD	0.63	0.73	1.25	0.63	0.40	0.66	1.05	0.67
Cortical thickness (mm)	M	1.63	1.93	2.09	2.30	1.54	1.94	1.95	2.05
	SD	0.25	0.29	0.29	0.24	0.19	0.42	0.46	0.22
Cortical area (mm^2)	M	28.88	42.47	53.34	59.90	20.53	32.62	36.56	40.12
	SD	5.86	8.45	13.57	9.02	3.43	9.39	17.66	6.61
Medullary area (mm^2)	M	13.01	20.55	29.01	28.37	5.77	9.39	12.18	14.26
	SD	4.18	6.16	12.26	6.17	1.80	3.58	7.56	4.60
Percentage Cortex (%)	M	44.77	43.28	41.71	43.48	53.56	53.04	50.67	49.45
	SD	6.11	5.37	5.87	3.70	5.23	9.07	6.77	5.10

* Indicates statistically sifnificant sex differences at 5% level of probability.
**N may vary by 1-3 in certain measurements.

males at all ages, but no statistically significant sex differences are observed except in Tibia at JII age group. The difference in the successive means in the younger ages is high at early ages and this declines in later age categories.

Percentage Cortex: The mean Cortical values appear to be constant not only within but also between sexes. No statistically significant sex difference was observed in any of the comparsion between and within the sexes.

DISCUSSION

Bone growth is a dynamic process in which elongation, increase in girth and remodelling takes place Elongation occurs at the two metaphyseal ends by the active proliferation of cells from epitphyseal discs which is followed by ossification. Bone circumference increases by deposition of new bone on their subperiosteal surface. At the same time absorption takes place from endosteal surface resulting in increased medullary cavity width. These two processes go on *parri passu*. The process of remodelling is necessary as the bone grows in order to maintain its morphology.

An examination of the Tables reveal that mean subperiosteal width rise sharply from JI to JII age groups. These is also a considerably high rise in mean medullary widths in the same time duration and this naturally leave a small rise in mean cortical width. This indicates that the subperiosteal and endosteal resorption takes place almost at the same rate.

From JI to AI the increase in both, mean subperiosteal and medullary widths are comparatively less and these leave a more or less

Table 4: Mean (M) and standard Deviation (SD) for subperiosteal width, medullary width and cortical thickness of femur and tibia by age and sex

		Males							
		Femur				*Tibra*			
Age		*JI*	*JII*	*AI*	*AII*	*JI*	*JII*	*AI*	*AII*
N**		16	44	18	2	15	42	19	5
Subperiosteal width (mm)	M	8.31	10.64	10.90	12.74*	6.34	7.98	7.81	8.22
	SD	0.94	1.28	1.43	0.03	0.52	0.80	1.14	0.60
Medullary width (mm)	M	4.90	6.31	6.40	7.02	3.43	4.32*	4.32	4.45
	SD	0.62	1.07	1.16	0.03*	0.33	0.78	1.03	0.64
Cortical thickness (mm)	M	1.71	2.17*	2.25*	2.86*	1.46	1.83*	1.75	1.88
	SD	0.28	0.34	0.38	0.03	0.23	0.31	0.27	0.39
Cortical area (mm^2)	M	35.77	58.07*	61.58	88.81*	22.45	35.26*	33.43	37.44
	SD	8.51	13.64	14.86	0.88	4.45	7.48	8.02	7.76
Medullary area (mm^2)	M	19.13	32.16	33.22	38.72	9.31	15.11*	15.43	15.83
	SD	4.93	10.78	12.25	0.31	1.74	5.73	7.39	4.47
Percentage Cortex (%)	M	40.93	40.87*	41.36*	44.90	45.74	45.95	45.18	45.65
	SD	4.81	5.71	5.60	0.34	5.15	7.00*	6.71	7.83
		Females							
N**		26	27	34	17	23	22	32	11
Subperiosteal width (mm)	M	8.18	10.11	10.71	11.11	6.51	7.23	7.93	7.77
	SD	0.82	0.96	1.25	0.59	0.61	0.42	0.91	0.54
Medullary width (mm)	M	5.07	6.35	6.80	6.59	1.59	3.90	4.37	4.28
	SD	0.96	0.85	1.02	0.72	0.31	0.55	0.72	1.75
Cortical thickness (mm)	M	1.56	1.88	1.95	2.26	1.59	1.66	1.78	1.75
	SD	0.34	0.25	0.27	0.22	0.31	0.17	0.73	0.17
Cortical area (mm^2)	M	32.26	48.84	54.17	62.82	24.67	29.00	34.68	33.25
	SD	7.61	9.29	12.34	6.44	5.99	3.16	7.55	5.13
Medullary area (mm^2)	M	20.87	32.23	37.14	34.48	8.85	12.16	15.40	14.14
	SD	7.80	9.31	11.45	8.10	2.24	3.70	5.23	1.74
Percentage Cortex (%)	M	38.30	37.32	36.60	40.82	48.69	46.20	45.05	44.91
	SD	8.32	4.18	4.30	4.23	6.95	5.18	4.78	2.02

* Indicates statistically sifnificant sex differences at 5% level of probability.
**N may vary by 1-3 in certain measurements.

constant value of cortical thickness.

Sex Differences: The major sex differences are observed mainly in four dimensions – Subperiosteal width, Cortical thickness, Cortical area and percentage cortex. Sexual dimorphism leads to differential development of bones in the two sexes. Males being subjected to more harder responsibilities (like protection of the social group) are liable to possess more ragged bones than females who perform much lighter work. Differences are only apparent in parameters which are exposed to maximum stress and strain. Like the weight of the body is borne by the cortex mostly and not by bone cavity. Males having comparatively heavier body weights, must therefore possess higher values of this parameter. Sex differences are statistically insignificant at younger ages and these become significant only in later ages. At the earlier ages both sexes appear to have similar bone dimensions and as the individual grows sexual dimorphism becomes distinct and the amount of variability increases thereafter.

The observations made in the present study throw light on the understanding of the dynamics of bone growth in *Macaca mulatta*. Although more elaborate longitudinal study followed from infantile till senile period is ideal for achieving the objectives of this study stated earlier, it is submitted that till such type of data become available, these data be used as a baseline information in the bone growth of *Macaca mulatta* through various ages and the influencing ecological factors therein.

KEY WORDS Radiography. Periosteal Width. Cortical Area. Estimated. Age Stages.

ABSTRACT The width, area of Cortex and Medullary cavity of the long bones measured on radiographs of various age group of 323 male and 376 female *Macaca mulatta* are presented in tables for Femur, Tibia, Humerus and Radius. The pattern emerging from the means in the Cortical bone dynamics is discussed. Generally, during the juvenile stages the mean Subperiosteal width and the mean Medullary width increase sharply from juvenile 1 to juvenile II stages. The sex differences are statistically insignificant at the younger ages but become significant in the later ages. The data may prove useful in judging impact of changing ecological pattern in the conservation of wild species.

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