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# A Study of Relationships Among Bone Density, Urinary Hydroxyproline, and the Metabolic Balances of Calcium, Phosphorus, and Nitrogen in Geriatric Women

Margaret Davies MacDuff
*University of Tennessee, Knoxville*

To the Graduate Council:

I am submitting herewith a thesis written by Margaret Davies MacDuff entitled "A Study of Relationships Among Bone Density, Urinary Hydroxyproline, and the Metabolic Balances of Calcium, Phosphorus, and Nitrogen in Geriatric Women." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Roy E. Beauchene, Major Professor

We have read this thesis and recommend its acceptance:

Rossie L. Mason, Jane R. Savage, Bernadine Meyer

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

May 17, 1973

To the Graduate Council:

I am submitting herewith a thesis written by Margaret Davies MacDuff entitled "A Study of Relationships Among Bone Density, Urinary Hydroxyproline, and the Metabolic Balances of Calcium, Phosphorus, and Nitrogen in Geriatric Women." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Roy E Beaudene
Major Professor

We have read this thesis
and recommend its acceptance:

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Accepted for the Council:

_____
Vice Chancellor for
Graduate Studies and Research

# A STUDY OF RELATIONSHIPS AMONG BONE DENSITY, URINARY HYDROXYPROLINE, AND THE METABOLIC BALANCES OF CALCIUM, PHOSPHORUS, AND NITROGEN IN GERIATRIC WOMEN

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A Thesis

Presented to

the Graduate Council of

The University of Tennessee

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Margaret Davies MacDuff

June 1973

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## ABSTRACT

Relationships among bone density, urinary hydroxyproline (HOP), and the metabolic balances of calcium, phosphorus, and nitrogen were investigated in 16 elderly women.

The subjects resided at Eastern State Psychiatric Hospital, Knoxville, Tennessee. They ranged in age from 68 to 82 years with a mean age of 74. The consent of each subject and that of her nearest relative or guardian and of the medical staff of the hospital was obtained before participation in the study was permitted.

Twenty-four-hour urine and fecal samples and food samples, including snacks, were collected over a 7-day period. Weighed food consumptions of each subject were obtained during the same period. Food, urine, and fecal samples were analyzed for calcium, phosphorus, and nitrogen content and urine was also analyzed for HOP and creatinine per 24 hours. In addition, HOP excretion was standardized for creatinine excretion and body surface area. The height, weight, and bone density of each subject was determined and body surface area ($m^2$) was calculated from a nomogram using the height and weight measurements.

The mean intakes for nitrogen, phosphorus, and calcium were 9.00 g, 1038 mg, and 770 mg, respectively. All 16 subjects were in positive nitrogen and phosphorus balance, while 12 out of 16 women were in negative calcium balance with mean balances of 2.29 g, 258 mg, and -52.4 mg, respectively. Urinary nitrogen and phosphorus excretions were significantly correlated with nitrogen ($r = 0.92$, $P < .001$) and phosphorus ($r = 0.61$, $P < .006$) intakes, respectively. Nitrogen, calcium, and

phosphorus intakes were positively correlated with nitrogen ($r = 0.74$, $P < .001$), calcium ($r = 0.58$, $P < .01$), and phosphorus ($r = 0.80$, $P < .001$) balances, respectively. There was a strong tendency for calcium ($r = -0.41$, $P = 0.57$) and nitrogen ($r = -0.31$, $P = .099$) intakes to decrease with age, while decrements in phosphorus intakes were significant with age ($r = -0.42$, $P < .05$).

The mean values for HOP excretion (24 mg) and for HOP excretion per square meter of body surface area (16 mg/24 hr/$m^2$) were normal, while the mean value for the HOP-creatinine ratio (41 mg/g) was high in comparison to values reported in the literature. Hydroxyproline excretion and HOP excretion per $m^2$ were both positively correlated with the urinary excretions of creatinine, nitrogen, calcium, and phosphorus ($P < .05$). In addition, HOP excretion ($r = 0.78$, $P < .001$) and HOP excretion per $m^2$ ($r = 0.62$, $P < .005$) were both positively correlated with body weight, and the HOP-creatinine ratio ($r = -0.50$, $P < .02$) and HOP excretion per $m^2$ ($r = -0.43$, $P < .05$) were both negatively correlated with age.

The mean bone density was 0.80 gram equivalents of alloy per cubic centimeter of bone, which was normal in comparison to values reported in the literature. The mean creatinine excretion was 0.607 g, which was low in comparison to values cited in the literature. Bone density was correlated with body weight ($r = 0.51$, $P < .02$), HOP excretion ($r = 0.45$, $P < .04$), and creatinine excretion ($r = 0.43$, $P < .05$), while the latter was correlated with body weight ($r = 0.70$, $P < .001$). No significant relationships were found between either bone density or creatinine excretion with age.

It was concluded that the quantity of osseous tissue was reflected in body weight and in HOP excretion. Also, from the relationships obtained among HOP excretion, bone density, body weight, and age, it was concluded that an age-associated decrement in body collagen turnover occurred.

TABLE OF CONTENTS


| CHAPTER | | PAGE |
|---|---|---|
| I. | INTRODUCTION | 1 |
| II. | REVIEW OF LITERATURE | 2 |
| | Osteoporosis | 2 |
| | Bone Density Studies | 4 |
| | Urinary Hydroxyproline | 5 |
| | Metabolic Balance Studies | 10 |
| III. | EXPERIMENTAL PROCEDURE | 12 |
| | General Plan | 12 |
| | Subjects | 12 |
| | Urine, fecal, and dietary collection | 13 |
| | Physical Measurements | 13 |
| | Height and weight | 13 |
| | Body surface area | 14 |
| | Bone density | 14 |
| | Biochemical Measurements | 15 |
| | Ashing | 15 |
| | Calcium method | 15 |
| | Phosphorus method | 16 |
| | Nitrogen method | 18 |
| | Urinary hydroxyproline method | 19 |
| | Creatinine method | 20 |
| | Statistical Analysis | 22 |

CHAPTER PAGE

IV. RESULTS AND DISCUSSION . . . . . 23

V. SUMMARY. . . . . 36

REFERENCES. . . . . 38

VITA. . . . . 45

LIST OF TABLES


| TABLE | | PAGE |
|---|---|---|
| 1. | Mean Values for Age, and Selected Physical and Urinary Measurements for the Elderly Women . . . . . | 24 |
| 2. | Correlation of Hydroxyproline Excretion with Creatinine, Nitrogen, Calcium, and Phosphorus Urinary Excretions . . | 27 |
| 3. | Correlation of Hydroxyproline Excretion per Square Meter of Body Surface with Creatinine, Nitrogen, Calcium, and Phosphorus Urinary Excretions. . . . . | 27 |
| 4. | Correlation of Nitrogen, Calcium, and Phosphorus Intakes with Their Respective Balances . . . . . | 28 |
| 5. | Correlation of Selected Factors with Age . . . . . | 30 |
| 6. | Correlation of Selected Factors with Weight. . . . . | 32 |
| 7. | Correlation of Selected Factors with Bone Density. . . . . | 34 |

# CHAPTER I

## INTRODUCTION

In recent years the well-being of America's senior citizens has been emphasized. With the lengthening of the average life span for man in the developed countries, this older population has consequently increased in number. This evolution has brought with it an awareness of the poor nutritional state which accompanies many elderly people.

In studying the nutritional status of aging individuals, many investigators have focused on the changes in bone with age. It is well established that both sexes experience a decrease in bone mass with age, but the pathogenesis of many bone disorders characterized by this change is unknown.

In the present study, bone density, urinary hydroxyproline, and metabolic balances of calcium, phosphorus, and nitrogen were measured in geriatric women. Many investigators have used one or more of the above parameters to study skeletal status, but few have used all of them. In the present study, the relationships which existed between these variables were investigated.

## CHAPTER II

## REVIEW OF LITERATURE

The following review of literature covers various studies which have used one or more of the above parameters mainly to evaluate bone tissue status and investigate bone disorders. For more meaning, this review is based against a short background of osteoporosis, a common bone disease found especially in the aged female population and which is the main basis for this study.

### Osteoporosis

The development of osteoporosis with advancing age in man is widespread (1-4). This disease has been shown to occur in both sexes, but elderly women tend to lose more bone than males (2, 5, 6). The minimal percentage of the radiographic incidence of clinically detectable osteoporosis has been estimated at 26% of women more than 60 years old (4, 7). The average loss of bone between youth and old age amounts to approximately 15%, but this loss involves a larger percentage of trabecular than cortical bone (1, 8).

In osteoporosis the bone present per unit volume is decreased in amount but normal in composition (8-10). The pathogenesis of osteoporosis is unknown, but it could be due to a decreased rate of new bone formation, an increased rate of resorption or a combination of the two (10). Albright (11) proposed that the development of osteoporosis was the result of a deficient formation of the protein matrix which caused a decrease in new bone formation while the resorption process continued at a normal rate. More

recently, however, Nordin (10) has postulated that a deficient calcium intake over a long period of time is the main determinant in osteoporosis. He suggested that pathological factors, such as intestinal malabsorption and excessive urinary or fecal excretion could contribute to the development of the disease; and he also reported that hormonal imbalance may contribute to the etiology of the disease.

Dallas and Nordin (12) observed through diet histories and X-ray scores that the mean calcium and protein intakes of 103 normal subjects were significantly higher than those of 123 osteoporotic subjects, but the mean calcium excretion was the same in both groups. An earlier study by Nordin (10) reporting the diet histories of 231 subjects indicated that a daily calcium intake over 1 g is rarely associated with osteoporosis, but that a lower intake could be associated with the disease depending upon whether an individual adapts to a low calcium intake. Also calcium excretion in the subjects with osteoporosis tended to be higher than in the control subjects. Caniggia et al. (13) administered an oral dose of $^{45}Ca$ to 13 osteoporotic subjects and 5 normal subjects to study the intestinal absorption of calcium. In 6 hours, 0.80 to 1.20% of the isotope was eliminated in the urine in normal subjects as compared to 0.15 to 0.55% in the osteoporotic subjects. But in the feces, collected over 3 days, the fraction of the dose administered was 28 to 38% in the normal subjects and 48 to 65% in the osteoporotics. The isotope appeared more slowly in the blood in osteoporotics and the levels of radioactivity reached were lower than in the normal subjects.

It has also been proposed that the increase in osteoporosis with age may partly represent the results of a life-long utilization of the buffering capacity of the basic salts of bone for the constant assault

against pH homeostasis. Diets in the United States usually include large amounts of meat, which is a primary source of acid-ash (4). Ellis and Ellis (14) reinforce this hypothesis in a study which showed a higher incidence of osteoporosis in omnivores than vegetarians.

These various studies support the view that multiple factors influence calcium retention, bone mass, and skeletal deformities which characterize the osteoporosis of aging (3).

Bone Density Studies

Perhaps the main reason the investigation of bone is used for the study of aging is due to the labile nature of bone in that changes in this tissue can be easily assessed radiologically and histologically (15). In studying the nutritional aspects of aging, several investigators have measured bone density. Multiple studies involving radiological and histological measurements have determined that after an increase in bone mass from infancy to puberty, there is a gradual but steady decrease in bone density beginning around the fourth decade and continuing throughout life (2, 3, 9, 14, 16-21). However, Baker and Angel (22) found no significant correlation between age and bone density. But it was found that the black male tended to have a higher bone density than the white male population and females in general had lower bone densities than males. Doyle (17) reported that in females cortical thickness was strikingly reduced in the age groups 40 to 49 and 60 to 69. Mason et al. (18) reported a decrease in calcium intake and bone densities in males and females after the fourth decade. Using X-rays taken with a photoelectric densitometer, Exton-Smith et al. (23) found that women with low skeletal densities weighed less than average and had low vitamin D intakes and low serum calcium values. Ellis

and Ellis (14) associated their bone density measurements with the acid-ash contents of diets. Though both vegetarians and omnivores displayed decreased bone densities with age, the omnivores showed a greater degree of decrease in all geriatric age groups, while the vegetarians showed no further decrease after approximately 69 years of age. Williams et al. (24) found that bone densities in young adults were not readily altered by changes in dietary calcium. In a radiographic survey of over 2000 ambulatory women, Smith and Frame (3) reported that bone density values were unrelated to calcium intakes. Morgan et al. (19) also reported insignificant correlations between bone density measurements and intakes of either calcium, protein, or ascorbic acid.

Much previous bone density work has been concerned with perfecting instrumentation and techniques. But with the various approaches to measurement, there is no uniform terminology used to express bone density. Colbert (25), in an overview, describes the different techniques which have been used to measure bone density and presents various suggestions for improvement in the future use of this parameter.

Urinary Hydroxyproline

The expectation that aging will result in a variety of demonstrable changes in bone collagen metabolism has led to the use of various measurements which might reflect these changes, such as urinary hydroxyproline (HOP) excretion. One of the distinguishing features of collagen is its high content of HOP (26). This amino acid was first noted in normal urine by Westall (27) who concluded that it was a degradation product of collagen. More recently, Klein et al. (28) have suggested that changes in bone collagen alone can greatly alter HOP excretion values. It has now become

generally accepted that bone collagen is the most important source of HOP since (a) bone collagen accounts for about 55% of total body collagen and (b) in most of the conditions characterized by high hydroxyprolinuria bone diseases have been shown to have a major influence (29).

Goidanich et al. (29) measured HOP excretion in various subjects affected with primary diseases of bone. In Marquio's disease and osteogenesis imperfecta HOP excretion was within normal limits, but in Paget's disease and in fibrous dysplasia of bone high levels of HOP were excreted. Dull and Henneman (30) also demonstrated in patients with Paget's disease and also in patients with hyperthyroidism a significant elevation in HOP excretion. In hyperparathyroidism, Klein et al (28) found increases in HOP excretion in the cases accompanied with bone lesions. Lee and Lloyd (31) measured 24-hour excretions of HOP in 80 people with various diseases. The highest values were found in the individuals with a localized disease of bone. Sjoerdsma et al. (32) reported an increased excretion of HOP in 7 out of 10 patients with Marfan's syndrome. Anderson et al. (33) have shown that the clinical and biochemical diagnosis of osteomalacia is associated with an increased output of HOP peptides. This increase has also been observed in patients with adult celiac disease and other malabsorption states where osteomalacia often develops as a secondary disorder (34). Collagen metabolism was studied by Langress and Behnke (35) in 16 patients with osteogenesis imperfecta, which is characterized by generalized osteoporosis and fragility of bone. Thirteen out of the 16 patients excreted more HOP than age-matched control subjects. Caniggia (36) has reported excretions of HOP to be greater in senile osteoporosis, often reaching the levels of osteolytic diseases. In a study by

Reshef et al. (2) the urinary excretion of HOP was positively correlated with osteoporosis in females but not in males. The older women excreted more HOP than did the younger females. In contrast, Moskowitz et al. (37) found no difference in HOP excretion between osteoporotic patients and normal controls. In patients with metastic carcinoma of bone, Platt et al. (38) found a correlation between HOP excretion and the degree of activity of the bony metastases. In patients with peripheral rheumatoid arthritis and patients with various collagen diseases, the mean daily excretion of HOP was shown to be elevated also (39).

The preceding results are consistent with the idea that increased urinary HOP excretion in the elderly may be indicative of abnormal bone or collagen metabolism.

Besides diseases which directly affect bone and collagen, various endocrine diseases have been associated with increased levels of urinary HOP. Benoit et al. (40) demonstrated the presence of elevated amounts of HOP in diseases associated with excess growth hormone, thyroid hormone, and pituitary gonadotropins. Only the hormones known to be concerned with bone metabolism were effective in altering excretion values.

In addition to disease states, HOP has been associated with other variables. Interest has existed for many years about the relationship between age and urinary HOP. Several investigators have shown higher excretion values in normal children than in adults (41-46). Jasin et al. (44) reported that HOP excretion was significantly higher in 10 to 14 year old children than in adults. Smiley and Ziff (42) have concluded that under normal conditions, highest values of HOP excretion are found during periods of growth.

In comparison to children, less information is available on normal excretion values in the elderly. Saleh and Coenegracht (45) determined HOP and calcium excretion in 80 patients between the ages of 10 to 90 years who were not known to be affected from diseases of the bone or collagen tissues. They reported a significant decrease in HOP and calcium excretion between the ages of 70 and 90. It was suggested that the lower values in the older age group were due to a diminished volume of metabolically active bone, the low turnover of insoluble collagen, and a decrease in the synthesis of soluble collagen.

Many studies have investigated the relationship between hydroxyproline excretion, bone formation, and bone resorption rates. Klein et al. (47) used calcium balance and kinetic studies to investigate the relationships among urinary HOP, serum alkaline phosphatase, calcium accretion and resorption rates. Pappas et al. (46) studied the patterns of HOP excretion to see if it would be effective in reflecting the skeletal metabolic stages in the maturational process in children. Both investigators found a positive correlation between urinary HOP and calcium resorption rates. Johnston and Deiss (48) have also concluded that an increase in urinary HOP following administration of parathyroid hormone was due to an increase in the resorption of bone. In contrast, Klein et al. (47) after finding a correlation between serum alkaline phosphatase and urinary HOP suggested that increases in HOP reflected bone collagen formation and not breakdown. But, in general, most authors agree that the urinary level of HOP represents bone destruction (30, 47-50).

The ingestion of large quantities of gelatin has been reported to greatly increase peptide-bound HOP excretion, while the ingestion of free

HOP results only in an increased excretion of the free amino acid. Non-peptide-bound urinary HOP normally constitutes 3% or less of the total urinary HOP excreted (38, 51). Ziff et al. (39) found that when subjects fed a normal diet were changed to a diet containing lower amounts of HOP and then later to a diet that was virtually free of HOP, there was no significant decrease in excretion of HOP. It was concluded that dietary HOP was not an important source of urinary HOP. In many of the studies which have been cited, the subjects were fed a HOP free diet, while other workers have felt no need for such a regimen and allowed their subjects an ad libitum diet. Because only slight increases in HOP excretion have been measured in people consuming a high-meat diet, it is likely that only the ingestion of gelatin leads to a significant absorption of HOP-peptides (39).

It is assumed that endogenous urinary HOP originates from the breakdown of collagen which is supported by reports which show that the amino acid composition of the peptides excreted are comparable to the amino acid composition of collagen (43, 49, 52). Prockop (53), using injections of proline-$^{14}C$, has suggested that the endogenous HOP-peptides excreted originate from both soluble and insoluble collagen degradation.

Along with differences in dietary regimens, studies have also varied in the manner employed to express HOP excretion. Many investigators have voiced the need to standardize HOP excretion values in relation to body size. These investigators have used ratios to express excretion values based on body surface area or urinary creatinine. Whitehead (54) has extensively used the hydroxyproline index as a measure of nutritional status of children in underdeveloped countries. In many of the studies

previously cited, urinary HOP was just expressed as the total mg excreted per 24 hours.

Metabolic Balance Studies

In seeking the pathogenesis of various bone disorders and evaluating mineral status in aging individuals, the use of metabolic balances is common. In 1953, Bogdonoff et al. (55) conducted calcium, phosphorus, nitrogen, and potassium balance studies in 7 aged males. At low levels of calcium intake the subjects were in negative calcium balance and did not reach calcium equilibrium until intakes averaged 850 mg of calcium daily. In two subjects with X-ray evidence of osteoporosis, who were storing nitrogen, phosphorus, and potassium, the retention of calcium was not significantly increased when large amounts of calcium were supplied. Nordin (10), reporting on 40 balance studies at different levels of calcium intake in 20 cases of osteoporosis, found that urinary calcium from the patients varied independently of their calcium intake. In contrast, the urinary phosphorus and nitrogen excretion in these patients varied with their phosphorus and nitrogen intakes, respectively. It was also reported that a patient with osteoporosis consuming a self-selected diet was yet to be found in negative nitrogen balance. Similar results were reported by Harrison et al. (56) who used calcium balance studies in osteoporotic patients to determine their responses to different levels of calcium intake. With higher intakes the patients did become more strongly positive, but not as much as the control subjects. Spencer et al. (57) reported similar results with calcium balance in 3 female patients with osteoporosis. With the additional aid of radioisotope data, it was shown that high fecal calcium during periods of high calcium intake was derived

principally from the ingested calcium and only to a minor extent from endogenous calcium. It was also reported that all nitrogen balances were positive. Ackerman and Toro (58) also found similar results in that an average of 80% of the calcium intake in 8 elderly women was excreted as fecal calcium. They determined an increased calcium intake was required for calcium balance in elderly women.

# CHAPTER III

# EXPERIMENTAL PROCEDURE

## I. GENERAL PLAN

This study is part of a more extensive investigation concerning the effect of diet and dietary supplements on the bone density and nutritional status of 80 elderly women. The subjects in the study were divided into 4 experimental groups approximately equal in number. One group was used as control; the other groups were administered the various supplements. Four women from each experimental group participated in a metabolic balance study. The measurements reported in this study are those obtained on these subjects before supplementation was begun.

### Subjects

The subjects were 16 geriatric women residing at Eastern State Psychiatric Hospital, Knoxville, Tennessee. They ranged in age from 68 to 82 years with a mean age of 74. Each of the participants received a letter of explanation concerning the study and indicated a desire to participate by signing a consent form. Likewise, the nearest relative or guardian of each subject received a letter of explanation and signed a similar consent form before the study began. All subjects were judged to be physically and mentally able to participate in the study by the medical staff of the hospital. All of the women ate the regular cafeteria diet served in their resident dormitory.

Urine, Fecal, and Dietary Collection

Twenty-four-hour urine and fecal samples were collected on each subject over a 7-day period in May of 1971. The volume of each urine sample was determined daily, and aliquots were pooled into a 7-day urinary sample. Also a portion of each 24-hour urine specimen was preserved for creatinine determinations. These urine samples were frozen until analyzed. All fecal samples collected over the 7-day period were also frozen in plastic cartons for later analyses. All the fecal samples of a subject were pooled and homogenized before analysis.

Seven-day records of weighed food intakes were used to obtain the dietary intake information. In the cafeteria with use of a one-pan balance,[a] each serving of food was weighed and recorded. At the end of each meal, the plates were collected and the amounts of the unconsumed foods were weighed and recorded. Using this procedure, the actual weight of each food consumed was determined by difference. The distribution and collection of plates were carried out as unobtrusively as possible to avoid any major change in the eating patterns of the subjects. Weights of all snacks consumed were also recorded during the 7-day period. Samples of all foods, including snacks, consumed by the subjects were collected daily and frozen for the later analyses of calcium, phosphorus, and nitrogen content. Each food was homogenized before analysis.

## II. PHYSICAL MEASUREMENTS

Height and Weight

The height of all subjects was recorded to the nearest one-fourth

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[a]Mettler P1000.

inch and weight was recorded to the nearest one-fourth pound with the approximate height of any noticeable shoe heels being taken into account for the final height determinations. The subjects were weighed wearing light indoor clothing and were asked to remove any sweaters or jackets before being weighed. Pounds and inches were later converted to kg and cm for expression of weight and height, respectively.

Body Surface Area

The body surface area ($m^2$) was calculated using height and weight values and a nomogram as described by Chaney and Ross (59). These values were determined so that the excretion of hydroxyproline (HOP) could be expressed in terms of mg HOP per 24 hours per square meter.

Bone Density

Bone density measurements were obtained at the same time the height and weight measurements were made. Measurements were made using the instrument and technique developed by the Department of Nutrition at the University of Tennessee (60).

A central pathway of the left phalanx 5-2 was marked and used as the site for the bone density measurement. Absorption curves for bone and flesh were drawn directly on graph paper as the finger was carried through a collimated X-ray beam. Anteroposterior and lateral scans were made to provide two measurements so that bone density could be calculated on the basis of an ellipse rather than a circle. Standard absorption curves were obtained by tracing an aluminum alloy wedge. Planimeter measurements were made on the absorption curves of flesh and bone and these measurements were referred to the standard absorption curve. Values of bone density are expressed as X-ray equivalent grams of alloy per cubic

centimeter of bone. The instrument and technique have been described more fully by Mason and Ruthven (60).

## III. BIOCHEMICAL MEASUREMENTS

### Ashing

Duplicate weighed food and pooled fecal samples and pipetted urine samples were put in numbered crucibles and dried under infrared lamps before being placed in a cold muffle furnace and ashed at 550° for 24 hours. The ashed samples were dissolved in concentrated HCl and were evaporated to dryness over water. Food and fecal samples were treated for the removal of silica by continued heating for 1 hour after drying. The samples were again dissolved in concentrated HCl and filtered through Whatman 42 ashless filter paper into volumetric flasks. Quantitative transfer was insured by rinsing the crucibles several times with demineralized water. After complete rinsing of the filter paper and funnels, the filtrate was diluted to the appropriate volume with demineralized water, stoppered and mixed by inversion. All ash solutions were stored in acid-rinsed polyethylene bottles and all equipment was rinsed in 10% HCl for 1 to 2 hours before future use.

### Calcium Method

The calcium content of the food, urine and fecal ash solutions was determined with a Perkin-Elmer Atomic Absorption Spectrophotometer 303 as described by the Perkin-Elmer manual (61). The energy source was a calcium-zinc hollow cathode tube and the sample solutions were aspirated into an acetylene-air flame to produce an atomic vapor from which absorbance values were determined.

Reagents.

1. Calcium Standard Solution (0.5 mg Ca/ml): 0.3121 g of $CaCO_3$ was diluted to 250 ml with 1 ml concentrated HCl and water. Ten ml of the stock solution was diluted up to 100 ml. Working standards containing 0.02 to 0.10 mg Ca/ml were prepared daily by diluting the latter solution with water.

2. Lanthanum, 5%: 5.9 g of $La_2O_3$ was dissolved in 25 ml concentrated HCl and diluted to 100 ml with water and filtered. The solution was used within 1 hour after preparation.

Procedure. A predetermined amount of ash solution known to contain between 0.02 and 0.10 mg Ca/ml was added to acid-rinsed test tubes. Demineralized water was added to bring the total volume to 9.0 ml. One ml of the lanthanum solution was added to each tube and mechanically mixed. The absorbances of the samples were then determined within 1 hour on an atomic absorption spectrophotometer[a] set at wavelength 421.2 nm. After referring the absorbances of the samples to the standard curve for sample concentrations, the following formula was employed:

$$\text{Mg Ca/24 hours} = \text{mg Ca/10 ml solution} \times \frac{\text{Dilution}}{\text{Factor}} \times \frac{\text{Mean 24-hour}}{\text{Urine Volume}}$$

Phosphorus Method

Phosphorus in ashed solutions was determined by a modification of the method of Dryer et al. (62). Ammonium molybdate oxidizes the phosphorus in the sample to phosphomolybdic acid which reacts with N-Phenyl-p-phenylenediamine monohydrochloride to give a blue color which can be measured spectrophotometrically.

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[a]Perkin-Elmer Model 303.

Reagents.

1. Sulfuric acid, 5N: 138 ml of concentrated $H_2SO_4$ was added to 750 ml of water which was then diluted to 1 liter with water.

2. Ammonium molybdate, 0.025 M: 15.45 g of $(NH_4)_6 MO_7 O_{24} \cdot 4 H_2O$ was diluted to 500 ml with water. Five-tenths ml concentrated $H_2SO_4$ was included to facilitate solubility.

3. N-Phenyl-p-phenylenediamine solution (PPD): a few drops of 95% ethanol was placed on 0.25 g of N-Phenyl-p-phenylenediamine monohydrochloride contained in a 500 ml volumetric flask, then diluted to volume with 1% aqueous sodium bisulfite ($NaHSO_3$), mixed, and filtered.

4. Phosphorus Standard Solution (0.5 mg P/ml): 0.4381 g of $KH_2PO_4$ was dissolved in water and diluted to 200 ml; 1, 2, 3, 4, and 5 ml of stock solution were diluted to 250 ml with water. These working standards provided 2, 4, 6, 8, and 10 ug of phosphorus per ml, respectively.

Procedure. Ash solution in an amount containing between 2 and 10 ug of phosphorus was placed in acid-rinsed test tubes. This volume was diluted to 1 ml with demineralized water. One ml of 5N $H_2SO_4$ was added to each tube followed with 1 ml of ammonium molybdate and then 2 ml of PPD. The tubes were mechanically mixed and allowed to stand at room temperature for 10 minutes after which the absorbances were measured against a reagent blank at 770 nm using a spectrophotometer.[a] The amount of phosphorus in each sample was calculated as follows:

$$\text{Mg P/5 ml solution} = \frac{\text{A of Sample X Conc. of Std.}}{\text{A of Std.}}$$

$$\text{Mg P/24 hours} = \text{Mg P/5 ml solution X } \frac{\text{Dilution}}{\text{Factor}} \text{ X } \frac{\text{Mean 24-hour}}{\text{Urine Volume}}$$

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[a] Beckman Model B.

Nitrogen Method

A modification of the macro-Kjeldahl method described by Hawk (63) was used to determine the nitrogen content of food, urine and fecal samples.

Reagents.

1. Sodium Hydroxide, 50%: 500 g of NaOH was dissolved in 750 ml of water in an ice bath.

2. Hydrochloric Acid, 0.1 N: 8.3 ml of concentrated HCl was added to 500 ml water and diluted to 1 liter with water and standardized.

3. Boric Acid, 4%: 55 g of $H_3BO_3$ was dissolved in 1 liter of water by heating.

Procedure. Weighed samples of homogenized food and pooled feces and samples of pipetted urine, prepared in triplicate and estimated to contain 10 to 15 mg of nitrogen, were transferred to 500 ml Kjeldahl flasks. To each flask, including 3 blanks, 5 g of sodium sulfate, concentrated $H_2SO_4$ (20 ml for food and feces and 10 ml for urine samples), 0.3 g of copper sulfate, 1 selenized Hengar crystal and 2 glass beads were added. The samples were digested for an additional 20 minutes after becoming clear and were diluted and mixed with 200 ml of water after cooling. After further cooling, 50 ml of 50% NaOH was added down the side of each flask, in a manner to avoid mixing, and each flask was immediately connected to the condenser after placement on the distilling rack. Each delivery tube was immersed in 50 ml of a 4% boric acid solution containing a few drops of methyl red-methyl blue indicator in an Erlenmeyer flask. Kjeldahl flasks were swirled to mix the 2 layers and the bunsen burners were immediately lit. The distillate was titrated

back to its original purple color with standardized HCl. The nitrogen content of each sample was calculated as follows:

g nitrogen in sample = ml HCl X N HCl X 0.014

Urinary Hydroxyproline Method

Urine was analyzed for total hydroxyproline (HOP) by a modification of the direct acid method described by Firschein and Shill (64). After acid hydrolysis the HOP in the urine sample was oxidized to a pyrrole by Chloramine T. The pyrrole was then condensed with p-dimethylaminobenzaldehyde to produce a red chromagen which was measured spectrophotometrically.

Reagents.

1. Sodium Carbonate, 5% (W/V): 25 g of $Na_2CO_3$ was diluted to 500 ml with water.

2. Citrate Buffer, 0.1 M, pH 6: 58.8 g of sodium citrate ($Na_3C_6H_5O_7 \cdot 2\ H_2O$) was diluted to 2 liters with water; 19.2 g of citric acid ($H_3C_6H_5O_7 \cdot H_2O$) was diluted to 1 liter with water. One hundred fifty ml of the citric acid solution was added to the 2 liters of the sodium citrate solution and the pH was adjusted to 6.0.

3. Chloramine T: 3.5 g of Chloramine T was dissolved in 50 ml of water and stored in the refrigerator.

4. Buffer Solution, pH 6: 57 g of sodium acetate ($CH_3COONa \cdot 3\ H_2O$), 37.5 g of sodium citrate ($Na_3C_6H_5O_7 \cdot 2\ H_2O$), 5.5 g of citric acid ($H_3C_6H_5O_7 \cdot H_2O$) and 385 ml of isopropanol were diluted to 1 liter with water.

5. Oxidizing Solution: Shortly before use, 1 part Chloramine T and 4 parts buffer solution, pH 6.0, were mixed.

6. Ehrlich's Reagent: On day of use, 20 g of p-dimethylamino-benzaldehyde was dissolved in 22 ml of concentrated HCl on a steam bath after which 128 ml of isopropanol was added.

7. Hydroxyproline Standard Solution (100 ug HOP/ml): 25 mg of L-hydroxyproline[a] was diluted to 250 ml with 0.001 N HCl. Ten ml of the stock solution was diluted to 100 ml with water daily. Working standards containing 1 to 10 ug of hydroxyproline were prepared by diluting the latter solution with water.

Procedure. Duplicate urine samples were placed in hydrolysis tubes with an equal volume of concentrated HCl. After hydrolysis for 18 hours at 125°C, the cooled samples were filtered through a sintered glass funnel. Duplicate 1 ml aliquots of each filtrate were neutralized with 6 ml of 5% $Na_2CO_3$ and 3 ml of 0.1 M citrate buffer. To test tubes, 1.5 ml of neutralized filtrate, 2 ml of isopropanol and 0.5 ml of oxidizing solution were added. After 4 minutes at room temperature, the samples were placed in an ice bath and 5 ml of Ehrlich's reagent was added. Then the samples were placed in a boiling water bath for 2 minutes and immediately cooled in an ice bath to room temperature. Standards containing 1 to 10 ug of hydroxyproline were also oxidized and treated in an identical manner. After standing at room temperature for 90 minutes, the absorbances of the samples and standards were measured at 565 nm on a spectrophotometer[b]

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[a] Nutritional Biochemicals Corporation, Cleveland, Ohio.

[b] Beckman Model B.

zeroed against a reagent blank. The amount of hydroxyproline was calculated as follows:

$$\text{ug HOP in sample} = \frac{\text{A of Sample} \times \text{Conc. of Std.}}{\text{A of Std.}}$$

$$\text{mg HOP/24 hours} = \text{mg HOP in Sample} \times \frac{\text{Dilution}}{\text{Factor}} \times \frac{\text{Mean 24-hour}}{\text{Urine Volume}}$$

Creatinine Method

The creatinine content of the daily collections of urine was determined by the ICNND method as described in the Manual for Nutrition Surveys (65). In alkaline solutions, creatinine reacts with picrate at room temperature and forms an orange color which can be measured spectrophotometrically.

Reagents.

1. Sodium Hydroxide, 10%: 100 g of NaOH was diluted to 1 liter with water.

2. Picric Acid, 1%: 10 g of picric acid was dissolved in 1 liter of water with heating.

3. Alkaline Picrate Solution, 1%: 100 ml of NaOH and 100 ml of picric acid were diluted to 1 liter with water.

4. Creatinine Standard Solution: 1.6106 g of crystalline creatinine zinc chloride was made up to 1 liter with 0.1 N HCl. Five ml of the stock solution was diluted to 100 ml with water. Working standards containing 0.05 to 0.25 mg creatinine were prepared daily by diluting the latter solution.

Procedure. Duplicate 0.1 ml urine samples were pipetted into 100 ml volumetric flasks and diluted with approximately 20 ml of water. To each flask 20 ml of alkaline picrate solution was added and samples were allowed to stand for 15 minutes after gentle mixing and then were made up to 100 ml with water. The absorbances of the samples were measured at 520 nm on a spectrophotometer[a] zeroed against a reagent blank. The creatinine content was calculated for each sample as follows:

$$\text{mg creatinine (Cr) in sample} = \frac{\text{A of Sample X Conc. of Std.}}{\text{A of Std.}}$$

$$\text{g Cr/24 hours} = \text{g Cr in sample X } \frac{\text{Dilution}}{\text{Factor}} \text{ X } \frac{\text{Mean 24-hour}}{\text{Urine Volume}}$$

## IV. STATISTICAL ANALYSIS

Using a SPSS computer program, means with standard deviations for all variables were calculated, and correlations (Pearson's r) were determined between all variables. These analyses were performed as described by Steel and Torrie (66).

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[a]Beckman Model B.

# CHAPTER IV

## RESULTS AND DISCUSSION

The mean values for the physical measurements and age for the 16 women are presented in Table 1. The mean value for the bone density was 0.80 ± 0.05 with a range of 0.57 to 1.25 gram equivalents of alloy per cubic centimeter of bone. The mean value agreed closely with that reported by Odland et al. (67) who found a mean bone density of 0.78 for females between the ages of 70 to 80. The mean age in the present study was 74.4 years while the mean height and weight values were 158.2 cm and 52.9 kg, respectively.

The mean daily excretion values for creatinine and hydroxyproline (HOP) are also given in Table 1 as well as the mean HOP-creatinine ratio and HOP excretion standardized for body surface area. The mean daily HOP excretion of 24.1 mg ± 1.7 was similar to those reported by other investigators. Saleh and Coenegracht (45) reported 25.3 mg, Ziff et al. (39) 21.8 mg, and Sjoerdsma et al. (32) 25.1 mg as normal mean values for HOP excretion per 24 hours in adult men and women. The highest mean value was reported by Benoit et al. (40) who found a daily excretion of 27.0 mg in adult female subjects. Dull et al. (68) found a daily excretion range of 8 to 31 mg in normal adults, while in the present study the range for the 16 subjects was 14.0 to 37.3 mg for 24 hours.

The mean daily creatinine excretion for the subjects in this study ranged from 0.32 to 1.0 g with a mean of 0.607 g ± 0.046. Figures varying from 1.0 to 1.8 g daily for men and 0.7 to 1.5 g for women are

TABLE 1

MEAN VALUES FOR AGE, AND SELECTED PHYSICAL AND URINARY MEASUREMENTS FOR THE ELDERLY WOMEN

| Variable | Mean ± SE |
|---|---|
| Age (years) | 74.4 ± 1.0 |
| **Physical Measurements** | |
| Height (cm) | 158.2 ± 1.8 |
| Weight (kg) | 52.9 ± 2.4 |
| Bone Density[a] | 0.80 ± 0.05 |
| **Urinary Measurements** | |
| HOP Excretion (mg/24 hr) | 24.1 ± 1.7 |
| Creatinine Excretion (g/24 hr) | 0.607 ± 0.046 |
| HOP-Creatinine Ratio (mg/g) | 40.8 ± 2.4 |
| HOP (mg/24 hr/$m^2$ body surface area) | 15.6 ± 0.9 |
| **Metabolic Balance** | |
| Nitrogen (g/24 hr) | 2.29 ± 0.27 |
| Calcium (mg/24 hr) | -52.4 ± 85.3 |
| Phosphorus (mg/24 hr) | 258.2 ± 52.5 |

[a]Expressed as X-ray equivalent grams of alloy per cubic centimeter of bone.

considered normal excretion values for creatinine by Cantarow and Schepartz (69). Fearon (70) has suggested 1.2 g, McGilvery (71) 1.7 g, and Harrison (72) 1.0 to 1.5 g per day as levels to be expected. In comparison to the values reported above the creatinine values in the present study were low.

Hydroxyproline-creatinine ratios (mg/g) ranged from 28.0 to 59.2 with a mean of 40.8 $\pm$ 2.41 (Table 1). This value tended to be higher than various values reported in the literature. Williams and Windsor (73) found a mean ratio of 31 in 35 normal elderly people aged 60 to 100. When the subjects in the latter study were categorized by specific age groups, the HOP-creatinine ratio of the 70 to 80 year old group, which comprised 15 out of the 35 subjects in the study, showed a range of 17 to 54 with a mean of 35. Lower ratios were reported by Allison et al. (74) who found a mean value of 28 in 6 adult women, and Crabbe and Isselbacher (34) who found a mean ratio of 19.0 $\pm$ 4.6 in 12 adults of both sexes. The higher HOP-creatinine ratios found in the present study can be explained in part by lower than normal creatinine values.

Unlike the mean HOP-creatinine ratio, the mean daily value of 15.6 $\pm$ 0.9 for HOP excretion, expressed as mg per 24 hours per $m^2$ of body surface, was normal in comparison to values reported in the literature. In 65 adult females, Anderson et al. (75) found a value of 16 for the corresponding ratio. Also in close agreement are results reported by Goidanich et al. (29) who found a mean daily excretion of 15.2 mg per $m^2$ of body surface in 6 adults aged 60 to 70.

All 16 subjects were in positive nitrogen and phosphorus balance with mean daily balance values of 2.29 g $\pm$ 0.27 and 258.2 mg $\pm$ 52.5,

respectively. There was a mean daily calcium balance of -52.4 mg ± 85.3 with 12 out of the 16 subjects in negative calcium balance. The mean daily intakes of calcium, phosphorus, and nitrogen were 770 mg, 1038 mg, and 9.00 g, respectively. These results are similar to those reported by Bogdonoff et al. (55) who, after determining calcium balances in 7 aged males, reported that the subjects did not reach equilibrium until intakes averaged 850 mg of calcium daily. Throughout the study, all 7 subjects were in positive nitrogen and phosphorus balance. Ohlson et al. (76) found 54% of 136 women between 30 and 85 years of age to be in negative calcium balance. She reported that negative calcium balances in the 60 to 69 age group decreased as intakes increased from 0.37 to 0.7 g per day. The subjects attained calcium equilibrium with intakes between 0.7 and 0.9 g of calcium per day. In another investigation Ohlson et al. (77) reported positive calcium, phosphorus, and nitrogen balances in 4 women aged 70 to 77 when the mean daily intakes were 680 mg, 1070 mg, and 8.94 g, respectively, which are similar to those intakes observed in the present study. Also in agreement with the present study are results by Nordin (10) and Harrison et al. (56) who have observed positive nitrogen balances in elderly but osteoporotic patients.

Hydroxyproline excretion and HOP excretion per $m^2$ were both significantly and positively correlated with urinary excretions of creatinine, nitrogen, calcium, and phosphorus (Tables 2, 3). Thus it would appear that as bone or collagen released HOP, there were concomitant losses of nitrogen, calcium, and phosphorus. But for this relationship to be generally acceptable, it would seem that the urinary excretions of nitrogen, calcium, and phosphorus should have been significantly correlated with age, weight, and bone density. Although the data are not presented, age

and bone density were not significantly correlated with either nitrogen, calcium, or phosphorus excretion, and only nitrogen and calcium urinary excretions were positively correlated with weight.

TABLE 2

CORRELATION OF HYDROXYPROLINE EXCRETION WITH CREATININE, NITROGEN, CALCIUM, AND PHOSPHORUS URINARY EXCRETIONS

| Factor | r Value | P Value |
|---|---|---|
| Creatinine | 0.70 | $<.001$ |
| Nitrogen | 0.68 | $<.002$ |
| Calcium | 0.51 | $<.02$ |
| Phosphorus | 0.62 | $<.005$ |

TABLE 3

CORRELATION OF HYDROXYPROLINE EXCRETION PER SQUARE METER OF BODY SURFACE WITH CREATININE, NITROGEN, CALCIUM, AND PHOSPHORUS URINARY EXCRETIONS

| Factor | r Value | P Value |
|---|---|---|
| Creatinine | 0.57 | $<.01$ |
| Nitrogen | 0.59 | $<.01$ |
| Calcium | 0.46 | $<.04$ |
| Phosphorus | 0.64 | $<.004$ |

As would be expected, nitrogen, calcium, and phosphorus intakes were significantly correlated with nitrogen, calcium, and phosphorus

balances, respectively (Table 4). These positive relationships would be expected in a normal individual, but an insignificant relationship might be expected between calcium intake and balance in an elderly osteoporotic individual (10).

TABLE 4

CORRELATION OF NITROGEN, CALCIUM, AND PHOSPHORUS INTAKES WITH THEIR RESPECTIVE BALANCES

| Intake vs. Balance | r Value | P Value |
|---|---|---|
| Nitrogen | 0.74 | $< .001$ |
| Calcium | 0.58 | $< .01$ |
| Phosphorus | 0.80 | $< .001$ |

In the present study, urinary nitrogen and phosphorus excretions were significantly correlated with nitrogen ($r = 0.92$, $P < .001$) and phosphorus ($r = 0.61$, $P < .006$) intakes, respectively, while urinary calcium excretion was not significantly correlated with calcium intake ($r = 0.15$, $P = .28$). These findings are in agreement with those of Nordin (10) who reported that urinary calcium in 20 osteoporotic patients varied quite independently of calcium intake, but in contrast urinary phosphorus and nitrogen tended to vary in accordance with phosphorus and nitrogen intakes, respectively. In another investigation, Nordin (78) also found no significant correlation between dietary calcium and the urinary excretion of calcium.

The r values for selected factors which were correlated with age are presented in Table 5. There was a tendency for urinary HOP to decrease with age ($r = -0.38$, $P = .07$). When the HOP excretion was corrected either for creatinine excretion ($r = -0.50$, $P < .02$) or for body surface area ($r = -0.43$, $P < .05$), the negative correlation became statistically significant. These results confirm the data of Saleh and Coenegracht (45) who reported a significant decrease in HOP excretion in elderly patients.

There was not a significant correlation between bone density and age ($r = 0.07$, $P = .40$). Though many investigators have reported a loss of bone during senescence, Baker and Angel (22) did not find a significant correlation between age and bone density in 48 cadavers, most of whom were over 65 years of age at the time of death.

There was no significant correlation between age and creatinine excretion in the present study ($r = 0.13$, $P = .32$). In elderly men, Solomon and Shock (79) have reported a decrease in creatinine excretion which was expressed as mg per minute per 1.73 square meters. Howell (80) reported a range of 0.035 to 1.0 g with a mean of 0.46 g for the daily creatinine excretion in 20 women aged 90 to 101. This value was considered to be low, but the creatinine excretion was correlated to the fluid output which was also low. It has been reported that urinary clearance of creatinine may fall with extreme oliguria which is a common condition in the elderly (80). In male rats past middle age, Everitt (81) found a decrease in creatinine excretion and creatinine per unit metabolic body size with increasing age. This decline in creatinine excretion was attributed to loss of muscle tissue as the body weight decreased in old age and senescence, but the decline was much greater than corresponding reductions in body weight and metabolic body size. In contrast to the above reports of

TABLE 5

CORRELATION OF SELECTED FACTORS WITH AGE

| Factor | r Value | P Value |
|---|---|---|
| Weight | -0.26 | NS[a] |
| Height | 0.21 | NS |
| Bone Density | 0.07 | NS |
| HOP Excretion | -0.38 | NS |
| Creatinine Excretion | 0.13 | NS |
| HOP-Creatinine Ratio | -0.50 | $< .02$ |
| HOP Excretion per $m^2$ | -0.43 | $< .05$ |
| Nitrogen Balance | -0.22 | NS |
| Calcium Balance | -0.05 | NS |
| Phosphorus Balance | -0.20 | NS |

[a]Not significant.

decreasing creatinine excretion with age, Verzár (82) has reported that creatinuria is a common phenomenon during senescence.

Though phosphorus and nitrogen balances were not related to age in the present study there was a significant negative correlation between phosphorus intake and age ($r = -0.42$, $P < .05$); and there was a strong tendency for nitrogen intakes to decrease with age ($r = -0.34$, $P = .09$). This is in agreement with Ohlson et al. (76) who found a decrease in nitrogen and phosphorus intakes with age in 136 women aged 30 to 85. Calcium intakes in the 136 women did not change significantly until after the age of 70. In the present study there was also a strong tendency for calcium intake to decrease with age ($r = -0.41$, $P = .06$). In a study by Reshef et al. (2) calcium consumption decreased significantly with age in males, but not in females. Swanson (83) in a study of 1072 women aged 30 to 90 found a decrease in protein and calcium intakes with age. The mean daily intake of calcium of all 1072 women was 0.5 g, and after age 70 the intakes ranged from 0.2 to 0.4 g per day. In the present study, the mean daily calcium intake was 0.77 g.

Both HOP excretion and HOP excretion standardized for body surface area were positively correlated with body weight (Table 6). This seems logical since body weight is related to osseous tissue weight (84) and the latter is the most important source of urinary hydroxyproline (29). Also in the present study bone density was positively correlated with weight ($r = 0.51$, $P < .02$). This finding agrees with Exton-Smith et al. (23) who reported in elderly women over 70 that a positive relationship existed between skeletal density and weight.

A highly significant correlation between weight and creatinine excretion was observed in the present study ($r = 0.70$, $P < .001$). This

TABLE 6

CORRELATION OF SELECTED FACTORS WITH WEIGHT

| Factor | r Value | P Value |
|---|---|---|
| Height | 0.05 | NS[a] |
| Bone Density | 0.51 | $< .02$ |
| HOP Excretion | 0.78 | $< .001$ |
| Creatinine Excretion | 0.70 | $< .001$ |
| HOP-Creatinine Ratio | -0.01 | NS |
| HOP Excretion per $m^2$ | 0.62 | $< .005$ |
| Nitrogen Balance | 0.15 | NS |
| Calcium Balance | 0.16 | NS |
| Phosphorus Balance | 0.25 | NS |

[a]Not significant.

relationship is well known and would be expected since the amount of creatinine excreted is a function of muscle mass (85). Even though this correlation was highly significant, there was no relationship found between weight and the HOP-creatinine ratio ($r = -0.01$, $P = .49$).

In Table 7 the correlations between bone density and selected variables are shown. Hydroxyproline excretion was significantly correlated with bone density ($r = 0.45$, $P < .04$). In normal individuals this relationship would be expected, since urinary HOP excretion should be related to the quantity of metabolically active bone present. For example, it has been suggested by Saleh and Coenegracht (45) that the lower HOP excretion values found in older age groups are due to diminished volumes of metabolically active bone. The significant negative correlation found between age and the HOP-creatinine ratio and the HOP excretion per $m^2$ support this hypothesis.

The lack of correlation between the HOP-creatinine ratio and body weight can be explained by the significant correlation of either of these excretory products with weight. Since the excretion of HOP and creatinine increased with weight, the net effect of computing the ratio nullified the relationship. A similar finding was obtained for bone density in that the individual excretory products were positively correlated but their ratio was not. In addition, the correlation between HOP excretion per $m^2$ of body surface and bone density was insignificant.

As indicated above, HOP excretion was positively correlated to body weight and bone density; in addition, the correlation between bone density and body weight was also significant. This indicated that HOP excretion was increased due to a larger quantity of skeletal mass which

TABLE 7

CORRELATION OF SELECTED FACTORS WITH BONE DENSITY

| Factors | r Value | P Value |
|---|---|---|
| Age | 0.07 | NS[a] |
| Weight | 0.51 | $< .02$ |
| Height | 0.19 | NS |
| HOP Excretion | 0.45 | $< .04$ |
| Creatinine Excretion | 0.43 | $< .05$ |
| HOP-Creatinine Ratio | -0.01 | NS |
| HOP Excretion per $m^2$ | 0.33 | NS |
| Nitrogen Balance | -0.22 | NS |
| Calcium Balance | 0.02 | NS |
| Phosphorus Balance | 0.06 | NS |

[a]Not significant.

in turn increased body weight. Hydroxyproline excretion was also negatively correlated with age. This decrease in HOP excretion could be interpreted as a loss of skeletal tissue (body collagen) with age or a decrease in collagen turnover with age. However, since the body weight, bone density, and creatinine excretion did not significantly change with age, it is unlikely that the body collagen decreased significantly. This leads to the postulation that the turnover of body collagen was decreased with age. This is consistent with the theory that an increase in the number of intra- and inter-molecular cross-linkings in aging collagen cause an increase in insoluble collagen (86). This, in turn, results in a slower turnover of collagen and therefore a decrease in HOP excretion with age.

# CHAPTER V

## SUMMARY

Relationships among bone density, urinary hydroxyproline (HOP), and the metabolic balances of calcium, phosphorus, and nitrogen were investigated in 16 elderly women aged 68 to 82 with a mean age of 74.4.

The mean daily intakes for nitrogen, phosphorus, and calcium were 9.00 g, 1038 mg, and 770 mg, respectively. All 16 subjects were in positive nitrogen and phosphorus balance, while 12 out of 16 women were in negative calcium balance with mean daily balances of 2.29 g, 258 mg, and -52.4 mg, respectively. Urinary nitrogen and phosphorus excretions were significantly correlated with nitrogen ($r = 0.92$, $P < .001$) and phosphorus ($r = 0.61$, $P < .006$) intakes, respectively. Nitrogen, calcium, and phosphorus intakes were positively correlated with nitrogen ($r = 0.74$, $P < .001$), calcium ($r = 0.58$, $P < .01$), and phosphorus ($r = 0.80$, $P < .001$) balances, respectively. There was a strong tendency for calcium ($r = -0.41$, $P = 0.57$) and nitrogen ($r = -0.31$, $P = .099$) intakes to decrease with age, while decrements in phosphorus intakes were significant with age ($r = -0.42$, $P < .05$).

The mean daily values for HOP excretion (24 mg) and for HOP excretion per square meter of body surface area (16 mg/24 hr/$m^2$) were normal, while the mean value for the HOP-creatinine ratio (41 mg/g) was high in comparison to values reported in the literature. Hydroxyproline excretion and HOP excretion per $m^2$ were both positively correlated with the urinary excretions of creatinine, nitrogen, calcium, and phosphorus ($P < .05$).

In addition, HOP excretion ($r = 0.78$, $P < .001$) and HOP excretion per $m^2$ ($r = 0.62$, $P < .005$) were both positively correlated with body weight, and the HOP-creatinine ratio ($r = -0.50$, $P < .02$) and HOP excretion per $m^2$ ($r = -0.43$, $P < .05$) were both negatively correlated with age.

The mean bone density was 0.80 gram equivalents of alloy per cubic centimeter of bone, which was normal in comparison to values reported in the literature. The mean creatinine excretion was 0.607 g, which was low in comparison to values cited in the literature. Bone density was correlated with body weight ($r = 0.51$, $P < .02$), HOP excretion ($r = 0.45$, $P < .04$), and creatinine excretion ($r = 0.43$, $P < .05$), while the latter was correlated with body weight ($r = 0.70$, $P < .001$). No significant relationships were found between either bone density or creatinine excretion with age.

It was concluded that the quantity of osseous tissue was reflected in body weight and in HOP excretion. Also, from the relationships obtained among HOP excretion, bone density, body weight, and age, it was concluded that an age-associated decrement in body collagen turnover occurred.

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# VITA

Peggy Davies MacDuff was born in West Chester, Pennsylvania on December 14, 1949. She attended primary school in Short Hills, New Jersey and Knoxville, Tennessee and graduated from Holston High School in June of 1967. In the summer of 1967, she entered the University of Tennessee and received a Bachelor of Science degree in Home Economics in June of 1971.

For Fall of 1971, she accepted a teaching assistantship from the Nutrition Department at the University of Tennessee and received a Master of Science degree with a major in nutrition in June of 1973. While in graduate school, she served as a graduate student representative to the College of Home Economics Executive Council and was chairman for the Home Economics Graduate Student Organization. She is a member of Omicron Nu.