THE EFFECT OF TWO LEVELS OF SODIUM FLUORIDE ON BONE DENSITY OF FEMURS IN WHITE RATS

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CHAPTER I

INTRODUCTION

Osteroporosis, a disease of bone quantity rather than bone quality (33), is widespread throughout the United States and is prevalent in all socio-economic classes. Long accepted as an inevitable accompaniment to aging, osteoporosis is one of the most common disorders of the bone (26). It is a disease in which there is no change in the chemical composition of the bone. Osteoporosis occurs with greater frequency in women than in men, and is essentially a disease of middle and old age. Lutwak and Whedon (25) estimated that four million cases of osteoporosis exist in the United States today. Disturbances in protein, mineral and hormonal aspects of bone metabolism have been implicated as causative factors. A review of the results obtained with experimental animals and with humans suggest that osteoporosis may result from an inadequate calcium intake probably of many years duration (26). Garn, Rohmann and Wagoner (12) regarded bone loss as a generalized phenomenon in man, beginning about the fifth decade and progressing about twice as fast in women as in men. Since bone loss appears to be delayed in taller individuals and persons with a high biophysical bone density, it is speculated that "the best natural protection against the sequlae of bone loss is a large skeletal mass to begin with" (25, p, 729). This implies that good nutrition during growth is important for the later prevention of osteoporosis.

Increasing attention to geriatrics and to the relatively high incidence of fractures and osteoporosis in the elderly has aroused considerable interest in the cause and treatment of osteoporosis. Although the cause or pathogenesis of osteoporosis is as yet unknown (26) several treatments have proved effective in battling this bone disorder. These treatments are as follows:

1. The oral ingestion of calcium in significant amounts so as to replace calcium loss from the bone (15).
2. The injection of female hormones, such as estrogen, in quantities at the level of natural secretion (24).
3. The injection and/or oral dosage of fluoride (35).

Perhaps the most controversial of these treatments is that of the fluoride dosage. The use of fluoride[1] to induce excess mineralization can be a painful method if administered in large quantities. The amount of fluoride that is adequate for mineralization differs from one individual to another. Therefore, a minimum dosage is needed which would accomplish adequate mineralization of the bone without the painful fluoride deposits in the joints and accompanying brittle bones.

Statement of the Problem

The author's interest in osteoporosis developed when she became aware that osteoporotic patients may consume large quantities of

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[1]Fluoride is used throughout this text as referring to the mineral which is a result of the gaseous form, fluorine, combining with another element or radical as well as the element fluorine when so described in related research.

calcium-rich foods and yet develop the metabolic disorder known as osteoporosis. There is some evidence of beneficial effects of giving varying dosages of sodium fluoride to these patients in the current literature. Therefore in this research the author wished to establish the amount of sodium fluoride which would induce an increased bone mineralization without fluorosis developing.

In this study it was decided to feed two different levels of fluoride to weanling, female rats for approximately six weeks. One control and two experimental groups of seven rats were selected randomly. The three groups were fed a ration containing all known nutrients in amounts needed for optimum nutrition in the white rat. Experimental Group I animals received distilled water containing 4 ppm of sodium fluoride ad libitum, while the animals in Experimental Group II received 8 ppm in their water supply. The Control Group of rats received distilled water ad libitum with no added sodium fluoride.

At the conclusion of this experiment the strength of the femur bones of animals in all three groups were compared as an index to the effect of the fluoride levels fed.

Assumptions

The following assumptions are accepted as true:

1. The albino rat is a suitable animal for demonstration of the effects of varying levels of fluoride on bone strength and development.
2. A dietary ration can be compounded which is adequate in all nutrients needed for the optimum nutrition of the female albino rat.

3. Calcium, phosphorus, protein and vitamin D are necessary for the development and repair of bones.
4. Fluoride is an essential element in animal metabolism.

Hypotheses

The following null hypotheses are postulated:

1. The inclusion of an increased amount of fluoride to the diet of the female albino rat will not significantly increase bone mineralization in the experimental group as determined by the amount of ash in the femurs.
2. The breaking point of the femurs of the two experimental groups of animals will not be significantly greater than those of the control group.
3. The overall body length of the two groups of experimental animals fed additional fluoride will not be greater than that of the control animals.

After approximately six weeks of feeding each rat will be evaluated according to: weight, animal length, femur densities and femur fragilities. Collected data will be analyzed statistically.

Definition of Terms

For the purpose of this study, the following terms are defined:

Osteoporosis - A disease in which there is a reduction in the amount of bone without change in its chemical composition (15).

Femur - The proximal bone of the hind or lower limb--commonly called the thigh-bone (44).

Mineralization - The impregnation or supply of minerals to the bone (8).

Stock diet - A diet which is adequate for normal growth and optimum nutrition in the white rat (14).

Bio-physical bone density - A measure of the density of bone mineralization (44).

Breaking point - That point at which the bone will break under a set weight (44).

Ash content - The amount of minerals concentrated in the femurs of the rats upon chemical ashing (18).

Animal body length - A measurement beginning at the tip of the nose and extending to the tip of the animal's tail (14).

Bone density - The amount of mineralization per unit of bone (44).

Ad libitum - To allow the animal to consume as much food and water as desired (20).

Fluoride - A mineral formed by the combination of fluorine with another element or radical (21).

Fractometer - An instrument designed to determine the breaking point of the rat femur.

CHAPTER II

REVIEW OF LITERATURE

In this chapter some of the literature which pertains to the study of bone loss from the skeleton is reviewed. A brief discussion of normal bone development is followed by a review of the interrelationship of calcium, phosphorus and vitamin D to normal calcification. The causes and effects of osteoporosis are followed by a discussion of the function and effects of fluoride on bone density in animals and humans. Finally a comprehensive discussion of the use of the rat as an experimental animal is covered.

Normal Bone Development

Bone tissue is in continuous flux throughout life. According to the change in the mechanical requirements of the skeleton, an internal reconstruction of bone tissue takes place. Throughout the life process of the animal and human various nutrients are needed for the normal growth and maintenance of the skeletal system.

Normal bone is a semi-rigid, highly specialized form of connective tissue (45) of which the greater part is composed of a strong interstitial substance or matrix, within which cells or osteocytes are embedded in small spaces called lacunae. Each osteocyte has many slender processes which penetrate fine passages known as canaliculi in the matrix. The latter has a complex structure consisting of bundles

of fibres similar to those of white fibrous connective tissue. The fibres are arranged in bundles of three to five millimicrons in thickness and are bound together by an amorphous material consisting largely of calcium salts (45). These fibres form about 30-40 per cent of adult bone and the inorganic salts in which they are embedded form 60 to 70 per cent of the dried interstitial material and consist mainly of a calcium salt of the apatite series (15).

In most parts of the body, bone formation takes place in relation to an existing cartilaginous model which it eventually replaces. In certain situations, however, the bone formation takes place in dense connective tissue (6). These two types of ossification are distinguished as endochondral and intramembranous respectively; the former is seen in the earlier stages of ossification in most bones of the body and the latter mainly in the skull and around the shafts of the long bones.

Bone tissue consists of two permanent elemental parts: specialized cells and intercellular substances. The cells are known as osteocytes and the intercellular substance is composed of fibrils and a calcified cementing substance. Two types of cells are observed during active stages of bone destruction or formation only and are, therefore termed transient elements of bone tissue as distinct from its permanent elements. The cells which are active in bone formation are known as osteoblasts while those causing resorption of bone are the osteoclasts (45).

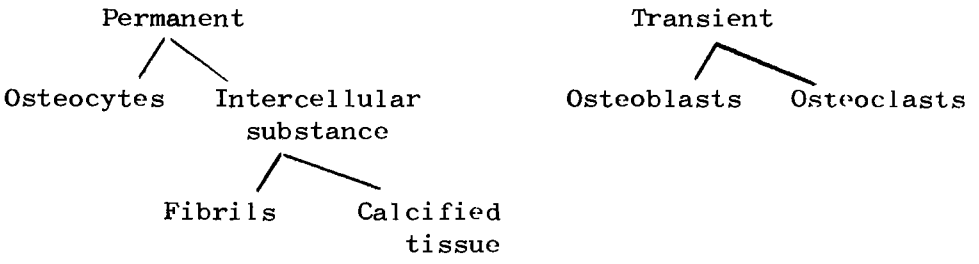

Figure 1. Elements of Bone Tissue (45, p. 18)

The structure of the long bone is such that it can be classified into two main categories, the shafts and the expanded ends. The shafts (diaphyses), are composed of compact bone and accounts for the rigidity or mechanical stability of the bone, whereas the expanded ends (epiphyses), are composed of spongious bone embedded in a thin layer of compact bone. The spongious bone serves as a buffer to maintain chemical homeostasis of the body fluids. In the compact bone most lamellae are arranged in cylindrical, concentrically laminated Haversian systems which are arranged around a central vascular channel, the Haversian canal (6). The spongy bone is a form in which the organic matrix is arranged in a network of rods, plates or tubes, such as the trabecula with the spaces filled with bone marrow (34).

It has been conclusively shown that except during periods of stress or disease the rate of bone deposition and bone absorption are equal to one another so that the total bone quantity remains the same (6, 11, 15). This has several important implications as an increase in exercise or physical labor seems to lead to a more localized or generalized strengthening of the bone. The bone becomes heavier and processes, crests and ridges, serving as attachments of muscles, are

enlarged and greatly strengthened. The pressure-bearing bones are likewise reinforced by the production of new bone (6, 15). Thus, it can be said that the rate of growth is influenced by the stresses which pass through these bones.

To do their part efficiently bones must be properly formed; that is, they must contain a sufficient quantity of mineral salts derived from food intake. They must also be exercised at more or less regular intervals in order to maintain their strength and structure.

In old age, the mineral constitution of the bone changes again. At this point, the bones may be brittle and less able to resist shock as well as having a reduced capacity on the body's part to repair a broken bone.

The Interrelationship of Calcium, Phosphorus and Vitamin D in Normal Bone Development

Of all the minerals in the human body, calcium is present in by far the largest amounts. It comprises about 1.5 to 2.0 per cent of the total body weight (11). Calcium is the most abundant and phosphorus the second most abundant of the mineral elements and are found in a 2:1 ratio in the body. Ninety-nine per cent of the calcium and about 80 per cent of the phosphorus in the body are in the bones and teeth (15). The remaining one per cent of calcium is found in the blood and extracellular tissues, whereas the 20 per cent of phosphorus is found mostly in the soft tissues with some phosphorus found in almost every cell and tissue (38).

Phosphorus performs an important role in combining with calcium in the formation and strengthening of the bony tissue. Inorganic

phosphates in the blood act as buffer substances that assist in maintaining body neutrality and the acid base balance of the blood (2).

The most labile supply of calcium and phosphorus in bones is found in the trabeculae-columns of crystalline calcium compounds that grow from the inner surface of the cavity at the bone's end and project toward the center in such a way as to act as braces in strengthening the end of the bone (2). Within the cavity, blood vessels and interstitial fluid come into close contact with the mineral material in the trabeculae, so that it may be readily taken up by the blood stream to meet minor changes in blood calcium. The more abundant the supply of calcium in the food, the greater is the development of bone trabeculae. In a low calcium diet, over a considerable period, these structures will be practically absent (34).

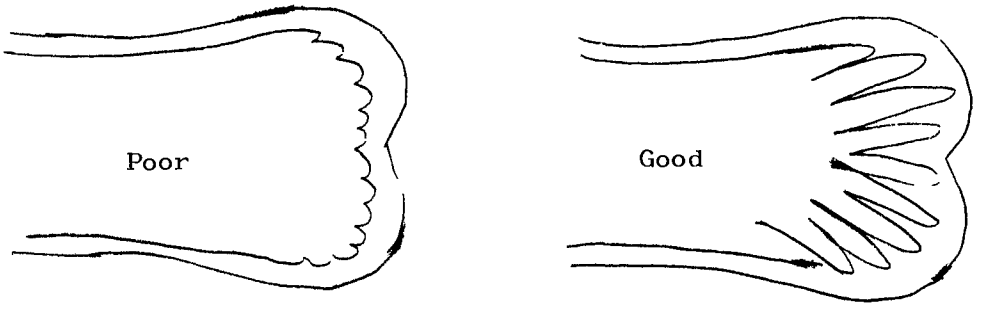

Figure 2. Diagrammatic Representation of Bone Trabeculae, Showing Poor or Good Development According to whether the Food Calcium Intake is Low or Liberal (40, p. 262)

Sherman has said, "The extra calcium received by the body is not only passively stored. It also adds to the body's working capital of blood-and tissue-regulating material" (40, p. 263).

Calcium and phosphorus in the digested food material are absorbed through the intestinal wall and carried by the blood in the form of acid phosphates to all parts of the body; the various tissues withdraw amounts needed and contribute mineral elements discarded by the cells. Any excess quantities of these two elements are excreted, in part, as soluble salts in the urine and in part by secretion across the intestinal membrane into the fecal material (46).

Phosphorus functions as an aid to calcification and mineralization of the bones and teeth (19). As phosphorus is closely related to calcium in human nutrition, it is constantly being deposited and reabsorbed in the process of bone formation. The absorption of phosphorus is closely related to that of calcium. Equal amounts of the two minerals in the diet is an optimal ration; excess of either causes increased fecal excretion of the other. Apparently the phosphorus is more efficiently absorbed than calcium, as only 30 per cent of the ingested phosphorus that is bound to calcium is excreted in the feces and about 70 per cent is absorbed, compared with only 10 to 30 per cent of dietary calcium (31).

The absorption of phosphorus is apparently secondary to that of calcium and the absorption of phosphorus follows (8). This absorption seems to take place by active transport across the proximal segment of the small intestine, and throughout the remainder of the intestine by passive diffusion. After the absorption of calcium and phosphorus through the intestinal wall, vitamin D continues to work in partnership

with calcium and phosphorus in the calcification aspect of bone formation (8). Tracer studies have conclusively shown that vitamin D directly increases the rate of mineral accretion and resorption in bone, by which the tissue is built and maintained (9).

Causes and Effects of Osteoporotic Disorders

Increasing attention to geriatrics and the relatively high incidence of fractures and osteoporosis in the elderly has aroused considerable interest in the cause and treatment of osteoporosis. When considering bone as a tissue, osteoporosis is considered a disease of bone quantity rather than bone quality (25). The effect of the disease is seen primarily upon the mechanical function of bone due to the weakening of the skeletal parts affected.

Although the pathogenesis of osteoporosis is unknown, several factors are recognized as being related to its origin. Prolonged and consistently low intakes of calcium, vitamin D, protein, and ascorbic acid are known to be detrimental to bone development and repair (12). An impaired or inadequate calcium-phosphorus ratio causes even greater problems of bone formation and repair. Normal human body requires an intake of two parts of calcium per each part of phosphorus, resulting in the ratio of 21:1 (24). This ratio remains relatively constant in health throughout the growth period.

Osteoporosis may be a localized or generalized disease. Local osteoporosis may arise as a result of inflammation or neoplasm of bone (1). The most common cause is immobilization. Disuse removes the stresses and strains which stimulate the osteoblasts to lay down new bone, while the osteoclasts continue to remove calcium salts. This

happens when movement is restricted by splinting, by inflammation or by pain, or when a patient is confined to bed. This restraint normally causes a slightly porous bone, depending on the length of restraint, which cannot be prevented by excess of nutrients in the diet. The only known relief is attained by allowing movement or posture of the individual.

Generalized osteoporosis is very common in old age and occurs in a variety of diseases. This disorder is characterized by porosity, thinness, and fragility of the bones. Apparently in the continuous remodeling of the bone, calcium has been withdrawn for body use or mandatory excretion over a long period and the calcium has not been adequately replaced. Recent work has established the fairly common occurrence of this disorder among older people both in the United States and elseqhere (3). A recent radiographic survey of 100 aged women revealed that 26 per cent of the subjects disclosed symptoms of osteoporosis and hip fractures in 15 per cent. Some cases of osteoporosis may respond to increased calcium intake by storing calcium, but it seems that in addition to calcium insufficiency, a lack of protein or sex hormones may be contributive factors (31).

The Function and Effects of Fluoride on Bone Density

In 1805 Gay-Lussac first detected fluorine in the animal body. Traces of this element are regularly present in human tissues, notably in the bones, teeth, thyroid gland and skin (10).

Most humans ingest between two and three mg. of fluorine daily. The chief source is usually drinking water, which, if it contains

one part million of fluorine, will supply one to two mg./ day. Soft waters may contain no fluorine while hard waters may contain over 10 ppm. Compared to this source, the fluorine in foodstuffs is of little importance. Very few foods contain more than one ppm; the exception is sea-fish which may contain relatively large amounts of the order of 5 to 10 ppm. Another significant source is tea, particularly China tea, which in the dry state may contain as much as 100 ppm. In Britain and Australia, where people consume large quantities of tea, the adult intake from this source may be as much as one mg. daily (22).

It is worth noting that the fluorine content of most plants bears little relation to the amount of fluorine in the soil on which they are grown; plants seem to be selective in the amount of this element that they absorb (43).

Since the late 1940's the use of fluorides for human health has been actively investigated. The present knowledge about fluoride absorption is based on numerous animal experiments and on observations and investigations in man (22, 29, 30, 31, 47). Adequate data with regard to the amount of ingested fluoride which will be absorbed can only be obtained by metabolic studies (16).

Fluoride, a prototype bone-seeker, in its skeletal deposition is also a cumulative element. Because excessive prolonged fluoride exposure leads not only to high skeletal concentrations but also to characteristic ill-effects, e.g., crippling fluorosis, more than ordinary significance is attached to evidence concerning fluoride elimination (8).

Fluoride is excreted in the urine, deposited in the skin which is shed, lost through the sweat, and excreted in the feces.

Fluoride occurs in traces in milk, in saliva, in hair and possibly in tears (48).

The principal excretion of fluoride is via the urine. Quantities of fluoride appear in the urine generally reflecting the daily intake but governed by other factors, several of which are known, such as (a) the total intake, (b) the form in which the fluoride is taken into the body, (c) the health status of the individual, especially in regard to advanced kidney disease, (d) to whether the individual is relatively unexposed or regularly exposed to fluoride. Hence, it can be seen that fluoride is certainly not irrevocably deposited in skeletal tissues. Experiments with rats of various ages have shown that there is an initial rapid decrease in the skeletally bound fluoride, followed by a more gradual removal (43). Hodge (17) pointed to a similar phenomenon in man. He offered an explanation that the escape of fluoride from mineral to tissue fluids by back-exchange with ions in the hydration shell could account for the relatively rapid loss of fluoride in the period immediately following incorporation.

Whereas there are good reasons to believe that the element will be removed relatively quickly as long as it is situated at the surface of crystallites, there is no direct experimental evidence in support of Hodge's hypothesis. According to Hodge, the remodeling of the bone by osteoclastic and osteoblastic activity are responsible for the more gradual, later phase of fluoride removal, accounting for a slower but considerable loss of the element. There is no doubt that some of the incorporated fluoride will eventually be buried deeply by crystal growth and subsequent apposition of new tissue. In this manner although fluoride could not escape from the mineral crystallites by

exchange, it could still be released by osteoclastic resorption.

Measurements of fluoride levels in bone or urine give no direct indication of the extent to which fluoride released may be reincorporated. Likins et al. (24) found that, although some of the fluoride present in the proximal metaphyses of the growing rat tibia had been lost during bone growth, there was considerable uptake in the adjacent developing bone segment. It seems, therefore, that fluoride released during remodeling did not necessarily enter the general circulation but redeposited in nearby sites of growth which were in active formation.

The evidence of fluoride removal from the human skeleton rests entirely upon measurements of urinary excretion rates. There is no way of directly detecting skeletal fluoride loss in man. Largent examined the urinary excretion of stored fluoride in persons who had ingested large amounts for long periods. For some time after discontinuation of fluoride administration, urinary excretion remained in excess of ingestion. Largent estimated that the decline of excess urinary fluoride reached its midpoint in 75 to 80 weeks and that a state of balance was reached in 200 to 225 weeks (21). He assumed that eventually the urinary fluoride concentration would reach a level similar to that of the drinking water. This was substantiated by the evidence gathered by McClure and Kinser in 1944, who found that the concentration of fluoride in human urine bore a linear relationship to the fluoride content of the domestic drinking water supply (28).

According to Largent (21) the total amount of fluoride deposited in the bones relates to the level of fluoride ingestion. So long as the level of ingestion remains unchanged, any further change will eventually be offset by the mobilization of some previously stored

fluoride. A balance appeared to be maintained between absorption and storage, on the one hand, and mobilization and excretion on the other. In the case of ten human subjects, he found that intake and output of fluoride were nearly equal. Largent's findings suggested that each person stored enough fluoride to reach a state of equilibrium. This seems to support the view that the fluoride concentration of bones increase with age for a time but that eventually a steady state is reached, after which there is no further rise in skeletal fluoride concentration (21). The ingestion of fluoride increases the size of the apatite crystals and reduces the bone solubility (39).

Lawrenz and Mitchell (22) in 1940 found that growing rats adapt themselves to the continuous ingestion of low levels of fluoride by excreting greater and greater proportions of the ingested fluoride in feces and urine, again indicating that less fluoride was retained by aging skeletal and dental tissues. However, Glock, Lowater and Murray (13) conducted a study involving the retention and elimination of fluoride in bones of rats. They found that fluoride fed to rats was absorbed by the bones in a gradually increasing fluoride content with increasing age. Zippin and McClure (49) combined previous research studies done in the field of fluoride retention and provided a comprehensive study of the effect of age on fluoride retention as well as comprehensive studies of periodic fluoride analyses of femurs, mandibles and teeth of rats undergoing continuous fluoride ingestion.

On the basis of previous studies, Rich, Ensinck and Ivanovich (37) studied the possible therapeutic effect of fluoride in osteoporosis and found that those subjects treated for osteoporosis with fluoride (1 mg. per kg. of body weight per day) improved with urinary excretion

of calcium reduced to near zero.

Patients suffering from osteoporosis and other demineralizing diseases have been treated with substantial amounts of sodium fluoride with reported beneficial effects upon back pains, bone density and calcium balance (7, 23, 32, 36, 37). Fluoride treatment appears to be a useful therapy for these conditions, but more research and experience are required to establish its efficacy and safety. Of wider importance is the findings of Leone and associates (23) that there is substantially less osteoporosis in a high-fluoride area in Texas (8 ppm fluoride in the water) than in a low fluoride area (0.09 pp, fluoride in the water) in Massachusetts. These workers suggested that fluoride ingestion might be important in the maintenance of the normal skeleton. Subsequently Bernstein et al. (4) examined approximately 1000 x-rays of the lower lumbar spine of adults over 45 living in two areas of North Dakota. In one area the water supply provided 0.15 - 0.3 ppm fluoride and in the other, 4-5 ppm. As expected, the number of individuals judged to have decreased bone density increased with age, in both the high- and the low-fluoride areas. At all ages there was substantially less osteoporosis in women in the high fluoride areas; the changes in men were in a similar direction but less obvious. The differences in women showing collapsed or distorted vertebrae were even greater, particularly those over 55. No such area effect was apparent in the men, who revealed a high incidence irrespective of fluoride intake (4). A fact that emerged from this study was the decreased calcification of the aorta in men in the high-fluoride area. This condition was approximately twice as common in men in the low fluoride area at all ages. No such differences were found in women.

The significance of this in terms of coronary heart disease or other forms of cardiovascular disease is unknown. It is pertinent that Leone et al. (23), in comparing mortality rates in high-and-low fluoride areas in Texas, found the only significant difference to be a somewhat lower incidence of death from heart attacks in the high fluoride area. It should be noted however, that the reported benefits in respect to the incidence of osteoporosis and collapsed vertebrae in women and of calcification of the aorta in men were obtained at levels of fluoride in the drinking water above those considered safe for children. It remains to be seen whether a level of one ppm of fluoride in the water is sufficient for the maintenance of a normal skeleton in the adult. A later study conducted in 1967 by Hegsted, Posner and Smith (16) supported the earlier study of Bernstein et al. by reporting that high levels of fluoride in drinking water seem to be better protection against normal, age related bone loss than high calcium diets.

A recent publication of the World Health Organization (48) states that "certain degrees of fluoride saturation, or possible other fluoride influences on the skeleton, may provide a partial protection against senile osteoporosis" (48, p. 14). Since the condition of osteoporosis is widespread and often leads to serious fractures and to invalidism, further knowledge of the role of fluorides in skeletal biology is needed.

The Rat as an Experimental Animal

Perhaps one of the most widely used of laboratory animals, the albino rat is selectively bred and reared and is highly desirable

for demonstrating the effects of various nutritional deficiencies, especially bone disorders. Among the many virtues that distinguish this animal as desirable for laboratory research in experiments involving nutrition or medical studies are the following:

1. The biological body processes, such as digestion, assimulation and circulation are comparable to those of the human (20).
2. The life cycle of the rat is approximately three years, therefore effects of nutritional deficiencies can be observed over a lifetime or several generations of the rat.
3. Care of the albino rat is inexpensive due to his size and minimal requirements for food.
4. The rat consumes and responds to different foods as the human often does, but does not require vitamin C and is thus, not a suitable animal for observing vitamin C deficiencies (14).
5. Albino rats thrive well on highly purified dietary rations. Therefore, the exact components of the diet can be analyzed for nutrients with greater ease than those diets containing natural foods (14).

When planning a dietary ration for the rat, it is of importance to know his daily requirements for the various nutrients which normally constitute a purified ration. According to Griffith and Farris the rat requires the following:

Nutrients	Amount Per Day
Protein	25-30 per cent
Calcium	40-50 mg.
Phosphorus	35-40 mg.

Sodium	0.5 per cent
Chlorine	5 mg.
Vitamin A	4 mg.
Thiamine	1 mcg.
Riboflavin	40 mcg.
Vitamin D	Not required if Ca:P is between 1:1 and 2:1

(14, p. 98)

It must also be considered that the albino weanling rat will consume approximately 15 to 20 grams per day. Based on this average one can calculate the amounts of dietary ration to mix for the duration of the research.

In preparing the research diet, one must be sure to have thoroughly mixed and ground all constituents of the ration. All mineral salts added to the ration should be ground with a mortar and pestle to insure a homogenous mixture of all ingredients in the diet. After mixing, the diet should be stored in brown bottles, covered with a lid and stored either in a freezer or a cool dry place.

Throughout the years of scientific research involving the sacrificing of the subjects of the experiment, the white rat has always been the ideal choice. In the study of osteoporosis or other bone disorders, it is ethical to sacrifice the animal in order to study the skeletal structure. Human studies on bone loss, however are limited to the methods of balance studies and roentgenological techniques.

Lutwak and Whedon (25) found that after long periods of fluoride exposure in vivo skeletal fluoride was removed with difficulty from the rat skeleton and appeared to be firmly fixed. Hodge and Smith (17)

found a linear relation between fluoride intake and urinary fluoride excretion in the albino rat as did studies done by Largent (21) which revealed an age-related increase of fluoride to bone density. These studies and experiments indicated that the white rat is a suitable animal for demonstrating the effect of fluoride administration upon bone loss.

CHAPTER III

METHOD OF PROCEDURE

Experimental Units

Female rats of the Holtzman strain,[1] weighing approximately 55 grams, were selected for this experiment. When the weanling rats arrived, they were randomly selected, numbered, marked and weighed. The initial body weight was recorded for future reference as shown in Appendix A.

The rats were marked as follows:

Rat 1 - one notch right ear
Rat 2 - one notch left ear
Rat 3 - two notches right ear
Rat 4 - two notches left ear
Rat 5 - two notches right ear, one notch left ear
Rat 6 - two notches left ear, one notch right ear
Rat 7 - two notches in both ears
Rat 8 - one notch right ear, one notch left ear
Rat 9 - no marks
Rat 10 - red color on head
Rat 11 - red color on tail
Rat 12 - blue color on head
Rat 13 - blue color on tail
Rat 14 - green color on head
Rat 15 - green color on tail
Rat 16 - red color on head and tail
Rat 17 - blue color on head and tail
Rat 18 - green color on head and tail
Rat 19 - yellow color on head
Rat 20 - yellow color on tail
Rat 21 - yellow color on head and tail

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[1]The rats were purchased from the Holtzman Company, 421 Holtzman Road, Madison, Wisconsin, 73711.

Randomization of Animals Into Groups

Slips of paper bearing the number of each rat were placed into a jar, shaken and one slip of paper drawn at a time. The animal having the first number drawn was then arbitrarily assigned to Experimental Group I, the second to Experimental Group II and the third to the Control Group. This process was repeated until each group contained seven rats. These animals were then placed so that their cages were together according to groups on the tiered metal carriers in the laboratory.

Composition of the Diet

A daily record of feeding and visual characteristics of each rat was kept for the entire length of the experiment. The complete record for each rat is included in Appendix A. The composition of the diet fed to the three groups of rats is presented in Table I.

The amount shown in Table I was calculated to provide 15 grams per day per animal for seven animals for seven weeks. Approximately one weeks ration was added to allow for spillage and deviations in eating patterns of individual animals. The diet was taken from Lamb's Manual for Nutrition Courses (20), and was fed tor a period of six weeks, ad libitum, to all animals.

TABLE I

COMPOSITION OF THE CONTROL DIET

Ingredients	Percentage	Grams
Casein, Technical	18	1,587.60
Cornstarch	48	4,233.60
Pure Fat[1]	8	705.60
Cod Liver Oil	2	176.40
Salt Mixture[2]	4	353.80
Yeast, Dried Brewer's	20	1,764.00
Total	100	8,821.00

[1]Cottonseed oil.

[2]Salt Mixture W purchased from Nutritional Biochemicals. Percentage: $CaCO_3$, 21.001; $CuSO_4$, $.5H_2O$, 0.039; $FePO_4$, 1.470; $MnSO_4$, 0.020; $MgSO_4$, 9.000; KAL $(SO_4)_2$, 0.009; KCL, 12.00; Kh_2PO_4, 31.000; KI, 0.005; NaCl, 10.500; NaF, 0.057; and Ca_3PO_4, 14.900.

Fluoridation of the Water Supply

The use of fluoridated water was arrived at after a careful review of the current literature indicated that this was an acceptable mode of administering fluoride. The amount and kind of fluoride to be used was based on an article published in 1968 entitled "Fluoride for the Elderly" (27).

The basis for the amount of sodium fluoride in parts per million for this experiment is shown in Table II.

TABLE II

PROPORTION OF FLUORIDE TO WATER IN EXPERIMENTAL GROUPS I AND II

Experimental Groups	Fluoride	Distilled Water	PPM
	mg	ml	
I	4.0	1000	4
II	8.0	1000	8

This fluoridated water was given to Experimental Groups I and II, as indicated above, ad libitum, for the duration of the six weeks feeding period. The Control Group received ad libitum distilled water.

Routine Feeding and Care

All animals were fed the semi-purified diet and were given distilled water ad libitum, which in the experimental groups contained an additional amount of fluoride. Random selection of the rats determined the order in which they were fed each day. The control diet was selected from Lamb's Manual for Nutrition Research (20) as it is adequate in known nutrients needed for optimum nutrition for the white rat. Addition of sodium fluoride to the distilled water was based on the literature advocating that fluoride is much better absorbed in a liquid form (27).

Before the rats arrived the diet was measured and mixed, after which it was tightly covered and stored in large brown bottles in a freezer. The daily diet was so measured that each animal received at least two more grams of diet than would be eaten for that day. This daily ration was based upon the previous day's diet consumption. Diet consumption was calculated by the amount of food left which was weighed each day before rations were given. Each day all water bubbles and food jars were washed and disinfected before being used again. The feces trays were emptied daily and fresh liners put in for each animal. The cages, screens, and feces trays were removed and scrubbed as well as disinfected once each week on a predetermined schedule.

Bone Density Analysis

After approximately six weeks of feeding the animals in all groups were randomly selected for sacrifice over a two day period. Before sacrificing, the final visual characteristics and weight of all animals were recorded. Sacrificing was accomplished by placing the animal in a large closed metal container in which an adequate amount of ether had been placed for inducing death. After death, the animals were removed from the container and while still limp were measured from the tip of the tail to the tip of the nose, for an accurate body measurement. The left and right femurs were then excised and all soft tissue carefully removed. The bones were then labeled according to animal number, group, and left and right location. The wet bone was then weighed and recorded and is given in Appendix B.

The breaking point of each bone was determined by an instrument

used by Sweeten in 1969 (42) called the fractometer.[2] A preliminary uniformity trial, testing the breaking load of toothpicks was conducted in order to acquaint the researcher with the fractometer. The breaking point of each femur was determined by placing the bone across two blunt knife edges of the fractometer so that the weight was equally distributed upon the center of the femur, the amount of number 8 shot used was weighed and recorded as the amount of weight needed to break the bone. After breaking, the bone was immersed in an individual airtight vial of ether for approximately 45 minutes to remove excess fat and to prepare the bone for future ashing. After breaking and immersion in ether, the bone was fastened to a sheet of metal for drying. The femurs had been carefully labeled according to animal number, group and location in the animal and were then placed in the drying oven at a temperature of approximately 90 degrees centigrade. After drying for 48 hours the bones were removed and weighed on a micro analytical balance and the weights recorded for future reference as seen in Appendix B.

Ashing of the Femurs

The ashing of the rat femurs was accomplished over a two day period. The dried and labeled bones were placed in platinum crucibles and arranged in a labeled and diagrammed order in a muffle furnace at 490 degrees centigrade. After a 48 hour period had elapsed, the bones

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[2]The fractometer was designed and constructed by Mr. Heinz Hall, Department of Physics and Chemistry, Oklahoma State University.

were removed and weighed with the crucibles. This weight was deducted from the previously recorded weight of the crucible and femur before ashing. The amount of ash for each group was triple checked on the micro analytical balance to insure a correct weight. This information can be found in Appendix B.

CHAPTER IV

RESULTS AND DISCUSSION

Visual Evidences of Health

A daily record of feeding and visual characteristics of each animal was kept for the entire length of the experiment. The complete record for each rat is included in Appendix A., Table III.

During the first week of the experiment, there were no obvious differences between the rats in Experimental Groups I and II or the Control Group. The majority of the rats seemed to be healthy and alert with a great deal of energy. Rat No. 4, however, seemed to be the exception. This animal appeared to be nervous and rigid to the touch and ate a daily average of 6.7 grams for the first week. The average food consumed for the rats in Experimental Group I, II and the Control Group was approximately 14 grams.

The rats in Experimental Group I showed no noticeable differences from their litter-mates in the Control Group during the second week. There was some indications that the Experimental Group II animals were consuming an increasingly larger proportion of the common diet. They also drank large amounts of the fluoridated water.

Beginning with the third week, all the rats in Experimental Groups I and II had outstripped their counterparts in food and water consumption. The rats in Experimental Group II had begun to exhibit a restless gnawing of their cage and food jar lids. Rats in these two

experimental groups also excreted increasing amounts of urine. This illustrates the expected urinary excretion of fluoride in the urine as the consumption of fluoridated water increased (43). These female rats were extremely active and often overturned water bubbles and food jars. By the end of the third week all water bubbles and food jars were wired down as well as the cage door and feces tray for one animal.

During the fourth week the control animals outweighed those animals in Experimental Groups I and II. Experimental Group I animals exhibited signs of extreme nervousness and seemed to suffer from some anorexia, although not as much as might have been expected (8, 21).

Those animals in the Control Group continued to exhibit a healthy and inquisitive nature during week five, consuming 24 grams of food per day. Experimental Group I animals were somewhat lethargic and exhibited some anorexia during the latter part of the week. Experimental Group II animals were active and alert, exhibiting a restless gnawing on cage screens. Those animals in Group I consumed an average diet of 21 grams per day and those in Group II, 20 grams per day.

During the final week of the experiment, the teeth of the rats in all groups were healthy and appeared to have no mottling or discoloration of any kind. Those animals in Experimental Group I were eager to eat the control diet but were not consuming large amounts of fluoridated water (8 ppm) although there was a noticeable drop in the amount of diet consumed. See Appendix A.

Body Weight and Length

The rats consuming the control diet and water supply attained the highest mean body weight, whereas those animals consuming 4 ppm of

fluoride in their water attained the lowest mean body weight.

The F value was employed for testing the hypotheses of the equality of the means. The calculated F value is the ratio of the estimate of the variance of the factor to be tested to an appropriate error mean square. This F value is then compared to the tabulated F value ($F_{m,n}$), where m and n are the degrees of freedom for the estimates of the variance present in the numerator and denominator (41).

An analysis of variance of final body weight data is given in Table IV. The results indicate that there was a significant difference at the 0.05 level, between the variables of initial weight, final weight and final length.

TABLE IV

ANALYSIS OF VARIANCE OF FINAL BODY WEIGHT

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F
Dry Weight of Femur	3	0.00593556	0.00118711	1.60315**
Error	16	0.01036681	0.00074049	
Corrected total	19	0.01630237		
Wet weight of Femur	3	0.01979072	0.00395814	1.52423**
Error	16	0.03635547	0.00259682	
Corrected total	19	0.05614619		
Ash Content	3	0.00863828	0.00172766	1.14042+
Error	16	0.03635547	0.00259682	
Corrected total	19	0.05614619		

**Significant at the 0.05 level.
+ Significant at the 0.10 level.

It was also found that the final weight in relation to the wet weight of the femur, the breaking point and ashing of the femurs was significant at the 0.05 level. This seems to indicate that the larger the animal the greater the level of bone mineralization that occurred during growth and bone formation.

Final mean weight gain and length of the rats in each group is given in Table V. The animals in Experimental Group I surpassed those in the Control Group and Experimental Group II in relation to final length. Neither Experimental Groups I and II reached the final weight of the Control Group.

TABLE V

MEAN WEIGHT GAIN AND FINAL LENGTH OF RATS

Rats	Final Weight	Final Length
Control Group	183.14	13.00
Experimental Group I	170.33	14.75
Experimental Group II	175.85	14.03

The differences in the Experimental Groups I and II, as well as the Control Group were not significant in relation due to diets or water consumption.

Femoral Characteristics

The femurs of the Control Group were healthy and well developed. The animals in Experimental Group I were healthy with the exception of animal No. 11, whose right femur was abnormal in weight and structure. Femurs of the animals in Experimental Group II were all healthy and well formed. There were no spontaneous fractures in any group. Spontaneous fractures could have been expected in Experimental Group II since the amount of fluoride ingested might have resulted in brittle or splintered bones. Although there were no spontaneous fractures, the abnormality of the right femur in animal No. 11, resulted in the use of a method developed by Yates (41) to facilitate the statistical analysis of the remaining data. The analysis of variance of the left and right femur differences is presented in Table VI.

TABLE VI

ANALYSIS OF VARIANCE OF LEFT AND RIGHT FEMUR DIFFERENCES

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F
Dry Weight	2	0.00058172	0.00029086	0.93213
Error	17	0.00530462	0.00031204	
Corrected total	19	0.00588634		
Wet Breaking Point	2	33446.3952	16723.1976	0.26573
Error	17	1069881.4047	62934.2002	
Corrected total	19	1103327.8000		
Ash Content	2	0.00025771	0.00012886	0.16571
Error	17	0.01321959	0.00077762	
Corrected total	19	0.01347730		
Wet Weight	2	0.00047515	0.00023757	0.32756
Error	17	0.01229224	0.00072307	
Corrected total	19	0.01276739		

Statistical analysis of the data revealed that there was no real difference between left and right femurs for each group of animals.

Mean weight, dry weight, and moisture content of femurs for rats, according to group and treatment, are presented in Table VII.

TABLE VII

MEAN WET WEIGHT, DRY WEIGHT, AND MOISTURE CONTENT OF FEMURS FOR RATS

Rats	Wet Weight gm.	Dry Weight gm.	Moisture Content %
Control Group	1.0663	0.7408	48
Experimental Group I	1.1049	0.7851	40
Experimental Group II	1.0904	0.7687	48

The percentage of moisture content in the Control Group femurs is equal to that of Experimental Group II, with Experimental Group I having the lowest mean moisture content of all groups.

Breaking Load of the Femurs

Femoral breaking loads are presented in Table VIII. These means are indicative that the femurs of the Experimental Groups I and II were stronger, hence more dense than the Control Group.

TABLE VIII

MEAN BREAKING LOADS OF THE FEMURS
FOR EACH GROUP OF RATS

Rats	Breaking Load gm.
Control Group	15793.8571
Experimental Group I*	17649.8333
Experimental Group II	16170.0000

* Note that in this group there were only six animals statistically analyzed due to the abnormality of animal No. 11.

The increment in breaking loads of the femurs was attributed to the presence of fluoride in the diet during the formation and growth of the bones. The analysis of femoral breaking load variance is shown in Table IX.

TABLE IX

ANALYSIS OF VARIANCE OF FEMORAL BREAKING LOADS

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F
Wet Weight	5	0.06614739	0.01322948	1.670
Error	4	0.11050890	0.00789349	
Corrected total	19	0.17664629		
Dry Weight of Femur	3	0.01979072	0.00395814	1.52423
Error	16	0.03635547	0.00259682	
Corrected total	19	0.05614619		
Ash Content	3	0.00863828	0.00172766	1.14042+
Error	16	0.03635547	0.00259682	
Corrected total	19	0.05614619		

+Significant at the 0.10 level.

Statistically, there was no significance at either the 0.01 or 0.05 level for the breaking loads of the femurs in the three groups.

Total Ash Content of the Femur

Dietary rations with an excess of fluoride increase the crystal apatite size and reduce the solubility of the bone to the resorption processes of the body and skeletal system. The result is a denser and stronger bone. Total ash content determinations of the femurs were the criteria used for indicating the bone density effects produced by the different treatments involving fluoridated water.

Individual mean values of ash content of the femurs are given in Table X.

TABLE X

MEAN ASH CONTENT OF FEMURS IN THREE GROUPS OF RATS

Group	Number of Animals	Mean Ash in Grams
Control Group	7	0.3871
Experimental Group I*	6	0.4807
Experimental Group II	7	0.4352

*Note that animal No. 11 was omitted due to the abnormal femur.

Ash content was recorded in grams weight and in percentages of the total dry weight of the bone. As shown in Table X, the total ash content of the bones of the control animals were smaller numerically than those of the Experimental Groups I and II although there was no level of significance statistically. Analysis of the ash content variance is given in Table XI.

TABLE XI

ANALYSIS OF VARIANCE OF ASH CONTENT

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F
Initial weight	3	0.00593556	0.00118711	1.60315
Error	16	0.01036681	0.00074049	
Corrected total	19	0.01630237		
Final weight	3	0.00103460	0.00128710	1.09635
Error	16	0.00332694	0.00006513	
Corrected total	19	0.00436154		
Length	3	0.00022828	0.00022828	0.30829
Error	14	0.00035240	0.00005060	
Corrected total	19	0.00058068		

There was no significant correlation of the variance of ash content to the three dependent variables, hence the ash content was not statistically significant.

CHAPTER V

SUMMARY AND CONCLUSIONS

The effect of two levels of sodium fluoride on bone density changes of femurs in white rats was investigated. The experimental units were the 21 albino rats, seven of which served as a Control Group and seven rats were in Experimental Groups I and II. The animals were randomly selected for each group and cages were placed together according to groups on the carrier. Experimental Groups I and II received 4 ppm and 8 ppm respectively of sodium fluoride in distilled water.

After approximately six weeks of feeding, the animals in each group were sacrificed and evaluated according to initial and final body weight, animal length and femoral characteristics which included the wet and dry weight of the bone, the breaking point and total ash of the femurs. Statistical analysis revealed no statistical significance at the 0.10 or the 0.05 level between the varying levels of fluoride administered to the animals.

Although the research did not statistically reveal any significance due to the administration of fluoride upon increased bone density, it did establish that the levels of 4 ppm and 8 ppm of fluoride added to the drinking supply was not toxic and could be increased without harming the animals. The study revealed a statistical significance at the 0.05 level in relation to the final body weight and

weights of the wet and dry femurs. No other findings were significant at either the 0.01 or 0.05 levels. However, there were definite numerical differences between the three groups. In relation to mean overall length, breaking load and bone ash content animals in Experimental Group I were consistently higher than Experimental Group II. The Control Group animals were lowest for all these characteristics. The mean final weight of the Control Group rats were highest, followed by Experimental Group II and Group I which was lowest. According to this study there was no statistical differences between groups attributed to fluoride intake.

Fluoride, whether ingested from food or fluoridated water, increases the strength of the bone through the enlargement of the apatite crystal and is seemingly less easily resorped by the body. It seemed reasonable to assume that this could be used as a bone strengthening element. As osteoporotic bone exists as a fragile, easily broken element, the use of fluoride seems to be a logical choice for future research.

Suggestions for Further Research

Some suggestion for further study are the following:

1. The importance of fluoride in conjunction with various minerals to bone formation in an animal other than the rat, i.e., the quinea pig.
2. The effect of ingesting varying levels of the somatropic hormone upon osteoblast supply.

3. The effect of fluoride and a phosphorus level of 1:1 with calcium during the early months of a pregnant and lactating animal, further to evaluate the bone density of both mother and offspring.

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APPENDIX A

DAILY RECORD OF ANIMAL FEEDINGS

TABLE III

DAILY RECORD OF ANIMAL FEEDINGS

Rat No. 1			Control Animals		
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.0	10.0	56.0	1 notch right ear
9/23-24	20.0	3.0	17.0		Healthy
9/25	12.0	1.5	10.5		
9/26	12.0	0.0	12.0		
9/27	14.0	2.0	12.0		Calm
9/28	15.0	2.3	12.7	98.0	
9/29	15.0	0.0	15.0		
9/30-10/1	30.0	2.0	28.0		Alert
10/2	17.0	1.0	16.0		
10/3	18.0	1.0	17.0		
10/4	19.0	2.0	17.0	130.0	Shiny Coat
10/5	20.0	1.4	18.6		
10/6	20.0	1.0	19.0		
10/7-8	40.0	3.9	36.1		Active
10/9	23.0	3.0	20.0		
10/10	23.0	1.5	21.5		
10/11	25.0	3.0	22.0		
10/12	25.0	2.0	23.0	154.0	Alert
10/13	25.0	3.0	22.0		
10/14-15	50.0	3.0	47.0		
10/16	25.0	5.0	20.0		
10/17	25.0	1.0	24.0		Healthy
10/18	26.0	2.0	24.0		
10/19	27.0	2.0	25.00	176.0	
10/20	28.0	3.0	25.0		
10/21-22	50.0	15.0	35.0		
10/23	25.0	2.0	23.0		
10/24	25.0	1.5	23.9		
10/25	26.0	3.0	23.0		Shiny Coat
10/26	26.0	2.0	24.0	176.0	
10/27	27.0	2.0	25.0		
10/28-29	50.0	10.0	40.0		
10/30	26.0	4.0	22.0		
10/31	26.0	2.0	24.0		
11/1	26.0	3.1	22.9		Calm
11/2	26.0	3.0	23.0	180.0	
11/3	26.0	2.0	24.0		
11/4-5	50.0	0.0	50.0		
11/6				185.0	Sacrificed

TABLE III (Continued)

Rat No. 2	Control Animals				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	3.0	7.0	52.0	1 notch left ear
9/23-24	20.0	2.0	18.0		
9/25	10.0	0.0	8.0		Restless
9/26	12.0	1.5	10.5		
9/27	14.0	2.0	12.0		Active
9/28	14.0	1.5	12.5	88.0	
9/29	15.0	0.0	15.0		
9/30-10/1	30.0	2.0	28.0		Healthy
10/2	17.0	0.0	17.0		
10/3	18.0	2.0	16.0		
10/4	18.0	2.0	16.0		Calm
10/5	18.0	1.5	16.5	120.0	
10/6	19.0	2.0	17.0		
10/7-8	40.0	3.2	36.8		Alert
10/9	23.0	2.5	20.5		
10/10	23.0	1.3	21.7		
10/11	24.0	3.0	21.0		Active
10/12	25.0	2.0	23.0	128.0	
10/13	25.0	2.3	22.7		Shiny Coat
10/14-15	50.0	2.0	48.0		
10/16	25.0	2.0	23.0		
10/17	26.0	2.0	24.0		Calm
10/18	27.0	2.0	25.0		
10/19	27.0	1.0	26.0	140.0	
10/20	27.0	3.0	24.0		Alert
10/21-22	50.0	2.0	48.0		
10/23	24.0	1.5	22.5		Healthy
10/24	25.0	1.0	24.0		Active
10/25	26.0	2.0	24.0		
10/26	27.0	3.4	23.6	174.0	
10/27	27.0	3.0	24.0		
10/28-29	50.0	3.0	47.0		Shiny Coat
10/30	28.0	3.0	25.0		
10/31	28.0	3.0	25.0		
11/1	30.0	2.0	28.0		
11/2	30.0	3.0	27.0	180.0	Alert
11/3	30.0	2.0	28.0		
11/4-5	50.0	3.5	46.5		Healthy
11/6				183.0	Sacrificed

TABLE III (Continued)

Rat No. 3	Control Animals				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.0	10.0	55.0	2 notches right ear
9/23-24	20.0	11.5	18.5		Healthy
9/25	12.0	2.0	10.0		Active
9/26	12.0	1.0	11.0		
9/27	13.0	1.5	11.5		
9/28	14.0	2.0	12.0	86.0	Alert
9/29	30.0	12.0	18.0		
9/30-10/1	30.0	12.0	18.0		
10/2	18.0	2.1	15.9		
10/3	18.0	2.0	16.0		Healthy
10/4	18.0	1.3	16.7		
10/5	19.0	0.0	19.0	130.0	Calm
10/6	20.0	0.0	20.0		
10/7-8	40.0	12.0	28.0		Shiny Coat
10/9	23.0	4.0	19.0		
10/10	23.0	3.3	19.7		
10/11	24.0	4.0	20.0		
10/12	24.0	3.4	20.6	152.0	Alert
10/13	24.0	3.0	21.0		
10/14-15	50.0	4.0	46.0		
10/16	25.0	4.1	20.9		Active
10/17	25.0	4.2	20.8		
10/18	25.0	3.3	21.7		
10/19	25.0	2.0	23.0	164.0	Healthy
10/20	25.0	1.6	23.4		
10/21-22	50.0	4.5	45.5		
10/23	26.0	0.0	26.0		Calm
10/24	27.0	2.3	24.7		
10/25	27.0	2.0	25.0		
10/26	27.0	2.1	24.9	180.0	
10/27	27.0	1.0	26.0		
10/28-29	50.0	3.0	47.0		Healthy
10/30	27.0	1.5	25.3		
10/31	28.0	3.0	25.0		
11/1	28.0	2.3	25.7		
11/2	28.0	2.1	25.9	184.0	Restless
11/3	28.0	2.0	25.0		Calm
11/4-5	50.0	0.0	50.0		
11/6				187.0	Sacrificed

TABLE III (Continued

Rat No. 4	Control Animals				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	6.0	4.0	55.0	2 notches left ear
9/23-24	20.0	8.0	12.0		Healthy
9/25	12.0	6.0	6.0		Active
9/26	12.0	5.0	7.0		
9/27	12.0	2.0	10.0		
9/28	12.0	2.0	10.0	87.0	Alert
9/29	12.0	3.0	9.0		
9/30-10/1	15.0	2.0	13.0		
10/2	15.0	2.3	12.7		
10/3	16.0	1.5	14.5		Calm
10/4	17.0	3.1	13.9		
10/5	17.0	3.0	14.0	121.0	
10/6	17.0	2.4	14.6		Healthy
10/7-8	40.0	4.0	36.0		
10/9	18.0	2.0	16.0		Active
10/10	18.0	1.6	16.4		
10/11	19.0	0.0	19.0		
10/12	20.0	1.1	18.9	150.0	
10/13	21.0	1.5	19.5		Restless
10/14-15	50.0	2.0	48.0		
10/16	25.0	2.3	22.7		
10/17	25.0	2.1	22.9		Shiny Coat
10/18	25.0	1.9	23.1		
10/19	26.0	0.0	26.0	150.0	
10/20	28.0	2.1	25.9		
10/21-22	50.0	2.0	48.0		Alert
10/23	30.0	3.1	26.9		
10/24	30.0	2.9	27.1		
10/25	31.0	4.0	27.0		
10/26	31.0	4.3	26.7	178.0	Healthy
10/27	31.0	4.0	27.0		
10/28-29	50.0	3.0	47.0		
10/30	30.0	2.8	27.2		
10/31	31.0	3.0	28.0		Active
11/1	31.0	2.5	28.5		
11/2	31.0	2.0	29.0	181.0	
11/3	31.0	2.6	28.4		
11/4-5	50.0	1.1	48.9		Healthy
11/6				184.0	Sacrificed

TABLE III (Continued)

Rat No. 5	Control Animals				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	1.0	9.0	56.0	2 notches right ear 1 notch left ear
9/23-24	20.0	1.5	18.5		
9/25	12.0	3.2	8.8		
9/26	12.0	1.4	10.6		Healthy
9/27	12.0	0.0	12.0		
9/28	14.0	1.2	12.8	86.0	
9/29	15.0	0.0	15.0		
9/30-10/1	30.0	4.0	26.0		
10/2	15.0	0.0	15.0		Alert
10/3	17.0	2.0	15.0		
10/4	18.0	1.3	16.7		
10/5	19.0	2.0	17.0	121.0	
10/6	19.0	1.0	18.0		
10/7-8	40.0	3.0	37.0		
10/9	23.0	1.0	22.0		Restless
10/10	23.0	2.1	20.9		
10/11	24.0	3.2	20.8		
10/12	24.0	3.2	20.8	148.0	Shiny Coat
10/13	24.0	0.0	24.0		
10/14-15	50.0	5.0	45.0		
10/16	25.0	1.0	24.0		
10/17	26.0	2.0	24.0		Active
10/18	27.0	1.3	25.7		
10/19	28.0	6.1	21.9	160.0	Restless
10/20	28.0	4.0	24.0		
10/21-22	50.0	4.0	46.0		
10/23	24.0	2.1	21.9		
10/24	25.0	3.2	21.8		
10/25	26.0	3.0	23.0		Calm
10/26	27.0	2.1	24.9	180.0	
10/27	27.0	2.0	25.0		
10/28-29	50.0	4.0	46.0		
10/30	26.0	2.0	24.0		Healthy
10/31	27.0	1.9	25.1		
11/1	28.0	2.0	26.0		
11/2	29.0	3.0	26.0	183.0	
11/3	30.0	1.8	28.2		Calm
11/4-5	55.0	3.0	52.0		
11/6	33.0	3.0	30.0		
11/7				184.0	Sacrificed

TABLE III (Continued)

Rat No. 6	Control Animals				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	1.0	9.0	52.0	2 notches left ear 1 notch right ear
9/23-24	20.0	2.0	18.0		Healthy
9/25	11.0	1.1	9.9		
9/26	11.0	2.0	8.9		
9/27	11.0	1.0	10.0		
9/28	12.0	1.1	10.9	88.0	Alert
9/29	14.0	2.0	12.0		Active
9/30-10/1	30.0	4.0	26.0		
10/2	15.0	3.0	12.0		Calm
10/3	17.0	4.0	13.0		
10/4	17.0	3.0	14.0	117.0	
10/5	17.0	2.5	14.5		Healthy
10/6	17.0	1.5	15.5		
10/7-8	40.0	0.0	40.0		Shiny Coat
10/9	21.0	2.0	19.0		
10/10	22.0	1.0	21.0		
10/11	23.0	2.0	21.0		
10/12	24.0	4.0	20.0	114.0	Calm
10/13	24.0	3.2	20.8		
10/14-15	50.0	2.1	47.9		
10/16	24.0	3.1	20.9		
10/17	24.0	3.0	21.0		
10/18	24.0	2.9	21.1		
10/19	25.0	1.0	24.0	160.0	
10/20	27.0	2.0	25.0		
10/21-22	50.0	5.0	45.0		Alert
10/23	26.0	2.0	24.0		
10/24	26.0	1.0	25.0		
10/25	27.0	3.0	24.0		Active
10/26	28.0	2.3	24.7	181.0	
10/27	28.0	5.0	23.0		
10/28-29	50.0	0.0	50.0		
10/30	28.0	2.0	26.0		
10/31	30.0	3.0	27.0		
11/1	30.0	3.2	26.8		Healthy
11/2	30.0	3.0	27.0	186.0	
11/3	30.0	3.0	27.0		
11/4-5	55.0	6.0	49.0		Healthy
11/6				187.0	Sacrificed

TABLE III (Continued

Rat No. 7	Control Animals				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.0	10.0	52.0	2 notches each ear
9/23-24	20.0	0.0	20.0		
9/25	12.0	2.0	10.0		
9/26	12.0	1.6	10.4		Healthy
9/27	12.0	0.0	12.0		
9/28	14.0	1.3	12.7	86.0	Active
9/29	14.0	0.0	14.0		
9/30-10/1	30.0	7.0	23.0		
10/2	15.0	0.0	15.0		Calm
10/3	18.0	1.0	17.0		
10/4	19.0	1.6	17.4		
10/5	19.0	3.0	16.0	123.0	
10/6	19.0	1.0	18.0		Active
10/7-8	40.0	0.0	40.0		
10/9	23.0	3.0	20.0		
10/10	23.0	1.6	21.4		
10/11	24.0	3.0	21.0		Alert
10/12	25.0	1.0	24.0	126.0	Healthy Fur
10/13	26.0	3.0	23.0		
10/14-15	50.0	0.0	50.0		
10/16	26.0	2.0	24.0		
10/17	27.0	1.5	15.5		
10/18	28.0	2.0	26.0		
10/19	28.0	2.0	26.0	158.0	
10/20	28.0	5.0	23.0		Active
10/21-22	50.0	5.0	45.0		
10/23	28.0	3.0	25.0		
10/24	28.0	3.0	25.0		
10/25	28.0	2.0	26.0		
10/26	28.0	2.1	25.9	173.0	Shiny Coat
10/27	28.0	3.0	25.0		
10/28-29	50.0	7.0	43.0		
10/30	26.0	2.0	24.0		
10/31	27.0	3.0	24.0		
11/1	27.0	2.1	24.9		
11/2	28.0	1.0	27.0	179.0	
11/3	29.0	2.0	27.0		
11/4-5	56.0	7.0	49.0		Calm
11/6	20.0	0.0	20.0	181.0	
11/7					Sacrificed

TABLE III (Continued)

Rat No. 8	Experimental Diet I				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.0	10.0	56.0	1 notch right ear 1 notch left ear
9/23-24	20.0	1.5	18.5		Healthy, sleek fur,
9/25	12.0	2.0	10.0		pert
9/26	12.0	1.5	10.5		Turned Water Tube
9/27	13.0	2.0	11.0		over
9/28	14.0	3.0	12.0	92.0	Alert
9/29	15.0	1.5	13.5		
9/30-10/1	30.0	2.1	27.0		Tried to get out of
10/2	17.0	1.0	16.0		cage
10/3	18.0	0.0	18.0		
10/4	20.0	1.8	18.2		
10/5	20.0	2.0	18.0	118.0	Active
10/6	20.0	1.3	18.7		
10/7-8	40.0	5.3	34.7		
10/9	20.0	0.0	20.0		Restless
10/10	23.0	2.0	21.0		
10/11	24.0	5.0	19.0		
10/12	24.0	2.0	22.0	130.0	Restless and wary of
10/13	25.0	2.0	23.0		being handled
10/14-15	50.0	5.0	45.0		
10/16	25.0	10.0	15.0		Tries to nibble on
10/17	20.0	2.0	18.0		fingers, gnawing
10/18	22.0	2.5	19.5		on cage door and
10/19	22.0	1.0	21.0	164.0	wire
10/20	23.0	3.0	20.0		
10/21-22	50.0	20.0	30.0		
10/23	25.0	12.0	13.0		
10/24	25.0	6.0	19.0		
10/25	25.0	5.0	20.0		Healthy fur, rest-
10/26	25.0	3.0	22.0	168.0	less
10/27	25.0	2.0	23.0		
10/28-29	50.0	12.0	38.0		
10/30	25.0	0.0	25.0		
10/31	26.0	3.0	23.0		
11/1	26.0	3.1	22.9		
11/2	26.0	2.0	24.0	170.0	Left eye is red and
11/3	28.0	6.0	22.0		runny
11/4-5	50.0	3.0	47.0		
11/6	23.0	0.0	23.0	170.0	Sacrificed

TABLE III (Continued)

Rat No. 9	Experimental Diet I				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	1.0	9.0	49.0	No marks
9/23-24	20.0	3.0	17.0		Healthy
9/25	10.0	0.0	10.0		
9/26	12.0	2.1	9.9		Nervous
9/27	12.0	3.0	9.9		
9/28	12.0	0.0	12.0		
9/29	14.0	11.0	3.0	90.0	Active
9/30-10/1	30.0	3.0	27.0		
10/2	15.0	2.0	13.0		
10/3	16.0	1.0	15.0		
10/4	17.0	1.0	16.0		Alert
10/5	18.0	1.3	16.7	130.0	
10/6	20.0	2.0	18.0		Restless
10/7-8	40.0	3.6	36.4		
10/9	23.0	3.0	20.0		
10/10	23.0	2.0	21.0		
10/11	24.0	2.5	21.5		
10/12	24.0	2.0	22.0	150.0	Calm
10/13	25.0	2.0	23.0		
10/14-15	50.0	6.0	44.0		
10/16	25.0	12.0	13.0		Extreme urination
10/17	20.0	2.0	18.0		
10/18	21.0	2.0	19.0		
10/19	21.0	5.0	17.0	160.0	
10/20	21.0	1.0	20.0		
10/21-22	50.0	20.0	30.0		
10/23	25.0	11.0	14.0		
10/24	24.0	7.0	18.0		Out of Cage
10/25	26.0	5.0	21.0		
10/26	26.0	2.0	24.0	170.0	
10/27	27.0	3.0	24.0		
10/28-29	50.0	14.0	36.0		
10/30	28.0	2.0	26.0		
10.31	29.0	3.0	26.0		Calm
11/1	29.0	3.0	26.0		
11/2	29.0	3.0	26.0	172.0	Alert
11/3	30.0	4.0	26.0		
11/4-5	50.0	0.0	50.0		Healthy
11/6				168.0	Sacrificed

TABLE III (Continued)

Rat No. 10	Experimental Diet I				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	2.0	8.0	52.0	Red Head
9/23-24	20.0	3.0	17.0		
9/25	12.0	3.0	9.0		
9/26	12.0	2.0	10.0		
9/27	12.0	2.0	10.0		
9/28	12.0	1.1	10.9	83.0	Calm
9/29	14.0	6.5	7.5		
9/30-10/1	30.0	1.1	28.9		
10/2	17.0	1.0	16.0		Alert
10/3	18.0	1.5	16.5		
10/4	19.0	2.0	17.0		Active
10/5	19.0	1.5	17.5	124.0	
10/6	20.0	0.0	20.0		
10/7-8	20.0	0.0	40.0		
10/9	23.0	3.0	20.0		
10/10	23.0	1.0	22.0		
10/11	24.0	2.0	22.0		
10/12	24.0	5.0	19.0	151.0	Alert
10/13	26.0	3.0	23.0		
10/14-15	50.0	0.0	50.0		Nervous
10/16	27.0	11.3	15.7		
10/17	20.0	3.0	17.0		
10/18	21.0	3.0	18.0		
10/19	21.0	3.0	19.0	160.0	
10/20	20.0	2.0	18.0		
10/21-22	50.0	31.1	18.9		
10/23	20.0	6.0	14.0		Out of Cage
10/24	20.0	3.0	17.0		
10/25	22.0	4.0	18.0		
10/26	23.0	3.9	19.1	172.0	
10/27	23.0	2.0	21.0		
10/28-29	50.0	4.0	46.0		
10/30	25.0	5.0	20.0		Out of Cage
10/31	25.0	3.0	22.0		
11/1	25.0	2.0	23.0		Alert
11/2	26.0	6.0	20.0	170.0	
11/3	26.0	5.0	21.0		
11/4-5	56.0	6.0	50.0		
11/6				172.0	Sacrificed

TABLE III (Continued)

Rat No. 11	Experimental Diet I				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.0	10.0	56.0	Red Tail
9/23-24	20.0	3.0	17.0		Healthy
9/25	10.0	2.0	8.0		
9/26	10.0	0.0	10.0		
9/27	12.0	0.0	12.0		
9/28	14.0	1.5	12.5	94.0	
9/29	14.0	010	14.0		
9/30-10/1	30.0	3.0	27.0		
10/2	17.0	2.0	15.0		
10/3	17.0	1.0	16.0		Shiny Coat
10/4	18.0	1.0	17.0		
10/5	19.0	2.0	17.0	130.0	
10/6	20.0	1.5	18.5		
10/7-8	40.0	5.0	35.0		
10/9	20.0	0.0	20.0		Alert
10/10	23.0	1.0	22.0		
10/11	24.0	2.0	22.0		
10/12	24.0	12.0	12.0	142.0	Out of Cage
10/13	25.0	0.0	25.0		
10/14-15	50.0	7.5	42.5		
10/16	26.0	3.0	23.0		Water Overturned
10/17	26.0	3.0	23.0		
10/18	26.0	2.0	24.0		
10/19	26.0	4.0	22.0	168.0	
10/20	28.0	3.0	25.0		
10/21-22	50.0	23.0	27.0		Animal Out of Cage
10/23	24.0	8.0	16.0		
10/24	24.0	6.0	18.0		
10/25	24.0	5.0	19.0		
10/26	25.0	4.0	21.0	174.0	Calm
10/27	25.0	3.0	22.0		
10/28-29	50.0	5.0	45.0		
10/30	25.0	13.0	12.0		
10/31	25.0	2.0	23.0		
11/1	26.0	2.0	24.0		
11/2	26.0	6.0	20.0	176.0	
11/3	26.0	3.0	23.0		
11/4-5	50.0	3.0	47.0		
11/6				176.0	Sacrificed

TABLE III (Continued)

Rat No. 12	Experimental Diet I				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	010	10.0	56.0	Blue Head
9/23-24	20.0	2.0	18.0		Healthy
9/25	10.0	0.0	10.0		
9/26	12.0	2.0	10.0		
9/27	12.0	10.0	2.0		Restless
9/28	14.0	2.0	12.0	94.0	
9/29	14.0	0.0	14.0		
9/30-10/1	30.0	4.0	26.0		
10/2	15.0	0.0	15.0		Shiny Coat
10/3	18.0	2.0	16.0		
10/4	18.0	1.6	16.4		
10/5	18.0	1.3	16.7	120.0	
10/6	18.0	0.0	18.0		
10/7-8	40.0	0.0	40.0		Nervous
10/9	23.0	3.0	20.0		
10/10	23.0	2.1	20.0		
10/11	24.0	2.3	21.7		
10/12	25.0	12.0	13.0	142.0	Very Active
10/13	26.0	2.0	24.0		
10/14-15	50.0	6.3	43.7		
10/16	26.0	8.0	18.0		Out of Cage
10/17	24.0	3.0	21.0		
10/18	24.0	1.0	23.0		
10/19	25.0	2.0	23.0	168.0	
10/20	26.0	6.0	20.0		
10/21-22	50.0	21.0	29.0		Active
10/23	25.0	5.3	19.7		
10/24	25.0	3.1	21.7		
10/25	26.0	3.0	23.0		
10/26	26.0	3.0	23.0	178.0	
10/27	26.0	4.0	22.0		Calm
10/28-29	50.0	10.0	40.0		
10/30	26.0	6.0	20.0		Healthy Coat
10/31	26.0	10.0	16.0		
11/1	26.0	6.0	20.0		
11/2	26.0	6.0	20.0	175.0	
11/3	26.0	4.0	22.0		
11/4-5	50.0	2.0	48.0		
11/6				176.0	Sacrificed

TABLE III (Continued)

Rat No. 13	Experimental Diet I				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.0	10.0	53.0	Blue Tail
9/23-24	20.0	1.5	18.5		Healthy
9/25	12.0	2.4	9.6		
9/26	12.0	3.0	9.0		Alert
9/27	12.0	2.6	9.4		
9/28	12.0	0.0	12.0	85.0	
9/29	14.0	2.0	12.0		
9/30-10/1	30.0	0.0	30.0		
10/2	17.0	1.0	16.0		
10/3	18.0	1.3	16.7		Active
10/4	19.0	2.0	17.0		
10/5	19.0	3.0	16.0	118.0	Shiny Coat
10/6	19.0	1.3	17.7		
10/7-8	40.0	3.0	37.0		
10/9	20.0	0.0	20.0		Active
10/10	23.0	2.0	21.0		
10/11	24.0	3.0	21.0		
10/12	24.0	10.0	14.0	138.0	
10/13	25.0	5.0	20.0		Restless
10/14-15	50.0	3.0	47.0		
10/16	26.0	2.0	24.0		
10/17	26.0	3.0	23.0		Shiny Coat
10/18	27.0	3.1	23.9		
10/19	27.0	4.0	23.0	150.0	
10/20	27.0	7.0	20.0		
10/21-22	50.0	15.0	35.0		
10/23	26.0	6.0	20.0		Gnawing on Cage
10/24	26.0	4.0	22.0		
10/25	27.0	2.0	25.0		
10/26	28.0	3.0	25.0	162.0	
10/27	28.0	3.0	25.0		Calm
10/28-29	50.0	3.0	47.0		
10/30	26.0	6.0	20.0		
10/31	26.0	5.0	21.0		Active
11/1	26.0	4.0	22.0		
11/2	26.0	4.0	22.0	165.0	
11/3	26.0	2.0	24.0		Restless
11/4-5	50.0	3.0	47.0		
11/6	25.0	5.0	20.0		
11/7				170.0	Sacrificed

TABLE III (Continued)

Rat No. 14			Experimental Diet I		
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	3.0	7.0	52.0	Green Head
9/23-24	20.0	3.0	17.0		
9/25	10.0	1.2	8.8		Healthy
9/26	12.0	2.0	10.0		
9/27	12.0	0.0	12.0		
9/28	14.0	1.5	12.5	86.0	Alert
9/29	15.0	0.0	15.0		
9/30-10/1	30.0	0.0	30.0		
10/2	17.0	1.5	15.5		Calm
10/3	18.0	0.0	18.0		
10/4	20.0	2.0	18.0		
10/5	20.0	3.0	17.0	130.0	
10/6	20.0	2.0	18.0		Shiny Coat
10/7-8	40.0	2.0	38.0		
10/9	23.0	2.0	21.0		Restless
10/10	24.0	1.0	23.0		
10/11	25.0	2.0	23.0		
10/12	23.0	1.0	22.0	126.0	
10/13	24.0	1.0	23.0		
10/14-15	50.0	3.1	46.9		
10/16	25.0	2.0	23.0		
10/17	25.0	3.0	22.0	126.0	
10/18	25.0	2.0	23.0		
10/19	26.0	3.0	23.0	128.0	
10/20	27.0	2.0	25.0		
10/21-22	50.0	13.4	36.6		Out of Cage
10/23	24.0	6.3	17.7		
10/24	24.0	6.0	18.0		
10/25	25.0	3.0	22.0		
10/26	25.0	5.0	20.0	150.0	
10/27	25.0	3.0	22.0		Cage was Open
10/28-29	50.0	6.0	44.0		
10/30	25.0	11.3	13.7		
10/31	25.0	8.0	17.0		Nervous
11/1	25.0	15.0	10.0		
11/2	25.0	3.0	22.0	153.0	
11/3	26.0	3.0	23.0		Alert
11/4-5	50.0	3.0	47.0		
11/6	23.0	3.0	20.0		Gnawing on Cage
11/7				155.0	Sacrificed

TABLE III (Continued)

Rat No. 15	Experimental Diet II				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.0	10.0	53.0	Green Tail
9/23-24	20.0	3.0	17.0		
9/25	10.0	2.0	8.0		Alert
9/26	10.0	0.0	10.0		
9/27	12.0	1.5	10.5		Appears healthy
9/28	14.0	2.0	12.0	94.0	
9/29	15.0	0.0	15.0		
9/30-10/1	30.0	3.0	27.0		Appears nervous
10/2	17.0	2.0	15.0		Restless
10/3	19.0	3.0	16.0		
10/4	19.0	2.0	17.0		
10/5	19.0	2.0	17.0	130.0	Calm
10/6	20.0	1.5	18.5		
10/7-8	40.0	4.0	36.0		
10/9	21.0	1.0	20.0		Out of cage
10/10	23.0	1.0	22.0		
10/11	25.0	2.0	23.0	125.0	
10/12	25.0	10.0	15.0		
10/13	26.0	2.0	24.0		
10/14-15	50.0	30.0	20.0		Restless
10/16	26.0	4.0	22.0		
10/17	26.0	3.0	23.0		
10/18	26.0	2.0	24.0		
10/19	27.0	7.0	20.0	148.0	
10/20	27.0	2.0	25.0		
10/21-22	50.0	20.0	30.0		Active
10/23	24.0	5.0	19.0		
10/24	24.0	3.0	21.0		
10/25	25.0	3.0	22.0		
10/26	25.0	5.0	20.0	165.0	Calm
10/27	26.0	3.0	23.0		
10/28-29	50.0	20.0	30.0		
10/30	26.0	6.0	20.0		
10/31	26.0	?	?		Food dumped
11/1	26.0	5.0	21.0		
11/2	26.0	6.0	20.0	168.0	
11/3	26.0	4.0	22.0		
11/4-5	50.0	3.0	47.0		
11/6				170.0	Sacrificed

TABLE III (Continued)

Rat No. 16	Experimental Diet II				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.0	10.0	52.0	Red Head and Tail
9/23-24	20.0	4.0	16.0		
9/25	10.0	2.5	7.5		Alert
9/26	10.0	1.5	8.5		
9/27	12.0	1.0	11.0		
9/28	13.0	2.0	11.0	86.0	Calm
9/29	14.0	1.0	13.0		
9/30-10/1	30.0	0.0	30.0		
10/2	17.0	1.0	16.0		Active
10/3	18.0	2.3	15.7		
10/4	18.0	0.0	18.0		
10/5	19.0	1.8	17.2	119.0	
10/6	20.0	1.0	19.0		
10/7-8	40.0	4.0	36.0		Shiny coat
10/9	21.0	1.0	20.0		
10/10	23.0	0.0	23.0		
10/11	25.0	3.0	22.0		
10/12	25.0	11.0	14.0	119.0	Calm
10/13	26.0	1.0	25.0		
10/14-15	50.0	5.0	45.0		
10/16	25.0	4.0	20.0		
10/17	25.0	4.0	21.0		Alert
10/18	25.0	2.0	23.0		
10/19	26.0	8.0	18.0	130.0	
10/20	26.0	6.0	20.0		
10/21-22	50.0	12.0	38.0		
10/23	26.0	7.0	19.0		
10/24	26.0	6.0	20.0		Active
10/25	26.0	4.0	22.0		
10/26	26.0	6.0	20.0	150.0	
10/27	26.0	3.0	23.0		
10/28-29	50.0	4.0	46.0		
10/30	26.0	10.3	15.7		Out of cage
10/31	26.0	3.0	23.0		
11/1	26.0	3.0	23.0	155.0	
11/2	26.0	3.0	23.0		
11/3	26.0	?	?		Food dumped
11/4-5	50.0	10.0	40.0		
11/6				156.0	Sacrificed

TABLE III (Continued

Rat No. 17	Experimental Diet II				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.0	10.0	50.0	Blue Head and Blue Tail
9/23-24	20.0	3.5	16.5		
9/25	10.0	2.0	8.0		
9/26	10.0	0.0	10.0		
9/27	12.0	2.0	10.0		
9/28	12.0	1.2	10.8	85.0	
9/29	13.0	0.0	13.0		Calm
9/30-10/1	30.0	5.0	25.0		Healthy
10/2	15.0	0.0	15.0		
10/3	17.0	2.0	15.0		
10/4	17.0	1.5	15.5		Active
10/5	18.0	2.0	16.0	119.0	
10/6	18.0	1.1	16.9		
10/7-8	40.0	2.0	38.0		
10/9	20.0	0.0	20.0		Shiny Coat
10/10	22.0	1.0	21.0		
10/11	23.0	1.5	21.0		
10/12	23.0	9.9	13.1	140.0	
10/13	24.0	2.0	22.0		
10/14-15	50.0	20.0	30.0	135.0	
10/16	25.0	13.0	12.0		Alert
10/17	25.0	3.0	22.0		
10/18	25.0	3.0	22.0		
10/19	26.0	3.0	23.0	158.0	Appeara to frighten easily
10/20	27.0	7.0	20.0		
10/21-22	50.0	8.0	42.0		Restless
10/23	25.0	3.0	22.0		
10/24	25.0	3.0	22.0		Alert
10/25	25.0	5.0	20.0		
10/26	25.0	4.0	21.0	160.0	
10/27	25.0	3.0	22.0		
10/28-29	50.0	10.0	40.0		
10/30	26.0	5.0	21.0		Calm
10/31	26.0	10.0	16.0		
11/1	26.0	8.0	18.0		
11/2	26.0	?	?	160.0	Food dumped
11/3	26.0	5.0	21.0		
11/4-5	50.0	5.0	45.0		
11/6	20.0	0.0	20.0		
11/7				180.0	Sacrificed

TABLE III (Continued)

Rat No. 18	Experimental Diet II				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	2.0	8.0	55.0	Green Head and Tail
9/23-24	20.0	0.0	20.0		Appears healthy
9/25	12.0	2.5	9.5		
9/26	12.0	0.0	12.0		
9/27	13.0	1.4	11.6		Alert
9/28	14.0	1.5	12.5	88.0	
9/29	15.0	0.0	15.0		
9/30-10/1	30.0	2.6	27.4		
10/2	17.0	1.5	15.5		Active
10/3	18.0	3.0	15.0		
10/4	18.0	2.0	16.0		
10/5	18.0	1.3	16.7	124.0	
10/6	19.0	1.0	18.0		Somewhat restless
10/7-8	40.0	0.0	40.0		
10/9	23.0	2.0	21.0		Nervous and
10/10	23.0	1.0	22.0		excited
10/11	24.0	2.0	22.0		
10/12	24.0	9.0	15.0	130.0	
10/13	24.0	2.0	22.0		Reasonably calm
10/14-15	50.0	20.0	30.0		
10.16	24.0	10.0	14.0		
10/17	25.0	5.0	20.0		Active
10/18	25.0	2.0	23.0		
10/19	26.0	7.0	19.0	150.0	
10/20	26.0	6.0	20.0		Gnawing on cage
10/21-22	50.0	11.0	39.0		
10/23	25.0	3.0	22.0		Alert
10/24	26.0	4.0	22.0		
10/25	26.0	8.0	18.0		
10/26	26.0	4.0	22.0	154.0	
10/27	26.0	4.3	21.7		Shiny coat
10/28-29	50.0	14.0	36.0		
10/30	25.0	4.1	20.9		Very active
10/31	25.0	11.3	13.7		
11/1	25.0	8.0	17.0		Restless
11/2	25.0	5.0	20.0	161.0	
11/3	26.0	4.0	22.0		
11/4-5	50.0	0.0	50.0		
11/6	23.0	3.0	20.0		Healthy
11/7				184.0	Sacrificed

TABLE III (Continued

Rat No. 19			Experimental Diet II		
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	2.0	8.0	56.0	Yellow Head
9/23-24	20.0	3.0	17.0		Healthy
9/25	12.0	1.5	10.5		
9/26	13.0	2.0	11.0		
9/27	14.0	1.2	12.8		
9/28	15.0	1.5	13.5		Active
9/29	16.0	1.5	14.5	88.0	
9/30-10/1	30.0	0.0	30.0		
10/2	18.0	1.0	17.0		
10/3	20.0	3.0	17.0		Restless
10/4	20.0	2.3	17.7		
10/5	20.0	2.0	18.0	130.0	Gnawing on cage
10/6	20.0	1.6	18.5		
10/7-8	40.0	2.0	38.0		
10/9	23.0	2.0	21.0		Shiny coat
10/10	23.0	0.0	23.0		
10/11	23.0	0.0	23.0		
10/12	25.0	2.0	23.0	160.0	Calm
10/13	25.0	1.5	23.5		
10/14-15	50.0	2.0	48.0		
10/16	28.0	8.0	20.0		Appears nervous
10/17	28.0	7.0	21.0		
10/18	28.0	5.0	23.0		
10/19	28.0	9.0	19.0	172.0	Restless
10/20	28.0	8.0	20.0		
10/21-22	50.0	10.0	40.0		
10/23	27.0	6.0	21.0		
10/24	27.0	3.0	24.0		Active
10/25	27.0	3.0	24.0		
10/26	28.0	3.0	25.0	173.0	
10/27	28.0	2.0	26.0		Very active
10/28-29	50.0	5.0	45.0		
10/30	26.0	7.4	19.6		Gnawing on cage
10/31	26.0	6.0	20.0		
11/1	26.0	5.1	20.9		
11/2	26.0	4.0	22.0	180.0	
11/3	26.0	3.0	23.0		Calm
11/4-5	50.0	1.5	48.5		
11/6	23.0	0.0	23.0		
11/7				200.0	Sacrificed

TABLE III (Continued)

Rat No. 20	Experimental Diet II				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	0.9	9.1	53.0	Yellow Tail
9/23-24	20.0	2.0	18.0		Healthy
9/25	11.0	0.5	10.5		Active
9/26	13.0	2.0	11.0		
9/27	13.0	0.5	12.5		
9/28	14.0	0.0	14.0	89.0	
9/29	16.0	2.0	14.0		Active
9/30-10/1	30.0	3.0	27.0		
10/2	17.0	2.0	15.0		Restless
10/3	18.0	0.0	18.0		
10/4	20.0	3.0	17.0		
10/5	20.0	2.3	17.7	134.0	
10/6	20.0	1.4	18.6		Calm
10/7-8	40.0	2.0	28.0		
10/9	23.0	2.0	21.0		
10/10	23.0	1.5	21.5		Calm
10/11	25.0	2.1	22.9		
10/12	25.0	8.0	17.0	150.0	
10/13	26.0	2.0	24.0		Shiny coat
10/14-15	50.0	30.0	20.0		
10/16	26.0	4.0	22.0		Nervous
10/17	26.0	6.0	20.0		
10/18	26.0	3.0	23.0		
10/19	26.0	5.0	21.0	158.0	
10/20	26.0	6.0	20.0		
10/21-22	50.0	15.0	35.0		Gnawing on cage
10/23	25.0	5.0	20.0		
10/24	25.0	3.0	22.0		
10/25	26.0	3.0	23.0		
10/26	26.0	3.0	23.0	166.0	
10/27	26.0	2.0	24.0		Gnawing on cage
10/28-29	50.0	5.0	45.0		
10/30	26.0	6.0	20.0		
10/31	26.0	8.0	18.0		Restless
11/1	26.0	5.0	21.0		
11/2	26.0	2.0	24.0	170.0	
11/3	26.0	3.0	23.0		
11/4-5	50.0	0.0	50.0		Calm, alert
11/6				170.0	Sacrificed

TABLE III (Continued)

Rat No. 21	Experimental Diet II				
Date	Food Given	Food Left	Food Eaten	Weight	Remarks
	grams	grams	grams	grams	
9/22	10.0	2.0	8.0	54	Yellow Head and Tail
9/23-24	20.0	3.0	17.0		
9/25	10.0	2.0	8.0		Alert
9/26	10.0	1.0	9.0		
9/27	12.0	1.5	10.5		
9/28	13.0	1.0	12.0	94.0	
9/29	14.0	0.0	14.0		
9/30-10/1	30.0	0.0	30.0		
10/2	17.0	1.0	16.0		Active
10/3	18.0	3.0	15.0		
10/4	19.0	3.0	16.0		Appears healthy
10/5	19.0	2.0	17.0	132.0	
10/6	20.0	2.0	18.0		Restless
10/7-8	40.0	2.0	38.0		
10/9	20.0	0.0	20.0		
10/10	23.0	1.0	22.0		
10/11	24.0	3.0	21.0		Calm
10/12	24.0	3.0	21.0	136.0	
10/13	24.0	1.0	23.0		Active
10/14-15	50.0	23.0	27.0	140.0	
10/16	25.0	4.0	21.0		Restless
10/17	25.0	5.0	20.0		
10/18	25.0	3.0	22.0		
10/19	25.0	6.0	19.0	150.0	Gnawing on cage
10/20	25.0	5.0	20.0		
10/21-22	50.0	35.0	15.0		
10/23	25.0	3.0	22.0		Restless
10/24	25.0	3.0	22.0		
10/25	25.0	?	:		Food dumped
10/26	26.0	3.0	23.0	165.0	
10/27	26.0	2.0	24.0		
10/28-29	50.0	13.0	37.0		
10/30	26.0	11.2	14.8		
10/31	26.0	4.0	22.0		Calm
11/1	25.0	3.4	22.6		
11/2	26.0	1.0	24.0	170.0	Calm
11/3	26.0	3.0	23.0		
11/4-5	50.0	1.5	48.5		Calm and alert
11/6				176.0	Sacrificed

APPENDIX B

COMPILATION OF RAW DATA

COMPILATION OF RAW DATA MEASUREMENTS

Animal Number	Group Number	Initial Weight	Final Weight	Final Length	Dry Weight		Wet Weight	
					Left	Right	Left	Right
		Grams	Grams	Inches	Grams	Grams	Grams	Grams
1	1	56	185	14.75	.4145	.4251	.5496	.5299
2	1	52	183	14.13	.3676	.3825	.5694	.5913
3	1	55	183	14.00	.3220	.3221	.4675	.4798
4	1	55	179	13.13	.3567	.3581	.5127	.4972
5	1	56	184	14.00	.3831	.3614	.5585	.5295
6	1	52	187	13.25	.3661	.3613	.5580	.5556
7	1	52	181	14.25	.3821	.3831	.5286	.5362
8	2	56	178	15.00	.4073	.4075	.5517	.5717
9	2	49	170	15.50	.4121	.4101	.5529	.5519
10	2	52	172	14.00	.4077	.4085	.5572	.5492
11	2	56	176	14.75	.4285	.5525	.5734	.8339
12	2	56	177	15.25	.3793	.3813	.5108	.5005
13	2	53	170	14.75	.3991	.3883	.5448	.5185
14	2	52	155	14.00	.3501	.3591	.6025	.6175
15	3	53	170	14.00	.3971	.3965	.5531	.5504
16	3	52	156	14.00	.3641	.3631	.5083	.5094
17	3	50	180	14.00	.3805	.3861	.6431	.5980
18	3	55	184	14.75	.3662	.3705	.5374	.5578
19	3	56	200	14.13	.4346	.4326	.6613	.6552
20	3	53	169	13.38	.3855	.3872	.4982	.5009
21	3	54	172	14.00	.3226	.3941	.4172	.4986

COMPILATION OF BONE MEASUREMENTS

Animal Number	Animal Group	Wet Breaking Point		Femur Ash	
		Left	Right	Left	Right
		Grams	Grams	Grams	Grams
1	1	7961	8150	.2495	.2400
2	1	7530	7210	.1875	.2565
3	1	7201	7168	.1270	.1165
4	1	8451	7993	.1475	.1605
5	1	8709	8721	.1735	.2050
6	1	8761	8895	.1975	.2050
7	1	6910	6897	.2220	.2220
8	2	8505	8790	.2360	.2325
9	2	9555	9560	.2616	.2375
10	2	9851	9421	.2265	.2875
11	2	8520	abnormal	.2355	abnormal
12	2	5954	5998	.2030	.2170
13	2	9983	9765	.2340	.2580
14	2	9161	9356	.2550	.2355
15	3	8870	8760	.2240	.2875
16	3	8902	8900	.1980	.2040
17	3	7651	7010	.2195	.2245
18	3	7761	7813	.1875	.1880
19	3	7832	7610	.2440	.2465
20	3	8103	8065	.2225	.2290
21	3	7901	8012	.1665	.2050

MEAN WEIGHT OF DRY FEMURS AND MEAN ASH WEIGHT
IN THREE GROUPS OF RATS

Group	Animal Number	Mean Weight of Dry Femurs	Mean Weight of Ash	Femur Ash Per Cent
		gram	gram	%
1	1	.41980	.24475	58
1	2	.37507	.22200	59
1	3	.32205	.12175	38
1	4	.35740	.15400	43
1	5	.37225	.18925	51
1	6	.36370	.20125	55
1	7	.38260	.22200	58
2	1	.40740	.23425	58
2	2	.41110	.24955	61
2	3	.40810	.25700	63
2	4	.49050	.26125	53
2	5	.38030	.21000	55
2	6	.39370	.24600	63
2	7	.35460	.24525	69
3	1	.39680	.25575	65
3	2	.36360	.20100	55
3	3	.38330	.22200	58
3	4	.36835	.18775	51
3	5	.43360	.24525	57
3	6	.38835	.22575	58
3	7	.35835	.18575	52

VITA

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Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF TWO LEVELS OF SODIUM FLUORIDE ON BONE DENSITY OF FEMURS IN WHITE RATS

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