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The objective of this research was to indirectly determine the existence of a possible retinal-endocrine pathway activated, or inhibited, by artificial fluorescent light as evidenced by it subsequent effects on bone metabolism. Forty-eight male Long-Evans post-breeder rats, half of which were bilaterally orbitally enucleated, were used in this experiment. A 21-day pretreatment diet contained calcium and phosphorus, each at a level of 0.2% of the diet, with no dietary vitamin D. During the last seven days of this period the animals were in complete darkness. For the 12-week experimental period, the diet contained 0.6% calcium and 0.4% phosphorus, and cholecalciferol was administered the first day by intubation. During this time, four groups of 12 animals each, half of which were bilaterally orbitally enucleated, were exposed to either ultraviolet lights, cool green lights, Vita-Lite (full spectrum) lamps, or kept in complete darkness in specially constructed environmental boxes. On the first and last day of the experimental period, the left femurs were x-rayed and blood was sampled. The serum of the animals was analyzed for alkaline phospha-

tase, total calcium and total phosphorus. Bone density was determined. Femur ash was measured for total calcium and total phosphorus. Under all lighting conditions, for both the sighted and enucleated rats, the parameters measured in the serum declined. For serum alkaline phosphatase, the sighted rats under the cool green lights had the least change; the sighted in darkness had the greatest decline. The least change in serum total calcium occurred for both the sighted and enucleated rats under the cool green lights, whereas the greatest decline was for the sighted rats under the Vita-Lite lamps and the enucleated under ultraviolet lights. The sighted rats under the cool green lights had the highest percent ash per gram of dry fat-free weight; the sighted under the Vita-Lite lamps had the lowest. The highest ash calcium was in the sighted rats under the cool green and the Vita-Lite lamps. By both methods of bone density measurements, specific gravity and densitometry, the sighted rats under the cool green lights had the greatest loss. Overall there appeared to be more significant differences among the sighted animals for the various lighting conditions than for the enucleated. Also the enucleated rats had less extreme variations from the mean than the sighted. The results of this study suggest that the light mediated by the photoreceptors of the retina of the sighted rats produced an effect on bone metabolism.

Influence of Various Wavelengths of Light on Bone Metabolism in Enucleated Rats

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX FIGURES

LIST OF APPENDIX TABLES

Influence of Various Wavelengths of Light on Bone Metabolism in Enucleated Rats

INTRODUCTION

Researchers have recently become aware of the possible adverse effects that artificial light may be having on modern man (23,50). With an increasing number of hours under artificial light, as man works, studies, and plays, the biological system is being deprived of the solar radiation in which man has evolved. Of particular interest is the large number of elderly who are confined to their homes and/or beds unable or unwilling to subject themselves to the sun's rays.

Neer et al. demonstrated that exposure to artificial lighting may improve intestinal absorption of calcium in humans. Elderly subjects who were exposed to Vita-Lite[1], a commercial fluorescent lamp which simulates natural sunlight, absorbed a higher percentage of an oral dose of ^{47}Ca than control elderly subjects who were exposed to cool white fluorescent light. Both groups of subjects received no exposure to sunlight and consumed a diet low in vitamin D (34).

Ott observed that certain wavelengths of light directly affected the pigment epithelial cells of a rabbit's eye. Having observed this while conducting drug toxicity studies, he commented:

> "From the beginning of the experiment it became apparent that there were far greater abnormal re-

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[1]Duro-Test Corporation, North Bergen, NJ.

> sponses in these cells, depending on the color of the filter placed in the light source of the phase contrast microscope, than from the drugs that were being tested. This would seem to indicate the need for serious considerations of what similar abnormal effects might result from placing various colored filters in the form of sunglasses, or tinted contact lenses, in the light source entering the human eye" (39, p.47).

Furthermore, Ott suggested

> "that the similar responses of the pigment granules in the epithelial cells, might be the photoreceptor mechanism that stimulates a retinal-hypothalamic-endocrine system in animals and influences the hormonal balance or body chemistry" (39, p.48).

In addition, Ott remarked that

> "any altered growth responses must be due to the absence of the wavelengths blocked by the filter, and that the lack of these wavelengths would cause a biochemical or hormonal deficiency in both plant and animal cells . . . . When certain wavelengths of light energy are missing in artificial light sources, or blocked from entering the eyes by eyeglasses and especially sunglasses of different colors or tinted contact lenses, then the various pigment granules in the epithelial cells that respond to these particular wavelengths that are missing will not be activated . . . nor is the endocrine system in animals being fully stimulated through the photoreceptor mechanism in the eye in which the pigment granules are . . ." (39, p.48).

Shipley stated that

> "not only is light itself of autonomic importance, but . . . its effects are wavelength-dependent. This dependency must somehow be mediated by neurochemical channels connecting the photoreceptors with the endocrine system. And these could involve photoreceptors with no visibility functions" (43, p. 161).

Wurtman believed that

> "light must activate photoreceptors which are connected to neural elements, and that special neuroendocrine organs must then transduce the resulting

> nervous stimuli into endocrine information" (49, p. 20).

Fiske and Greep felt that evidence of tracts that carry light information directly from the mammalian retina to the hypothalamus is inadequate, but it has been found that light information from the retina eventually finds its way to the hypothalamus (20). Once in the hypothalamus, light information could be converted directly to an endocrine output or could be transmitted to other neuroendocrine effector organs (49). However, Wurtman warned that a cardinal tenet of photoneuroendocrinology is that "an endocrine event can be modified by experimental light [but] does not constitute proof that light normally regulates the event" (49, p.21).

My position in this argument is that artificial light for man is not a normal situation, thus resulting in possible abnormal biological responses via a retinal-endocrine system. As the elderly are increasingly confined to artificial light, the consequences of hormonal imbalances effected by missing wavelengths could result. I propose that this imbalance could affect indirectly the activity of vitamin D, as a hormone (14,31), and its metabolic activities, especially involving bone metabolism.

Indoor lamps have been designed to produce brightness and to accommodate the retinal photoreceptors for vision in the yellow-green range of visible light (50). It is this output of yellow-green emissions on which light is measured in footcandles (ft-c). Wurtman stated that

> "it should be apparent that for physiologists studying the metabolic effects of light that

> depend on other portions of the spectrum . . . footcandle measurements give misleading information . . . the intensities of visible light emissions [should] be measured in absolute irradiance units, such as microwatts per square centimeter ($\mu W/cm^2$) for each spectral band" (50, p. 469).

The average artificially lighted room has a light intensity at eye level around 160 to 320 $\mu W/cm^2$ for cool white fluorescent light. This is equivalent to 50 to 100 ft-c which in turn is equivalent to less than 10% of the light intensity in the shade outdoors. At mid-latitude around solar noon in direct sunlight, the earth's surface receives approximately 6400 to 8800 ft-c, i.e., 34 to 47 mW/cm^2 for wavelengths between 290 and 770 nm. No wavelengths shorter than 290 nm penetrate the earth's atmosphere (50).

The objective of the research reported in this thesis was to indirectly determine the existence of a possible retinal-endocrine pathway activated or inhibited by artificial light as evidenced by its subsequent effects on bone metabolism. Postbreeder male Long-Evans rats, half of which were bilaterally orbitally enucleated, were exposed to different wavelengths of artificial lights to determine the effects on bone metabolism. The serum of the animals was analyzed for alkaline phosphatase, total calcium and total phosphorus. Also bone density and bone ash, calcium, and phosphorus were measured.

REVIEW OF LITERATURE

The effect of light on humans and animals

Over four hundred years ago, Sir Isaac Newton wrote in his book, Opticks (36, p. 22):

> "This Image of Spectrum PT was coloured, being red at its least refracted end T, and violet at its most refracted end P, and yellow green and blew in the intermediate spaces."

He was discussing his observations of a beam of white sunlight which had been dispersed by a glass prism.

This spectacular array of colors has been observed since the beginning of time as the sun's light strikes a cloud of raindrops left hanging in the air after a shower. The raindrops act as prisms that separate the sunlight, fanning the whole spectrum of visible colors across the sky.

This visible light is only a tiny fraction of the whole electromagnetic spectrum. Above the visible violet in the spectrum are shorter, invisible near ultraviolet rays, then far ultraviolet, x-rays, gamma rays and cosmic rays. Below the visible red are longer invisible infrareds, microwaves, radar, television, nuclear magnetic resonance, radio waves and electric current (19).

No wavelengths shorter than 290 nm, in the far ultraviolet range, penetrate the earth's atmospheric shield. On the other end of the visible spectrum, the infrared waves gradually decrease in intensity as they reach the earth's surface. Also, there are geographic and

seasonal variations in the amount of ultraviolet wavelengths that reach the earth's surface. At midday the solar spectrum has a peak intensity in the blue-green range from 450 to 500 nm (50).

The wavelength limits required for human vision range from approximately 380 nm in the visible violet region to 770 nm in the visible red (45). The photoreceptors in the human retina were first identified by Bairati and Orzalesi (3). They are most sensitive at 555 nm, having maximum absorption in the yellow-green range (47). In the rat the maximum absorption curve is around 500 nm for the rods. The second absorption peak for the rat is around 600 nm (red) (22). However, Cardinali, Larin and Wurtman have not acknowledged this second peak or so-called modulator (9).

Modern technology has prolonged the hours of light for mankind by providing incandescent and fluorescent lamps which emit wavelengths of varying intensity, predominantly in the yellow-green range (555 nm) (50). However, in the last two decades lamp manufacturers have attempted to produce lamps that more closely simulate the spectrum of natural sunlight (46). The diverse effects that natural light and artificial illuminants have on various forms of life have been reviewed at length by Hollwich (23) and by Wurtman (50,51).

Not only does the type of light, but also the duration of light and/or darkness appears to affect living forms. Axelrod, Wurtman and Snyder felt that the exaggerated alterations in the size of the rat's pineal gland experienced during constant light and constant darkness were indicative of a diurnal pattern of a pineal melatonin-forming enzyme (2).

The effect of diurnal light on the outer segments of the photoreceptors is a constant shedding and renewal process. In constant light, shedding is eliminated and the outer segments continue to elongate, whereas in constant darkness, shedding is reduced but renewal continues in a circadian manner. In other words, a normal photoperiod is essential to maintain the metabolism of the photoreceptors, i.e., the disc addition (renewal) and disc loss (shedding) (24). Therefore, throughout the adult life, lighting conditions influence continuously the maintenance of the constant outer segment length of the photoreceptors.

In other studies, rats have been exposed to lights of various spectra which have been found to have different, and sometimes very definite effects, on their systems or organs (9,32,42,52). For instance, studies by Cardinali et al. suggest that the same or closely related photopigments mediate both the visual and the neuroendocrine effect of light in rats. These researchers discovered that green (530 nm) light was the most effective in decreasing the enzyme activity of pineal gland functions, blue (435 nm) and yellow (590 nm) bands of the spectra were 75% and 46% as effective, respectively. In contrast, neither red (660 nm) nor ultraviolet (360 nm) light significantly suppressed the enzyme activity (9). Also, McGuire, Rand and Wurtman found a spectral dependence on the entrainment of body temperature rhythm in rats that was most sensitive to the green (530 nm) portion of the visual spectrum, with low sensitivity to the red (660 nm) and blue (455 nm) ends (32).

Under various colored fluorescent lamps, Saltarelli and Coppola

studied the sensitivity to changes in lighting of the weights of various body organs of male and female mice (42). In the male mice they found significant increases in weight in the pituitary gland under ultraviolet light (300 to 400 nm, 350 nm peak), in the kidneys under full spectrum light (350 to 700 nm) and in the adrenal glands under pink (550 to 700 nm, 620 nm peak), and significant decreases in the prostate gland under pink light. In the female mice there were significant increases in weight in the adrenal glands under ultraviolet and blue (350 to 650 nm, 450 nm peak) lights, in the thyroid gland under cool white light (350 to 700 nm, 475 and 575 nm peaks), and significant decrease in the pineal gland under full spectrum and pink lights.

Wurtman and Weisel believed that various wavelengths of light might influence neuroendocrine responses. Their studies showed that the sizes of the gonadal glands in both the male and female rats were significantly smaller and the spleens significantly larger after exposure to cool white fluorescent lamps, compared to those exposed to full spectrum Vita-Lite fluorescent lamps (no wavelengths given) (52).

Experiments on effect of light on humans

A few human experiments have been performed in which lights, both artificial luminants and natural sunlight, have revealed changes in body systems (34,40,53). Neer et al. studied human male subjects exposed to full spectrum (Vita-Lite) fluorescent lamps (assuming 5% of its wavelengths ranged from 290 to 380 nm), while control subjects were exposed to cool white fluorescent lamps (no wavelengths given). During

the experimental period all the subjects received no sunlight and a diet relatively low in vitamin D. Intestinal absorption of an oral dose of radioactive calcium in the experimental subjects exposed to full spectrum (Vita-Lite) lamps was significantly greater than in the control subjects exposed to cool white fluorescent lamps (34).

Studies by Parry and Lister suggested that increased sunlight was the prime cause of hypercalciuria in a group of soldiers studied in the Persian Gulf area (40). In soldiers arriving in the Gulf from the United Kingdom during the hot season, urinary calcium excretion increased significantly compared to the soldiers arriving during the cool season. However, the level of urinary calcium excretion in the latter group of soldiers reached similar levels during the following hot season.

Zacharias and Wurtman studied a group of blind girls who were mostly suffering from retrolental fibroplasia (an eye disorder in low birthweight premature infants exposed to high concentrations of supplemental oxygen). In these girls, Zacharias and Wurtman found that this total absence of light perception produced menarche earlier than in blind girls who could perceive light, and much earlier than girls with normal vision. They concluded that the absence of retinal response to light was associated with an acceleration of menarche in proportion to the degree of loss of vision (53).

Vitamin D, parathyroid hormone, calcium and phosphorus

Vitamin D metabolism has been extensively reviewed by DeLuca et al.

(15,16,44). The two major sources of vitamin D are from the diet and by exposure of the skin to ultraviolet light or sunlight. These forms are not metabolically active. The 7-dehydrocholesterol in the skin is converted by ultraviolet light to cholecalciferol (vitamin D). In the liver cholecalciferol is converted to the main circulatory form of vitamin D, 25-hydroxycholecalciferol. This is further hydroxylated in the kidney to form 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$). In nephrectomized animals $1,25(OH)_2D_3$ stimulates increased intestinal absorption of calcium, increased bone calcium mobilization and increased intestinal phosphate retention. These results suggest that $1,25(OH)_2D_3$ is the biologically active form of vitamin D. Since the action sites of $1,25(OH)_2D_3$ are removed from its site of synthesis, this metabolically active form of vitamin D is also referred to as a hormone.

The synthesis of $1,25(OH)_2D_3$ is regulated by the parathyroid hormone (PTH) which controls the 1-hydroxylation of $25(OH)D_3$ in the kidney. The feedback mechanism involving PTH alters the levels of ionized serum calcium, maintaining a set serum calcium value within a narrow range by increasing or decreasing the release of the PTH. Lowered serum calcium increases the rate while a raised value decreases the rate of the PTH secretion. PTH in concert with $1,25(OH)_2D_3$ mobilizes calcium from previously formed bone to elevate serum calcium levels. Renal reabsorption of calcium may also be aided by PTH (17). In addition, the $1,25(OH)_2D_3$ acts directly without PTH on the intestine to increase calcium absorption. These phenomena result in increased serum calcium levels which suppress PTH secretion and the formation of $1,25(OH)_2D_3$.

Hypophosphatemia also stimulates the formation of $1,25(OH)_2D_3$, but in the absence of PTH. Phosphate in addition to calcium mobilizes $1,25(OH)_2D_3$.

There have been no maintenance requirements established for calcium or phosphorus in the diet of the rat. Draper, Sie and Bergan recommend a ratio of 2:1 of calcium to phosphorus for the prevention of osteoporosis in rats (18).

Bone mineral

The skeleton contains 60% of the body collagen which makes up the matrix for the bone minerals, calcium and phosphorus. From two-thirds to one-half of the volume of the bone mineral is in the form of hydroxyapatite. If the Ca:P ratio for the apatitic fraction falls below 1.67, the bone is considered calcium-poor. There are varying amounts of trace elements in bone mineral, such as magnesium, fluorine, sodium, and potassium, as well as the anions, citrate and carbonate. According to Jowsey, the variation in bone mineral density for the rat ranges from a low density of 0.565 g Ca/cm^3 to a high density of 0.678 g Ca/cm^3 measured by microradiographic methods. In this method of measuring bone density, the degree of absorption of the x-ray beam is related to the density of the bone, with most of the absorption energy of the beam used by the calcium (26).

Alkaline phosphatase

The origin of alkaline phosphatase in the serum is mainly from the tissues in the intestine, liver, lungs, bone and placenta. High serum alkaline phosphatase levels occur when the mineralization process is at a high level (26). Norman et al. have shown that the rate of increase of alkaline phosphatase activity is directly correlated with the rate of increase in calcium absorption (38). Also Wasserman and Corradino observed that a change in calcium metabolism can be a result of an increase in alkaline phosphatase activity (48). In the past the state of vitamin D nutrition was evaluated by measuring serum alkaline phosphatase activity (5). Clinically, vitamin D deficiency is one of the pathological states indicated by an increase in alkaline phosphatase activity in the serum. In adults, serum alkaline phosphatase levels reflect liver and some bone activity of the enzyme (27).

MATERIALS AND METHODS

Table 1 presents the protocol for the experiment, including the schedule for the time period, the lighting conditions, the diet and the procedures.

Animals

Prior to the experiment, 48 seven-month old Long-Evans postbreeder male rats[2] were kept in quarantine. They initially weighed between 500 and 600 g. Their fur coloring ranged from solid black, grey or white to various degrees of mottling. After one week in quarantine, half of the animals underwent bilateral orbital enucleation[3]. To minimize stress to the animals, they remained in quarantine three more weeks to recover from the operation.

After the fourth week the rats were randomly assigned to environmental boxes (Appendix A) where they remained in complete darkness for one week. The purpose of the environmental boxes for the experimental period was to confine the animals to specific types of light while maintaining comfortable atmospheric conditions. In each environmental box were three cages of sighted rats and three of enucleated rats.

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[2]Obtained from Simonsen Laboratories, Gilroy, CA.

[3]Performed by Dr. Nephi M. Patton and his assistants, Laboratory Animal Resource Center, Oregon State University, Corvallis, OR.

Table 1. Experimental protocol.

Situation	Time period	Light	Diet	Procedure
1. Pretreatmsnt				
A. Quarantins	35 days	diurnal standard laboratory lighting	stock diet - 14 days pretrsatment diet (↓Ca, ↓P, no vitamin D) - 21 days	weighed ear punched enucleated
B. Environmental boxes	7 days	all boxes: constant darkness	prstreatment diet (↓Ca, ↓P, no vitamin D	weighed
2. Treatment				
A. Environmental boxes	first day of 12-week period	3 boxee: constant light - ultraviolet cool green or Vita-Lits 1 box: constant darkness	vitamin D intubation treatment diet (↑Ca, ↑P, no vitamin D)	weighed blood sampled x-rayed
B. Same ae 2A	12 weeks	same as 2A	treatment diet (↑Ca, ↑P, no vitamin D)	weighed weekly
C. Sams as 2A	last day of 12-wsek psriod	same as 2A	same as 2B	weighsd blood sampled x-rayed
3. Posttreatment				
A.	first day after 12-wsek period			sacrificed sxcised left and right femurs, and thyroid glands

Each cage contained two rats. During routine cage cleaning the cages were rotated by one position clockwise from previous placement in the box.

At the beginning and at the end of the experiment, each rat had blood withdrawn by cardiac puncture. The serum was assayed for alkaline phosphatase, total calcium and total phosphorus. Also at the beginning and at the end of the experiment, the left femur of each rat was x-rayed while the rat was under total anesthesia. These x-rays were read with a microdensitometer (description follows) to obtain bone density values. The animals were weighed weekly and prior to obtaining blood samples.

At the end of the 12-week experiment, the animals were sacrifioed using carbon dioxide gas. Their left and right femurs, and thyroid glands were removed. The femurs were preserved at -20°C until assayed. Physical density was determined by water displacement from the left femurs. The right femurs were ashed for the determination of calcium and phosphorus. Also obtained from each of the left femurs were ten 1-mm cross-section serial slices and a medial 100-μ cross-section slice.

The thyroid glands, with the parathyroid glands attached, were fixed in a 4% glutaraldehyde solution. After two days the glutaraldehyde solution was poured off, replaced with a 0.1 M phosphate buffer (pH 7.4) solution and stored at 4°C. The parathyroid glands were subsequently excised and embedded in Spurr blocks for electron micro-

scopy[4]. The findings on the femur cross-sections and the parathyroid glands will be discussed in a later paper.

Lights

Groups of animals were exposed to one of three types of fluorescent lights: black ultraviolet lamps[5], cool green lamps[6], and full spectrum lamps[7]. In addition, one group was kept in complete darkness. During the 12-week treatment period these lamps in the illuminated environmental boxes were constantly lit; the fourth box remained in constant darkness. A 10-watt red light was used during brief periods of weighing and cage changes for the rats in the darkness and under the ultraviolet lights.

Prior to the experiment the lamps were measured with a spectroradiometer (Appendix B) to determine the wavelengths of the light emitted. The receptor of the spectroradiometer was centered under each pair of lamps, 22 cm off the floor of the environmental box, to coincide with the light received at the top level of the plastic animal cages. It was noted that the visible radiation increased with the door of the

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[4]Funded by NHLBI Program Project Grant HL-04664, Dr. Earl H. Wood, Principal Investigator, Biodynamics Research Unit, Mayo Clinic and Mayo Foundation, Rochester, MN.

[5]F40BLB, Sylvania General Telephone & Electronics, Danvers, MA.

[6]F40CG, Sylvania General Telephone & Electronics, Danvers, MA.

[7]40W Vita-Lite, Duro-Test Corporation, North Bergen, NJ.

environmental box closed due to reflection from the interior white walls and ceiling. For example, the cool green lamps had approximately a 7% increase at 590 nm. This increase was minimal, however, compared to the field variation from the center to the outside edge. Appendix Table B.1 summarizes the irradiance obtained from the experimental fluorescent lamps relative to irradiance of standard lamps at specific wavelengths. The spectral distribution for these lamps appears in Appendix Figure B.1.

Diet

The first 14 days in quarantine the rats were fed a stock diet[8]. The next 21 days they received a pretreatment diet, containing per kilogram: 15% vitamin-free casein[9]; 72% sucrose; 5% cottonseed oil; 4% mineral salt mixture (Table 2); 1% vitamin D-free vitamin mixture (Table 3); and 3% Alphacel[10]. The pretreatment diet contained 0.2% calcium and phosphorus each. For the 12-week treatment period they received a diet with the same ingredient proportions as the pretreatment diet, except the calcium was 0.6% and the phosphorus was 0.4%[11]

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[8]Purina Rat Chow, Ralston Purina Company, St. Louis, MO.

[9]Nutritional Biochemical Corporation, Cleveland, OH.

[10]ICN Pharmaceuticals, Inc., Life Science Group, Cleveland, OH.

[11]Jones and Foster salt mixture, ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH.

Table 2. Mineral salt mixture (25)[a]

Salt	g/kg diet
NaCl	5.573
KH_2PO_4	15.559
$MgSO_4$	2.292
$CaCO_3$	15.258
$FeSO_4 \cdot 7H_2O$	1.078
KI	0.032
$MnSO_4 \cdot 2H_2O$	0.178
$ZnCl_2$	0.010
$CuSO_4 \cdot 5H_2O$	0.019
$CoCl_2 \cdot 6H_2O$	0.001

[a]Modification of above formula for pretreatment diet: KH_2PO_4 was decreased by 43.5%; $CaCO_3$ was decreased by 67.2%, resulting in a minimal dietary source of 0.2% phosphorus and 0.2% calcium.

Table 3. Vitamin D-free vitamin mixture (10)[a]

Vitamin[b]	g/kg diet
Vitamin A powder, 20,000 IU/g[c]	1.000
Menadione	0.001
Thiamine hydrochloride	0.025
Pyridoxine hydrochloride	0.012
Nicotinic acid (niacin)	0.150
D-Pantothenic acid (hemi-calcium salt)	0.080
Choline chloride, crystalline	7.500
Cyanocobalamin, crystalline vitamin B_{12}[d]	0.002
Riboflavin	0.025
Folic acid (pteroylglutamic acid)	0.020
D-Biotin	0.002
D-alpha-Tocopherol Type III acetate crystallized	0.300
Sucrose	0.883

[a]The vitamin mixture was the same as formulated by Cheeke, Kinzell and Pedersen (10) except the 200 IU of vitamin D_3 was not added.

[b]All vitamins were obtained from Sigma Chemical Company, St. Louis, MO unless indicated otherwise.

[c]ICN Pharmaceuticals, Inc., Life Science Group, Cleveland, OH.

[d]Merck & Company, Inc., Rahway, NJ.

of the diet. All diets and water were fed ad libitum. Food and water were withheld for a 24-hour period prior to obtaining blood samples. In order to ensure that each rat had ingested adequate vitamin D, on day one of the treatment period each rat received by intubation 1.0 ml of cholecalciferol solution containing 1.875 mg cholecalciferol[12]/ml cottonseed oil.

X-ray procedures for left femur

Prior to x-raying, each rat was anesthetized with 3.5 mg Nembutal[13] /100 g body weight, given intraperitoneally, and a blood sample was obtained by cardiac puncture. The anesthetized rat was taped to the x-ray film[14] with masking tape onto a clipboard. They were individually exposed for ten minutes at 30 kv and 3 ma (total of 1800 mas) in a x-ray machine[15] without an intensifying screen. The left femur of the animal was positioned in the center of the x-ray cone, 30 cm from its source. The film was processed with a 90-second instant developer[16].

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[12]Compliments of D. Broida, Sigma Chemioal Company, St. Louis, MO.

[13]Abbott Laboratories, North Chicago, IL.

[14]Kodak Safety Film XM-2, Eastman Kodak Company, Rochester, NY.

[15]Faxitron 805, Field Emission Corporation, McMinnville, OR.

[16]Kodak RPX-omat Processor, Eastman Kodak Company, Rochester, NY.

Bone density determination from x-ray film

The x-ray film optical densities were measured with a scanning microdensitometer[17]. One hundred densitometer readings were read in steps along a line (Figure 1) transversal to the femur, beginning 1.2 cm from the distal end of the condyles. Within each step, which was 0.1 mm wide and 0.57 mm high, 100 adjacent measurements with a width of 0.001 mm were averaged. Each line covered a length of 1.0 cm, beginning and ending outside the lateral and medial edges of the bone, progressing proximally by 1.0 mm intervals to obtain six lines of densitometer readings. The data from the third line from the distal end was used to obtain the approximate bone density determinations from the x-ray film. See Appendix C for conversion of densitometer readings to bone density values.

Physical density determination of left femur[18]

The left femurs were scraped clean of adhering flesh, defatted with an ether-acetone solution, dehydrated with absolute alcohol, and air-dried at room temperature to a constant weight. Each femur was sprayed

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[17]Grant Instrument Company, Berkeley, CA. This densitometer is capable of resolving 16,384 optical density (OD) values in the range of 0.0 to 2.0 OD.

[18]Under the direction of James T. Bronk, laboratory technician, Orthopaedic Research, and in the laboratory of and under the auspices of Dr. Jenifer Jowsey, director of Orthopaedic Research, Mayo Clinic and Mayo Foundation, Rochester, MN.

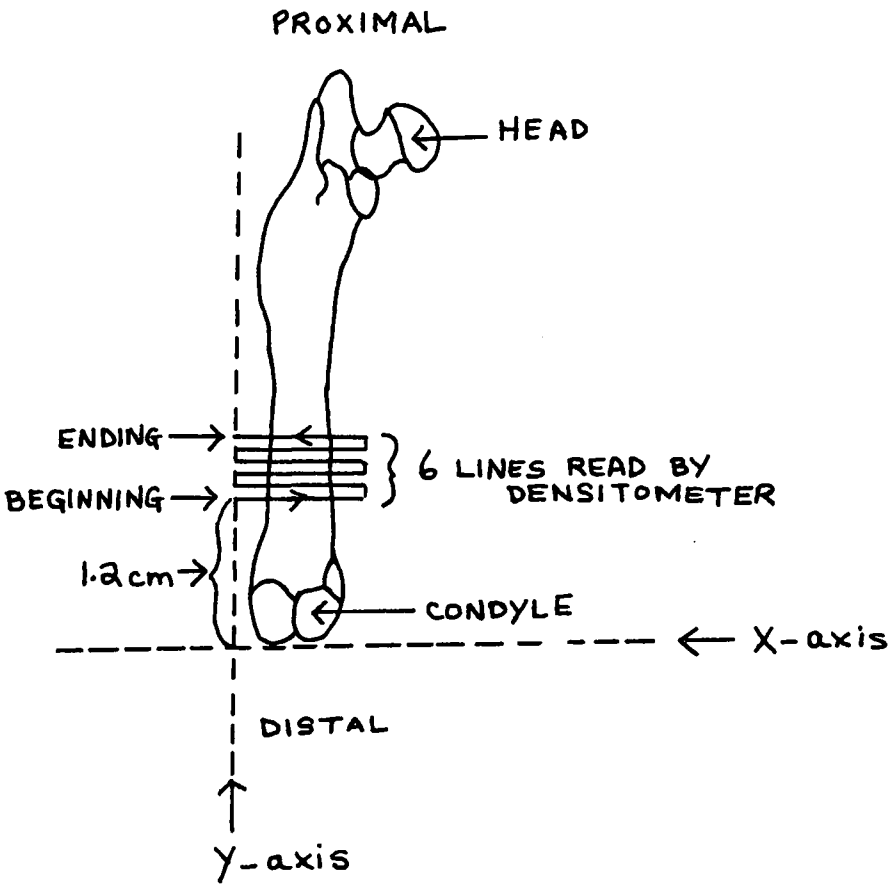

Figure 1. Flexor surface of rat left femur as it appeared in x-ray film (not to scale) showing lines of the densitometer readings beginning and ending on the y-axis, 1.2 cm above the x-axis and proceeding in 1.0 mm intervals, for 6 lines.

with Cover Glass[19] to prevent water absorption when placed in a 25-ml graduated cylinder to measure the volume of water displaced. Density was obtained by dividing the femur weight by the displaced water volume.

Serum assays

Serum alkaline phosphatase was determined with a spectrophotometer[20] according to the method of Kind and King (28) as modified by Powell and Smith (41). Serum total calcium was assayed[21,4] with an atomic absorption spectrophotometer[22] according to the method supplied by the manufacturer (1). Serum total phosphorus was assayed[23,4] according to the method of Frings, Rahman and Jones (21) with an automatic analyzer[24].

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[19]Trycolac Liquid Cover Glass, Lab-Line Biomedical Products, Inc., Melrose Park, IL.

[20]Beckman Model DU, Beckman Instruments, Inc., Fullerton, CA.

[21]Assayed by the Metals Laboratories, Mayo Clinic, Rochester, MN.

[22]Model 303, Perkin-Elmer Corporation, Norwalk, CT.

[23]Assayed by the Clinical Chemistry Laboratories, Mayo Clinic, Rochester, MN.

[24]Technicon AutoAnalyzer, Technicon Instruments Corporation, Chauncey, NY.

Ash assays for calcium and phosphorus[18]

The right femurs were scraped clean of adhering flesh, defatted with an ether-acetone solution, dehydrated with absolute alcohol, and air-dried at room temperature to a constant weight. Each femur, in individual porcelain crucibles, was placed in an ashing oven[25] for 24 hours with a maximum temperature of 850°C. The ashed weight of each femur was obtained. Each ashed femur was placed in a 25 ml volumetric flask to which 5 ml concentrated HCl were added, and allowed to stand for 24 hours. Distilled water was added to each flask to below volume level and allowed to stand for another 24 hours, after which time all traces of ash had dissolved. After distilled water was added to volume level, the solution was assayed for total calcium and total phosphorus as described previously under "Serum assays".

Statistical analysis

The Statistical Package for the Social Sciences (Version 7.0) was used to analyze the experimental data. The experimental variables (serum assays, bone density measurements and ash values) were statistically analyzed by analysis of variance (ANOVA) (Appendix D). This analysis revealed by the F-test if there was a significant two-way interaction between the light types (darkness, ultraviolet, cool green and Vita-Lite) and the rat types (sighted and enucleated) for any of

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[25]Thermolyne ashing oven, Sybron Corporation, Arden, NC.

the variables. Also Pearson correlation coefficients (r) were determined for each of the experimental variables. The significance of light and rat type effects within each rat type and within each light type was determined by Student's t-test.

RESULTS

Statistical determinations

ANOVA tables. The ANOVA tables are presented in Appendix D. These were computed with and without the interim gain in weight (Table 4) as a oovariate. Since both analyses of variance were essentially the same, the ANOVA data listed are without the animals' weight as a covariate. Significanoe was considered among the light types and between the rat types, as well as the interaction between the light and the rat types. Only significant values are discussed below.

Significant interaction was found between light types and rat types for the bone density variables (Appendix Tables D.4,5,6). Also there was a significant effect on physical bone density in accordance with rat type (Appendix Table D.6), and on ash calcium with rat type (Appendix Table D.8). There was a significant effect from light on the percent ash per gram of dry fat-free weight (Appendix Table D.7).

Pearson correlation coefficients. The Pearson oorrelation coefficients in Table 5 show an overall relationship of the experimental variables for the total population, without regard to the effect on subpopulations. The following experimental variables correlated: ash calcium and ash phosphorus ($r = 0.39$, $p < 0.01$), physical bone density (by water displacement) and bone density after treatment (by x-ray) ($r = 0.37$, $p < 0.01$), ash calcium and physical bone density (by water displacement) ($r = -0.32$, $p < 0.05$), ash calcium and percent ash per gram of dry fat-free weight ($r = -0.29$, $p < 0.05$), ash phosphorus and percent

Table 4. Rat weights (g) before and after treatment with interim gain in weight (mean ± 1 SD) (6 rats per group)

	Light	Before treatment	After treatment	Gain[a]
Sighted:	Darkness	516 ± 19	569 ± 21	53 ± 9[b]
	Ultraviolet	563 ± 23	627 ± 62	65 ± 52
	Cool green	572 ± 26	658 ± 56	86 ± 39[b]
	Vita-Lite	556 ± 24	631 ± 30	75 ± 26
Enucleated:	Darkness	559 ± 30	617 ± 35	58 ± 25
	Ultraviolet	560 ± 41	628 ± 63	68 ± 29
	Cool green	557 ± 19	626 ± 50	69 ± 43
	Vita-Lite	553 ± 25	634 ± 39	81 ± 22

[a]Values with same superscript were significantly different (≤ 0.05).

[b]$t = -1.768$, $p < 0.05$

Table 5. Pearson correlation coefficients (r) for total population for the experimental variables One-tailed test (Number in parentheses indicates sample size)

Experimental variables	Change in serum Alk Pase	Change in serum total Ca	Change in serum total P	Bone density after treatment (x-ray)	Physical bone density (water displ.)	% ash/dry fat-free weight	Ash Ca
Change in serum total Ca	0.10 (40)	-[a]					
Change in serum total P	-0.16 (35)	0.12 (36)	-				
Change in bone density (x-ray)	-0.21 (40)	-0.06 (40)	-0.23 (35)	-			
Physical bone density (water displ.)				0.37[b] (46)	-		
% ash/dry fat-free weight				-0.02 (46)	0.21 (48)	-	
Ash Ca				0.09 (46)	-0.32[c] (48)	-0.29[c] (48)	-
Ash P				-0.06 (46)	-0.10 (48)	-0.33[c] (48)	0.39[b] (48)

[a] dependent variables

[b] $p < 0.01$

[c] $p < 0.05$

ash per gram of dry fat-free weight ($r = -0.33$, $p < 0.05$).

These "r" values do not reveal any information on the effect of the light types on the rat types. Although the examination of these relationships is useful in analyzing causal relationships, they do not imply that causal patterns can be established between the variables (35). In other words, one cannot predict the value "y" from the value "x" on the basis of highly correlated "r" values without further examination of the data. For example, even though the two methods for obtaining bone density after treatment have an $r = 0.37$ ($p < 0.01$), the coefficient of determination (r^2) is only 0.14. In order for there to be a direct correlation, "r^2" has to be near or equal to one.

In examining Table 6, the effect of the light types on the rat types is examined at the subpopulation level. It is apparent that the correlation coefficients between the various experimental variables which were significant for the population are significant only for a portion of the population. Also, in some cases where significance was not evident at the population level, it is significant for sectors of the subpopulation.

Serum assays

Serum alkaline phosphatase. There was a decline in mean serum alkaline phosphatase activity under all lighting conditions for both the sighted and the enucleated rats (Table 7). There were no significant differences (t-test) between the sighted and the enucleated rats under the various lighting conditions. Among the sighted rats, the ones

Table 6. Pearson correlation coefficients (r) for subpopulations for the experimental variables. One-tailed test. (6 rats per group unless indicated otherwise in parentheses)

	Darkness		Ultraviolet		Cool green		Vita-Lite	
	Sighted	Enucleated	Sighted	Enucleated	Sighted	Enucleated	Sighted	Enucleated
Chg in serum alkaline phosphatase Chg in serum total calcium	+1.00[a] (5)	-0.21 (5)	+0.75 (5)	-0.17	-0.82 (3)	+0.13	-0.49 (5)	+0.18 (5)
Chg in serum alkaline phosphatase Chg in serum total phosphorus	-0.72 (4)	-0.88[b] (5)	-0.75 (4)	+0.28	NV[c] (2)	+0.39 (5)	-0.67 (5)	+0.97[a] (4)
Chg in serum alkaline phosphatase Chg in bone density (x-ray)	+0.71	+0.35 (5)	-0.05 (5)	+0.02	+0.08 (3)	-0.38 (5)	-0.55 (5)	-0.16 (5)
Chg in serum total calcium Chg in serum total phosphorus	-0.78 (4)	+0.48 (5)	-0.88[b] (5)	-0.54	NV (2)	+0.79 (5)	+0.78 (5)	+0.11 (4)
Chg in serum total calcium Chg in bone density (x-ray)	+0.68 (5)	-0.81[b] (5)	-0.09	-0.72	+0.50 (3)	+0.01 (5)	+0.29 (5)	-0.58 (5)
Chg in serum total phosphorus Chg in bone density (x-ray)	-0.88[b] (4)	-0.56 (5)	+0.02 (5)	+0.53	NV (2)	-0.40 (4)	+0.58 (5)	-0.54 (4)
Bone density after treatment (x-ray) Physical bone density (water displ.)	+0.58	+0.01	+0.17	-0.37	+0.48	-0.60 (5)	+0.80 (5)	+0.20
Bone density after treatment (x-ray) % ash/g dry fat-free weight	+0.05	+0.64	+0.37	+0.64	-0.40	-0.80 (5)	+0.74 (5)	+0.35
Bone density after treatment (x-ray) Ash calcium	-0.32	+0.21	+0.56	-0.47	+0.50	+0.72 (5)	+0.20 (5)	+0.56
Bone density after treatment (x-ray) Ash phosphorus	-0.49	-0.62	-0.06	-0.05	-0.21	+0.68 (5)	+0.33 (5)	+0.25
Physical bone density (water displ.) % ash/g dry fat-free weight	+0.61	-0.21	+0.80[b]	-0.47	+0.04	+0.63	+0.43	+0.31
Physical bone density (water displ.) Ash calcium	-0.56	-0.38	-0.31	+0.96[a]	-0.24	-0.77[b]	-0.15	-0.66
Physical bone density (water displ.) Ash phosphorus	-0.18	-0.19	-0.80[b]	+0.91[a]	+0.30	-0.43	-0.12	-0.70
% ash/g dry fat-free weight Ash calcium	-0.77[b]	-0.40	-0.41	-0.62	-0.23	-0.53	-0.10	+0.11
% ash/g dry fat-free weight Ash phosphorus	-0.10	-0.77[b]	-0.92[a]	-0.30	+0.95[a]	-0.89[a]	+0.69	-0.13
Ash calcium Ash phosphorus	+0.71	+0.58	+0.53	+0.87[a]	+0.16	+0.46	+0.25	+0.78[b]

[a] $p < 0.01$ [b] $p < 0.05$ [c] not valid

Table 7. Serum alkaline phosphatase (K.A.U./100 ml) before and after treatment with changes ascribed to treatment (mean ± 1 SD) (6 rats per group unless indicated otherwise in parentheses)

	Light	Before treatment	After treatment	Changes[a]
Sighted:	Darkness	16.77 ± 3.40	0.65 ± 0.61	-16.12 ± 3.42[b]
	Ultraviolet	14.12 ± 7.94	0.50 ± 0.89 (5)	-14.51 ± 8.66 (5)[c]
	Cool green	7.92 ± 2.51 (3)	1.04 ± 0.77	- 6.48 ± 2.14 (3)[b,c]
	Vita-Lite	13.44 ± 11.29	1.00 ± 0.30	-12.44 ± 11.29
Enucleated:	Darkness	11.43 ± 2.80	0.99 ± 0.67 (5)	-10.87 ± 2.90 (5)
	Ultraviolet	12.67 ± 5.63	0.89 ± 0.19	-11.78 ± 5.49
	Cool green	11.34 ± 2.68	1.16 ± 0.72	-10.18 ± 2.38
	Vita-Lite	12.29 ± 6.62 (5)	1.12 ± 0.56	-11.02 ± 6.29 (5)

[a]Values with same superscript were significantly different (≤ 0.05).

[b]$t = -2.14$, $p < 0.05$

[c]$t = -1.73$, $p < 0.05$

exposed to the cool green lights had a significantly smaller change in alkaline phosphatase activity than those in darkness ($p < 0.05$) and under ultraviolet lights ($p < 0.05$). Since this difference was not observed among the enucleated rats this suggests there was an interaction between the wavelengths of the cool green light and the change in activity of alkaline phosphatase in the sighted rats under the cool green light (Figure 2). On the other hand, this could have been due to the lower baseline value of serum alkaline phosphatase activity for the sighted rats under the cool green lights (Table 7). However, from the ANOVA (Appendix Table D.1), the effect of light type, rat type or interaction was not significant.

The standard deviations of the means of the enucleated animals was smaller than among the sighted, except for the rats under the cool green lights. This suggests that sight may have had a variable effect on the activity of serum alkaline phosphatase. Also there was possibly more variation among the individual rats within the light types of the sighted rats than the enucleated, especially for the sighted rats under ultraviolet and Vita-Lite lamps. At the subpopulation level a high correlation appeared between the change in serum alkaline phosphatase and total serum calcium for the sighted rats in darkness ($r = 1.00$, $p < 0.01$) (Table 6). Also when the change in serum alkaline phosphatase is compared to the change in serum total phosphorus the following significant correlations occur at the subpopulation level: enucleated rats under the Vita-Lite lamps ($r = 0.97$, $p < 0.01$), and enucleated rats in darkness ($r = -0.88$, $p < 0.05$) (Table 6).

Serum total calcium. Under all lighting conditions, for both the

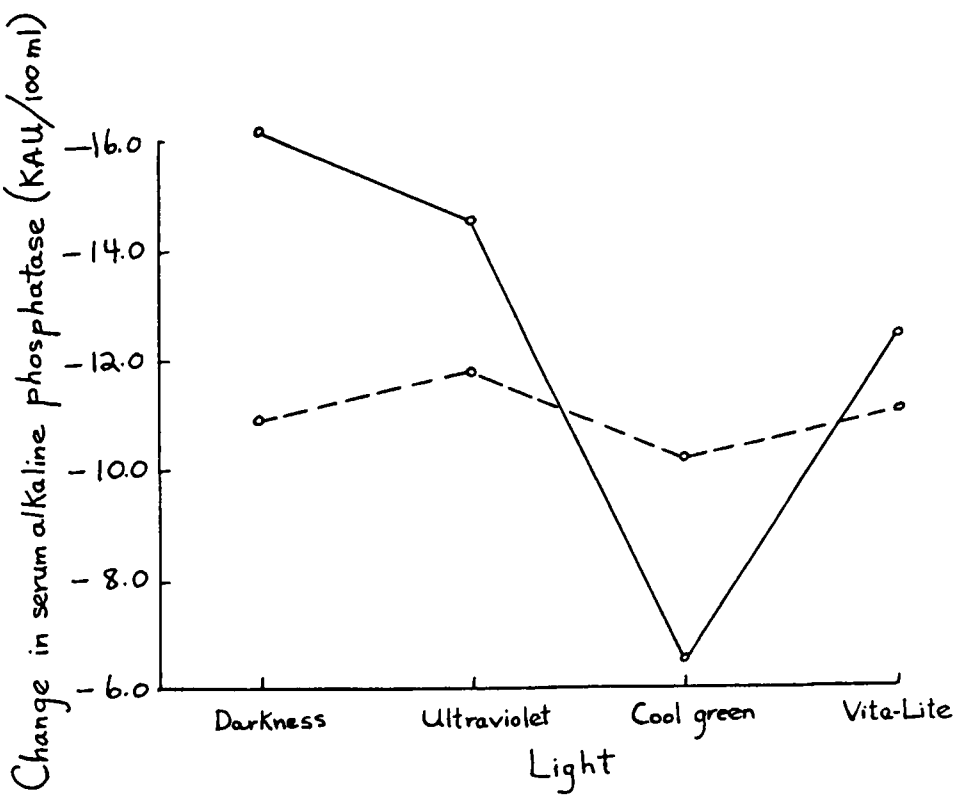

Figure 2. Light and rat type effects, with interactions, for the change in serum alkaline phosphatase. No significant differences between the means within the light types. Rat types: (———) sighted; (- - -) enucleated.

sighted and enucleated rats, there was a decline in mean serum total calcium (Table 8). The loss evidenced for the sighted rats under the Vita-Lite lamps was significantly greater than for those exposed to the cool green lights ($p < 0.05$). In the enucleated rats under ultraviolet light the loss was significantly greater than for those under cool green lights ($p < 0.05$), in darkness ($p < 0.05$), and under Vita-Lite lamps ($p < 0.05$). Under the Vita-Lite lamps there was a significantly greater decline in total serum calcium in the enucleated rats than in the sighted rats ($p < 0.05$). In several groups the standard deviation was greater than the mean. The data depicted in Figure 3 suggests an interaction between light and rat types as it affects the level of serum total calcium. However, ANOVA (Appendix Table D.2) revealed no significant effect of light type, rat type or interaction between these types. At subpopulation level the change in serum total oalcium had a significant negative correlation with the change in serum total phosphorus for the sighted rats under the ultraviolet lights ($r = -0.88$, $p < 0.05$), and the change in serum total calcium had a significant negative correlation with the change in bone density (by x-ray) for the enucleated rats in darkness ($r = -0.81$, $p < 0.05$) (Table 6).

Serum total phosphorus. The mean serum total phosphorus declined under all lighting conditions for both the sighted and the enucleated rats (Table 9). None of the changes were signifioant. The standard deviation in most groups was greater than the mean, which shows the wide variation in responses. Figure 4 illustrates the lack of interaction between light and rat types for serum total phosphorus. There was a significant negative correlation between the change in serum

Table 8. Serum total calcium (mg/dl) before and after treatment with changes ascribed to treatment (mean ± 1 SD) (6 rats per group unless indicated otherwise in parentheses)

	Light	Before treatment	After treatment	Changes[a]
Sighted:	Darkness	10.3 ± 0.4 (5)	9.9 ± 0.3	-0.4 ± 0.4 (5)
	Ultraviolet	10.2 ± 0.2	9.7 ± 0.3	-0.5 ± 0.3
	Cool green	10.1 ± 0.1 (3)	9.7 ± 0.4	-0.2 ± 0.3 (3)[b]
	Vita-Lite	10.1 ± 0.3 (5)	9.4 ± 0.2	-0.7 ± 0.3 (5)[b,c]
Enucleated:	Darkness	9.8 ± 0.2	9.5 ± 0.5 (5)	-0.3 ± 0.6 (5)[d]
	Ultraviolet	10.1 ± 0.2	9.4 ± 0.4	-0.7 ± 0.5[d,e,f]
	Cool green	10.1 ± 0.3	9.9 ± 0.3	-0.2 ± 0.3[e]
	Vita-Lite	9.9 ± 0.2 (5)	9.7 ± 0.3	-0.3 ± 0.3 (5)[f,c]

[a]Values with same superscript were significantly different (≤ 0.05).

[b]$t = 1.90$, $p < 0.05$

[c]$t = 1.81$, $p < 0.05$

[d]$t = 1.74$, $p < 0.05$

[e]$t = -2.18$, $p < 0.05$

[f]$t = -1.82$, $p < 0.05$

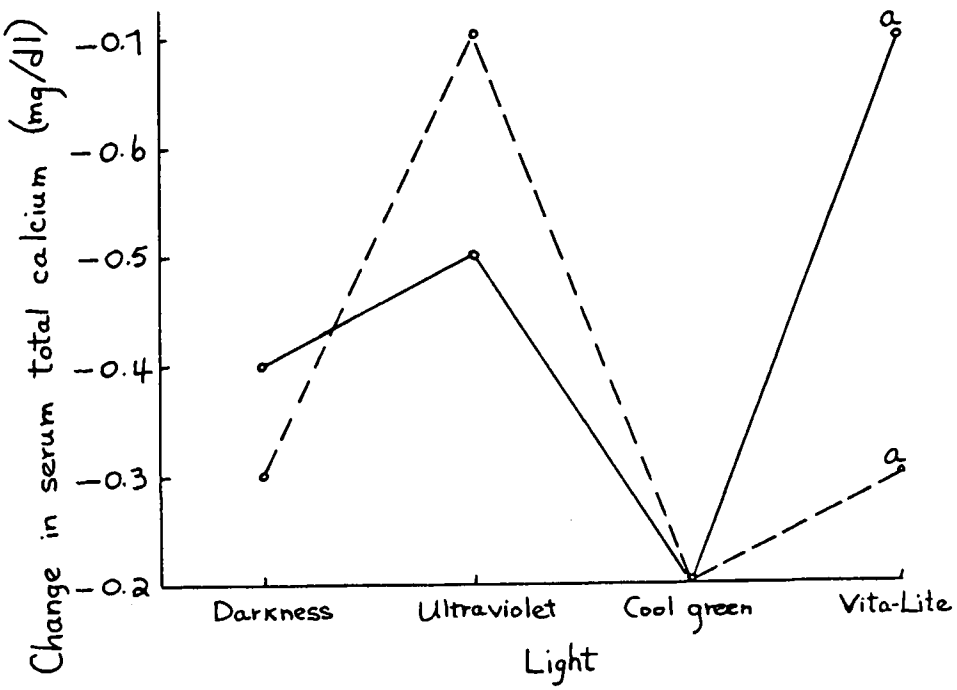

Figure 3. Light and rat type effects, with interactions, for the change in serum total calcium within light types. Vita-Lite means (a) were significantly different ($t = 1.81$, $p < 0.05$). Rat types: (———) sighted; (- - -) enucleated.

Table 9. Serum total phosphorus (mg/dl) before and after treatment with changes ascribed to treatment (mean ± 1 SD) (6 rats per group unless indicated otherwise in parentheses)

	Light	Before treatment	After treatment	Changes[a]
Sighted:	Darkness	7.4 ± 0.8 (4)	7.2 ± 2.3	-0.3 ± 2.0 (4)
	Ultraviolet	8.2 ± 2.0 (5)	6.0 ± 1.5	-2.1 ± 2.7 (5)
	Cool green	7.2 ± 0.8 (2)	6.0 ± 1.0 (5)	-0.9 ± 0.3 (2)
	Vita-Lite	8.0 ± 0.9 (5)	5.7 ± 0.7	-2.1 ± 0.6 (5)
Enucleated:	Darkness	6.9 ± 0.6	7.0 ± 1.1 (5)	0.0 ± 1.1 (5)
	Ultraviolet	7.1 ± 0.9	6.4 ± 0.6	-0.8 ± 0.4
	Cool green	7.2 ± 0.8 (5)	6.8 ± 1.3	-0.2 ± 1.8 (5)
	Vita-Lite	8.8 ± 2.5 (4)	6.9 ± 1.0	-1.7 ± 2.1 (4)

[a]No significant differences (≤ 0.05).

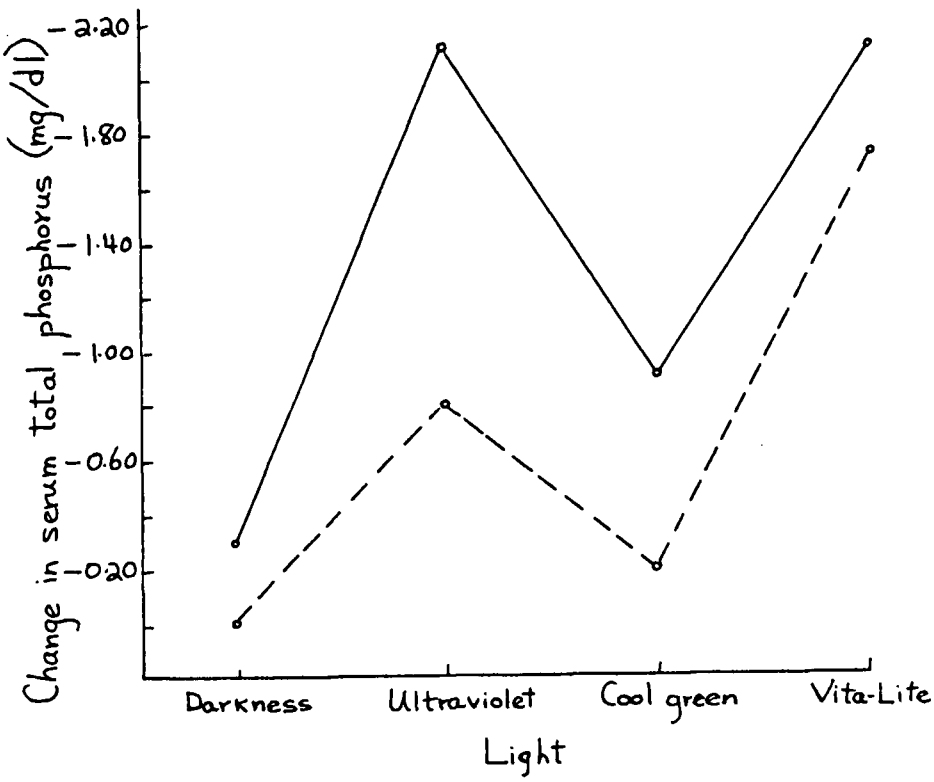

Figure 4. Light and rat type effects, with interactions, for the change in serum total phosphorus. No significant differences between the means within the light types. Rat types: (———) sighted; (- - -) enucleated.

total phosphorus and the change in bone density (by x-ray) for the sighted rats in darkness ($r = -0.88$, $p < 0.05$) (Table 6).

Ash values of right femur

Percentage of ash per gram of dry fat-free weight. The mean percentage of ash per gram of dry fat-free weight of the right femurs showed differences between the means among the sighted rats (Table 10). The sighted rats exposed to cool green lights had a higher percentage of ash than those under ultraviolet lights ($p < 0.01$), and Vita-Lite lamps ($p < 0.01$). The sighted rats in darkness had a significantly higher percentage of ash than those under ultraviolet lights ($p < 0.05$), and Vita-Lite lamps ($p < 0.01$). No significant differenoes were seen among the enucleated rats or within the light types. There appears to be an effect on the percentage of ash per gram of dry fat-free weight from the interaction between light and rat types (Figure 5). From ANOVA (Appendix Table D.7), this interaction between light and rat type was not signifioant. However, effect of light type was significant ($p < 0.01$). When comparing the values for the percentage of ash per gram of dry fat-free weight with the other experimental variables, the following correlations occur: with physical bone density (by water displacement) for the sighted rats under the ultraviolet lights ($r = 0.80$, $p < 0.05$), with ash calcium for the sighted rats in darkness ($r = -0.77$, $p < 0.05$), with ash phosphorus for the enucleated rats in darkness ($r = -0.77$, $p < 0.05$), for the enucleated rats under cool green lights ($r = -0.89$, $p < 0.01$), for the sighted rats under cool green lights ($r =$

Table 10. Percent ash per gram of dry fat-free weight of right femur after treatment (mean ± 1 SD) (6 rats per group)

	Light	After treatment[a]
Sighted:	Darkness	$60.9 \pm 1.3^{b,c}$
	Ultraviolet	$60.0 \pm 0.8^{b,d}$
	Cool green	$61.2 \pm 1.0^{d,e}$
	Vita-Lite	$59.6 \pm 0.6^{c,e}$
Enucleated:	Darkness	60.5 ± 0.6
	Ultraviolet	60.6 ± 0.2
	Cool green	60.8 ± 0.9
	Vita-Lite	60.0 ± 0.4

[a]Values with same superscript were significantly different (≤ 0.05).

[b]$t = 1.86$, $p < 0.05$

[c]$t = 2.64$, $p < 0.01$

[d]$t = 2.68$, $p < 0.01$

[e]$t = 3.46$, $p < 0.01$

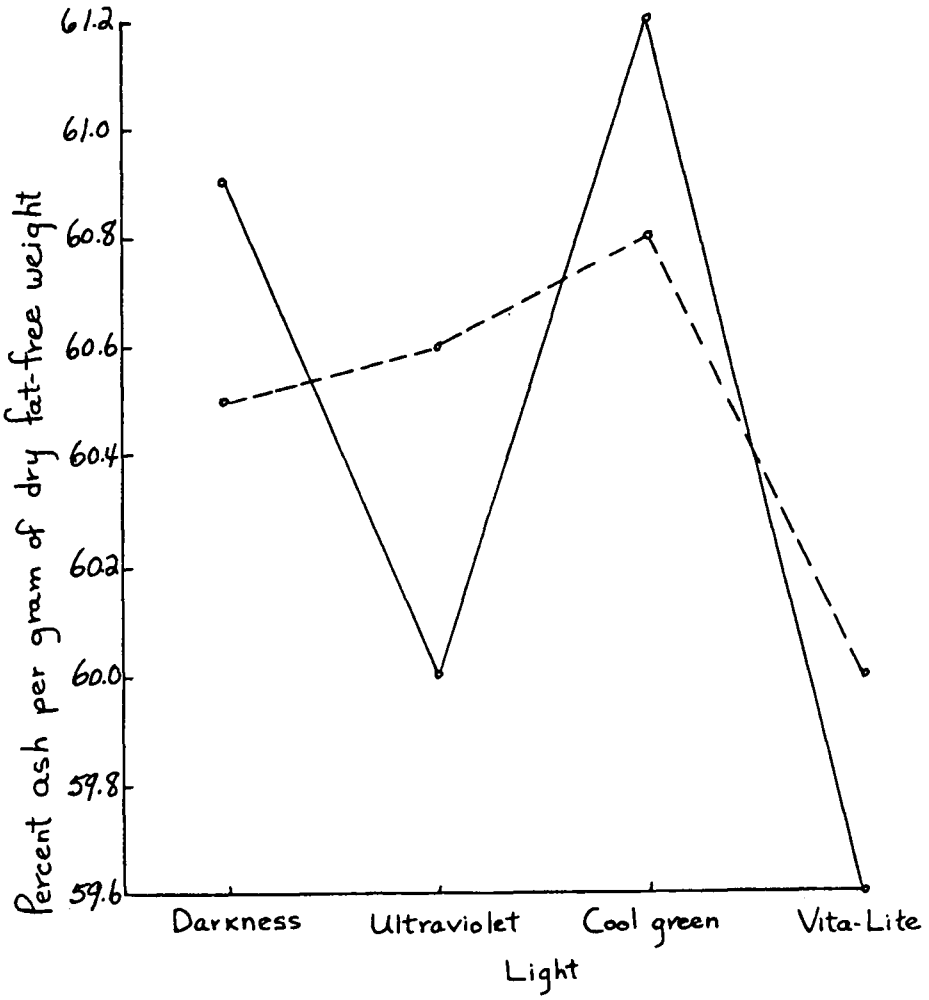

Figure 5. Light and rat type effects, with interactions, for percent of ash per gram of dry fat-free weight of right femur after treatment. No significant differences between the means within light types. Rat types: (———) sighted; (- - -) enucleated.

0.95, $p < 0.01$), and for the sighted rats under ultraviolet lights ($r = 0.92$, $p < 0.01$) (Table 6).

Ash calcium. There was a lower level of ash calcium in the right femurs of the sighted rats under ultraviolet lights than those under the cool green lights ($p < 0.05$), and the Vita-Lite lamps ($p < 0.05$) (Table 11). No effect of light appeared among the enucleated rats or within the light types. There was a little interaction between the light and rat types for ash calcium (Figure 6). ANOVA (Appendix Table D.8) revealed no significant interaction. However, the effect of rat type on ash calcium was significant ($p < 0.05$). Except for the enucleated rats under ultraviolet light ($r = 0.96$, $p < 0.01$), there is a negative correlation between the physical bone density and ash calcium values of the right femurs and is significant for the enucleated rats under cool green lights ($r = -0.77$, $p < 0.05$) (Table 6). There is no ready explanation for this incongruent relationship. Positive correlations occur between ash calcium and ash phosphorus and are significant at the subpopulation level for the enucleated rats under the Vita-Lite lamps ($r = 0.78$, $p < 0.05$), and for the enucleated rats under ultraviolet lights ($r = 0.87$, $p < 0.01$).

Ash phosphorus. There were no significant differences among the means for ash phosphorus content of the right femurs either within the rat types or within the light types (Table 12). Figure 7 suggests an interaction between light and rat types as it affects ash phosphorus. However, from ANOVA (Appendix Table D.9) no significant effects are apparent. Although there is an overall significant negative correlation between ash phosphorus and percentage of ash per gram of dry

Table 11. Ash calcium content (mg/g) of right femur after treatment (mean ± 1 SD) (6 rats per group)

	Light	After treatment[a]
Sighted:	Darkness	344.4 ± 5.5
	Ultraviolet	341.9 ± 7.6[b,c]
	Cool green	348.7 ± 6.9[b]
	Vita-Lite	348.5 ± 5.5[c]
Enucleated:	Darkness	340.3 ± 5.8
	Ultraviolet	340.4 ± 5.8
	Cool green	342.9 ± 8.9
	Vita-Lite	343.1 ± 3.6

[a]Values with same superscript were significantly different (≤ 0.05).

[b]$t = -1.84$, $p < 0.05$

[c]$t = -1.78$, $p < 0.05$

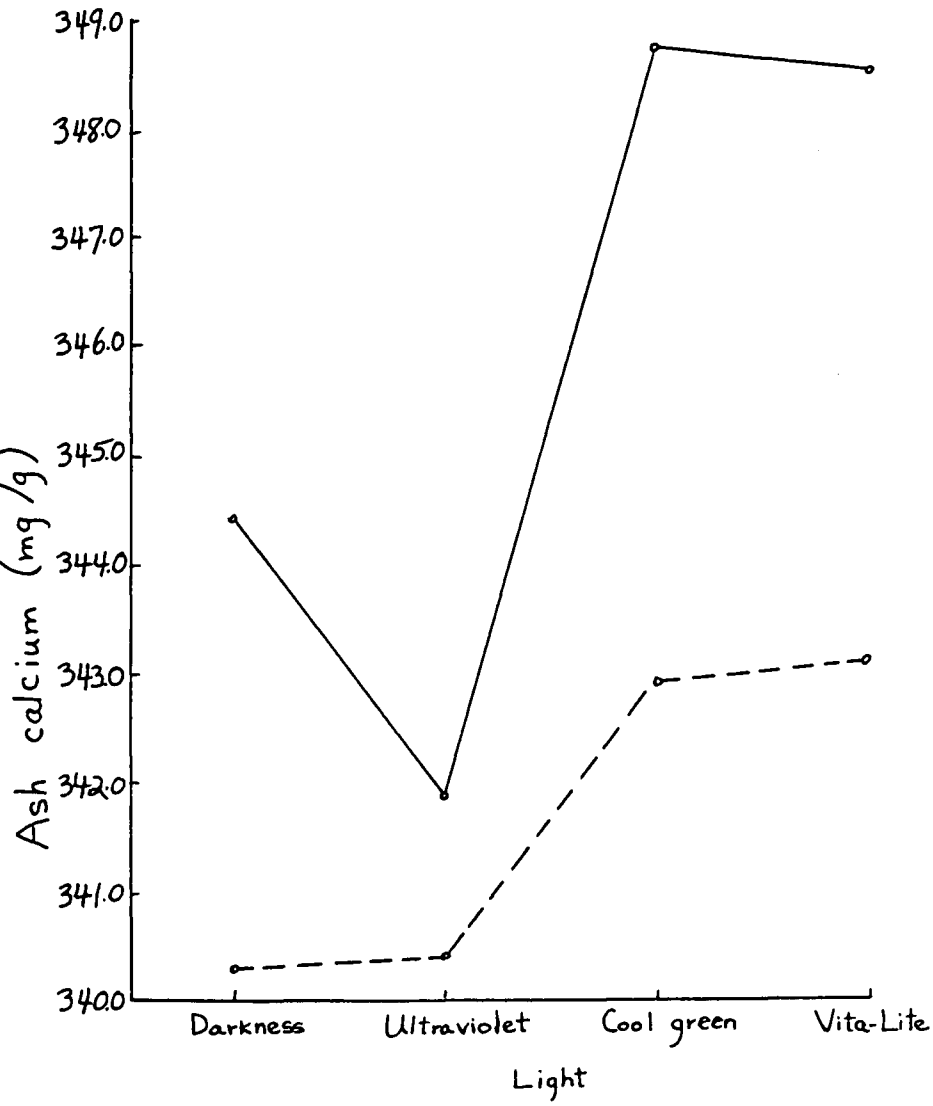

Figure 6. Light and rat type effects, with interactions, for ash calcium of right femur after treatment. No significant differences between the means within light types. Rat types: (——) sighted; (- - -) enucleated.

Table 12. Ash phosphorus content (mg/g) of right femur after treatment (mean ± 1 SD) (6 rats per group)

	Light	After treatment[a]
Sighted:	Darkness	190.6 ± 8.9
	Ultraviolet	182.5 ± 14.6
	Cool green	184.6 ± 3.1
	Vita-Lite	193.4 ± 7.7
Enucleated:	Darkness	194.6 ± 21.0
	Ultraviolet	186.1 ± 7.4
	Cool green	185.3 ± 9.5
	Vita-Lite	191.2 ± 8.0

[a]No significant differences (≤ 0.05).

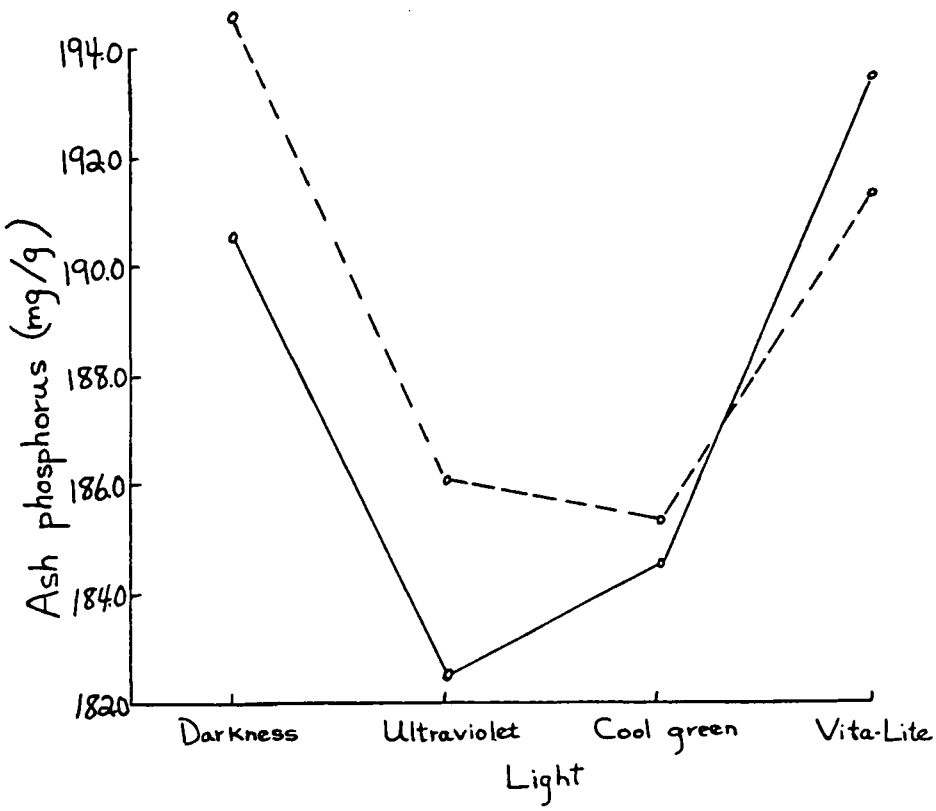

Figure 7. Light and rat type effects, with interactions, for ash phosphorus of right femur after treatment. No significant differences between the means within light types. Rat types: (——) sighted; (- - -) enucleated.

fat-free weight, the subpopulations have mixed negative and positive significant correlations (Table 5,6). Again there appears to be no explanation for this phenomena. Regardless of the relationship to the percentage of ash per gram of dry fat-free weight to ash calcium or to ash phosphorus, the latter two are positively correlated even at the subpopulation level. Also at this level reversed significant correlations occur under the ultraviolet lights when ash phosphorus is compared with physical bone density (by water displacement). The enucleated rats positively correlate ($r = 0.91$, $p < 0.01$, whereas the sighted negatively correlate ($r = -0.80$, $p < 0.05$) (Table 6).

Bone density measurements of left femur

Physical bone density determined by water displacement. The sighted rats under cool green lights and Vita-Lite lamps had the lowest bone density compared to all the other animals (Table 13). There were significant differences between the means under each lighting condition between the sighted and the enucleated rats: under cool green lights a significantly greater density in the enucleated ($p < 0.01$), under Vita-Lite lamps a significantly greater density in the enucleated ($p < 0.01$), under ultraviolet lights a significantly greater density in the enucleated ($p < 0.05$), and in the darkness a significantly greater in the sighted ($p < 0.01$). Among the sighted rats, those in darkness had a significantly greater density than those under cool green lights ($p < 0.01$), than those under Vita-Lite lamps ($p < 0.01$), and than those under ultraviolet lights ($p < 0.01$). Among the enucleated rats, those

Table 13. Physical bone density (wt/crude volume) determined by water displacement of left femur after treatment (mean ± 1 SD) (6 rats per group)

	Light	After treatment[a]
Sighted:	Darkness	$1.35 \pm 0.06^{b,c,d,e}$
	Ultraviolet	$1.20 \pm 0.07^{b,f}$
	Cool green	$1.11 \pm 0.10^{c,g}$
	Vita-Lite	$1.13 \pm 0.11^{d,h}$
Enucleated:	Darkness	$1.20 \pm 0.12^{i,e}$
	Ultraviolet	$1.32 \pm 0.11^{i,f}$
	Cool green	1.25 ± 0.06^{g}
	Vita-Lite	1.29 ± 0.08^{h}

[a]Values with same superscript were significantly different ($\leqslant 0.05$).

[b] $t = 2.89$, $p < 0.01$

[c] $t = 4.47$, $p < 0.01$

[d] $t = 4.19$, $p < 0.01$

[e] $t = -2.73$, $p < 0.01$

[f] $t = 2.26$, $p < 0.05$

[g] $t = 2.54$, $p < 0.01$

[h] $t = 3.10$, $p < 0.01$

[i] $t = -2.11$, $p < 0.05$

in darkness had significantly less density than those under ultraviolet lights ($p < 0.05$). There was a reverse effect for the two rat types kept in darkness compared to the interaction of the other light types with the two rat types (Figure 8). From ANOVA (Appendix Table D.6), the effect of interaction between light and rat types on bone density is significant ($p < 0.01$).

Bone density determined from x-ray. The sighted rats under the cool green lights had the greatest loss in bone density compared to all the other animals (Table 14). The change in bone density derived from the optical density of the x-ray films of the left femurs revealed significantly greater loss for the sighted rats under cool green lights than in darkness ($p < 0.01$), under ultraviolet lights ($p < 0.05$), and the enucleated rats under cool green lights ($p < 0.05$). Also the sighted rats in darkness not only gained significantly more bone density than those under the cool green lights, but more than those under Vita-Lite lamps ($p < 0.05$), and more than the enucleated rats in darkness ($p < 0.01$). These changes in bone density show a wide variation from the mean, and the standard deviations for all groups of animals were larger than the mean values. Further evidence that interaction occurred between the light and rat types resulting in a change of bone density is depicted in Figure 9. The effect of this interaction is significant ($p < 0.05$) as revealed by ANOVA (Appendix Table D.5). In comparing the bone density after treatment as determined from x-ray film (Figure 10) with the effects of interaction between light and rat types for physical bone density measured by water displacement (Figure 8), there is a similarity in patterns except for the bone density of

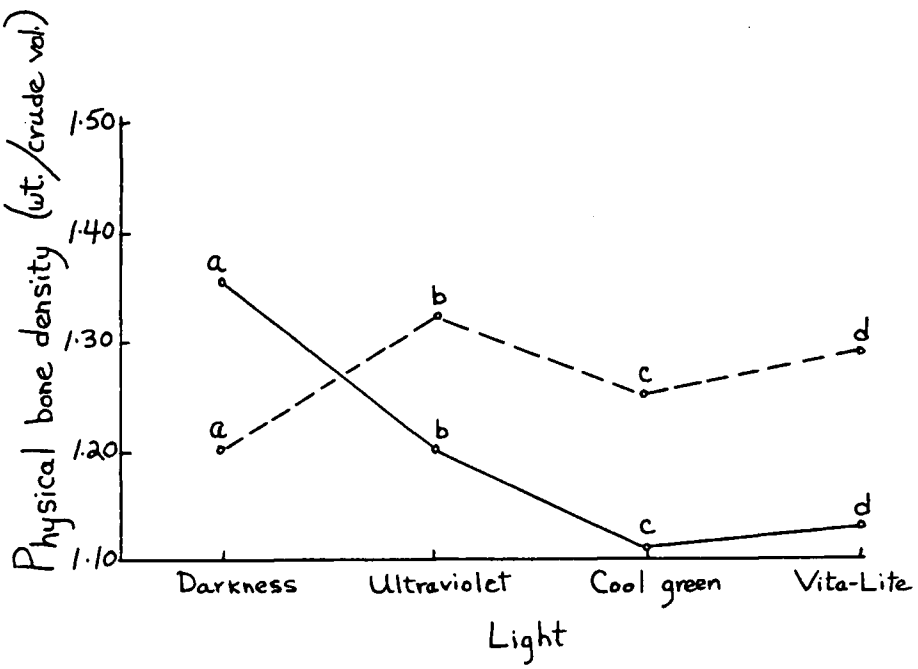

Figure 8. Light and rat type effects, with interactions, for physical bone density of left femur determined by water displacement after treatment. All light means were significantly different within the light types: darkness (a) $t = -2.73$, $p < 0.01$; ultraviolet (b) $t = 2.26$, $p < 0.05$; cool green (c) $t = 2.54$, $p < 0.01$; Vita-Lite (d) $t = 3.10$, $p < 0.01$. Rat types: (——) sighted; (- - -) enucleated.

Table 14. Bone density determined from x-ray film of left femur before and after treatment with changes ascribed to treatment (mean ± 1 SD) (6 rats per group unless indicated otherwise in parentheses)

	Light	Before treatment	After treatment	Changes[a]
Sighted:	Darkness	154.5 ± 33.1	196.7 ± 14.4	42.2 ± 43.4[b,c,d]
	Ultraviolet	167.1 ± 21.0	191.8 ± 22.6	28.1 ± 30.0[e]
	Cool green	181.3 ± 18.0	166.1 ± 36.3	-15.2 ± 37.9[b,e,f]
	Vita-Lite	180.5 ± 20.7 (5)	181.7 ± 23.5 (5)	1.1 ± 30.5[c]
Enucleated:	Darkness	182.5 ± 22.8	179.6 ± 11.1	-2.9 ± 23.4[d]
	Ultraviolet	183.7 ± 32.3	191.8 ± 23.2	8.1 ± 26.1
	Cool green	173.6 ± 35.3 (5)	201.1 ± 11.5 (5)	27.1 ± 36.9 (5)[f]
	Vita-Lite	187.0 ± 13.8	188.7 ± 19.5	1.6 ± 21.5

[a]Values with same superscript were significantly different (≤ 0.05).

[b]$t = 3.11$, $p < 0.01$

[c]$t = 2.12$, $p < 0.05$

[d]$t = -2.45$, $p < 0.01$

[e]$t = 2.35$, $p < 0.05$

[f]$t = 2.19$, $p < 0.05$

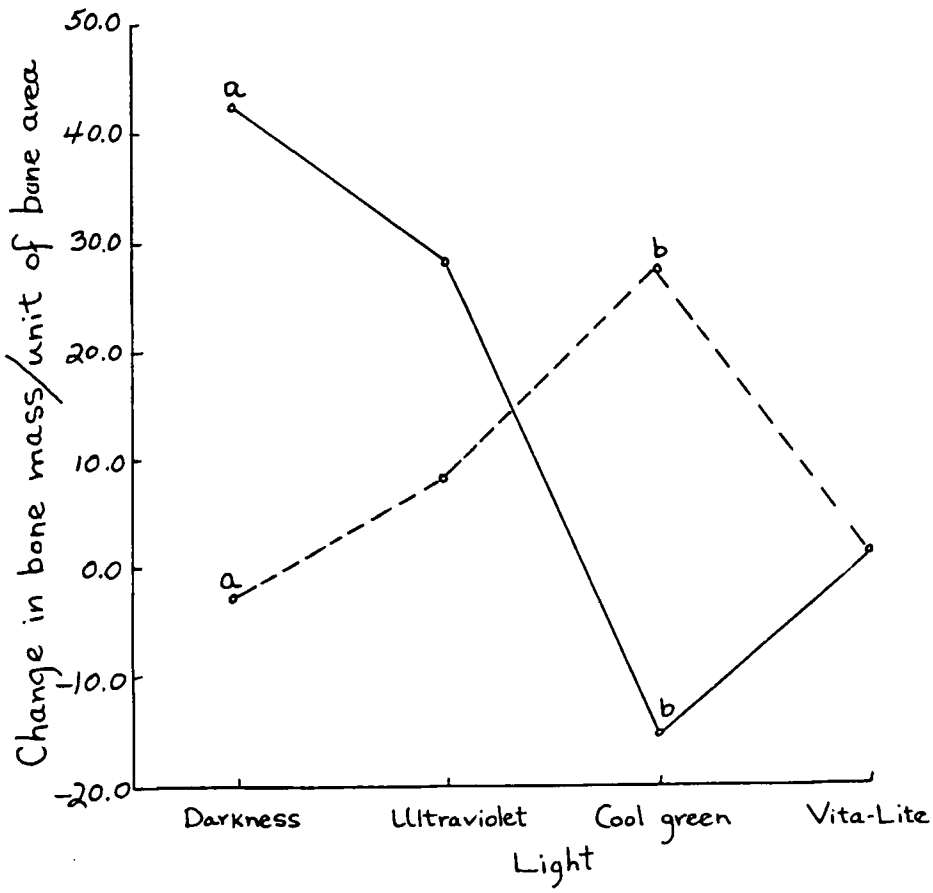

Figure 9. Light and rat type effects, with interactions, for the change in the bone density determined from x-ray film of the left femur within light types. Darkness means (a) were significantly different ($t = -2.45$, $p < 0.01$); and cool green light means (b) were significantly different ($t = 2.19$, $p < 0.05$). Rat types: (——) sighted; (- - -) enucleated.

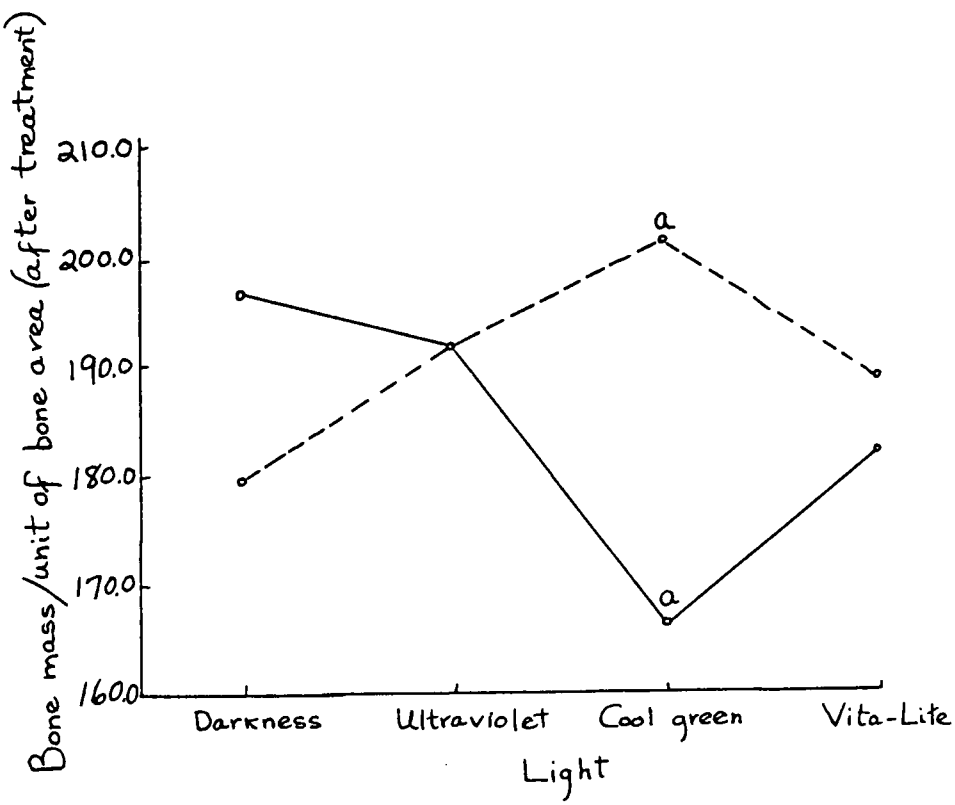

Figure 10. Light and rat type effects, with interactions, for bone density after treatment determined from x-ray film of left femur within light types. Cool green light means (a) were significantly different ($t = 2.65$, $p < 0.01$). Rat types: (——) sighted; (- - -) enucleated.

the emucleated rats under the cool green lights. As mentioned previously there is significant correlation between these two methods of measuring bone density at the population level ($p < 0.01$) (Table 5), however, this is not apparent for either rat types under any of the lighting conditions when examined at the subpopulations level (Table 6).

DISCUSSION

Many elderly are confined to an environment in which they are constantly exposed to artificial light. This experimenter was concerned about the bone metabolism of such individuals and whether it could be affected by such a light source, not as related to skin exposure, but rather, to the light received by the photoreceptors of the eye. To demonstrate if there was any possible effect, Long-Evans rats, which have pigmented epithelia in the retinae, were utilized. Colonies of sighted and enucleated postbreeder rats were exposed to various wavelengths from different artificial lights. Parameters of their sera and femurs were assayed to determine effects, if any, on their bone metabolism. As would be expected for any elderly population, a wide variation in values resulted, which complicated the interpretation of the data. However, certain consistencies did occur, and these are discussed below.

Serum assays

After the treatment period alkaline phosphatase activity declined to lower levels under all the light types for nearly all the rat types (Table 7). This polarization of values could have been due to the effect of the diet. The basal blood samples were taken at the end of a 21-day period which involved a diet without vitamin D and was low in calcium and phosphorus (Table 1). Thus there could have been some stimulation of alkaline phosphatase to mobilize bone mineral. The

decline in the activity of this enzyme evidently occurred when calcium and phosphorus dietary levels were returned to a normal range, along with administration of vitamin D.

King and Armstrong stated that any values for serum alkaline phosphatase below 3.0 units or above 13.0 units should be viewed with suspicion (29). However, their experiments on humans, dogs, sheep and rabbits included neither elderly subjects, nor rats. Neither were they able to explain why they had values outside of that range (3.0 to 13.0 units/ml), since none of their animals exhibited any abnormalities. In addition, their values did not reflect any manipulation of dietary components, such as calcium, phosphorus and vitamin D, as in the present study.

For young rats, the normal range for serum total calcium is 10.3 $\pm$ 0.5 mg/dl, and for total phosphorus 10.2 $\pm$ 0.9 mg/dl (26). Serum phosphorus is known to decline with advancing age in humans (27). The ubiquitous decline in serum total calcium in the present study (Table 8) could again have been the effect of the diets, although circulating calcium is usually independent of dietary intake due to the feedback control mechanism of the parathyroid gland (12). Since all the rats were healthy and gained weight generally at a comparable rate (Table 4), it would be improbable that any disease factors could have been the cause of the hypocalcemia. When fasting plasma phosphate is normal or low, as in this experiment (Table 9), in conjunction with hypocalcemia, this could indicate a vitamin D deficiency or calcium malabsorption (13). However, in vitamin D deficiency, as seen in osteomalacia when hypocalcemia and hypophosphatemia are present, serum alkaline phospha-

tase is elevated (13). This was not the case in the present study. Therefore, it is assumed that the "hypo-states" of the serum were due to decreased intestinal absorption. Since adult rats were used in this experiment, there could have been a decline in intestinal absorption of dietary components, as is evidenced with increasing age, especially in fat absorption (4). If there was some decrease in the absorption of fat, then there would be a decrease in vitamin D absorption, and a consequential decrease in calcium absorption. However, the rats were not vitamin D deficient if elevation of serum alkaline phosphatase is used as an indicator. Also the sighted rats in darkness had the greatest decline in alkaline phosphatase, and endogenous vitamin D could not be produced without ultraviolet light, therefore, there must have been an adequate exogenous source from the intubation of vitamin D at the beginning of the experiment. Also lower serum levels of calcium would be expected to stimulate the parathyroid glands to produce the parathyroid hormone which triggers the synthesis of $1,25(OH)_2D_3$, derived from exogenous cholecalciferol, in the kidney. The hormone, $1,25(OH)_2D_3$, could have mobilized calcium from the bones and concurrently produced phosphate diuresis (14). But if there was inadequate mobilization of calcium, along with decreased calcium absorption, then low levels of serum calcium would remain, along with decreasing levels of serum phosphorus. The correlation between the change in serum alkaline phosphatase and serum total calcium is significant (Table 6) only for the sighted rats in darkness which had the greatest decline in serum alkaline phosphatase (Table 7). However, they did not have the greatest decline in serum total calcium. Evident-

ly this correlation occurred only by chance. When the change in serum alkaline phosphatase is compared to the change in serum total phosphorus, the reason for the significant reverse correlations for the enucleated rats in darkness ($r = -0.88$, $p < 0.05$) and under Vita-Lite lamps ($r = 0.97$, $p < 0.01$) is not understandable. Neither does there appear to be any apparent reason for the other significant correlations at the subpopulation level for sera values.

The significant differences in serum total calcium (Table 8) between the sighted rats under the cool green lights and those under the Vita-Lite lamps may have been due to the difference between the full spectrum (Vita-Lite) which contained some ultraviolet rays, and the cool green lights which had very little (Appendix Table B.1). This would suggest that the photoreceptors were mediating this difference, since this did not occur for the enucleated rats under these same lights.

Ash values of right femur

There were significant differences between values in the ash assays (Tables 10, 11). Biltz and Pellegrino (6) gave ash calcium content for rat femurs as 39% of total ash, and phosphorus as 18%. Mezey, Potter and Merchant (33) had 47% of the ash as calcium, and 13% as phosphorus. In the present experiment, involving the total population, the average ash calcium content of the right femurs was 57%, and ash phosphorus was 31% of the total ash, with a 1.84 Ca/P ratio. Since $CaCO_3$ is considered to be the variable factor in the

apatite in this ratio (6), then the rats in this experiment had less ash calcium in relation to ash phosphorus than the others cited. This difference in ratio, as well as in the difference in percentage of mineral content, compared to the other experiments could be due to differences in assay methods or in the age of the rats. However, the Ca/P ratio of 1.84 is above the theoretioal ratio of 1.67 (6). The latter value is based on the apatitic fraction as $Ca_9(PO_4)_6 \cdot CaCO_3$ and possibly on younger animals. None of the individual experimental groups under the various light conditions calculated out to be less than 1.67 Ca/P, indicating none of the femurs were calcium-poor.

Even though there was an irregularity of effect from the various lights on the ash components, it appears that these lights have a differing effect on the animals with their photoreceptors intact and no differenoes in effect was evidenced for the enucleated animals. In this regard the sighted rats under the cool green lights had the highest percent ash per gram of dry fat-free weight (Table 10), while the sighted under the Vita-Lite lamps had the lowest. Again, as with the serum total calcium, this could have been due to the lower amount of ultraviolet rays in the cool green lights (Appendix Table B.1) and its reception by the photoreceptors, sinoe this difference did not occur among the enucleated rats for the same lights. Again, as with the sera values, there appears to be no ready explanation why the variables compared at the subpopulation level to the ash values significantly correlate for some rat and light types and not for others. Nor is it explicable why some are positively correlated while others are negative.

When analyzed by ANOVA there was a significant effect on ash calcium ($p < 0.05$) (Appendix Table D.8) depending on whether the rats were sighted or enucleated. However, when compared by the t-test (Table 11), there were no significant differences between the rat types. Also there was a significant effect dependent on light type on the percent ash per gram of dry fat-free weight ($p < 0.01$) (Appendix Table D.7). This effect of light was significant only for the sighted, not the enucleated, rats (Table 10). This suggests an effect of light on the calcium content of the bone via the photoreceptors.

Bone density measurements

It is not known whether serum alkaline phosphatase activity reflects bone formation (11) or bone resorption (8). However, the sighted rats in darkness had the greatest decline in serum alkaline phosphatase (Table 7), but the greatest increase in bone density (Tables 13,14); whereas the sighted rats under the cool green lights had the least decline in serum alkaline phosphatase (Table 7), but a marked loss in bone density (Table 13,14). Since green light (500 nm) is most readily absorbed by the rat retina (22), the light (cool green light) or non-light (darkness) could have in some way affected bone metabolism via the retina. It is known that the rod outer segment in the photoreceptors of the rat shed their disks in a circadian rhythm in cyclic lighting (30). In constant darkness this shedding is reduced but renewal of the disks (elongation) continues in a circadian manner (24). But in constant light the shedding of the disks is eliminated and the

rod outer segments of the retina continue to elongate (24). Due to this disruption of the circadian rhythm of shedding and elongating, which has been suggested to be involved in higher centers of cerebral activity (30), effect via the retina on bone metabolism may be occurring. Also, since the rat is a nocturnal animal, its bone metabolism may be affected accordingly. Therefore, the difference between the bone densities (Tables 13,14) in the sighted rats in darkness and the enucleated rats in darkness, could have been due to the presence or the absence of their photoreceptors, even though no light was being received by their retinae. This in turn may have affected their bone metabolism, since the circadian rhythm was eliminated by the loss of the photoreceptors in the enucleated rats in darkness.

Further statistical evidence that light was affecting bone density dependent on rat type is revealed by ANOVA, wherein the interaction between light and rat types is significant for the following variables: bone density after treatment (by x-ray) ($p < 0.05$), change in bone density (by x-ray) ($p < 0.05$), and physical bone density (by water displacement) ($p < 0.01$) (Appendix Tables D.4,5,6, respectively). Particularly significant was the differing effect of light between the sighted and enucleated rats on the physical bone density ($p < 0.01$) (Appendix Table D.6). Additional substantiation of this effect by light on rat types is revealed when these parameters are examined by the t-test (Tables 13,14). This would again suggest that there was an effect of light on the density of the bone via the photoreceptors.

SUMMARY AND CONCLUSIONS

Groups of sighted and enucleated postbreeder rats were exposed to various artificial light sources, and to darkness, to determine the possible effect on bone metabolism due to the presence or absence of the photoreceptors of the retina.

The experiment was preceded by a 21-day pretreatment period in which the calcium and phosphorus content of the diet was each 0.2%, with no dietary vitamin D. The animals, during the last seven days of this pretreatment period, were in total darkness. During the 12-week experimental period, four groups of 12 rats each, half of which were bilaterally orbitally enucleated, were exposed to either ultraviolet lights, cool green lights, Vita-Lite (full spectrum) lamps, or kept in complete darkness. Their formula diet for this time included 0.6% calcium and 0.4% phosphorus. Cholecalciferol was administered the first day by intubation.

Blood was sampled by cardiac puncture and the left femurs were x-rayed the first and last day of the 12-week experimental period. The serum was assayed for alkaline phosphatase, total calcium and total phosphorus. The optical densities of the x-ray films of the left femurs were determined by microdensitometry. This density was compared to the density (by water displacement) of the excised left femurs. The excised right femurs were ashed and assayed for percent ash per gram of dry fat-free weight, and calcium and phosphorus content.

Under all lighting conditions, for both the sighted and enucleated rats, there was a decline in serum alkaline phosphatase, serum total

calcium and serum total phosphorus (Tables 7,8, and 9, respectively). The sighted rats under the cool green lights had the least change in alkaline phosphatase, whereas the sighted in darkness had the greatest decline (Table 7). Both the sighted and the enucleated rats under the cool green lights had the least change in serum total calcium (Table 8). The sighted animals under the Vita-Lite lamps and the enucleated under the ultraviolet lights had the greatest decline. The presence of photoreceptors seemed to make a difference for the rats under the Vita-Lite lamps, since the serum total calcium levels were significantly lower ($p < 0.05$) for the sighted than for the enucleated animals. The lights appeared to have no different effect on the level of serum total phosphorus for the enucleated than for the sighted rats (Table 9).

Differences appeared among the sighted rats for percent ash per gram of dry fat-free weight of the right femur (Table 10) and for ash calcium (Table 11). There were no significant differences between the sighted and enucleated rats for any of the ash assays (Tables 10,11,12).

The differential effect between the sighted and enucleated is seen in the bone density results (Tables 13,14). The changes in bone density evidenced from the x-ray film of the left femur indicated that the sighted rats in darkness had the greatest increase in bone density, while the sighted rats under the cool green lights had the greatest loss (Table 14). This also was the case when bone density was measured by water displacement (Table 13). In this latter method the significant differences between the bone density of the sighted and the enucleated rats is especially evident.

Overall there appeared to be more significant differences among

the sighted rats for the various lighting conditions than for the enucleated. Also the enucleated tended to have less extreme variations from the mean than the sighted. This could have been by chance or it could have been due to the effect of light via the photoreceptors of the rats' retinae. If it were mediated by the light reception of the photoreceptors, then the eyes appear to have a dual role, i.e., one for vision and one for non-vision metabolism. Assuming this is so, then one should be concerned about the types of artificial lights to which one is exposed.

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APPENDICES

Appendix A

Environmental boxes

Four boxes (Figure A.1), one for each light type, were constructed to each house six plastic animal cages, measuring the standard 37.5 cm x 32.5 cm x 15.0 cm size, with a wire top which held the self-dispensing water bottle. The overall size of each environmental box was 90.0 cm deep, 120.0 cm wide and 90.0 cm high. They were constructed with A/C 1.2 cm plywood and reinforced at each inside corner with 2.5 cm^2 posts. On the inside bottom of each box, a rack was installed 7.0 cm off the floor to hold the individual plastic animal cages in place at a 5.0 cm distance from each other (Figure A.2). Also a 5.0 cm space was allowed between both the front, the back and one of the side walls from the adjacent cages. On the other side of the box was a 10.0 cm space allowance which was partitioned with a plywood deflector 7.0 cm off the floor and 15.0 cm higher than the tops of the plastic cages. The purpose of this deflector was to prevent the air flow from the inlet blowing directly onto the animal cages. The environmental boxes maintained a complete air change at the rate of 25 times each hour by squirrel fans[26] mounted exteriorly on the end of curved 5.0 cm black plastic pipe near the top of one side as the air outlet. On the outside of the box on the opposite side near the bottom, curved 5.0 cm black plastic pipes were mounted with inlet fittings (Figure A.3). The

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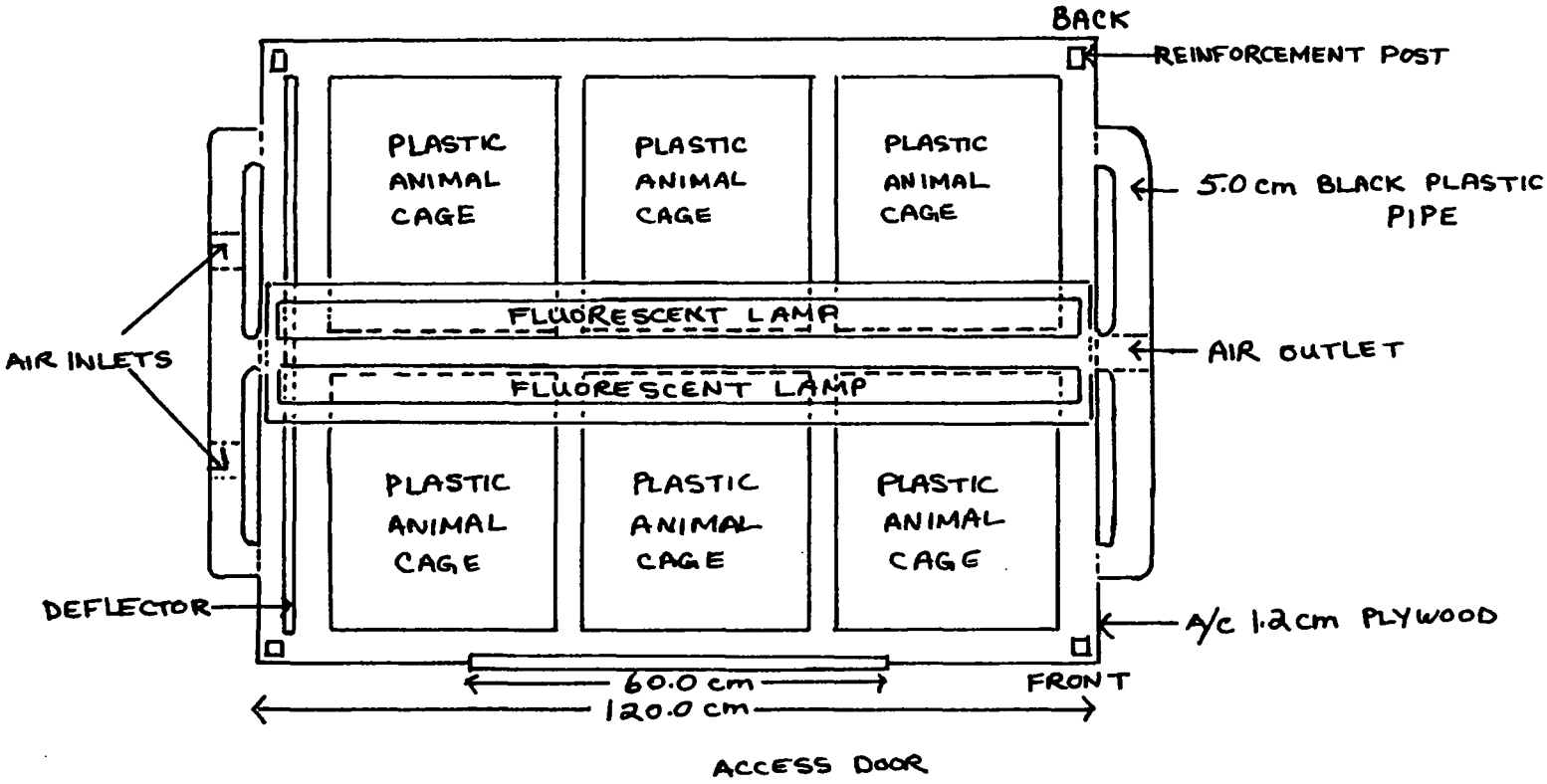
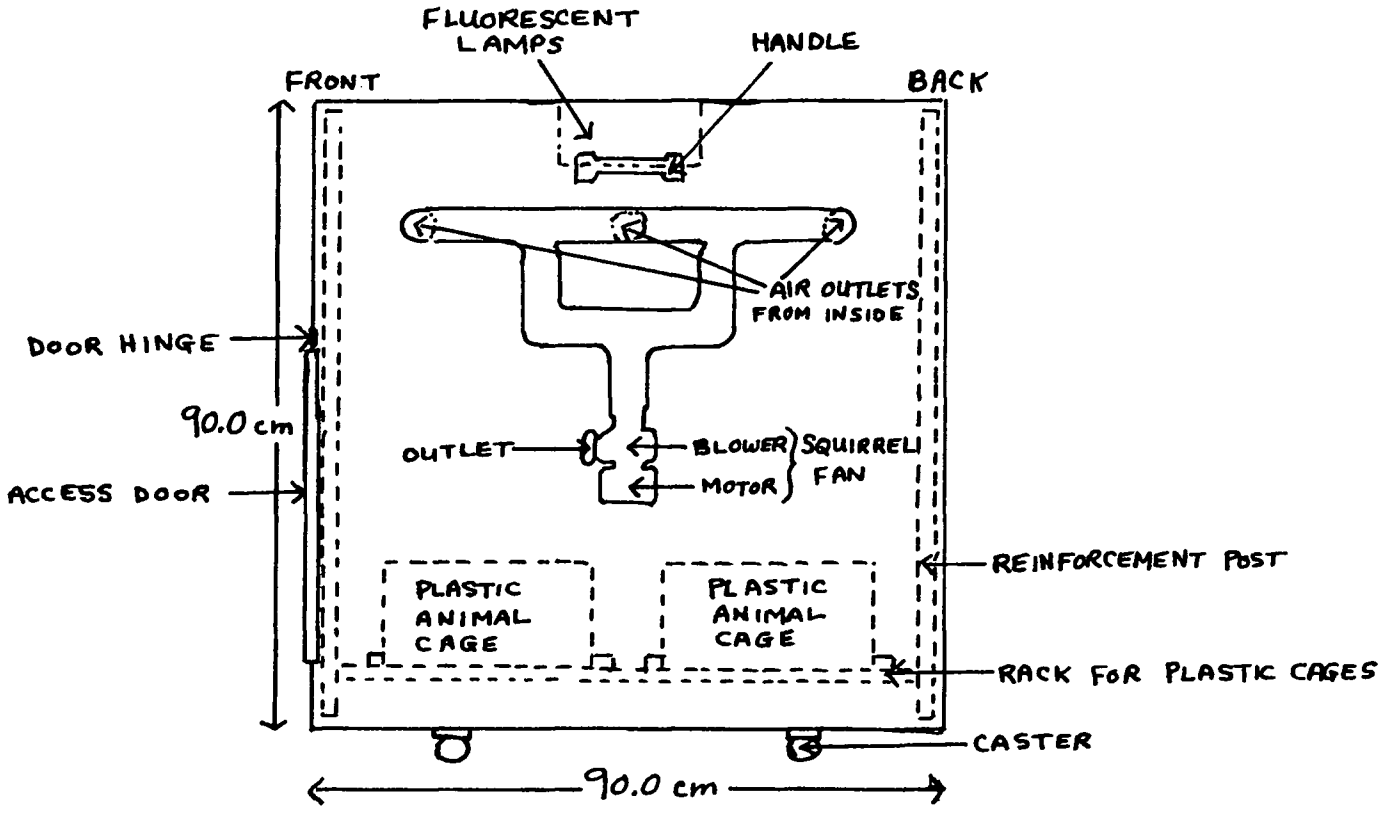
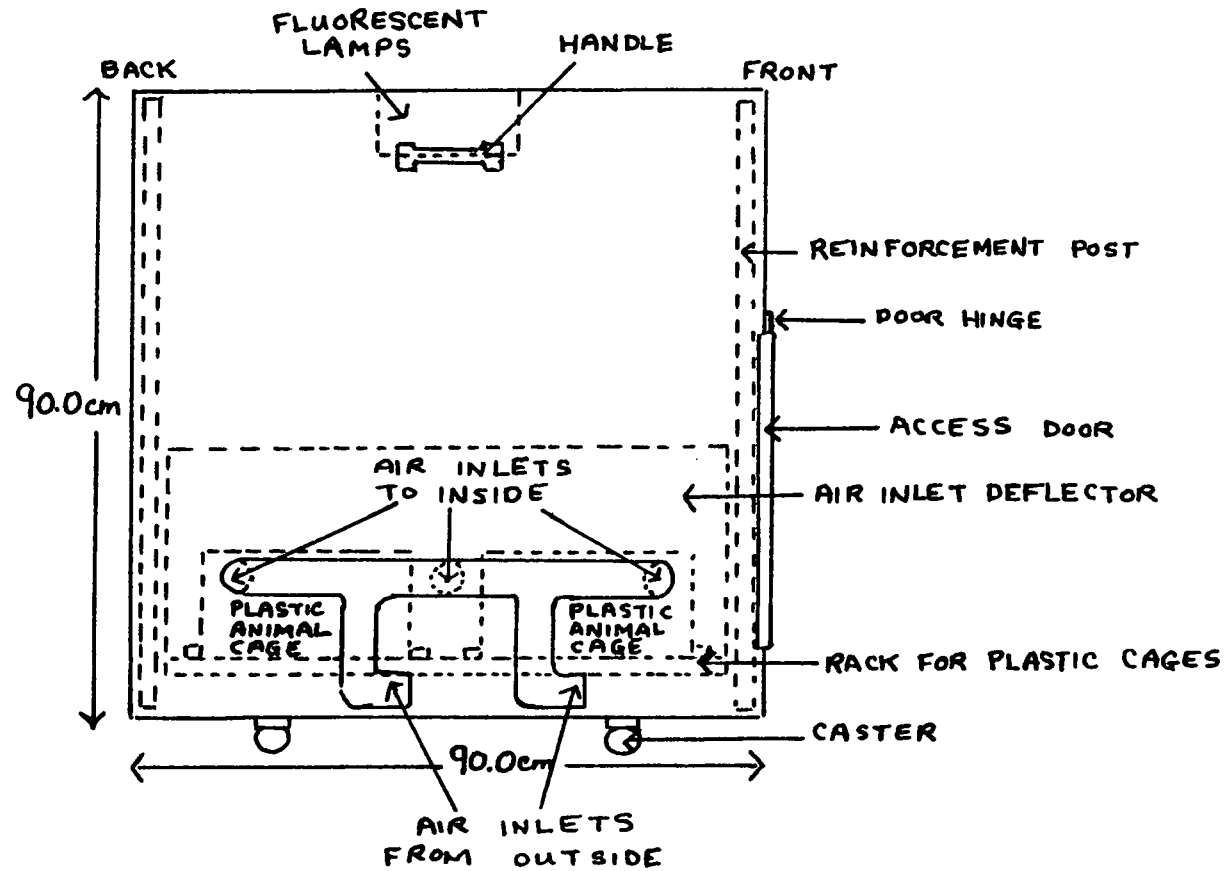
[26]Model no. 2C782, Dayton Electric Manufacturing Company, Chicago, IL.



Appendix Figure A.1. Environmental box, top view.



Appendix Figure A.2. Environmental box, outlet side view.



Appendix Figure A.3. Environmental box, inlet side view.

curved black plastic pipe was used to prevent infiltration of outside light while maintaining constant air circulation. A minimum/maximum thermometer[27] was hung in each box in order to monitor daily the interior temperature which was manually recorded simultaneously with the temperature of the air-conditioned laboratory room in which the boxes were housed. The front of the environmental boxes each had recessed doors which were fitted with hinges on the top edge, enabling the door to be swung open from the bottom adjacent to the animal cage for routine care. Also each box was fitted with casters and handles to facilitate in moving them.

On the ceiling of three boxes fluorescent luminaires[28] were mounted into which two 120.0 cm fluorescent lamps (described previously) were installed. The fourth box had no lighting; its interior was painted black. The interiors of the aforementioned three boxes were painted white.

The boxes were housed in an air-conditioned room having an average daily temperature of 21°C. The three illuminated environmental boxes each averaged 27°C throughout the experiment; and the box remaining in darkness averaged 23°C.

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[27]Model no. 5458, Taylor Instrument, Division of Sybron Corporation, Arden, NC.

[28]Model C-240, Keystone Lighting Corporation, Bristol, PA.

Appendix B

Spectroradiometer[29]

The system used to measure the wavelengths emitted by the three types of fluorescent lamps installed in the environmental boxes was a spectroradiometer[30] which had been modified and characterized by the National Bureau of Standards (NBS), Washington, D.C. A Teflon[31] diffuser was substituted for the "fused silica" diffuser in order to give better cosine response and greater sensitivity in the ultraviolet region of 286 to 320 nm. The wavelength readability and short-term precision were improved to approximately 0.1 nm by adding a helipot dial for wavelength indications. The NBS made a wavelength calibration, determined the spectral responsivity of the instrument in the region from 275 to 350 nm, and determined the slit function. Standard lamps, traceable to NBS, were used to check irradiance measurements. The ultraviolet standard lamp[32] was calibrated from 180 to 400 nm; the

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[29]Personal communication, Dr. Robert C. Worrest, General Science, Oregon State University, Corvallis, OR.

[30]Model 2900 SR, Gamma Scientific, Inc., San Diego, CA.

[31]Dupont registered trademark, E.I. Dupont DeNemours, Wilmington, DE.

[32]Model UV-40, Optronic Laboratories, Orlando, FL.

visible standard lamp[33] was calibrated from 380 to 1100 nm; and two sunlamps[34] were calibrated from 275 to 350 nm.

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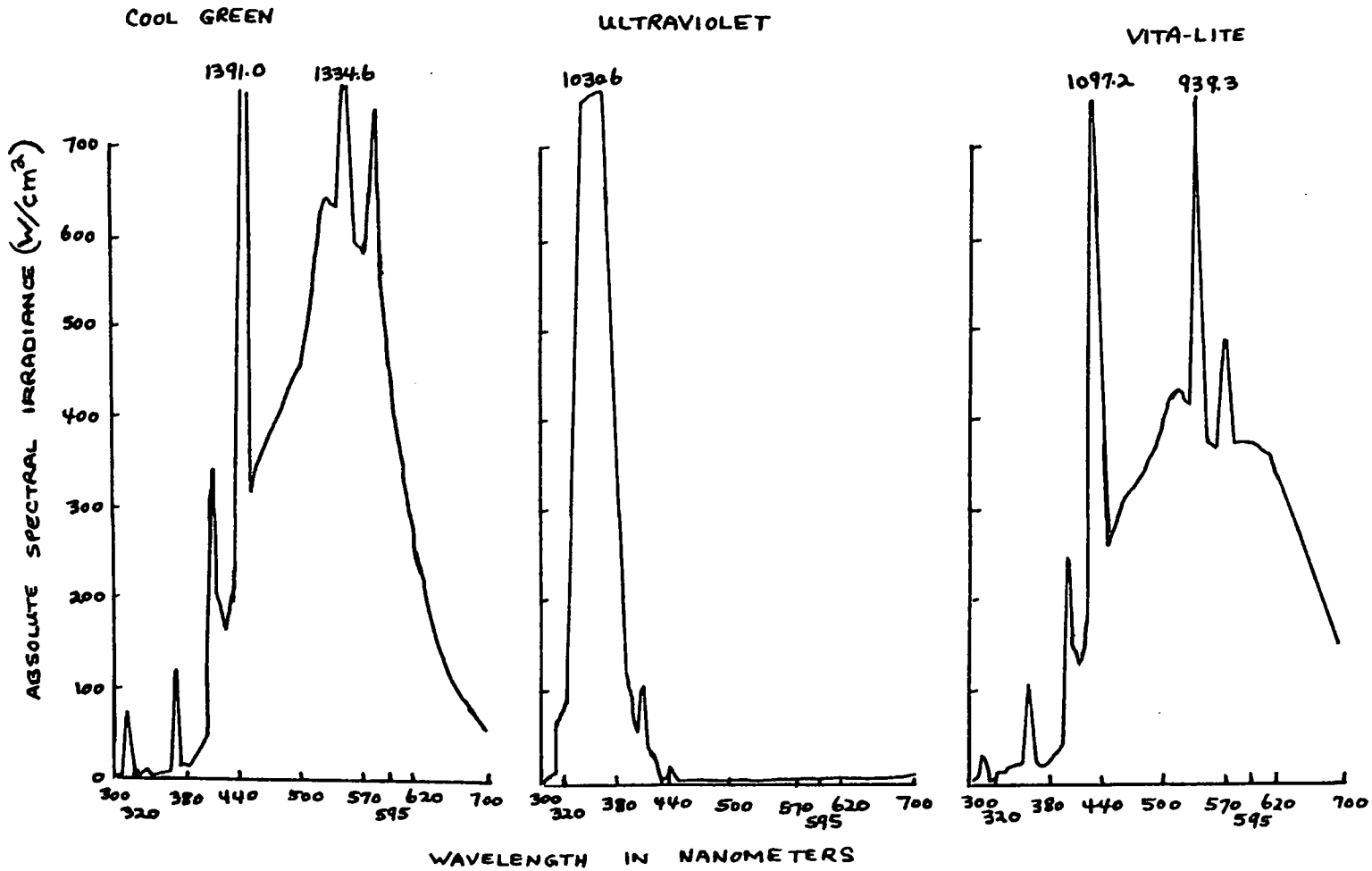
[33]Model 220-9A, Gamma Scientific, Inc., San Diego, CA.

[34]Model BZS, Westinghouse Electric Corporation, Bloomfield, NJ.



Appendix Figure B.1. Spectral distribution curves of the three types of fluorescent lamps in the environmental boxes. Spectra 300 to 320 nm - middle UV; 320 to 380 nm - near UV; 380 to 440 nm- violet; 440 to 500 nm - blue; 500 to 570 nm - green; 570 to 595 nm - yellow; 595 to 620 nm - orange; and 620 to 700 nm - red.

Appendix Table B.1. Absolute spectral irradiance of the fluorescent lamps.

Wavelength nm	Cool green Sylvania F40 CG W/cm^2	Vita-Lite Duro-Test 40W W/cm^2	Ultraviolet Sylvania F40 BLB W/cm^2
300	0.4	0.2	1.7
310	0.2	0.4	9.7
320	0.2	1.6	88.0
330	0.3	6.0	327.0
340	0.5	13.8	743.8
350	1.3	20.8	1030.6
360	3.7	22.2	944.4
370	14.7	25.7	592.4
380	13.2	19.7	283.5
390	25.0	27.2	128.0
400	46.2	39.6	49.7
410	207.0	159.0	51.4
420	156.0	126.0	4.9
430	219.0	178.0	1.4
440	883.0	688.0	1.0
450	313.0	258.0	0.5
460	350.0	288.0	0.4
470	381.0	314.0	0.4
480	398.0	331.0	0.3
490	420.0	345.0	0.3
500	444.0	358.0	0.3
510	502.0	383.0	0.3
520	591.0	422.0	0.3
530	635.0	435.0	0.3
540	620.0	413.0	0.3
550	940.0	613.0	0.3
560	585.0	375.0	0.3
570	571.0	368.0	0.3
580	730.0	491.0	0.3
590	496.0	374.0	0.3
600	434.0	378.0	0.4
610	364.0	375.0	0.0
620	299.0	370.0	0.1
630	239.0	358.0	0.1
640	192.0	338.0	0.2
650	154.0	314.0	0.5
660	123.0	285.0	0.9
670	99.0	252.0	1.5
680	79.5	219.0	2.3
690	68.2	187.0	3.6
700	53.8	157.0	4.6
546.5	1334.6	939.3	0.4
437.0	1391.0	1097.2	17.9
406.5	336.6	253.2	108.9
365.0	115.0	111.9	874.8
334.0	7.5	14.8	478.4
313.0	69.9	29.3	65.5

Appendix C

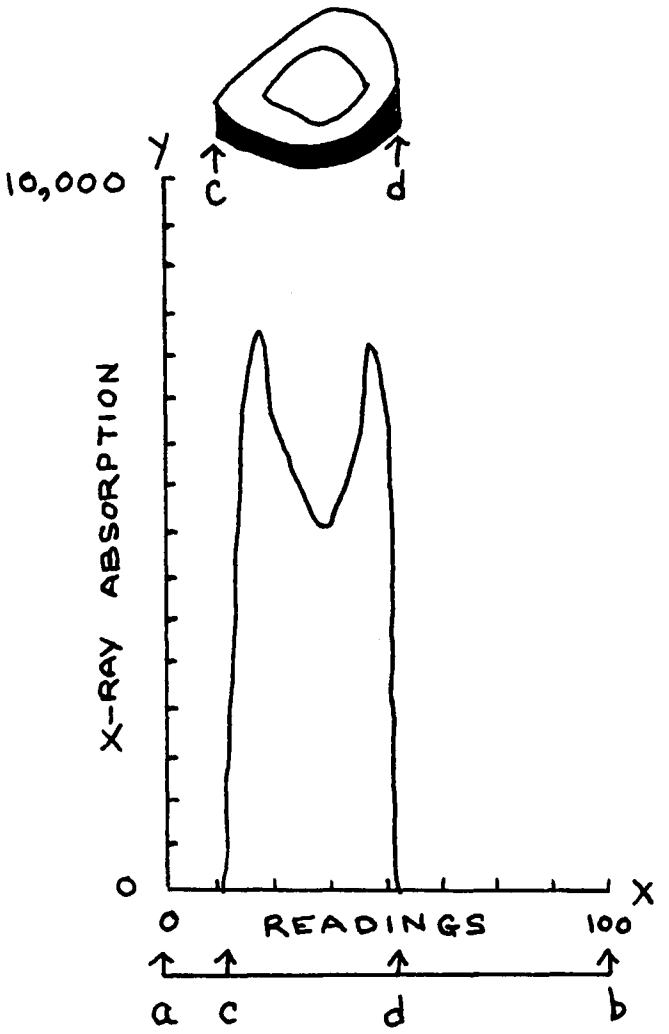
Conversion of densitometer readings to bone density values

There was an input into the computer of the actual densitometer readings determined by the densitometer from the optical density of the x-ray film of the left femur. A correction factor for the subtraction of the soft tissue around the femur was incorporated into the computer program. This resulted in an output of adjusted densitometer readings which were used as the data to compute the bone density measurements.

The formula used to determine the approximate bone density of the left femur was:

$$\sum_{i=1}^{100} y_i \Big/ \pi \left(\frac{d-c}{2}\right)^2$$

where $\sum_{i=1}^{100} y_i$ represents the sum of the 100 adjusted densitometer readings between points a and b (Appendix Figure C.1). Each of the values y is the approximate total mass along the line from the point x on the film to the x-ray source, so $\sum_{i=1}^{100} y_i$ is the approximate total mass of the cross-section. And $\pi \left(\frac{d-c}{2}\right)^2$ represents the approximate area of the bone, assuming the cross-section of the bone is circular, which of course it is not. C and d represent the points at either side of the bone. Therefore, d minus c is the diameter of the femur on the line read by the densitometer.



Appendix Figure C.1. Diagram of a densitometer absorption trace along a line of 100 densitometer readings (a to b) from the x-ray film of the left femur of a rat. The shaded area (c to d) of the femur cross-section represents the side of the bone which was on the plane of the x-ray film (not to scale).

Sample calculation:

if $\sum_{i=1}^{100} y_i = 217,654$

$c = 13$

$d = 52$

$$\frac{\sum_{i=1}^{100} y_i}{\pi\left(\frac{d-c}{2}\right)^2} = \frac{217,654}{\pi\left(\frac{52-13}{2}\right)^2}$$

= 182.2 bone mass/unit of bone area

or

an approximate bone density of 182.2

Appendix D

Analysis of variance

Appendix Table D.1. Variable: Change in serum alkaline phosphatase

	DF	Mean square	Significance of F
Light type	3	49.002	-[a]
Rat type	1	20.335	-
Interaction	3	31.939	-
Residual	34	40.453	

Appendix Table D.2. Variable: Change in serum total calcium

	DF	Mean square	Significance of F
Light type	3	0.307	-
Rat type	1	0.088	-
Interaction	3	0.180	-
Residual	33	0.147	

Appendix Table D.3. Variable: Change in serum total phosphorus

	DF	Mean square	Significance of F
Light type	3	5.527	-
Rat type	1	3.522	-
Interaction	3	0.542	-
Residual	28	2.657	

Analysis of variance (continued)

Appendix Table D.4. Variable: Bone density after treatment (x-ray)

	DF	Mean equare	Significance of F
Light type	3	150.899	-
Rat type	1	440.969	-
Interaction	3	1336.385	0.05
Residual	38	477.652	

Appendix Table D.5. Variable: Change in bone deneity (x-ray)

	DF	Mean equare	Significance of F
Light type	3	919.950	-
Rat type	1	354.146	-
Interaction	3	3879.143	0.05
Residual	38	1018.823	

Appendix Table D.6. Variable: Physical bone density (water displacement)

	DF	Mean equare	Significance of F
Light type	3	0.024	-
Rat type	1	0.058	0.01
Interaction	3	0.063	0.01
Reeidual	40	0.009	

Analysis of variance (continued)

Appendix Table D.7. Variable: Percent ash per gram of dry fat-free weight

	DF	Mean equare	Significance of F
Light type	3	3.181	0.01
Rat type	1	0.075	-
Interaction	3	0.856	-
Residual	40	0.654	

Appendix Table D.8. Variable: Ash calcium

	DF	Mean square	Significance of F
Light type	3	69.091	-
Rat type	1	211.260	0.05
Interaction	3	10.807	-
Residual	40	40.769	

Appendix Table D.9. Variable: Ash phosphorus

	DF	Mean square	Significance of F
Light type	3	247.841	-
Rat type	1	28.060	-
Interaction	3	24.884	-
Residual	40	126.228	

[a]Below 5% level of significance