INCIDENCE OF DECREASED BONE DENSITY AMONG MALE MENTALLY RETARDED GERIATRIC SUBJECTS

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We hereby recommend that the thesis prepared under our supervision by Maranda Maier entitled INCIDENCE OF DECREASED BONE DENSITY AMONG MALE MENTALLY RETARDED GERIATRIC SUBJECTS be accepted as fulfilling this part of the requirements for the Degree of

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CHAPTER I

INTRODUCTION

Osteoporosis is a common cause of prolonged disability in the aged. It is becoming a major economic problem because the number of the elderly is increasing at a rate much faster than that for the general population.

Due to the relatively high incidence of osteoporotic fractures in the elderly and the difficulties of caring for the aged who are disabled, studies have been conducted in state supported institutions on dietary intake and the general nutritional state of the elderly in an effort to determine the etiology of osteoporosis. However, the mentally retarded geriatric subject presents several unique challenges. First, the mentally retarded geriatric subject is at times erratic and disinterested in his surroundings--an attitude resulting in problems related to both feeding and exercising. Secondly, there may be genetic differences or biochemical changes in the mentally retarded subject which result in alterations in bone density, differences in adaptation to altered hormone balance or in the metabolic response to varying calcium intake levels over a period of years. Therefore research is needed to determine if a relationship does exist between mental retardation and an increased incidence of decreased bone density.

REVIEW OF LITERATURE

An increased geriatric population and the relatively high incidence of osteoporotic fractures in the elderly have aroused considerable interest in the etiology of osteoporosis. Through an unexplained failure of the normal mechanism that governs skeletal homeostasis, a progressive decrease in the amount of mineralized tissue occurs with a marked thinning of cortical bone and a reduction in the number and size of trabeculae in cancellous bone. However, the structure of the bone is apparently normal as assessed by histological and chemical analyses (35). The reduction in bone mass occurs slowly with a 25 to 30 per cent loss of mineral over a period of 60 years (11, 23).

Estimates of the incidence of reduction in bone mass in the general population range from 18 to 25 per cent (20, 23, 38). Examples of typical studies include those of Gershon-Cohen (20). In 1953 this author investigated 54 male ambulatory subjects, residents in a home for the aged, ranging in age from 63 to 92 years. Of the group, 20 per cent showed moderate to advanced osteoporosis as determined by X-ray examination and low excretion of 11-deoxy 17 ketosteroids. Vincent (61) also studied male subjects with an average age of 66 years. All subjects were ambulatory without serious disease. X-ray examinations revealed 18 per cent of the subjects had moderate to advanced osteoporosis. Vincent also stated that the average male with symptomatic osteoporosis was five years younger than the average male with non-symptomatic osteoporosis. The author concluded that age had no significant relationship to the degree of severity of osteoporosis in the male subjects investigated. Vincent also stated that the occurance of symptomatic osteoporosis did not increase after age 65. A possible explanation for these findings would be that few subjects survived past the age range of 65 to 70 years.

For many years osteoporosis was thought to be due primarily to faulty metabolism of the bone matrix. The pathogenetic mechanism of osteoporosis was explained as an insufficiency of osteoblasts in the germinal tissue of the mesenchyma and in the deficient replacement of bone tissue resulting in a progressive loss of bone substance. This phenomenon is especially conspicuous in places where the rate of exchange is higher, as in cancellous bone, and therefore in sections of the skeleton where spongious bone is predominant, namely the trunk. This is the site of the most rapidly advancing atrophy (11, 13, 59). However, similar aspects are observed in other bones. Macroscopically, one receives the impression that beyond a certain age the bone tissue that is reabsorbed fails to be replaced completely, so that the bones grow lighter and more fragile. On the whole, histologically, osteoporosis demonstrates the loss of osteoformative capacity at an age in which reabsorption is very marked: this explains the formation of large very thin-walled osteons in the bone adjoining the medullary canal (4).

Recent evidence, based on tetracycline estimated appositional rates, has shown that bone formation is impaired in many osteoporotics (4). However, many investigators in recent years have produced evidence suggesting that the pathogenetic mechanism of osteoporosis may involve other factors. It has been possible in recent years to apply techniques such as radioactive calcium kinetic analysis and microradiography in the evaluation of bone formation and resorption rate in osteoporotic patients (14, 39). Whedon (66), Munson (39), and Epstein (14), among others, used radioactive isotopes to show that the rate of osteogenesis in aged subjects with osteoporosis is the same as in young people. Potts (43) and Foster (16) demonstrated through calcium kinetic studies that absolute bone resorption rates are not greatly increased when compared to rates in normal adults, corrected for body size. However, resorption may exceed formation by 50 to 100 mg of calcium per day. Analysis by microradiography, on the other hand, has shown a marked increase in the resorptive surface, suggesting that since formative surfaces were normal in area, that osteoporosis resulted from excessive resorption. Lafferty (35) interpreted radioactive calcium disappearance curves to show that bone formation is normal in osteoporotics. This investigator states that since bone is lost, bone resorption must be accelerated. It should be noted that the accretion rates estimated from radioactive calcium disappearance data greatly exceed those estimated from tetracycline labeling (18).

The conflicting nature of these findings and the small magnitude of the changes detected by calcium kinetic methods present difficulty in interpretation of these studies. Firm conclusions are impossible and one must be content with the implication that osteoporosis is a multifactorial disease, characterized by an increase of bone reabsorption processes beyond the normal capacity of repair of an essentially normal bone matric (43, 66).

In recent years considerable interest has been evidenced in the calcium and phosphorus metabolism of osteoporotic subjects. Whedon (65) found that the mean calcium intake at which equilibrium occurs in patients with osteoporosis is 1100 mg. Likewise Ackerman (1) demonstrated a mean equilibrium of 18.5 mg per kg a day for elderly male osteoporotics; whereas, Steggarda (59) stated that 520 mg a day is the mean for normal subjects. Similarly Ackerman reported 457 mg a day as being essential for normal subjects. Bertolini (4) found that radioactive calcium administered orally to healthy adult subjects, including elderly people, reached the plasma in 10 to 15 minutes and then increased to a peak of between 2.0 and 2.5 per cent of the administered dose per liter of plasma. In osteoporosis, radioactivity appears later in the plasma (20 to 30 minutes after administration); the increase of radioactivity is slower and the peak values are in the region of 1.0 to 1.5 per cent of the dose per liter of plasma.

Caniggia (9) has also shown a defect in the intestinal absorption of calcium in osteoporotics. This author states that both calcium and vitamin D are absorbed more slowly and to a lesser extent than in normal subjects. Likewise Nordin (41) found that 15 per cent of patients with osteoporosis were also suffering from steatorrhea and that a form of osteomalacia was frequently superimposed upon osteoporosis in these subjects. Related observations were reported by Shuster (51) and Scarborough (50) who found that the skin collagen content was decreased in patients with osteoporosis and that the incidence of senile purpura was greater.

Whedon (66) reported a relative resistance to calcium absorption in certain patients as shown by the very high calcium intake levels required to approach or obtain positive balance, suggesting that in some patients there has developed a gastrointestinal absorption defect for calcium.

Confirming this data Heaney (26) found in a study of active (acutely developing) osteoporotics and chronic (static inactive) osteoporotics that in spite of a lower mean calcium intake, the patients classified as chronic absorbed twice as much calcium as did patients in the acute group. Heaney concluded that gastrointestinal absorption is impaired during the acute phase of osteoporosis. Heaney also reported bone formation rates, from normal to twice normal, and bone resorption rates, from two to three times normal, in the acute phase patients as compared to the chronic group.

Spencer (58) conducted a study on the metabolic effects of long term supplementation of calcium in the diets of patients with osteoporosis. This investigator found that calcium supplementation 5 to 10 times the previous intake of 119 to 154 mg a day improved calcium balance in the osteoporotic patients. However, this improvement was considerably less than in persons without osteoporosis. Nordin (42) stated that the negative calcium balance of osteoporosis can be reversed with an appropriate diet containing large amounts of calcium. This author also reported increased bone density with calcium treatment. It was interesting that Nordin was the only investigator reporting consistently positive calcium balance and increased bone density with calcium treatment. Harris (25) and Heaney (27) attributed Nordin's results to the use of calcium glycerophosphate--a calcium preparation not used in the United States. Harris stated that perhaps Nordin's results were due more to the phosphate than to the calcium. Garn (19), using cortical thickness as a standard, stated that the intake of dietary calcium in levels above 1500 mg a day does not seem to be "protective" and that levels of calcium intake below 300 mg a day are not demonstrably associated with bone loss. Garn concluded that whatever may be true in patients with malabsorption syndromes, dietary calcium is not primarily and directly relatable to the osseous state in man. Garn's findings are confirmed by those of Smith (56) and Hegsted (29). Smith states that long-term follow up studies, such as those conducted by Garn, fail to demonstrate relationships between bone loss and calcium intake. Potts (44) also concluded that the calcium metabolism of most patients with osteoporosis is normal when compared with that of normal subjects of a similar age and sex. Furthermore this investigator stated that long-term calcium supplementation does not result in remineralization of the skeleton.

Bertolini (4) reports a comparative study conducted in 100 subjects with osteoporosis and in 40 aged but healthy controls. The calcium and phosphorus levels in the blood were normal but low in the urine in both groups. The lowering of calciuria and phosphaturia was thought to be age and sex related by Bertolini, increasing with advancing years.

The proteinemia was also lower in the osteoporotic group; whereas, the alkaline phosphatase activity was more often reduced in the healthy control group than in the osteoporotic group. Perhaps the higher alkaline phosphatase activity in the osteoporotic group reflects the continued enzymatic activity necessary to convert the mesenchymal cells to osteoblasts--an indication of a disturbance in the equilibrium between bone formation and bone resorption.

Nordin (41) reports studies on renal clearance of calcium. He found that the urinary calcium decreases with age, from an average of 226 mg a day in young subjects to 126 mg a day in subjects over 60 years of age. This reduction could also result from increased calcium requirements in old age.

Caniggia (9) reports that the urinary excretion of radioactive calcium is defective; whereas, the normal subject eliminates 1.1 per cent of the administered dose in six hours, the patient with osteoporosis eliminates only 0.15 to 0.5 per cent. This is also confirmed by the data on fecal elimination. The patients with osteoporosis eliminate as much as 50 to 65 per cent of the dose in three days while normal subjects eliminate only 30 per cent.

Heaney (26) investigated serum calcium, phosphorus, and alkaline phosphatase in a series of normal and paralyzed older males. During the period of observation, the serum calcium, phosphorus and alkaline phosphatase all remained constant and except for phosphorus elevations were not appreciable abnormal. Mean serum calcium in patients with osteoporosis was 10.6 mg per cent with values as high as 13.9 mg per cent sporadically observed in individual patients from time to time. Serum inorganic phosphorus averaged 4.8 mg per cent with individual values as high as 6.1 mg per cent. The serum alkaline phosphatase averaged 9.3 King-Armstrong units. The high serum phosphorus, together with normal to high serum calcium, has been reported by others and is compatible with the suggestion that renal phosphate clearance is reduced as a result of suppression of endogeneous parathyroid secretion (27, 33).

Another factor that modifies the maintenance of a positive calcium balance is physical activity. It has been known for many years that immobilization results in a decrease in bone tissue mass--for the maintenance of normal skeletal metabolism is dependent upon mechanical factors, such as muscle pull or weight bearing. When these factors are decreased or removed, bone mass decreases to about 50 per cent of normal if immobilization is complete (19, 37).

If the activity is limited, the refilling of osteones during turnover is only a third to a half complete, while if there is full activity even in senile osteoporotics, osteones are completely refilled. There appears to be a need for muscular activity in order to retain bone. The ratio of muscle mass to bone mass is relatively constant, and the amount of bone regenerated from the osteoporotic state is proportional to the amount and intensity of muscle action (32, 36, 37).

Another factor that has a role in the alteration of calcium balance is hormone imbalance. This theory has many supporters including Reifenstein (47) who cites evidence in support of the hypothesis that the immediate cause of osteoporosis is an adrenal imbalance--an imbalance in the anabolic-catabolic equilibrium. He states that the level of production of catabolic hormones is fairly constant during life, although a temporary increase is noted in times of stress. However, the anabolic steroids are not so uniform. Both gonadal and adrenal anabolic steroids come into prominance during adolescence. In males, the adrenal anabolic and gonadal steroids decrease in the age range of 60 to 80 years. However, osteoporosis can occur in young and middle-aged adults with normal hormonal function. Although short-term improvements have been noted during the administration of androgen, long term results have not been achieved (11, 39, 43). Munson (39) states that current evidence does not support the view that gonadal deficiency is a principal factor in the pathogenesis of osteoporosis.

Parathyroid hormone and thyroidcalcitonin (PTH and TCT) are known to play an important role in the control of bone resorption; PTH stimulates and TCT inhibits bone resorption. Although both of these peptide hormones are continuously secreted at normal blood calcium concentrations and must therefore exert a continual influence on the rate of bone remodeling, most evidence suggests that their primary function is control of calcium homeostasis (11, 43). The secretory rate of either hormone is controlled by blood calcium concentration; the physiological role of these hormones therefore seems less important for skeletal homeostasis than for calcium homeostasis (43). Deficiency or excess of these hormones would be expected only in response to abnormal blood calcium concentration, and hypo or hyper-calcemia is not characteristic of osteoporosis. To assign PTH or TCT any causative importance in osteoporosis it would be necessary to postulate a primary disorder in the production of either hormone or an altered sensitivity to its effects. There is at present no convincing evidence for these possibilities (44).

As these studies show the data on calcium requirements and calcium absorption are controversial, being reported as decreased, increased or unchanged. Whatever the etiology of osteoporosis may be, the data may be interpreted to indicate that many osteoporotics are unable to absorb calcium with efficiency. There is no doubt that osteoporosis is a multi-factorial disease, produced when bone is forced to provide calcium which the organism fails to obtain from its environment. The realization that bone formation and resorption are not independent but are homeostatically linked, is a key factor in the understanding of osteoporosis. Ultimately, the degree of parathyroid activity and consequently bone resorption will depend on how well intestinal absorption balances excretory loss: if body intake balances body output, bone resorption will balance bone formation and bone mass will not change. Bone resorption is highly sensitive, flexible and responds rapidly to changing levels of PTH.

Most of the theories concerning the causes of osteoporosis can be reconciled when visualized in the "homeostatic" control context. Dietary calcium deficiency, intestinal malabsorption syndrome and excessive renal loss may produce osteoporosis simply by presenting the organism with less mineral than needed for minimal daily losses; osteoporosis results, not because of homeostatic derangement, but because of homeostatic efficiency.

PURPOSES

One purpose of this study was to determine if the incidence of low bone density in mentally retarded geriatric males at the Travis State School in Austin, Texas would be similar or dissimilar to that found among normal subjects of the same age and sex. Another purpose was to make a preliminary evaluation of possible related bionutritional factors.

A third purpose of this study was to determine if a diet, planned in accordance with the Recommended Dietary Allowances established by the Food and Nutrition Board of the National Research Council and prepared and dispensed under the supervision of a trained Food Service Supervisor, does result in normal calcium and phosphorus plasma levels and does reflect healthy bone as determined by densitometric analyses of mentally retarded geriatric subjects.

The specific objectives of this study were:

1) To determine the frequency of subnormal bone density in mentally retarded geriatric males between the ages of 50 and 82 years.

2) To ascertain by dietary histories and by evaluation of plate waste the intake of 265 mentally retarded geriatric males.

3) To determine the relationships between a subnormal bone density and:

    a) Dietary intake
    b) Age
    c) Physical activity
    d) Biochemical parameters related to the metabolism of bone.

The subjects in this study consisted of 265 ambulatory and semi-ambulatory male residents of the Travis State School in Austin, Texas, who were mentally retarded and ranged in age from 50 to 82 years.

This study took place during the spring and summer of 1970. Primary data included laboratory analyses of serum calcium, phosphorus, alkaline phosphatase, urea nitrogen, urinary protein, hemoglobin, creatinine, and serum glutamic-oxaloacetic transaminase. Bone density was determined by X-ray techniques. These analyses were conducted in the Bio-nutrition and Electron Microscopy Laboratories of the Research Institute of Texas Woman's University. The investigator also conducted personal interviews with the Hospital Administrator, the Medical Director, the Food Service Director, and the Therapeutic Dietitian. Information obtained concerning dietary intake was evaluated according to the Recommended Dietary Allowances established by the Food and Nutrition Board of the National Research Council.

CHAPTER II

PLAN OF PROCEDURE

The 265 mentally retarded males were randomly chosen by the Medical Director of Travis State School to be participants in a study conducted by the Research Institute of Texas Woman's University. Liaison between the dormitory directors in Austin and the research personnel in Denton was accomplished through efforts of one of the members of the nursing staff at the Travis State School. Selection of residents from 10 of the 23 dormitories was limited to those who did not require special therapeutic diets and who were relatively easy to control physically. All the subjects were considered to be in good physical condition. After the initial screening the number of subjects was narrowed, for various reasons, to 181. Written consent for participation in the study was obtained from the guardian of each subject.

Facilities and X-ray equipment at the Travis State School were used during week-end periods. All personnel and supplies were furnished by the Texas Woman's University Research Institute and the College of Household Arts and Sciences. The data obtained at the Travis State School were compared with standard deviation classes established by the

Texas Woman's University Research Institute using normal males in the same age range.

BONE DENSITOMETRIC ASSESSMENT

The subjects were taken to the radiology unit of Travis State School where X-rays of the os calcis and phalanx 5-2 were taken under controlled exposure conditions. Photographic processing techniques were equally well controlled. All were conducted by registered X-ray technicians under the supervision of a board certified Radiologist. Quantitative analyses of bone mineralization were achieved through use of a photometric computing apparatus which consisted of a microphotometer, recorder, digital computer, analog-to-digital converter and teletype tape for immediate output of data (63). The instrument is capable of reproducability within 1.0 per cent and the overall error is less than 3.0 per cent. In view of the recently published findings of Goldsmith (22) and Vose (63) stating that the os calcis, which undergoes constant muscle stress, does not reflect vertebral osteoporosis well in the normal ambulent person, only data obtained from measurement of the phalanx 5-2 will be presented. The subjects were classified into "Standard Deviation Classes" according to their deviation from the normal regression of bone density with age as shown in Table I.

TABLE I

STANDARD DEVIATION CLASSES OBTAINED FROM NORMAL

AGE-BONE DENSITY* REGRESSION SLOPE**

Standard Deviation	Age Groups					
	50-54	55-59	60-64	65-69	70-74	75+
+4.0	0.385	0.380	0.370	0.355	0.335	0.315
+3.0	0.355	0.350	0.340	0.330	0.310	0.290
+2.0	0.330	0.325	0.310	0.300	0.280	0.260
+1.0	0.305	0.300	0.290	0.275	0.255	0.235
+0.5	0.290	0.285	0.270	0.260	0.240	0.220
0.0	0.275	0.270	0.265	0.250	0.235	0.215
-0.5	0.265	0.260	0.250	0.240	0.225	0.205
-1.0	0.250	0.245	0.235	0.225	0.210	0.190
-2.0	0.225	0.220	0.210	0.195	0.180	0.160
-3.0	0.195	0.190	0.180	0.170	0.150	0.130
-4.0	0.165	0.160	0.150	0.140	0.125	0.105

*Bone Density recorded as "Aluminum Equivalency Units (22).

**From data stored in the Computer Center, Texas Woman's University.

DIETARY ASSESSMENT

The menus in use at the time the study began, the ingredients of prepared dishes, and approximate plate wastes were recorded. The menus for the Travis State School are planned in three-month cycles by a Therapeutic Dietitian and evaluated according to the Recommended Dietary Allowances established by the Food and Nutrition Board of the National Research Council (RDA). Menus for all state institutions are centrally planned and distributed to the Food Service Managers in each facility for use in food purchasing. Provisions are made to adapt the menu for special diets and for residents with masticatory problems. The Food Service Director stresses, through in-service training sessions, that diets should be adapted to individual needs to avoid obesity and to maintain healthy residents. The Food Service Director has developed a serving program using trained food service personnel to assist the residents at meal times. Most facilities for long-term patient care use the nursing staff to supervise patient meal service. A Food Service Supervisor was assigned to each dining hall to encourage quality food control and quality patient care.

The complete dietary served during the period of this study was assessed according to values of Watt and Merrill (64). In calculating the dietary, allowances were made for trimming and cooking losses. Protein, calories, vitamin A, thiamine, riboflavin, ascorbic acid, vitamin D, calcium and iron were recorded for use in the study because it was felt that these nutrients were closely allied with osteoblastic activity. The nutritional accounting was done on a quarterly basis because the menus were planned quarterly. After obtaining totals for all nutrients for the quarterly period, daily averages were calculated for comparison with the RDA. Meats were divided as to type, vegetables as to green and yellow, high in ascorbic acid or starchy, fruits as to citrus, dried or other.

BIOCHEMICAL ASSESSMENT

Various biochemical parameters, which were chosen initially because of their importance in the medical screening and selection of subjects for continued study, were also used to determine whether there was a consistent relationship between the dietary intake and nutritional status as reflected by these parameters. Blood samples were withdrawn by venipuncture approximately one to three hours postprandial. The tubes containing the blood specimens were immediately placed in an ice bath and maintained between 0 and 5°C, until completion of centrifugation. The sera thus obtained were immediately frozen and stored in a deep freeze until thawed for analyses. To determine the effect of this method of handling on the activity of enzymes being assayed, two different blood samples were divided into each of four tubes and processed as follows:

Tube 1: Immediately iced and kept between 0-5°C for 48 hours, centrifuged in a refrigerated centrifuge, serum analysed for enxymatic activity.

Tube 2: Blood allowed to clot at room temperature, centrifuged at room temperature and serum analyzed (the procedure used in most clinical laboratories).

Tube 3: Blood allowed to clot at room temperature, centrifuged at room temperature, serum frozen for 48 hours, thawed and analyzed.

Tube 4: Blood immediately iced and kept between 0-5°C, centrifuged in a refrigerated centrifuge, serum frozen for 48 hours, thawed and analyzed (procedure used in this study).

The data presented in Table II indicate that freezing has no deleterious effect on the enzymes being assayed, nor is there any indication that the procedure used to transport samples from Austin for processing in Denton was any less acceptable than that used in routine clinical research laboratories. On the contrary, the values obtained for serum acid phosphatase, one of the most labile enzymes, were significantly higher than those obtained by conventional techniques.

TABLE II

EFFECT OF TEMPERATURE ON SERUM ENZYMATIC ACTIVITY

Enzyme	Iced (Tube 1)	Room Temperature (Tube 2)	Room Temperature (Tube 3)	Iced/Frozen (Tube 4)
Acid Phosphatase*				
Subject 1	1.06	0.57	0.69	1.46
Subject 2	1.92	0.43	0.51	1.75
Alkaline Phosphatase*				
Subject 1	2.28	2.16	2.39	2.15
Subject 2	1.41	1.28	1.37	1.56
Serum Glutanic Oxaloacetic Transaminase**				
Subject 1	19.80	19.80	18.50	17.60
Subject 2	20.30	16.30	13.20	17.60

*Unit of phosphatase activity: amount of enzyme necessary to liberate 1.0 μm of p-nitrophenol per hour at 30°C.

**Unit of transaminase activity: amount of enzyme which will cause a decrease in optical density (at 340 mμ) of 0.001 per minute per centimeter of light path at 25°C.

It is assumed that the concentrations of other substances being assayed were protected similarly.

Equipment and solutions necessary for the determination of hemoglobin in the blood were transported to Austin and the analyses made immediately after withdrawal of the specimens. Urinalyses were also performed at the installation in Austin. Urine samples were collected and refrigerated for no longer than three hours prior to analyses. No attempt to obtain 24-hour samples was made.

Calcium is one of the essential ions of the body, and in conjunction with phosphorus, constitutes the major portion of the mineral content of bone. Most of the body's calcium is in the bone and the ratio of calcium to phosphorus, while not entirely constant, is nearly so. Appreciable losses or gains of one of these elements by the body is usually accompanied by comparable but inverse changes in the other.

Calcium was determined by atomic absorption spectrophotometry, the techniques and principles of which have been fully described by Ramirez-Munoz (46) and Robinson (48). The procedure involved the conversion of the calcium ion to elemental calcium which is then capable of absorbing radiant energy having a very narrow wave length span. The amount of radiant energy absorbed is proportional to the number of calcium atoms present. This may be quantitated through use of appropriate spectrophotometric instrumentation. The calcium analyses reported in this study were obtained through use of the Perkin-Elmer Atomic Absorption Spectrophotometer. Acetylene served as the reducing flame, air as the oxidizer, and the multi-element calcium-magnesium lamp as the radiation source. A solution of lanthanium chloride, 0.5 per cent by weight, was used as the diluent to prevent precipitation of plasma protein and to prevent interference by sodium, potassium and phosphate ions present in the serum. Only 0.2 ml of serum was required for each analysis. The method was reproducible within 1.0 per cent. Internal standards were used with each series of analyses in order to determine and correct for any variability in technique or instrument performance.

Inorganic phosphorus forms the principle anionic constituent of bone. Metabolically, phosphorus is directly associated with calcium metabolism and is also involved in many other physiological reactions, particularly those related to energy metabolism.

The procedure used for the determination of inorganic phosphorus involved the removal of serum protein by addition of trichloroacetic acid. The inorganic phosphorus present in the protein-free filtrate was determined by reaction with ammonium molybdate in acid solution. The intensity of the blue color of phosphomolybdate formed by the addition of a reducing agent which produced a colloidal solution of molybdenum blue was measured spectrophotometrically (30).

Hemoglobin, the essential oxygen carrier of the blood, reflects the body's adaptation to varying intakes of protein, iron, folic acid, and ascorbic acid. The procedure for the determination of hemoglobin involved dilution of freshly drawn blood with a commercially prepared solution of potassium ferricyanide solution which changes hemoglobin to methemoglobin. The latter was then converted into a red cyanmethemoglobin complex, the intensity of which could be determined spectrophotometrically. The procedure was standardized by use of a freshly prepared commercially calibrated standard approved by the Board of Clinical Pathologists of the American Medical Association. The precision of this method was approximately 3.0 per cent. All reagents and the hemoglobin standard were prepared and standardized by the Ames Company (30).

Serum Urea Nitrogen is a reflection of protein catabolism and renal function. Under normal conditions, the kidney readily excretes urea so that the blood urea concentration is relatively low. However, in certain kidney disorders (nephrosis, nephritis) and in dehydration the ability to excrete urea may be impaired and the concentration of urea nitrogen in the blood increases. Conversely, an abnormally low concentration of urea nitrogen in the blood may indicate an inadequate nitrogen (protein) intake.

The determination of serum urea nitrogen involved the incubation of an aliquot sample of serum for 30 minutes at 37°C with a freshly prepared solution of urease which hydrolyzes urea to ammonia and carbon dioxide. At the end of the incubation period, a protein-free filtrate was prepared using solutions of zinc sulfate and barium hydroxide. An aliquot of the protein-free centrifugate was treated with a solution of mercuric iodide which reacts with ammonia to form a yellow complex, the absorbance of which may be measured spectrophotometrically (53). All reagents used in this procedure were prepared and standardized by Sigma Chemical Company.

The excretion of creatinine is a measurement of kidney function. Creatinine is derived from the utilization of high-energy creatine phosphate by the muscle. The amount produced per day is relatively constant, providing physical activity is also relatively constant, and is normally not reabsorbed by the renal tubules. Serum creatinine was determined by the classic procedure of Jaffe which involves the formation of a red alkaline picrate which may be quantitated spectrophotometrically (30).

Semi-quantitative urinalyses were performed on freshly collected urine specimens using diagnostic paper strips commercially available from the Ames Company. The tests included determination of protein, pH, and glucose. Microscopic examinations were also made at the Austin facility.

The procedure for determining serum glutamic-oxaloacetic transaminase is based on the coupling of the reactions shown below:

Reaction 1.

L-aspartic acid + Ketoglutaric acid $\xrightarrow{\text{G-ot}}$
oxaloacetic acid + L-glutamic acid

Reaction 2.

oxaloacetic acid + reduced B-nicotinamide $\xrightarrow{\text{MDH}}$
malic acid + B-NAD adenine dinucleotide

Coupled Reaction Used in the Determination of Serum Glutamic Oxaloacetic Transaminase

In reaction (2), reducted B-NADH, which absorbs ultraviolet light having a wave length of 340 mμ, is converted to the oxidized form which does not absorb light at that wave length. Enzymatic activity is expressed as change in optical density per minute at 30°C (54). All of the reagents for this procedure were obtained in pre-packaged form from Sigma Chemical Company.

The determination of alkaline phosphatase is used in the diagnosis of several diseases of the bone. Normally, there is a small amount of alkaline phosphatase in the serum; however, it has been demonstrated that elevated levels occur in patients with diseases of the bone which are characterized by increased osteoblastic activity (alkaline phosphatase levels rise in proportion to the rate of formation of new bone cells). However, the primary clinical application of alkaline phosphatase is as a measure of liver function.

The procedure for the analysis of alkaline phosphatase involved the use of an alkaline buffer solution and the reagent sodium p-nitrophenyl phosphate. Sodium p-nitrophenyl phosphate is a colorless compound; however, with the splitting off of the phosphate group, the chromogenic salt of p-nitrophenol is liberated. This product of the reaction served directly as a measure of enzymatic activity (52).

CHAPTER III

DISCUSSION OF FINDINGS WITH PRESENTATION OF DATA

As previously stated, the major purposes of this study were: first, to determine if the incidence of hypomineralization among mentally retarded geriatric males would be similar to that found among normal subjects of the same age and sex; and, second, if dissimilar, to make a preliminary evaluation of possible related bio-nutritional factors. This is part of a larger study being conducted by the staff of the Texas Woman's University Research Institute into the effectiveness of certain regimens for improvement of bone density.

This study included a total of 265 men between the ages of 50 and 82 years. The age distribution of the experimental group is given in Table III. The mean age was 58.6 years with 67 per cent of the sample falling within the age range of 50 to 59 years. The mode age for this group of 265 men was 52 years and the median age was 57.4 years.

BONE DENSITOMETRIC FINDINGS

As indicated in the previous chapter, the subjects in this study were classified according to the magnitude of

TABLE III

AGE DISTRIBUTION BY BONE DENSITY

Standard Deviation Classes	Percentage of Age Group					
	50-54 (n=86)	55-59 (n=80)	60-64 (n=50)	65-69 (n=31)	70-74 (n=14)	75+ (n=4)
-4	9.2	8.8	4.0	3.2	-	-
-3	25.6	17.5	8.0	9.7	21.4	-
-2	23.2	36.3	44.0	19.3	35.7	-
-1	30.2	25.0	36.0	51.3	28.6	50.0
0	3.5	7.5	2.0	3.2	-	25.0
1	7.0	5.0	4.0	12.9	14.3	25.0
4	1.2	-	2.0	-	-	-
Per cent of Total Sample	32.5	30.2	18.9	11.7	5.3	1.5

deviation in mineralization of the phalanx 5-2 from that of the normal male population within the same age bracket. A t-test was used to compare the sample mean with the population mean for each age group. The differences were significant for only two of the age groups. In both instances the mean was significantly lower for the experimental group. In a sample of 86 subjects between the ages of 50 and 54 years, 40.7 per cent fell within ±1.0 standard deviations from the mean of a random sample selected from a normal population. This difference was significant at the 0.05 level. Similarly 37.5 per cent of 80 subjects between the ages of 55 and 59 years fell within ±1.0 standard deviations and had a significantly lower mean than for the general population ($P < 0.01$). In the subjects between the ages of 60 and 64, 42.0 per cent fell within ±1.0 standard deviations; and 42.9 per cent of those between the ages of 70 and 74 years fell within the same range of ±1.0 standard deviation units. This is approximately 25 per cent less than would be expected in a normal population distribution. However, results of a t-test for the age groups 60 through 64 and 70 through 74 indicated the means did not differ significantly from the means for the normal male population. After the age of 75 years, 100 per cent of the subjects fell within ±1.0 standard deviation units, but the sample consisted of only four men.

Within the age range of 65 to 69 years, 76.4 per cent of the 31 subjects had a bone density within ±1.0 standard deviation units from the mean of a random sample of a male geriatric population. This is consistent with the findings of Vincent (61) who, after studying ambulatory male subjects with a mean age of 66 years, concluded that age had no significant relationship to the degree of severity of osteoporosis.

Figure 1 shows the frequency distribution of standard deviation units from the norm, and in essence, is a reflection of the frequency distribution of bone density, in terms of aluminum equivalency units, in a sample consisting of mentally retarded geriatric males. The frequency distribution tends to follow a normal bell shaped probability curve, but is shifted to the left of the norm in that 55.1 per cent of the experimental group falls at least 1.0 or more standard deviation units below the mean of the normal population.

The discussion to follow is concerned with the overall assessment of various factors, both dietary and physiological, which might be related to bone density, either directly or indirectly, and which might offer some explanation for the findings presented above.

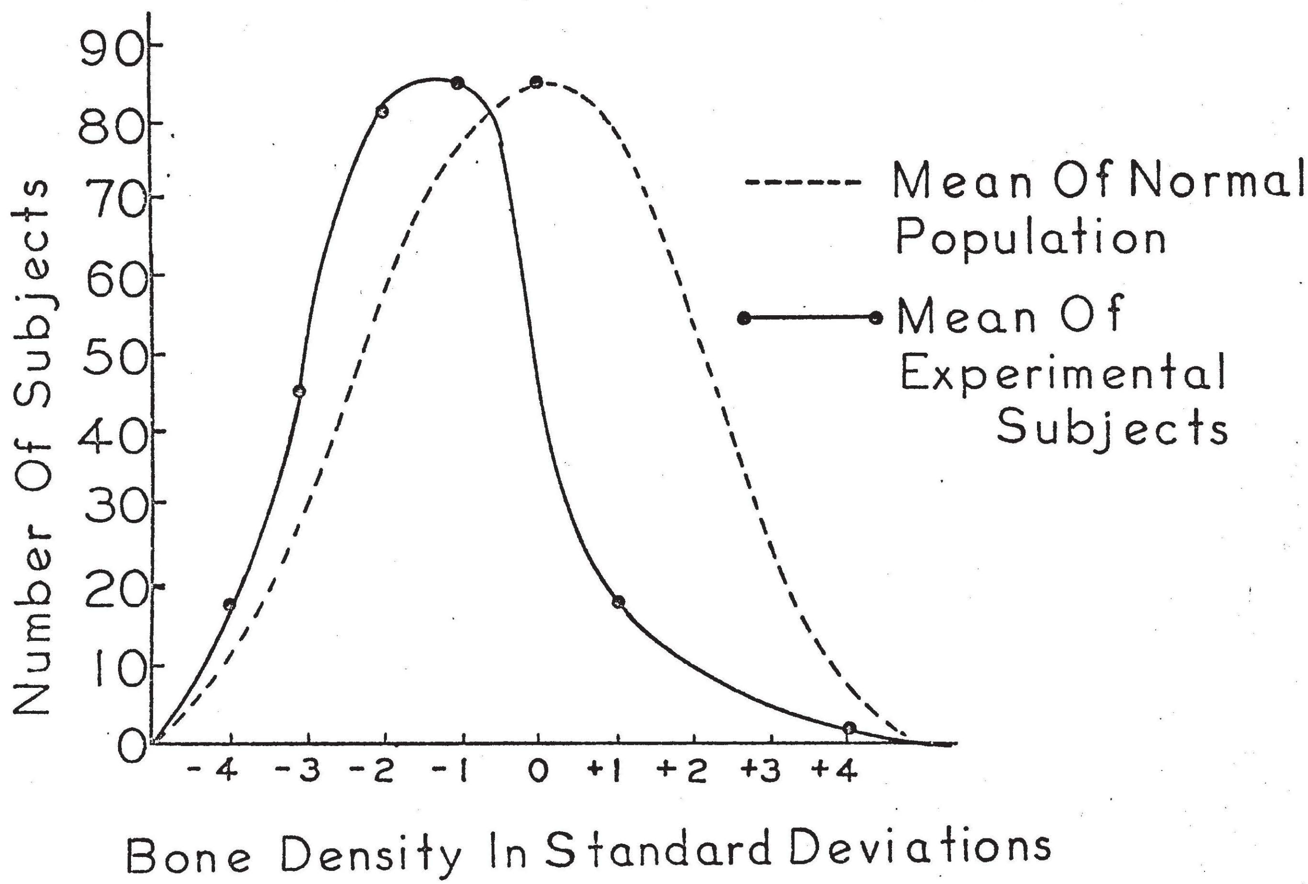

Figure 1

Comparison of Bone Density of Mentally Retarded Geriatric Males With Normal Geriatric Males

DIETARY FINDINGS

As described in Chapter II, menus for all state institutions are planned and distributed by a centralized facility and implemented at the individual installations by Food Service Managers. Plate waste at Travis State School is minimal; the real problem is in controlling the quantity of intake and thus the weight of the residents. The average daily intake, assessed by interview and observation is given in Table IV. As can be seen, all of the Recommended Dietary Allowances (RDA) were met and many nutrients such as calcium, iron, protein and ascorbic acid, exceeded the RDA by 50 per cent or more. It is questionable, however, that the actual amount of available ascorbic acid remaining after food preparation was as high as estimated. Nevertheless, the amount of ascorbic acid available from citrus fruits alone averaged 47 mg per day, and the amount available from the combined sources of citrus fruits and tomatoes averaged 56 mg per day. Likewise a nutritional breakdown of calcium reveals an average of 0.96 gms per day from dairy products. An analysis of protein sources shows that 65 per cent of the average daily protein intake was derived from animal sources. Although vitamin D is usually not included in a dietary assessment such as this, this vitamin was included to emphasize that residents over 60 years of age received twice the amount of milk (one quart) as did those under 60 years.

TABLE IV

AVERAGE DAILY INTAKE OF SELECTED NUTRIENTS BY MENTALLY RETARDED MALES AS COMPARED WITH RECOMMENDED DIETARY ALLOWANCES

Nutrient (Units)	Age Range (Years)			
	50-59		60-75+	
	Intake	Per cent RDA	Intake	Per cent RDA
Food energy (Kcal)	2448	94	2720	113
Protein (gm)	93	143	111	170
Calcium (gm)	1.2	158	1.8	230
Iron (mg)	17.3	173	17.5	175
Thiamine (mg)	1.46	112	1.6	133
Riboflavin (mg)	2.78	164	3.6	212
Niacin (mg equivalents)	17	100	17	100
Vitamin A (I.U.)	10543	218	10543	218
Ascorbic acid (mg)	120	200	122	203
Vitamin D (I.U.)	200	-	400	-

BIOCHEMICAL FINDINGS

Theoretically, calcium intake should correlate with both bone stability and serum calcium levels: low calcium intake being reflected in bone loss and low serum calcium levels, and high calcium intake being reflected in bone stability and high serum calcium levels. However, at present no satisfactory evidence exists to show true bone loss consequent upon consumption of a low calcium diet. Likewise, no satisfactory evidence exists to show a correlation between bone apposition and high dietary calcium (18, 19, 55). This study confirmed the above theories that high dietary calcium intake together with high intakes of vitamin D and ascorbid acid (intakes more than 50 per cent above the RDA) did not result in high serum calcium. In fact, the calcium values obtained in this study (Table V) were low normals.

In an effort to ascertain the reason for the relatively low calcium values, analytical and clinical studies were conducted to determine any pathological conditions that could be responsible for the low calcium levels. Inorganic phosphorus in serum was analyzed because calcium and phosphorus are usually considered together, since disturbances of one usually result in disturbances of the other. As a rule, they bear a reciprocal relationship to each other, a rise in one resulting in a fall of the other. This is not the case invariably, however. The results obtained in this study were

TABLE V

EFFECT OF AGE ON THE SERUM CALCIUM CONCENTRATION OF MENTALLY RETARDED MALES*

Age Range (Years)	Calcium mg/per cent		
	Mean	Standard Deviation	Range
50-54 (n=58)	9.17	±0.80	5.72-12.48
55-59 (n=60)	9.15	±0.42	8.19-12.48
60-64 (n=37)	9.32	±0.53	7.68-11.30
65-69 (n=14)	9.30	±0.77	8.02-10.90
70-74 (n=10)	9.46	±0.67	8.11-10.20
75+ (n=2)	9.60	±0.68	9.50- 9.70

*Normal concentration 9-11.5 mg/100 ml

all within normal limits for serum inorganic phosphorus, as seen in Table VI.

Serum glutamic oxaloacetic transaminase and alkaline phosphatase were determined to ascertain hepatic efficiency. Results on these tests were all within normal limits. Routine urinalyses and tests for creatinine were conducted to ascertain renal function. Results of both urinalyses and creatinine were within normal limits.

The storage of protein in the body is limited to the relatively small amount present as albumin in the interstitial and vascular space. The remaining unused amino acids are catabolized via routes of carbohydrate metabolism (as a result of transamination) or through conversion to urea which is excreted in the urine. The concentration of urea in the blood, expressed as blood urea nitrogen (BUN) is therefore primarily a reflection of protein catabolism and renal function. As shown in the data presented in Table VII, the mean value for each of the six groups was above the upper limit of the range accepted as normal under fasting conditions. These evelated levels were interpreted as a reflection of protein intake rather than renal dysfunction because the creatinine levels were not elevated. Addis (2) reported that the average BUN increases from 8.8 to 21.0 mg per cent as the protein intake, in grams per kilogram body weight, increases from 0.5

TABLE VI

EFFECT OF AGE ON THE SERUM INORGANIC PHOSPHORUS CONCENTRATION OF MENTALLY RETARDED MALES*

Age Range (Years)	Inorganic Phosphorus mg/per cent		
	Mean	Standard Deviation	Range
50-54 (n=58)	3.91	±0.64	2.1-6.2
55-59 (n=60)	3.72	±0.75	2.3-6.2
60-64 (n=37)	3.79	±0.69	2.1-5.7
65-69 (n=14)	3.22	±0.17	2.6-4.1
70-74 (n=10)	3.65	±0.37	3.0-4.1
75+ (n=2)	3.95	±0.35	3.8-4.1

*Normal concentration 3-4.5mg/100 ml

TABLE VII

EFFECT OF AGE ON THE SERUM UREA NITROGEN CONCENTRATION OF MENTALLY RETARDED MALES

Age Range (Years)	Urea Nitrogen Concentration (mg/100 ml)*			
	Mean	Range	Individuals Above 20 mg	
			Number	Per cent
50-54 (n=58)	19.8	13.2-27.9	13	22.4
55-59 (n=60)	19.3	12.4-31.6	19	31.6
60-64 (n=37)	19.9	13.2-29.4	14	37.8
65-69 (n=14)	17.1	12.4-28.3	2	14.3
70-74 (n=10)	18.9	13.0-23.0	2	20.0
75+ (n=2)	29.8	20.2-39.5	1	50.0

*Normal range 8.0-16.0 mg/100 ml

to 2.5 grams. The subjects in this study were receiving approximately 1.4 grams of protein per kilogram of body weight per day.

The results of these biochemical tests led this investigator to conclude that the low normal serum calcium values obtained in this study were possibly the result of intestinal malabsorption. Bullamore, Wilkinson, and Nordin (8) studied calcium absorption in 75 men aged 20 to 95 years. Absorption of calcium fell with age after about age 60, and everyone over 80 years had a significant degree of intestinal malabsorption.

The use of hemoglobin concentration in the blood as an index of nutritional adequacy is based on the fact that the synthesis of this particular protein is dependent, not only upon the availability of those nutrients necessary for production of energy, but also upon an adequate source of amino acids, vitamin B_{12}, folic acid and ascorbic acid. Adequacy of vitamin E, which prevents excessive red blood cell fragility, also has an important bearing on hemoglobin concentration. Data were obtained from 181 subjects ranging in age from 50 to 82 years and are given in Table VIII. Standard deviations were not determined because, with the exception of the value obtained from the oldest group, there was no variation in hemoglobin concentration with age. These findings are in agreement with those of Greendyke (24), who studied

TABLE VIII

EFFECT OF AGE ON THE BLOOD HEMOGLOBIN CONCENTRATION OF MENTALLY RETARDED MALES

Age Range (Years)	Blood Hemoglobin Concentration (gms/100 ml)*			
	Mean	Range	Individuals Above 20 mg Number	Individuals Above 20 mg Per cent
50-54 (n=58)	13.4	11.3-16.2	19	33.0
55-59 (n=60)	13.3	9.9-14.9	19	32.0
60-64 (n=37)	13.4	11.1-15.7	12	32.0
65-69 (n=14)	13.3	10.2-14.7	4	29.0
70-74 (n=10)	13.1	11.7-14.8	4	40.0
75+ (n=2)	12.4	11.9-12.9	2	100.0

*Normal range 13-18 gms/100 ml

1150 men between the age of 17 and 63 years and found no significant variation in hemoglobin concentration. However, Speck and Hawkins (57) state that in a study of 1308 males between the ages of nine and 98 years higher values for hemoglobin tend to occur among younger subjects. After the age of 50 years, these investigators found a progressive decline in hemoglobin values.

PHYSICAL ACTIVITY FINDINGS

Since the classic work of Deitrick, Whedon, and Shorr (10) in the 1940's, the effect of bed rest on loss of calcium from the body has been recognized. More recently, the numerous studies by Mack and her associates (38) at the Texas Woman's University Research Institute have verified, by bone densitometric techniques, that immobilization results in a significant loss of calcium from the bone mineral deposits. On this basis attempts were made to quantitate the relative amount of physical activity in which the subjects of this study were engaged and to determine whether there was any correlation between physical activity and bone mineralization. One of the members of the nursing staff at Travis State Hospital and the attendant for each participant were asked to rate the activity level of each of 129 subjects using a scale of 1.0 through 4.0 to indicate sedentary, moderately active, active and hyperactive, respectively. The lack of a significant correlation between bone density and physical activity, as assessed by this technique, is shown in Figure 2. The sedentary and moderately active patients were almost equally distributed among all standard deviation classes, and one of the highest bone densities was registered by a patient who was confined to a wheel chair. One of the difficulties in interpretation may lie in the fact that approximately 80 per cent of the subjects were classified as either sedentary or moderately active, although most subjects were ambulant. Preliminary trials using an "actometer" attached to the wrist were unsatisfactory also as a measure of physical activity.

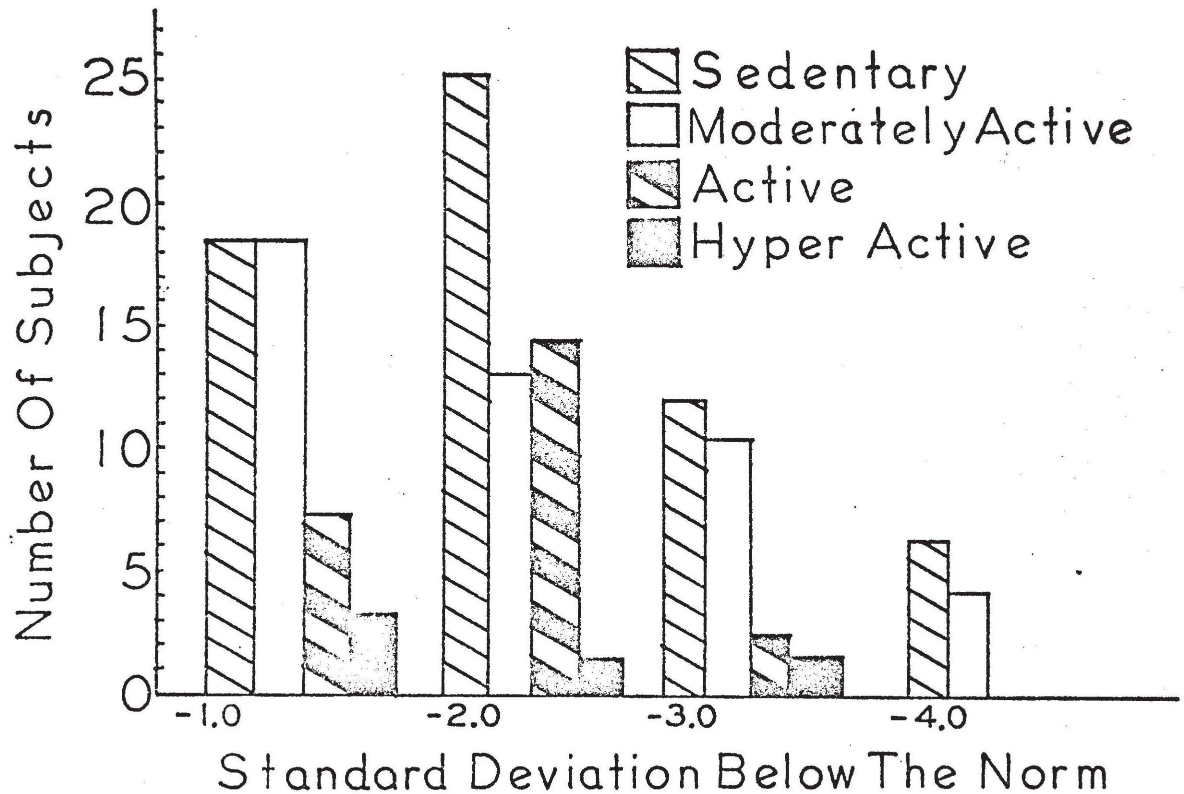

Figure 2

Relationship Between Physical Activity

and Relative Bone Density

CHAPTER IV

CONCLUSIONS AND RECOMMENDATIONS

The major purposes of this study were: first, to determine if the incidence of hypomineralization among mentally retarded geriatric males was similar to that found among normal subjects of the same age and sex; and second, if dissimilar, to make a preliminary evaluation of possible related bio-nutritional factors. A third purpose was to determine if a diet, planned in accordance with the Recommended Dietary Allowances (RDA) established by the Food and Nutrition Board of the National Research Council, did reflect healthy bone mineralization as determined by densitometric analyses of the phalanx 5-2 of the mentally retarded geriatrics.

The 265 subjects were residents of the Travis State School at Austin, Texas. All participants ranged in age from 50 to 82 years with a mean age of 58.6 years.

As indicated previously, the subjects in this study were classified according to the magnitude of deviation in mineralization of the phalanx 5-2 from that of a normal male population within the same age bracket. A mean of 40.8 per cent of 230 subjects between the ages of 50 and 64 years and

70 and 74 years fell within ±1.0 standard deviations from the mean of the "normal random" population. This is approximately 25 per cent less than would be expected in a normal population distribution. After the age of 75 years, 100 per cent of the subjects fell within the ±1.0 standard deviations from the "normal" mean. However, the sample of subjects 75 years and older consisted of only four subjects.

Within the age range of 65 to 69 years, 67.4 per cent of the 31 subjects had a bone density within ±1.0 standard deviation units from the mean of a random male geriatric population. These findings are consistent with those of others who, after studying ambulatory male subjects of the same age, concluded that age had no significant relationship to the degree of severity of osteoporosis (39, 61).

In an effort to ascertain a possible related bio-nutritional relationship with bone loss, dietary intake and clinical studies were conducted. In view of the fact that the Recommended Dietary Allowances were met in all instances, and in most subjects were exceeded by at least 50 per cent, the low normal means for serum calcium and hemoglobin may confirm the conclusions of other investigators that low serum calcium and low hemoglobin values may be the result of intestinal malabsorption (27, 40, 41).

Additional clinical analyses were made to rule out other causes of low serum calcium. Serum glutamic oxalo-acetic transaminase and alkaline phosphatase values were determined to ascertain hepatic efficiency. Renal function was determined by routine urinalyses and creatinine. Results of all these tests were within normal limits. Blood urea nitrogen (BUN) analyses were also conducted. The elevated levels obtained were interpreted as a reflection of protein intake rather than renal dysfunction, because the creatinine levels were not elevated. The dietary and clinical findings of this study are consistent with those of Garn (19) and Smith (56) who concluded that dietary calcium is not simply primarily and directly relatable to the osseous state. Naturally these data from an ambulatory sample do not mean that clinical osteoporosis could not in some individuals stem from inadequate calcium intake, or at least calcium intake inadequate for the individual's level of absorptive efficiency.

Two attempts were made to assess the importance of physical activity on bone retention. Preliminary trials using an "actometer" attached to the wrist were not satisfactory. The second method of assessing physical activity of the subjects involved the use of activity level ratings (sedentary, moderately active, active, hyperactive). Again, no significant correlation between bone density and physical activity was found. The sedentary and moderately active patients were almost equally distributed among all standard deviation classes, and one of the highest bone densities was registered by a patient who was confined to a wheel chair. One of the difficulties in interpretation was that approximately 80 per cent of the subjects were classified as either sedentary or moderately active.

Since the interrelationships between the factors involved in bone formation and bone destruction are not clearly understood, further metabolic research is needed to determine the controlling mechanisms of calcium absorption.

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