EPITHELIAL AND BONE TISSUE MAST CELL POPULATIONS IN THE FEMALE RAT AS INFLUENCED BY CALCIUM AND VITAMIN D DEFICIENCIES, OVARIECTOMY, AND ESTROGEN

BY

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to my mother

who would have thoroughly enjoyed

observing this entire experience

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Effects of dietary deficiencies on mast cell populations in the bone marrow and vaginal epithelial tissue of rats were investigated. Additionally, effects of exogenous estrogen, bilateral ovariectomy, and a combination of the two treatments on mast cell populations were observed in these two tissues in rats on normal and deficient diets.

Two-month old, female Sprague-Dawley rats were fed cal-calcium- and vitamin D-deficient diets for five weeks. One group of rats was given estradiol by injection (100 µg/.1 ml) three times a week for the duration of the experiment, another group was ovariectomized, a third group received both treatments, while a fourth, untreated group served as a control group.

Dietary-induced osteopenia was evidenced by densitometric measurements and bone ash of the rat femur. Significant decreases in bone mineral content ($P<.005$) due to diet were observed. Bone ash values were also significantly low due to diet ($P<.0005$).

Femur length, measured by photonabsorptiometry, was found to be decreased due to (a) dietary deficiencies of calcium and vitamin D ($P<.005$); (b) estrogen administration ($P<.005$); and (c) ovariectomy ($P<.005$).

Contrary to expectations, bone marrow mast cell populations were not altered by dietary deficiencies in untreated rats. This result may be due to the age of the rat when begun on the deficient diet (two months old) and to the duration of the imposed diet deficiencies (five weeks). Dietary deficiencies reduced marrow mast cell counts in estrogen treated rats, however ($P<.05$). Ovariectomy induced a reduction of mast cells in the bone marrow of calcium- and vitamin D-deficient rats ($P<.01$), which suggests that bone is an estrogen sensitive tissue even though estrogen receptors are not present in bone.

Vaginal tissue mast cells were not significantly altered in number by dietary deficiencies except when rats were estrogen treated ($P<.05$). The most significant finding was that of mast cell population increases in vaginal tissue as a result of ovariectomy ($P<.01$) in all dietary groups.

The removal of endogenous estrogen by ovariectomy in the female rat was found to affect both bone marrow and vaginal tissue mast cell populations. The relevance of this finding remains to be determined.

CHAPTER I
INTRODUCTION

Rapid bone loss with resultant osteoporosis affects 25 to 30% of all postmenopausal women (Urist, 1971) and about 75% of women who have undergone a bilateral ovariectomy. The significance of this condition is associated not only with pathological fractures and potential invalidism but also with the premature death of these women. The early diagnosis of this condition and measurement of treatment response present two problem areas in clinical management. To date there is no universally applicable, noninvasive method for detecting the early stages of rapid bone loss in humans.

Research has shown that mast cells, found in increased numbers in the bone marrow of osteoporotic and calcium-deficient subjects, accompany local loss of bone mass. The present study seeks to determine whether vaginal epithelial mast cell populations, as well as bone marrow populations are affected by dietary deficiencies of calcium and vitamin D, exogenous estrogen, and ovariectomy in the female rat. Should such alterations be found in the female rat, similar changes in populations might be expected in the osteoporotic human female. Tissue biopsy of vaginal epithelium to determine mast cell populations could provide a relatively noninvasive method for detecting osteomalacia and conditions

involving increased bone resorption such as osteoporosis. The procedure might also be used to assess treatment efficacy.

Therefore, two hypotheses were tested in this investigation:

(a) Dietary calcium- and vitamin D-deficiencies produce changes in mast cell populations in female rat bone marrow and vaginal epithelium.

(b) Exogenous estrogen, bilateral ovariectomy, or a combination of the two treatments alters mast cell populations in bone marrow and vaginal tissue of calcium- and vitamin D-deficient female rats.

Other parameters considered and investigated in this study were:

(a) weight changes of the laboratory animals during the experimental period

(b) serum calcium concentration

(c) bone mineral content, density, and length

(d) torsion and deformation of the rat femurs

(e) ash content of the femurs.

CHAPTER II
REVIEW OF THE LITERATURE

Addressed in the literature review are a description of the mast cell and the activity of two constituents, histamine and heparin. Research involving the mast cell in bone marrow of the rat and human is reviewed. Effects of dietary deficiencies, specifically those of calcium and vitamin D, on the rat mast cell in bone tissue are examined. Also cited are the few studies concerning mast cells in vaginal tissue and those relating to the effects of gonadal hormones on skeletal and vaginal tissues.

Description of the Mast Cell

Naming the specialized histiocytes as mast cells has been credited to Paul Ehrlich when he suggested, in 1877, that the cells arose from connective tissue cells which had been well-fed, or 'masted' (Wilhelm et al., 1978). The German masten (to feed) or mastzellen (mast cells), from which the name originated, appropriately describes their usually full appearance. This is due to a high content of cytoplasmic granules.

Pathak and Goyal (1973) state that two separate types of mast cells occur (1) large spindle shaped, fusiform or cylindrical cells with or without elongated processes and (2) small, round, or elliptical cells. Riley (1959) also

described differences in rat mast cells and distinguished between two types based on granule maturation. Differences also exist when mast cells are examined with electron microscopy (Combs et al., 1965).

Rat peritoneal fluid, the most common source of mast cells for laboratory investigation, exhibits round mast cells, 13.5 to 17 μm in diameter, each with a round or oval nucleus. Cytoplasmic granules are approximately .7 μm in diameter (Yong et al., 1975).

In an extensive quantitative analysis of rat mast cell structure, Helander and Bloom (1974) report an average mast cell diameter as 11 μm, granule diameter as .78 μm, with the nucleus occupying 10.7% of the cell. The size, shape, staining properties, and distribution of mast cells vary with the tissue and species of animal studied (Wilhelm et al., 1978). Rat tissues abound in mast cells, whereas tissues of the rabbit contain related basophils often referred to as blood mast cells. Tissues in man and the guinea pig exhibit both mast cells and basophils.

A well-known characteristic of both mast cell and basophil leukocyte granules is an exhibition of metachromasia upon treatment with certain basic dyes such as toluidine blue, methylene blue, alcian blue, and azure A. These stains are used to identify and demonstrate the mast cells in tissues. Since many differences in mast cell reactivity towards these dyes occur within and between species, an extensive investigative interaction among histological studies, identification of cell constituents, and physiological

functions of the mast cells exists. The constituents of the mast cell granules, specifically the acidic mucopolysaccharides, are responsible for the various staining properties of the cells. However, partly because of the chemical diversity of the granular contents, mast cell functions in health or disease remain an enigma.

In 1937, the metachromatic component of the mast cell was reported to be heparin (Jorpes et al., 1937); and since then, the mast cell in hepatic tissue has been considered as the only endogenous source of heparin production (Riley, 1962). A protein matrix is thought to bind anionic heparin in the mast cell granule, possibly by sulfate groups. Heparin, in turn, binds histamine and other basic nitrogen-containing compounds such as serotonin (Schubert, 1968). Histamine levels in tissues correlate with the mast cell count and a very large proportion of rat histamine formation takes place in the bone marrow.

The close histological relationship of mast cells and blood and lymph vessels is well-known. Small vessels are the prime target of histamine-mediated inflammatory reactions. Histamine is known to cause contraction of part of smooth muscle (mainly the bronchioles), dilate blood capillaries, and increase their permeability (Rahima and Soderwall, 1977).

In-vitro laboratory studies of the mast cells (in certain tissues and species) demonstrate that histamine is released by liberators such as stilbamidine, 48/80, or protamine sulfate by displacing the heparin-bound histamine (Schubert, 1968).

Sudden degranulation of mast cells may cause adverse reactions since large amounts of histamine are released into the extracellular space. This release usually occurs in response to a type I antigen-antibody reaction on the surface of mast cells that have been previously sensitized by cell-bound Ig E antibody (Coombs and Gell, 1975). Allergic rhinitis, allergic asthma, urticaria, angioedema, and mastocytosis constitute some manifestations of extensive degranulation. An editorial by Kaliner (1979) details this aspect of the mast cell's varied activities.

Mast Cells in Bone Marrow

In addition to its role in immediate hypersensitivity reactions, histamine has been reported to affect bone remodeling and maturation (Norton et al., 1969). Systemic mastocytosis in urticaria pigmentosa has been accompanied by marked bone remodeling, bone hypertrophy (Sagher et al., 1956), and osteosclerosis (Kruse et al., 1973).

In this same regard, the acid glycosaminoglycan, heparin, in addition to its anticoagulant property, has been considered as a bone resorbing and osteoporosis producing agent. This is especially true with the use of high doses of the anticoagulant for long periods of time (Goldhaber, 1965; Griffith et al., 1965; Jaffe and Willis, 1965; Wise and Hall, 1980). Heparin has been reported to stimulate bone collagenase activity in the rat (Asher and Nichols, 1965) and to potentiate the action of parathyroid hormone (Goldhaber, 1965), suggesting inducement of osteoporosis. A review of the relationship

between mast cells, heparin, and osteoporosis has been provided by Hegsted (1969).

Many reports of heparin use with resulting skeletal problems involved the use of heparin for control of blood coagulation. It has been documented that heparin extracted and purified from tissues rich in mast cells and reinjected by the physician behaves differently from endogenous heparin (Jaques et al., 1977). Only in the dog has the hepatic release of heparin been shown to have a rapid anticoagulant effect in the circulation. Several species have no heparin in the mast cell; its metachromasia is attributed to other sulfated mucopolysaccharides.

Osteoporosis has been viewed by some as a sequel to diminished blood flow through the marrow (Burkhardt, 1973). Contracted arterioles in mastocellular lesions of the bone marrow in human osteoporotics provide evidence for this concept (te Velde et al., 1978).

Increased numbers of bone marrow mast cells have been reported in osteoporosis (Frame and Nixon, 1968; Kruse et al., 1973; Peart and Ellis, 1975). Two theories have been have been expressed (1) mast cells induce porosis (Frame and Nixon, 1968) and (2) mast cells oppose the porosis (Kruse et al., 1973).

Increased mast cell numbers have been associated with bone resorption in regenerating parts of the marrow. Gillman (1958) noted increased mast cells in long bone marrow of rats fed sweet pea seeds containing a lathyrogenic agent. He

distinguished between newly formed and old femoral shafts, with the increased number found in the marrow of the newly formed shaft. Severson (1969) showed that mast cells secrete a factor necessary for hydrolytic enzyme release in regions of increased resorption and remodeling. Walker (1970) reported an eight-fold increase of mast numbers in regenerating rat femoral marrow after mechanical disruption when compared to the unoperated contralateral femur. Hypophysectomy resulted in an even greater increase and longer effect.

Extensive studies in bone repair (Lindholm et al., 1967, 1969) demonstrated active involvement by mast cells, increased mast cell numbers in callus formation, and mast cell provision of alkaline phosphatase, phosphorylase, and other enzymes essential for endochondral ossification.

Human alveolar bone resorption in chronic periodontal disease is associated with increased mast cell counts in gingival tissue (Shapiro et al., 1969; Riley, 1959; Sognnaes, 1965). Other investigators (Carranza and Cabrini, 1955; Calonius, 1960; Dummet et al., 1961) failed to confirm this finding.

In hyperparathyroidism, both the resorption and the formation of bone are stimulated, but greater increases in bone resorption occur (Bonucci et al., 1978). Mast cells have been reported in fibrotic marrow spaces in human hyperparathyroid patients. Other researchers (Rockoff and Armstrong, 1970) found that low doses of parathyroid hormone chronically administered to rats produced mast cell hyperplasia in the

tibial metaphyseal marrow, without alterations of serum calcium or phosphorous.

Secondary hyperparathyroidism is known to result from lowered serum calcium levels and is thought to be a mechanism whereby low dietary calcium intakes promote mast cell increases in bone.

Effects of Diet

As early as 1922, increase of tissue basophils in the immediate vicinity of the bone trabeculae and marked resorption of bone in rats on calcium deficient diets were reported (Shipley and Park, 1922). Urist and McLean (1957) identified those basophils as mast cells. They also maintained rats on low calcium, low vitamin D, and high phosphorus diets which produced rickets, osteoporosis, and osteitis fibrosa as well as increased endosteal mast cells. Cass et al. (1958) confirmed the results of increased bone mast cells in rats fed calcium-deficient diets and found an increase in bone marrow content of histamine and 5-hydroxytryptamine, another mast cell mucopolysaccharide. Rockoff and Armstrong (1970) also administered a calcium-deficient but vitamin D-adequate diet to a group of rats, with bone marrow mast cell hyperplasia resulting in all test animals. In providing hypocalcemia-inducing vitamin D-deficient and calcium- and vitamin D-deficient diets to rats, Rasmussen (1972) observed significant increases in tibial metaphyseal bone marrow mast cells. Parathyroidectomy caused a significant reduction in mast cells, again suggesting secondary hyperparathyroidism as a

mechanism for increased mast cell populations in bone. Other rats given low calcium and high phosphorus diets with and without vitamin D exhibited hypocalcemia, rachitic bone changes, increased bone resorption and increased mast cells in metaphyseal areas of long bone but not in the epiphyses or caudal vertebrae (Feik and Storey, 1979); however, it was not possible to relate the mast cell increases to specific areas of bone formation or resorption, as had been planned.

In dietary calcium- and vitamin D-deficient rats with induced fracture callus, mast cell counts in the callus approximated 200 to 300 cells per mm^2 which rose to 1,900 cell per mm^2 until 35 days after fracture. These mast cells were mostly degranulated. Normal rats exhibited strongly granulated mast cells, 2,000 to 4,000 mm^2 for the first two-month period with remarkably decreased levels thereafter. Mast cell numbers were correlated with mineralization after fracture (Lindholm et al., 1972).

Accumulations of mast cells in healing sockets or extracted mandibular first molars were found in rats fed calcium-deficient diets, with control rats exhibiting only an occasional mast cell (Smith, 1974). Besides the fact that mast cell numbers were examined in different bones in the two studies, the contradictory findings were not explained.

Other dietary deficiencies have also affected mast cell populations in bone marrow. Bélanger (1978) found a significant increase of bone marrow mast cells in rats on zinc

deficient diets, and also on magnesium-deficient diets (1977). Concurrent decreases in skin mast cell numbers of the magnesium-deficient rats agree with the findings of Bois (1962).

Mast Cells in Vaginal Tissue and Influences of Gonadal Hormones

Vaginal tissue mast cell population studies in the rat are essentially nonexistent. Salvi (1952) found mast cells more abundant and with greater metachromatic properties in the mouse vagina than in the uterus. After daily estrogen administration, adult mouse vaginal tissue revealed a considerable increase in the number of mast cells (Arvy, 1955). Westin and Odeblad (1956) also investigated the influence of ovarian hormones on mast cells in the mouse vagina. Darker metachromasia in the vagina than in the uterus and difficulty in detecting granules were experienced. The control group had the highest number of vaginal tissue mast cells and also the highest variation in number per field examined. The estrogen treated groups had a significantly reduced number; intermediate numbers were observed when estrogen plus progesterone was administered. Mast cells of the skin remained constant. The estrogen effect on the mast cells was considered a local process within the reproductive organs. Zwillenberg (1958) noted a variable occurrence of mast cells in the vaginal epithelium of human subjects.

Discrepancies in results among studies may be due, in part, to differences in estrogen dosage. Iversen (1962)

notes that, while small doses of estradiol decreased the number of uterine mast cells in the guinea pig, prolonged treatment with large doses had an opposite effect.

Although there are mast cell studies which investigated the effect of gonadal hormones in various tissue (Constantinides and Rutherdale, 1954; Asboe-Hansen, 1956; Johansson and Westin, 1958; Smith and Lewis, 1958; Schiff and Burn, 1961; Kameswaren et al., 1978), few reported on bone tissue. Bélanger (1977), in his study with magnesium deprived rats, administered large doses of testosterone to males and estradiol to females. This treatment depressed the mast cell population increase in the bone marrow and moderated skin mast cell loss.

Similarly, there is no reference in the literature concerning the effect of ovariectomy on mast cells in bone or vaginal tissue. The mice in the study of Westin and Odeblad (1956) were all spayed so that effect of ovariectomy could not be compared to control groups. Two studies reported that ovariectomy has no effect on uterine mast cells in guinea pigs or hamsters (Iversen, 1962; Harvey, 1964).

Because of the differences in mast cell populations, structure, function and activity in various species (and in tissues within the same species), information cannot be extrapolated from one species to another. Because of the various mast cell constituents and their resulting diverse functions and actions in tissues, inconsistencies in results will continue to be reported. However, in the recent past,

much new information on the mast cell has been brought forth. The particular role of the mast cell in the pathogenesis of the osteoporoses and other demineralizing bone diseases remains in need of further investigation.

CHAPTER III
MATERIALS AND METHODS

Animals and Treatments

To monitor the care, treatment, and use of laboratory animals at the University of Florida, the All University Committee on the Care and Use of Laboratory Animals requires specific information pertaining to research involving laboratory animals. The application requesting use of laboratory animals for this particular research project as submitted to the Committee and its approval are found in Appendix B.

One hundred thirty-six female Sprague Dawley rats,[1] 9 weeks of age and weighing approximately 180 g at the start, were used for the research.

The Health Center Animal Resources Department, University of Florida, provided housing for the animals. The rats were kept in galvanized wire cages, two to a cage, in a room maintained constantly at 24°C and 60% humidity. The rats were weighed at least once a week for the 4 to 5 week experimental period. Appendix C contains data on the animal weights.

The animals were divided into the following dietary groups:

[1]Outbred laboratory Sprague-Dawley rats were obtained from Harlan·Sprague-Dawley, Madison, WI 53711.

I. Normal (control) rats (semipurified complete diet)

II. Calcium-deficient (-Ca) rats (calcium-deficient diet)

III. Vitamin D-deficient (-D) rats (rachitogenic diet; calcium to phosphorus ratio of 4.2:1)

IV. Calcium- and vitamin D-deficient (-Ca, -D) rats (custom formulated calcium- and vitamin D-deficient diet).

Appendix D contains information on dietary formulations.

Each dietary group consisted of 32 rats, except for group II, in which there were 40. The diets (pelleted) and distilled water were offered ad libitum. Group III rats were borderline vitamin D-deficient at the beginning of the experimental period. One rat in group IV was rejected because of a malfunctioning eye.

Upon receipt of the animals and prior to further treatment, each rat was tested by vaginal smear daily for an 8 day period to determine presence of estrous cycling. Each rat presented with at least once cycle, demonstrating reproductive capability and ascertaining endogenous circulating estrogen.

Half the rats in each group were bilaterally ovariectomized. Sham ovariectomies were performed on the remaining rats in each group. The combination of ketamine and xylazine (Van Pelt, 1977), at a concentration of 87 mg ketamine and 13 mg xylazine per kg body weight of rat, was used to induce surgical anesthesia. One rat in group II died as a result of the surgery.

One-half of the ovariectomized and one-half of the sham-operated rats in each group were injected three times per week under the dorsal skin with .1 ml of a solution of estradiol valerate (100 µg/.1 ml) in sesame oil (12 injections per rat total). Concurrently, the remaining rats in each group were injected with .1 ml of sesame oil as a control measure.

After the five to six week experimental period, all animals were killed by decapitation.

Table III.1 summarizes the research design described.

TABLE III.1
STUDY DESIGN

	Diet Group			
	Normal (N)	-Ca (N)	-D (N)	-Ca, -D (N)
Treatment				
Ovariectomized + Estrogen	8	12	8	8
Ovariectomized	8	12	8	8
Estrogen	8	8	8	8
Normal (no treatment)	8	8	8	8

Analytical Methods

Blood was obtained at the time of decapitation by exsanguination. Total calcium in serum was determined by atomic absorption spectrophotometry (AAS). The procedure followed was that of Fick et al. (1979). Data on serum calcium values are found in Appendix E.

Immediately after the animals were killed, uterine and vaginal tissues were removed, hind extremities were disarticulated at the acetabulum, and femurs were dissected free. The femurs were cleaned of adherent tissues. The uterine and vaginal tissues and femurs were fixed for 24 hours in 10% aqueous formalin.

Bone densitometric measurements[1] using direct photon absorptiometry were made on one femur from each animal. Values of bone mineral content in grams per centimeter length of bone, linear bone density in grams per square centimeter, and bone length in centimeters were obtained. Appendix F contains information on these paremeters.

The same femurs were subjected to torque and deformation testing[2] to determine the effect of treatment modality on these biomechanical properties. The procedure followed was that of Puhl et al. (1972). Explanation of this testing procedure is found in Appendix G.

Ashing of these same femurs was done as described by Fick et al. (1979). Appendix H contains data on ash analysis.

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[1]Norland Digital Bone Densitometer, Model 278, Norland Corporation, Ft. Atkinson, WI 53538.

[2]Rapid Load Torsional Testing Machine, Biomechanics Laboratory, Department of Mechanical Engineering, University of Florida, Gainesville, FL 32611.

Histology

The alternate femurs of all animals were demineralized in a 10% solution of di-sodium-ethylene-diamine-tetracetic acid (EDTA) for a 7 to 14 day period. The solution was kept at 5°F with changes of solution every 2 to 3 days (Bélanger et al., 1965).

The demineralized femurs and vaginal tissues were dehydrated in 80% acetone for one-half hour and in 100% acetone for another half-hour. Clearing was accomplished with two changes of xylene (15 and 45 minutes); subsequently the tissues were embedded in paraffin. Medial sagittal sections of bone and cross sections of vaginal tissue were cut at 8 μm in a microtome-cryostat, floated on water, slipped onto slides prepared with Haupt's solution, and air-dried.

Toluidine blue stain was chosen for mast cell quantitation purposes (Pathak and Goyal, 1973). With this stain, mast cells appear purple or reddish-purple against a general blue background. The slides were subjected to the following staining procedure:

(1) 2 changes of xylene (4 minutes each)
(2) acetone (several dips)
(3) water rinse
(4) .2% toluidine blue
(5) water rinse
(6) acetone (several dips)
(7) 2 changes of xylene (4 minutes each)

Alternate slides were stained with .1% alcian blue in 3% acetic acid for thirty minutes followed by a water rinse and .1% safranin in 1% acetic acid for five minutes in place of step #4 (Spicer, 1960). Using this staining procedure, the maturity of mast cells can be determined. Analysis of these slides is planned for a future time.

After air-drying, cover slips were applied to the slides with mounting medium.

Mast cells were counted in vaginal tissue and in the distal part of the femoral metaphysis and in the bone marrow of the diaphysis. Care was taken to avoid bone trabeculae and sinuses. Counts were made over five fields in each of five sections (25 fields per rat) for each type tissue at a magnification of X400.[1] Each field measured .458 mm in diameter, representing a surface area of .165 mm^2 and a total surface area of 4.1 mm^2 for each tissue per rat. Counts were adjusted to 1 mm^2 surface area. Appendix I contains mast cell quantitation data.

Data Analysis

The statistical evaluations for tests of significance were carried out on the parameters using analysis of variance and applying the t test (Steel and Torrie, 1960). The tables list mean values and standard error of the mean ($SE = s/\sqrt{n}$), and indicate level of significance.

[1]Labophot-Laboratory & Clinical Microscope, Nikon Instrument Division, 623 Stewart Ave, Garden City, NY 11530.

Effects of diet were determined statistically by comparing the means in each deficient diet group with means in the normal diet group for each treatment.

Effects of treatment were determined statistically by comparing means for each treatment with the mean of the untreated rats within each diet group.

CHAPTER IV
RESULTS AND DISCUSSION

The experimental animals used in this investigation were at least three months old at termination of the experiment and regarded as young adults. Reproductive capability was determined prior to treatments by vaginal cell sampling. One-half the population underwent ovariectomy, thereby removing the source of estrogen production.

The diet used for the vitamin D-deficient group of rats was also notably deficient in phosphorus, with a calcium to phosphorus ratio of 4.2 to 1. Calcium to phosphorus ratios of all the diets are given in Appendix D.

As a matter of information, the calcium content of the complete diet (normal diet group) was 11.5 g/kg and the phosphorus content was 10.1 g/kg. The calcium-deficient diet contained 1.6 g/kg calcium and 26.6 g/kg phosphorus. The rachitogenic vitamin D-deficient diet noted as being low in phosphorus contained 12.4 g/kg calcium and 2.9 g/kg phosphorus. The calcium- and vitamin D-deficient diet contained 1.6 g/kg calcium and 26.6 g/kg phosphorus (identical to the calcium-deficient diet but with omission of vitamin D_3).

In addition, the protein in the vitamin D-deficient diet provided by whole yellow maize and as gluten, was of poor quality, lacking in essential amino acids. Therefore,

when evaluating effects of this diet, the deficiency in protein and phosphorus must also be considered. Effects of diet were not due solely to lack of vitamin D.

Body Weights

Body weight changes were observed in the rats. In Table IV.1 is recorded the average weight gain for each cell. The % weight gain is listed below the mean. The untreated rats fed a normal diet, and used as a control group, increased their body weight by 34% during the experiment. Lower weight gains were observed as due to calcium deficiency ($P < .05$). The vitamin D-deficient group also had lower weight gains as an effect of diet ($P < .01$).

Administration of estrogen to both intact and ovariectomized rats decreased body weight gains in the normal, calcium-deficient, and the calcium- and vitamin D-deficient groups ($P < .05$ to $.005$). Similar effects occured with rats on a normal diet (Cruess and Hong, 1979).

Ovariectomy increased the weight gains in the same three dietary groups ($P < .005$) (Fig. IV.1).

This supports the findings of Cruess and Hong (1979) and Lindgren and Lindholm (1979) in that removal of ovaries subjected rats to high increases in body weight. This effect has been associated with a higher food intake (Aitken et al., 1972). However, significant decreases in body weight of young castrated male rats have been observed (Scow, 1952; Gumbreck, 1957; Saville, 1969; Wink and Felts, 1980).

TABLE IV.1
BODY WEIGHT GAIN AS AFFECTED
BY DIET AND TREATMENT

Treatment	Diet							
	Normal	(N)	-Ca	(N)	-D	(N)	-Ca,-D	(N)
Ovariectomy + Estrogen	20.9±4.1[c] 11.3%	8	17.3±2.1[c] 9.5%	12	26.1±2.7[b] 17.3%	8	19.1±2.5[c] 9.8%	8
Ovariectomy	115.4±9.1[c] 60.4%	7	108.3±4.9[c] 59.2%	8	53.3±4.6[f] 30.8%	8	101.8±12.5[c] 51.8%	8
Estrogen	30.4±4.9[c] 16.2%	7	39.2±3.2[a] 21.0%	7	35.6±3.5 23.5%	8	15.9±8.6[c] 7.9%	8
No treatment	66.1±4.1 34.1%	8	53.4±4.5[d] 29.3%	8	44.3±6.1[e] 27.5%	8	57.1±3.1 27.7%	7

Results are given as the mean ± SE.

Means are expressed in grams and % gain.

Significance of difference between treated and untreated groups: [a]P<.05, [b]P<.01, [c]P<.005

Significance of difference between deficient diet and normal diet: [d]P<.05, [e]P<.01, [f]P<.005

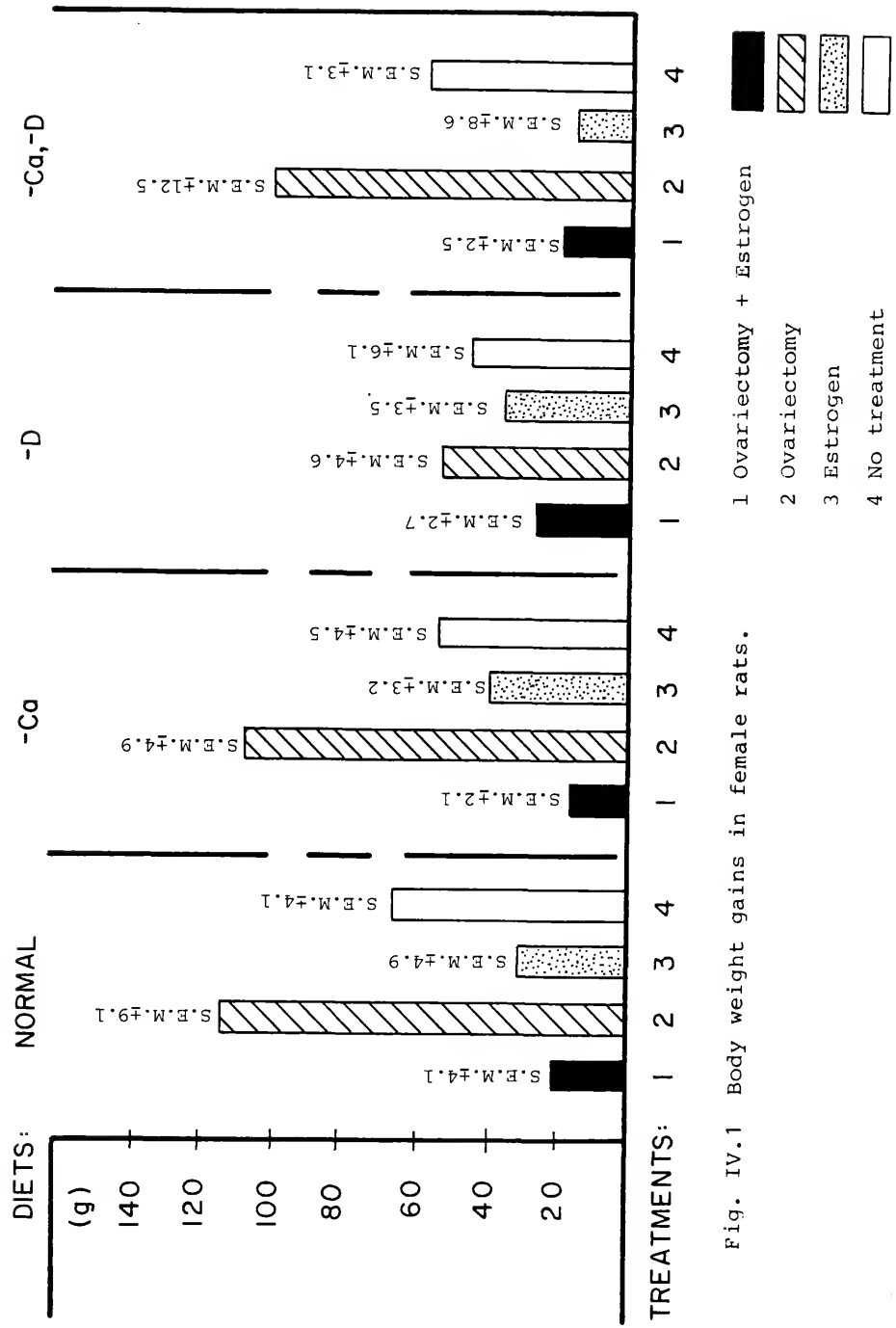


Fig. IV.1 Body weight gains in female rats.

Results indicate a gain of weight in all cells of the study. A calcium-deficient diet and a combination protein, phosphorus, and vitamin D-deficient diet caused rats to gain less weight.

Estrogen inhibited the rate of weight gain, which is an effect not well understood. This effect was seen with both intact and ovariectomized rats. Ovariectomy clearly increased weight gain and estrogen administration reduced that gain to below the normal gain. The same pattern of this hormonal effect was observed in the deficient diet groups.

Serum Calcium

Table IV.2 outlines the changes in serum calcium observed in rats on four diet regimes and four treatments. Untreated rats on a normal diet had a mean serum calcium of 9.63 mg/100 ml. Rats on deficient diets which had been given estrogen had significantly lower serum calcium ($P<.01$ to .0005) than normally fed rats given estrogen. Rockoff and Armstrong (1970) and Feik and Storey (1979) also observed significant decreases in serum calcium with untreated, calcium- and vitamin D-deficient rats ($P<.1$ to .01).

Estrogen increased the serum calcium in the intact and ovariectomized, normally fed rats ($P<.01$ to .001) on the present study. Cruess and Hong (1979) found no consistent change in serum calcium concentration when estrogen was administered to intact, normally fed female rats over a 12 month period, but observed significant increases at one and six months ($P<.05$).

TABLE IV.2
EFFECT OF DIET AND TREATMENT
ON SERUM CALCIUM

Parameter and Treatment	Diet							
	Normal	(N)	-Ca	(N)	-D	(N)	-Ca,-D	(N)
Ovariectomy + Estrogen	12.55±.30[c]	8	8.87±.19[e]	12	8.94±.32[e]	8	8.05±.34[c,e]	8
Ovariectomy	11.43±.64[b]	6	10.24±.13[a,d]	12	9.50±.65[d]	8	8.45±.39[c,e]	8
Estrogen	11.70±.25[c]	7	9.39±.17[e]	7	9.05±.30[e]	8	5.45±.68[e]	8
No treatment	9.63±.47	8	9.09±.13	8	9.38±.33	8	4.34±.68[e]	7

Results are given as the mean ± SE.

Significance of difference between treated and untreated groups: [a] $P < .05$, [b] $P < .01$, [c] $P < 0.005$

Significance of difference between deficient diet and normal diet: [d] $P < .01$, [e] $P < .0005$

Ovariectomy significantly increased serum calcium in all dietary groups ($P<.05$ to .001) except in the vitamin D-deficient group. Lindgren and Lindholm (1979) did not observe an increase in serum calcium in normally fed, ovariectomized rats. Others (Cruess and Hong, 1979) found ovariectomy to significantly decrease the serum calcium ($P<.05$).

Results indicate that calcium- and vitamin D-deficient diets including deficiencies in phosphorus and protein may not have an effect on calcium concentration in the blood. The rat may compensate for lack of dietary nutrients by bone resorption of calcium and phosphorus to maintain normal blood calcium levels. Evidence for bone resorption was found in lower bone mineral content and lower ash values in rats deficient in calcium and vitamin D in the present study (Tables IV.3 and IV.5).

Estrogen increased serum calcium in normally fed intact and ovariectomized rats. However, because of conflicting findings in the several studies mentioned, no firm conclusions can be made on the effect of estrogen on serum calcium in normal rats.

Ovariectomy effects on serum calcium have also been noted to vary among studies so that no conclusions can be made. Unknown factors may be influencing these two treatments, which causes findings to be inconsistent.

Bone Densitometry

Femur lengths. Statistically significant differences in femur lengths due to diet and to treatments were observed. Values are found in Table IV.3.

The bone lengths of the normally fed rats were significantly longer than those of calcium-deficient and calcium- and vitamin D-deficient rats in all treatment groups ($P<.05$ to .005).

Estrogen administered to intact and ovariectomized rats reduced bone length in the normally fed group and the calcium- and vitamin D-deficient group ($P<.05$ to .005).

Ovariectomy increased femur lengths in the normally fed group ($P<.05$). Lindgren and Lindholm (1979) found femur length unaffected by oophorectomy. The sensitive photon-absorption method (Norland, 1980) used in the present study may have been responsible for detecting length differences.

Deficient diets used in this study clearly bring about a decrease in femur length in female rats during the growth period.

Estrogen tends to cause a shorter bone length, especially in normally fed rats and rats both calcium- and vitamin D-deficient. It is thought that estrogen causes the epiphyses to close prematurely which results in a shorter bone. Use of estrogen in young human females has this effect.

Confirming evidence of the preceding is observed in Fig. IV.2. Ovariectomy, or absence of estrogen, caused bone

TABLE IV.3
EFFECT OF DIET AND TREATMENT ON
DENSITOMETRIC MEASUREMENT OF RAT FEMURS

Parameter and Treatment	Diet							
	Normal	(N)	-Ca	(N)	-D	(N)	-Ca,-D	(N)
Bone Length (cm)								
Ovariectomy + Estrogen	3.21±.12[a]	8	3.09±.12[d]	11	3.10±.06[d]	7	3.13±.08[e]	6
Ovariectomy	3.50±.11[a]	6	3.27±.13[f]	10	3.23±.09[f]	7	3.36	1
Estrogen	3.26±.07[b]	7	3.09±.11[f]	6	3.11±.07[f]	8	3.15±.04[e,f]	6
No treatment	3.37±.12	7	3.13±.07[e]	2	3.16±.11[f]	8	3.30±.09	5
Bone Mineral Content (g/cm)								
Ovariectomy + Estrogen	.108±.002[b]	8	.104±.001[d]	11	.096±.001[e,f]	8	.092±.002[e,f]	8
Ovariectomy	.118±.002	7	.110±.001[b,f]	11	.101±.002[e,f]	8	.009±.002[f]	8
Estrogen	.109±.002[a]	7	.106±.001	6	.109±.003	8	.099±.001[f]	7
No treatment	.116±.002	8	.102±.003	2	.113±.001	8	.099±.001[f]	7
Bone Density (g/cm^2)								
Ovariectomy + Estrogen	.317±.006	8	.294±.004[f]	11	.296±.005[e,e]	8	.274±.005[f]	8
Ovariectomy	.313±.004	7	.289±.004[f]	11	.288±.004[e,f]	8	.263±.003[f]	8
Estrogen	.315±.004	7	.292±.004[f]	6	.314±.006	8	.275±.005[f]	7
No treatment	.311±.006	8	.275	1	.318±.005	8	.269±.004[f]	7

Results are given as the mean ± SE.

Significance of difference between treated and untreated groups: [a]P<.05, [b]P<.01, [c]P<.005

Significance of difference between deficient diet and normal diet: [d]P<.05, [e]P<.01, [f]P<.005

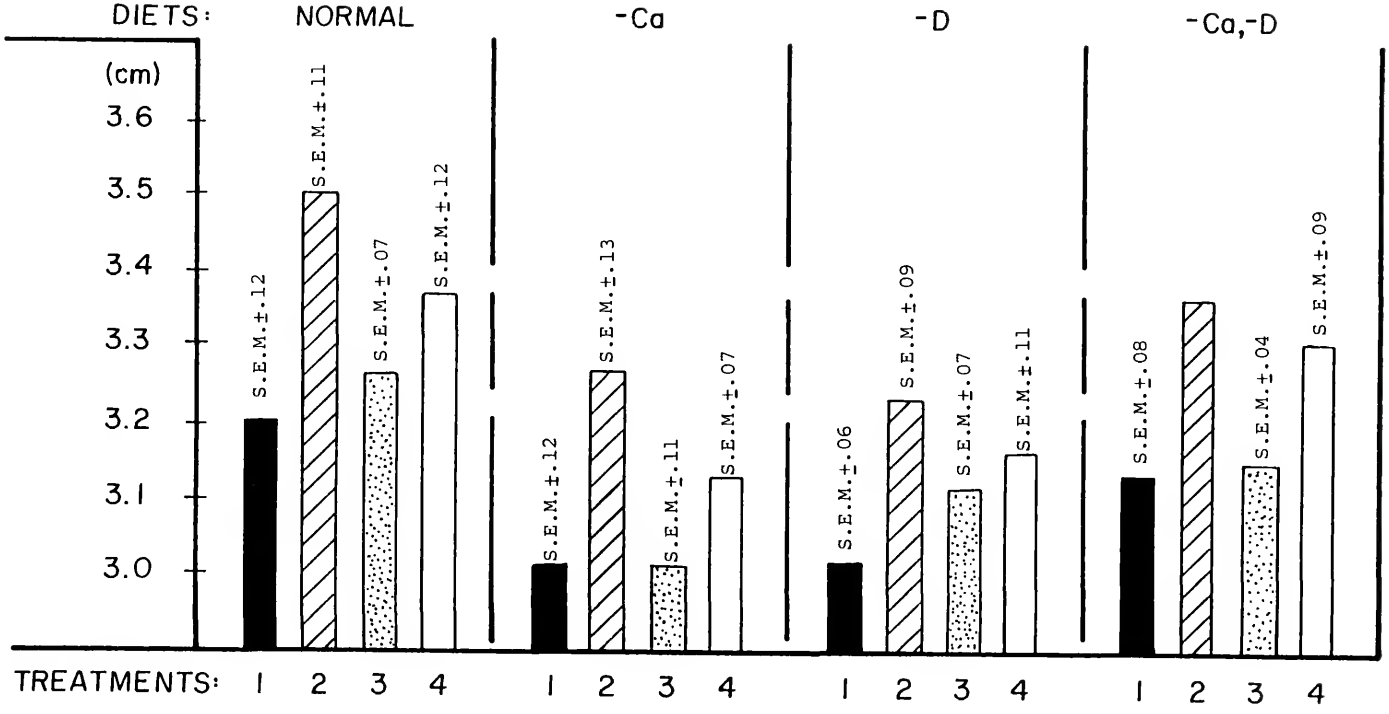


Fig IV.2 Femur length by photonabsorptiometry.

1 Ovariectomy + Estrogen
2 Ovariectomy
3 Estrogen
4 No treatment

lengths to increase in normal rats. Addition of estrogen depressed bone length to normal or below normal.

Bone mineral content. Means and standard errors of the mean for mineral content of the femur as measured by the photonabsorption method (Norland, 1980) are listed in Table IV.3. Statistically significant differences in mineral content of the femur due to dietary deficiencies and to treatments were found. The calcium-deficient diet and the vitamin D-deficient diet lowered bone mineral content ($P<.005$).

When administered estrogen, the normally fed intact rats had decreased bone mineral content ($P<.05$) but other dietary groups showed no effect from estrogen.

Ovariectomy caused a higher bone mineral content in calcium-deficient rats ($P<.01$), and a lower bone mineral content in vitamin D-deficient rats ($P.<005$); the latter effect most probably was due to the multiple deficiencies of the diet.

When rats were ovariectomized and estrogen was added, bone mineral content decreased in the normally fed group ($P<.01$), and in both the vitamin D and calcium- and vitamin D-deficient groups ($P<.005$).

The results indicate that diet does cause decreases in bone mineral content in the female rat. When the rat is depleted of certain nutrients, osteopenia results. That the rat is a suitable model for this premise has been established by this and other studies. Extrapolation of the

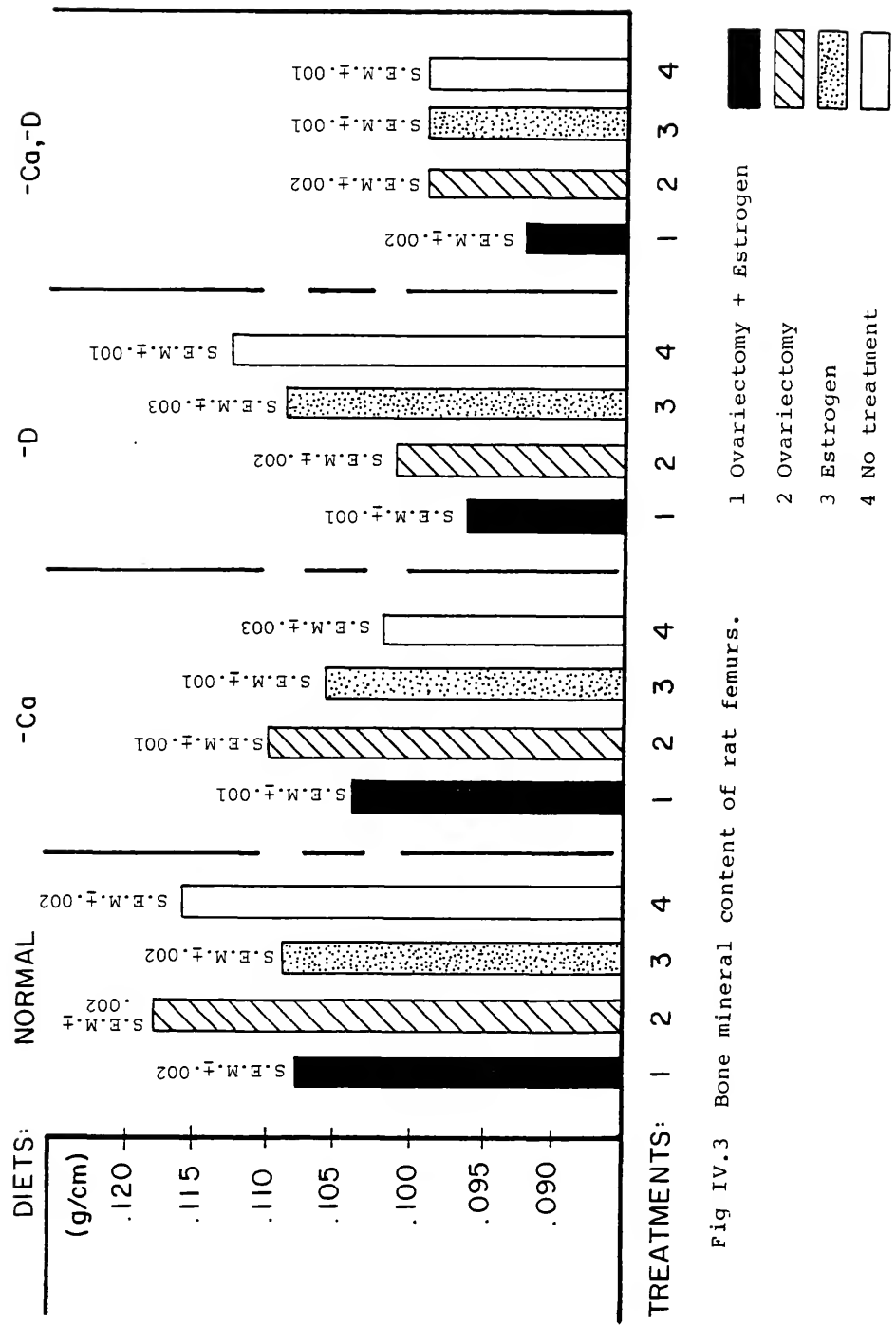


Fig IV.3 Bone mineral content of rat femurs.

conclusion to the human female is difficult, however, the corollary does exist.

Sanchez et al. (1981) measured bone mineral mass in vivo in normally fed, untreated rats with a Norland-Cameron model 178 bone mineral analyzer. They found highly significant positive correlations between femoral mineral mass, femoral ash weight, and body weights. Similar statistical correlation tests are planned for the data in the present study.

Bone density. The bone density measurement is a ratio of the bone mineral content and the femur width, so that differences between bone mineral content and bone density were due to bone width and did not vary with bone mineral content, since the measurements were done simultaneously.

The means and standard errors of the means for bone density values are found in Table IV.3.

Dietary deficiencies signficantly decreased bone density in several treated and untreated groups ($P<.05$ to .005). Calcium- and vitamin D-deficiency especially affected density ($P<.005$) (Fig. IV.4). Only one intact femoral bone from untreated calcium-deficient rats was available; therefore it was not used for statistical comparisons.

Estrogen treatment showed no effect on bone density, whether given to intact or ovariectomized rats.

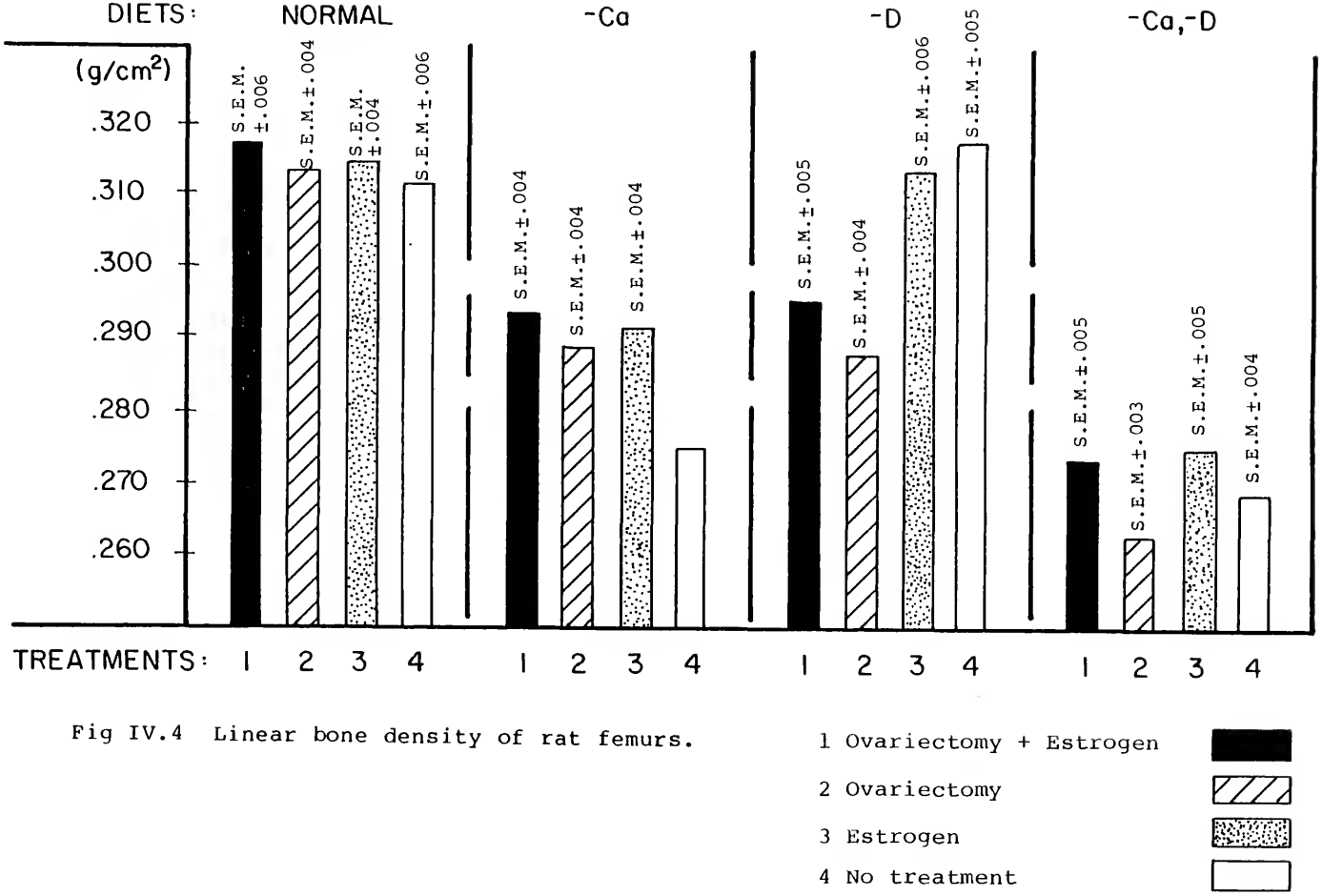


Fig IV.4 Linear bone density of rat femurs.

Ovariectomy also did not affect bone density. However, within the vitamin D deficient group, which also was deficient in protein and phosphorus, bone density was decreased by estrogen and by ovariectomy ($P<.005$).

Burkhart and Beresford (1978) castrated 1 1/2-year-old male rats and reported decreased femoral density 3 to 6 months later. A Joyce-Loebel photodensitometer was used to measure the density. Wink and Felts (1980) also reported density decreases in male castrates ($P<.01$) and femoral osteoporosis four months after castration in year-old male rats.

Biomechanical Tests

Table IV.4 lists the mean and standard errors of the torque required to fracture the femurs and of the deformation undergone by the bones at fracture.

Torque. In all treatments, the calcium-deficient group and the vitamin D-deficient group required the least torque ($P<.01$) for fracture to occur (Fig. IV.5).

Estrogen administered to intact rats significantly lowered torque in the normally fed group ($P<.05$) and raised it in the vitamin D-deficient group ($P<.05$).

Ovariectomy decreased the torque value significantly in the calcium- and vitamin D-deficient group ($P<.05$).

When estrogen was given to ovariectomied rats, torque values were reduced significantly in the normally fed group ($P<.05$) and raised significantly in the calcium-deficient group ($P<.05$).

TABLE IV.4
EFFECT OF DIET AND TREATMENT ON
BIOMECHANICAL PROPERTIES OF RAT FEMURS

Parameter and Treatment	Diet							
	Normal	(N)	-Ca	(N)	-D	(N)	-Ca,-D	(N)
Torque (kg-cm)								
Ovariectomy + Estrogen	3.75±.39[a]	7	2.49±.08[a,f]	12	2.61±.19[e]	8	3.06±.20	7
Ovariectomy	4.62±.24	6	2.49±.15[f]	9	3.07±.25[f]	7	2.72±.17[a,f]	6
Estrogen	3.86±.19[a]	5	2.58±.22[f]	4	3.04±.18[a,e]	7	3.24±.10[f]	7
No treatment	4.46±.20	8	1.70[f]	7	2.56±.18[f]	7	3.15±.17[f]	6
Deformation (degrees)								
Ovariectomy + Estrogen	14.41±1.77	7	15.96± .98	12	17.00±1.37	8	14.43±1.48	7
Ovariectomy	16.20± .58	5	17.94±1.24	9	17.43±1.36	7	14.92± .89	6
Estrogen	12.90± .56[c]	5	18.00±2.17	4	16.50± .53[f]	7	13.50± .29[c]	7
No treatment	16.00± .54	8	17.00	1	17.14±1.53	7	16.50± .58	6

Results are given as the mean ± SE.

Significance of difference between treated and untreated groups: [a] $P<.05$, [b] $P<.01$, [c] $P<.005$

Significance of difference between deficient diet and normal diet: [d] $P<.05$, [e] $P<.01$, [f] $P<.005$

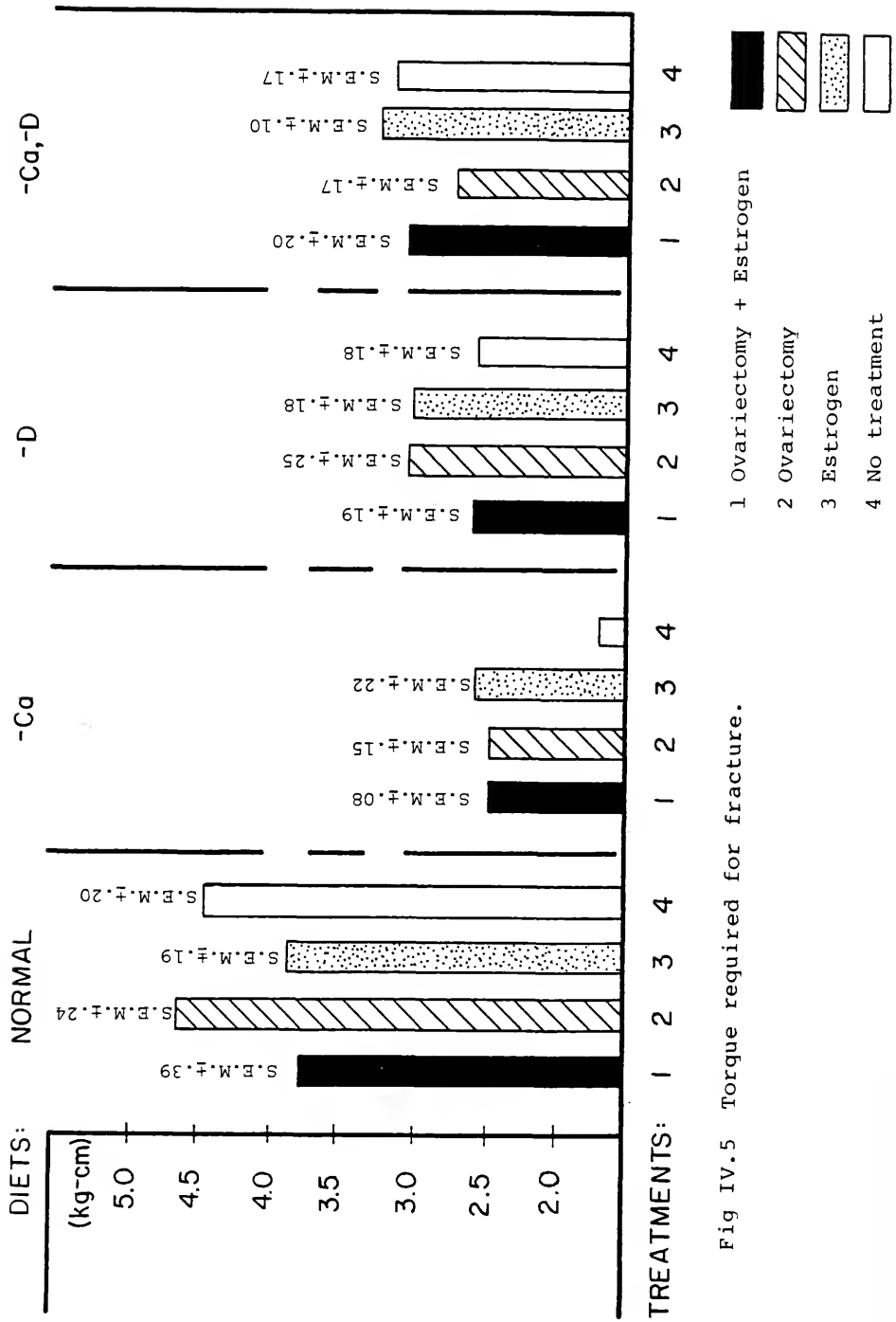


Fig IV.5 Torque required for fracture.

Deformation. Dietary deficiencies had no effect on deformation of the rat femurs.

When estrogen was administered to intact rats in each group, the normally fed group and the calcium- and vitamin D-deficient group showed significant decreases in deformation values ($P < .005$). This effect can be interpreted to mean a harder bone with less bending ability resulting from estrogen administration.

Ovariectomy did not affect deformation of the femurs in any group.

Fig IV.6 represents the femoral deformation values for the rats in the study.

Bone Ash

Values for bone ash as % of dry, fat-free femoral bone are listed as means ± SE in Table IV.5. Differences in bone ash content between rats on deficient diets and those adequately fed were statistically significant in all treatments ($P < .01$), with highest values in the adequately fed group. Decreases in skeletal ash weight of rats on calcium-deficient diets have been previously observed ($P < .001$) (Rockoff and Armstrong, 1970).

Estrogen administration did not alter bone ash in any dietary group, however, ovariectomy decreased % ash content in the calcium-deficient group. The effect of ovariectomy and estrogen was an increase in bone ash in vitamin D-deficient and calcium- and vitamin D-deficient groups ($P < .05$).

TABLE IV.5
EFFECT OF DIET AND TREATMENT ON
ON BONE ASH

Parameter and Treatment	Diet							
	Normal	(N)	-Ca	(N)	-D	(N)	-Ca,-D	(N)
Ovariectomy + Estrogen	65.5±.42	8	61.3±1.50[g]	12	63.2±.48[b,e]	8	61.9±.27[a,g]	8
Ovariectomy	63.9±.47	7	58.8± .64[g]	12	60.7±.47[f]	8	60.0±.72[e]	8
Estrogen	64.8±.62	7	62.3± .63[d]	7	60.8±.60[f]	8	61.8±.44[e]	8
No treatment	65.5±.65	7	61.6± .57[f]	8	61.0±.57[f]	8	60.6±.54[g]	7

Results are given as the mean ± SE.

Significance of difference between treated and untreated groups: [a]P <.05, [b]P <.01, [c]P<.005

Significance of difference between deficient diet and normal diet: [d]P <.01, [e]P <.005, [f]P < .0005,[g]P<.0001

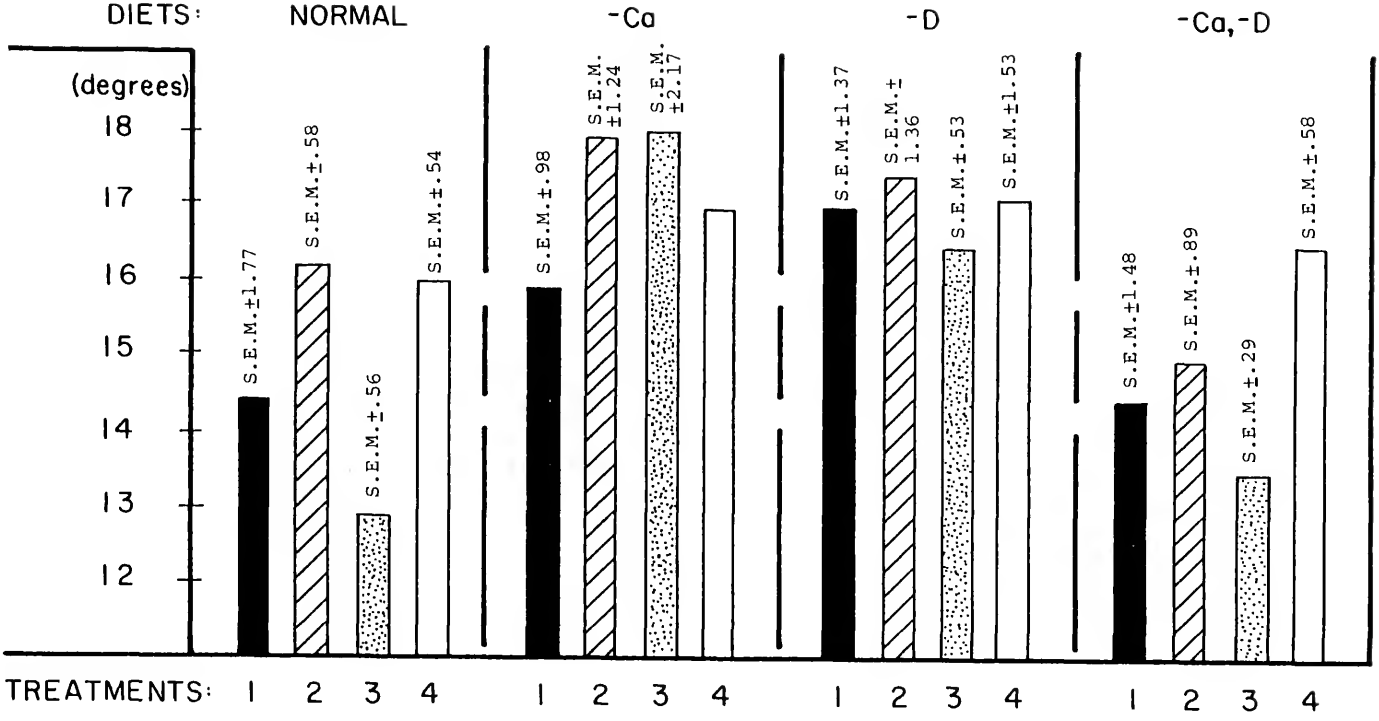


Fig IV.6 Femoral deformation prior to fracture.

1 Ovariectomy + Estrogen

2 Ovariectomy

3 Estrogen

4 No treatment

Other normally fed animals have shown no alteration in ash content of bones after estrogen administration, but oophorectomy caused a significant decrease ($P<.05$) in ash content which was reversed by estrogen ($P<.05$) (Cruess and Hong, 1979). Other significant decreases in ash weight as % of femur dry weight have also been reported with oophorectomy ($P<.01$), even though the rats also had a high body weight gain ($P<.05$) (Lindgren and Lindholm, 1979). By castrating male rats and maintaining a normal diet, a decrease in % ash was observed after 3 to 6 months (Burkhart and Beresford, 1978; Wink and Felts, 1980). It is probable that a longer period than one month must be observed post ovariectomy in order to detect significant differences in bone ash in rats on a normal diet.

Mast Cells

Bone Marrow Mast Cells.

Means of mast cell counts in the distal metaphyseal and diaphyseal portions of the femoral bone marrow are presented in Table IV.6. Means for each group were obtained and adjusted to an area of 1 mm^2. The range of mast cell counts is also given as counts per 1 mm^2 surface area. Appendix I contains photomicrographs of bone marrow mast cells (Fig I.1-I.4). Figure IV.7 provides a visual representation of bone marrow mast cell populations observed in this study.

TABLE IV.6
MAST CELL POPULATIONS AS AFFECTED
BY DIET AND TREATMENT IN THE FEMALE RAT

Parameter and Treatment	Diet							
	Normal	(N)	-Ca	(N)	-D	(N)	-Ca,-D	(N)
Bone Marrow Mast Cells								
Ovariectomy + Estrogen	131.6±34.9[a] 26.4-286.1	7	162.1±22.3 44.5-330.9	12	168.1±25.0 80.8-241.0	7	179.5±16.9 104.2-249.2	8
Ovariectomy	112.7±42.7[a] 19.2-288.5	6	118.6±15.9[c] 42.7-201.9	10	290.±56.2[a,e] 116.6-519.8	7	140.9±11.4[b] 103.8-188.4	8
Estrogen	328.3±58.6[a] 192.0-624.0	7	187.4±20.8[e] 97.7-258.2	7	181.5±34.2[b] 79.0-339.9	7	169.5±13.6[a,e] 113.5-217.5	8
No treatment	202.9±20.7 139.9-323.6	8	183.4±25.3 90.4-341.6	8	157.7±40.1 46.5-386.1		273.3±51.7 168.2-524.8	7
Vaginal Tissue Mast Cells								
Ovariectomy + Estrogen	38.7±3.9[b] 25.2-58.4	8	27.8±2.2[b,e] 16.5-154.0	12	42.6±6.2 28.0-80.5	8	38.5±3.2[a] 25.9-50.4	8
Ovariectomy	43.8±6.0[b] 19.4-58.9	6	51.4±7.2[c] 30.8-124.4	12	35.5±3.3[a] 26.7-55.0	8	55.8±6.5[c] 41.0-82.2	8
Estrogen	26.6±1.9 22.7-33.8	7	20.2±1.8[e] 13.9-27.0	7	41.1±10.4 25.0-112.2	8	28.3±4.2 16.7-49.0	8
No treatment	27.2±2.6 14.5-39.3	8	21.7±1.7[d] 15.3-29.2	7	54.4±9.4[e] 26.5-31.1	8	29.5±3.1 19.4-43.2	7

Results are given as the mean ± SE of mast cells/mm^2 surface area.
The range of mast cells/mm^2 is stated below the mean for each group.
Significance of difference between treated and untreated groups: [a] $p<.10$, [b] $p<.05$, [c] $p<0.01$
Significance of difference between deficient diet and normal diet: [d] $p<.10$, [e] $p<.05$

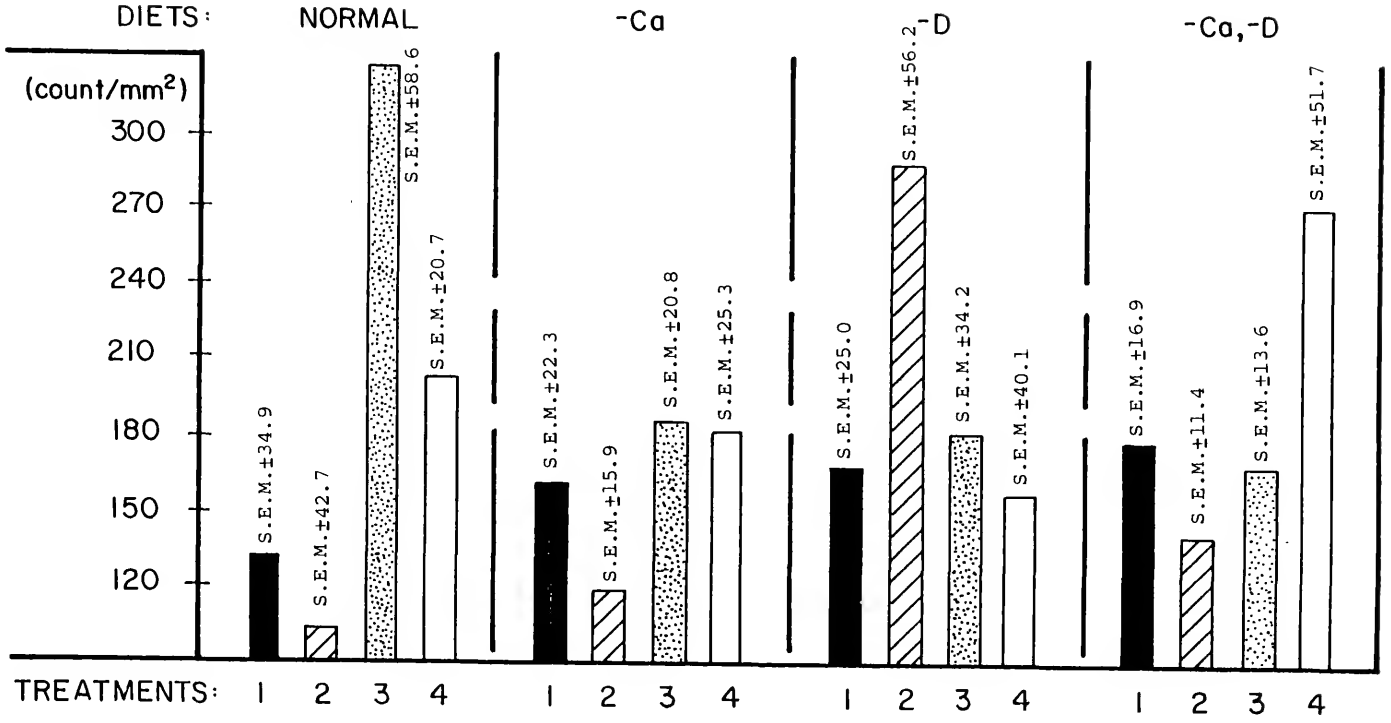


Fig IV.7 Bone marrow mast cell populations in the female rat.

1 Ovariectomy + Estrogen
2 Ovariectomy
3 Estrogen
4 No treatment

Normal diet group. The mean number of mast cells per mm^2 in the bone marrow of these normal, untreated young rats was 202.9±20.7. Bélanger (1977) recorded mast cell populations of 123±16 per mm^2 bone marrow in normally fed rats.

Estrogen increased the count in normally fed, intact rats ($P<.10$)(Fig. IV.7). Bélanger (1977) found no change in count when estrogen was used in rats on normal diets.

Ovariectomy produced a decrease in mast cell count ($P<.10$). When estrogen was given to ovariectomized rats, the count continued to remain below that of the untreated rat ($P<.10$).

Calcium-deficient group. The calcium-deficient, untreated rats in this study showed no change in bone marrow mast cell count from that of the adequately fed rats. This finding was somewhat surprising. The well-known study by Urist and McLean (1957) describes extensive increases in calcium-deficient rat bone marrow. However, no statistical evidence was reported. Their rats were weaned at three weeks to a calcium-deficient diet, whereas the rats on this study began the deficient diet at two months of age. Greatest increases in mast cell counts in their rats were reported as occurring after six to 15 weeks. The rats on the present study were fed a calcium-deficient diet for only six weeks. Rockoff and Armstrong (1970) also experienced marked mast cell hyperplasia in calcium-deficient rats. However,

mean number per field and distribution of cells in calcium-deficient rats did not vary from the normally fed rats in the study of Rasmussen (1972). It may well be that age of rat and duration of calcium deficiency play an important role in mast cell population changes, if alterations do, in fact, occur.

In contrast to normally fed animals, calcium-deficient rats administered estrogen did not show altered counts. However, calcium-deficient, estrogen treated rats did show increased counts when compared to normally fed, estrogen treated rats ($P < .05$).

Ovariectomy produced a significantly decreased mast cell count in this group ($P < .01$).

Vitamin D-deficient group. A combined lack of protein, phosphorus, and vitamin D produced no changes in bone marrow mast cell count in the untreated animals. Estrogen significantly increased the marrow count in the intact rats ($P < .05$) and ovariectomy also increased the count ($P < .10$). Ovariectomized rats also had an increased bone marrow mast cell count when compared to ovariectomized, normally fed rats ($P < .05$) indicating an effect of diet.

Contrary to the above finding, Rasmussen (1972) reported marrow mast cells in a vitamin D-deficient group to be higher than those of normally fed rats. The statistical significance level was not given.

Calcium- and vitamin D-deficient group. Effect of diet in untreated rats in this group was not observed. Rasmussen

(1972) reported significantly higher numbers of marrow mast cells per field in a calcium- and vitamin D-deficient group when compared with a normally fed group.

In the present study, when rats in this dietary group were treated with estrogen, the mast cell count decreased from that of the estrogen treated, normally fed group ($P < .05$) and from the untreated rats in the same calcium- and vitamin D-deficient group ($P < .10$) indicating an effect of diet and treatment.

Ovariectomy also caused a significant decrease in mast cell marrow count ($P < .05$).

It has been suggested that mast cell increases in bone marrow are due to secondary hyperparathyroidism caused by hypocalcemia (Rasmussen, 1972). As was mentioned previously in the serum calcium section, dietary deficiencies did not consistently cause hypocalcemia in the present study nor in other studies. As stated, diet did not affect serum calcium or bone marrow mast cell numbers in the present study.

The rats given estrogen were made hypocalcemic in all deficient diet groups, but did not exhibit mast cell increases. The reverse was found with normally fed rats, i.e., an increased mast cell count was observed in estrogen treated rats with normal serum calcium levels. Hormone treatment may have interfered with the theory mentioned above, even though estrogen receptors are not known to occur in bone.

A consistent decrease in marrow mast cell number was observed in calcium- and vitamin D-depleted, estrogen deficient rats (ovariectomized). With the addition of estrogen, marrow mast cell numbers were returned to the normal range. This finding clearly indicates the presence of hormonal activity in bone.

Vaginal Tissue Mast Cells

The mean number of mast cells per mm^2 vaginal tissue in the different groups of rats is given in Table IV.5 and is illustrated graphically in Fig. IV.8. Photomicrographs of vaginal tissue mast cells observed in this study are contained in Appendix I (Fig. I.5-I.8).

Normal diet group. Vaginal mast cells in the untreated control group numbered 27.2±2.6 with a range of 14.5 to 39.3. Estrogen given to intact rats in this group did not alter the count. However, ovariectomy did increase the count significantly ($P<.05$). Estrogen given to ovariectomized rats, however, did not return the count to a normal range.

Estrogen given to intact mice increased vaginal mast cells substantially (Westin and Odeblad, 1956) again suggesting species difference; however, Johannson and Westin (1959) report estrogen as suppressing true mast cell numbers in mouse vaginal tissue.

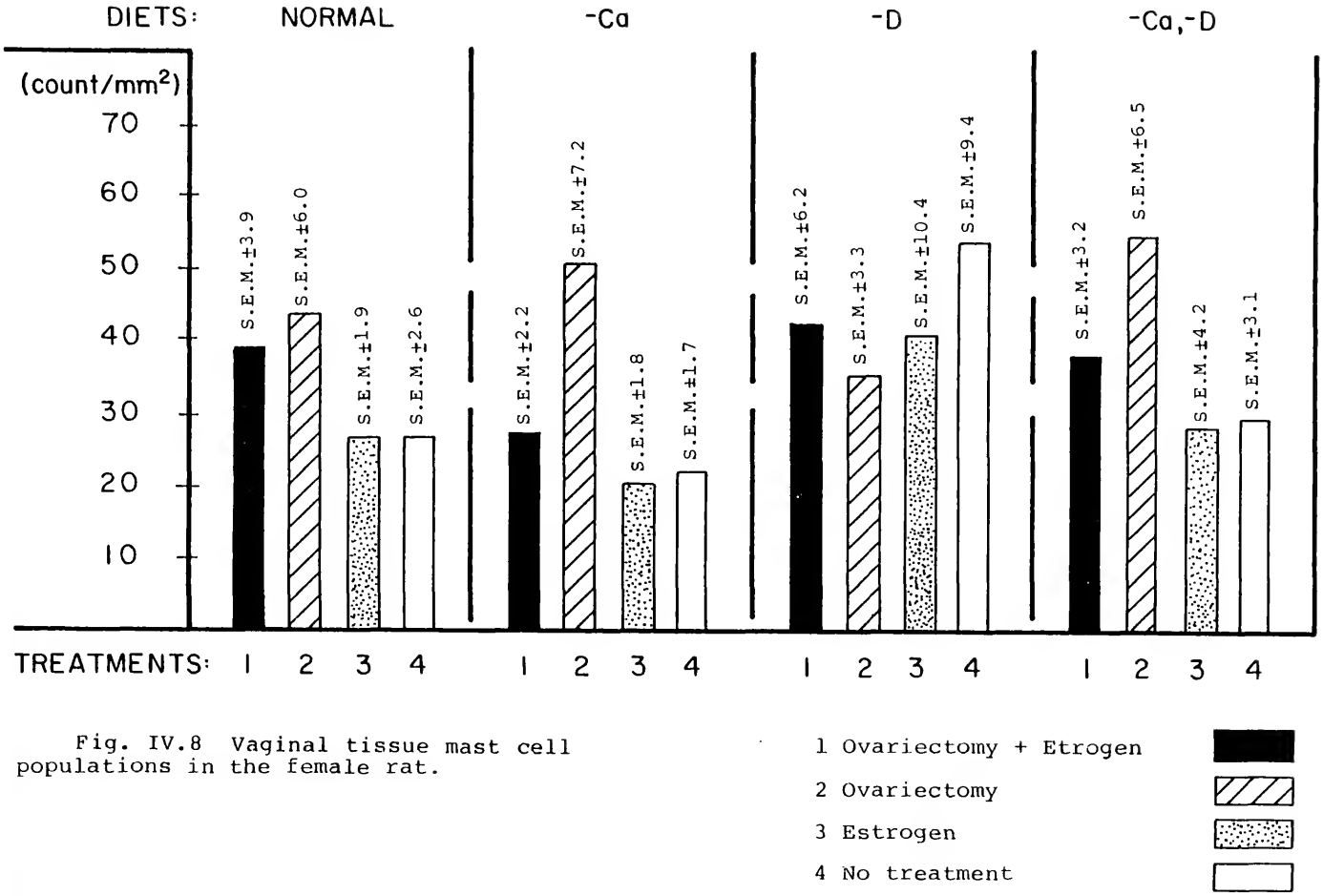


Fig. IV.8 Vaginal tissue mast cell populations in the female rat.

Calcium-deficient group. Calcium deficiency produced a decrease in the vaginal tissue mast cell count at the $P<.10$ level, indicating a very weak, almost non-existent effect.

Calcium deficiency also produced a decrease ($P<.01$) in estrogen treated rats. Likewise, the deficient diet showed an effect of decreasing the mast cell count when these rats were ovariectomized and given estrogen (compared with normally fed rats of similar treatment ($P<.05$).

Within the group, when compared with untreated rats, ovariectomized rats exhibited an increased vaginal tissue mast cell count ($P<.01$). With the addition of estrogen, the cell count was reduced to the normal range of a calcium-deficient animal.

Vitamin D-deficient group. Untreated rats in this group had higher vaginal tissue mast cell counts than normally fed rats ($P<.05$). Treated rats did not display an effect of diet deficiencies on mast cell numbers.

Estrogen did not alter mast cell counts; but lack of estrogen caused a reduction in number ($P<.10$). These rats were given a diet deficient in protein and phosphorus as well as vitamin D; results would possibly be affected by the lack of those nutrients.

Calcium- and vitamin D-deficient group. Diet had no effect on mast cell count in this group.

Within the group, estrogen given to intact rats produced no changes in vaginal tissue mast cell counts. Ovariectomy, however, caused an increase in cell numbers ($P<.01$).

Estrogen added to the ovariectomized rats maintained a count higher than normal for this group ($P<.10$). Overall, mast cell counts in rat vaginal tissue were much less variable within groups than in bone marrow.

Summarizing the influence of diet and treatment on vaginal tissue mast cell populations, one observes that depletion of bone in the rat by dietary calcium deficiency, as evidenced by densitometric bone analysis and bone ash levels, has no effect on the vaginal tissue mast cell number. This is a finding which has not been supported by the literature since studies of vaginal tissue mast cells do not exist.

It is important to examine the pattern of hormonal effects. Estrogen given to intact rats did not affect mast cells in the vaginal epithelium, an estrogen sensitive tissue. But when the rats were deprived of estrogen by ovariectomy, mast cells increased above normal, irrespective of diet. When estrogen was added, the increased populations were maintained above normal, but a hormonal effect of reducing the numbers may be a possibility.

The vitamin D-deficient group does not fit this pattern, most probably because of the effects of additional dietary deficiencies. As an effect of this diet, however, mast cells did increase in vaginal tissue. With ovariectomy a decrease was observed.

CHAPTER V
CONCLUSIONS

Change (reduction) in mast cell numbers in rats made osteopenic by dietary deficiencies of calcium and vitamin D as evaluated by bone densitometry and bone ash content was observed in vaginal tissue as an effect of a calcium-deficient diet at the $P < .10$ level of significance. Because of the weak evidence of mast cell reduction occurring, the first hypothesis is not proven. This finding is not documented in the literature as studies concerning mast cell populations in rat vaginal tissue do not exist.

Bone marrow mast cell populations did not vary as a result of dietary deficiencies. That significant changes in mast cell populations did not occur in the bone marrow was surprising because other studies have indicated a substantial increase in bone marrow mast cells with calcium deficiency and osteoporosis (Urist and McLean, 1957; Frame and Nixon, 1968; Rockoff and Armstrong, 1970; te Velde et al., 1978).

Two possible explanations for lack of change in marrow mast cell numbers are suggested. The rats in the present study were at least two months old before being fed deficient diets. It was desirable to have rats with estrous

cycles in order to observe the effect of removing the circulating estrogen. The rats in the aforementioned studies were weanlings.

In addition, the duration of dietary deficiencies may have been too short to overcome the effect of age, even though osteopenia was indicated. The rats in the present study were kept on deficient diets for a period of five weeks which approximated .05% of their life span. With dietary deficiencies extended over a longer period of time, significant alterations in marrow mast cell numbers may have been seen.

The effects of exogenous estrogen were seen to reduce bone marrow mast cell numbers in rats both calcium and vitamin D deficient, which supports the second hypothesis of this study, in part. An opposite effect of estrogen administration was seen in normally fed rats: the bone marrow increased significantly in mast cells. This inconsistency is not understood. However, these results strongly suggest that bone is an estrogen sensitive tissue, even though estrogen receptors have not been found in bone.

Bilateral ovariectomy significantly affects both vaginal tissue and bone marrow with respect to mast cell populations. Removal of estrogen production in the rat reduces bone marrow mast cells in rats normally fed and also in those deficient in calcium and vitamin D. An opposite effect of estrogen removal is seen in the vaginal tissue. Both observations support the hypothesis that ovariectomy

alters mast cell populations in bone marrow and vaginal tissue of calcium- and vitamin D-deficient rats.

In absolute terms, ovariectomized rats resupplied with estrogen demonstrated increases in vaginal tissue mast cells and a decrease in marrow mast cells. These are changes similar to those stated as an effect of ovariectomy alone. However, upon observing Figs. IV.7 and IV.8, one can detect that replacement of estrogen may be causing a reversal of mast cell population change due to ovariectomy. Statistical analyses between ovariectomized groups with and without estrogen will be performed in the near future to determine whether added estrogen changes mast cell populations in ovariectomized rats.

It is important to consider again the possibility that both age of the rat when begun on a deficient diet and duration of feeding a deficient diet may greatly influence mast cell populations in the two tissues examined in this study.

Ovariectomy, the procedure which removes the endogenous gonadal hormone supply has known consequences relating to bone loss in the human female. Whether mast cell changes similar to those in the rat occur in the human is not known. Considering the results of the present study, a quantitative investigation of mast cell populations in vaginal tissue of ovariectomized women may provide information useful in the study of osteopenia and bone resorption.

It has also become apparent that qualitative investigation of the mast cell populations, including histologic bone evaluations, needs to be considered from data obtained in the present study. The need for further examination of correlations between bone densitometric measurements, bone ash, serum calcium, biomechanical tests and the mast cell populations is also immediate. Statistical analysis is scheduled for these parameters.

APPENDIX A

SIGMA XI GRANT-IN-AID OF RESEARCH

SIGMA XI
THE SCIENTIFIC RESEARCH SOCIETY OF NORTH AMERICA
For the Encouragement of Scientific Research

Grants-in-Aid of Research

Grants-in-Aid of Research are supported by voluntary contributions to the Research Program from the membership of SIGMA XI. Awards are normally made in amounts ranging from $100 (or less) to a maximum of $1,000.

Research awards may be made to support scientific investigation in any field. Each award is made payable to the individual recipient. No part of a grant may be used for the payment of any indirect costs to the recipient's institution–all of the funds must be expended directly in support of the proposed investigation. All equipment purchased shall be the property of the institution. Grants normally are not made for expenses of publication, salary or tuition, travel to meetings, or usual and routine institutional obligations. Priority is usually given to applicants who are in an early stage of their scientific careers.

The Committee on Awards meets on or about the first of March, June, and December of each year and applicants are notified of the Committee's decisions within six weeks. In order to be considered, applications must be received by February 1 for the March meeting, May 1 for the June meeting, and November 1 for the December meeting at Sigma Xi National Headquarters, 345 Whitney Avenue, New Haven, Connecticut 06511, Attention: Committee on Awards.

Franklyn B. Van Houten

Franklyn B. Van Houten, *Chairman*

APPLICANT: *Please fill in these three items only.*

APPLICANT: Tesar (LAST NAME) Rogene (FIRST NAME) E. (MIDDLE NAME)

FIELD: Nutrition and Osteoporosis

TITLE OF STUDY: The Relationship Between Epithelial and Connective Tissue Mast Cell Populations in the Female Rat

FOR COMMITTEE USE ONLY

Date Received.....

Amount Requested:

.....

Action:

Not Granted

Granted:

Full ($)
Partial ($)
Conditional ($)

Date of mailing award:

.....

Date of receipt of final report:

.....

COMMENTS AND RECOMMENDATIONS	
COMMITTEE ACTION	
DATE OF MEETING	
ADDITIONAL REMARKS	

Please type or print all information

APPLICATION FOR GRANT-IN-AID OF RESEARCH

Name Tesar (LAST NAME) Rogene (FIRST NAME) E. (MIDDLE NAME)

Address 6916 N.W. 20th Place, Gainesville, Florida 32605

Age 42

Present position and institution Graduate Assistant, Department of Obstetrics and Gynecology, University of Florida

Degrees, institutions conferring them, dates B.Sc. (Home Economics) Kansas State University, 1962; B.Sc. (Food Science) University of Florida, 1977; M.Ag. (Human Nutrition) University of Florida, 1979; Ph.D. (in progress) University of Florida

Membership in SIGMA XI non-member

Please attach a list of titles of articles published during the last five years, with names of periodicals and dates: List of titles appended

Title of proposed investigation: The Relationship Between Epithelial and Connective Tissue Mast Cell Populations in the Female Rat

Proposed investigation, described in non-technical language:

Previous studies suggest a relationship between bone marrow mast cell (MC) activity and local bone loss. There is also evidence that changes in skin MC activity may be indicative of bone loss. The proposed study is designed: 1) to determine whether a correlation exists in female rats between MC activity in bone marrow and vaginal tissue and 2) to examine the effects of calcium- and vitamin D-deficient diets, exogenous estrogens and removal of the ovaries on this relationship.

The following hypotheses will be tested: 1) dietary calcium and vitamin D deficiences produce an increase in bone marrow MCs and a decrease in vaginal epithelial MCs in female rats; 2) administration of exogenous estrogens alters the bone and vaginal tissue MC activity in the osteoporotic female rat; and 3) removal of the ovaries (removal of primary source of endogenous estrogens) produces changes in bone and vaginal tissue MC activity in the female rat.

Should the correlation be shown to exist, a similar correlation in the pre-osteoporotic and osteoporotic human female could be suggested.

At present there is no universally applicable, non-invasive method for evaluation of bone resorption and formation. Evaluation of MC activity in vaginal tissue may prove useful as a non-invasive means of detecting increased bone resorption (indicative of osteoporosis associated with endogenous or exogenous excess of corticosteroids, hyperthyroidism, hyperparathyroidism and osteomalacia). In a similar manner, the method could be used to assess treatment efficacy.

Locations where problem will be studied: Department of Animal Resources, J. Hillis Miller Health Center, University of Florida; Department of Animal Science, College of Agriculture, University of Florida.

Nature of assistance desired and amount of grant needed, itemized:

Purchase of 128 Sprague-Dawley female rats	$544.00
Feed and bedding for above rats for 5 wk. period	394.25
TOTAL	$938.25

Institutional support for study of problem: Remaining necessary support to carry out entire research project: anesthesia, estrogen, stains and chemicals, microscope slides and use of all equipment.

Previous grants received from SIGMA XI and others: none

Other applications pending: none

Attach a list giving name of each assistant or co-worker, if any, engaged in the investigation:

List attached X *Number of co-workers* 7

Names and addresses of at least two specialists in this field who will be ASKED BY THE APPLICANT to send to Sigma Xi National Headquarters statements indicating (1) the importance of the proposed investigation and (2) the qualifications of the investigator.*

Morris Notelovitz, M.D., Ph.D., Dept. of Obstetrics and Gynecology, Box J-294 JHMHC, College of Medicine, U of F, Gainesville, FL 32610

J.P. Feaster, Ph.D., Dept. of Animal Science, 20 Nutrition Lab, IFAS College of Agriculture, U of F, Gainesville, FL 32610

**If applicant is a degree candidate, one must be that faculty or research staff member supervising his research.*

Applicant's Signature [signature]

Date October 28, 1980

Title: The Relationship Between Epithelial and Connective Tissue Mast Cell Populations in the Female Rat

Investigator: Rogene Tesar

Assistants and Co-workers Engaged in Investigation:

Morris Notelovitz, M.D., Ph.D.	Advisor - Endocrine Functions
J.P. Feaster, Ph.D.	Advisor - Dietary
A.F. Moreland, D.V.M.	Laboratory Assistance
Laboratory Technician of Dr. Moreland	Laboratory Assistance
Marsha Ware	Laboratory Assistance
Lynda McKenzie, R.N.	Laboratory Assistance
Cindy Soroski	Statistical Assistance

The University of Florida College of Medicine

Department of Obstetrics and Gynecology

BOX J-294, JHMHC
GAINESVILLE, FLORIDA 32610
TELEPHONE: 904-392-2893

November 26, 1980

Committee on Awards
Sigma Xi
National Headquarters
345 Whitney Avenue
New Haven, CT 06511

RE: Application by Rogene Tesar for grant to investigate the relationship between epithelial and connective tissue mast cell population in the female rat.

Sirs,

The above application relates to the potential role that mast cell activity may have in the development of osteoporosis, a condition that affects some 25% of menopausal women and about 75% of women who have undergone a surgical menopause. Osteoporosis is a significant disease since it is not only associated with pathologic fractures and potential invalidism, but can frequently result in the premature death of elderly women. One of the problems in the clinical management of this condition is the difficulty of its diagnosis and measurement of its response to treatment. Mrs. Tesar's research could provide the answer to this problem.

I have been acquainted with Mrs. Tesar for approximately two years and am currently an advisor to her for her Ph.D. requirements. She is a highly competent research worker, and I am confident that she will be able to accomplish all the goals of her research project. I have no hesitation in supporting her request.

Warm kind regards.

Yours sincerely,

Morris Notelovitz, M.D.
(RAND), Ph.D., F.R.C.O.G.,
F.A.C.O.G.
Director of the Center for
Climacteric Studies

MN:mlh

SIGMA XI, THE SCIENTIFIC RESEARCH SOCIETY

OFFICE OF THE
COMMITTEE ON AWARDS

345 WHITNEY AVENUE
NEW HAVEN, CONNECTICUT 06511
(203) 624-9883

20 April 1981

Ms. Rogene E. Tesar
6916 N.W. 20th Place
Gainesville, FL 32605

Dear Ms. Tesar:

I am happy to inform you that at a recent meeting of the Committee on Awards a Grant-in-Aid of Research of $250.00 was given you to further the work in your application: The Relationship Between Epithelial and Connective Tissue Mast Cell Populations in the Female Rat. Please complete the enclosed acceptance form so that we may write and forward your check.

This award is one of eight made possible this year from the income of a specific gift to the Research Fund by Mrs. Daisy Yen Wu in memory of her husband, Dr. Hsien Wu.

It is understood that in accepting this award you will at the close of the academic year (1981-82) submit a report of the work done to the Committee on Awards, Sigma Xi, The Scientific Research Society, 345 Whitney Avenue, New Haven, Conn. 06511. This should be a short one or two-page summary of the work accomplished with your Sigma Xi grant.

It is further understood that all published reports of your work will contain a statement that the research was aided by a Grant-in-Aid of Research from Sigma Xi, The Scientific Research Society. Also, any equipment purchased with the funds which have been made available is to be considered the property of the institution where the research is being carried on. It is also to be understood that no indirect costs are to be paid to your institution from this grant.

It is a great pleasure to express the Committee's hope for your continued success in scientific research.

Sincerely yours,

F. B. Van Houten

Franklyn B. Van Houten
Chairman

FVH/ia
Enclosure

Date April 27, 1981

Committee on Awards
Sigma Xi, The Scientific Research Society
345 Whitney Avenue
New Haven, CT 06511

Gentlemen:

I have received your letter stating that a grant has been awarded to me by the Committee on Awards.

I. (X) I accept the award in the amount of $250.

( ) I cannot accept the award because _____

_____

( ) I shall let you know by _____ whether or not I can accept it.

II. The name of the President or Chancellor or Chief Executive Officer of my institution is:

President: Robert Q. Marston

III. The name of the Head or Chairman of my Department is:

Animal Science: H.D. Wallace, Chairman
Center for Climacteric Studies:
M. Notelovitz, M.D., Ph.D.

IV. Will you please have Sigma Xi, the Scientific Research Society, forward to me a check made payable for the amount of the award. I understand that it will be sent to my institutional address only, and I have made arrangements for its being forwarded if necessary.

NAME (Please Print): Rogene Tesar, R.D., M.Ag.

INSTITUTIONAL ADDRESS ONLY: Center for Climacteric Studies
Univ. of Fla., 901 N.W. 20 Pl.
Suite B-1 Gainesville, FL 32601

Rogene Tesar
Signature

SIGMA XI, THE SCIENTIFIC RESEARCH SOCIETY

OFFICE OF THE
EXECUTIVE DIRECTOR

345 WHITNEY AVENUE
NEW HAVEN, CONNECTICUT 06511
(203) 624-9683

10 June 1981

Ms. Rogene E. Tesar
University of Florida
Center for Climacteric Studies
901 N.W. 20th Place
Suite B-1
Gainesville, FL 32601

Dear Ms. Tesar:

Enclosed please find our check in the amount of $250.00, which represents the Grant-in-Aid of Research award made to you by the Sigma Xi Committee on Grants-in-Aid at their March meeting.

This award is one of eight made possible this year from the income of a specific gift to the Research Fund by Mrs. Daisy Yen Wu in memory of her husband, Dr. Hsien Wu.

Upon completion of your research a report of your findings should be forwarded to the Committee on Grants-in-Aid, 345 Whitney Avenue, New Haven, CT 06511.

May I take this opportunity to wish you every success with your research.

Sincerely,

Thomas T. Holme
Executive Director

TTH/ia
Enclosure

cc: Office of the President
Department Chairman

APPENDIX B

LABORATORY ANIMAL USE

All University Committee on the Care and Use
of Laboratory Animals

In order to comply with DHEW policy and all federal, state and local rules and regulations concerning the care, treatment and use of laboratory animals, the following information is necessary for grants to be processed by the Division of Sponsored Research. Instructional programs and research projects supported internally using laboratory animals must also complete this questionnaire and return to the Committee completing the appropriate parts.

1. Principal investigator: Rogene E. Tesar
   Department Obstetrics and Gynecology Telephone 392-3184 Date Nov. 17, 1980
   College College of Medicine

2. Proposal submitted to: Sigma Xi (Name) 345 Whitney Ave., New Haven, Conn. 06511 (Address)

3. Starting date: December 15, 1980; conclusion: August 30, 1981

4. Proposal title:

   The Relationships Between Epithelial and Connective Tissue Mast Cell Populations in the Female Rat

5. Animal species: Rat
   Strains Sprague-Dawley
   Numbers 137
   Sex Female
   Age 3 weeks and 2 months
   Size 50 gram and 120 gram

6. Is the animal-model appropriate?
   Explain Yes. The female rat has been used in many experiments involving the mast cell. The metabolism of the rat closely relates to that of the human and will produce results due to the treatment given which can be implicated as occurring in the human.

7. Abstract of animal use:
   (Use continuation sheet if needed)
   137 rats will be utilized to observe differences in population and activity of the mast cell in bone and vaginal tissue. Of these rats, 12 will be utilized for a preliminary trial to define whether mature rats or weanlings are to be used for treatment (a) castration. Treatments are as follows: a) castration (70 rats) at beginning of study b) estrogen (LV.) (67 rats) 3 X week for 5 weeks, c) normal diet (32 rats), d) calcium deficient diet (32 rats) e) vitamin D deficient diet (32 rats), f) calcium and vitamin D deficient diet (32 rats), (continued)

8. Care and location of animals:
   The rats will be maintained at the Animal Resources Department, JHMHC, University of Florida in individual cages with daily care and feeding.

9. Is the study designed to avoid inflicting needless pain and/or suffering including the appropriate use of tranquilizers, analgesics and/or anesthetics? If not, explain: Yes.
   Anesthesia will be used for surgery purposes.

10. Method(s) of euthanasia to be used:
    At the end of a maximum 5 week feeding period, the rats will be killed by decapitation

Rogene Tesar
Principal Investigator
Rogene Tesar, M.A., R.D.

Eduard G. Friedrich, Jr.
Department Chairman
Eduard G. Friedrich, Jr., M.D.

All University Committee on the Care and Use
of Laboratory Animals
Continuation Sheet

7. h) photon absorptiometry of the femur and tibia during the 5 week diet period. Castration (removal of ovaries) will be performed under anesthetization. At the termination of the 5 week diet period the rats will be killed by decapitation. Bone and vaginal tissue specimens will be obtained for histological purposes.

University of Florida
All-University Committee for
The Care and Use of
Laboratory Animals

1. Acknowledge receipt of your form for the care and use of laboratory animals entitled:

   "The Relationship Between Epithelial and Connective Tissue Mast Cell Populations in the Female Rat" submitted 11/17/80.

2. Review Results

   Approval: XX

   Disapproval: _____

   Incomplete-please provide _____

3. For questions, please call: Dr. Halliwell at (904) 392-4751.

APPENDIX C
EXPERIMENTAL ANIMAL BODY WEIGHTS

TABLE C.1
NORMAL DIET

Sample ID	0W	1W	2W	3W	4W	5W
1	192	197	195	191	195	196
2	175	184	189	197	200	204
3	184	198	202	208	213	212
4	180	186	187	190	192	193
5	185	198	198	201	204	209
6	183	183	189	196	199	200
7	186	187	195	197	197	198
8	197	204	237	231	234	237
9	195	195	227	259	276	280
10	189	196	251	299	321	332
11	185	196	240	264	282	294
12						
13	212	205	248	291	308	315
14	195	198	253	282	297	309
15	177	175	222	257	270	278
16	184	195	259	298	325	337
17	177	182	206	217	219	217
18	183	187	197	205	209	206
19	183	202	199	228	231	230
20	197	191	215	209	214	216
21	191	196	206	214	204	206
22						
23	184	190	190	208	204	209
24	201	209	254	242	241	245
25	194	203	225	234	244	250
26	206	204	230	238	255	264
27	196	209	228	247	251	255
28	200	209	242	267	275	285
29	171	188	201	217	226	228
30	180	191	231	250	256	263
31	209	214	241	267	273	275
32	194	199	226	245	252	259

Weights expressed in grams

TABLE C.2
-Ca DIET

Sample ID	0W	1W		2W		3W		4W		5W	6W
1	161	179	176	179	177	169	171	176	176	175	181
2	187	204	197	197	196	195	196	202	196	195	205
3	181	192	189	190	197	193	198	196	198	197	195
4	183	202	201	197	196	196	193	199	199	208	209
5	175	175	176	187	190	188	196	200	201	202	200
6	175	187	185	185	184	181	188	188	195	195	197
7	177	194	192	182	173	185	187	189	188	186	190
8	183	198	205	210	210	210	215	213	214	212	215
1a	211	211		211		215		216		222	
2a	197	201		213		212		213		211	
3a	182	187		187		194		200		194	
4a	188	196		203		203		209		211	
9	181	191	210	225	239	247	255	261	275	274	290
10	167	174	190	205	220	226	236	244	250	259	265
11	190	204	222	248	262	275	286	295	301	305	310
12	204	210	233	264	282	295	310	320	328	336	342
13	170	178	203	221	238	242	253	259	268	275	285
14	168	184	200	228	247	257	270	276	279	290	294
15	194	212	226	241	255	260	262	263	258	265	265
16	185	199	217	242	258	274	291	299	303	310	317
5a	191	192		251		275		292		298	
6a	177	177		214		253		269		278	
7a	186	196		243		283		295		306	
8a	183	191		239		275		291		299	
17	184	207	192	195	194	200	203	213	208	215	218
18	196	195	201	205	205	209	215	224	223	233	228
19	187	201	204	215	219	229	233	233	234	242	235
20	202	217	213	220	229	236	233	239	239	234	244
21	172										
22	167	186	184	192	190	191	191	201	203	207	207
23	183	198	197	206	207	215	230	215	215	214	204
24	200	216	217	225	219	230	216	235	232	234	220
25	181	189	189	199	199	202	205	210	209	210	210
26	184	198	210	220	225	228	239	246	246	245	249
27	187	195	203	214	219	224	230	233	236	242	241
28	181	188	194	201	206	210	212	216	219	222	223
29	173	190	203	208	216	217	223	228	228	235	235
30	177	198	211	223	225	228	234	239	242	245	247
31	185	204	210	219	226	231	242	247	239	242	245
32	191	204	213	221	228	232	241	248	242	245	249

Weights expressed in grams

TABLE C.3
-D DIET

Sample ID	0W		1W	2W	3W	4W
1	144	161	165	164	172	168
2	157	166	167	167	170	176
3	146	173	171	167	174	174
4	158	181	179	186	196	198
5	146	165	166	165	176	180
6	151	173	177	171	175	174
7	154	171	169	168	173	177
8	154	180	175	172	171	172
9	156	180	187	207	223	232
10	175	190	193	208	224	234
11	161	173	174	189	204	214
12	153	165	172	182	200	216
13	203	192	196	213	231	250
14	184	183	184	203	210	219
15	173	166	164	179	201	213
16	177	176	179	203	220	230
17	202	209	199	196	210	218
18	134	145	146	149	161	163
19	136	151	157	158	170	176
20	142	165	169	169	173	177
21	148	174	177	181	195	193
22	161	189	190	189	203	206
23	139	157	158	163	169	172
24	152	177	181	183	190	194
25	133	149	158	168	173	188
26	154	176	182	188	201	210
27	149	172	171	185	197	208
28	155	181	184	203	210	217
29	150	169	174	182	191	199
30	175	171	174	182	193	197
31	187	180	179	195	211	219
32	184	183	185	191	191	203

Weights expressed in grams

TABLE C.4
-Ca, -D DIET

Sample ID	0W	1W	2W	3W	4W	5W
1	204	217	211	223	229	228
2	204	215	210	224	225	217
3	199	207	199	213	224	231
4	176	184	180	187	194	196
5	194	202	190	197	207	206
6	187	192	190	201	203	202
7	218	225	216	230	239	242
8	184	185	186	194	196	197
9	205	205	234	269	294	315
10	171	183	214	251	272	287
11	212	194	258	303	329	343
12	191	200	238	278	305	318
13	187	198	223	262	280	300
14	197	214	239	283	306	323
15	212	223	224	240	248	258
16	197	208	204	224	228	242
17	196	205	209	246	222	177
18	196	209	224	214	252	253
19	221	229	234	244	258	246
20	195	197	209	224	234	215
21	198	210	209	212	200	190
22	201	212	216	230	232	231
23	210	212	215	234	245	234
24	200	201	202	220	229	198
25	211	224	248	246	268	268
26						
27	217	225	237	243	259	266
28	205	213	220	231	259	257
29	214	229	243	257	270	277
30	194	201	219	225	253	247
31	194	203	221	229	241	247
32	207	208	242	225	268	280

Weights expressed in grams

APPENDIX D
COMPOSITION OF EXPERIMENTAL DIETS

AIN-76 Semipurified Diet (Ca:P 1.14:1)*

Casein	20.0%	
DL-Methionine	0.3%	
Cornstarch	15.0%	
Sucrose	50.0%	
Fiber	5.0%	
Corn Oil	5.0%	
AIN Mineral mix	3.5%	g/kg Mixture
Calcium Phosphate, Dibasic ($CaHPO_4$)		500.0
Sodium Chloride (NaCl)		74.0
Potassium Citrate, Monohydrate $(HOC(COOK)CH_4COOK)_4 \cdot H_2O$		220.0
Potassium Sulfate (K_2SO_4)		52.0
Magnesium Oxide (MgO)		24.0
Manganous Carbonate (43-48% Mn)		3.5
Ferric Citrate (16-17% Fe)		6.0
Zinc Carbonate (70% ZnO)		1.6
Cupric Carbonate (53-55% Cu)		0.3
Potassium Iodate (KIO_3)		0.01
Sodium Selenite ($Na_2SeO_3 \cdot 5H_2O$)		0.01
Chromium Postassium Sulfate ($CrK(SO_4)_2 \cdot 12H_2O$)		0.55
Sucrose, finely powdered		118.0
All Vitamin mix	1.0%	per kg Mixture
Thiamine HCl		600 mg
Riboflavin		600 mg
Pyridoxine HCl		700 mg
Nicotinic Acid		3 mg
D-Calcium Pantothenate		1.6 mg
Folic Acid		200 myl Acetate
(Vit. E), Pre-mix		20 g
Cholecalciferol (Vit. D_3)		2.5 mg
Menaquinone (Vit. K)		5.0 mg
Sucrose, finely powdered		972.9 g
Choline bitrate	0.2%	

*Obtained from Nutritional Biochemicals, Cleveland, OH

Calcium Deficient Diet (Ca:P .07:1)*

Casein (purified)	24.0%	
Sucrose	68.0%	
Corn Oil	5.0%	
Calcium Free Salt Mixture	3.0%	
Dipotassium Phosphate		52.873%
Monosodium Phosphate		10.313%
Magnesium Sulfate		8.188%
Sodium Chloride		23.125%
Ferric Citrate		4.500%
Potassium Iodine		0.130%
Manganese Sulfate		0.741%
Zinc Chloride		0.080%
Copper Sulfate		0.050%

Plus Special ICN Vitamin Diet Fortification Mixture:

	gm/kg
Vitamin A Concentrate (200,000 units/gm)	.1
Vitamin D Concentrate (400,000 units/gm)	.006
Alpha Tocopherol	.1
Ascorbic Acid	1.0
Inositol	.1
Choline Chloride	1.7
Menadione	.05
-Aminobenzoic Acid	.1
Niacin	.1
Riboflavin	.02
Pyridoxine Hydrochloride	.02
Thiamine Hydrochloride	.02
Calcium Pantothenate	.007
	mgs/kg
Biotin	.4
Folic Acid	2.0
Vitamin B-12	.03

Rachitogenic U.S.P. No. 2 Diet (Ca:P 4.23:1)*

Ground Gluten	20%
Ground Whole Yellow Maize	76%
Calcium Carbonate	3%
Sodium Chloride	1%

Custom Vitamin D and Calcium Deficient Diet (Ca:P .07:1)*

Based on the Calcium-Deficient Diet
with omission of Vitamin D Concentrate

* Obtained from Nutritional Biochemicals, Cleveland, OH

** Formulated and obtained from Nutritional Biochemicals, Cleveland, OH

APPENDIX E
SERUM CALCIUM ANALYSIS

Sample Collection and Procedure[1]

Blood was obtained at decapitation of the laboratory rats by exsanguanation. The blood was collected in tubes, allowed to clot and centrifuged. Serum was drawn off using Pasteur glass pipettes, transferred to clean tubes, labeled, and frozen for future determination.

A protein-free filtrate was required for analysis of calcium. After thawing the serum samples at room temperature for one half-hour, precipitation of serum protein was accomplished as follows:

(a) 9 ml 10% (w/v) trichloracetic acid (TCA) were delivered into labeled test tubes.

(b) 1 ml serum from each well-mixed sample was pipetted into the TCA.

(c) the solutions were mixed on a vortex mixer, allowed to stand for 10 minutes, and centrifuged 10 minutes at 2,500 rpm. This filtrate represented a 1→10 dilution of the serum samples.

(d) 1 ml of the supernatant was diluted to 5 ml with 1% lanthanum. The dilution factor was 10 x 5 = 50.

[1] Fick et al., 1979

This procedure provided the appropriate serum calcium concentration for the reading of absorbance by the Perkin-Elmer 306 atomic absorption spectrophotometer (AAS), which has a linear working range of 7 ppm for calcium. Confirmation of the above is shown by the following calculation:

$$\text{calcium concentration} = \frac{(\text{ppm calcium expected for original sample}) \times (\text{ml sample})}{\text{sample dilution}}$$

$$\text{calcium concentration} = \frac{(100 \text{ ppm}) \times (1 \text{ ml})}{50 \text{ ml}}$$

calcium concentration = 2 ppm.

Calcium standards of 0, 2, 3, 4, 5, and 7 µg/ml were prepared in 100 ml volumetric flasks. The 1,000 ppm stock standard calcium solution was first diluted to 100 ppm. Each standard was made to contain 18 ml of 10% TCA to match the final dilution of serum and 16 ml of 5% lanthanum. Table E.1 lists the concentration and absorbance of the standards, as read by AAS.

TABLE E.1
SERUM CALCIUM STANDARDS

Standard µg/ml	Readout Absorbance (A)	Calculated Slope (a)
0	.000	.000
2	.074	.037
3	.120	.040
4	.164	.041
5	.184	.037
7	.268	.038

Calcium in the sample solutions was then measured for absorbance by AAS and concentration was calculated as mg/100 ml serum as follows:

$$\text{sample ppm} = \frac{\text{(absorbance)} \times \text{(dilution factors)}}{\text{(slope)} \times \text{(sample weight)}}$$

This equation is derived from Beer's Law, which states that

$$A = abc$$

where

A = absorbance (optical density)

a = absorptivity or slope of the standard

b = length of the light path (always constant)

c = concentration

The absorbance reading was obtained from the machine. The slope was determined as an average of the slopes of the standards (a = A/c from Beer's Law). The slope used in calculations for samples was .0386, obtained from Table E.1.

Tables E.2 through E.5 contain serum calcium concentration values of the laboratory rats.

TABLE E.2
SERUM CALCIUM ANALYSIS DATA

Rat Diet Normal Date 2/16/82
Total Dilution 50 Technician R. Tesar

Sample ID	Readout Absorbance	ppm	mg/100 ml
1	.100	129.5	13.0
2	.099	128.2	12.8
3	.096	124.4	12.4
4	.097	125.6	12.6
5	.097	125.6	12.6
6	.102	132.1	13.2
7	.102	132.1	13.2
8	.082	106.2	10.6
9	.103	133.4	13.3
10	.067	86.8	8.7
11	.096	124.4	12.4
12			
13	.084	108.8	10.9
14			
15	.088	114.0	11.4
16	.092	119.2	11.9
17	.082	106.2	10.6
18	.088	114.0	11.4
19	.091	117.9	11.8
20	.092	119.2	11.9
21	.098	126.9	12.7
22			
23	.088	114.0	11.4
24	.093	120.5	12.1
25	.062	80.3	8.0
26	.074	95.9	9.6
27	.092	119.2	11.9
28	.070	90.7	9.1
29	.068	88.1	8.8
30	.068	88.1	8.8
31	.073	94.6	9.5
32	.087	112.7	11.3

TABLE E.3
SERUM CALCIUM ANALYSIS DATA

Rat Diet -Ca Date 2/16/82
Total Dilution 50 Technician R. Tesar

Sample ID	Readout Absorbance	ppm	mg/100 ml
1	.074	95.9	9.6
2	.064	82.9	8.3
3	.068	88.1	8.8
4	.060	77.7	7.8
5	.074	95.9	9.6
6	.074	95.9	9.6
7	.064	82.9	8.3
8	.074	95.9	9.6
1a	.069	89.4	8.9
2a	.071	92.0	9.2
3a	.064	82.9	8.3
4a	.064	84.2	8.4
9	.082	106.2	10.6
10	.082	106.2	10.6
11	.076	98.4	9.8
12	.093	120.5	12.1
13	.076	98.4	9.8
14	.073	94.6	9.5
15	.076	98.4	9.8
16	.095	123.1	12.3
5a	.074	95.9	9.6
6a	.056	72.5	7.3
7a	.084	108.8	10.9
8a	.082	106.2	10.6
17	.077	99.7	9.9
18	.072	93.3	9.3
19	.071	92.0	9.2
20	.071	92.0	9.2
21			
22	.071	92.0	9.2
23	.068	88.1	8.8
24	.078	101.0	10.1
25	.071	92.0	9.2
26	.073	94.6	9.5
27	.065	84.2	8.4
28	.070	90.7	9.1
29	.073	94.6	9.5
30	.070	90.7	9.1
31	.070	90.7	9.1
32	.068	88.1	8.8

TABLE E.4
SERUM CALCIUM ANALYSIS DATA

Rat Diet -D
Total Dilution 50
Date 2/16/82
Technician R. Tesar

Sample ID	Readout Absorbance	ppm	mg/100 ml
1	.080	103.6	10.4
2	.059	76.4	7.6
3	.065	84.2	8.4
4	.065	84.2	8.4
5	.067	86.8	8.7
6	.077	99.7	10.0
7	.070	90.7	9.1
8	.068	88.1	8.8
9	.057	73.8	7.4
10	.047	60.9	6.1
11	.078	101.0	10.1
12	.076	98.4	9.8
13	.082	106.2	10.6
14	.078	101.0	10.1
15	.079	102.3	10.2
16	.090	116.6	11.7
17	.079	102.3	10.2
18	.077	99.7	10.0
19	.066	85.5	8.6
20	.064	82.9	8.3
21	.073	94.6	9.5
22	.073	94.6	9.5
23	.061	79.0	7.9
24	.065	84.2	8.4
25	.082	106.2	10.6
26	.068	88.1	8.8
27	.079	102.3	10.2
28	.067	86.8	8.7
29	.064	82.9	8.3
30	.081	104.9	10.5
31	.072	93.3	9.3
32	.066	85.5	8.6

TABLE E.5
SERUM CALCIUM ANALYSIS DATA

Rat Diet -Ca, -D Date 2/16/82
Total Dilution 50 Technician R. Tesar

Sample ID	Readout Absorbance	ppm	mg/100 ml
1	.059	76.4	7.6
2	.070	90.7	9.1
3	.071	92.0	9.2
4	.058	75.1	7.5
5	.057	73.8	7.4
6	.053	68.7	6.9
7	.072	93.3	9.3
8	.057	73.8	7.4
9	.053	68.7	6.9
10	.063	81.6	8.2
11	.079	102.3	10.2
12	.062	80.3	8.0
13	.057	73.8	7.4
14	.069	89.4	8.9
15	.065	84.2	8.4
16	.074	95.9	9.6
17	.031	40.2	4.0
18	.067	86.8	8.7
19	.043	55.7	5.6
20	.043	55.7	5.6
21	.031	40.2	4.0
22	.060	77.7	7.8
23	.036	46.6	4.7
24	.025	32.4	3.2
25	.055	71.2	7.1
26			
27	.038	49.2	4.9
28	.023	29.8	3.0
29	.031	40.2	4.0
30	.017	22.0	2.2
31	.023	29.8	3.0
32	.048	62.2	6.2

APPENDIX F
DENSITOMETRIC BONE ANALYSIS

Instrumentation and Procedure

A Norland Digital Bone Densitometer Model 278 (Norland Instruments, Ft. Atkinson, WI) was used to measure bone mineral content, bone width, and bone length in the excised, cleaned rat femur by direct photon absorptiometry. This technique measures the attenuation of a beam of gamma radiation by calcified tissue and is based on the concept that the mass of bone mineral present is directly proportional to the attenuation by bone (Sanchez et al., 1980).

The densitometer consisted of a scanner module and a computer module (Fig. F.1). The scanner transported a highly collimated beam of monoenergetic gamma rays from a radioactive sealed source of ^{I25}I across the bone being measured. A 1/16" diameter detector collimator and a threshold setting of 85% were used to enhance accuracy. The computer module calculated the bone mineral content (BMC) and bone width (BW) values from the resulting absorption curve. These values and the bone profiles were displayed on the computer module screen. See Fig. F.2.

BMC and BW measurements were made at six distinct scan sites of the femoral bone, beginning at the edge of the

lesser trochanter (proximal end) and progressing to the widening of the distal end. Scans were made perpendicular to the bone axis.

The quantity measurement of BMC is a linear mass density of g/cm length of bone, an average linear density over the approximately 4 mm wide scan path. In terms of another explanation, BMC is the grams of mineral which would be obtained if a 1 cm thick crosswise slice were cut out of the bone and this slice were heated in a crucible to burn away all non-mineral material (Norland Corp., 1980).

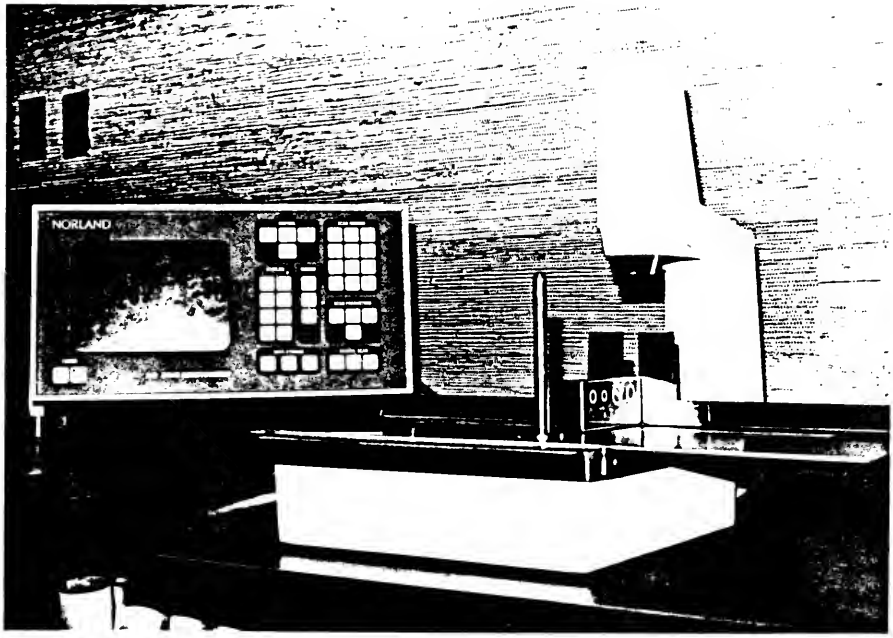
BW represents the distance in cm from one longitudinal edge of the bone to the other.

The value of BMC/BW, calculated by the computer module, provides a measurement of linear bone density in cm^2. The entire depth of the bone, i.e., the distance from top surface to bottom surface of a bone lying flat, is included in this measurement. The BMC, BW, and BMC/BW values are recorded in Tables F.1 through F.4.

Bone lengths were measured by placing each of the bones in a longitudinal position along the scan path of the scanner module. Data on these lengths are recorded in Table F.5.

Fig. F.1 The Norland Digital Bone Densitometer, Model 278, with computer module at left. The densitometer scanner module transports a highly collimated beam of monenergetic gamma rays from a radioactive sealed source (^{125}I) across the bone to be measured. The computer module calculates the values from the resulting absorption curve and displays the results on the screen. A calibration standard is shown on the scanner deck.

Fig. F.2 Printout display of rat femur profile. Measurement results are BMC (bone mineral content-mass) expressed in grams per cm, BW (bone width) in cm, BMC/BW in grams per cm^2.


NORLAND

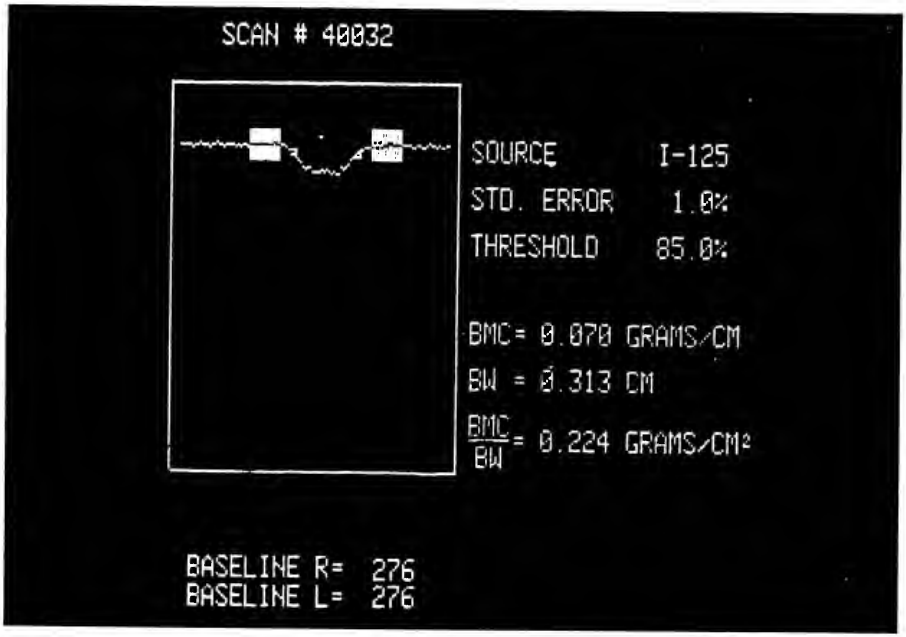

SCAN # 40032
SOURCE I-125
STD. ERROR 1.0%
THRESHOLD 85.0%
BMC= 0.070 GRAMS/CM
BW = 0.313 CM
BMC/BW = 0.224 GRAMS/CM²
BASELINE R= 276
BASELINE L= 276

TABLE F.1
DENSITOMETRIC BONE ANALYSIS
BMC (g/cm) AND BMC/BW (g/cm^2)

Bone Rat Femur
Rat Diet Normal

Date 12/10/ 81
Technician R. Tesar

Sample ID	Measurement											
	1		2		3		4		5		6	
	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW
1	.118	.321	.108	.342	.102	.318	.100	.290	.106	.301	.111	.321
2	.126	.334	.112	.350	.104	.324	.101	.310	.102	.330	.113	.290
3	.128	.286	.118	.335	.103	.360	.093	.352	.099	.304	.099	.308
4	.120	.284	.102	.313	.097	.273	.093	.290	.091	.280	.104	.257
5	.117	.359	.112	.344	.104	.346	.101	.316	.100	.319	.106	.292
6	.130	.318	.113	.300	.109	.300	.101	.294	.101	.301	.105	.296
7	.115	.340	.104	.370	.098	.349	.100	.338	.100	.318	.110	.299
8	.158	.309	.132	.349	.107	.338	.107	.329	.103	.321	.114	.278
9	.130	.322	.115	.358	.106	.344	.106	.306	.108	.306	.108	.310
10	.136	.356	.119	.349	.106	.355	.110	.299	.108	.291	.114	.267
11	.120	.372	.112	.347	.109	.324	.105	.329	.112	.301	.115	.267
12												
13	.131	.332	.114	.353	.105	.345	.109	.299	.110	.303	.114	.266
14	.139	.308	.142	.297	.130	.330	.113	.318	.113	.307	.116	.256
15	.126	.345	.110	.324	.105	.294	.103	.309	.106	.281	.187	.270
16	.157	.324	.123	.302	.114	.330	.108	.304	.109	.291	.114	.275
17	.124	.291	.102	.328	.099	.324	.097	.305	.096	.317	.105	.294
18	.122	.293	.108	.344	.101	.336	.097	.317	.097	.300	.101	.289
19	.125	.340	.109	.362	.102	.347	.101	.314	.102	.306	.113	.300
20	.122	.272	.114	.339	.101	.327	.091	.313	.091	.298	.100	.289
21	.138	.359	.118	.369	.110	.318	.105	.323	.102	.333	.120	.297
22												
23	.138	.273	.117	.353	.103	.319	.101	.317	.097	.312	.111	.271
24	.138	.331	.115	.343	.111	.290	.107	.312	.108	.283	.119	.284

TABLE F.1 Continued

Sample ID	Measurement											
	1		2		3		4		5		6	
	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW
25	.153	.310	.123	.329	.110	.348	.111	.310	.115	.299	.118	.297
26	.142	.207	.113	.328	.108	.334	.106	.318	.110	.286	.107	.285
27	.126	.358	.115	.337	.111	.317	.110	.296	.107	.290	.117	.265
28	.131	.341	.116	.354	.111	.324	.106	.344	.110	.297	.112	.288
29	.120	.338	.111	.384	.103	.350	.098	.334	.103	.307	.108	.277
30	.134	.307	.123	.344	.103	.350	.107	.326	.106	.300	.106	.290
31	.136	.334	.126	.325	.112	.309	.107	.331	.113	.288	.118	.260
32	.144	.321	.127	.332	.114	.333	.110	.307	.113	.134	.118	.272

TABLE F.2
DENSITOMETRIC BONE ANALYSIS
BMC (g/cm) AND BMC/BW (g/cm^2)

Bone Rat Femur
Rat Diet -Ca

Date 12/10/ 81
Technician R. Tesar

Sample ID	Measurement											
	1		2		3		4		5		6	
	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW
1	.116	.305	.103	.289	.096	.318	.098	.336	.098	.287	.100	.254
2	.116	.298	.105	.292	.099	.283	.101	.307	.096	.314	.096	.282
3	.119	.294	.112.	.303	.101	.318	.101	.318	.099	.307	.096	.280
4	.106	.279	.102	.306	.099	.174	.101	.307	.094	.290	.093	.255
5												
6	.112	.291	.107	.306	.103	.294	.097	.322	.096	.302	.090	.299
7	.119	.275	.108	.278	.101	.314	.100	.299	.098	.302	.096	.277
8	.128	.308	.112	.315	.103	.332	.107	.323	.104	.310	.103	.281
1a	.123	.296	.120	.308	.110	.324	.106	.320	.109	.307	.106	.270
2a	.113	.292	.103	.328	.099	.325	.097	.291	.098	.298	.094	.265
3a	.123	.258	.113	.292	.105	.311	.105	.274	.101	.295	.098	.280
4a	.108	.292	.104	.290	.104	.280	.102	.286	.102	.271	.101	.256
9	.124	.279	.115	.328	.106	.342	.112	.329	.107	.319	.101	.262
10	.113	.298	.109	.313	.105	.279	.104	.331	.106	.286	.096	.267
11	.125	.250	.112.	.301	.109	.333	.110	.284	.108	.285	.105	.244
12	.119	.277	.119	.287	.118	.293	.112	.306	.110	.283	.104	.228
13	.136	.302	.116	.331	.107	.329	.105	.285	.104	.286	.100	.270
14	.110	.334	.109	.293	.105	.297	.107	.306	.101	.292	.096	.292
15	.122	.297	.121	.281	.116	.293	.110	.286	.112	.270	.109	.245
16	.125	.262	.115	.309	.112	.308	.114	.299	.109	.277	.100	.250
5a	.132	.283	.120	.263	.117	.298	.104	.329	.106	.291	.108	.235
6a	.113	.304	.108	.310	.103	.313	.108	.303	.104	.269	.099	.231
7a			.111	.302	.101	.344	.103	.344	.105	.263		
8a	.123	.259	.112	.296	.103	.296	.107	.292	.109	.257	.105	.232

TABLE F.2 continued

Sample ID	Measurement											
	1		2		3		4		5		6	
	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW
17	.117	.287	.106	.294	.105	.297	.100	.313	.103	.287	.095	.271
18	.118	.287	.108	.306	.104	.304	.101	.299	.096	.267	.098	.278
19	.121	.299	.105	.323	.105	.307	.102	.291	.099	.282	.101	.260
20	.140	.270	.109	.286	.104	.320	.096	.324				
21												
22	.120	.291	.107	.330	.099	.325	.103	.336	.098	.291	.098	.279
23	.120	.312	.111	.339	.108	.303	.105	.311	.102	.298	.101	.115
24	.129	.270	.112	.301	.109	.291	.107	.306	.108	.283	.099	.274
25	.124	.277	.112	.292	.105	.315	.101	.327	.093	.272		
26	.111	.290	.103	.311	.104	.287	.100	.302	.102	.287		
27	.121	.279	.115	.336	.110	.303	.109	.304	.111	.295		
28	.132	.266	.090	.246	.071	.313	.092	.288	.105	.311	.105	.257
29							.103	.317	.105	.279	.101	.268
30												
31	.121	.307	.114	.314	.110	.314	.111	.328				
32	.119	.282	.112	.269	.100	.286	.105	.284	.098	.283	.097	.245

TABLE F.3
DENSITOMETRIC BONE ANALYSIS
BMC (g/cm) AND BMC/BW (g/cm^2)

Bone Rat Femur
Rat Diet -D

Date 12/10/ 81
Technician R. Tesar

Sample ID	Measurement											
	1		2		3		4		5		6	
	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW
1	.114	.268	.106	.346	.092	.342	.090	.292	.084	.298	.087	.264
2	.127	.263	.102	.318	.091	.300	.087	.280	.084	.301	.083	.260
3	.114	.306	.097	.319	.090	.307	.086	.274	.086	.283	.115	.296
4	.101	.301	.099	.307	.096	.299	.089	.289	.085	.273	.094	.282
5	.113	.260	.097	.331	.092	.285	.085	.254	.087	.260	.089	.228
6	.104	.317	.096	.344	.093	.329	.094	.305	.091	.290	.086	.314
7	.121	.324	.103	.344	.093	.297	.088	.353	.087	.261	.090	.282
8	.114	.329	.107	.285	.093	.326	.091	.290	.086	.273	.089	.257
9	.114	.281	.104	.299	.101	.314	.097	.276	.093	.285	.097	.245
10	.113	.287	.100	.314	.101	.299	.097	.302	.103	.278	.103	.249
11	.111	.283	.097	.320	.090	.328	.094	.284	.090	.295	.091	.261
12	.112	.305	.096	.339	.089	.310	.088	.321	.085	.273	.095	.249
13	.137	.281	.118	.286	.111	.273	.102	.273	.104	.259	.104	.227
14	.118	.269	.105	.313	.101	.316	.098	.283	.090	.283	.091	.216
15	.115	.307	.106	.296	.096	.317	.095	.278	.089	.301	.089	.255
16	.119	.307	.106	.310	.097	.332	.098	.305	.095	.295	.094	.264
17	.135	.286	.109	.329	.101	.321	.097	.290	.092	.293	.095	.262
18	.108	.266	.095	.325	.091	.312	.086	.300	.086	.279	.081	.250
19	.123	.312	.117	.336	.108	.323	.104	.323	.103	.310	.102	.301
20	.129	.333	.114	.341	.109	.322	.108	.304	.104	.301	.101	.290
21	.140	.328	.123	.348	.110	.320	.105	.319	.106	.286	.104	.274
22	.119	.385	.112	.368	.105	.360	.106	.343	.105	.292	.102	.295
23	.131	.302	.123	.272	.109	.345	.108	.319	.104	.311	.101	.294
24	.138	.342	.127	.343	.120	.350	.111	.344	.106	.328	.109	.318

Table F.3 continued

Sample ID	Measurement											
	1		2		3		4		5		6	
	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW
25	.122	.362	.110	.341	.107	.318	.103	.320	.099	.309	.102	.276
26	.123	.378	.112	.365	.111	.329	.111	.366	.103	.364	.110	.286
27	.123	.314	.114	.354	.108	.350	.106	.295	.100	.332	.109	.294
28	.127	.318	.113	.334	.118	.318	.108	.314	.114	.292	.124	.265
29	.130	.312	.115	.319	.108	.308	.107	.345	.103	.327	.109	.278
30	.132	.317	.112	.345	.110	.344	.108	.325	.112	.287	.110	.278
31	.136	.328	.119	.324	.110	.296	.111	.319	.111	.306	.110	.279
32	.138	.295	.119	.346	.108	.334	.108	.306	.110	.271	.113	.272

TABLE F.4
DENSITOMETRIC BONE ANALYSIS
BMC (g/cm) AND BMC/BW (g/cm^2)

Bone Rat Femur
Rat Diet -Ca, -D

Date 12/10/ 81
Technician R. Tesar

Sample ID	Measurement 1		2		3		4		5		6	
	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW
1	.102	.279	.101	.275	.099	.286	.093	.270	.091	.249	.092	.239
2	.106	.274	.097	.287	.090	.294	.093	.256	.090	.241	.091	.248
3	.100	.278	.094	.304	.093	.311	.089	.265	.089	.259	.089	.225
4	.095	.252	.090	.291	.085	.272	.088	.260	.083	.280	.090	.238
5	.102	.296	.090	.315	.089	.303	.088	.289	.087	.280	.092	.261
6	.095	.313	.093	.317	.087	.304	.086	.299	.085	.299	.094	.243
7	.117	.271	.101	.275	.097	.266	.096	.269	.095	.245	.094	.231
8	.099	.293	.089	.280	.083	.300	.086	.270	.080	.268	,080	.237
9	.102	.271	.097	.272	.099	.287	.098	.263	.100	.248	.097	.212
10	.095	.273	.094	.280	.089	.284	.090	.280	.086	.268	.086	.217
11	.110	.267	.105	.246	.100	.287	.105	.258	.098	.231	.093	.218
12	.108	.284	.104	.287	.103	.259	.103	.261	.098	.235	.096	.220
13	.104	.267	.093	.262	.096	.274	.095	.301	.093	.270	.093	.213
14	.111	.273	.100	.314	.106	.279	.098	.306	.106	.191	.094	.278
15	.120	.260	.107	.255	.098	.274	.098	.286	.099	.248	.103	.221
16	.102	.276	.093	.260	.092	.278	.093	.292	.091	.286	.087	.269
17	.102	.291	.098	.278	.094	.297	.096	.280	.101	.283	.129	.312
18	.102	.266	.100	.287	.097	.283	.095	.250	.093	.238	.097	.253
19	.111	.304	.102	.313	.095	.291	.098	.303	.097	.285	.104	.279
20	.111	.259	.106	.274	.096	.299	.094	.263	.093	.248	.101	.246
21	.110	.252	.097	.296	.094	.287	.090	.262	.087	.288	.094	.253
22	.111	.249	.099	.271	.099	.266	.090	.292	.096	.261	.090	.218
23	.111	.302	.099	.289	.093	.284	.090	.287	.095	.255	.094	.238
24							.093	.271	.093	.275	.094	.249

Table F.4 continued

Sample ID	Measurement											
	1		2		3		4		5		6	
	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW	BMC	BMC/BW
25	.110	.264	.102	.249	.099	.273	.089	.265	.095	.277	.089	.239
26												
27	.114	.248	.104	.269	.097	.267	.093	.292	.091	.241	.097	.229
28	.107	.270	.103	.285	.102	.295	.103	.273	.102	.239	.102	.232
29	.106	.288	.103	.282	.098	.308	.103	.252	.095	.247	.098	.218
30	.102	.264	.099	.281	.094	.297	.095	.277	.097	.264	.102	.234
31	.104	.256	.094	.289	.092	.309	.089	.304	.093	.243	.096	.231
32	.102	.341	.100	.303	.101	.282	.102	.297	.101	.279	.095	.241

TABLE F.5
DENSITOMETRIC BONE ANALYSIS
BONE LENGTH (cm)

Bone Rat Femur Collimator 1/16" Date 12/10/81

Threshold 85% Technician R. Tesar

Sample ID	Normal	-Ca	-D	-Ca, -D
1	3.081	3.113	3.098	3.143
2	3.237	3.018	3.169	3.209
3	3.203	3.142		3.118
4	3.207	3.097	3.118	2.994
5	3.244		2.994	3.119
6	3.235	2.970	3.207	
7	3.047	2.974	3.122	3.207
8	3.453	3.210	3.165	
1a		3.308		
2a		3.076		
3a		2.914		
4a		3.220		
9		3.370		
10	3.457	3.097	3.205	
11	3.301	3.197		
12		3.487	3.118	
13	3.563	3.234	3.292	
14	3.581	3.316	3.297	
15	3.518	3.130	3.123	3.364
16	3.609	3.260	3.235	
5a		3.197		
6a				
7a				
8a		3.423		
17	3.148	3.039	3.207	
18	3.220	3.085	3.010	3.148
19	3.199	3.197	3.083	3.169
20	3.254		3.100	3.201
21	3.337	3.082	3.138	3.109
22			3.159	3.160
23	3.301	2.928	3.039	3.101
24		3.222	3.163	
25	3.489		3.010	3.373
26	3.342		3.163	
27	3.413		3.092	3.216
28	3.426	3.082	3.405	3.417
29	3.139		3.159	3.292
30	3.342		3.134	
31			3.147	3.222
32	3.471	3.184	3.204	

APPENDIX G
BIOMECHANICAL TESTING

One femur from each rat was used in a biomechanical testing procedure (Puhl et al., 1972) to determine torque and deformation values at time of fracture. This testing procedure is based on a dynamic test, with load being applied very rapidly. More reliability of results is expected to be obtained than by use of static methods which would allow creep to occur in the molded ends of the bones.

In preparation, continually keeping the bones moist, both ends of each femur were embedded in soft, pliable methyl methacrylate, placed in a special mold, and allowed to harden. After the bones were removed from the mold, they were individually mounted in the torsional testing machine shown in Fig. G.1. The specimens were loaded to fracture in external torsion about their longitudinal axis. A torsional pendulum provided the load by engaging the bone midway in its free fall.

A coupled oscilloscope traced out a torque-deflection diagram as each bone was twisted to fracture. A permanent record of each torque-deflection trace was obtained by an electronically triggered camera. The photographs provided a means for determining the value of torque (kg-cm) and deformation, or twist angle (degrees), as shown by representations in Fig. G.2. Values are recorded in Tables G.1 through G.4.

Fig. G.1 The Rapid Loading Torsional Testing Machine, with the recording oscilloscope at left. The bone specimen is loaded in dynamic torsion between the headstock and tailstock of the machine, indicated by the arrow. Biomechanics Laboratory, University of Florida.

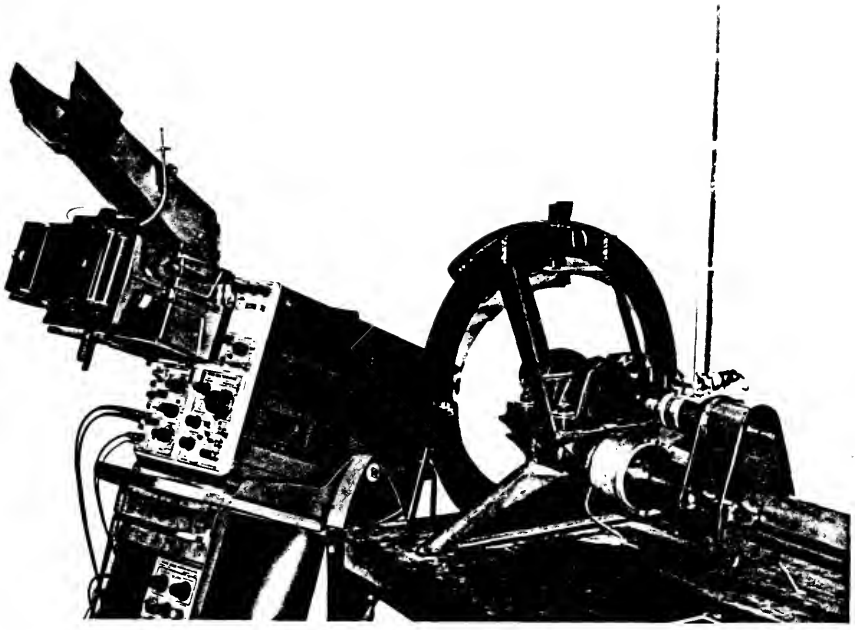
1. Normal Diet

2. -Ca

3. -D

4. -Ca, -D

Fig. G.2 Representative torque - deflection curves. The horizontal scale is 10 degrees/division (deformation); the vertical scale is 1 kg force-cm/division (torque).



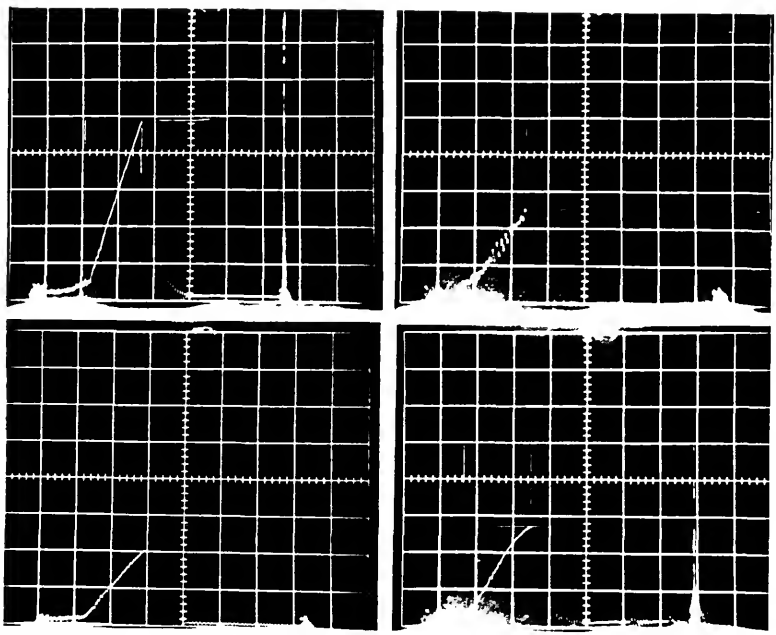


TABLE G.1
BIOMECHANICAL PROPERTIES

NORMAL DIET

Sample ID	Deformation (degrees)	Torque (kg-cm)
1	22.0	3.6
2	6.9	1.8
3	13.5	4.5
4	15.5	3.0
5	17.5	4.2
6		
7	13.5	4.5
8	12.0	4.6
9	16.0	4.6
10	14.5	5.0
11	17.5	5.0
12		
13		
14	15.5	5.2
15	15.0	3.6
16	17.5	4.3
17		
18	14.0	3.5
19	14.0	3.7
20	11.0	3.5
21	13.0	4.4
22		
23		
24	12.5	4.2
25	14.0	4.2
26	15.5	4.8
27	15.0	5.1
28	15.0	3.8
29	16.0	3.7
30	17.0	4.2
31	16.5	5.1
32	19.0	4.8

TABLE G.2
BIOMECHANICAL PROPERTIES

-CA DIET

Sample ID	Deformation (degrees)	Torque (kg-cm)
1	15.0	2.4
2	13.5	3.0
3	12.0	2.4
4	17.0	2.4
5	12.5	2.2
6	21.0	3.0
7	17.5	2.2
8	16.0	2.6
1a	10.5	2.7
2a	19.5	2.5
3a	16.5	2.4
4a	20.5	2.1
9	22.5	2.5
10	18.5	1.8
11	16.5	2.1
12	14.5	2.4
13	16.0	3.3
14	23.5	2.6
15	13.5	3.0
16	21.5	2.4
5a		
6a		
7a		
8a	15.0	2.3
17	19.0	2.5
18	23.0	2.2
19	12.5	2.4
20		
21		
22		
23		
24	17.5	3.2
25		
26		
27		
28		
29		
30		
31		
32	17.0	1.7

TABLE G.3
BIOMECHANICAL PROPERTIES

-D DIET

Sample ID	Deformation (degrees)	Torque (kg-cm)
1	12.0	2.2
2	15.5	2.6
3	21.0	2.8
4	13.5	3.1
5	15.5	2.7
6	15.5	1.5
7	23.0	3.0
8	20.0	3.0
9	14.0	3.2
10	17.5	4.0
11		
12	17.0	2.1
13	16.0	3.6
14	25.0	3.2
15	16.0	2.4
16	15.0	3.0
17	17.0	3.6
18	14.0	2.7
19	16.5	2.8
20	19.0	3.8
21	14.0	2.9
22	16.0	2.9
23	17.0	2.6
24		
25	17.5	2.6
26	23.0	2.6
27	21.0	2.4
28	10.5	2.4
29	15.5	3.0
30	16.5	3.2
31	13.5	
32	16.0	1.7

TABLE G.4
BIOMECHANICAL PROPERTIES

-CA, -D DIET

Sample ID	Deformation (degrees)	Torque (kg-cm)
1	13.0	3.3
2	19.0	4.0
3	10.0	2.6
4	9.0	2.4
5	15.5	3.0
6	18.5	3.0
7		
8	16.0	3.1
9		
10	12.0	2.0
11	12.5	3.0
12	15.0	2.6
13	16.5	2.7
14	17.0	2.8
15	16.5	3.2
16		
17	12.0	3.2
18	14.0	3.6
19	14.0	3.0
20	13.5	3.3
21	14.0	2.8
22	13.0	3.5
23	14.0	3.3
24		
25	17.5	3.5
26		
27	17.0	2.8
28	15.0	3.6
29	18.0	2.6
30	14.5	3.0
31	17.0	3.4
32		

APPENDIX H
BONE ASH ANALYSIS

Ashing Procedure[1]

One femur from each rat was dried at 100°C for 24 hours. After cooling, each bone was wrapped in cloth and labeled. Fat was removed by the ether extraction in a Soxhlet extractor for 36 hours. After extraction, the bones were placed under a hood until the odor of ether was no longer detectable. The bone samples were then placed in an oven to dry at 100°C for 24 hours.

Clean, demineralized crucibles were placed in a drying oven (100°C) for two hours. The crucibles were removed from the oven and cooled in a dessicator for two hours. The crucibles were then weighed to four decimal places on a digital anlytical balance. While on the balance, a bone sample was weighed into each crucible.

Crucibles containing the dry samples were placed in a muffle furnace and the temperature (200°C to begin) was rasied 100° every hour until 550°C was reached. Ashing proceeded overnight. The crucibles were removed from the furnace after they cooled partially to an oven at 100°C. After one hour,

[1]Fick et al., 1979

they were removed to dessicators to cool for two hours. The crucibles and bone ash were then weighed. The % ash of dry, fat-free bone was calculated for each sample. Recorded weights and % ash are found in Tables H.1 through H.4.

TABLE H.1
BONE ASHING ANALYSIS DATA

Diet Normal Date 3/5/82 Technician R. Tesar

	Dry Weight				Ash Weight		
Sample ID	Crucible Number	Crucible Weight	Crucible + Sample	Sample Weight	Crucible + Sample	Sample Weight	% Ash
1	184	18.2898	18.6612	0.3714	18.5312	0.2414	65.00
2	5	28.3107	28.7312	0.4205	25.5832	0.2725	64.80
3	104	17.0868	17.4124	0.3256	17.2990	0.2122	65.17
4	23	18.5401	18.8534	0.3133	18.7397	0.1996	63.71
5	138	19.0462	19.4760	0.4298	19.3261	0.2799	65.12
6	12	51.6655	52.0592	0.3937	51.9306	0.2651	67.73
7	51	16.8971	17.2489	0.3518	17.1286	0.2315	65.80
8	536	42.7861	43.2509	0.4648	43.0941	0.3080	66.27
9	201	17.2556	17.5954	0.3398	17.4702	0.2146	63.15
10	100	49.9888	50.3968	0.4080	50.2513	0.2625	64.34
11	39	18.0128	18.3995	0.3867	18.2633	0.2505	64.78
12							
13	33	23.5589	24.0029	0.4440	23.8343	0.2754	62.03
14	36	23.4544	23.9334	0.4790	23.7549	0.3005	62.73
15	4	18.9853	19.3499	0.3646	19.2232	0.2379	65.25
16	8	14.9008	15.3453	0.4445	15.1890	0.2882	64.84
17	153	18.6800	18.9089	0.2289	18.8246	0.1446	63.17
18	2	43.4218	43.7554	0.3336	43.6401	0.2183	65.44
19	176	19.5214	19.9193	0.3979	19.7918	0.2704	67.96
20	23	23.9153	24.2643	0.3490	24.1386	0.2233	63.98
21	19	17.8583	18.3249	0.4666	18.1623	0.3040	65.15
22							
23	141	19.2044	19.6626	0.4582	19.4946	0.2902	63.33
24	37	17.8953	18.3231	0.4278	18.1715	0.2762	64.56

Table H. 1 continued

	Dry Weight				Ash Weight		
Sample ID	Crucible Number	Crucible Weight	Crucible + Sample	Sample Weight	Crucible + Sample	Sample Weight	% Ash
25	19	23.5955	24.0299	0.4344	23.8837	0.2882	66.34
26	505	50.1260	50.5545	0.4285	50.4027	0.2767	64.57
27	529	49.4547	49.9368	0.4821	49.7615	0.3068	63.64
28	4	42.5948	43.0484	0.4536	42.8950	0.3002	66.18
29	3	17.0024	17.3338	0.3314	17.2197	0.2173	65.57
30	61	15.4402	15.6788	0.2386	15.5922	0.1520	63.70
31	29						
32	165	17.6847	18.0507	0.3660	17.9355	0.2508	68.52

TABLE H.2
BONE ASHING ANALYSIS DATA

Diet -Ca Date 3/5/82 Technician R. Tesar

	Dry Weight				Ash Weight		
Sample ID	Crucible Number	Crucible Weight	Crucible + Sample	Sample Weight	Crucible + Sample	Sample Weight	% Ash
1	23	18.1672	18.4845	0.3173	18.3637	0.1965	61.92
2	144	17.2092	17.4755	0.2663	17.3751	0.1659	62.30
3	20	18.3832	18.6752	0.2920	18.5634	0.1802	61.71
4	204	17.6650	17.9307	0.2657	17.8325	0.1675	63.04
5	400	47.0558	47.3505	0.2947	47.2292	0.1734	58.84
6	200	17.4986	17.7676	0.2780	17.6597	0.1701	61.19
7	179	18.6759	18.8586	0.1827	18.7936	0.1177	64.42
8	58	48.2376	48.5548	0.3172	48.4254	0.1878	59.21
1a	2	50.2486	50.5370	0.2884	50.4230	0.1744	60.47
2a	409	51.0690	51.3575	0.2885	51.2468	0.1778	61.63
3a	31	23.1199	23.4350	0.3151	23.3146	0.1947	61.79
4a	25	27.2608	27.5066	0.2458	27.4059	0.1451	59.03
9	20	17.6923	17.9631	0.2708	17.8527	0.1604	59.23
10	15	17.4653	17.7380	0.2727	17.6235	0.1582	58.01
11	33	49.6344	49.9335	0.2991	49.8035	0.1691	56.54
12	30	17.1003	17.3915	0.2912	17.2721	0.1718	59.00
13	54	18.1439	18.4784	0.3345	18.3561	0.2122	63.44
14	6	16.8860	17.1341	0.2481	17.0361	0.1502	60.54
15	206	17.5213	17.7988	0.2775	17.6891	0.1678	60.47
16	26	17.9924	18.2903	0.2979	18.1607	0.1683	56.50
5a	9	15.7380	15.8878	0.1498	15.8205	0.0825	55.07
6a	32	24.4516	24.6765	0.2249	24.5824	0.1308	58.16
7a	129	19.1535	19.4665	0.3130	19.3416	0.1881	60.10
8a	8	53.4614	53.7376	0.2762	53.6235	0.1621	58.69

Table H.2 continued

Sample ID	Dry Weight				Ash Weight		
	Crucible Number	Crucible Weight	Crucible + Sample	Sample Weight	Crucible + Sample	Sample Weight	% Ash
17	224	50.1478	50.4623	0.3145	50.3354	0.1876	59.65
18	13	18.0007	18.3053	0.3046	18.1884	0.1877	61.62
19	1	18.3815	18.6580	0.2765	18.5534	0.1719	62.17
20	51	17.0252	17.2128	0.1876	17.1454	0.1202	64.07
21							
22	203	17.1148	17.3769	0.2621	17.2770	0.1622	61.88
23	4	23.0054	23.2955	0.2901	23.1849	0.1795	61.88
24	3	42.8336	43.1481	0.3145	43.0370	0.2034	64.67
25	34	23.5061	23.7377	0.2316	23.6524	0.1463	63.17
26	172	19.0238	19.3697	0.3459	19.2280	0.2042	59.03
27	29	18.2194	18.5018	0.2824	18.3918	0.1724	61.05
28	151	17.9494	18.1626	0.2132	18.0826	0.1332	62.48
29	14	16.3204	16.5040	0.1836	16.4306	0.1102	60.02
30	512	48.2088	48.3297	0.1209	48.2846	0.0758	62.70
31	2	18.4815	18.7075	0.2260	18.6249	0.1434	63.45
32	193	20.8541	21.1278	0.2737	21.0203	0.1662	60.72

TABLE H.3
BONE ASHING ANALYSIS DATA

Diet -D Date 3/5/82 Technician R. Tesar

	Dry Weight				Ash Weight		
Sample ID	Crucible Number	Crucible Weight	Crucible + Sample	Sample Weight	Crucible + Sample	Sample Weight	% Ash
1	5	43.0434	43.3778	0.3344	43.2518	0.2084	62.32
2	1	43.7819	44.0750	0.2931	43.9653	0.1834	62.57
3	145	18.6987	18.9727	0.2740	18.8699	0.1712	62.48
4	2	18.3669	18.7218	0.3549	18.5987	0.2318	65.31
5	15	16.8110	17.1427	0.3317	17.0148	0.2038	61.44
6	53	50.6808	50.9873	0.3065	50.8755	0.1947	63.52
7	30	24.1104	24.4661	0.3557	24.3352	0.2248	63.20
8	49	16.9877	17.3196	0.3319	17.2037	0.2160	65.08
9	9	16.4565	16.7861	0.3296	16.6577	0.2012	61.04
10	143	18.0463	18.3742	0.3279	18.2424	0.1961	59.80
11	16	17.7680	18.0187	0.2507	17.9249	0.1569	62.58
12	18	16.4956	16.7641	0.2685	16.6560	0.1604	59.74
13	136	49.2820	49.6883	0.4063	49.5249	0.2429	59.78
14	531	50.1828	50.5128	0.3300	50.3894	0.2066	62.61
15	21	16.9532	17.2831	0.3311	17.1478	0.1958	59.14
16	207	16.6047	16.9737	0.3690	16.8294	0.2247	60.89
17	12	28.7179	29.0893	0.3713	28.9531	0.2352	63.35
18	3	18.2296	18.5332	0.3036	18.4091	0.1795	59.12
19	117	18.5312	18.8652	0.3340	18.7316	0.2004	60.00
20	149	18.6037	18.9499	0.3462	18.8086	0.2049	59.19
21	40	17.1239	17.5079	0.3840	17.3596	0.2357	61.14
22	24	23.7742	24.1366	0.3624	23.9924	0.2182	60.21
23	102	18.1196	18.4441	0.3245	18.3148	0.1952	60.15
24	410	48.3175	48.7070	0.3895	48.5644	0.2469	63.39

Table H.3 continued

Sample ID	Dry Weight				Ash Weight		
	Crucible Number	Crucible Weight	Crucible + Sample	Sample Weight	Crucible + Sample	Sample Weight	% Ash
25	8	42.7545	43.0812	0.3267	42.9511	0.1966	60.18
26	10	16.2414	16.6204	0.3790	16.4635	0.2221	58.60
27	13	27.1791	27.5305	0.3514	27.3870	0.2079	59.16
28	151	17.9494	18.1626	0.2132	18.0826	0.1332	62.48
29	208	18.7331	19.0440	0.3109	18.9262	0.1931	62.11
30	205	20.4791	20.8260	0.3469	20.6948	0.2157	62.18
31	23	27.8907	28.2390	0.3483	28.1094	0.2187	62.79
32	401	50.1781	50.5432	0.3651	50.3985	0.2204	60.37

TABLE H.4
BONE ASHING ANALYSIS DATA

Diet -Ca, -D Date 3/5/82 Technician R. Tesar

	Dry Weight				Ash Weight		
Sample ID	Crucible Number	Crucible Weight	Crucible + Sample	Sample Weight	Crucible + Sample	Sample Weight	% Ash
1	21	28.0174	28.3449	0.3275	28.2198	0.2024	61.80
2	25	16.8127	17.1425	0.3298	17.0184	0.2057	62.37
3	11	19.7364	20.0467	0.3103	19.9252	0.1888	60.84
4	38	17.0910	17.3731	0.2821	17.2658	0.1748	61.96
5	106	18.6055	18.9469	0.3414	18.8170	0.2115	61.95
6	5	43.3968	43.6863	0.2895	43.5736	0.1768	61.07
7	102	52.6066	52.7396	0.1330	52.6893	0.0827	62.18
8	150	17.9878	18.2411	0.2533	18.1481	0.1603	63.28
9	21	21.1292	21.3970	0.2678	21.2911	0.1619	60.05
10	27	16.5363	16.7990	0.2627	16.6922	0.1559	59.35
11	185	17.9227	18.3031	0.3104	18.1726	0.1799	57.96
12	17	17.1807	17.4880	0.3073	17.3642	0.1835	59.71
13	30	51.6462	51.9420	0.2958	51.8157	0.1695	57.30
14	32	17.2939	17.6168	0.3229	17.4891	0.1952	60.45
15	26	19.5140	19.8932	0.3792	19.7446	0.2306	60.81
16	140	18.3426	18.6059	0.2633	18.5113	0.1687	64.07
17	530	51.5959	51.9786	0.3827	51.8350	0.2391	62.48
18	35	23.3998	23.7334	0.3336	23.6078	0.2080	62.35
19	188	17.5655	17.9532	0.3877	17.8069	0.2414	62.26
20	32	49.8432	50.1835	0.3403	50.0586	0.2154	63.30
21	223	48.3179	48.6517	0.3338	48.5220	0.2041	61.14
22	18	23.9219	24.2511	0.3292	24.1172	0.1953	59.33
23	9	25.4091	25.7420	0.3329	25.6142	0.2051	61.16
24	136	19.7376	20.0580	0.3204	19.9380	0.2004	62.55

Table H.4 continued

Sample ID	Dry Weight				Ash Weight		
	Crucible Number	Crucible Weight	Crucible + Sample	Sample Weight	Crucible + Sample	Sample Weight	% Ash
25	584	47.7644	48.0678	0.3034	47.9446	0.1802	59.39
26							
27	126	18.1805	18.5009	0.3204	18.3754	0.1949	60.83
28	16	28.8193	29.2337	0.4144	29.0672	0.2479	59.82
29	28	17.2979	17.6345	0.3366	17.4979	0.2000	59.42
30	4	28.0820	28.3689	0.2787	28.2595	0.1775	61.67
31	131	19.2032	19.4842	0.2810	19.3713	0.1681	59.82
32	25	16.6611	16.9443	0.2832	16.8402	0.1791	63.24

APPENDIX I
MAST CELLS

TABLE I.1
MAST CELL COUNT--VAGINAL TISSUE

NORMAL DIET

Rat ID	Section				
	1	2	3	4	5
1	6	5	14	19	2
	1	4	3	8	2
	3	5	5	2	3
	6	3	2	6	9
	2	4	5	6	10
2	5	11	10	7	8
	12	10	6	2	4
	5	5	4	6	2
	4	8	2	6	4
	4	6	6	9	2
3	4	6	5	4	3
	5	5	7	8	3
	8	14	8	3	2
	6	2	16	11	3
	21	17	3	21	13
4	6	3	1	3	5
	8	2	5	2	2
	4	3	8	3	2
	4	9	4	3	6
	4	7	2	4	4
5	6	9	3	9	12
	2	3	4	18	5
	6	9	13	16	9
	6	12	9	7	8
	28	11	2	10	24
6	2	4	11	3	6
	3	4	2	3	1
	1	7	5	4	8
	11	6	3	5	3
	2	7	5	4	2

Table I.1 continued

Rat ID	Section				
	1	2	3	4	5
7	6	4	9	5	13
	10	5	7	3	7
	10	9	5	12	12
	5	5	5	5	12
	3	9	2	9	7
8	5	7	6	4	5
	6	5	6	8	7
	12	8	8	3	8
	11	5	4	7	5
	6	3	7	7	6
	17	16	6	8	10
	11	9	7	5	4
	8	9	13	6	11
	13	4	5	8	8
	19	3	13	8	22
10	13	8	9	6	10
	16	6	9	14	7
	5	11	8	20	5
	7	5	4	6	7
	8	9	2	7	11
11	8	8	5	7	8
	11	5	9	6	6
	3	16	13	3	4
	5	7	8	6	8
	11	5	3	11	9
12					
13	3	4	6	11	8
	8	5	10	19	18
	3	6	7	9	12
	5	9	8	12	12
	6	6	6	12	19
14	2	0	2	2	0
	9	3	0	0	0
	0	4	2	0	0
	5	0	0	0	0
	2	1	0	0	0
15					
16	5	5	1	11	13
	2	6	3	9	11
	2	3	3	5	5
	4	1	3	6	6
	5	4	5	8	14

Table I.1 continued

Rat ID	Section				
	1	2	3	4	5
17	5	4	4	5	9
	2	1	7	0	0
	3	3	4	2	1
	7	4	3	3	1
	6	4	4	2	2
18	7	6	5	9	6
	0	2	0	3	4
	6	2	2	0	2
	1	3	0	6	2
	8	6	1	6	1
19	12	4	4	6	4
	1	1	4	3	0
	0	6	5	1	6
	5	5	3	1	5
	3	2	1	4	1
20	8	9	2	6	5
	2	4	6	3	2
	5	4	1	1	2
	5	5	7	12	0
	10	5	1	24	5
21	6	4	2	4	10
	2	2	1	9	6
	3	4	4	1	4
	3	5	3	3	1
	1	9	5	4	2
22					
23	6	12	4	4	3
	2	7	2	1	6
	1	7	3	4	3
	7	4	2	3	2
	3	4	4	2	2
24	5	8	3	9	6
	7	7	4	2	8
	11	6	5	6	3
	8	7	4	5	3
	8	2	7	2	3
25	8	6	15	3	12
	0	4	8	0	4
	0	0	4	6	2
	3	4	2	0	7
	2	2	2	8	3

Table I.1 continued

Rat ID	Section				
	1	2	3	4	5
26	11	4	2	2	6
	4	5	4	4	0
	5	5	3	1	1
	3	0	11	1	2
	9	1	0	4	5
27	8	11	3	4	6
	0	1	3	4	9
	2	1	1	0	4
	3	3	1	2	7
	12	6	1	1	4
28	4	4	2	8	5
	4	0	2	1	1
	1	3	1	10	1
	2	2	3	0	1
	8	6	2	5	5
29	4	9	8	4	6
	0	0	10	11	3
	1	2	10	0	0
	2	1	3	1	6
	4	0	3	2	7
30	1	2	3	4	2
	0	2	3	0	0
	0	0	2	0	0
	2	3	4	1	4
	1	1	5	2	1
31	4	9	3	10	16
	4	12	2	19	3
	1	5	0	8	11
	7	3	4	9	0
	7	5	1	3	3
32	11	9	6	8	6
	2	7	2	6	2
	4	1	7	2	5
	2	2	7	1	6
	5	3	2	10	9

TABLE I.2
MAST CELL COUNT--VAGINAL TISSUE

-CA DIET

Rat ID	Section				
	1	2	3	4	5
1	5	4	11	5	3
	0	1	0	1	0
	0	0	1	3	3
	5	0	0	0	1
	2	1	1	6	3
2	13	0	3	3	3
	0	10	11	3	3
	4	5	2	5	4
	2	2	5	22	17
	1	21	1	10	4
3	1	3	1	3	1
	1	2	1	5	0
	3	0	2	2	0
	0	3	1	1	4
	1	3	2	2	15
4	6	7	7	8	2
	3	10	3	4	3
	8	3	1	3	10
	9	3	3	6	5
	5	4	3	6	4
5	3	3	2	2	4
	0	4	2	2	3
	0	3	3	0	8
	2	22	5	5	2
	5	13	8	27	1
6	5	3	6	5	2
	2	1	3	8	3
	8	1	6	2	5
	2	8	3	5	10
	7	2	6	4	2
7	2	6	5	2	4
	1	5	6	10	1
	7	4	6	10	8
	4	11	5	6	7
	8	6	7	6	4
8	0	2	0	1	0
	2	2	3	8	2
	5	2	3	1	1
	1	3	2	6	4
	0	0	6	4	2

Table I.2 continued

Rat ID	Section				
	1	2	3	4	5
1a	7	3	2	2	2
	10	8	7	2	4
	4	10	1	7	1
	3	3	12	2	1
	3	8	14	6	4
2a	6	4	2	1	4
	0	2	4	3	3
	5	0	2	3	4
	1	0	1	1	1
	11	2	5	1	6
3a	5	7	7	2	4
	1	7	13	4	7
	7	3	5	1	1
	8	4	4	3	4
	7	4	3	3	3
4a	12	6	2	5	4
	5	7	9	4	3
	6	4	2	8	1
	3	6	8	3	5
	9	3	11	6	4
9	14	5	8	4	4
	4	8	10	2	4
	3	7	8	2	3
	7	11	8	7	3
	5	3	6	4	2
10	5	6	4	7	5
	6	5	8	12	12
	5	3	6	4	4
	4	6	4	3	5
	5	2	2	2	2
11	5	10	7	5	8
	8	5	6	4	12
	7	5	3	5	10
	9	10	3	5	6
	5	4	2	4	4
12	9	6	7	4	19
	9	3	10	6	11
	7	12	8	13	28
	5	9	6	13	3
	2	8	19	14	11

Table I.2 continued

Rat ID	Section				
	1	2	3	4	5
13	7	3	4	8	5
	6	5	3	6	8
	5	7	4	7	7
	4	4	3	9	0
	7	12	10	8	11
14	9	3	9	4	5
	13	12	13	2	22
	9	16	12	9	9
	6	7	10	19	10
	3	11	6	10	10
15	5	5	6	7	9
	5	7	13	10	3
	4	5	3	3	4
	4	7	6	8	7
	6	5	9	5	4
16	5	5	8	7	7
	3	7	10	6	10
	6	12	10	4	10
	11	4	8	10	16
	6	4	9	3	13
5a	11	4	11	9	11
	5	5	5	6	8
	12	5	6	5	4
	8	6	8	10	7
	10	9	28	12	6
6a	15	9	9	6	5
	14	7	5	5	6
	6	5	11	7	7
	5	3	7	9	14
	9	6	8	4	4
7a	18	61	14	13	16
	15	37	5	19	10
	19	25	6	8	27
	16	36	33	13	15
	26	28	34	11	8
8a	8	5	4	33	7
	5	7	4	18	3
	3	3	3	8	8
	6	6	10	6	10
	28	6	17	5	18

Table I.2 continued

Rat ID	Section				
	1	2	3	4	5
17	5	6	7	2	6
	3	1	2	2	2
	4	3	3	6	4
	0	4	4	2	3
	2	2	1	3	4
18	9	7	2	4	12
	6	1	0	3	5
	3	4	0	2	2
	2	2	0	9	5
	3	3	1	3	3
19	5	4	4	3	1
	0	0	1	2	0
	3	0	1	1	2
	1	2	2	0	2
	0	0	1	0	4
20	6	7	4	6	3
	2	0	2	1	0
	3	1	2	3	2
	1	2	4	0	3
	2	0	3	2	0
21	4	2	6	1	5
	5	3	1	6	1
	1	1	6	5	5
	3	3	2	0	4
	6	2	5	4	2
22					
23	17	5	5	5	6
	0	2	0	5	7
	3	5	2	1	5
	0	7	4	1	1
	1	6	5	1	4
24	1	4	7	5	4
	3	2	3	1	4
	1	2	1	2	4
	4	2	3	3	1
	3	1	2	1	2

Table I.2 continued

Rat ID	Section				
	1	2	3	4	5
25	5	6	1	3	1
	1	0	7	6	3
	2	2	0	3	4
	1	6	4	0	1
	1	3	3	2	2
26	5	4	6	6	6
	4	2	2	2	2
	2	1	1	6	3
	0	0	5	5	2
	3	1	0	0	4
27					
28	4	14	17	4	5
	4	0	0	1	3
	3	8	5	2	3
	3	2	0	6	2
	6	3	2	4	5
29	7	3	5	6	3
	4	5	4	1	4
	7	7	0	1	2
	5	3	5	5	6
	3	5	2	3	6
30	5	5	2	1	8
	0	1	2	1	1
	0	1	0	0	8
	1	2	4	0	2
	1	1	1	0	1
31	5	4	7	2	8
	5	3	3	2	1
	1	5	3	1	0
	0	1	12	0	2
	2	0	5	2	1
32	6	2	3	9	5
	1	0	0	1	5
	1	4	3	0	4
	6	2	5	2	5
	3	2	2	0	2

TABLE I.3
MAST CELL COUNT--VAGINAL TISSUE

-D DIET

Rat ID	Section				
	1	2	3	4	5
1	10	4	6	5	6
	5	14	7	4	4
	10	5	6	7	6
	14	6	7	4	4
	7	6	7	8	3
2	8	6	19	6	16
	17	4	4	21	13
	17	5	4	12	35
	21	6	33	4	4
	16	4	32	20	5
3	3	5	5	6	4
	3	8	5	6	5
	3	2	3	6	3
	4	5	2	4	6
	5	6	4	0	8
4	8	5	3	3	9
	5	10	2	3	10
	4	6	13	4	3
	6	3	2	8	5
	4	4	1	7	5
5	5	6	13	24	16
	10	11	7	9	9
	8	9	5	4	5
	5	4	13	2	7
	9	6	3	13	3
6	6	7	11	12	10
	6	11	10	10	5
	6	6	15	5	2
	8	15	1	5	26
	6	13	1	5	2
7	4	9	7	5	6
	6	2	10	2	5
	2	4	5	5	7
	2	6	4	11	3
	3	7	4	5	2
8	7	8	7	5	4
	13	2	3	7	5
	6	6	3	3	5
	5	6	2	5	2
	6	2	3	4	4

Table I.3 continued

Rat ID	Section				
	1	2	3	4	5
9	42	8	4	5	8
	9	5	5	10	2
	4	4	4	17	9
	6	5	5	6	22
	4	12	3	7	21
10	4	4	5	5	6
	3	6	8	4	4
	12	10	8	6	4
	4	8	5	8	3
	4	4	5	8	7
11	3	3	6	6	4
	3	3	8	7	4
	4	2	4	5	7
	1	2	8	2	5
	4	7	5	9	2
12	6	4	2	4	5
	2	3	5	17	5
	2	4	4	5	9
	3	3	4	3	3
	4	3	5	3	2
13	3	3	3	13	9
	2	4	6	6	7
	3	3	7	8	8
	3	3	7	10	5
	2	3	4	6	4
14	25	5	5	31	7
	16	2	2	3	4
	8	1	2	9	11
	3	2	4	2	5
	3	1	1	5	2
15	2	5	3	5	10
	8	3	4	8	3
	7	4	5	1	4
	10	5	3	4	6
	3	4	3	4	6
16	6	6	11	17	5
	6	5	6	11	6
	9	4	7	5	4
	5	5	7	5	3
	4	10	8	6	3

Table I.3 continued

Rat ID	Section				
	1	2	3	4	5
17	5	10	2	4	5
	0	2	2	2	0
	2	3	3	6	7
	3	7	5	5	3
	1	5	5	4	4
18	10	5	3	9	4
	5	0	1	7	3
	0	8	3	3	9
	1	3	8	11	1
	4	3	1	18	2
19	11	10	3	6	3
	9	8	3	5	4
	4	4	4	6	4
	7	3	8	5	0
	5	11	3	4	5
20	11	2	12	7	3
	5	3	0	13	6
	4	1	6	6	5
	3	0	2	1	4
	3	8	8	45	3
21	4	6	8	4	6
	4	2	7	5	7
	3	5	5	0	3
	3	4	8	4	5
	7	2	2	1	2
22	42	5	18	21	11
	8	1	5	8	6
	6	7	1	52	6
	42	17	1	24	24
	40	25	4	81	8
23	3	9	5	4	16
	0	0	2	7	6
	5	0	3	3	5
	0	3	5	2	7
	3	2	0	3	1
24	1	4	5	12	5
	1	3	0	7	5
	14	1	4	0	6
	7	3	6	1	3
	3	2	2	3	0

Table I.3 continued

Rat ID	Section				
	1	2	3	4	5
25	11	3	0	9	6
	2	11	0	12	8
	1	0	5	4	1
	5	5	2	2	1
	6	10	2	4	3
26	13	7	27	15	12
	66	7	15	2	11
	16	25	26	4	10
	37	44	6	7	4
	16	34	13	9	15
27	10	43	13	12	5
	7	11	7	13	16
	5	14	9	13	29
	3	26	5	16	9
	8	23	21	15	11
28	4	5	3	8	7
	5	3	4	7	10
	2	3	7	10	4
	9	3	2	9	8
	13	7	7	7	5
29	19	19	12	8	4
	6	3	3	6	14
	9	4	7	4	12
	6	7	1	3	8
	3	2	0	10	5
30	12	2	11	3	3
	3	9	4	6	5
	1	4	7	6	9
	4	7	8	7	12
	9	8	14	3	1
31	7	11	6	8	13
	4	6	5	2	1
	4	13	6	4	12
	10	6	7	8	21
	2	11	8	5	9
32	16	17	11	4	3
	9	7	18	5	11
	6	3	5	5	4
	1	2	6	8	8
	9	25	7	10	2

TABLE I.4
MAST CELL COUNT--VAGINAL TISSUE

-CA, -D DIET

Rat ID	Section				
	1	2	3	4	5
1	1	5	3	5	8
	4	6	4	6	7
	7	4	8	3	4
	2	2	3	5	7
	10	10	2	4	6
2	4	6	7	12	5
	6	4	8	5	4
	10	11	7	7	2
	9	3	6	4	3
	5	5	10	3	3
3	4	5	2	6	6
	7	3	10	3	4
	6	5	6	2	7
	5	5	10	3	6
	7	7	5	2	8
4	3	2	4	7	6
	6	4	1	7	3
	2	5	5	4	3
	4	6	5	3	2
	5	2	3	10	5
5	4	5	6	11	6
	11	4	7	9	5
	7	6	3	10	12
	23	6	18	5	2
	12	3	4	7	12
6	3	9	7	2	2
	5	3	2	17	16
	13	2	8	6	12
	11	9	3	4	6
	8	13	12	16	6
7	8	3	5	9	6
	6	3	7	2	3
	12	1	7	20	3
	5	1	4	6	15
	5	7	5	7	4
8	9	7	5	4	7
	3	11	3	3	7
	6	8	3	38	6
	10	12	8	28	8
	3	5	4	5	5

Table I.4 continued

Rat ID	Section				
	1	2	3	4	5
9	6	6	4	7	9
	12	3	6	5	11
	5	3	15	5	6
	10	9	3	4	9
	10	7	8	10	8
10	6	5	6	6	14
	3	4	5	5	5
	7	9	3	9	2
	5	14	3	14	2
	6	12	9	6	9
11	13	17	9	7	14
	24	21	6	15	8
	28	24	6	6	5
	8	5	21	5	14
	5	9	4	11	7
12	10	6	4	4	8
	7	4	12	10	11
	5	7	13	7	6
	5	6	5	7	4
	7	7	5	10	12
13	5	8	15	18	10
	8	6	5	3	7
	5	3	7	6	4
	3	4	2	8	14
	4	5	9	10	4
14	19	3	6	4	40
	4	9	11	4	36
	5	4	42	8	15
	9	11	17	3	8
	4	6	31	13	20
15	15	11	15	12	17
	39	14	6	11	22
	33	6	24	10	20
	7	8	14	4	12
	10	12	5	8	4
16	5	4	14	7	6
	4	5	12	5	7
	8	4	13	5	8
	12	2	3	6	6
	12	6	9	5	7

Table I.4 continued

Rat ID	Section				
	1	2	3	4	5
17	7	5	6	3	4
	7	4	5	3	0
	5	6	4	2	1
	8	4	8	0	4
	5	5	6	9	1
18	4	3	11	4	7
	16	4	15	6	3
	11	14	8	11	0
	8	16	8	10	13
	8	4	4	2	4
19	2	4	5	4	1
	6	3	1	0	3
	2	1	4	0	2
	7	2	4	0	2
	2	3	2	5	2
20	4	5	12	1	2
	1	1	2	4	3
	2	3	9	0	7
	1	2	4	6	1
	3	5	2	0	1
21	12	7	26	13	6
	2	0	21	0	1
	3	1	2	0	2
	1	0	0	1	2
	0	4	3	3	2
22	2	6	1	4	1
	2	0	1	3	5
	1	2	2	4	1
	6	1	4	5	0
	2	2	6	2	2
23	5	3	4	5	3
	4	2	2	0	0
	1	1	6	0	3
	5	2	2	1	3
	1	0	1	1	3
24	4	7	12	14	9
	2	5	4	11	8
	1	0	5	3	6
	8	7	8	11	8
	3	2	2	7	5

Table I.4 continued

Rat ID	Section				
	1	2	3	4	5
25	4	7	10	11	7
	4	12	6	6	5
	2	3	0	4	5
	1	5	2	5	4
	6	4	3	7	5
26					
27	3	13	6	6	3
	5	8	5	4	4
	7	2	9	4	1
	0	10	1	5	2
	2	5	0	3	3
28	3	9	4	11	2
	3	5	3	1	9
	2	2	2	2	4
	1	4	7	1	8
	1	8	3	1	7
29	27	4	4	7	0
	5	1	2	1	0
	2	1	3	2	0
	2	1	0	0	1
	1	2	2	4	1
30	12	6	9	11	8
	8	8	9	3	5
	3	7	4	5	5
	17	4	9	7	2
	16	7	2	0	4
31	1	5	3	3	5
	4	1	1	2	3
	0	1	1	4	7
	3	4	9	3	1
	5	1	4	4	2
32	4	6	11	15	4
	7	7	8	6	5
	5	8	3	2	3
	6	13	1	5	5
	9	4	2	4	1

TABLE I.5
MAST CELL COUNT--BONE MARROW

NORMAL DIET

Rat ID	Section				
	1	2	3	4	5
1	24	1	1	6	0
	29	0	1	1	0
	3	0	0	0	0
	6	0	0	0	1
	0	33	0	0	0
2	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
3	21	22	22	35	92
	7	5	10	42	62
	11	32	17	57	103
	17	14	40	49	38
	31	19	16	52	25
4	0	0	0	3	2
	0	0	0	0	5
	0	0	0	4	4
	0	2	0	0	0
	0	0	4	0	0
5	36	31	23	26	34
	39	27	24	31	32
	42	22	22	32	17
	28	20	6	31	34
	39	23	0	34	15
6	10	3	0	32	36
	39	8	2	10	11
	6	12	0	21	2
	8	7	18	28	18
	0	19	46	38	17
7	0	5	15	15	4
	0	8	2	9	5
	0	11	21	8	5
	0	12	14	12	5
	26	5	17	0	12
8	109	27	49	82	33
	64	58	29	101	13
	56	33	9	107	16
	78	18	11	92	15
	73	21	32	47	7

Table I.5 continued

Rat ID	Section				
	1	2	3	4	5
9	5	0	0	0	1
	3	4	0	0	2
	0	6	0	0	0
	2	4	4	0	0
	2	3	2	0	0
10	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
11	41	54	15	32	31
	18	48	15	22	23
	37	101	41	17	29
	10	52	30	14	34
	14	37	26	33	15
12					
13	0	0	8	20	26
	0	7	10	14	13
	9	2	11	4	15
	0	3	4	9	9
	0	2	1	21	6
14	64	88	40	42	33
	36	67	27	32	58
	53	49	24	48	60
	40	53	31	36	25
	66	76	42	55	45
15	25	14	10	16	6
	9	10	14	5	11
	11	3	8	9	5
	23	9	9	20	16
	17	10	5	18	11
16	2	11	2	3	5
	3	4	4	9	13
	5	8	9	14	10
	2	8	4	0	32
	2	12	0	5	18

Table I.5 continued

Rat ID	Section				
	1	2	3	4	5
17	48	75	26	27	91
	41	80	16	33	62
	57	65	26	26	75
	84	56	24	20	83
	102	66	37	29	28
18	32	31	20	33	68
	21	21	21	32	24
	35	23	13	25	63
	21	27	19	26	70
	15	27	34	34	57
19	32	53	52	104	61
	55	111	83	126	85
	68	101	70	50	23
	47	54	100	78	62
	93	47	96	142	54
20	52	29	59	43	41
	62	39	80	34	38
	32	56	50	22	30
	29	76	20	17	34
	20	63	50	39	42
21	45	37	13	52	51
	60	49	21	59	66
	43	33	37	43	29
	64	32	20	38	48
	38	38	38	37	55
22					
23	84	117	103	112	168
	105	137	90	77	89
	97	142	99	71	132
	91	102	117	90	90
	62	120	118	109	52
24	64	31	39	35	45
	41	34	41	29	43
	53	26	28	47	38
	36	48	32	17	39
	21	52	35	14	31

Table I.5 continued

Rat ID	Section				
	1	2	3	4	5
25	47	35	25	32	66
	42	24	37	39	48
	36	27	23	22	58
	25	33	33	17	68
	72	33	19	26	49
26	41	45	52	26	28
	40	31	28	29	26
	63	30	40	35	24
	51	15	38	11	25
	34	30	58	32	34
27	60	34	18	32	23
	41	21	16	8	28
	25	22	17	14	21
	30	12	27	11	15
	37	11	15	19	20
28	18	38	40	42	54
	31	29	34	34	25
	60	39	41	36	21
	37	39	60	40	17
	33	27	49	40	18
29	8	12	9	78	47
	16	22	14	46	42
	23	15	29	44	40
	17	10	16	50	56
	21	32	22	28	51
30	41	15	18	18	16
	24	11	10	31	23
	18	27	26	21	19
	34	19	25	24	32
	20	28	43	22	24
31	59	56	78	33	36
	51	29	67	120	24
	42	47	49	59	72
	60	72	26	58	48
	59	46	57	52	35
32	57	20	23	34	22
	84	13	32	21	13
	25	18	37	16	36
	33	23	54	31	12
	13	22	59	26	20

TABLE I.6
MAST CELL COUNT--BONE MARROW

-CA DIET

Rat ID	Section				
	1	2	3	4	5
1	20	2	24	45	8
	35	0	9	44	21
	20	0	10	42	15
	20	0	21	21	52
	68	0	11	14	12
2	40	44	32	43	30
	25	43	48	56	17
	47	42	47	53	17
	32	34	50	51	21
	47	48	61	45	34
3	2	29	13	42	46
	4	27	18	17	10
	35	40	18	17	20
	29	20	23	22	22
	17	27	27	16	28
4	31	41	42	15	43
	25	71	44	27	32
	51	57	52	35	31
	23	85	75	15	35
	42	56	32	36	41
5	23	22	11	20	20
	15	17	21	16	29
	27	13	9	33	20
	26	25	21	18	14
	21	9	22	17	15
6	28	96			
	24	63			
	38	64			
	45	77			
	78	33			
7	28	26	21	24	17
	27	32	18	23	44
	34	27	27	25	26
	21	31	14	26	17
	33	20	16	26	25
8	24	13	26	29	36
	12	12	20	27	21
	14	9	32	7	9
	9	20	33	16	11
	8	7	20	8	24

Table I.6 continued

Rat ID	Section				
	1	2	3	4	5
1a	17	16	12	24	26
	21	33	21	25	12
	31	41	26	23	21
	20	32	32	17	24
	18	26	37	25	26
2a	37	13	33	14	12
	23	17	22	17	53
	31	30	18	15	19
	20	30	28	15	15
	20	28	22	12	14
3a	25	8	3	2	15
	13	5	4	3	2
	15	3	0	1	14
	11	1	10	5	1
	8	1	9	4	7
4a	16	16	19	30	30
	21	14	5	18	29
	1	36	17	9	20
	13	28	24	19	17
	19	25	24	15	20
9	36	22	33	32	30
	30	23	30	36	40
	24	39	53	35	23
	33	27	52	27	55
	17	24	26	39	47
10	29	37	40	21	15
	24	11	39	16	12
	26	25	41	19	19
	27	12	32	13	18
	17	22	16	16	18
11	10	4	17	6	8
	12	6	9	4	4
	5	6	9	8	3
	10	3	13	9	2
	5	0	6	8	2
12	18	16	29	26	10
	9	17	12	21	14
	7	7	19	12	44
	22	17	29	27	15
	22	28	14	27	31

Table I.6 continued

Rat ID	Section				
	1	2	3	4	5
13					
14	49	26	18	27	37
	41	28	60	10	25
	25	64	30	6	13
	55	27	33	51	30
	21	22	20	28	16
15	12	33	18	20	10
	1	28	12	23	12
	4	13	9	5	16
	3	1	5	18	10
	2	6	17	24	19
16	7	13	26	0	16
	9	16	2	15	0
	8	10	8	12	5
	15	8	9	8	11
	11	19	12	16	6
5a	14	7	18	32	7
	18	0	12	24	5
	16	4	11	12	2
	11	13	15	48	0
	11	9	16	17	4
6a	0	20	18	21	15
	16	20	17	18	8
	6	16	12	21	7
	24	26	12	38	8
	30	20	20	46	17
7a	21	14	26	18	21
	19	13	42	47	19
	20	15	20	31	36
	13	18	34	20	33
	27	20	26	18	25
8a	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0

Table I.6 continued

Rat ID	Section				
	1	2	3	4	5
17	52	33	50	29	21
	41	34	44	31	42
	18	32	50	46	64
	31	24	25	41	61
	29	29	23	54	31
18	60	34	25	54	37
	19	52	43	26	32
	15	34	21	21	28
	42	40	26	18	22
	48	26	23	22	28
19	46	40	45	43	23
	21	32	44	25	16
	39	63	33	46	16
	50	46	27	27	32
	27	43	57	44	28
20	27	51	38	61	49
	18	54	41	88	42
	37	40	51	31	31
	39	53	43	36	39
	24	66	37	33	36
21					
22	71	63	17	20	20
	51	20	12	61	23
	32	18	18	23	21
	29	19	24	27	25
	21	22	36	21	20
23	29	33	22	38	41
	11	26	36	21	22
	16	17	17	20	19
	23	22	17	33	24
	16	12	23	21	25
24	17	38	21	19	19
	9	11	15	12	39
	7	8	7	5	61
	9	12	16	9	17
	6	10	15	8	13

Table I.6 continued

Rat ID	Section				
	1	2	3	4	5
25	51	30	27	26	33
	20	50	26	14	22
	25	24	30	16	8
	15	8	16	33	35
	25	21	27	15	18
26	62	54	68	70	71
	57	106	87	61	40
	43	52	48	40	41
	53	54	51	53	49
	45	71	55	29	49
27	12	8	29	37	58
	10	4	12	21	32
	8	15	16	31	20
	20	10	26	12	54
	21	14	24	15	11
28	58	19	25	41	27
	49	18	25	25	18
	40	17	19	25	40
	31	23	22	29	18
	26	31	33	24	38
29	25	9	21	22	9
	9	12	17	19	7
	17	10	11	17	11
	13	35	17	21	10
	15	19	10	6	11
30	31	39	23	21	14
	5	20	25	32	30
	37	31	31	33	42
	30	28	21	48	35
	39	30	36	26	57
31	26	34	16	60	41
	39	30	28	49	30
	35	36	26	23	27
	33	25	17	29	18
	49	25	31	33	35
32	43	53	26	28	34
	38	44	23	22	19
	24	34	49	61	28
	20	37	49	26	21
	25	35	30	22	64

TABLE I.7
MAST CELL COUNT--BONE MARROW

-D DIET

Rat ID	Section				
	1	2	3	4	5
1	0	36	16	22	38
	0	37	44	20	90
	4	21	18	23	34
	75	60	65	15	24
	77	32	29	30	38
2	16	23	17	31	47
	6	38	45	22	32
	12	26	35	18	13
	14	23	39	11	58
	34	44	21	23	38
3	23	63	41	51	19
	37	58	42	72	24
	46	21	46	57	15
	67	38	19	80	22
	27	52	16	14	44
4	19	17			
		4			
5	52	26	24	24	34
	31	31	29	39	20
	26	44	51	46	28
	20	70	47	39	51
	20	66	64	27	59
6	15	34	8	31	5
	15	20	19	32	26
	18	13	48	32	18
	28	14	13	27	17
	18	7	35	26	19
7					
8	2	10	15	56	10
	3	8	26	15	40
	8	6	29	6	10
	33	12	21	10	13
	24	16	17	7	26

Table I.7 continued

Rat ID	Section				
	1	2	3	4	5
9	65	16	52	56	32
	84	29	56	24	80
	31	32	47	86	54
	54	60	46	68	37
	41	44	42	44	58
10	133	95	55	93	74
	63	88	83	76	85
	92	86	57	113	94
	52	42	112	101	104
	101	69	90	70	116
11	36	37	20	37	
	42	39	18	24	
	14	37	16	35	
	18	16	21	18	
	35	32	16	15	
12	58	30	39		
	26	21	39		
	52	16	32		
	38	39	26		
	42	42	48		
13	36	70	22	62	44
	46	17	16	22	27
	43	56	22	53	48
	63	86	30	76	32
	38	56	36	30	
14	50	83			
	99	72			
	79	67			
	79	72			
	58	90			
15	6	20	5	31	31
	36	20	13	28	33
	24	14	30	26	26
	20	7	24	19	12
	17	2	5	10	22
16					

Table I.7 continued

Rat ID	Section				
	1	2	3	4	5
17	45	0	36	12	5
	18	0	42	0	34
	8	3	29	7	17
	32	2	28	29	36
	12	26	0	0	22
18					
19	44	35	13	46	24
	36	36	28	45	35
	35	25	39	38	30
	21	10	50	36	38
	35	45	31	23	55
20	5	4	18	37	21
	8	2	20	11	29
	5	5	11	14	23
	9	18	5	27	28
	4	24	4	21	27
21	26	73	45	91	11
	26	45	22	64	41
	25	26	21	59	33
	40	45	43	37	46
	23	32	19	34	37
22	9	20	7	22	21
	21	15	19	19	13
	7	16	21	8	4
	4	8	9	9	12
	3	10	16	15	18
23	48	63	112	38	28
	58	31	70	29	63
	60	60	89	75	59
	56	30	50	97	43
	39	35	47	85	37
24	21	17	21	54	24
	34	37	19	33	12
	29	18	38	14	38
	72	20	55	14	37
	82	19	25	17	13

Table I.7 continued

Rat ID	Section				
	1	2	3	4	5
25	30	8	2	2	
	11	28	2		
	7	5	1		
	9	1	9		
	11	3	1		
26	21	28	20	32	104
	18	47	9	56	59
	24	57	8	24	30
	50	46	8	46	50
	68	14	14	49	18
27	15	19	16	15	29
	29	33	44	31	15
	30	31	27	11	47
	8	13	36	11	32
	30	43	36	24	9
28	9	2	7	12	11
	18	5	13	29	
	16	5	21	0	
	14	10	15	18	
	15	10	2	5	
29	40	12	15	45	26
	17	47	24	14	33
	9	9	43	22	
	9	30	4	16	
	30	10	16	16	
30	3	40			
	34	18			
	10	44			
	21	54			
	28	24			
31	2	9	10	14	6
	1	14	26	1	19
	2	7	10	14	2
	0	16	4	2	3
	2	9	3	3	5
32	36	24	55	20	36
	21	26	48	22	17
	11	26	42	23	37
	9	45	58	36	46
	18	63	30	26	58

TABLE I.8
MAST CELL COUNT--BONE MARROW

-CA, -D DIET

Rat ID	Section				
	1	2	3	4	5
1	30	57	42	28	15
	27	39	51	12	26
	85	33	43	32	16
	70	39	56	43	47
	69	50	57	23	38
2	19	34	9	33	11
	11	6	31	18	23
	35	17	43	21	20
	13	25	13	20	25
	35	25	45		19
3	25	65	37	53	23
	22	55	24	59	18
	15	24	20	23	14
	33	56	19	19	21
	19	18	11	45	17
4	23	18	33	60	16
	18	33	31	51	49
	23	32	49	50	64
	41	26	38	21	52
	39	34	20	14	63
5	28	39	39	25	21
	69	45	17	17	38
	12	24	37	37	27
	32	60	31	41	33
	22	62	37	29	42
6	31	35	15	46	31
	23	38	22	50	25
	21	38	37	39	31
	33	43	16	30	15
	34	28	17	42	48
7	17	35	14	14	13
	4	15	16	12	12
	14	22	3	13	28
	8	10	7	22	20
	42	57	4	15	13
8	21	15	20	23	50
	10	14	25	28	36
	15	28	5	22	43
	40	23	34	15	37
	30	23	31	22	38

Table I.8 continued

Rat ID	Section				
	1	2	3	4	5
9	28	10	17	39	25
	34	19	17	37	9
	25	25	8	25	29
	23	20	9	9	7
	13	17	13	11	12
10	26	46	35	47	35
	14	15	32	18	48
	32	16	40	34	27
	17	36	19	21	33
	20	23	43	24	37
11	29	19	35	34	24
	13	20	23	25	31
	34	43	51	40	26
	44	37	40	20	33
	38	31	32	30	25
12	15	12	17	10	14
	21	14	10	15	13
	17	15	14	12	14
	24	21	17	15	14
	25	37	22	19	21
13	23				
	23				
	23				
	33				
	35				
14	11	7	58	14	55
	7	10	15	37	15
	9	7	10	16	17
	13	16	37	19	7
	16	26	9	34	23
15	24	17	12	20	20
	21	20	27	20	23
	16	27	14	14	14
	15	27	16	20	22
	24	25	16	27	20
16	13	21	17	52	30
	27	26	20	60	8
	29	26	11	34	7
	26	15	25	26	8
	30	8	10	5	18

Table I.8 continued

Rat ID	Section				
	1	2	3	4	5
17	40	28	14	28	15
	14	11	23	16	22
	37	34	46	24	17
	67	24	20	14	16
	32	38	35	11	14
18	49	54	27	10	19
	32	19	11	15	10
	20	15	11	14	16
	19	14	8	9	16
	18	15	12	24	11
19	29	38	23	36	31
	50	17	53	39	43
	27	28	23	22	49
	23	27	17	33	43
	51	16	45	2	30
20	20	78	31	14	40
	37	30	48	18	23
	40	28	23	32	25
	41	63	26	28	31
	35	64	23	28	52
21	16	79	12	16	7
	110	39	23	20	21
	35	57	26	17	50
	51	52	53	18	26
	33	67	23	24	22
22	23	6	14	20	92
	8	11	35	9	71
	14	11	30	33	
	11	6	10	15	
	15	17	5	69	
23	19	11	24	20	18
	26	11	15	9	19
	30	11	66	20	17
	16	18	29	29	18
	13	12	27	41	25
24	18	19	24	21	42
	28	29	51	27	51
	24	46	25	18	39
	33	36	24	54	47
	19	19	27	27	26

Table I.8 continued

Rat ID	Section				
	1	2	3	4	5
25	22	27	53	22	22
	26	37	57	35	39
	43	45	60	75	36
	19	23	57	26	27
	23	43	40	37	20
26					
27	5	70	16		
	8	42	32		
	15	16	50		
	20	49			
	41	18			
28	23	28	28	27	67
	10	22	11	8	20
	22	32	29	10	12
	100	26	23	13	20
	20	66	25	33	19
29	153	123	74	92	
	110	84	59	59	
	72	78	103	50	
	61	143	62	80	
	78	78	75	98	
30	38	12	28	37	26
	60	13	35	61	69
	39	32	45	50	25
	46	26	9	33	34
	32	43	13	36	21
31	111	68	70	75	65
	48	92	58	59	43
	38	58	76	89	80
	56	50	50	68	44
	62	97	58	47	110
32	66	17	21	63	17
	17	52	33	35	56
	27	14	14	44	25
	31	13	36	45	48
	57	21	25	34	39

Fig. I.1. Photomicrographs showing mast cells in 8 μm sections of femoral bone marrow in female rats fed a normal diet. Age of rat was 3 1/2 months. Original magnification: X400. Toluidine blue stain. 1. Ovariectomized and estrogen treated rat. The mast cells are small (< 10 μm in diameter), round, darkly stained and difficult to distinguish because of blending in with background. 2. Ovariectomized rat. Mast cells are dark and round. 3. Estrogen treated, intact rats. 4. Untreated rat. Mast cells stained very light and were difficult to distinguish.

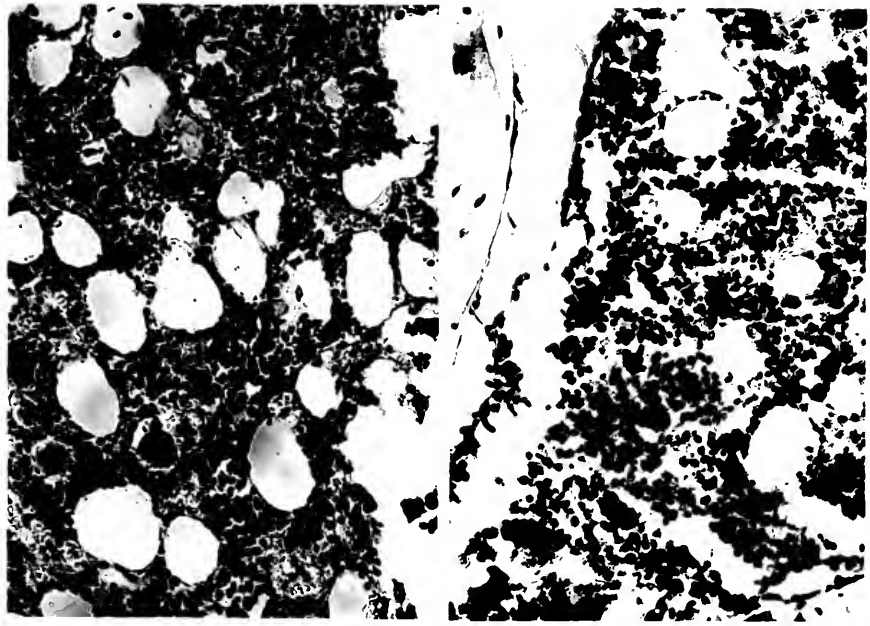


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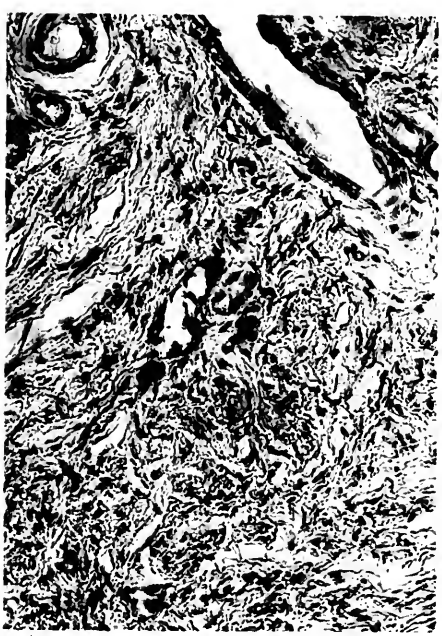
Fig. I.2. Photomicrographs showing mast cells in 8 μm sections of epithelial and connective tissue in female rats fed a calcium-deficient diet. Original magnification X400. Toluidine blue stain. 1. Ovariectomized and estrogen treated rat. Vaginal tissue. Mast cells are small and darkly stained. 2. Ovariectomized rat. Granules of bone marrow mast cells stained dark and light purple. 3. Estrogen treated, intact rat. Bone marrow mast cells stained faintly. 4. Untreated rat. Bone marrow mast cells were small and stained reddish purple.



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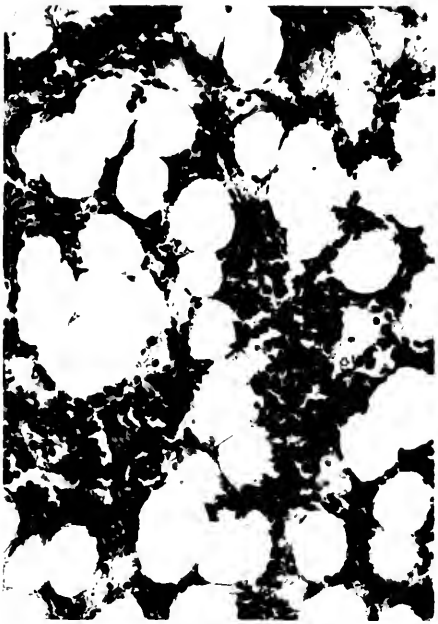

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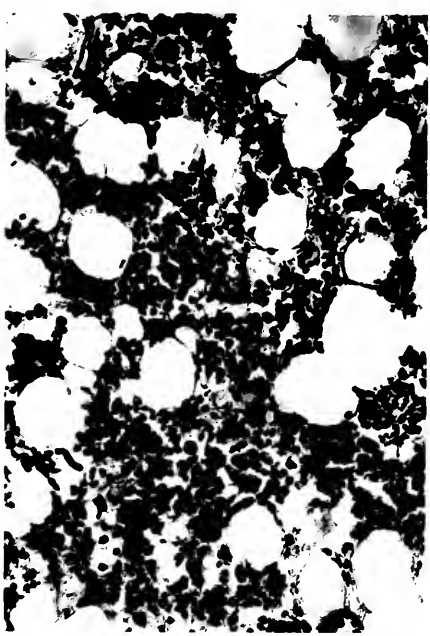

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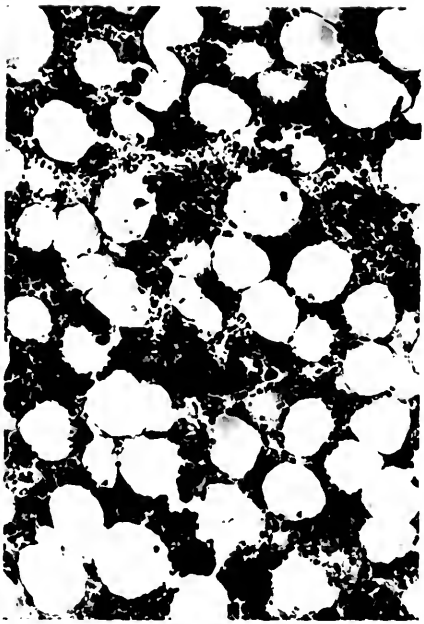

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Fig. I.3. Photomicrographs showing mast cells in 8 μm sections of femoral bone marrow in female rats fed a vitamin D-deficient diet. Original magnification: X400. Toluidine blue stain. 1. Ovariectomized and estrogen treated rat. 2. Ovariectomized rat. 3. Estrogen treated, intact rat. 4. Untreated rat.

Mast cells in the bone marrow of vitamin D-deficient rats stained deep purple and occasionally a reddish-purple. They were difficult to distinguish from the stained background.

1

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Fig. I.4 Photomicrographs showing mast cells in 8 μm sections of femoral marrow in female rats fed a calcium- and vitamin D-deficient diet for 5 weeks. Original magnification: X400. Toluidine blue stain. 1. Ovariectomized and estrogen treated rat. The mast cells are round and deeply stained with dense granules. 2. Ovariectomized rat. Mast cells are very small and difficult to distingiush from the darkly stained background. 3. Estrogen treated, intact rats. Mast cells are darkly stained and small (approximately 10 μm in diameter). 4. Untreated rat. Mast cell "nests" found within bone trabeculae are shown.

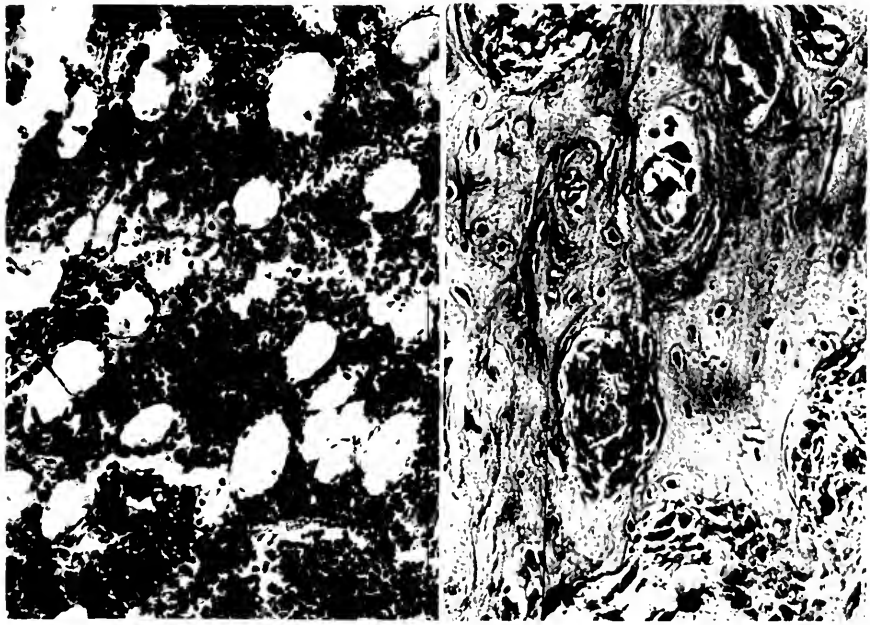

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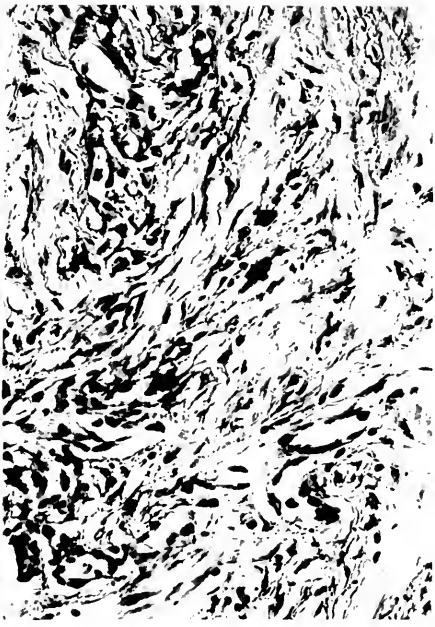
Fig. I.5. Photomicrographs showing mast cells in 8 μm sections of vaginal tissue in rats fed a normal diet Original magnification: X400. Toluidine blue stain. 1. Ovariectomized and estrogen treated rat. Mast cells stained deep purple. All mast cells were slightly degranulating, with at least 3 to 4 granules around a cell. 2. Ovariectomized rat. Mast cells appeared in various shapes with slight degranulation. 3. Estrogen treated, intact rat. Note the various sizes and shapes of mast cells. 4. Untreated rat. Mast cells stained deep purple and the nucleus is obliterated by the stain. Mast cells are of various sizes with streaks of granules nearby.

2



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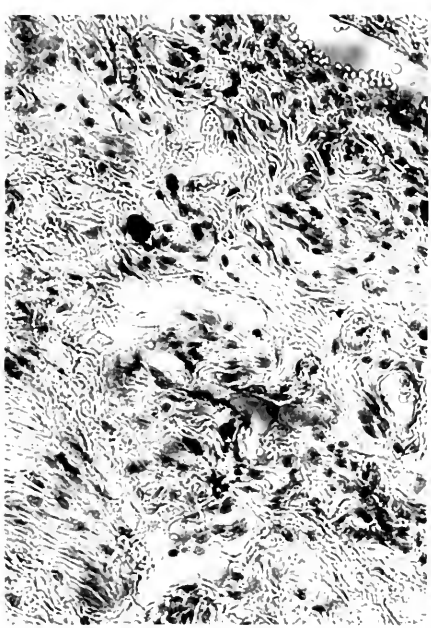

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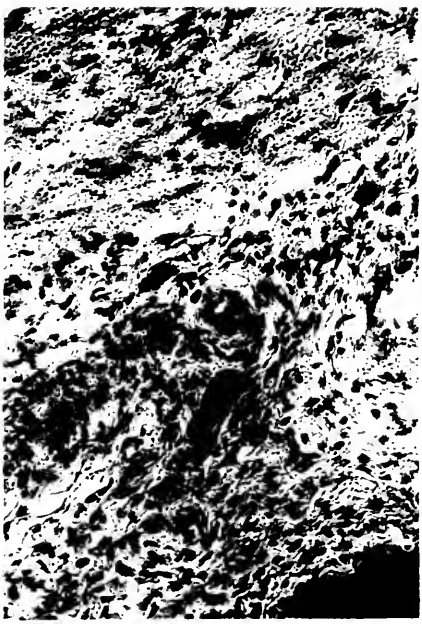
Fig. I.6. Photomicrographs showing mast cells in 8 μm sections of vaginal tissue in rats fed a calcium-deficient diet. Original magnification: X400. Toluidine blue stain. 1. Ovariectomized and estrogen treated rat. Mast cells stained deep purple. A few granules were seen around the cells. 2. Ovariectomized rat. Degranulation of mast cells was common. Note the perivascular locations of the mast cells. 3. Estrogen treated, intact rat. The mast cells stained dark and exhibit irregular shapes. 4. Untreated rat. Irregular shape, perivascular location, and sparse occurrence predominate.

2



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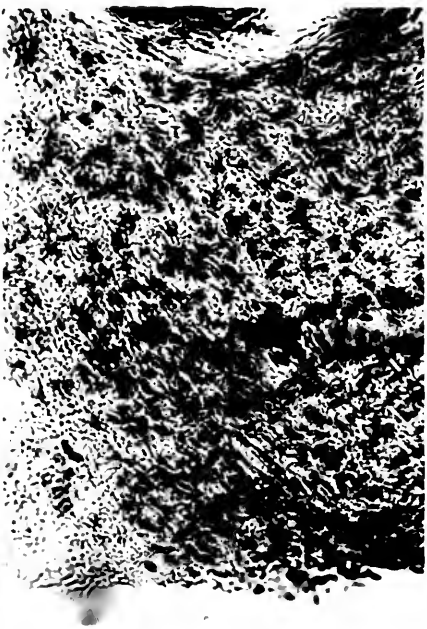

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Fig. I.7. Photomicrographs showing mast cells in 8 μm sections of vaginal tissue in rats fed a vitamin D-deficient diet. Original magnification: X400. Toluidine blue stain. 1. Ovariectomized and estrogen treated rat. Mast cells were not well shaped, and often degranulating. 2. Ovariectomized rat. Mast cells were small (approximately 10 to 15 μm in diameter, sparse, and of various shapes. 3. Estrogen treated, intact rat. Mast cells were small, round, and dark. 4. Untreated rat. Mast cells were found in "areas" of degranulation and granule "streaking" was common.

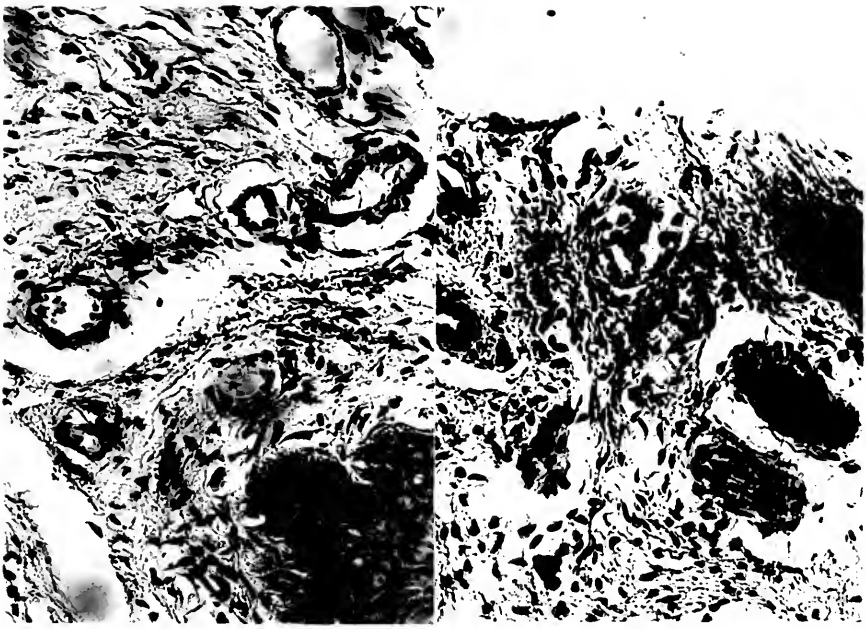

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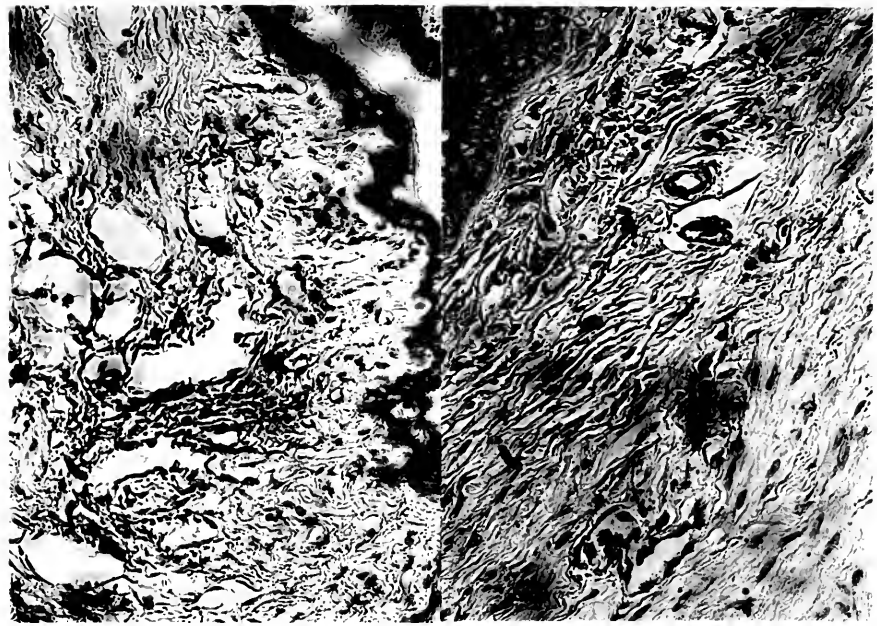
Fig. I.8. Photomicrographs showing mast cells in 8 μm sections of vaginal tissue in rats fed a calcium- and vitamin D-deficient diet. Original magnification: X400. Toluidine blue stain. 1. Ovariectomized and estrogen treated rat. Mast cells exhibit various shapes and degranulation. 2. Ovariectomized rat. 3. Estrogen treated, intact rat. 4. Untreated rat. Mast cells tend to occur in groups and near arterioles and blood vessels.



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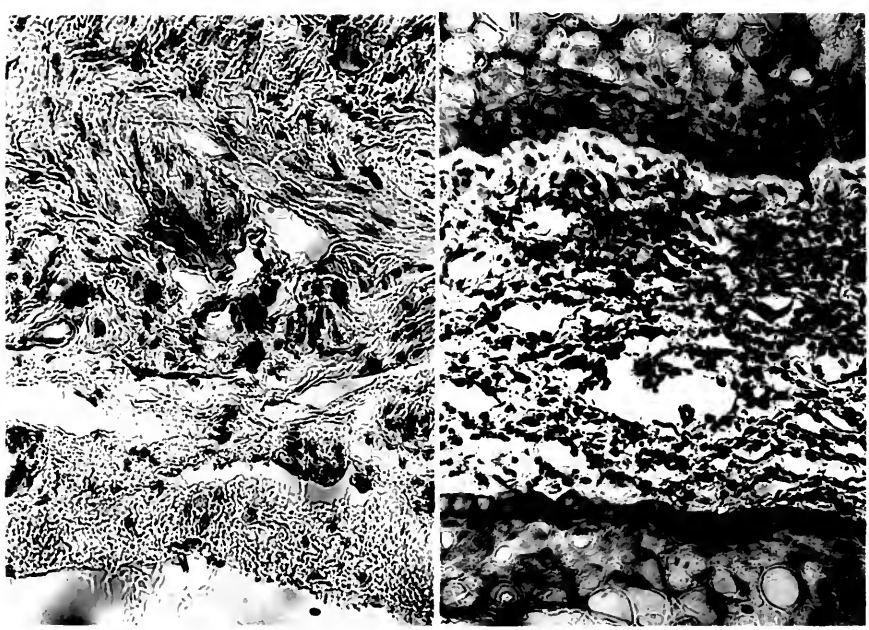

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BIOGRAPHICAL SKETCH

Rogene E. Kresak Tesar was born on June 5, 1938, on a farm near Western, Nebraska. After attending and graduating from Milligan High School, Milligan, Nebraska, as valedictorian of her class in 1956, she attended the University of Nebraska at Lincoln for two years. In 1957 she married Delbert Tesar; two daughters were born to this union. Upon moving to Manhattan, Kansas, she continued her education and obtained a Bachelor of Science in Home Economics degree in 1962.

That same year, Rogene and her family moved to Atlanta, Georgia. She taught in the DeKalb County Public School System for two years.

The next eight years included living for a year in Vienna, Austria, moving to Gainesville, Florida, having two additional children, and living for a year in Cheshire, England.

In 1973, she enrolled at the University of Florida and completed another Bachelor of Science degree in 1977; the major was food science. Immediately, she began graduate studies and graduated with a Master of Agriculture degree in food science and human nutrition at the University of Florida in 1979. At this time, she also became a Registered Dietitian after training at North Florida Regional Hospital.

Continuing graduate work in the Department of Animal Science specializing in nutrition, she obtained a graduate research assistantship at the University of Florida and became involved in research and professional counseling, principally concerning bone loss and osteoporosis, at the Center for Climacteric Studies. In December, 1980, she became a candidate for the Ph.D. After completing the required research and dissertation, she received the Doctor of Philosophy degree in May, 1982.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J.P. Feaster, Chairman
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Morris Notelovitz, Cochairman
Associate Professor of Obstetrics and Gynecology

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Clarence B. Ammerman
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James S. Dinning
Research Scientist
Food Science & Human Nutrition

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 1982

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Dean, College of Agriculture

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