THE EFFECT OF GRADED DOSES OF CORTICOSTEROIDS ON REGIONAL BODY CALCIUM IN THE CEBUS MONKEY: AN ANALYSIS WITH IN VIVO NEUTRON ACTIVATION

A Thesis

by

SCOTT HOWARD LOEFFLER

Submitted to the Graduate College of
Texas A&M University
in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

August 1984

Major Subject: Epidemiology

THE EFFECT OF GRADED DOSES OF CORTICOSTEROIDS ON REGIONAL BODY CALCIUM IN THE CEBUS MONKEY: AN ANALYSIS WITH IN VIVO NEUTRON ACTIVATION

A Thesis

by

SCOTT HOWARD LOEFFLER

Approved as to style and content by:

Leon H. Russell (Chairman of Committee)	Archie I. Flowers (Member)
William F. McCulloch (Member)	Dan Hightower (Member)
Lloyd W. Rooney (Member)	Norman D. Heidelbaugh (Head of the Department)

August 1984

ABSTRACT

The Effect of Graded Doses of Corticosteroids
On Regional Body Calcium in the Cebus Monkey:
An Analysis with In Vivo Neutron Activation. (August 1984)
Scott Howard Loeffler, B.S., Vanderbilt University;
Drs., State University of Utrecht
Chairman of Advisory Committee: Dr. Leon H. Russell

Eight adult Cebus apella/Cebus albifrons were studied to determine the effects of an 11-week graded dosage treatment with cortisone acetate followed by a 5-week treatment with hydrocortisone. Four experimental monkeys received sequentially an aqueous suspension of cortisone acetate in daily injections of 5 mg/kg body weight (b.w.) for four weeks, 10 mg/kg b.w. for three weeks, and 35 mg/kg for four weeks. This was immediately followed by a 5-week, daily treatment with aqueous hydrocortisone succinate at a dose of 26 mg/kg b.w./day. All drugs were given by intramuscular injection. Body calcium in the leg and spinal regions was monitored by regional activation analysis employing a ^{252}Cf neutron source. Serum cortisol, serum electrolytes (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}), urinary calcium and a few routine hematological indices (Ht, Hb, RBC, WBC) were tested during this period. Control animals received no injections, but were tested in the same manner. Serum cortisol was elevated ($p < .05$) and weight losses occurred in the treated group. Bone calcium (expressed as

corrected counts) was unchanged ($p < .05$), although urinary calcium excretion appeared to be elevated in the treated group. An experimental animal which died during the last weeks of the experiment displayed atrophy and fatty infiltration of the adrenal glands at post-mortem histological examination. There were no changes in serum electrolytes or hematological indices as measured except for an occasional upward fluctuation of the hematocrit in experimental monkeys. Regional *in vivo* activation analysis for measurement of spinal and leg calcium had a coefficient of variation of 3.25%.

ACKNOWLEDGMENTS

A long process of finishing my professional veterinary degree in the Netherlands has resulted in an extraordinary prolongation of the final copy of this thesis. Hopefully, the time in which I have gained more insight into the endocrinology of bone metabolism as well as a fresh dive into the literature (which now seems much less frightening) has resulted in a better product. The thanks and appreciation for those who supported me during this work and gave me the freedom to engage in a first research experience seem even more meaningful now, in retrospect.

Grateful appreciation is extended to Dr. Leon H. Russell for his friendship, sincere interest in my work and the guidance of my graduate education. Instrumental in this project were my co-investigators, Dr. Adrian LeBlanc and Dr. Harlan J. Evans of the Nuclear Medicine Department, Baylor College of Medicine. The project would have been wholly impossible without their technical and advisorial support. Thanks also to Dr. Archie I. Flowers and Dr. Norman Heidelbaugh and the Department of Veterinary Public Health for providing the animal colony, arrangements for transportation and research funds.

It is my pleasure to thank Dr. Lloyd Rooney of the Department of Soil and Crop Sciences for allowing me to use his cereal lab for the preparation of animal diets; Dr. Bennie J. Camp of the Department of Veterinary Physiology and Pharmacology for the use of his atomic

absorption spectroscopy equipment; Dr. Ronnie Green for the preparation and evaluation of the radiographs; and Ms. Vernel Festerman of the National Aeronautics and Space Administration (NASA), Endocrinology Division, for the cortisol and calcium analyses of serum and the calcium analysis of urine.

Dr. Dan Hightower of the Department of Veterinary Physiology and Pharmacology and Dr. Gary Joiner of the Department of Veterinary Public Health were helpful with suggestions or alternative ideas throughout the project. Ms. Modene Botter was a lifesaver in finding needed tubes, needles or other equipment when no one else could imagine where they were located. Dr. Ken Samonds of the Harvard School of Public Health, Nutrition Department, offered both encouragement and Monkey Vitamin Mix whenever they were needed. W.A. Landers Company fabricated (cost-free) the urine metabolism pans according to my specifications.

Last, but not least, I wish to thank Dr. William McCulloch for serving on my committee; fellow students Soussan Toutouchian, Bill Yates, Don Scarborough, John Zettelmoyer, Steve Waring, Duane Hilborn and Gary Harwel for their help; and Evelien and my family for all of their encouragement.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In 1968, the Food and Nutrition Board of the National Academy of Sciences cited osteoporosis as a major cause of human illness, especially among the elderly (1). More prevalent among post-menopausal women (2,3), this disease has been estimated to afflict from ten to thirty percent of those people in western countries above the age of fifty, depending upon age and sex. This phenomenon called osteoporosis is also associated with factors independent of age, such as: bedrest or immobilisation (4,5,6), spaceflight (7,8), relative or absolute deficiencies in calcium uptake (9,10), exogenous or endogenous elevations of plasma glucocorticoid levels (11,12), renal insufficiency (13), thyroid disease (14), acromegaly (15) as well as other metabolic diseases. To say the least, the relationship of bone metabolism to osteoporosis is complex and the reader is referred to two review articles (16,17).

Current research on osteoporosis can legitimately be broken down into three principal divisions: 1) the methodological improvement for the early detection of bone changes; 2) endocrinological and cellular research on bone metabolism; and 3) clinical and experimental studies to arrest or reverse bone changes once they begin to occur.

Various studies have shown that the limited physical activity and weightlessness associated with spaceflight have a negative effect on

---

This paper is styled after The Journal of Clinical Investigation.

bone (18,19). These changes were sometimes accompanied by a rise in plasma cortisol levels (18). Likewise, tests in rabbits given a daily dose of cortisone showed increased resorption activity (20). At the time of these experiments there were no publications, to the author's knowledge, on the relation between glucocorticoid treatments and osteoporosis in monkeys.

The objectives of this thesis were as follows: 1) to investigate the Cebus monkey as a possible model for corticosteroid- or spaceflight-induced osteoporosis in humans; 2) to determine the calcium changes on a regional basis using neutron activation analysis in an animal with a skeletal structure similar to man; and 3) to obtain serum and urine electrolyte values as well as serum cortisol values during the therapy for comparison with controls. The Cebus monkey was chosen because of its small size and availability.

Abbreviations used in this paper: BMC, bone mineral content; CS-OP, corticosteroid-induced osteoporosis; TBNAA, total-body neutron activation analysis; PBNAA, partial-body neutron activation analysis; TBBM, total body bone mineral; TBCa, total body calcium; TBV, trabecular bone volume; SPA, single-photon absorptiometry; and DPA, dual-photon absorptiometry.

CHAPTER II

LITERATURE REVIEW

Osteoporosis in Animal Models

Because experiments on human subjects cannot be done for ethical and practical reasons (control of diet, etc.), it is important that animal models be available for research on human diseases. While mice, rats, monkeys, dogs and other animals have proven themselves as reliable models for various human disease states (for example, mammary tumors by the bitch), there is no especially suited animal for osteoporosis research. Even more exasperating is the conclusion in a recent book concerning animal models for human disease: there are no animals listed for bone metabolism studies (21)! The question then presents itself, "How does one induce a bone loss in animals which resembles osteoporosis in man?" In this section, the author will examine several methods which have been used.

Calcium deficiency. The most obvious and perhaps the oldest method of inducing bone resorption is the feeding of a diet, complete in all nutrients except calcium. Bone mineral is composed of about 39% calcium, the inorganic matrix resembling closely that of calcium hydroxyapatite ($Ca_{10}(PO_4)_6 \cdot (OH)_2$) (22). Lack of calcium in the diet causes the body to call on its reserves in bone via a parathyroid hormone (PTH)-directed mechanism. Bone loss via this pathway has been used successfully in experiments with monkeys (10,23) and rats (24-26). The question arises, however, as to whether or not this is the

same kind of osteoporosis which frequently occurs in western man. The presence of sufficient calcium in the diets of those who have experienced senile, immobilization or spaceflight osteoporosis and the fact that calcium when given intravenously does little to restore osteoporotic bone to its normal state (27), would suggest that: 1) either other factors play a role in osteoporosis, and/or, 2) as Bollet suggests (28), we are dealing with different types of osteoporoses.

Immobilization. Immobilization or disuse is a second manner to induce osteoporosis in rats (29,30), rabbits (31,32), man (33), dogs (34-36), and monkeys (37,38). This is usually accomplished by either providing the subject with a very limited space (38), suspension of the hindquarters in a sling (30), casting an extremity in plaster (32,34), or resection of a motoric nerve (36). This method of induction is consistent with the theory of Urist and Adams (39) that bone formation is regulated by mechanical stress. A disadvantage to this method is the extreme discomfort and psychological stress (especially to the very active normal monkey) inflicted upon the animal. It is clear from research by Mack (38) that earthbound controls who were similarly restrained lost less bone than experimental monkeys in spaceflight. It is unclear as to whether or not this increased bone loss is due to other factors or merely to less mechanical stress encountered during weightlessness.

Spaceflight. In man (40,41), monkeys (38) and rats (42) there is a loss of bone density which occurs during spaceflights of greater than one week duration. This method of induction is obviously beyond the costs of all research institutes not affiliated with a space

program; and even for those laboratories, the possibilities for large animal groups and frequent measurements of various metabolic functions are beyond the current technical capabilities. However, it is noteworthy for research into the endocrinology of osteoporosis that scientists have speculated that psychological stress could be an important factor in the differences found between space-bound apes and their controls (38). A support for the theory that these animals undergo a stress is the fact that rats develop a hypertrophy of the adrenal glands during spaceflight (42). The adrenal glands are also the centers of production for endogenous glucocorticoids.

Corticosteroid-induced osteoporosis (CS-OP). In 1958, Urist (43) pointed out that osteoporosis had only been produced in rabbits and birds using a cortisone or a combination of cortisone and ACTH (an adrenal stimulator released by the pituitary). Excluding man, this list has not been expanded, as yet. Jaffe and Epstein (44) noted generalized muscle atrophy and bone loss in rabbits after five weeks of a nine-week study using cortisone. Likewise, Thompson et al. (45) showed significant body weight and bone mineral losses in a rabbit study of a similar duration at doses of 15 mg/kg b.w./day. Other authors (46-48) have also induced bone losses in rabbits.

Yasamura et al. (49), on the other hand, were unable to produce bone losses in rats given hydrocortisone at a rate of 12 mg/kg b.w./alternate days. In fact, these rats showed an increase in bone density as measured by TBNAA. These findings are consistent with those of Follis (50) who demonstrated that glucocorticoids had an inhibitory effect on bone resorption in rats. Moreover, neither was

Beneton et al. (51) able to produce bone changes in cynomolgus monkeys with doses of a synthetic product (deflazacort) analogous to cortisone. In dogs, although Cushing's syndrome occurs naturally as well as after excessive cortisone treatments, to the author's knowledge, there is no documentation on osteoporosis as a result.

Corticosteroid Interactions: Theories of CS-OP

Since Cushing's original study of adrenal hypercortisonism in 1932 (52), many researchers have studied the effects of glucocorticoids on bone. Since 1948, with the introduction of cortisone as drug, the side-effects of these powerful compounds have become apparent. In 1957, Pietrogrande and Mastromarino (53) noted the relationship between steroid therapy and osteoporosis in a report of a 43-year-old man who had been treated continuously over a four year period with cortisone. Murray (54), three years later, noted significant radiological changes in patients receiving corticosteroids. Several authors (44,55) have shown that CS-OP is a result of both low bone formation and high bone resorption rates. Bressot et al. (56) concluded that humans receiving long-term corticosteroids display a general loss of trabecular bone volume (TBV) as a result of increased osteoid resorption and a decrease in osteoblastic appositional rate when iliac crest biopsies were examined. He attributed these changes to a total increase in basic multicellular units (BMU), combined with a relative decrease in the activity of the osteoblasts, giving the osteoblasts in each BMU an advantage.

How these changes in the number of cells and their activity arise is a subject of intense speculation. The central issue is: 1) do corticosteroids act directly on the cells involved, or 2) do corticosteroids act via circulating hormones such as vitamin D_3, parathyroid hormone, estrogens and calcitonin, or 3) both of the above. The degree to which effects on bone occur is no doubt related to the biological properties of the specific corticosteroids. In Table 1 some of these properties are listed.

Meunier et al. (55) noted a decrease in serum 25-OH D_3 (vitamin D precursor) and a slight rise in serum PTH values in 70 osteoporosis patients on corticosteroid regimens. Bernini et al. (57) found a significantly depressed response (to calcium infusion) of calcitonin in patients who had received prednisone and betamethasone prior to the response test. This depressed response was not observed in the patients who had received deflazacort therapy. A "bone-saving" effect has also been attributed to deflazacort by others (58,59). Russell and Avioli (59) found that prednisone (in vitro) inhibited the glycolytic pathway, which is necessary for bone calcification in epiphyseal cartilage, but deflazacort did not. These findings must be taken into account when drawing conclusions from the monkey experiment cited earlier (51).

Support for the theory that corticosteroids act directly on cells was provided by Chen and Feldman (60). In cultured bone cells, dexamethasone had the effect of potentiating the cAMP production by PTH; increased amounts of $1,25(OH)_2D_3$ in bone cells were found as well. A similar result was described by Teitelbaum (61), who not only

TABLE 1

Properties of Corticosteroids

Steroid name	Half-life time (min)	Relative anti-imflammatory effect (potential)
Cortisol	80 - 100	1.0
Corticosterone	60 - 80	0.4
Cortisone	30	0.8
Prednisone	60	4.0
Dexamethasone	200	25.0
Deflazacort	Not available	Not available

(Biological data in man)

(Adopted from data in: Peterson, R.E. Metabolism of Corticosteroids in Man. Ann. N.Y. Acad. Sci. 82:846; Gilman, G.G., L.S. Goodman, and A. Gilman (eds.), The Pharmacological Basis of Therapeutics, 6th ed. MacMillan Publishing Co., N.Y., 1980.)

recognized an increased production of cAMP in the presence of cortisol, but also noted that the longevity of cytosol receptors for $1,25(OH)_2D_3$ was increased. In this case, the fall in serum vitamin D may be secondary.

It is also possible that cortisol may regulate cell activity via prostaglandins. Raisz and Chyun (62) showed that a depressed cell proliferation and DNA synthesis in rat calvaria (induced by cortisol) could be reversed by the addition of PGE_2 (prostaglandin) to the culture medium. For further details on this subject, the reader is referred to a review by Ibbotson et al. (17).

Methods of Diagnosis

In order to recognize small bone changes caused by osteoporosis it is necessary to make sensitive and frequent measurements during its early stages. There are several methods available to measure these changes, the most important of which are discussed below.

Radiology. Until the 1960's, radiography was the only widely available method for measuring bone density without taking a biopsy. The problems with this method are that photographic standardization is difficult (due to small differences in film quality and development techniques) and density changes of at least 15% must take place in order to be detected easily. Moreover, any such visual evaluation is dependent upon intra- and inter-observer errors. Because of the subjectivity involved in evaluating hypertransparency of films, some authors (55) accept only vertebral crush fractures or femoral neck, wrist or rib fractures as valid radiological evidence of osteoporosis.

Kovarik and Kuster (63) discuss some of the uses of this method in a recent article.

Radiology with film densitometry. One means of overcoming the problems of standardization and observer error is with the method employed at the Texas Women's University (40). A normal radiograph can be calibrated by placing an aluminum wedge (see Figure 1) next to the bone during film exposure. This alloy wedge exhibits an X-ray absorption coefficient similar to bone and can be standardized to mass-equivalents of calcium hydroxyapatite. Bone mass can be determined as wedge mass-equivalency by means of a densitometer which scans the wedge and bone sections on the developed X-ray film. This method has the advantage of: 1) the fact that positioning of the bone is not extremely critical because the complete bone section can be analyzed by the scanning densitometer; 2) rem exposure to the patient is not greater than with a normal radiograph; and 3) the system may be used in conjunction with several Roentgen machines as long as they are calibrated with phantoms (mineral substances impregnated in an organic matrix) and aluminum wedges are used. The disadvantage of this set-up probably lies in the costs of a densitometer-computer assembly in addition to the fact that accuracy is limited by a maximum film quality.

Bone biopsy-histomorphometry. A widely used method of determining the osteoporotic status of a subject is the iliac crest biopsy combined with the microscopic measurement of various parameters. An obvious advantage of this method is that one is able to measure

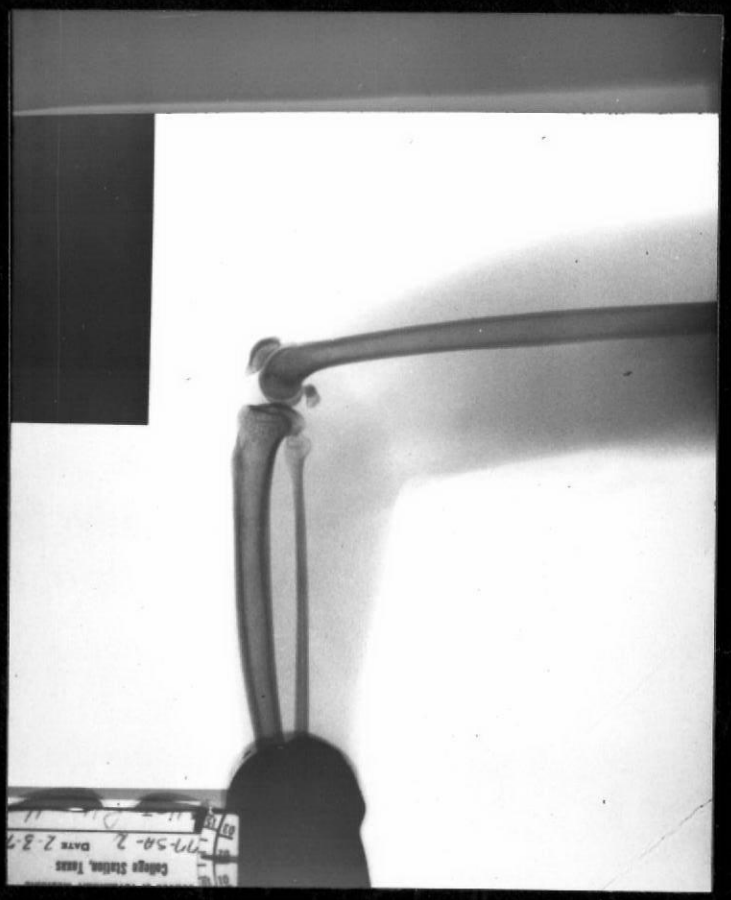


FIGURE 1 Reproduction of a radiograph of experimental monkey (12B) taken prior to the experimental period. The aluminum step-wedge (upper left-hand corner) and the right femur and tibia are shown.

simultaneously a large number of parameters such as: TBV, calcification rate, osteoid thickness, and resorption surface (55). The trabecular bone volume (TBV), for example, is measured by determining the percentage of trabecular bone in the total spongiosa by means of an image analyzing computer which scans the section with a light beam. Meunier et al. (55) has noted that a high degree of correlation existed between vertebral crush fractures and a TBV of less than 11%. This method of measuring trabecular bone seems to have slowly replaced the microradiography method of Jowsey and Riggs (64). The disadvantages of bone biopsies are: 1) a slight change in location may produce a significant change in the measurement; 2) the difficulty/discomfort of frequent biopsies; and 3) the questionable representativity of the iliac crest (chosen primarily for ease of access) for other body areas.

Single photon absorptiometry (SPA). This method of bone mineral measurement first described by Cameron and Sorenson (65) and commercially available (Norland Instruments, WI) has the advantage of being cheaper and more portable than, for example, DPA and PBNAA techniques. The linear bone density, expressed as bone mineral content (BMC) over a 1/8" thick scan site of the mid-distal radius, is usually measured. Using photon energy from an ^{125}I source with collimator, the bone is scanned over its width and the baseline value due to soft tissue absorption is subtracted. The limited penetration of the beam from the source and the need to place the extremity in a soft tissue equivalent medium during measurements, limit the use of this machine to forearm, hands and feet (or perhaps a child's leg).

It has been shown that the BMC values of bones are linearly related to the breaking strength (66,67); yet this method measures BMC at a point (mid-distal radius) which is >75% cortical bone while the vertebrae are predominantly trabecular bone. Riggs et al. (2) have shown that trabecular bone in the axial skeleton loses proportionately more mineral in osteoporosis and that either whole body or extremity (radius) mineral measurements are less discriminatory than vertebral measurements.

Dual photon absorptiometry (DPA). This recent improvement on SPA employs a source, ^{153}Gd, with a dichromatic beam and a greater tissue penetration. It can be used for either whole body (68) or regional body (69) scans. In short, this method differs from the above-mentioned Cameron technique by transmitting and measuring the absorption of two separate photon energies which travel through bone and soft tissue. This method eliminates the need for a constant soft tissue thickness and bones which are more thickly covered with tissue can also be measured. Edges of bone and vertebral disks can be distinguished by a computer which makes point by point determinations of density; thus, problems with positioning can be more easily corrected than with SPA. Riggs et al. (2) found a coefficient of variation (C.V.) of 2.3% in multiple measurements on five volunteers. The same author had less precision in radius measurements with SPA (C.V. 3-5%), but others (70,71) have shown the precision of measurements with SPA to be of the same order (C.V. 1-3%) when water baths are used and care is taken with repositioning. The advantages of regional BMC measurements of the vertebrae have already been

mentioned (as a disadvantage of SPA). Further, DPA shares with SPA the advantage of a small radiation dose to the subject. To cite two practical comparisons: 1) a set of four scans of the radius with a Norland-Cameron Bone Mineral Analyzer exposes the patient to about 1/100 of the dose delivered to a patient for a radiograph of the forearm; and 2) the radiation exposure for a whole body TBBM measurement with DPA was 500-5000 times smaller than for a TBNAA measurement for calcium (71,72). These low doses allow for frequent measurements with little radiation exposure.

Neutron activation analysis (NAA). The technique of neutron activation analysis of body elements in vivo has been extensively described by various authors (72-81). The method utilizes the fact that a living subject contains certain quantities of elemental nuclides which can be activated (used to generate small quantitites of detectable radioactive isotopes) by collision with neutrons (n). A few of the generated nuclides are: $^{48}Ca(n,\gamma)^{49}Ca$; $^{23}Na(n,\gamma)^{24}Na$; $^{37}Cl(n,\gamma)^{38}Cl$; $^{14}N(n,2n)^{13}N$ and $^{39}K(n,2n)^{38}K$. These radionuclides decay at various (half-life) rates, giving off energy which can be measured. By determining the gamma energy output of a given radionuclide in its specific millivolt range per time unit, one can estimate the amount of element present in the irradiated body part for a given neutron dosage by standardization procedures with phantoms (known amounts of mineral embedded in a matrix shaped to the geometric form of the body part to be measured). It is, of course, necessary that the neutron beam directed towards the subject creates an homogenous distribution of neutrons (neutron flux) within the area to

be measured in order for all elementary atoms in the activated body region to have a statistically "equal chance" to become activated. Body thickness, distance from the point source to the body surface, and the type of neutron source (determines the percentage of neutrons which are of sufficient energy to pass through several inches of flesh and bone) are all factors which must be considered in developing facilities and using NAA methods.

Basically, the techniques used in human and animal studies are either of the whole-body (TBNAA) (72-77) or partial-body (PBNAA) (78-81) type. The advantage of TBNAA lies in the determination of total Na, K, N, P and Cl besides the already mentioned measurement of calcium for osteoporosis status. Local or regional measurement of the former elements has little use unless the results can be converted to whole body equivalents. Total body potassium can be used for estimating body fat, nitrogen for estimating body protein and sodium for electrolyte studies (75). Total body Ca (TBCa) measurements may be the only feasible measurement in small animals such as rats (82), but, in man, the axial skeleton is thought to be the best indicator of osteoporosis associated with old age (2). The high correlation between bone mineral measurements in the distal radius and total body Ca ($r = 0.93 - 0.98$) would seemingly make the greater radiation exposure to subjects inherent in TBNAA unnecessary (80,83). This finding emphasizes the importance of regional methods for measuring axial bone mineral.

The advantage of a smaller radiation dose to the subject with PBNAA in comparison to TBNAA is made possible by the smaller source to

skin distance which allows a weaker neutron source to be employed (to radiate a large object adequately, one must employ a stronger source at a greater distance). This is important with respect to the total neutron exposure of the subject. The growing concern over neutron exposure can best be expressed by a recent statement of The National Council on *Radiation Protection* (NCRP): "Although, at present, there is a recommended limit on neutron dosage, this may soon be reduced to an even smaller limit."(84) An accurate assessment of vertebral mineral and calcium content is currently available with regional DPA and PBNAA, respectively. Regional trunk calcium as measured by activation analysis and regional trunk mineral as measured by DPA are about equally precise (C.V. 1-2%) and correlation between the two is high ($r = 0.97$) (85). The disadvantage of a greater radiation exposure with PBNAA when compared to regional DPA is probably less than the 500-5000x difference stated above for whole body measurements. Because of the growing importance of regional measurements in man and the similarity in skeletal structure between the species, the monkey seemed an ideal model for testing PBNAA.

CHAPTER III

METHODS

Animals

Eight adult Cebus monkeys, confirmed by epiphyseal control of the appendicular skeleton with radiographic examination, were housed in individual cages in a room with stabilized temperature and humidity controls. Animals had been laboratory reared and shown to be negative with respect to tuberculosis. The monkeys were identifiable by a tatoo on the chest. Table 2 gives a breakdown of the animals in terms of age, weight, and sex which were the criteria used to divide them into two groups of four. The experimental group (Group B) was chosen at random.

Caged animals were transported to Houston for neutron activation bi-weekly. They were housed overnight on test days at Baylor College of Medicine. After testing, animals were returned to the vivarium at Texas A&M University, College of Veterinary Medicine.

Animal Diet

Two months prior to the beginning of the experimental period, monkeys were changed from a commercial diet (Wayne Animal Diets, Chicago) to a modification of the diet as described by Corey and Hayes (86). The only modification was a partial substitution of the sucrose with cornstarch (Argo Products Inc., Argo, IL).

TABLE 2

Monkey Background Data

Controls	16A	33A	33B	3B	$\bar{X}$	S.D.
Sex	M	F	M	F		
Age (months)	92	71	46	50	65	21.2
Weight (kgs)	3.2	1.8	2.0	1.8	2.2	0.7
Experimentals	**9B**	**12B**	**52A**	**36A**	$\bar{X}$	**S.D.**
Sex	F	F	M	M		
Age (months)	85	67	50	56	64.5	15.4
Weight (kgs)	1.7	2.3	2.2	2.0	2.1	0.5

The diet (Table 3) was mixed dry in 5 kg batches. The oil was mixed into the powder, followed by 4 l of a hot 2.5% agar-in-water solution. The slurry was then poured into a 20" x 36" fiberglass tray and stored at 4°C until feeding. The cake-like substance was cut into cubes of approximately 110 g and fed to the animals (one cube/animal) twice daily. Calcium from the mineral mix was calculated to be 0.55% of the dry diet. Because the casein contributes about the same amount of calcium to the diet, the total calcium percentage was 1.1% of the dry diet. Each animal also received two orange slices and two raw peanuts per day. Distilled water was given ad libitum.

Drug Treatment

Four experimental monkeys received sequentially an aqueous suspension of cortisone acetate (Upjohn) in daily intramuscular injections of 5 mg/kg b.w. (body weight) for four weeks, 10 mg/kg b.w. for three weeks, and 35 mg/kg b.w. for four weeks. This was followed by a five-week daily treatment with hydrocortisone succinate (Upjohn) (i.m.) at 26 mg/kg b.w. Survivors (three monkeys) were slowly withdrawn from treatment by step-wise reduction of hydrocortisone over a period of 60 days after the experimental period.

Neutron Activation

Holding device. A restraining apparatus was constructed from plexiglass to assure better reproducibility of the animal positioning during irradiation and counting as depicted in Figures 2 and 3. Although the regional activation analysis involves a greater bone area

TABLE 3

Synthetic Diet Composition

Ingredients	g/100 g of diet
Casein	20.0
Sucrose	33.4
Cornstarch	9.1
Cottonseed-soybean oil	10.0
Vitamin mix	0.5
Mineral mix	4.0
Choline chloride	0.3
Inositol	0.1
Cellulose	21.6

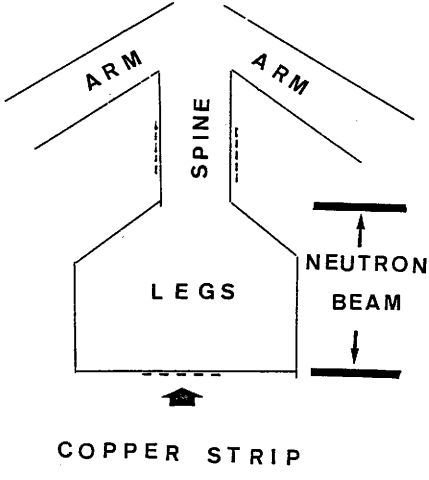


FIGURE 2 Sketch of monkey holder showing position of copper strips and neutron beam for leg irradiation.

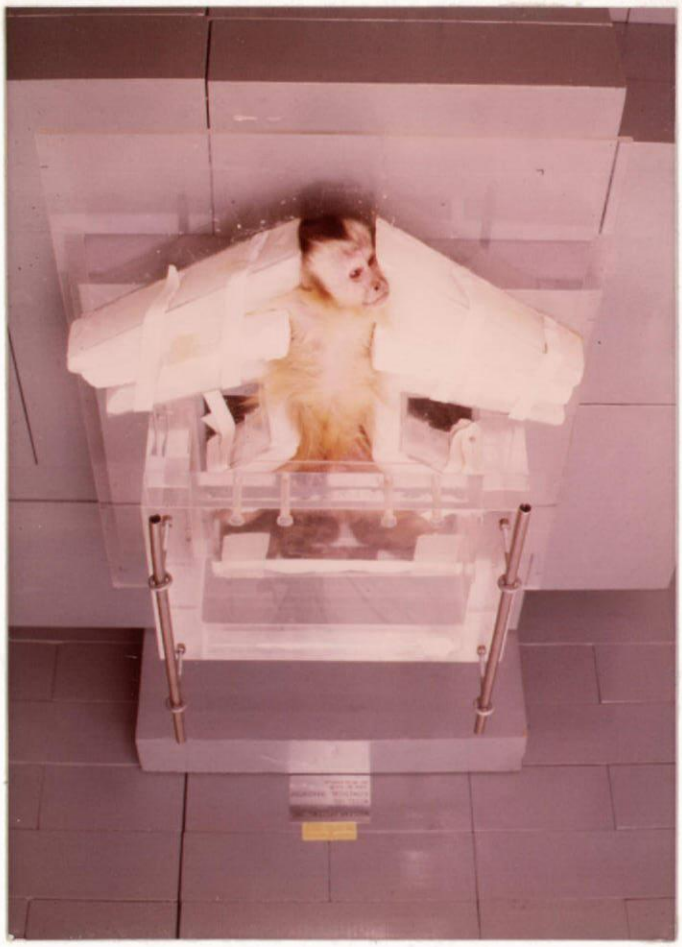


FIGURE 3 Photograph of the monkey in holder for leg irradiation. Here shown with a plexiglass instead of a paraffin moderator.

(in this case the vertebral column or the lower extremities) than is the case with the Cameron single photon absorption technique (±5 mm x 2 cm) (71), it is quite important to measure calcium changes in one area of bone with respect to time and not calcium differences between two adjacent areas of bone. The holder was placed in the same position over the irradiation port for consecutive irradiations by positioning marks on the port across from corresponding marks on the holder. This allowed the animal to maintain about the same body geometry from one sequence to the next. Molded paraffin was built into the holder lateral to leg and spinal regions and a removable paraffin piece was placed above the given region during irradiations. This caused a better neutron flux uniformity throughout the irradiated region by thermalizing "fast" neutrons and reflecting others back towards the bone. Strips of copper were placed in both the spinal and appendicular regions of the holder (Figure 2) as a control for variations in positioning. In this manner, detectable amounts of ^{66}Cu were created and could be counted simultaneously with ^{49}Ca. Variations in corrected copper counts were then used to calculate the precision in positioning of the holder. There was no doubt also some minor variation in the positioning of the animals in the holder; yet, as each monkey was immobilized with 25 mg ketamine-HCl (Bristol Labs, Syracuse, NY) and subsequently strapped into the holder, this was kept to a minimum.

Neutron source. The 3 mg ^{252}Cf source at the Baylor College of Medicine was supplied by the Energy Research and Development Administration (ERDA). The source was small and had a relatively high

activity (approximately 4.4 x 10^9 neutrons per second per Curie). It produced a sufficient quantity of neutrons for low-density studies at short distances, and, according to Morgan et al. (87), ^{252}Cf is the preferred source for regional activation analysis in man. The source was shielded by a 7.5 cm lead barrier in addition to 60 cm of 1.0% water extended polyester (WEP) to minimize streaming. The irradiator, also constructed of WEP blocks, was built with an exit port of 20 cm x 30 cm with a source to port distance of 41 cm. For the vertebral activation, a plexiglass collimator with a 20 cm x 10 cm exit port was employed (Figure 4). Monkeys were irradiated for 10 minutes for each region for a total of 20 minutes. The spinal region was activated and counted the first day, the leg region the following day, in order to make region to region interference negligible while counting. The same protocol was repeated for each test period.

Counting facility. Following the irradiation of an animal, it was removed immediately to the counting facility. Figure 5 is a block diagram showing the basic parts of the system and their commercial sources. This facility consisted of two opposing single crystal, sodium iodide detectors (Figure 6), each with three photomultipliers which were connected to a pre-amplifier, stabilizing amplifier, mixer-router, pulse-height analyzer and teletype. Monkeys remained in the above-mentioned holder for a 1000 second count in the supine position by placement of the holder between the detectors (dorsal and ventral to the monkey). The two photons in the range of 1.10 to 3.5 MeV were analyzed during this time by the multi-channel analyzer. After subtraction of background and correction for the contributions in this

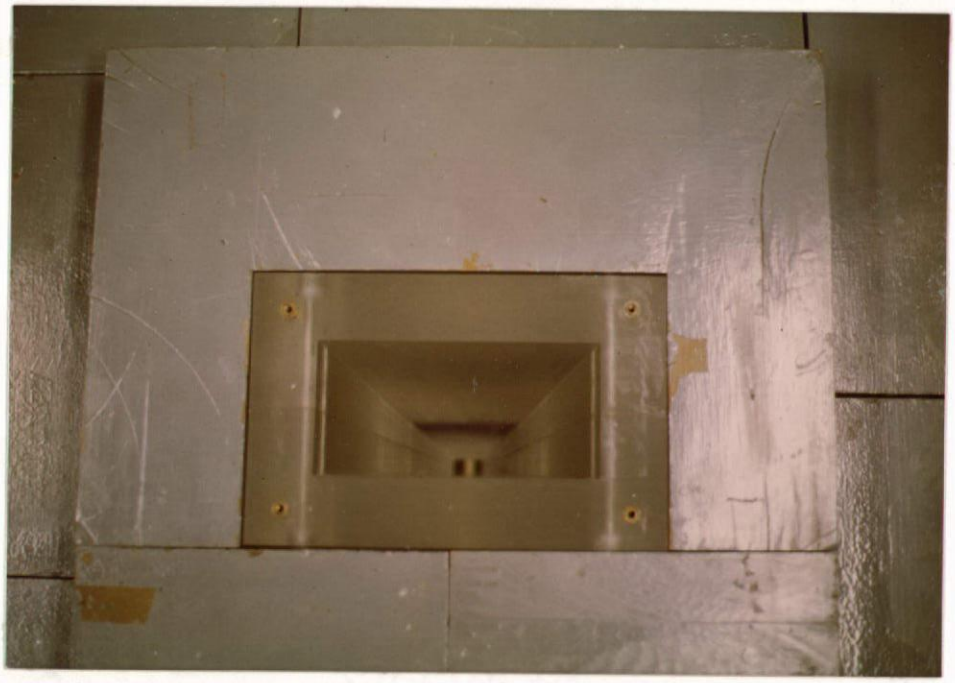
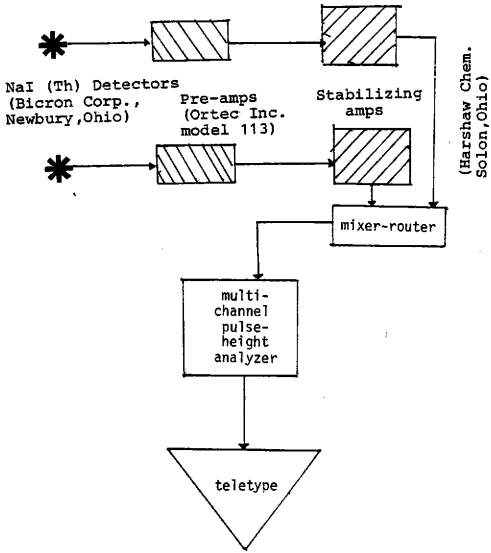


FIGURE 4 Exit port (20 cm x 10 cm) with plexiglass collimator.



The last three items in the block diagram were purchased together as one unit from Tracor Northern Co.

FIGURE 5 Block diagram of counting facility.

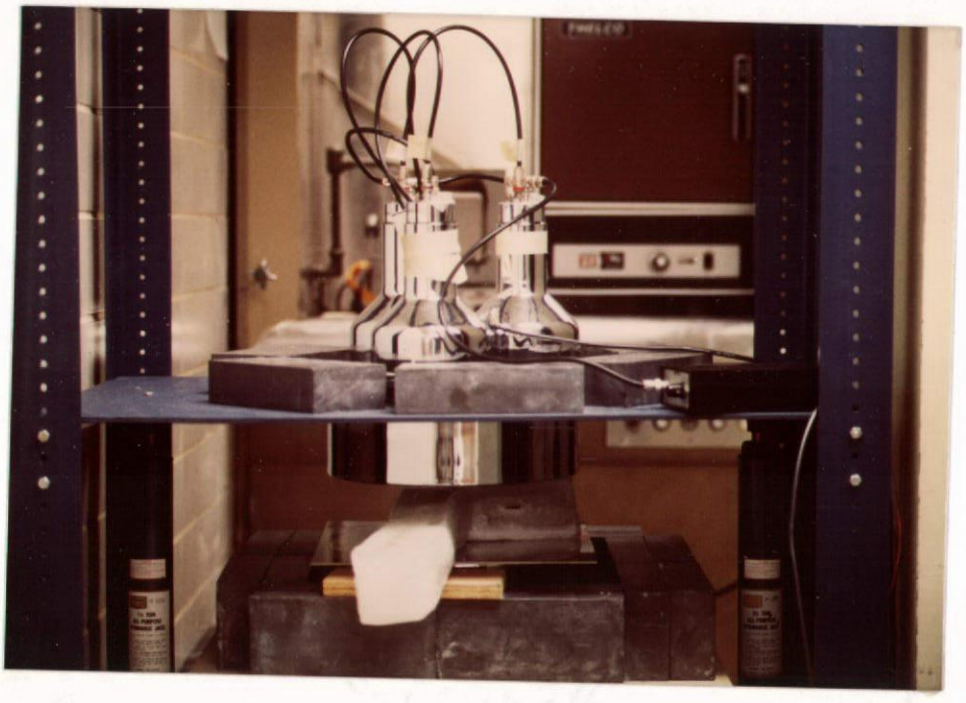


FIGURE 6 Photograph of two opposing sodium iodide detectors.

area by ^{38}Cl and ^{24}Na (Figures 7 and 8), the number of ^{49}Ca counts was computed. The relative contributions to the total counts in the area of 3.10 MeV (^{49}Ca peak) had been previously determined with the use of phantoms (paraffin moulds in the geometry of monkey bones containing known amounts of the elements: sodium, chlorine and calcium). Thus, the "corrected calcium counts" shown in the results are directly proportional to grams of calcium. The purpose of this study, however, was not to determine absolute calcium values, but to test the precision of the method and the relative changes occuring in regions tested. For a following study, the authors wish to be able to express results in grams (Ca) after applying a cross-correction on the basis of the calcium ash content of the measured bones of the dead monkey (16A in Table 2, p. 18).

Urine Collection

A metabolism pan for the collection of urine was constructed of stainless steel and designed to fit beneath the monkey cages. The pan was designed with a locking urine collection cup beneath the drainage port. A wire screen was secured across the open face of the pan to prevent food and feces from entering the cup. A 24-hour urine collection was made from each animal periodically. Before removal of the cup at the end of this period, the pan surface was rinsed with a small quantity of distilled water. The washings were added to the collected urine and the total calcium per day was measured. Urine to be measured was first filtered with low-grade filter paper and samples were stored at -25°C in labelled plastic vials.

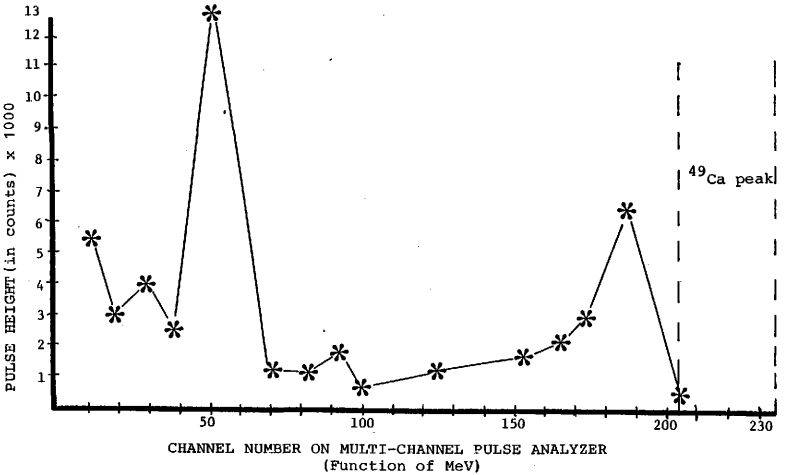


FIGURE 7 Pulse-height of ^{24}Na at various channel settings on PHA.
(Calcium photopeak occurs between channels 205 and 234.)

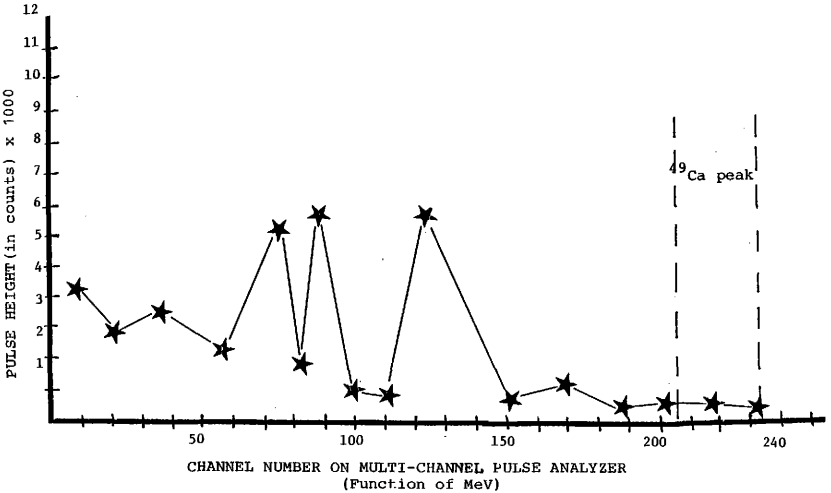


FIGURE 8 Pulse-height of ^{38}Cl at various channel settings on PHA.
(Calcium photopeak occurs between channels 205 and 234.)

Blood Collection

Animals were bled from the femoral vein approximately every two weeks during the experiment. If assistance was available, the animals were bled unanesthetized; otherwise, the monkeys were given an intramuscular injection of ketamine-HCl at 7 mg per kg body weight prior to bleeding. No anticoagulants were used. Serum was removed from the clot and stored at -25°C in labelled plastic vials. Bleedings always took place in the early afternoon in order to minimize the effects of daily biological rhythms.

Biochemical Analyses

Serum sodium, potassium and magnesium were determined on the Varian Techtron Model 1000 Spectrophotometer (Varian Ltd., Melbourne). Serum and urinary calcium were performed by a similar technique at the NASA laboratories. Blood tests for Ht, Hb, RBC and WBC were performed at the Clinical Pathology Laboratory of the Texas A&M College of Veterinary Medicine using the microhematocrit, hemoglobin spectrophotometer and Coulter Counter (Coulter Electronics, Hialeah, FL) methods. Serum cortisol was measured at the NASA labs according to the method of Foster and Dunn (88).

Radiographic Examination

As a comparison study, all animals were radiographed before and after the experimental period. Using a 300 ma X-ray machine (H.G. Fischer Inc., Chicago, IL), radiographs were taken of the right stifle (mediolateral), as well as the lower vertebrae (lateromedial and

ventrodorsal). Films were taken at 52, 54 and 60 kV, respectively (1500 mAs). Kodak G Film with Finescreen (Kodak Co., Rochester, NY) was employed. An aluminum step-wedge was placed next to the bone for standardization.

Pathological Examination

Necropsies of monkeys which died during the experiment (16A and 52A in Table 2, p. 18) were performed by the Department of Pathology of the Texas A&M College of Veterinary Medicine. Adrenal glands (see Results) of number 52A were fixed in 4% formalin and embedded in paraffin blocks. Microtome sections were stained with hemotoxylin and eosin according to standard techniques (89) and examined by means of a microscope with a photographic attachment.

Statistical Methods

Computation of $\bar{X}$, S.D., t tests and Wilcoxon matched-pairs signed-rank tests were performed on a Hewlett-Packard HP-11C programmable calculator. The t test for two means was according to Ostle (90) and the Wilcoxon test was performed as described by Siegel (91). Methods for the correction of calcium counts have been described earlier by Evans et al. (81). The sign test used for the evaluation of calcium count trends was also according to Siegel (91).

CHAPTER IV

RESULTS

Data reported here is a compilation of 11 bleedings, 11 weighings, 22 neutron activations, and approximately 18 urinalyses per subject. Data was not separated into subgroupings such as male and female for evaluation due to the small number of subjects.

Animal Weight Changes

The scale used to weigh the animals was a standard physicians model accurate to one-eight pound (0.057 kg). Changes of 0.10 kg or greater were assumed to be significant. Table 4 depicts the weight changes of all animals. Three out of four (75%) of the experimental animals lost weight, while none of the controls lost weight. Although there were no measurements made of muscle circumference in this study, muscle atrophy is a common side-effect of corticosteroid therapy (92) and may be responsible for weight losses.

Routine Hematological Tests

The routine hematological tests of hematocrit, hemoglobin, and red and white cell counts were included in the study (Table 5) as a check for extraneous factors such as anemia or leukocytosis associated with illness which might have influenced the outcome of the experiment. The variation expressed as coefficient of variation (C.V. equals the standard deviation in percentage) was the greatest for the leukocytes. One may calculate a t test value (90) by assuming that

TABLE 4

Monkey Weight Chart

Week	Monkeys' weight (kg)							
	33A	16A	3B	9B*	36A*	52A*	12B*	33B
-6	1.8	3.1	1.9	1.8	2.0	2.2	2.3	2.0
-4	1.8	3.2	1.8	1.8	2.0	2.1	2.2	1.9
-2	1.8	3.2	1.8	1.8	2.0	2.2	2.3	1.9
0	1.8	3.4	1.8	1.7	2.0	2.1	2.2	1.9
2	1.8	3.1	1.9	1.7	2.0	2.0	2.2	1.9
4	1.8	3.1	1.8	1.7	2.0	---	2.2	1.9
6	1.8	3.2	1.8	1.7	2.0	2.0	2.3	---
9	1.8	3.2	1.9	1.7	2.0	---	2.4	1.9
11	1.8	3.3	---	1.7	2.0	2.1	2.4	2.0
13	1.8	§	1.8	1.8	1.9	2.0	2.5	---
15	1.8		1.9	1.8	2.0	§	2.5	2.0
Net change	---	+0.2	---	---	-0.2	-0.2	-0.2	---

* Experimental monkeys.

§ Died.

Begin treatment = 0.

TABLE 5

Routine Hematological Indices

Week	Animals (n)	Ht (%)	Hb (g/100 ml)	RBC ($x10^{12}$/L)	WBC ($x10^{9}$/L)
			Controls		
0	4	48.8±2.2	15.4±0.9	5.8±0.4	8.8±2.5
5	4	49.1±3.0	14.2±2.0	5.4±0.6	6.9±2.7
10	4	48.2±2.0	14.8±2.2	5.6±0.4	5.8±3.6
15	3	51.5±2.4	14.0±1.3	5.9±0.3	8.4±3.0
Average	15	49.4±2.2	14.6±1.5	5.7±0.4	7.5±2.6
			Experimentals		
0	4	46.1±3.3	14.5±2.1	5.6±0.3	7.1±2.6
5	4	45.0±3.0	15.1±1.1	5.2±0.5	7.0±2.3
10	4	53.2±1.1	13.9±1.6	5.4±0.3	6.4±3.2
15	3	*51.5±2.4	15.4±1.2	5.9±0.4	8.0±2.3
Average	15	50.1±3.2	14.7±1.4	5.5±0.4	7.1±2.2
Total $\bar{X}$	30	50.0±3.0	14.7±1.5	5.6±0.4	7.3±1.7
C.V.		6%	10%	7%	23%

*$t = (58.2 - 50.0)/(2.1/2) = 7.9$.

The deviation is significant for df = 7.

the total mean approximates the expected mean for normal distribution according to the formula: $t = (\bar{x}_i - \bar{x}_{total})/(s/N^{\frac{1}{2}})$. Using this computation, the value for week 15 of the hematocrit is outside the expected range. A mild polycythemia is reported to accompany dogs during hyperadrenocorticism in a small percentage of animals (93), which could explain the deviation.

Serum Electrolytes

As can be seen from Table 6, serum electrolytes displayed no alarming changes. Calcium, magnesium and potassium paired means (for each week) were compared by means of the t statistic. There was no significant difference between the control and the experimental groups ($p < .01$). Because serum sodium was not measured for the experimental group in the week prior to the beginning of drug treatment, the paired test could not be applied. By applying the measurement for the comparison of two means, however, a t value could be computed. Comparing the total means for experimentals vs controls, the difference was insignificant ($p < .01$) for sodium also.

Pathological Examination

One control animal died in the 13th week after a trip to and from Houston. The animal suffered from hyperthermia due to a malfunction of the air-conditioner in the van. Attempts to cool the animal were in vain. At necropsy, the pathologist found characteristic petechial hemorrhage in the heart and brain as well as acute degeneration of the heart, liver and kidneys. These findings are consistent with

TABLE 6

Serum Electrolytes

Week	Animals (n)	Calcium (mg/100 ml)	Magnesium (mg/100 ml)	Sodium (mmole/l)	Potassium (mmole/l)
			Controls		
-1	7	9.3±0.8	2.5±0.6	106±14	3.1±0.3
1	4	9.1±0.6	3.8±0.7	126± 2	3.3±0.2
3	4	10.8±0.3	3.8±0.7	129± 4	3.0±0.2
5	4	9.5±0.3	2.6±0.3	138± 3	2.8±0.4
9	4	9.9±0.4	3.3±0.5	136±18	3.8±0.5
11	4	9.4±0.3	2.5±0.1	135± 3	3.3±0.2
16	6	9.5±0.3	2.1±0.2	137± 4	4.8±0.9
Average control		9.6±0.5	2.9±0.6	130± 9	3.4±0.6
			Experimentals		
-1	4	9.8±0.6	2.4±0.2	---	3.5±0.4
1	4	9.1±0.2	3.7±0.2	123±14	3.8±0.5
3	4	9.9±1.0	3.1±0.1	131± 4	2.6±0.3
5	4	9.5±0.3	2.7±0.4	142± 6	3.0±0.1
9	4	9.6±0.4	2.7±0.5	138± 2	3.0±0.2
11	3	8.9±0.7	3.0±0.5	138± 4	3.3±0.9
16	5	9.5±0.6	2.3±0.4	134± 5	4.9±1.1
Average exper.		9.5±0.6	2.8±0.4	134± 7	3.4±0.7
t values		1.0	0.7	*0.9	0
df		6	6	11	6

*t value for a comparison of two means. Begin treatment on week 1.

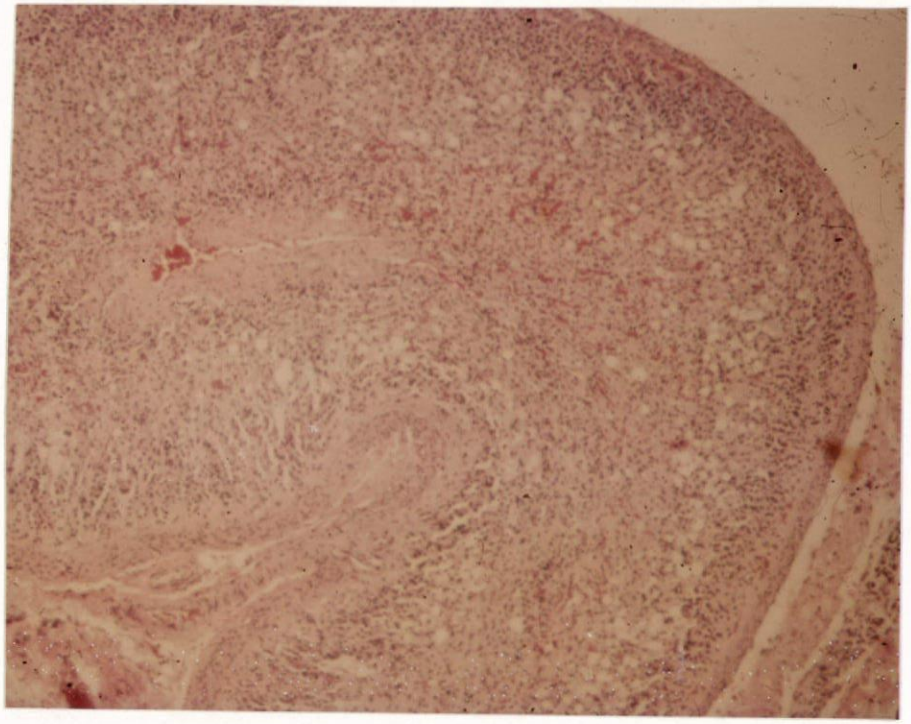
cardiovascular crisis after hyperthermia. An experimental monkey suffered an acute onset of illness just prior to death in the 15th week of the experiment. The only abnormality described at necropsy was a severe atrophy of the adrenal glands, characterized by a diminished size and fatty infiltration (Figure 9). It is probable that this animal died from an Addisonian crisis (92) of adrenal insufficiency in spite of the daily cortisol injections given. Focal skin pigmentation, a frequent symptom of hyperadrenocorticism (93), was also noted.

Radiograph Examination

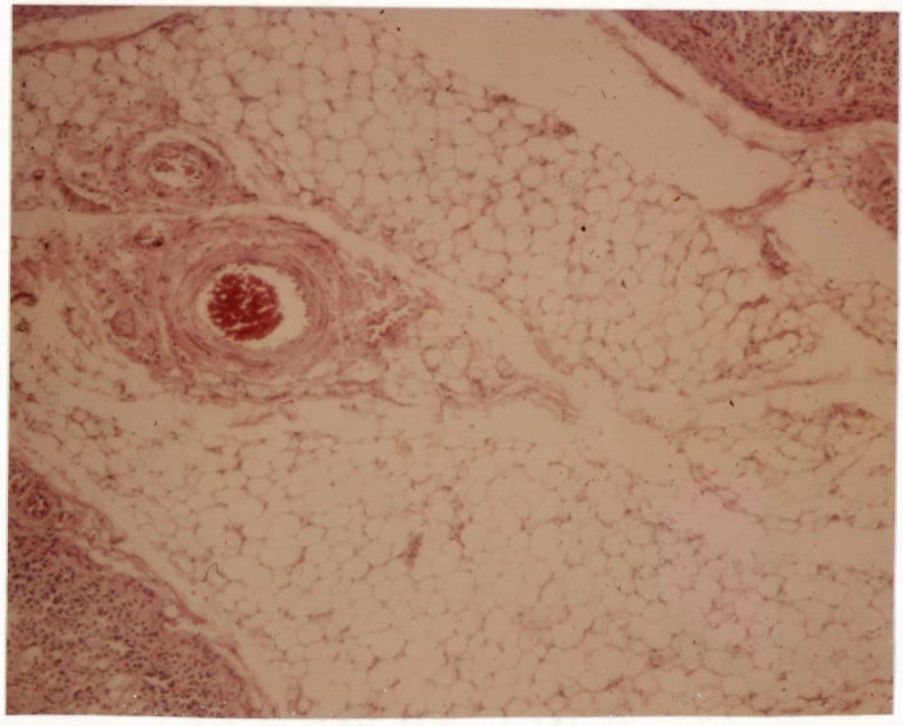
No differences in bone density could be determined by examination of radiographs taken just prior to and at the end of the experimental period. Figures 1 (p. 11) and 10 are reproductions of, respectively, BEFORE and AFTER radiographs made for an experimental monkey. Contrast duplication of the aluminum step-wedge was difficult, a fact which further substantiates the problems involved when one attempts to visually evaluate small bone density differences by means of radiographs with a step-wedge.

Bone Calcium Measurement with PBNAA

The bone calcium values for both the leg and spine regions are reported as corrected counts and as a percentage change for each animal's total mean (Tables 7-16). These changes were assumed to be due to random errors in positioning and counting geometry. This assumption was partially confirmed bythe fact that calcium counts



(40x)



(100x)

FIGURE 9 Adrenal gland in paraffin section (H.E.) showing fatty infiltration and local atrophy at 40x and 100x in microphotographs.

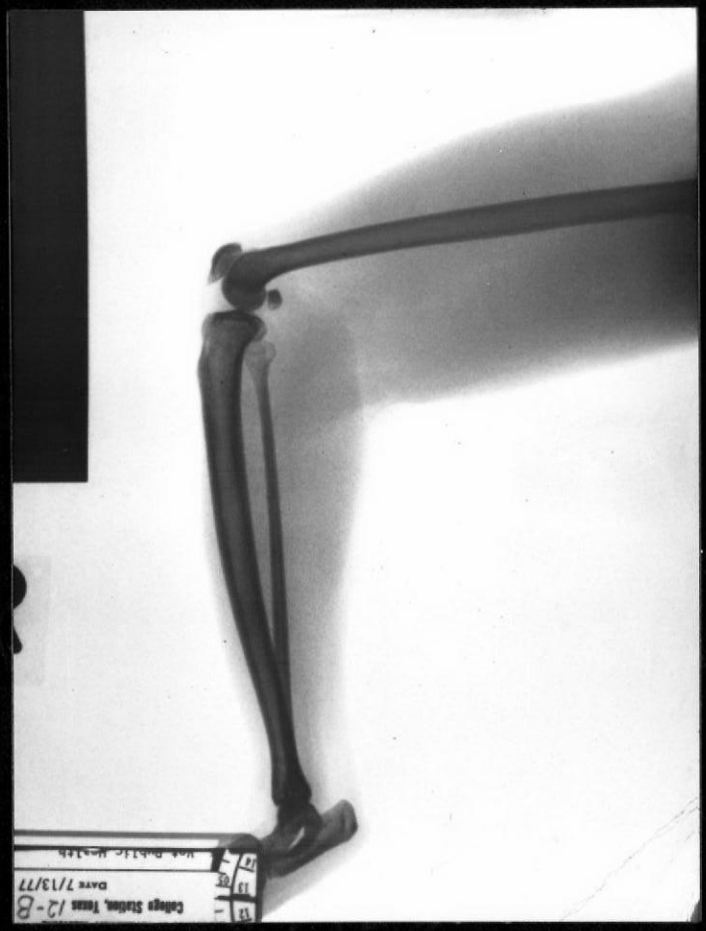


FIGURE 10 Reproduction of a radiograph of experimental monkey (12B) taken after the experimental period. The aluminum step-wedge (upper left-hand corner) and the right femur and tibia are shown.

TABLE 7

Experimentals - Spine Position

Normalized Calcium

	Monkey and percent change			
Week	9B (%)	12B (%)	36A (%)	52A (%)
-6	15976 + 4.3	18530 - 1.6	14959 + 0.9	15190 - 3.3
-4	14873 - 2.8	19197 + 1.9	14590 - 1.6	15124 - 0.8
-2	14780 - 3.5	18763 - 0.4	14828 - 1.6	15586 + 0.8
*0	15653 + 2.3	18480 - 1.9	15186 + 2.4	15799 + 3.7
2	15107 - 1.2	18591 - 1.3	14589 - 1.6	15021 - 1.4
4	15541 + 1.6	18040 - 4.4	14660 - 1.1	14098 - 7.5
6	14924 - 2.5	18535 - 1.6	14876 + 0.3	15579 + 2.2
9	14692 - 4.1	18960 + 0.7	15935 + 7.4	14841 - 2.6
11	15188 - 0.7	18982 + 0.8	14444 - 2.6	15927 + 4.5
13	15778 + 3.1	19581 + 3.8	14998 + 1.1	
15	15730 + 2.8	19483 + 3.4	14053 - 5.3	
	C.V. = 3.0%	C.V. = 2.5%	C.V. = 3.2%	C.V. = 3.7%

*Begins experimental period.

TABLE 8

Controls - Spine Position

Normalized Calcium

	Monkey and percent change							
Week	3B	(%)	33A	(%)	16A	(%)	33B	(%)
-6	14847 -	1.9	13788 -	5.8	21410 +	2.1	14171 -	5.1
-4	14987 -	1.0	14725 +	0.5	20797 -	0.8	15126 +	1.3
-2	15194 +	0.4	15039 +	2.7	20993 +	0.1	15086 +	1.0
*0	15033 -	0.6	15063 +	2.8	21461 +	2.4	14791 -	0.9
2	14674 -	3.0	14518 -	0.9	21638 +	3.2	14849 -	0.5
4	15625 +	3.3	15043 +	2.7	20785 -	0.8	14063 -	5.8
6	14738 -	2.6	14824 +	1.2	20299 -	3.2	15364 +	2.9
9	15145 +	0.1	14815 +	1.2	21214 +	1.2	15037 +	0.7
11	15419 +	1.9	14112 -	3.6	20061 -	4.3	13888 +	7.0
13	15658 +	3.5	14476 -	1.1			15855 +	6.2
15			14695 +	0.3			15991 +	7.1
	C.V. = 2.2%		C.V. = 2.8%		C.V. = 2.5%		C.V. = 3.7%	

*Begins experimental period.

TABLE 9

Monkey 52A - Leg Position

No. of weeks	% Change	Normalized Ca^{++}	Normalized Cu^{++}
-6	+ 0.9	31069	---
-4	+ 2.0	31417	459299
-2	- 1.6	30292	453164
*0	+ 1.7	31337	443026
2	+ 5.0	32342	457161
4	+ 0.5	30969	450529
6	- 2.9	29892	435430
9	- 1.9	30201	439169
11	- 0.2	30726	---
13	- 3.4	29769	443946
		C.V. = 2.5%	C.V. = 3.7%

Correlation (r = 0.69).

*Begins experimental period.

TABLE 10

Monkey 36A - Leg Position

No. of weeks	% Change	Normalized Ca^{++}	Normalized Cu^{++}
-6	- 4.2	28438	450859
-4	+ 1.9	30262	448830
-2	+ 2.0	30288	461941
*0	- 0.1	29673	461565
2	+ 1.7	30198	452260
4	- 1.0	29398	454033
6	+ 5.9	31460	468671
9	+ 0.1	30091	470711
11	- 5.1	28187	444563
13	+ 0.7	29898	438735
15	- 3.2	28757	446043
		C.V. = 4.0%	C.V. = 2.2%

Correlation (r = 0.42).

*Begins experimental period.

TABLE 11

Monkey 12B - Leg Position

No. of weeks	% Change	Normalized Ca^{++}	Normalized Cu^{++}
-6	- 0.8	40343	474201
-4	- 2.4	39695	474970
-2	+ 1.2	41147	497989
*0	+ 0.4	40828	474314
2	+ 1.7	41364	492089
4	- 1.4	40099	480899
6	- 3.3	39330	464969
9	+ 0.4	40851	473846
11	- 1.6	40023	462746
13	+ 1.5	41283	477924
15	+ 4.3	42434	477924
		C.V. = 2.1%	C.V. = 2.2%

Correlation (r = 0.52).

*Begins experimental period.

TABLE 12

Monkey 9B - Leg Position

No. of weeks	% Change	Normalized Ca^{++}	Normalized Cu^{++}
-6	+ 9.0	32267	467231
-4	+ 1.8	30156	438760
-2	+ 8.9	32244	443051
*0	- 0.2	29560	460440
2	+ 1.7	30110	465281
4	- 3.4	28598	437104
6	- 3.0	28723	453583
9	- 1.2	29252	453026
11	- 5.1	28085	447879
13	- 2.0	29015	434296
15	- 6.5	27681	447172
		C.V. = 5.1%	C.V. = 2.5%

Correlation (r = 0.33).

*Begins experimental period.

TABLE 13

Monkey 3B - Leg Position

No. of weeks	% Change	Normalized Ca^{++}	Normalized Cu^{++}
-6	+ 5.2	30708	482723
-4	+ 2.5	29897	456754
-2	- 0.2	29124	440978
*0	- 0.3	29081	444408
2	+ 1.0	29495	450431
4	+ 2.6	29955	446922
6	- 1.8	28666	439224
9	- 5.8	27486	437265
11	- 2.4	28481	432854
13	- 3.9	28029	435687
15	+ 3.0	30062	447939
		C.V. = 3.3%	C.V. = 3.2%

Correlation (r = 0.81).

*Begins experimental period.

TABLE 14

Monkey 33B - Leg Position

No. of weeks	% Change	Normalized Ca^{++}	Normalized Cu^{++}
-6	- 6.3	28551	428141
-4	- 1.3	30066	447086
-2	+ 3.5	31544	---
*0	- 0.0	30469	467293
2	- 3.1	29538	441865
4	+ 0.4	30617	495086
6	- 1.4	30053	450788
9	+ 0.9	30754	467252
11	+ 1.8	31021	449534
13	+ 2.6	31261	452966
15	+ 2.9	31364	446043
		C.V. = 2.8%	C.V. = 4.0%

Correlation (r = 0.47).

*Begins experimental period.

TABLE 15

Monkey 16A - Leg Position

No. of weeks	% Change	Normalized Ca^{++}	Normalized Cu^{++}
-6	+ 0.4	45127	478540
-4	+ 0.2	45066	476511
-2	+ 1.7	45728	---
*0	+ 3.5	46540	483238
2	+ 1.7	45728	485413
4	- 2.6	43794	454033
6	+ 0.4	45132	480093
9	- 8.6	41107	481526
11	+ 3.2	46404	490089
		C.V. = 3.9%	C.V. = 2.3%

Correlation ($r = 0.35$).

*Control animal received no treatment in experiment period.

TABLE 16

Monkey 33A - Leg Position

No. of weeks	% Change	Normalized Ca^{++}	Normalized Cu^{++}
-6	- 4.2	28438	433712
-4	+ 1.9	30262	435143
-2	+ 2.0	30288	---
*0	- 0.1	29673	447609
2	+ 1.7	30198	444195
4	- 1.0	29398	438277
6	+ 5.9	31460	450029
9	+ 0.1	30091	456025
11	- 5.1	28187	445518
13	+ 0.7	29898	437874
15	- 3.2	28757	443102
		C.V. = 3.3%	C.V. = 1.6%

Correlation (r = 0.49).

*Control animal received no treatment in experiment period.

from legs (x) and copper counts from the strip placed in the holder (y) (see METHODS) had a positive correlation for all eight animals when tested by linear regression. The correlation coefficients ranged from (r =) 0.33 to 0.81. For calcium counts, the mean standard deviation when expressed in percentage (coefficient of variation = C.V.) was 3.2% for controls versus 3.3% for experimentals. The calcium count C.V. for the leg region was higher (3.4%) than that for the spinal region (3.1%) for all measurements as a whole. The total C.V. for both regions was 3.25%.

To test for significant changes in either of the groups during the corticosteroid therapy, the sign test was done on the last five measurements for both regions. If bone losses were to occur in this period, one can expect a distribution of +/- changes (from $\bar{X}$) significant ($p < .01$) from a ($p = 0.5$) distribution. This was not the case for either group. Only experimental monkey 9B showed an unexpected negative trend from $\bar{X}$ during the last measurements ($p = .016$ for $n = 6$), however, this trend was not to be repeated for the same animal in the spinal region. Bone losses occurring in only one animal in one area seems unlikely. At any rate, the leg region of 9B showed the poorest correlation ($r = 0.33$) between calcium and copper values which argues for the fact that positioning was not the major factor causing the large C.V. (= 5.1%).

Serum Cortisol Changes

In Table 17 and Figure 11, the means and standard deviations for the serum cortisol values are depicted. Although there was a large

TABLE 17

Monkey Serum Cortisol

No. of weeks	Controls $\bar{X}$ S.D.	Experimentals $\bar{X}$ S.D.	Value 30 min p.i.
-2	121 ± 58	153 ± 87	*800
0	97 ± 70	60 ± 17	
2	115 ± 46	157 ± 47	
3	68 ± 22	148 ± 76	
5	76 ± 28	130 ± 33	
8	229 ± 227	360 ± 142	
11	88 ± 37	81 ± 25	
15	114 ± 47	140 ± 73	

All values are expressed in µg/100 ml.

t test: Average difference = 154; S.D. difference = 91; $t = 4.8$; $df = 7$.

*This was a serum cortisol value from a blood sample taken ±30 min after a subcutaneous injection of cortisol at a dose of 26 µg/kg b.w. This value was not used in the calculation of means.

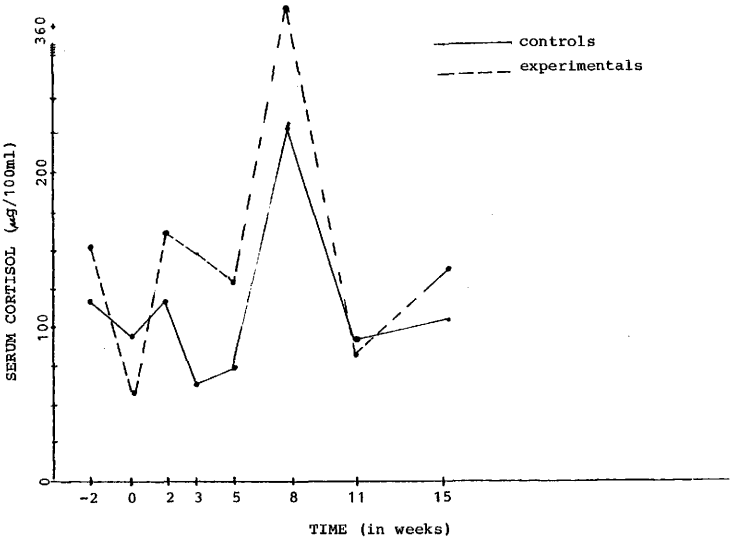


FIGURE 11 Serum cortisol values as means vs time. (15h p.i.)

intra-measurement variation, it was evident that the daily cortisone/cortisol injections had a positive effect ($p < .05$) on the experimental group when either the paired t test or the Wilcoxon matched-pairs signed-rank test were employed. Urinary cortisol values were measured, but are not included in the results due to the fact that the S.D. often exceeded the mean for groups measured. The author assumes that interfering components in the urine were too variable in concentration (dependent not only upon the animal's concentrating ability, but also upon the amounts of distilled water used to rinse the metabolism pans--see METHODS) to use the very sensitive radioimmunoassay for serum as a method for measuring urine cortisol.

Urinary Calcium Excretion

Because the spectrophotometric measurement of calcium is less sensitive to interferences than the RIA (radioimmunoassay), variations in total calcium in the urine/24 hours were acceptable. As depicted in Table 18, urinary calcium in the experimentals was slightly elevated.

TABLE 18

Monkey Urinary Calcium

No. of weeks	Controls n	$\bar{X}$ S.D.	Experimentals n	$\bar{X}$ S.D.
-2	8	.084 ± .06	7	.11 ± .05
0	13	.14 ± .07	14	.13 ± .07
1	9	.09 ± .05	8	.13 ± .06
3	11	.12 ± .08	11	.21 ± .29
5	8	.11 ± .05	10	.10 ± .03
7	10	.10 ± .04	8	.34 ± .70
12	10	.35 ± .18	9	.39 ± .27
15	6	.26 ± .22	12	.27 ± .12

All values are expressed in mg/24 hours.

Average of differences = .05; S.D. of difference = .08; $t = 1.8$; $df = 7$.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Osteoporosis, as a side-effect of long-term glucocorticosteroid therapy in humans, often takes years to develop (53). On the other hand, in experimental studies in rabbits, bone loss occurred following several weeks of glucocorticosteroid therapy (47). Moreover, in mature rats, there has been no negative effect on bone as a result of similar therapies (49). Where the monkey should be placed within this range of possibilities is as yet unsolved. In the only other study reported in which bone parameters were examined in monkeys during a corticosteroid insult, the effects were insignificent (51). These authors administered deflazacort, an oxazoline derivative of prednisolone, to both juvenile and mature cynomolgus monkeys during a one year period. In this previous study, no dose-dependent changes were found for the following parameters: plasma calcium, phosphate, alkaline phosphatase, 25-hydroxy-vitamin D and urinary calcium excretion. Neither did bone volume, bone circumference nor bone cell counts have changes. These reports coincide with the results of the present study in Cebus monkeys. However, before drawing conclusions concerning the susceptibility of monkeys to the effects of glucocorticosteroids on bone, the following should be considered.

Firstly, deflazacort has been shown to have remarkably few effects on bone when compared to other corticosteroids. LoCascio et al. (94) compared patient groups who received either prednisolone or deflazacort. Iliac crest biopses were examined before and after a

six-month treatment period for both groups. Bone biopsies had a significant reduction in the total bone volume (TBV) in the prednisolone group (n = 13), whereas, the changes for the deflazacort-treated group were insignificant (n = 14). Lund et al. (58) in Denmark also showed deflazacort to cause less calcium release than prednisolone *in vitro*.

In the present study, the length of the treatment period may have been a decisive factor in the absence of calcium changes. Elevated serum cortisol, adrenal glands showing atrophy at post-mortem examination, weight losses and focal hyperpigmentation of the skin suggest that the dosage of glucocorticosteroids, as given, had a measurable effect on the endocrinological homeostasis, but evidently not on bone metabolism. This could be solely due to the length of the treatment period if one compares the results to those of Nagant de Deuxchaisnes and Devogelaar (95). If the young adult Cebus in this study had lost bone calcium at the same rate as the young adult men in the experiment by Nagant de Deuxchaisnes (1.71 - 2.26%/year in the radius), the mineral loss would not have been detected in a 16-week experiment (2.26 x (16/52) = 0.69%) which lay under the detection range in this experiment with eight animals. An ideal but expensive study would be to examine regional calcium changes in larger groups of Cebus monkeys for a longer period, while comparing several different glucocorticosteroid compounds with each other.

A third factor which may have played a role in the results of the above-mentioned experiments with monkeys is the CBG (cortisol binding globulin) interaction with the drug used. Assandri et al. (96) have

shown that deflazacort inhibits or prevents CBG plasma binding to itself in man, rats, dogs and cynomolgus monkeys, which tends to reduce its biological effect. The present author suggests that there is a positive correlation between the CBG binding to a particular glucocorticosteroid and the effect of this corticosteroid on bone. This would also explain differences seen in the effects of cortisone on man and Cebus monkeys. According to Seal and Doe (97), the latter is only able to bind a small percentage of cortisol with CBG (3.3 µg/100 ml as compared to 22 µg/100 ml in man). If this hypothesis is true, one would expect to find the same response in squirrel (Saimiri scireus) and howler (Alouatta palliata) monkeys as was observed in the Cebus due to the relatively low CBG binding of cortisol in these two species (5.0 µg/100 ml and 1.4 µg/100 ml, respectively). Furthermore, an interesting fact is that estrogen also binds to CBG (98). After menopause, it is logical that there would be more CBG available to bind to cortisol, a fact which may yet help to explain why post-menopausal women lose bone at much higher rates than men (2).

Lastly, concerning the in vivo regional activation analysis of the Cebus monkey, the results were similar to those in the literature cited by Mazess et al. (83). The Cebus monkey proved itself a suitable animal in terms of size, manageability and feasibility for regional calcium measurements.

REFERENCES

1. McBean, L.D., and E.W. Speckmann. 1974. A recognition of the interrelationships of calcium with various dietary components. Am. J. Clin. Nutr. 27:603.

2. Riggs, B.L., H.W. Wahner, W.G. Dunn, R.B. Mazess, and K.P. Offord. 1981. Differential changes in bone mineral density of the appendicular and axial skeleton with aging. J. Clin. Invest. 67:328.

3. Cohn, S.H., A. Vaswani, I. Zanzi, and K.J. Ellis. 1976. Effect of aging on bone mass in adult women. Am. J. Physiol. 230:143.

4. Semb, H. 1966. Experimental disuse osteoporosis. Acta Soc. Med. Ups. 71:98.

5. Fleisch, H., R.G.G. Russell, B. Simpson, and R.C. Muhlbauer. 1969. Prevention by diphosphonate of immobilization osteoporosis in rats. Nature 223:211.

6. Griffith, A.P. 1971. Immobilization hypercalciuria: treatment and a possible pathophysiologic mechanism. Aerospace Med. 42: 1322.

7. Rambaut, P.D., M. Smith, P. Mack, and F.S. Vogel. 1975. Biomedical results of Apollo. NASA Report, Wash., DC.

8. Douglass, W.R. 1970. The calcium metabolism problem in space medical science. Space Life Sci. 2:151.

9. Griffith, H.J., R. Hunt, R. Zimmera, H. Finberg, and J. Cutting. 1975. Role of calcium and fluoride in osteoporosis in rhesus monkeys. Invest. Radiol. 10:263.

10. Anderson, M.P. 1977. Long-term effect of low dietary calcium to phosphorus ration on the skeleton of the Cebus albifrons. J. Nutr. 107:834.

11. Murray, R.O. 1960. Radiological bone changes in Cushing's syndrome and steroid therapy. Brit. J. Radiol. 33:1.

12. Kemple, K.L., and M.N. Mueller. 1975. Corticosteroid therapy in rheumatoid arthritis. Clin. Res. 23:324.

13. Cohn, S.H., K.J. Ellis, A. Martino, and S. Asad. 1976. Loss of calcium from axial and appendicular skeleton in patients with chronic renal failure. Calcif. Tiss. Res. 21:216.

14. Nordin, B.E.C. 1973. Metabolic Bone and Stone Disease. Churchill-Livingstone, London.

15. Riggs, B.L., R.V. Randall, H.W. Wahner, J. Jowsey, P.J. Kelly, and M. Singh. 1972. The nature of metabolic bone disorder in acromegaly. J. Clin. Endocr. Metab. 34:911.

16. Seville, P.D. 1973. Osteoporosis, a review. In Clinical Aspects of Metabolic Bone Disease, B. Frame, A. Parfitt and H. Duncan (eds.). Excerpta Med., Amsterdam.

17. Ibbotson, K.J., S.M. D'Souza, J.A. Kanis, D.L. Douglas, and R.G.G. Russell. 1980. Physiological and pharmacological regulation of bone resorption. Metab. Bone Dis. and Rel. Res. 2:177.

18. Leach, C.S., P.C. Johnson, and P.C. Rambaut. 1976. Metabolic and endocrine studies-2nd manned space lab mission. Aviat. Space Environ. Med. 47:402.

19. Rambaut, P.C., and R.S. Johnston. 1979. Prolonged weightlessness and calcium loss in man. Acta Astron. 6:1113.

20. Hansen, C.A., H. Duncan, and A. Curtiss. 1970. Corticosteroid induced osteoporosis in rabbits. Clin. Res. 18:455.

21. Mitruka, B.M., and H.M. Raunsly. 1976. Animals for Medical Research, Models for the Study of Human Disease. J. Wiley and Sons, New York.

22. Peppler, W.W., and R.B. Mazess. 1981. Total body bone mineral and lean body mass by dual photon absorptiometry. I. Theory and measurement procedure. Calcif. Tiss. Int. 33:353.

23. Jubb, K.V., and P.C. Kennedy. 1970. Pathology of Domestic Animals. Vol. 1, 2nd ed. Academic Press, New York.

24. Stauffer, M., D. Baylink, and J. Wergedal. 1973. Decreased bone formation, mineralization and enhanced resorption in calcium deficient rats. Am. J. Physiol. 225:269.

25. Goulding, A. 1980. Effects of dietary supplement on parathyroid function, bone turnover and bone composition in rats taking restricted amounts of calcium. Min. Electr. Metab. 4:203.

26. Orny, A., I. Wolinsky, and K. Guggenheim. 1974. Structure of long bones of rats and mice fed a low-calcium diet. Calcif. Tiss. Res. 15:71.

27. Dudl, R.J., J.W. Ensink, and J.W. Baylink. 1973. Evaluation of intravenous calcium as therapy for osteoporosis. Am. J. Med. 55:631.

28. Bollet, A.J. 1968. Osteoporosis or the osteoporosities. Am. J. Med. Sci. 256:271.

29. Fleisch, H., R.G.G. Russell, B. Simpson, and R.C. Muhlbauer. 1969. Prevention by diphosphonate of immobilization osteoporosis in rats. Nature 223:211.

30. Wranski, T.J., and E.R. Morey. 1982. Skeletal abnormalities in rats induced by simulated weightlessness. Metab. Bone Dis. and Rel. Res. 4:69.

31. Semb, H. 1966. Experimental disuse osteoporosis. Acta Soc. Med. Ups. 71:98.

32. Geiser, M., and J. Trueta. 1958. Muscle action, bone rarefaction and bone formation. J. Bone J. Surg. 40B:282.

33. Allison, N., and B. Brooks. 1921. Bone atrophy. An experimental and clinical study of changes in bone which result from non-use. Surg. Gyn. Obstet. 33:250.

34. Burkhar, J.M., and J. Jowsey. 1967. Parathyroid and thyroid hormones in the development of immobilization osteoporosis. Endocrinology 81:1053.

35. Semb, H. 1966. The chemical composition of microscopic cortical bone structures from immobilized dogs. Acta Soc. Med. Ups. 71:197.

36. Landry, M., and H. Fleisch. 1964. The influence of immobilization on bone formation as evaluated by osseous incorporation of tetracyclines. J. Bone J. Surg. 46B:764.

37. Mack, P.B., R.A. Hoffman, and A. Al-Shawi. 1968. Physiologic and metabolic changes in Macaca nemestrina on two types of diets during restraint and non-restraint: II. Bone density changes. Aerospace Med. 39:704.

38. Mack, P.B. 1971. Bone density changes in a Macaca nemestrina monkey during the Biosatellite III project. Aerospace Med. 42:828.

39. Urist, M.R., and J. Adams. 1969. Bone dynamics. J. Bone J. Surg. 544:1120.

40. Mack, P.B., and F.B. Vogt. 1970. Skeletal density measurements during Apollo VII and VIII missions. Report of Texas Women's University to NASA Manned Spaceflight Center Contr. 9-8888.

41. Douglas, W.R. 1970. The calcium metabolism problem in space medical science. Space Life Sci. 2:151.

42. Portugalov, V.V., E. Savina, and A.S. Koplansky. 1976. Effect of spaceflight factor on the mammal. Aviat. Space Environ. Med. 47:813.

43. Urist, M.R. 1958. The problem of osteoporosis. Clin. Res. 6:377.

44. Jaffe, N.L., and M. Epstein. 1972. The effect of cortisone on femoral and humoral heads in rabbits. Clin. Orthop. 82:221.

45. Thompson, J.S., R. Palmieri, R. Crawford, and A. Elial. 1972. The effect of porcine calcitonin on osteoporosis induced by adrenal cortical steroids. J. Bone J. Surg. 54A:1120.

46. Freehafer, A.A. 1969. Effect of hydrocortisone and gonadal hormones on growing rabbit bone. J. Surg. Res. 9:35.

47. Adams, P., and J. Jowsey. 1967. Effect of calcium on cortisone induced osteoporosis in rabbits. Endocrinology 81:152.

48. Hansen, L.A., H. Duncan, and A. Curtiss. 1970. Corticosteroid induced osteoporosis in rabbits. Clin. Res. 18:455.

49. Yasamura, S., K.J. Ellis, and S.H. Cohn. 1976. Effect of hydrocortisone on total body calcium in rats. J. Lab. Clin. Med. 68:834.

50. Follis, R.H. 1951. The effect of cortisone on the growing bones of rats. Proc. Soc. Exp. Biol. Med. 76:722.

51. Beneton, M., L. Harvey, D. Guilland-Cumming, B. Imbimbo, R.G.G. Russell, and J.A. Kanis. 1982. Effects of deflazacort on skeletal metabolism in cynomolgus monkeys. Calcif. Tiss. Int. Suppl. 34:63.

52. Cushing, H. 1932. The basophil adenomas of the pituitary body and their clinical manifestations. Bull. Johns Hopkins Hosp. 50:137.

53. Pietrogrande, V., and P. Mastromarino. 1957. Osteopatia da prolongato trattamento cortisonico. Ortop. Traum. 25:791.

54. Murray, R.D. 1960. Radiological bone changes in Cushing's syndrome and steroid therapy. Brit. J. Radiol. 33:1.

55. Meunier, P.J., P. Courpron, C. Edouard, and J. Bernard. 1973. Physiological senile evolution and pathological rarefaction of bone, quantitative and comparative histological data. Clin. Endocr. Metab. 2:239.

56. Bressot, C., P.J. Meunier, M.C. Chapuy, E. Lejeune, C. Edouard, and A.J. Darby. 1979. Histomorphometric profile, pathophysiology and reversibility of corticosteroid-induced osteoporosis. Metab. Bone Dis. Rel. Res. 1:303.

57. Bernini, P. Nardi, and L. Fusi. 1982. Plasma levels of calcitonin in glucocorticoid treated patients. Calcif. Tiss. Int. Suppl. 34:69.

58. Lund, B., J.G. Pederson, and C. Egsmad. 1982. In Proceedings of the 1st International Colloquium on Glucocorticoid Effects. Univer. Sienna, Italy.

59. Russell, J.E., and L.V. Avioli. 1982. Effect of prednisone, deflazacort and the 1-6485 component on glycolytic activity in a growing animal. Calcif. Tiss. Int. Suppl. 34:22.

60. Chen, T.L., and S. Feldman. 1979. Glucocorticoid receptors and actions in subpopulations of cultured rat bone cells. Mechanism of dexamethasone potentiation of parathyroid hormone-stimulated cyclic AMP. J. Clin. Invest. 63:750.

61. Teitelbaum, S.L. 1982. Effects of glucocorticoids on osteolysis. Calcif. Tiss. Int. Suppl. 34:10.

62. Raisz, L.G., and Y.S. Chyun. 1982. Inhibition of bone growth in vitro by cortisol and its reversal by prostaglandin E_2. Calcif. Tiss. Int. Suppl. 34:62.

63. Kovarik, J., and W. Kuster. 1981. Clinical relevance of radiological examination and bone density measurement in osteoporosis of old age. Skelet. Radiol. 7:37.

64. Jowsey, J., and B.L. Riggs. 1972. Assessment of bone turnover by microradiography and autoradiography. Semin. Nucl. Med. 2:3.

65. Cameron, J.R., and S. Sorenson. 1963. Measurement of bone mineral in vivo: an improved method. Science 142:230.

66. Wilson, C.R. 1972. Abstract of doctoral thesis, University of Wisconsin. Madison, Wisconsin.

67. Chalmers, J., and J.K. Weaver. 1966. Cancellous bone and its strength and changes with ageing and an evaluation of some methods for measuring its mineral content. J. Bone J. Surg. 48A:299.

68. Mazess, R.B., M. Ort, P. Judy, and W. Mather. 1970. Absorptiometric bone mineral determination using ^{153}Gd. In Proceedings of Bone Measurement Conference, J.P. Cameron (ed.). U.S. AEC 700515:308, Wash., D.C.

69. Wilson, C.R., and M. Madsen. 1977. Dichromatic absorptiometry of vertebral bone content. Invest. Radiol. 12:180.

70. Mazess, R.B. 1971. Estimation of bone and skeletal weight by direct photon absorptiometry. Invest. Radiol. 6:52.

71. Questions and answers about bone mineral analysis. 1977. Bulletin. Norland Instruments. Ft. Atkinson, Wisconsin.

72. Mazess, R.B., W.W. Peppler, C.H. Chestnut, W.B. Nelp, S.H. Cohn, and I. Zanzi. 1981. Total body bone mineral and lean body mass by DPA. Comparison with total body calcium by neutron activation. Calcif. Tiss. Int. 33:361-364.

73. Cohn, S.H., and C.S. Dombrowski. 1971. Measurement of total body Ca, Na, Cl, N, and P in man by in vivo neutron activation analysis. J. Nucl. Med. 12:499.

74. Palmer, H.E., W.E. Nelp, R. Murano, and C. Rich. 1968. The feasibility of in vivo neutron activation analysis of total body calcium and other elements of body composition. Phys. Med. Biol. 13:269.

75. Williams, E.D., K. Boddy, and J.J. Brown. 1982. Whole body elemental composition in patients with hypertension. Eur. J. Clin. Invest. 12:321.

76. Chamberlain, M.J., J.H. Fremlin, and D.K Peter. 1968. Total body Ca by whole body neutron activation. Brit. J. Med. 2:581.

77. Kennedy, N.S., and R. Estell. 1982. Total body neutron activation analysis of calcium: calibration and normalisation. Phys. Med. Biol. 27:697.

78. Harrison, J.E. 1979. Bone mineral measurements of the central skeleton by neutron activation analysis for routine investigation of osteoporia. Radiology 14:27.

79. Maziere, B., D. Kuntz, and D. Camor. 1979. In vivo analysis of bone Ca by local activation of the hand. J. Nucl. Med. 20:85.

80. Kuntz, D., Maziere, B., and Knipper, M. 1981. Analyse in vivo du calcium osseux par activation neutronique localisee de la main chez l'osteoporotique. Revue Du Rhum 48:487.

81. Evans, H.J., A.D. LeBlanc, and P.C. Johnson. 1976. Feasibility study. In vivo neutron activation for regional measurement of calcium using ^{252}Cf. Med. Phys. 3:148.

82. Evans, H.J. and A. LeBlanc. Unpublished data.

83. Mazess, R.B., W.W. Peppler, C.H. Chestnut, W.B. Nelp, S.H. Cohn, and I. Zanzi. 1981. Total body bone mineral and lean body mass by DPA. Comparison with total body calcium by neutron activation. Calcif. Tiss. Int. 33:365.

84. NCRP on dose limit. 1980. Radiology 135:746.

85. Mazess, R.B., W.W. Peppler, J.E. Harrison, and K.G. McNiell. 1981. Total body bone mineral and lean body mass by DPAIII. Comparison with trunk Ca by neutron activation. Calcif. Tiss. Int, 33:365.

86. Corey and Hayes. Diet for the adult cebus monkey. 1973. Report of the Nutrition Dept., Harvard University, Boston, MA.

87. Morgan, W., K.J. Ellis, and S.H. Cohn. 1981. A comparison of Cf, Pu and Be neutron sources for partial body in vivo neutron activation analysis. Phys. Med. Biol. 26:413.

88. Foster, L.B., and R.T. Dunn. 1971. Single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. Clin. Chem. 20:365.

89. Humason, G.L. 1962. Animal Tissue Techniques. Freeman and Co., San Francisco.

90. Ostle, B. 1963. Statistics in Research. Iowa State University Press, Ames, Iowa.

91. Siegel, S. 1956. Non-Parametric Statistics. McGraw-Hill, Kogakusha, Japan.

92. Gilman, G.G., L.S. Goodman, and A. Gilman (eds.). 1980. The pharmacological basis of therapeutics, 6th ed. MacMillan, New York.

93. Ettinger, S.E. 1983. Textbook of Veterinary Internal Medicine. W.B. Saunders, Philadelphia.

94. LoCascio, V., E. Bonucci, S. Adami, and P. Ballenti. 1982. Glucocorticoid-induced osteopenia in long-term treated patients: prednisolone vs deflazacort. Calcif. Tiss. Int. Suppl. 34:29.

95. Nagant de Deuxchaisnes, C., and J. Devogelaar. 1982. Effect of glucocorticoids on bone. Calcif. Tiss. Int. Suppl. 34:8.

96. Assandri, A., P. Fernari, and E. Mortinelli. 1982. Disposition and metabolism of a glucocorticoid, deflazacort, in rats, dogs, monkeys and man. Calcif. Tiss. Int. Suppl. 34:49.

97. Seal, U., and R. Doe. 1965. Vertebrate distribution of corticosteroid-binding globulin and some endocrine effects on concentration. Steroids 5:827.

98. Wilson, E., and M. Jawed. 1981. CBG as a biological assay for endogenous estrogen in patients with and without endometrial carcinoma. Am. J. Obst. Gynecol. 139:661.

VITA

Scott Howard Loeffler was born on February 24, 1953 in Oklahoma City, Oklahoma. The son of Mr. and Mrs. W.B. Loeffler, he attended Casady School in the same city. In 1971, he began course work at Vanderbilt University (Nashville, Tennessee) from which he received his Bachelor of Science degree in Chemistry. In the spring of 1976, he began a course of study in the Department of Veterinary Public Health at the Texas A&M College of Veterinary Medicine. His research during this period was under the guidance of Dr. Leon Russell and is the subject of this thesis. After working a short time at the U.S. Army Natick Research Labs (Natick, Massachusetts), he began the professional veterinary curriculum at the State University of Utrecht (Utrecht, The Netherlands). In August 1981, he received the Drs. degree (= American D.V.M.). Since this time he has done research at the Small Animal Clinic at the State University of Utrecht and completed a specialization in Tropical Animal Health. He is now working on a research project on rabies in Mexico at the Instituto Nacional de Investigaciones Pecuarias. His permanent address is: 1425 Westchester, Oklahoma City, Oklahoma 73120.