A QUANTITATIVE STUDY OF SKELETAL COMPONENTS OF THE PIG-TAIL MONKEY (MACACA NEMISTRINA) AS MEASURED BY BONE DENSITOMETRIC AND CHEMICAL ANALYSES

A THESIS

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INTRODUCTION

The study described in this thesis was conducted in the Texas Woman's University Research Institute as a part of a broader research project sponsored by the National Aeronautic and Space Administration.

Four primates of the Macaca nemestrina species (the Pigtail Monkey) were used in this study for the purpose of finding the possible relationships of skeletal analyses in different bones in the body.

The main objectives of this study have been the following:

a. To dissect out and clean the major bones from the carcasses of the four animals in the study, as follows: leg - os calsis; tibia; fibula; femur; pelvic area; arm - radius; ulna; humerus; spine - lumbar vertebra 4; and lumbar vertebra 3.

b. To determine the dry and the ashed weight of each of the above bones.

c. To find by analysis the quantity of calcium and of phosphorus in each of the ashed bones, and to calculate the percentages of these two components in the bones.

d. To find by statistical analysis the correlations between certain of the pairs of factors determined by analysis in a., b., and c. above.

e. To find the bone density of representative bones included in the study, and to determine by statistical analysis possible correlations between this factor and other factors analyzed in the investigation.

REVIEW OF LITERATURE

Literature Descriptions of the Primates Used in the Study

As noted, the animals used in this study were pig-tail monkeys (Macacas Nemistrina). The young adult, male, was employed.

The pig-tail monkey in the world state occurs in Southeast Asia, ranging from Borneo, Java, and Sumatra northward along the Malay Peninsula to Southern Burma. It also is known as the coconut monkey, the term derived from its use by the Malays for harvesting coconuts from the trees. The pig-tail monkey is somewhat larger at maturity than the better known rhesus monkey (Macaca Mulatta). Also, it is more placid, considerably less raucous and more amenable to training. It has been stated by Pace and associates (1) that Macaca Nemestrina can learn to understand and distinguish between commands consisting of as many as seven syllables. The authors have found that 60 to 90 days of training are required before an animal can be used as a satisfactory subject. It should be pointed out, however, that considerable variation in behavior pattern is manifest among individual animals, some being substantially more docile and rapidly trainable than others. Also, old males are reputed to be savage, unpredictable and difficult to train, according to these investigators.

Employment of Macaca Nemestrina in the Scientific Area

Bassett and Pawluk (2) have used Macaca Nemestrina for the study of effects of electric currents on bone in vivo. Jensen, et al. (3) have used eight mother-infant pairs of pig-tail monkeys for the study of the effects of environment on the relationship between mother and infant. These monkeys were housed in bare cages located in a sound-proof room, with others in the laboratory in cages containing toys. Interactive behavior was observed during the first 15 weeks of the infants' lives. Pairs in the privation environment spent more time in physical contact. Infants of these pairs oriented more behavior toward themselves and less toward the environment, locomoted less, did less climbing, and showed less differentiation in their activity toward their mothers.

Weir, et al. (4) made a study of the effects of continuous inhalation of high concentrations of oxygen at ambient pressure and temperature. These investigators used mice, rats, guinea pigs, dogs, and monkeys in a study in which they were exposed continuously to 96–99 per cent oxygen for 240 hours. Better research results came from the monkey groups.

According to the primate behavior study of Devore (5), the facial expressions in the Macaca Nemestrina, similar to those of baboons, include prominent use of the eyes as signals, embellished further by eyelid fluttering, in which raising of the brows and lowering of the lids serve to show a perceived signal.

Hoffman, Dozier, Mack and Hood (6) used four groups of male Macaca Nemestrina ranging in weight from 7.4 to 8.4 kilograms within their study. The primates were fed two diets which were similar in provision of calories, but which differed in content of major nutrients. Diet A surpassed Diet B in protein, but was exceeded by Diet B in fat, carbohydrate, and major minerals. Calcium was approximately three times as high in the second diet.

Two groups of animals were put on the respective diets and were placed in restraint on couches for 35 days followed by 35 days of reconditioning. One group of animals on each diet was non-restrained throughout the study. All primates, restrained, were exposed to a biosatellite simulated reentry profile with centrifugation to 12 g. on the thirty-fifth day of the study. The Diet A restrained primates lost a higher percentage of weight during restraint and exposure to the reentry profile than did the Diet B animals. Although the two groups consumed approximately the same quantity of food and the same amount of energy based on initial body weights of the primates, the four groups of animals differed in urinary excretion of nitrogen, creatine, and creatinine.

Earlier Studies in the TWU Research Institute Involving the Macaca Nemistrina Primate

Brooks (7) studied the effect of exercise on the preservation of the integrity of the skeleton of the Macaca Nemistrina primate. She stated that, while the animals were exercising, the density of the bones in the 18 sites of the skeletons of six animals used in the study behaved in an opposite manner in comparison with that which took place with the restrained animals. Bone mass increased in all anatomic locations during the exercise period, and decreased during the ambulatory period.

Aliya N. Al-Shawi (8) studied the effects of diet and restraint on the bone in 17 anatomical sites of the same type of primates as those used in this investigation. Her findings with respect to bone mass when the restrained animals were compared statistically with those which were not restrained showed that the parts of the body which were most incapable of movement by virtue of the restraint while the animals were on their couches showed the greatest statistically significant difference between the restrained and the non-restrained animals. As a result, the lumbar spine showed the greatest reduction in density of the restrained primates, with the os calsis ranking next. The medial femur and medial tibia showed some results of the restraint, with the cortical vertebrae and the phalanx small finger showing lesser change in this factor.

Dorothy Chen (9) studied the urinary excretion of 17-hydroxycorticosteroid from four healthy male primates of this type. One of the interesting findings of her study was the fact that the amount of 17-hydroxycorticosteroids excreted in the urine varied from primate to primate. In spite of individual differences between primates, however, a statistically significant increase was obtained during the Bed Rest Periods as compared with the value during the Pre-Bed Rest phase. A statistically significant decrease also was found during the Post-Bed Rest Period as compared with the value obtained during the Bed Rest Period.

Marina Eiland (10) studied the urinary hydroxy-proline excretion by four primates of this type as influenced by immobilization. She concluded from her study that there was a highly significant increase in excretion of urinary hydroxyproline during the Restraint Period as compared with the Pre-Restraint period, although one of the four experimental animals did not increase in this respect significantly. During the Post-Restraint Period, only two primates remained in the study. The increase in bone density in these primates during the Restraint Period was significantly higher as compared to that obtained during the Pre-Restraint Period.

Ila Varia (11) studied the urinary creatine and creatinine excretion in four pig-tail monkeys during restraint and non-restraint. She stated that, in all four primates, daily creatine excretion during the Pre-Bed Rest Period was surpassed by that of the Bed Rest Period by a difference which was highly significant. Two of the primates experienced very little activity when they were returned from their couches to their metabolism cages. This fact would explain the reason why primates excreted higher amounts of creatine during the Post-Bed Rest Period as compared to the Pre-Bed Rest Period. With regard to creatinine, there was no statistically significant change among the primates in any of the different periods of the study.

Dorothy Van Zandt (12) studied the effect of a new type of restraint garment on bone mass and on the calcium, phosphorus, and nitrogen metabolism of Macaca Nemestrina Primates. She found that all animals lost bone mass in all paralled sections across lumbar Vertebrae 4 and 3, in the pelvic area, in the head of the femur, and in Cervical Vertebrae 1 and 2. Contrary to the findings of previous studies, the elimination of calcium, phosphorus, and nitrogen decreased at the same time that loss of bone mass and of body weight was taking place. This is attributed to the fact that the food intake by these primates was very low, and was insufficient to maintain metabolism adequately.

Siada Tolba (13) conducted a study on the nitrogen metabolism of Macaca Nemestrina primates during immobilization and ambulation. The results showed that these primates excreted the highest amount of urinary nitrogen and total nitrogen (including that in urine and feces) during the Restraint Period, with the lowest amount during the Pre-Restraint Period.

Aliya Al-Shawi (14) determined the quantitative measurements of the mineral in the skeleton of a squirrel monkey and the correlation of the method of determining bone mass by means of radiographic bone densitometry with biochemical analyses of bone components.

Various skeletal sites of the monkeys were X-rayed in vivo while under an anesthetic. The animals were sacrificed and 108 bones were dissected out of each for the determination of dry weight, ash weight, and per cent ash. This experimenter stated that the weights of the bone ash were relatively proportional to the dry weights. The per cent of ash of the various bones was relatively close. Sixty-nine per cent of the bones contained from 55 to 65 per cent ash.

Forty-seven bones were analyzed for calcium. The amount of calcium in the bones varied according to the size of the bones. The greatest amount of calcium was found in the skull.

The mass of 24 bones was determined by the means of densitometric traces of X-rayed portions of the skeleton. The bone density and the content of ash in the bone were correlated with the quantitative measurements determined by means of biochemical analysis.

Charlene Varner (15) studied a problem designed primarily to find the relationship between the bone mass in terms of calibration wedge equivalency of certain anatomical sites in four primates of the species Macaca Nemistrina and the bone ash and calcium found in the bones which were analyzed by radiographic bone densitometry.

The animals were X-rayed "in situ" and then certain bones were extirpated, cleaned, and X-rayed again as in the "in situ" position. The same bones were ashed, and were X-rayed a third time. Then the radiographs in each case were scanned in certain carefully selected sites to find the relationship between the bone density of the same site in situ, in the dry form, and in the ashed form.

The per cent of the bone ash and the quantity of calcium of each of the ashed bones was determined by chemical analysis.

Certain findings of the author's study are compared with previous Texas Woman's University Research Institute studies, and are discussed later in this thesis.

PLAN OF PROCEDURE

PRIMATES INCLUDED IN STUDY

The animals used in this study were four male pig-tail monkeys (Macaca Nemestrina). They were numbered as follows: P-21, P-25, P-30, P-69. The study was done after the animals had been sacrificed and maintained by freezing.

MEASUREMENT OF BONE DENSITY

The bone density of the primates was measured in certain anatomical sites in situ, in the same bones extirpated and cleaned, and in the same bones ashed. The quantity of calcium in the ashed bones was determined by chemical analysis.

The radiographic bone densitometric technique used in this study was that developed by Mack and colleagues of the Texas Woman's University Research Institute. (18)(19)(20) It consisted of a Digital Computer method based on an Analogue Computer system.

Analogue System. The equipment of this system consists of four basic units as follows:

a. A modified Knorr-Albers scanning unit;

b. A Speedomax Model G transmitting recorder which produces the film density curve and contains within the same panel a series of potentiometers used for the purpose of correcting the nonlinear density curve;

c. A Speedomax Model G recorder for displaying the curve; and

d. An Instron Integrator geared to the output of the second recorder.

The sequence of operations needed to achieve linearization of a density curve and to integrate the area under the curve of a bone on the same radiograph is as follows:

a. The wedge X-ray image first is scanned for the purpose of providing the density curve of that film on the first recorder.

b. Then the technologist measures the displacement from a standard trace which conforms to the straight-line slope of the wedge at 20 points along the chart baseline, and sets the respective potentiometers in the panel associated with each of the points. This is facilitated by the use of a scale which shows the deviation of the scan from linearity at each point, thus indicating the correct potentiometer setting required to obtain the needed resistance for a section of a major slidewire within the instrument geared to each of the 20 points on the curve mentioned above.

c. The wedge image is traced again on a chart on the second recorder, in order to check the linearity of the corrected curve.

d. When the wedge tracing displayed on the second panel is corrected to a straight line, the desired section of the bone then is scanned with its trace also shown on the second recorder. The scan is evaluated simultaneously by means of a readout count obtained from an Instron Integrator situated in the lower part of the second record panel of the instrumentation.

<u>Addition of Digital Computer</u>. The Texas Woman's University bone densitometric system has received further automation by the addition of a digital computer to the Analogue System.

The computer has been implemented to perform computations similar to the functions performed by the previous Analogue System. First, the wedge image is scanned and the resulting light transmission data are stored in terms of distance along (or thickness of) the wedge. Second, the bone image is scanned and the resulting light transmission data are stored. After both wedge and bone scans have been completed, the computer calibrates the stored bone scan in terms of equivalent wedge thickness by using the stored wedge scan data. The calibrated bone data then are integrated along the scan by using a trapezoidal approximation integration formula. (21) See Figure 1 for Bone Densitometer.

Standardization of X-Ray Units and Radiographs. Three methods of standardization are used: (a) an aluminum alloy calibration wedge is exposed on each film adjacent to the bone; (b) a Victoreen roentgen-meter is used immediately before making a radiograph to determine the calibrated kilovoltage which would produce identical X-ray beam qualities with all X-ray units used; and (c) at each testing period, a phantom composed of bone imbedded in an organic matrix is exposed.

DETERMINATION OF DRY WEIGHT OF BONES

Thawing each animal was the first step in this study. After thawing the animals, the bones needed were dissected from the carcass, and as much of the soft tissue was removed as possible. All adhering tissue and cartilage were moved from the bones by means of scissors and chamois skin. After the dissection of the various bones, they were labeled and were placed in an oven to dry at 85°C for 24 hours, or longer if necessary, to obtain a constant weight. Then they were weighed.

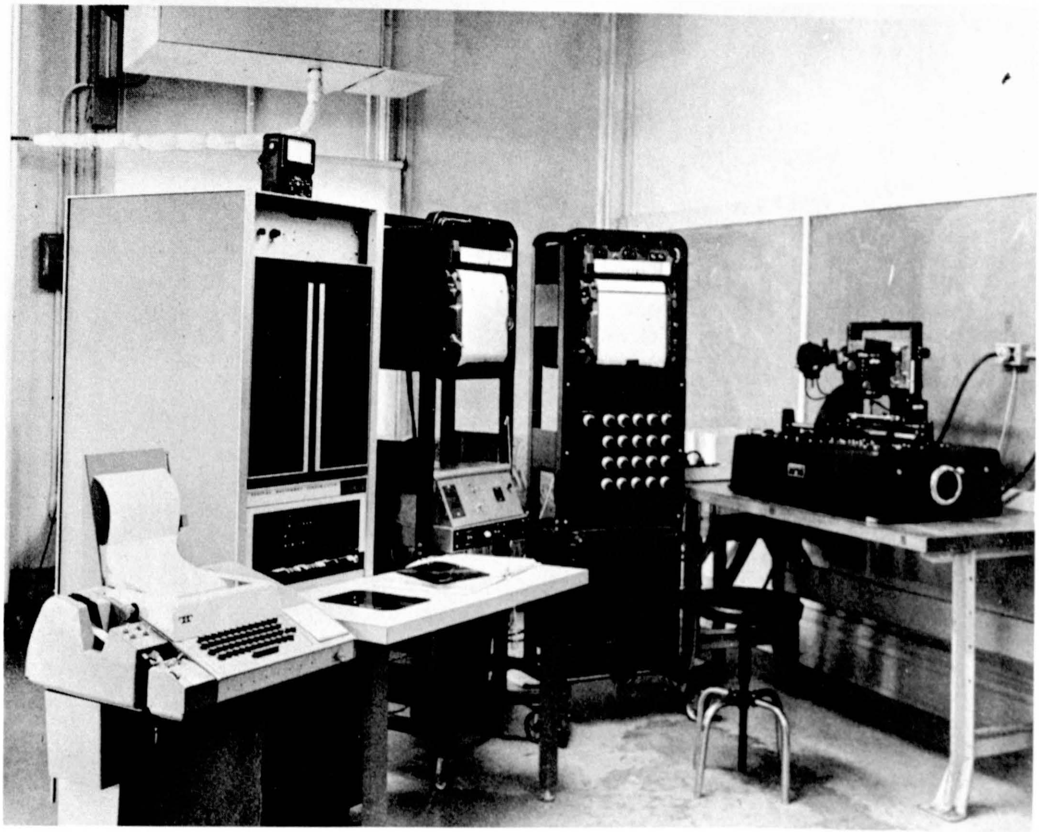

Figure 1. Digital-Analogue Computer Assembly. This is the bone densitometer assembly used in scanning sections of bone radiographs in order to evaluate their density in the Apollo VII tests. At the extreme right is the densitometer through which the calibration wedge is scanned by means of a standard light beam, after which the bone section to be measured is scanned. The two central sections are a part of the analogue computer assembly. At the extreme left is the digital computer, by means of which the test is programmed so that the density of the bone section is obtained without manual work on the two central recorders. The entire analogue assembly is used only periodically for calibration purposes.

DETERMINATION OF ASH

Approximately 48 hours were required to ash the bones. This was accomplished by placing the various bones in individually marked porcelain crucibles of proper size, which previously had been brought to constant weight. The bones were ashed in a muffle furnace regulated at 600°C until constant weight was obtained. After cooling, the ashed bones were weighed and X-rayed.

By dividing the ashed weight by the dry weight and multiplying by 100, the percent ash of each bone was calculated.

DETERMINATION OF CALCIUM IN BONE ASH

The calcium determination was made by the method of Ferro and Ham. (22)(23) The accuracy of the method has been established in the laboratories in which the work was done, with the following procedure.

The reagents used in the calcium determination were as following:

Chloranilic Acid. Four grams of sodium hydroxide were dissolved in 600-700 milliliters of distilled water containing 11 grams of chloranilic acid. Enough distilled water was added to make one liter. The solution was agitated for 10 minutes or until all of the acid was dissolved. Sodium hydroxide or chloranilic acid was employed to adjust the solution to pH 7.0.

Isopropyl Alcohol (50 per cent).

Methyl Red as an indicator.

Calcium Standard (10 milligrams per 100 milliliters).

Nine milliliters of 1 N HCl were added to exactly 0.2497 gram of reagent grade calcium carbonate, which was placed in a liter volumetric flask. The mixture was allowed to stand until all of the calcium carbonate had dissolved. A dilution of one liter was made by adding sufficient water.

The ashed bones were dissolved in 6 N HCl or diluted HCl, and diluted to a volume of 200 or 100 milliliters depending upon the weight of the ashed bones. An aliquot of this bone solution was obtained and placed in a volumetric flask where volume was diluted so that the calcium concentration was approximately 10 milligram per 100 milliliters. Some drops of methyl red solution were added to flasks which were filled about three-fourths full with distilled water. After adjusting the pH of the orange neutral point (pH 4.5-5.0) with HCl and NH_4OH, the flasks then were filled to the mark.

Two milliliter aliquots of the final bone solution, a water blank, and the standard were pipetted into 15 milliliter plastic centrifuge tubes. To each tube was added one milliliter of the chloranilic acid reagent. This was permitted to stand for approximately two hours.

The tubes were centrifuged for 10 minutes at 1500 r.p.m. The supernatant liquids were discarded with care, so that none of the precipitate was lost. The tubes were inverted and allowed to drain for at least five minutes. The precipitates then were washed with isopropyl alcohol (50 per cent), recentrifuged, and drained as before. This washing step was repeated and the tubes were allowed to drain as dry as possible. Two drops of distilled water were added to each tube, followed by minor shaking, with six milliliters of the EDTA solution added, after which the tubes were shaken thoroughly.

The EDTA dissolved all precipitates. Then the absorbance of each solution was determined at 520 mu by use of the Coleman Jr. spectrophotometer. A EDTA solution as a blank was used for comparison.

The concentration of the diluted bone solution was obtained by using the formula:

$$\frac{\text{Concentration of Standard}}{\text{Optical Density of Standard}} = K$$

K x Optical density of sample x Dilution x Volume of Sample = milligrams of calcium

DETERMINATION OF PHOSPHORUS IN BONE ASH

Phosphorus was determined by the method of Fiske and Subbarow (24) as modified by Dryer, et al. (25).

The reagents used in the phosphorus determination were as following:

Phosphorus Stock Standard. 500 ppm. 0.4381 g of reagent grade KH_2PO_4 is dissolved in water in a 200 ml volumetric flask and the solution is diluted to volume.

Phosphorus Working Standards. The phosphorus stock solution is diluted to prepare working standards containing 0.25, 0.50, 1.00 and 1.50mg/100 ml of phosphorus, respectively.

Sulfuric Acid. 5 N. 135 ml of concentrated sulfuric acid is diluted to 1 liter.

Ammonium Molybdate Solution. 0.025 M. 30.8 g of ammonium molybdate is placed in a liter volumetric flask containing approximately 800 ml of water and 0.8 ml of concentrated sulfuric acid. After the salt is dissolved, the contents of the flask are diluted to volume with water. The reagent is stored in the refrigerator.

Sodium Bisulfite Solution. 20 grams of sodium bisulfite (1 per cent) is dissolved in water and diluted to 2 liters.

P.P.P.D. Solution. 1.00 g of N-phenyl-p-phenylenediamine monohydrochloride (Semidine) is placed in a 2 liter flask and moistened with ethyl alcohol. The contents of the flask are diluted to volume with 1.0 per cent sodium bisulfite solution. The contents of the tube are shaken well before making up to volume. The solution must be filtered. The reagent is stored in the refrigerator.

Sample Solution. The samples are diluted so that the diluted solution contains an amount of phosphorus which will fall within the range of the standards. A range of 250 to 5000 dilution is used for ashed bones.

1. Place 2 ml of each working standard, each sample solution, and a water blank in a medium size test tube.
2. Add 2 ml of 5 N sulfuric acid to each tube and mix.
3. Add 2 ml of ammonium molybdate solution to each tube and mix.

4. Add 4 ml of P.P.P.D. solution to each tube and mix.
5. Allow to stand 15 minutes.
6. Pour into 19 mm. Coleman cuvettes and read in the Coleman Spectrophotometer at 700 mu versis a water blank which has been carried through the complete procedure.

STATISTICAL TREATMENT OF DATA

All values for each bone were recorded on charts and punched on IBM cards in the statistical laboratory of the Texas Woman's University Research Institute. The cards then were run at the TWU Data Processing Center, by means of an IBM 1620 computer.

P R E S E N T A T I O N   O F   F I N D I N G S

RESULTS OF BONE ANALYSES

The results of the analyses of dry weights and ashed weights of the bone, together with milligrams of calcium and of phosphorus in the bone are given in Table I - A to D for the respective primates in the study.

The percentages of calcium and of phosphorus as calculated from the dry weights and ashed weights of the individual bones appear in Table II - A to D. All tables are given in the Appendix of this Report.

The values for the various components of the bone factors are clearly related to the sizes of the bones involved. Thus the pelvic area, which includes the largest overall mass of skeletal material, has the highest values for dry and ashed bone weights, together with the highest weights of calcium and of phosphorus. This is followed by the femur, which is the largest individual bone. The smallest quantities of dry and ashed bone and of the two major components of bone are found in the os calsis, the smallest bone studied. This pertains to each of the four animal skeletons in the study.

STATISTICAL COMPARISONS OF PAIRS OF THE VALUES FOUND IN THE ANALYSIS OF DRY AND ASHED WEIGHTS OF THE DESIGNATED BONES, AND OF CALCIUM AND PHOSPHORUS IN THE BONES

FEMUR

Correlation coefficients calculated for comparisons between the four factors analyzed on the composition of the femur as shown in this study are given in Table III, Part A. Correlation coefficients between the ashed weight of the bone and calcium in the bone, between ashed weight and phosphorus in the bone, and between calcium in the bone and phosphorus in the bone were highly significant ($P < 0.001$).

Correlation coefficients between dry weight and ashed weight of the bone, and between dry weight and phosphorus in the bone were significant at the 2.0 per cent level, while that calculated between dry weight and calcium in the bone was significant at the 1.0 per cent level.

The data from all four primates in the respective bones for study were pooled for these statistical calculations.

HUMERUS

As in the case of the femur calculations, the coefficients of correlation for ashed weight and calcium in the bone, for ashed weight and phosphorus in the bone, and for calcium in the bone and phosphorus in the bone were highly significant ($P < 0.001$). On the other hand, dry weight compared with ashed weight, dry weight compared with calcium in the bone, and dry weight compared with phosphorus in the bone gave coefficients of correlation which were significant at the 1.0 per cent level. See Table III, Part B.

RADIUS

Table III, Part C gives the correlation coefficients and the Probabilities of Significance resulting from the comparisons of the analytical factors found for the radius.

Findings from the statistical analyses for the radius included results which deviated from those found for the two heavier bones, the femur and the humerus. In this case, the comparisons of the dry weight and calcium in the bone, of the dry weight and phosphorus in the bone, and of the ashed weight and both the calcium and the phosphorus in the bone were not statistically significant.

On the other hand, comparisons of phosphorus in the bone and the per cent of phosphorus in dry weight of bone, and of phosphorus in the bone and per cent of phosphorus in ashed weight were highly significant ($P < 0.001$). Calcium in the bone and phosphorus in the bone were distinctly significant ($P < 0.01$), while calcium in the bone compared with per cent of calcium in dry weight of bone, and of calcium in the bone and per cent of calcium in ashed bone weight gave coefficients of correlation which were significant at the 5.0 per cent level.

ULNA

The ulna likewise differed from the heavier long bones, femur and humerus, in the significance of the coefficients of correlation which were calculated for the pairs of analytical factors. See Table III, Part D. These two closely related bones of the forearm were similar with respect to the correlation coefficients for comparisons of dry weight and phosphorus in the bone (not significant in both cases), in ashed weight and phosphorus in the bone (also not significant in both cases), in phosphorus in the bone and per cent of phosphorus in dry weight, and again in ashed weight (coefficients of correlation highly significant in both cases ($P < 0.001$).

The correlation coefficients derived from the comparisons of dry weight and ashed weight were significant both for the radius and ulna, with the former significant at the 1.0 per cent level and the latter at the 5.0 per cent level.

The remainder of the comparisons were not similar in the radius and ulna. The reasons could be that the two bones differ in size and in their content of marrow, with the relatively higher per cent of phosphorus than of calcium in bone marrow possibly a factor.

TIBIA

Highly significant correlation coefficients were found for the tibia when ashed weight of the bone was compared with the quantity of phosphorus in the bone, and when the amounts of calcium and of phosphorus in the bone were compared with each other ($P < 0.001$ in both comparisons).

The coefficient of correlation between ashed bone weight and calcium in the bone was significant at the 1.0 per cent level, while dry bone weight and quantity of calcium in the bone had a correlation coefficient significant at the 5.0 per cent level.

The coefficient of correlation for the comparison of dry bone weight and phosphorus in the bone was significant only at the 10.0 per cent level. That for the comparison of dry weight and ashed weight for the tibia was not significant. See Table III, Part E.

FIBULA

The correlation between dry weight and ashed weight of the fibula was shown to be highly significant ($P < 0.001$). This was in contrast with the findings for the tibia with respect to this comparison. This can be accounted for by the fact that the proportion of mineral in the fibula is higher than that in the tibia.

Phosphorus in the bone was correlated with per cent of phosphorus in the dry weight of bone, with a correlation coefficient which was significant at the 1.0 per cent level. The same level of significance was found in the correlation coefficient calculated between the amount of phosphorus in the bone and the per cent of phosphorus in the ashed bone.

Dry weight and ashed weight of the fibula were related by a correlation coefficient which was significant at the 5.0 per cent level. The dry weight of this bone and the quantity of calcium in the bone, the ashed weight and calcium in the bone, and the amounts of calcium and phosphorus in the bone also gave correlation coefficients which were significant at the 5.0 per cent level.

The comparison of ashed weight and the amount of phosphorus in the bone proved to be not significantly correlated. See Table III, Part F.

OS CALSIS

Only one of the correlations between the various bone factors for the os calsis which were computed was found to be significant. This was the comparison between ashed weight and quantity of calcium in the bone ($P < 0.01$). See Table III, Part G.

PELVIC AREA

The pelvic area which was extirpated was composed of extensive cancellous material, some cortical bone and some bone marrow. The composition of this bone therefore differs considerably from that of a bone such as the femur.

When calculations were made in order to determine possible correlations between pairs of factors which had been analyzed in this study, no significance was found in three of the comparisons which were made, namely - that between dry weight and calcium in the bone, that between ashed weight and calcium in the bone, and that between calcium in the bone and phosphorus in the bone.

The ashed weight of the pelvic area and the level of phosphorus in the bone gave a correlation coefficient which was highly significant ($P < 0.001$).

When dry weight of the skeletal material in this area was compared with ashed weight, the correlation coefficient was distinctly significant ($P < 0.01$). The comparison of dry weight with phosphorus in the bone gave a correlation coefficient which was somewhat significant ($P < 0.05$). See Table III, Part H.

LUMBAR VERTEBRAE 3 AND 4

The dry weights of the two lumbar vertebrae which were included in the study were not obtained, and hence these factors could not be included in the comparisons which were made to find whether correlations were found. See Table III, Parts I and J.

For both of these bones the ashed weight of the bone and the quantity of calcium in the bone were found to be related, with highly significant coefficients of correlation ($P < 0.001$).

Only one additional calculated correlation proved to be significant for either vertebra. This was the relationship between phosphorus in the bone and per cent of phosphorus in the bone. The correlation coefficients in both cases were significant at the 1.0 level.

RELATIONSHIP OF BONE DENSITY TO CERTAIN SKELETAL FACTORS

Correlations were determined in order to show possible relationships between bone density of representative bones and certain skeletal factors derived from this study analytically. Only three of the experimental animals had radiographic bone densitometric determinations made.

The bone density values are in terms of Integrator counts from the computer, which is closely related to bone density.

Pelvic Area. The pelvic area, which included the skeletal material of the greatest weight, in the study, exhibited statistically significant correlations between bone density and the following analytical factors considered in the study:

	Correlation Coefficient	Level of Significance
Bone density and dry weight of bone . . .	0.9633	$P < 0.01$
Bone density and ashed weight of bone . .	0.9861	$P < 0.01$
Bone density and per cent of calcium in dry weight of the bone . . .	0.9258	$P < 0.05$
Bone density and per cent of phosphorus in dry weight of bone . . .	0.9999	$P < 0.001$
Bone density and per cent of calcium in ashed weight of bone	0.9387	$P < 0.02$
Bone density and per cent of phosphorus in ashed weight of bone . .	0.7683	$P < 0.05$

Femur. The femur, the largest of the long bones in the study, was found to show various significant correlations between bone density and bone factors for which analyses were run, as follows:

	Correlation Coefficient	Level of Significance
Bone density and dry weight of bone . . .	0.9562	$P < 0.02$
Bone density and ashed weight of bone	0.9487	$P < 0.02$

Bone density and per cent of calcium in dry weight of bone	0.9783	$P < 0.01$
Bone density and per cent of calcium in ashed weight of bone	0.9946	$P < 0.01$
Bone density and quantity of phosphorus in dry bone	0.9967	$P < 0.001$

Radius. The radius exhibited some statistically significant correlation coefficients, although a few of the correlations had a relatively low level of significance, as shown in the following:

	Correlation Coefficient	Level of Significance
Bone density and dry weight of bone	0.9560	$P < 0.02$
Bone density and ashed weight of bones	0.9363	$P < 0.02$
Bone density and per cent of calcium in dry weight of bone	0.8255	$P < 0.10$
Bone density and per cent of calcium in ashed weight of bone	0.8117	$P < 0.10$
Bone density and per cent of phosphorus in dry weight of bone	0.8999	$P < 0.05$
Bone density and per cent of phosphorus in ashed weight of bone	0.9015	$P < 0.05$

Ulna. Contrary to the radius, the ulna, a smaller bone, failed to show statistical significance in any of the coefficients of correlation which were calculated between comparisons of bone density and other analytical bone factors.

Tibia. The tibia, on the other hand, was found to have significant relationships between bone density and four other skeletal factors as shown below:

	Correlation Coefficient	Level of Significance
Bone density and per cent of calcium in dry weight of bone	0.9535	$P < 0.02$
Bone density and per cent of calcium in ashed weight of bone . .	0.8977	$P < 0.05$
Bone density and per cent of phosphorus in dry weight of bone . . .	0.9999	$P < 0.001$
Bone density and per cent of phosphorus in ashed weight of bone . .	0.9853	$P < 0.01$

Lumbar Vertebra 4. Lumbar vertebra 4 exhibited three statistically significant correlations between bone density and other skeletal factors. These were the following:

	Correlation Coefficient	Level of Significance
Bone density and ashed weight of bone	0.9924	$P < 0.001$
Bone density and quantity of calcium in the bone .	0.9747	$P < 0.01$
Bone density and per cent of calcium in ashed bone .	0.8641	$P < 0.10$

Lumbar Vertebra Three and other of the smaller bones which were tested did not show significant correlations between the bone density and other analytical bone factors which were analyzed. There were various reasons why this was the case. The bones were small and the data on the various analyses probably was inadequate for a satisfactory comparison with the bone density scans, with only a few scans possible on the smaller bones. The os calsis, for example, permitted only one computer scan because of its size.

COMPOSITE ANALYSIS OF POOLED DATA

When data for all bones analyzed for all three experimental primates having bone density measurements were pooled, highly significant correlation coefficients were obtained for two major comparisons. The correlation coefficient for the comparison between composite bone density and overall per cent of calcium in the dry weight of bone was 0.7725 with a level of statistical significance of $P < 0.001$. The correlation coefficient for the comparison of overall bone density with per cent of phosphorus in the dry weight of bone was 0.6151 which is highly significant ($P < 0.001$). The reason why the level of significance was higher for lower correlation coefficients than were obtained in some other comparisons is the fact that a larger group of data were available for many bones of all animals than was able to be used for one bone of one animal.

DISCUSSION

The results from this study bear a strong relationship to those from two similar investigations carried on in the Texas Woman's University Research Institute, namely--Al Shawi (14) and Varner (15). The purpose of the work by Al Shawi was to obtain quantitative measurements of the mineral in the skeleton of a squirrel monkey and to correlate the method of determining bone mass by means of radiographic bone densitometry with biochemical analysis.

Of 108 bones which were given partial study, 47 bones were analyzed for calcium. The amount of calcium in the bones varied according to the size and density of the bones. The greatest amount of calcium was found in the skull which contained 1305.0 milligrams.

The simple linear correlation coefficients between the bone mass as determined by densitometric traces from the in situ X-rays and the dry bone weight, the ash weight, and the calcium content in the 24 selected bones were highly significant ($P < 0.001$). The correlation coefficient between the bone mass and the per cent ash was statistically significant at the two per cent level of confidence.

The following summary gives the correlation coefficients obtained by this investigator when pooled data from bone density evaluations obtained from the 24 bones were compared with dry bone weight, bone ash weight, per cent of bone ash, and two calcium determinations from the ash.

Dry Bone Weight	Bone Ash Weight	Per Cent Bone Ash	Calcium Determination I	Calcium Determination II	Calcium Average
0.980	0.999	0.498	0.998	0.998	0.998

Significant at the 0.01 level of confidence (r = 0.496)
Significant at the 0.001 level of confidence (r = 0.608)

The dissertation by Varner (15) cited above was based on a problem designed primarily to find the relationship between the bone mass in terms of calibration wedge equivalency of certain anatomical sites in four primates of the species Macaca nemestrina and the bone ash and calcium found in the bones which were analyzed by radiographic bone densitometry.

The animals were X-rayed "in situ" and then certain bones were extirpated and cleaned, and were X-rayed again as in the "in situ" position. The same bones were ashed, with the ash maintained in the original shape, and were X-rayed a third time. Then the radiographs in each case were scanned in certain carefully selected sites to find the relationship between the bone density of the same site in situ, in the dry form, and in the ashed form.

The content of each of the ashed bones was determined by chemical analysis.

The per cent of the bone ash and the quantity of calcium in the ash was found to differ in the different bones, and for the same bone in the four primates to a certain extent.

Coefficients of correlation were found in the 12 sites investigated, ranging from 0.9758 to 0.99986, denoting a high degree of correlation between the values found for bone mass by the method of radiographic bone densitometry and the quantity of calcium in the bones as determined by chemical analysis.

SUMMARY AND CONCLUSIONS

This study involved four primates of the Macaca Nemistrina species, which had as its purpose to confirm and to extend previous research which had been conducted in these laboratories on the interrelationships of various skeletal factors of this type of primate, including the comparison of bone density data as obtained by the TWU method of radiographic bone densitometry and that secured by chemical analysis.

This work extended the findings of two previous TWU research workers in that it included the amounts and percentages of calcium and of phosphorus in the dry weight and ashed weight of a large number of bones, as well as that only in the ash. Also, intercorrelations were determined between pairs of the factors of dry weight and ash weight of the bone, calcium and phosphorus in the dry bone, and calcium and phosphorus in the ashed bone. This was in addition to finding correlations between each of these factors and bone density, the latter being the prime purpose of the two previous investigations.

In this study, significant correlations were determined in comparisons made between pairs of the enumerated analytical bone factors in 10 anatomical sites. The highest levels of statistical significance were found in the larger bones.

In addition, significant correlations were found between bone density by the radiographic bone densitometric technique developed by research workers in the Texas Woman's University Research Institute and certain of the bone factors. The most consistently high coefficients of correlation were found between bone density and the levels of calcium and of phosphorus in the bones studied.

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A P P E N D I X

TABLE I-A

DRY WEIGHT, ASHED WEIGHT, CALCIUM AND PHOSPHORUS CONTENT OF DESIGNATED INDIVIDUAL BONES FROM PRIMATE 21

Bone	Dry Weight (grams)	Ashed Weight (grams)	Calcium in Bone (milligrams)	Phosphorus in Bone (milligrams)
Femur	38.1065	22.0310	8429	3908
Humerus	30.7972	18.9783	7332	3257
Ulna	15.2484	9.3282	3551	3354
Fibula	5.9100	3.4428	1714	1187
Tibia	24.4005	13.4687	5165	2286
Radius	15.0563	8.9106	3228	3103
Os calsis	4.5130	2.1164	762	366
Pelvic Area	86.6272	38.3269	14618	6651
Lumbar Vertebra 4	7.7257	3.6643	1273	322
Lumbar Vertebra 3	8.4355	3.8002	1383	321

TABLE I-B

DRY WEIGHT, ASHED WEIGHT, CALCIUM AND PHOSPHORUS CONTENT OF DESIGNATED INDIVIDUAL BONES FROM PRIMATE 25

Bone	Dry Weight (grams)	Ashed Weight (grams)	Calcium in Bone (milligrams)	Phosphorus in Bone (milligrams)
Femur	48.5580	25.4396	10040	4329
Humerus	34.9164	20.4764	8104	3474
Ulna	17.7650	9.8127	3838	1746
Fibula	7.9184	4.3590	1784	740
Tibia	28.3348	15.1560	6050	2599
Radius	18.1310	10.2340	3354	1741
Os calsis	4.6580	2.0340	771	238
Pelvic Area	154.8368	51.4592	13360	8042
Lumbar Vertebra 4	X	4.5773	1757	790
Lumbar Vertebra 3	X	4.8939	1749	823
Lumbar Vertebra 2	X	4.9855	1851	847

TABLE I-C

DRY WEIGHT, ASHED WEIGHT, CALCIUM AND PHOSPHORUS CONTENT OF DESIGNATED INDIVIDUAL BONES FROM PRIMATE 30

Bone	Dry Weight (grams)	Ashed Weight (grams)	Calcium in Bone (milligrams)	Phosphorus in Bone (milligrams)
Femur	32.9233	17.1550	6771	2953
Humerus	24.8360	13.2740	5247	2250
Tibia	23.2720	11.1135	4484	1895
Ulna	13.5950	6.9206	2726	1211
Radius	11.4480	6.3710	2484	1106
Fibula	5.1552	2.7727	1197	467
Os calsis	4.7590	1.7004	664	296
Pelvic area	69.3940	26.4814	10530	4466
Lumbar Vertebra 4	X	2.3715	859	402
Lumbar Vertebra 3	X	2.6364	969	432

T A B L E I - D

DRY WEIGHT, ASHED WEIGHT, CALCIUM AND PHOSPHORUS CONTENT OF DESIGNATED INDIVIDUAL BONES FROM PRIMATE 69

Bone	Dry Weight (grams)	Ashed Weight (grams)	Calcium in Bone (milligrams)	Phosphorus in Bone (milligrams)
Femur	32.0628	20.2399	7462	3385
Humerus	24.5199	15.7107	5852	2632
Tibia	22.2568	13.5342	5058	2245
Ulna	12.5852	7.8723	2888	6513
Radius	10.3070	6.3311	4717	5452
Fibula	4.7797	2.9105	1067	239
Os calsis	3.4800	1.8613	697	310
Pelvic area	83.4681	35.9187	13497	5999
Lumbar Vertebra 3	X	3.4980	1249	1179
Lumbar Vertebra 2	X	3.6273	1300	1182

TABLE II-A

PER CENT CALCIUM AND PHOSPHORUS CALCULATED FROM THE DRY WEIGHT AND ASHED WEIGHT OF VARIOUS INDIVIDUAL BONES FROM PRIMATE 21

Bone	Dry Weight (grams)	Ashed Weight (grams)	Calcium in Dry Weight (per cent)	Calcium in Ashed Weight (per cent)	Phosphorus in Dry Weight (per cent)	Phosphorus in Ashed Weight (per cent)
Femur	38.1065	22.0310	22.12	38.26	10.26	17.74
Humerus	30.7972	18.9783	23.81	38.63	10.58	17.16
Ulna	15.2484	9.3282	23.29	38.07	21.99	35.96
Fibula	5.9100	3.4428	29.00	49.79	20.09	34.48
Tibia	24.4005	13.4687	21.17	38.35	9.37	16.97
Radius	15.0563	8.9106	21.44	36.23	20.61	34.82
Os calsis	4.5130	2.1164	16.88	36.00	8.11	17.30
Pelvic Area	86.6272	38.3269	16.87	38.14	7.67	17.35
Lumbar Vertebra 4	7.7257	3.6643	16.48	34.74	4.17	8.78
Lumbar Vertebra 3	8.4355	3.8002	16.39	36.39	3.81	8.45

TABLE II-B

PER CENT CALCIUM AND PHOSPHORUS CALCULATED FROM THE DRY WEIGHT AND ASHED WEIGHT OF VARIOUS INDIVIDUAL BONES FROM PRIMATE 25

Bone	Dry Weight (grams)	Ashed Weight (grams)	Calcium in Dry Weight (per cent)	Calcium in Ashed Weight (per cent)	Phosphorus in Dry Weight (per cent)	Phosphorus in Ashed Weight (per cent)
Femur	48.5580	25.4396	20.68	39.47	8.92	17.02
Humerus	34.9164	20.4764	23.21	39.58	9.95	16.97
Ulna	17.7650	9.8127	21.60	39.11	9.83	16.97
Fibula	7.9184	4.3590	22.53	40.92	9.35	16.97
Tibia	28.3348	15.1560	21.35	39.92	9.17	17.19
Radius	18.1310	10.2340	18.49	32.77	9.60	17.01
Os calsis	4.6580	2.0340	16.55	37.91	5.11	11.70
Pelvic Area	154.8368	51.4592	8.63	25.96	5.20	15.63
Lumbar Vertebra 4	X	4.5773	X	38.38	X	17.26
Lumbar Vertebra 3	X	4.8939	X	35.74	X	16.82
Lumbar Vertebra 2	X	4.9855	X	37.13	X	16.99

TABLE II-C

PER CENT CALCIUM AND PHOSPHORUS CALCULATED FROM THE DRY WEIGHT AND ASHED WEIGHT OF VARIOUS INDIVIDUAL BONES FROM PRIMATE 30

Bone	Dry Weight (grams)	Ashed Weight (grams)	Calcium in Dry Weight (per cent)	Calcium in Ashed Weight (per cent)	Phosphorus in Dry Weight (per cent)	Phosphorus in Ashed Weight (per cent)
Femur	32.9233	17.1550	20.57	38.14	8.97	17.21
Humerus	24.8360	13.2740	21.13	39.53	9.06	16.95
Tibia	23.2720	11.1135	19.27	40.35	8.14	17.05
Ulna	13.5950	6.9206	20.05	39.39	8.91	17.49
Radius	11.4480	6.3710	21.70	38.99	9.66	17.36
Fibula	5.1552	2.7727	23.22	43.17	9.06	16.84
Os calsis	4.7590	1.7004	13.95	39.05	6.22	17.41
Pelvic area	69.3940	26.4814	15.17	39.76	6.44	16.86
Lumbar Vertebra 4	X	2.3715	X	36.22	X	X
Lumbar Vertebra 3	X	2.6364	X	36.75	X	X

TABLE II-D

PER CENT CALCIUM AND PHOSPHORUS CALCULATED FROM THE DRY WEIGHT AND ASHED WEIGHT OF VARIOUS INDIVIDUAL BONES FROM PRIMATE 69

Bone	Dry Weight (grams)	Ashed Weight (grams)	Calcium in Dry Weight (per cent)	Calcium in Ashed Weight (per cent)	Phosphorus in Dry Weight (per cent)	Phosphorus in Ashed Weight (per cent)
Femur	32.0628	20.2399	23.27	36.87	10.56	16.72
Humerus	24.5199	15.7107	23.87	37.25	10.73	16.75
Tibia	22.2568	13.5342	22.73	37.37	10.09	16.59
Ulna	12.5852	7.8723	22.95	36.69	51.75	82.73
Radius	10.3070	6.3311	45.76	74.51	52.89	86.11
Fibula	4.7797	2.9105	22.32	36.66	5.00	8.21
Os calsis	3.4800	1.8613	20.03	37.45	8.91	16.66
Pelvic area	83.4681	35.9187	16.17	37.58	7.19	16.70
Lumbar Vertebra 4	X	3.4980	X	35.71	X	33.70
Lumbar Vertebra 3	X	3.6270	X	35.84	X	32.59

TABLE III

CORRELATION COEFFICIENTS FOR PAIRS OF FACTORS FOR DESIGNATED BONES

PART A. FEMUR

Factors Compared	Correlation Coefficient	Probability of Significance
Dry Weight and Ashed Weight	0.9028	P<0.02
Dry Weight and Calcium in the Bone . . .	0.9643	P<0.01
Dry Weight and Phosphorus in the Bone . .	0.9002	P<0.02
Ashed Weight and Calcium in the Bone . . .	0.9842	P<0.001
Ashed Weight and Phosphorus in the Bone . .	0.9882	P<0.001
Calcium in the Bone and Phosphorus in the Bone	0.9784	P<0.001

TABLE III

CORRELATION COEFFICIENTS FOR PAIRS OF FACTORS FOR DESIGNATED BONES, CONTINUED

PART B. HUMERUS

Factors Compared	Correlation Coefficient	Probability of Significance
Dry Weight and Ashed Weight	0.9338	$P<0.01$
Dry Weight and Calcium in the Bone . . .	0.9725	$P<0.01$
Dry Weight and Phosphorus in the Bone . .	0.9383	$P<0.01$
Ashed Weight and Calcium in the Bone . . .	0.9914	$P<0.001$
Ashed Weight and Phosphorus in the Bone . .	0.9989	$P<0.001$
Calcium in the Bone and Phosphorus in the Bone	0.9926	$P<0.001$

TABLE III

CORRELATION COEFFICIENTS FOR PAIRS OF FACTORS FOR DESIGNATED BONES, CONTINUED

PART C. RADIUS

Factors Compared	Correlation Coefficient	Probability of Significance
Dry Weight and Ashed Weight	0.9892	$P < 0.001$
Dry Weight and Calcium in the Bone . . .	Not Significant	
Dry Weight and Phosphorus in the Bone . .	Not Significant	
Ashed Weight and Calcium in the Bone . . .	Not Significant	
Ashed Weight and Phosphorus in the Bone . .	Not Significant	
Calcium in the Bone and Phosphorus in the Bone	0.9369	$P < 0.01$
Calcium in the Bone and per cent of Calcium in Dry Weight	0.8733	$P < 0.05$
Calcium in the Bone and per cent of Calcium in Ashed Weight	0.8544	$P < 0.05$
Phosphorus in the Bone and per cent of Phosphorus in Dry Weight	0.9760	$P < 0.001$
Phosphorus in the Bone and per cent of Phosphorus in Ashed Weight	0.9760	$P < 0.001$

TABLE III

CORRELATION COEFFICIENTS FOR PAIRS OF FACTORS FOR DESIGNATED BONES, CONTINUED

PART D. ULNA

Factors Compared	Correlation Coefficient	Probability of Significance
Dry Weight and Ashed Weight	0.8382	$P<0.05$
Dry Weight and Calcium in the Bone . . .	0.9211	$P<0.01$
Dry Weight and Phosphorus in the Bone . .	Not Significant	
Ashed Weight and Calcium in the Bone . . .	0.9831	$P<0.001$
Ashed Weight and Phosphorus in the Bone . .	Not Significant	
Calcium in the Bone and Phosphorus in the Bone . .	Not Significant	
Phosphorus in the Bone and per cent of Phosphorus in Dry Weight	0.9942	$P<0.001$
Phosphorus in the Bone and per cent of Phosphorus in Ashed Weight	0.9909	$P<0.001$

TABLE III

CORRELATION COEFFICIENTS FOR PAIRS OF FACTORS FOR DESIGNATED BONES, CONTINUED

PART E. TIBIA

Factors Compared	Correlation Coefficient	Probability of Significance
Dry Weight and Ashed Weight	Not Significant	
Dry Weight and Calcium in the Bone . . .	0.8642	$P<0.05$
Dry Weight and Phosphorus in the Bone . .	0.7741	$P<0.10$
Ashed Weight and Calcium in the Bone . . .	0.9611	$P<0.01$
Ashed Weight and Phosphorus in the Bone . .	0.9931	$P<0.001$
Calcium in the Bone and Phosphorus in the Bone	0.9844	$P<0.001$

TABLE III

CORRELATION COEFFICIENTS FOR PAIRS OF FACTORS FOR DESIGNATED BONES, CONTINUED

PART E. FIBULA

Factors Compared	Correlation Coefficient	Probability of Significance
Dry Weight and Ashed Weight	0.9790	$P < 0.001$
Dry Weight and Calcium in the Bone . . .	0.8541	$P < 0.05$
Dry Weight and Phosphorus in the Bone . .	Not Significant	
Ashed Weight and Calcium in the Bone . . .	0.8681	$P < 0.05$
Ashed Weight and Phosphorus in the Bone . .	Not Significant	
Calcium in the Bone and Phosphorus in the Bone . .	0.8505	$P < 0.05$
Phosphorus in the Bone and per cent of Phosphorus in Dry Weight of Bone	0.9571	$P < 0.01$
Phosphorus in the Bone and per cent of Phosphorus in Ashed Weight of Bone . . .	0.9606	$P < 0.01$

TABLE III

CORRELATION COEFFICIENTS FOR PAIRS OF FACTORS FOR DESIGNATED BONES, CONTINUED

PART G. OS CALCIS

Factors Compared	Correlation Coefficient	Probability of Significance
Dry Weight and Ashed Weight	Not Significant	
Dry Weight and Calcium in the Bone . . .	Not Significant	
Dry Weight and Phosphorus in the Bone . .	Not Significant	
Ashed Weight and Calcium in the Bone . . .	0.9628	$P<0.01$
Ashed Weight and Phosphorus in the Bone . .	Not Significant	
Calcium in the Bone and Phosphorus in the Bone	Not Significant	

TABLE III

CORRELATION COEFFICIENTS FOR PAIRS OF FACTORS FOR DESIGNATED BONES, CONTINUED

PART H. PELVIC AREA

Factors Compared	Correlation Coefficient	Probability of Significance
Dry Weight and Ashed Weight	0.9485	P<0.01
Dry Weight and Calcium in the Bone . . .	Not Significant	
Dry Weight and Phosphorus in the Bone . .	0.8917	P<0.05
Ashed Weight and Calcium in the Bone . . .	Not Significant	
Ashed Weight and Phosphorus in the Bone . .	0.9878	P<0.001
Calcium in the Bone and Phosphorus in the Bone	Not Significant	

TABLE III

CORRELATION COEFFICIENTS FOR PAIRS OF FACTORS FOR DESIGNATED BONES, CONTINUED

PART I. LUMBAR VERTEBRA 3

Factors Compared	Correlation Coefficient	Probability of Significance
Ashed Weight and Calcium in the Bone . . .	0.9995	P<0.001
Ashed Weight and Phosphorus in the Bone . .	Not Significant	
Calcium in the Bone and Phosphorus in the Bone	Not Significant	
Phosphorus in the Bone and per cent Phosphorus in the Bone	0.9204	P<0.01

PART J. LUMBAR VERTEBRA 4

Ashed Weight and Calcium in the Bone . . .	0.9887	P<0.001
Ashed Weight and Phosphorus in the Bone . .	Not Significant	
Calcium in the Bone and Phosphorus in the Bone	Not Significant	
Phosphorus in the Bone and per cent of Phosphorus in the Bone	0.9203	P<0.01