THE EFFECTS OF HIGH LEVELS OF VITAMIN D_2 ON YOUNG CALVES

by

Richard Fredrick Bristol

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Pathology

Iowa State University
Of Science and Technology
Ames, Iowa

1966

TABLE OF CONTENTS

INTRODUCTION

The increase in the use of vitamin D as a feed additive and as a readily available therapeutic agent has also increased the danger of the overuse of this vitamin. Therefore, the possible toxic effects of vitamin D become increasingly important in the diagnosis and treatment of animal disease conditions.

In animals, the primary source of natural vitamin D is in the action of the ultra violet rays of the sun upon the sterols contained in the epidermis, Reed et al. (1939). It is obvious, then, that the vitamin D made available to an animal varies with the weather, cloud cover, exposure to sunlight, animal covering, and the season of the year. The ingestion of animal, fish or vegetable nutrients can further vary the amount of vitamin D made available to a given animal.

The feed industry, in an attempt to stabilize the amount of known vitamin D that is available to an animal, has included in their formulation of supplements, concentrate, and complete feeds various forms of vitamin D. Through advances in manufacturing techniques the industry has developed vitamin D supplements[1] that contain as much as 80,000,000 units of vitamin D_2 for each pound of base.

Injectable products are available which contain 1,000,000

---

[1]Irradiated Dry Yeast type 178F. Standard Brands, Inc., New York, New York.

units of vitamin D per gram, Reed et al. (1939, p. 3). One gram of this substance contains enough vitamin D_2 to supplement the diet of a 100 pound calf for approximately 3,333 days.[1]

It is fortunate that the range between the therapeutic dose of vitamin D and the known amount which produces disturbances in the body is larger than any known potent pharmaceutical.[2]

Little work has been done on the effects of vitamin D_2 in the young calf since the work of Duncan (Duncan and Huffman, 1934). Yet there is clinical evidence to indicate that vitamin D_2 intoxication may be a complicating factor in disease conditions of the young calf.[3]

This study was undertaken to determine what possible effects high levels of vitamin D had on the young calf. The objectives were to establish a basis for further studies in regard to clinical diagnosis, histopathologic characteristics, radiographic characteristics, and clinical pathologic characteristics.

---

[1]The National Research Council recommends the supplementation of 300 I.U. of vitamin D_2 to each 100 pounds of body weight, National Research Council (1958).

[2]Clinically up to 1,000,000 units of vitamin D have been used in so-called shock therapy and no ill effects observed, Reed et al. (1939, p. 430).

[3]Stange Memorial Clinic hospital case number 09649.

REVIEW OF LITERATURE

It is unfortunate that during the early work on vitamin D and in the experimental work that followed, the unit of dosage varied and the defined limits of the amount of vitamin D active product used were confusing. This made interpretation of the results of the experimental effort almost impossible. It was not until the League of Nations Health Organization established the vitamin D standard unit in 1935 that interpretation of experimental results could follow a definite pattern, League of Nations Health Organization (1935).

For this reason the literature review is divided into two sections -- work prior to 1935 and work since that time.

Part I. Cited Work Prior to 1935

Early recognition that large amounts of cod liver oil would cause alterations in the metabolic pattern of animals followed soon after the discovery of the antirachitic property of the unsaponifiable fraction of the oil by Zucker and others, Zucker *et al*. (1922).

The earliest reference to knowledge on the toxic limit of irradiated ergosterol was made by Rosenheim and Webster who reported that 10,000 times the effective dose for rickets was not lethal to rats, Rosenheim and Webster (1927). Kreitmair and Moll using what was termed tremendous doses described pathological findings for several species and observed

differences in species susceptibility, Kreitmair and Moll (1928). Varela and his collaborators observed that rats died within 6 weeks when fed a high calcium rickets producing diet, plus 50,000 times an overdosage of activated ergosterol, Varela et al. (1930). Harris and Moore in 1928 noted that rats receiving .1% irradiated ergosterol in their daily diet lost weight rapidly and were dead after a period of 20 days. They demonstrated that 1000 times the antirachitic dose was harmless to rats, whereas 100,000 times the antirachitic dose was rapidly fatal, Harris and Moore (1929). Light and his associates stated that 100,000 times an overdosage was fatal, while 10,000 times the antirachitic dose had no effect on growth, Light et al. (1929).

In 1930 Bills and Wirick in a long term study involving large numbers of rats from infancy to maturity found that 1000 times the overdosage was rarely harmful, 4000 times the overdosage definitely injurious and 40,000 times the overdosage definitely toxic to rats on a "normal" diet, Bills and Wirick (1930).

That the composition of the diet exerts an important influence upon the effects produced by vitamin D was stated and confirmed by numerous authors. Calcium salts and high calcium intake seem to increase the severity of the lesions produced, Bills and Wirick (1930), Harris (1930), Hoyle (1930a, b), Duguid (1930a), Harris and Innes (1931), Jones et al. (1930), Shelling (1931), Hauch (1934),

Gough et al. (1933), and Underhill (1928).

Toxic levels

Man As much as 40 times the ordinary dose of irradiated ergosterol has been administered to babies for periods of six months with no adverse effects other than a tendency toward colic and constipation, Hess et al. (1930). The before mentioned authors did note an increase in appetite over this period, Hess et al. (1930). Three hundred times the normal dose was administered to patients for several days to correct parathyroid tetany with the tetany being alleviated and no toxic symptoms being observed, Reed and Seed (1933). It has been shown that doses this large given to apparently normal adults can induce mild toxic effects when continued for several weeks, Rappaport and Reed (1933). These results were confirmed by other workers, Crimm (1932), Crimm et al. (1933). These investigators working with tubercular patients found that 150 times the overdosage, continued for a period of time, resulted in a hypercalcemia; further treatment caused toxic symptoms, Crimm (1932), Crimm et al. (1933).

Shelling and Hopper found that the production of toxic symptoms depends upon the daily dosage, duration of dosage, route of administration, calcium and phosphorus content of the diet, age and physical activity of the subject, individual variation in susceptibility and other unknown factors,

Shelling and Hopper (1934), Bills (1935). In general, the toxic effects appear at approximately 200 times the ordinary dose if administered daily over several weeks, Crimm (1932), Reed and Seed (1933).

The exact lethal dose for man is not known. Large doses were given by various workers to treat hay fever, impotency and other ailments. These doses approximated 920 times the ordinary dose of irradiated ergosterol. These doses produced no cases of serious poisoning, Rappaport and Reed (1933), Reed (1934). Larger doses were administered by Spies and Hanzal to terminal patients. The maximum given was about 6,000 times the ordinary dose, Spies and Hanzal (1934). This produced a hypercalcemia but no calcification of the tissues, Spies and Hanzal (1934).

Other species Kreitmair and Moll compared the susceptibility of rats, mice, guinea pigs, rabbits, cats, dogs, chickens and axolotls. They found that cats and rabbits were the most sensitive, with chickens and axolotls extremely resistant, Kreitmair and Moll (1928). Others have compared rats, mice, chickens, guinea pigs, rabbits, cats and dogs. The order of listing is the usual order of their increasing susceptibility, Taylor et al. (1931). The above authors also stated that the young seemed to be more susceptible than the old, Taylor et al. (1931). This was confirmed by Harris and Moore, Harris and Moore (1929). Hauch, however, felt the older animal to be more susceptible, Hauch (1934).

Freeman and Farmer found that 26,000 rat units were toxic to rabbits if administered for 15 days or longer, Freeman and Farmer (1930). Reed, in an experiment with dogs, found 12,000 I.U. per kilo of body weight intoxicated 11 out of 14 in 15 days; at 4,000 units per kilo 7 out of 12 were intoxicated in 15 days, Reed (1938).

Bovine Agduhr noted that calves became apathetic with a concurrent diarrhea upon being fed .6 to 1.6 cc of cod liver oil per kilogram of body weight, Agduhr (1927). The interpretation of his results is difficult, however, because he was using a proprietary product known as "Vigantol" which is known to contain toxisterol and the toxicity manifested may have been due to this product, Bills (1935).

Duncan and Huffman, in experiments with one to two month old calves noted that the degree of toxicity of irradiated ergosterol is partially dependent upon the calcium phosphorus ratio in the diet. They were able to produce death by feeding 25 ml of Viosterol[1] for a period of 27 days, Duncan and Huffman (1934).

The clinical signs of vitamin D intoxication

Man In man the clinical signs have been described as a sense of well being with an increased appetite changing to nausea and loss of appetite. Vomition, cramps, diarrhea and

---

[1]Mead Johnson, New York, New York.

polyuria follow. Dizziness, muscular weakness, headache, lapse of memory and occasional numbness are usually associated with the intoxication, Crimm (1932), Rappaport and Reed (1933), Reed (1934).

Other species In rats, inappetence, diarrhea, greasy hair coat, failure of conception, reabsorption of feti, failure of growth, loss of body weight, weakness, labored breathing and convulsive tremors, albumin in the urine, hypercalcemia, and lack of ovulation have been noted and recorded by many authors, Pfannenstiel (1928), Harris and Moore (1929), Light et al. (1929), Harris and Stewart (1929), Harris and Moore (1929), Ashford (1930), Spies and Glover (1930), Duguid (1930b), Van Donk (1933).

In rabbits further signs were noted such as spontaneous fractues, soft pliable ribs and skull bones that were soft and easily damaged, Schmidtmann (1930).

In dogs, Jones found a hypercalcemia and a greatly increased water consumption, Jones and Robson (1932), Light et al. (1931).

Bovine Apathy, diarrhea, polyuria, eczema, cardiac infections, with a tendency toward diseases of the lungs were noted by Agduhr, Agduhr (1927). Duncan and Huffman recorded that symptoms were not particularly noticeable during the first seven days of their experiment. After that time the calves became drowsy and apathetic. They became indifferent to food. The feces were soft and contained

some blood but a severe diarrhea was not observed. No other clinical signs were recorded, Duncan and Huffman (1934).

Necropsy and histopathologic observations

Man During the early years of the discovery of vitamin D intoxication there was a lack of reports in the literature of deaths that might have been caused by vitamin D.

Other species In the rat, excessive growth of the incisor teeth has been noted, Light et al. (1929). Grossly observable calcification of the lungs, kidney, aorta, heart and pylorus of the stomach, Hojer (1926), Harris and Moore (1929), Harris and Stewart (1929), Duguid (1934), Hoyle (1930a, b), Spies (1930), Ashford (1930). The spleen and thymus were found to be atrophic by Hoyle (1930a, b), Harris and Moore (1929). Calcareous deposits were found in the bladder, Harris and Moore (1929), Harris and Stewart (1929). The histopathologic alterations of the kidney were observed to be calcareous deposits in the afferent tubules and calcification in the medulla, cortex, and pelvis, Harris and Moore (1929), Light et al. (1931), Hoyle (1930a, b), Spies (1930), Gough (1933).

The heart muscle showed areas of calcification with the small arteries of the heart thickened and calcified. There was one calcified mass obscuring all structural detail of the

heart, Harris and Moore (1929), Hoyle (1930a, b), Spies (1930), Shohl et al. (1930), Duguid (1930a, 1934), Shelling and Asher (1932), Gough et al. (1933). There was calcification in the cartilagenous tissues of the lungs, Harris and Moore (1929), Hoyle (1930a, b), Spies (1930).

The duodenum showed calcification of the reticular tissues, Harris and Moore (1929). Further, calcification of the ovaries and adrenals was noted by Spies and Glover (1930).

The aorta and smaller arteries were extensively calcified, Vanderveer (1931), Harris and Innes (1931), Hoyle (1930a, b), Shohl et al. (1930), Spies and Glover (1930). Hoyle described the lesions in the aorta in this manner, "The aorta many times was converted into a severe and brittle tube", Hoyle (1930a, b).

A description of the histopathologic alteration was given by Harris and Moore as follows: "The calcareous deposits were found in the kidneys, heart muscle, pylorus of the stomach and aorta. There was extensive calcification of the tunica media. The small arteries of the heart muscles were thickened and calcified, and calcified cartilaginous tissue was found in the lungs. There was calcium deposition in progress in the small intestine", Harris and Moore (1929).

In further work Harris and Innes found in addition, the production of densely calcified overgrowth at the ends of

the long bone and the compact bone became spongy, Harris and Innes (1931). Vanderveer found that the arterial lesions observed were not those of a specific type but resembled the lesions produced by the administration of epinephrine hydrochloride, barium chloride, and digitalin. Inflammatory factors did not seem to be a factor in the involvement, Vanderveer (1931). Duguid described vitamin D arterial sclerosis as a muscle degeneration and calcification of the media resembling that seen in Mönokeberg sclerosis of the peripheral arteries of man, Duguid (1934). He further described the kidney alterations as a chronic nephritis characterized by hyaline degeneration, a thickening of Bowman's capsule, atrophy and finally calcification, Duguid (1934), Hoyle (1930a, b).

The changes in the skeletal tissues were described by Jones and Robson in 1933. Osteoclasts were found to be very numerous. The inner surface of the bone was covered by a thin layer of osteoid. The epiphyseal cartilage was unchanged. The spongy bone of the metaphysis showed irregular bony spicules with areas of resorption. The appearance of cortical bone and spongy bone was one of osteoclastic resorption with accompanying new bone formation. In none of the sections studied did they find filling of defects with fibrous tissue. Where bone disappeared the defect was filled with marrow. All animals under the conditions of their experiment showed a definite reduction in ash content

of the bone when compared to the controls, Jones and Robson (1932). Shohl and his co-workers found that chemically there was a demineralization of bone, Shohl et al. (1930). Schmidtmann found similar lesions in rabbits, Schmidtmann (1930). Ham and Lewis, however, found that the prolonged and excessive feeding of vitamin D to young rats not only prevents calcification from progressing normally but also prevents the proper development of the long bones. They did not feel that osteoclasts were the agency responsible in causing decalcification or in preventing normal calcification, Ham and Lewis (1934).

Reed and his associates found that the calcium content of any tissue is significantly increased in rats, Reed et al. (1933).

Bovine On necropsy, Agduhr found that Peyer's plaques were enlarged, the red bone marrow atrophied with a disintegration of the blood forming elements. The spleen was enlarged and pigmented. The adrenal glands showed a pigment atrophy which appeared with regularity. There were ulcerations in the mucosa of the fundus and pylorus. Miliary hemorrhages were present in the lungs. There was chronic passive congestion present. Nephrosis was usually present.

Histopathologic alterations were observed in the liver which showed a central necrosis, yellow atrophy of the liver cells and a degeneration of the Kupffer cells. The pancreas contained areas of necrosis. There was a pigment atrophy

of the heart with vacuolization of the muscle cells. The lungs showed atelectasis and edema, Agduhr (1927). Duncan and Huffman found a marked hemorrhagic gastritis. The kidneys of both calves were soft and enlarged; in addition, there were large reddish gray spots. One animal had enlarged lymph nodes along the backbone. No abnormal calcification was discernable upon microscopic examination of the tissues, Duncan and Huffman (1934).

Radiographic observations

Man Review of the literature did not reveal reports of any radiographic lesions present in humans as a result of vitamin D intoxication.

Other species In 1933 Jones and Robson used an undescribed densitometer to demonstrate the comparative density of the long bones of rats fed large doses of irradiated ergosterol, Jones and Robson (1932). They found an increase in density at the ends of the long bones of those animals on a high irradiated ergosterol intake, Jones and Robson (1932), Shohl et al. (1930).

Physiologic observations

Man and other species Early workers on rickets realized that vitamin D in some way regulates the passage of calcium and phosphorus across the intestinal wall, Harris and Innes (1931), Harris (1932). These investigators held the view that vitamin D exerts its action by regulating

the blood calcium and/or phosphate, Harris and Innes (1931). As the dosage of vitamin D becomes larger and larger, it would seem that there would be an increased absorption from the gut and an increased retention by the blood with a subsequent increase in excretion by the kidney, Bills (1935). Crimm and Strayer found that massive doses of vitamin D increased both the diffusible and nondiffusible fractions of calcium in human blood, Crimm and Strayer (1934). Crimm and his associates reported that soon after the administration of vitamin D there was a decrease in urinary calcium. This they felt might be related to renal and bladder stones sometimes associated with overdosage, Crimm et al. (1933). The increase in urinary calcium has been noted by many investigators in other species, Dixon and Hoyle (1928), Harris and Moore (1929), Hoyle and Buckland (1929), and Hoyle (1930a, b). Ashford found that during hypervitaminosis D there is an increase in the excretion of inorganic phosphorus by the kidney and a greatly increased excretion of calcium, Ashford (1930), Watchorn (1930). According to Shohl, blood serum calcium was higher in groups of rats receiving irradiated ergosterol, Shohl (1930). Jones found that dogs developed a hypercalcemia within two weeks after receiving the first oral administration of irradiated ergosterol, Jones et al. (1930). Light et al. reported an increase in blood serum calcium and phosphorus, Light et al. (1929).

Bovine Duncan and Huffman did not report a hypercalcemia in young calves receiving large doses of Viosterol. The serum calcium increased moderately within 72 hours after Viosterol was first fed and then returned to its former level. This finding was also true of blood phosphorus levels except that phosphorus seemed to peak at about 24 hours after Viosterol was first fed. All returned to pre-Viosterol feeding levels after 14 days on experiment, Duncan and Huffman (1934). They further reported an increase in urinary calcium excretion, Duncan and Huffman (1934).

Hess and co-workers noted a slight increase in serum calcium and a marked increase in serum phosphorus in adult cows fed varying amounts of vitamin D, Hess et al. (1932).

The only report of blood cellular element changes comes from Agduhr who reports a decrease in the number of red blood cells with an increase in hemoglobin and large numbers of erythroblasts visable. He further reported an increase in the number of white blood cells and the appearance of a large form of a blood platelet. There was an increase in lymphocytes with a gradual decrease in polymorphonuclear cells. If dosing continued for a period of time, a leukopenia appeared. The white blood cells decrease to 1/10 the normal value, Agduhr (1927).

Part II. Cited Work After 1935

Between 1935 and 1940 there is a pronounced gap in the literature in regard to intoxications due to vitamin D. Research seemed to be at a standstill until further interest in vitamin D intoxication was stimulated by the use of massive doses of the vitamin for the treatment of infant rickets and arthritis, Reed et al. (1939).

Toxic levels

Man Danowski and Winkler reported on two cases of hypercalcemia and renal failure produced by massive doses of vitamin D for arthritis therapy. The two fatal cases reported received 50,000 I.U. to 500,000 I.U. daily for 8 weeks, Danowski et al. (1945). These workers cautioned against administering vitamin D to patients in amounts sufficient to produce hypercalcemia, Danowski et al. (1945). Covey and Whitlock report that vitamin D causes parencymal degeneration in the kidney when doses greater than 150,000 units per day are used, Covey and Whitlock (1946). Hyde and Hyde reported on a case of an adult who had taken 200,000 units per day for 17 months with resultant metastatic calcification and death, Hyde and Hyde (1947). Other deaths were reported due to overdosage of vitamin D by the following authors: Bevans and Taylor (1947), Walsh and Howard (1947), Kaufman et al. (1947), Donegan et al. (1949), Davies (1960). Anning and coworkers noted that in a series of 200 patients treated with

large doses of calciferol, 19 per cent developed intoxication, with no fatalities reported. A dose of more than 1,100 units per kilogram may cause symptoms indicative of toxicity, Anning et al. (1948).

Other species A case of hypervitaminosis D in monkeys at the Radiobiological Laboratory of the University of Texas and the United States Air Force Base, Austin, Texas is reported by Kent and co-workers, Kent et al. (1958). An error in making up the diet of the entire colony of monkeys caused them to receive excessive amounts of calcium, phosphorus and vitamin D. A number of deaths resulted before a diagnosis was established. These animals received 162,000 U.S.P. units of vitamin D per animal per day, Kent et al. (1958).

Clinical signs

Man In man the signs of intoxication due to vitamin D are a hypercalcemia, vertigo, nausea, diarrhea, and generalized renal failure, Davies (1960), Donegan et al. (1949), Howard and Meyer (1948), Kaufman et al. (1947), Bevans and Taylor (1947), Covey and Whitlock (1946). In addition, Walsh and Howard reported crystals in the bulbar conjunctiva with bilateral band keratitis, Walsh and Howard (1947), Howard and Meyer (1948).

Other species In monkeys the first clinical evidence of hypervitaminosis D is an increased incidence of

upper respiratory infection and diarrhea, Kent et al. (1958). There was a weight loss that was progressive until the toxic diet was terminated, Kent et al. (1958). In gravid rats it has been found that pregnancy offers some degree of protection from hypervitaminosis D, Potvliege (1962). In dogs, weakness, lassitude, nausea, anorexia, polydipsia, polyuria, and diarrhea were the clinical signs produced by Becks in experiments to determine the effect of high doses of vitamin D on the dental structure, Becks (1942). These clinical signs were produced while giving the dogs 10,000 I.U. of vitamin D per kilogram of body weight per day, Becks (1942). Dogs in another experiment showed a 32-61 per cent loss in body weight, Mulligan and Stricker (1948).

Bovine Greig noted that when 20 ml of a water miscible preparation of vitamin D_3 was given to dairy cows intravenously, they developed a vague discomfort, reduced appetite and a weak, fast pulse. Later cardiac irregularity developed. Cardiac output diminished. These signs persisted for a week or more, Greig (1963).

Swan administered orally 10,000,000 units of vitamin D for 10 days to an adult cow. He observed no clinical symptoms and no lesions when the animal was killed two months later. He further gave 20,000,000 units daily for ten days. There were no symptoms, but lesions of calcification were detected in the left heart and aorta when the animal was killed two

months later. A cow was given 10,000,000 units daily for three days before calving. There were no symptoms, but there was a slight calcification of the posterior half of the aorta when the cow was killed two months later. Upon subcutaneous injection of equivalent amounts of material, he noted no symptoms in the animal given 10,000,000 units of vitamin D but did note lesions of calcification in the posterior one-half of the aorta. Another animal receiving 20,000,000 units daily for 10 days showed diarrhea, cessation of lactation and rapid weight loss. The animal was killed seven weeks after administration of the vitamin D. Lesions of calcification were found in the lungs, kidneys, pleura, peritoneum, both auricles, aorta and pulmonary vein. A four weeks embryo was present and showed signs of imminent abortion.

Upon intramuscular injection, an animal receiving 10,000,000 units daily for 10 days failed to show symptoms or lesions. Another animal received 20,000,000 units of vitamin D daily intramuscularly for 10 days and conceived one month after treatment but aborted three months prior to term. Lesions of calcification were present in the posterior portion of the aorta, Swan (1952).

Necropsy and histopathologic alterations

Man The main necropsy and histopathologic observations in humans was an overwhelming metastatic calcification

with extensive renal damage, Walsh and Howard (1947), Donegan et al. (1949), Davies (1960), Bevans and Taylor (1947), Kaufman et al. (1947), Covey and Whitlock (1946), Howard and Meyer (1948).

Other species Kent and his co-workers reported that in monkeys the characteristic lesions found consisted of mineral deposits with and without associated inflammation. These deposits were found in kidney, heart, lungs, and, in two animals the liver was also affected in this manner. The kidneys of each animal that died showed some mineral deposition. The earliest lesions consisted of occasional small deposits in the tubules, often in the basement membranes. As the intoxication progressed, severe lesions were noted. These consisted of grossly visible yellowish, white flecks in the cortex which were visible on postmortem roentgenograms. Microscopically the deposits appeared in the tubules or the surrounding interstitial tissue, the thinner portion of the loop tubules was involved more frequently. No discernable lesions were found in the proximal tubules and medullary collecting tubules. Foreign body type giant cells and mononuclear cells were found most frequently in the surrounding tissue. In several cases deposits were noted in the glomeruli and walls of small arteries and veins. No stones were found in the urinary tract. The lungs were often affected but not as regularly as were the kidneys. Mineral deposits were seen in the basement membranes of the

small bronchi and alveolar ducts. The cell types found were mononuclear and foreign body giant cells. Further multiple granulomas were found in many advanced cases. In older animals laryngeal cartilages were often calcified. Bronchial cartilages were frequently calcified.

The cardiovascular system showed a greenish yellow mottling of the myocardium. Myofibril reaction was demonstrated microscopically. Lesions were found more frequently on the left ventricle, never in the right atrium. Mineral deposits were also found in the aortas of many of the animals. The deposits were in the media along the elastic fibers. Small vessels were not frequently affected.

The submaxillary salivary glands were frequently mineralized. Mineral deposits were also noted in the mucosa of the stomach in some animals.

No mineral deposits were noted in the pituitary, thyroid or pancreas. However, laminated deposits were frequently noted in the cortex of the adrenal gland.

Few changes were noted in the musculoskeletal system, Kent et al. (1958).

In the rabbit, hypervitaminosis D induced widespread calcification unaccompanied by increase in serum calcium according to experiments conducted by Eisenstein and others, Eisenstein et al. (1962).

Experiments in rabbits conducted by Hass and his associates showed that hypervitaminosis D caused the bones to

become more brittle than normal due to resorption of cortical bone. It resembled human osteosclerosis. In animals severely affected, there were calcium deposits in the aorta and its major branches. The deposits were most frequently found in the media. Calcium deposits were less frequently found in the kidneys. Tracheal and bronchial cartilages, gastric mucosa, and muscle were often rigid and calcified. Thin plaques of calcium were often found in the mucosa of the larger respiratory passages.

The deposition of calcium in the heart was associated with the internal elastic membrane of the coronary arteries, smooth muscle of the coronary arteries and the fibroelastic tissue of the endocardium.

The cardiac orifice of the alimentary tract contained calcium deposits. The kidneys showed calcification of the convoluted tubules. There were calcium deposits in the thyroid and thymus, Hass et al. (1958).

In rats, Thomas and Morgan reported extensive bone changes in animals fed 400,000 units per kilogram per day. Examination of the tibia showed some decrease in the zone of proliferative cartilage due to persistence of calcified cartilage matrix surrounded by new bone and excessive osteoid tissue. The metaphysis were much wider than normal. In rats on experiment 14 days, active bone resorption had rendered the mid-metaphyseal area almost devoid of bone.

This process was characterized by the presence of partially destroyed trabeculae, large vascular spaces and numerous osteoclasts. Throughout the tibia there was abundant osteoid tissue. These changes were less marked in those animals on experiment for 7 days.

Rats given 100,000 I.U. per kilogram per day were hypercalcemic, but the changes in the bones were much less striking, Thomas and Morgan (1958).

In 1942 Becks conducted a series of experiments on dogs to determine the effect of overdosage of vitamin D on the dental and paradental structures. He concluded that overdosage of vitamin D leads to malocclusion and malformation of the teeth, osteosclerosis of the jaw bone and paradental structures. He found the formation of multiple pulp stones. Further, the marrow cavities were closed by increased bone formation or amorphous calcium deposits. There was calcification of the peridental membrane and gum tissue, Becks (1942), Becks et al. (1945), Hendricks (1947).

In monkeys, Kent et al. reported a significant rise in serum calcium and phosphorus as well as blood urea nitrogen in those animals accidentally poisoned, Kent et al. (1958).

Rats showed increased serum calcium levels, Eisenstein and Groff (1957), Opper (1941), Potvliege (1962), Williams et al. (1962).

In rabbits, Eisenstein noted an increase in serum phosphorus but no increase in serum calcium levels,

Eisenstein et al. (1962).

Swine Quarterman and co-workers made these observations in swine fed 250,000 I.U. of vitamin D daily: "The aorta was the only organ that showed microscopic lesions. These were infrequent and took the form of small irregular raised areas roughly elliptical in shape and each with a slightly depressed center. The vascular endothelium remained smooth and the lesions evidently subintimal."

The microscopic lesions observed by these investigators were described as follows, "All kidneys from pigs killed at time 0 showed pathological depositions of calcium salts in the cortex and in the medulla. Numerous small foci were present, most commonly in/or adjacent to the epithelial cells of the renal tubules. Areas of calcification in the wall of a tubule were commonly overgrown by epithelial cells of normal appearance, resulting in the development of knob-like protuberances into the lumen. There was calcification of glomerular capillaries of the epithelium of Bowmans capsule and also in the interstitial tissue. There were foci of calcification in the walls of the larger renal arteries which showed local necrosis of smooth muscle cells in the vessel wall in the affected areas. The noncalcified tubular epithelium appeared normal, but cystic dilation of the renal tubules was common and there was considerable disorganization of the renal cortex. There were occasional groups of mononuclear cells and a general enlargement of

interstitial cells of fibroblast type. Sections stained by Van Gieson's method showed an early, diffuse fibrosis", Quarterman et al. (1964). These same investigators found foci of calcification in the bronchial cartilage, bronchial mucosa and the bronchial smooth muscle of all animals receiving vitamin D. The aortas of two of six pigs showed calcified areas in the vessel wall. These foci caused a distortion of the vessel wall but did not affect the endothelium. The hearts of four out of six of those pigs receiving vitamin D showed cardiac damage. This consisted of multiple lesions in the coronary arteries in all chambers of the heart. The auricles were most severely affected and the left ventricle least severely. Adjacent elastic membrane was destroyed.

Clinically the animals showed periods of inappetence, a poor growth rate and elevated blood citrate and blood calcium levels, Quarterman et al. (1964).

Bovine Conrad and Hansard, after feeding 5,000,000 units of vitamin D for 5 days to six month old Hereford calves, observed increased deposition of calcium in areas of new bone formation and increased calcium deposition in the kidney and esophagus, Conrad and Hansard (1957). Mac Donald, after feeding a baby calf 1,000,000 units a day for 60 days noticed active and fibrosing medullary lesions in the kidney. Glomeruli appeared to be affected. In another calf, glomeruli were severely affected, Mac Donald

(1958). Cole and his associates reported a case of Viosterol poisoning in a Jersey cow as a result of overdosage of vitamin D in studies on the prevention of milk fever. The animal was given 30,000,000 units of Viosterol per day for 20 days prior to calving, Cole et al (1957).

Hibbs and co-workers reported no increases in serum calcium or serum phosphorus levels of the blood in cows fed 1,000,000 units per day of vitamin D, Hibbs et al. (1946). When the dosage was increased to 2,000,000 units per day, there was an increase in serum calcium and phosphorus values, Hibbs et al. (1946). In the latter trial, there was a concurrent increase in magnesium with the decrease in phosphorus and calcium, Hibbs et al. (1946). Alkaline phosphatase is variable under both levels of feeding, Hibbs et al. (1946). Conrad and Hansard, in six month old calves fed 5,000,000 units for a 5 day period noted a transitory increase in blood calcium, Conrad and Hansard (1957). They did observe an increase in blood phosphorus which was also transitory and preceded the blood serum calcium increase, Conrad and Hansard (1957). Greig noted that injections of vitamin D were invariably followed by an increase in serum calcium reaching its peak in about 7 days but returning to normal in two to three weeks. The serum inorganic phosphorus also increased, reaching a peak in about 14 days and dropping slowly after that time. Serum magnesium concentration showed an inverse trend, Greig (1963).

Blood cellular elements

In monkeys, a decrease in erythrocytes and hemoglobin occurred during and after the period of excessive vitamin D intake, Kent et al. (1958), Melville et al. (1960). A similar finding has been reported in humans, Scharfman and Proff (1956). Melville and others reported a lower white blood cell count in monkeys subject to acute hypervitaminosis D, Melville et al. (1960).

MATERIALS AND METHODS

Clinical Materials and Methods

Three groups of six each, uncastrated grade Holstein male calves ranging in age from three to four days and weighing from 80 to 100 pounds were purchased from Woodland Dairy Farms, Story City, Iowa.

The calves had all received colostrum for three days, were weaned and transported to the Stange Memorial Clinic on the third or fourth day after birth.

As the calves were unloaded from the truck they were selected at random to determine whether they would be controls, high level (1,250,000 I.U. of Vitamin D_2 per day) or low level (300,000 I.U. of Vitamin D_2 per day). Two calves were placed in each classification (two controls, two low level, two high level). The experimental number was tattooed on the inside of the left ear. The calves were started on treatment 12 hours after being placed on experiment (Figure 1).

Upon arrival they were given a complete physical examination which consisted of the following:

I. General appearance
   - A. Behavior
   - B. Condition
   - C. Posture
   - D. Locomotion
   - E. Visible deformities

Experimental number	Department of pathology number	Amount of vitamin D_2 administered per day	Number of days on experiment
1	64-P-853	1,250,000 I.U.	14
2	64-P-854	300,000 I.U.	14
3	64-P-855	CONTROL	14
0	64-P-856	1,250,000 I.U.	14
5	64-P-857	300,000 I.U.	14
6	64-P-858	CONTROL	14
7	64-P-859	1,250,000 I.U.	28
8	64-P-860	1,250,000 I.U.	28
9	64-P-861	300,000 I.U.	28
10	64-P-862	300,000 I.U.	28
11	64-P-863	CONTROL	28
12	64-P-864	Calf died on experimental day 2	
13	64-P-865	1,250,000 I.U.	56
14	64-P-866	1,250,000 I.U.	56
15	64-P-867	300,000 I.U.	56
16	64-P-868	300,000 I.U.	56
17	64-P-869	CONTROL	56
18	64-P-870	CONTROL	56

Figure 1. Identification of experimental calves

F. Appetite

G. Mastication

H. Rumination

I. Weight

II. Physical inspection

A. Rectal temperature

B. Pulse

C. Head

1. Mouth

2. Salivation

3. Breath

4. Teeth

5. Tongue and pharynx

6. Eyes

7. Ears

8. Poll

9. Intermandibular space

10. External lymph glands

11. Nostrils

D. Neck

1. Jugular pulse

2. Prescapular lymph nodes

3. Larynx

E. Thorax

1. Heart

a. Rate

b. Character

c. Abnormal sounds

2. Respiration

a. Rate

b. Character

c. Abnormal sounds

F. Abdomen

1. Size

2. Ausculation

a. Right paralumbar fossa

b. Left paralumbar fossa

3. Palpation

4. Prefemoral lymph nodes

5. Umbilicus

G. Skin

1. Hair coat

2. Parasites

3. Condition

H. Testes

1. Shape

2. Size

I. Feet and legs

1. Joints

2. Abnormalities

J. Character of feces

K. Character of urine

The calves were housed in pens four feet wide by ten feet long. Two calves, each receiving the same level of vitamin D_2, were put in each pen. They were bedded with a liberal quantity of mixed wood shavings which were cleaned of gross debris twice daily. The calves received fresh water, changed twice daily, ad libitum. A good quality alfalfa-brome grass hay was fed free choice after the first seven days on experiment. A grain mixture designed by Iowa State University Department of Animal Science for use in a dairy calf heifer replacement program was weighed and fed ad libitum after the first seven days on experiment, Van Horn and Jacobson (1963). The milk replacer was purchased through a commercial source and fed according to the directions on the label.[1]

The vitamin D_2 used in this experiment was obtained from Standard Brands, Inc. and designated type 178F[2] containing 80,000,000 international units of vitamin D_2 per pound. The determined daily dose of vitamin D_2 for each calf was weighed, placed in a gelatin capsule and administered with a balling gun. The controls received empty gelatin capsules. The vitamin D was given immediately after each morning's feeding of milk replacer.

---

[1]Doughboy calf milk replacer - Formula number 308, Doughboy Industries Inc., Ames, Iowa.

[2]Standard Brands, Inc., New York, New York. Potency confirmed by Pharmatox Laboratories, Ames, Iowa.

All utensils, water and feed containers used for the calves, were washed with soap and water, rinsed with hot water and immersed in a 1:1000 solution of chlorhexidine.[1]

The milk replacer contained twenty per cent milk protein, twenty per cent fat, 20,000 I.U. of vitamin A per pound, 50 grams of chlortetracycline per ton[2], and 4000 I.C. units of vitamin D_3 per pound. The specific ingredients of the milk replacer were dried skim milk, dried buttermilk, animal fat (preserved with butylated hydroxyanisole, propyl gallate, citric acid, prophylene glycol), soy lethicin, vitamin A palmitate (in gelatin) D-activated animal sterol (source of vitamin D_3), dicalcium phosphate, riboflavin, niacin, D pantothenic acid, vitamin B_{12} supplement, vitamin E supplement, 0.25 per cent salt, choline chloride, copper oxide, cobalt carbonate, calcium iodate, ferrous carbonate, zinc oxide, and dried whole whey.

The directions on the label for feeding were:

Age of calf	Cups of milk formula	Pints of warm water
5-7 days	1	2
8-14 days	1	2
15-21 days	1	2 1/2
22-28 days	1	3
29-34 days	1	3
35-37 days	1	3
38-56 days	1	3

---

[1]Nolvasan, Fort Dodge Laboratories, Fort Dodge, Iowa.

[2]Aureomycin, American Cyanamide Inc., Princeton, New Jersey.

These amounts were fed twice daily.

In general, the feeding and management instructions were followed as set forth in the cooperative extension service pamphlet 253, Van Horn and Jacobson (1963).

The pens were of the open variety constructed of two feet by ten feet wood planks with four inches of spacing between each plank. The door closure was solid core three-fourths inch construction plywood. The size was four by ten feet with a small hay manger in one end. Two calves were placed in each pen. The building in which they were kept was heated by a steam blower and kept at a temperature of 56° F. There were six windows and a large door the width of one end of the building, which was half glass panes eight inches by eight inches square.

Two calves from each group were placed on 1,250,000 I.U. of vitamin D_2 per day per os. Two were placed on 300,000 I.U. of vitamin D_2 per day per os. Two calves were designated as controls and received the basal ration plus an empty gelatin capsule per os.

Calves from Group I were kept on experiment 14 days. Calves from Group II were kept on experiment 28 days. Calves from Group III were kept on experiment for 56 days.

The calves were killed and necropsied the day following the last dose of vitamin D_2. In the first group, final physical examination, clinical laboratory procedures and radiographic examination were concluded approximately eight

hours before necropsy. These animals were killed on day 15 of the experiment. For Group II and III the same procedure was followed. However, they were killed and necropsied on the 29th and 57th days respectively.

Radiographic Materials and Methods

Radiographs were taken of all calves prior to the initial administration of vitamin D_2, at each weekly physical examination and immediately after termination of the experiment. The areas radiographed were the radius and ulna, carpus and metacarpus of the left leg, anterior-posterior view.

A Picker Meteor x-ray machine[1], style F10 was used to take the radiographs. Par speed intensifying screens mounted in cassettes were utilized. Kodak medical x-ray film[2] was used in all instances.

The time-temperature method of development as described by Carlson was used in processing the film, Carlson (1961). The films were fixed for at least one-half hour and washed for at least one hour before being dried[3].

---

[1]Picker X-Ray Corporation, White Plains, New York.

[2]Eastman Kodak Company, Rochester, New York.

[3]All radiographs were developed by Mrs. Ellen Houser.

The radiographs were taken with the calves in a standing position and from an anterior posterior view.[1]

A densitometer was used for all radiographs after the first series of pictures of Group I calves[2]. (Figure 2). For purposes of radiographic study the radiolucent area between the metaphyseal side of the diaphysis and the distal epiphysis of the left ulna was measured. Cortical thickness measurements were taken of the left large metacarpal bone (mc 3 and 4) at a point half the distance in length and opposite the fusion of the third and fourth metacarpals. The measurements were made at the thickest part of each cortex of the radiographs of the left metacarpus and the results averaged. Density measurements were taken with a Densometer Photometric Scale[3].

Pathologic Materials and Methods

Necropsy procedure

Each animal was destroyed by electrocution and exsanquinated by an incision in the right jugular vein.

Samples of tissue were taken from the distal end of the metacarpus, adrenal glands, kidneys, aorta (abdominal and

---

[1]Radiographs were taken by Dr. Paul Neubauer.

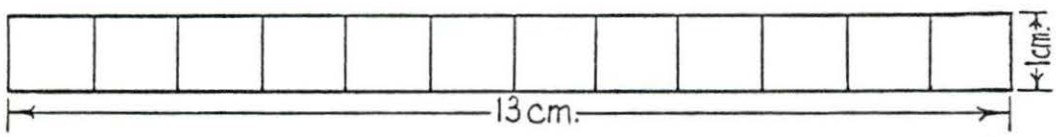
[2]Emmerson, M. A. Iowa State University Department of Clinical Sciences. Personal Communication. October, 1964.

[3]Photovelt Corporation, New York, New York.

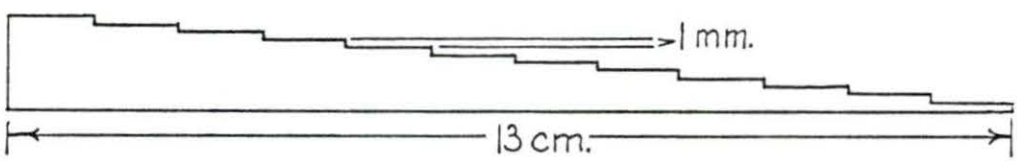
Figure 2. Densitometer used to evaluate portions of the radiographic parts of this experiment. It consisted of a bar of rolled aluminum, 13 centimeters long and one centimeter wide. The thickness was graduated in one millimeter steps and progressed from 1 millimeter up to 12 millimeters in depth

DENSITOMETER USED IN RADIOGRAPHS

TOP VIEW



SIDE VIEW



thoracic), liver, proximal end of the tibia, costochondral junction of the 5th and 6th rib, right central incisor tooth, parathyroid glands, thyroid gland, pituitary gland and thymus gland. These were immediately placed in a solution of 10 per cent buffered formalin. Tissues were left in this solution for 10 days. At this time the collected samples were trimmed and the bone samples placed into nitric acid decalcifying solution under a negative pressure of approximately 18 mm of mercury.[1] The other samples (liver, adrenals, pituitary, heart, aorta, thyroids, parathyroids, kidneys and thymus) were embedded in paraffin.

After imbedding, samples were cut to a thickness of six microns. One sample of the soft tissues was stained with Harris hematoxylin and eosin. A sample of kidney, aorta (abdominal and thoracic) and heart was stained with Von Kossa's stain.

Samples of lung tissue were preserved, embedded, stained and examined only if the lung showed gross pneumonic lesions.

All staining procedures were performed as outlined in the Armed Forces Institute of Pathology Manual, Armed Forces Institute of Pathology (1960).

---

[1]E. D. Roberts, Assistant Professor, Department of Veterinary Pathology, Iowa State University of Science and Technology, Ames, Iowa. A faster method of decalcifying bone. Personal communication, October 1964.

Bacteriologic examinations were made only if experimental animals died during the experiment or showed gross evidence of a disease process at necropsy.

Areas of evaluation

For purposes of microscopic evaluation the following tissues were used: Sections of bone were studied from the central one-third of the distal end of the left metacarpus, the central one-third of the proximal end of the left tibia and the costochondral junctions of the left 5th and 6th rib. The trabecular portions of the rib were taken from approximately three inches dorsal to the epiphyseal plate. The zona compacta of the left tibia was evaluated from pieces of bone removed three to four inches ventral to the proximal epiphyseal plate.

Alveolar bone formation and tooth development were evaluated from sections of the right central incisor tooth.

The articular surfaces were microscopically examined from the central one-third of the proximal articulating surface of the tibia.

Clinical Pathologic Materials and Methods

Thorough physical examinations were conducted prior to placing the animals on experiment, at weekly intervals while on experiment and at the termination of the experiment. Included in the examination were blood studies. Thirty ml of whole blood were taken by an aseptic venipuncture of the

right jugular vein from each calf. Fifteen cc of the blood were prepared with an anticoagulant and the remaining blood was allowed to clot at room temperature for one hour and then refrigerated at 42° F until the following day. The blood prepared with the anticoagulant[1] was used for PCV, hemoglobin determination by acid hematin method, total leukocyte counts and differential leukocyte counts.

The cells were classified according to the Committee on Classification of Cells and Diseases of the Blood and Blood Forming Organs of the American Society of Clinical Pathologists, American Society of Clinical Pathologists (1948).

All tests were performed in duplicate and the results averaged.

The serum fraction of the nonoxalated blood sample was used for serum calcium determinations according to the method of Harper, Harper (1959); inorganic phosphorus according to the method of Fiske and Subbarow, Fiske and Subbarow (1925); and serum magnesium based on the method of Hirschfelder, Hirschfelder (1934). These tests were also run in duplicate and the results averaged.

Sections of the thoracic aorta at the thoracic arch and the abdominal aorta three to four inches caudad to the aortic hiatus were examined histologically.

---

[1] Vacutainer, Becton, Dickenson and Company, Columbus, Nebraska.

Further sections for microscopic examination were taken from the kidney, liver, adrenal gland, parathyroid, thyroid, thymus, pituitary gland, and heart.

RESULTS

Radiographic Interpretations[1]

The calves in the first group (two weeks experimental administration of 1,250,000 I.U. of vitamin D_2 per day) showed a progressive reduction in mineral content of the metacarpals (Mc. 3+4). However, one calf (experimental calf number one) had a greater density in the distal ulnar epiphysis at the end of the experiment than at the beginning (Figure 8).

The calves in Group 2 (four weeks of experimental administration of vitamin D_2 1,250,000 I.U. per day) showed an increased mineralization (increased density) in the area of the epiphyseal plate on the diaphyseal side. This increased density began to show at the end of the second week of the experiment. The density increased on each succeeding radiographic examination and was most pronounced at the end of the experiment. The epiphyseal plate was beginning to ossify at the time the last radiograph was taken (four weeks).

Group 2 calves on the low level (300,000 I.U. D_2) showed a detectable widening of the epiphyseal plate at the end of the first week of the experiment. This widening continued through the third week, but began to disappear during the last week of the experiment. The distal epiphyseal line

---

[1] All radiographic opinions were confirmed by Dr. M. A. Emmerson, Dept. of Radiology, Iowa State University, Ames, Iowa.

of the radius showed signs of closure at the time the last radiograph was taken (four weeks).

Radiographs of the control calf failed to show widening of the epiphyseal plate at the distal end of the radius, and there was no evidence of beginning closure when the experiment was terminated.

Two calves in Group 3 had a definite widening of the distal radial epiphyseal plate that began to appear within two weeks of initiation of experiment. These calves received high levels of vitamin D_2 (1,250,000 I.U. per day). In calf number 13, this widening appeared at the end of the second week of the experiment, whereas in calf number 14, it appeared at the end of the third week of the experiment.

The epiphyseal plate was about doubled in width at the end of the fifth week of the experiment in calf number 13. In calf number 14, the doubling width of the epiphyseal plate did not occur until the seventh week of the experiment. The undulations of the distal radial diaphyseal-epiphyseal junction plus the increased width of the epiphyseal plate made the eighth week radiograph of calf number 13 appear as though three epiphyseal plates were present (Figures 5 and 6). This apparent triple plate was not seen in the radiographs of calf number 14.

Beginning closure of the distal radial epiphyseal-diaphyseal juncture was radiographically detectable at the end of the third week of the experiment in calf number 13,

but did not become evident in calf number 14 until the end of the fourth week.

In the two calves receiving 300,000 units of vitamin D_2 per day, a well defined epiphyseal zone appeared at the end of the second week, with a double transverse zone appearing in the terminal radiograph (end of eighth week of experiment). The beginning closure of the epiphyseal line was visible at the end of the third week.

In the control animals, a double epiphyseal density zone appeared in the radiograph of calf number 17 at the end of the sixth week. This was a sharp clean area without the waffling effect that appeared in the radiographs of the animals on vitamin D_2 supplementation. In calf number 18, no double zone was apparent in the radiographs. The beginning of the epiphyseal line closure was visible at the end of the fourth week in calf number 17, and at the end of the sixth week in calf number 18.

Radiographic Measurements

There was no appreciable difference between the width of the ulnar gap of the control animals and the same area in those whose diet was supplemented with vitamin D_2.

Measurements of the thickness of the shaft cortex did not demonstrate any marked difference between those calves on supplemental vitamin D_2 and the control animals (Figures 3 and 4).

In Group 2, the animals that had been given the highest level of vitamin D_2 (1,250,000 I.U. per day) showed a decrease in the density of the cortices as determined by the use of the densitometer or aluminum step ladder when compared with cortical densities in the control animals (Figure 4).

Pathologic Results

Macroscopic observations

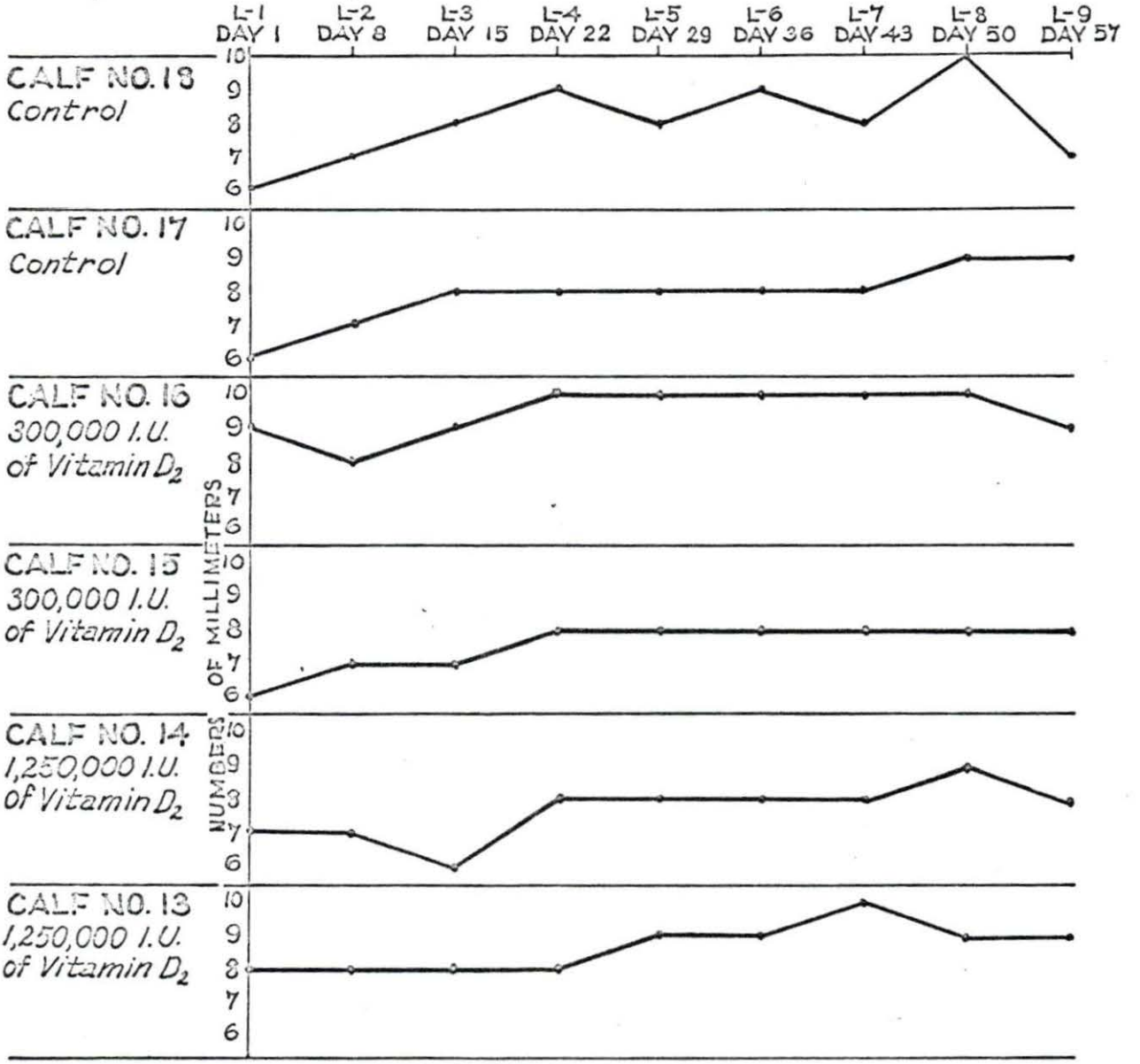
No gross abnormalities were observed in the skin, subcutis or special sense organs of the calves.

Small pneumonic lesions were seen in experimental calf number 6 (Pathology number 64-P-858), calf number 1 (Pathology number 64-P-853), experimental calf number 15 (Pathology number 64-P-867), calf number 18 (Pathology number 64-P-870), and calf number 14 (Pathology number 64-P-866). A severe lobar pneumonia was observed involving the intermediate, right apical, right cardiac, the ventral one-half of the left apical and left cardiac lobes of experimental calf number 3 (Pathology number 64-P-855). Little evidence of any gross abnormalities was found in the cardiovascular system except in experimental calf number 16 (Pathology number 64-P-868). A small plaque was observed in the abdominal aorta. Experimental calf number 18 (Pathology number 64-P-870) displayed a red mottling of the aorta in the region adjacent to the heart. Experimental calf

Figure 3. Results of density measurements conducted on Group 3 calves

CORTICAL BONE DENSITY EQUIVALENT IN MILLIMETERS OF ALUMINUM

Group Three – 57 days on experiment

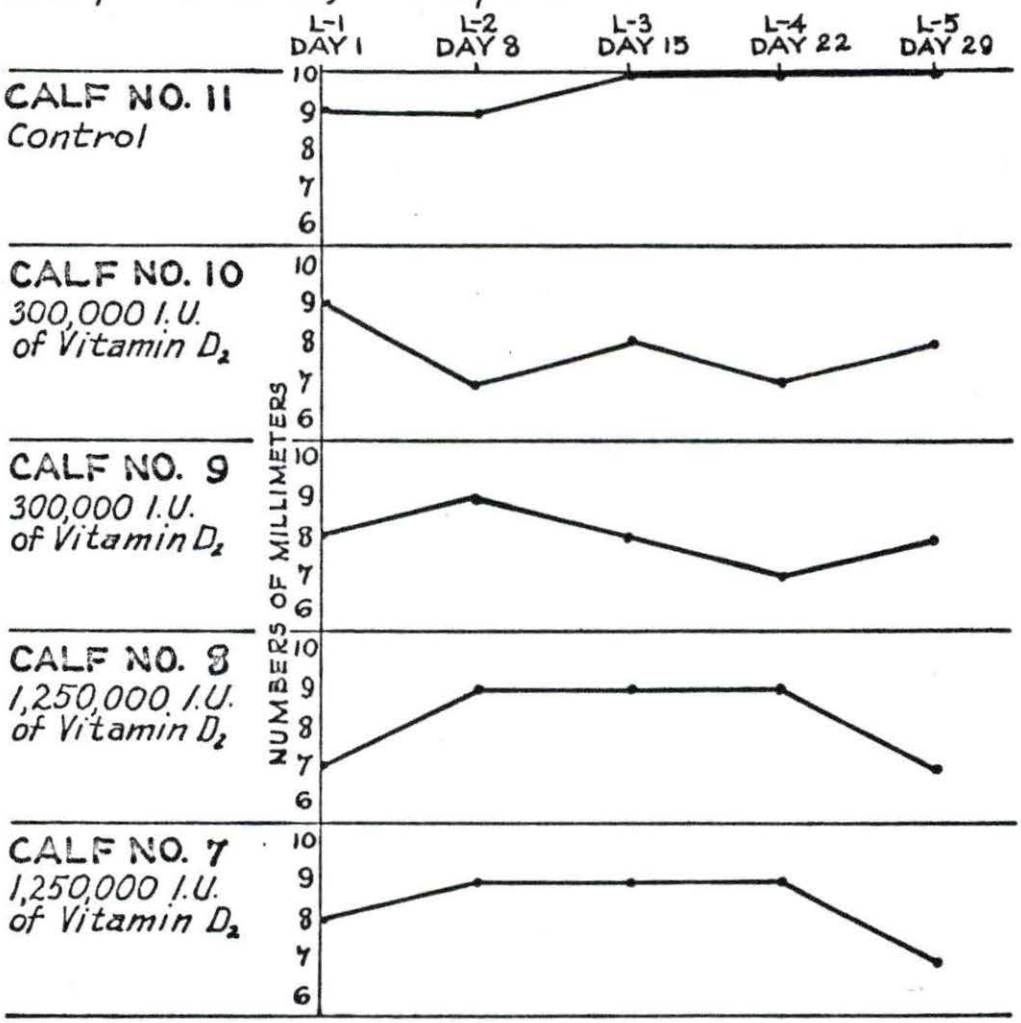


ALL ANIMALS			
Kvp	74	Exposure	0.15
Ma	10	Film size	7"x17"
F.T.D.	36"		

Figure 4. Results of density measurements conducted on control and experimental animals on Group 2 calves

CORTICAL BONE DENSITY EQUIVALENT IN MILLIMETERS OF ALUMINUM

Group Two—29 days on experiment



ALL ANIMALS			
Kvp	74	Exposure	0.15
Ma	10	Film size	7"x17"
F.T.D.	36"		

Figure 5. Photographic reproduction of radiograph showing the distal ends of the left radius and ulna, the carpus and the metacarpals of calf number 13. This calf received 1,250,000 I.U. of vitamin D_2 per day for four weeks. Note the layered appearance (three layers visible) of the widened epiphyseal-diaphyseal plates at the distal end of the radius with intervening areas of increased density (arrow)

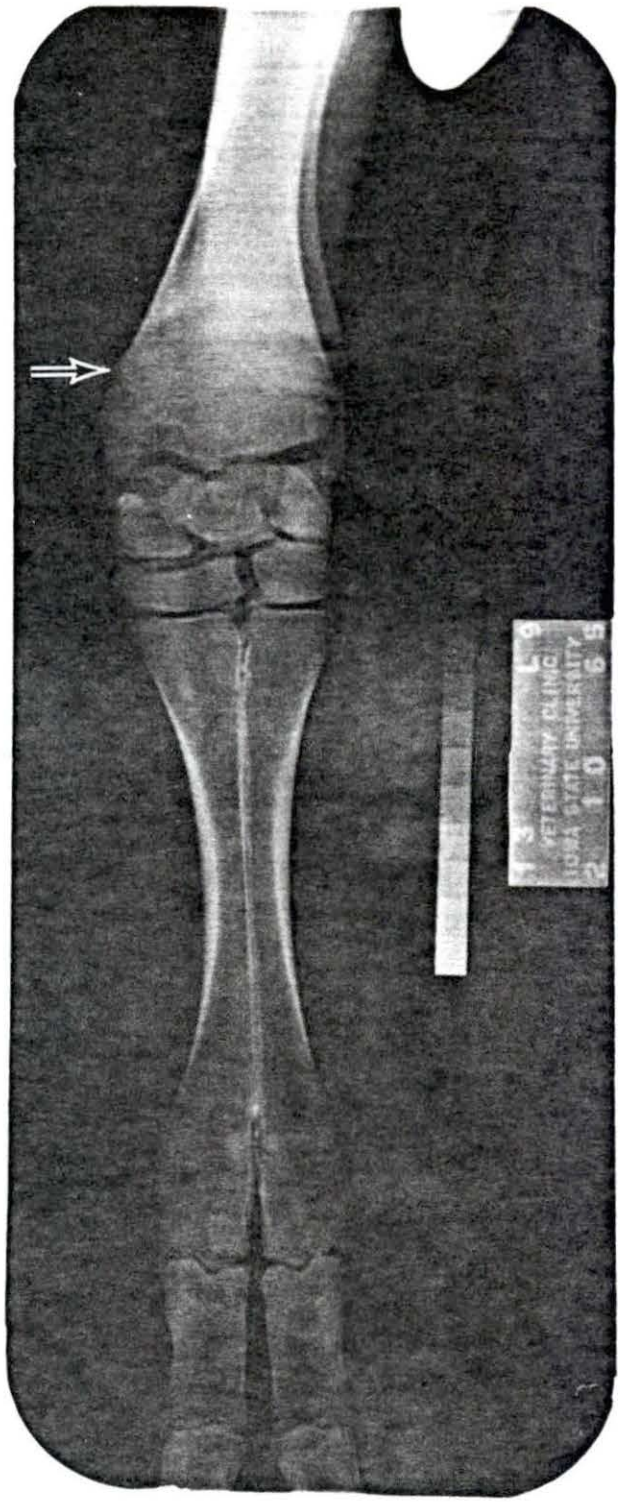

VETERINARY CLINIC
IOWA STATE UNIVERSITY

Figure 6. An enlargement of the radiograph in Figure 5. A photograph of a radiograph of the carpus of experimental calf number 13. This calf was given 1,250,000 I.U. of vitamin D_2 per day for 28 days. Note the outlined irregular triple lines of density in the epiphyseal zone and the increased density on the epiphyseal side of the ulnar gap (arrow)

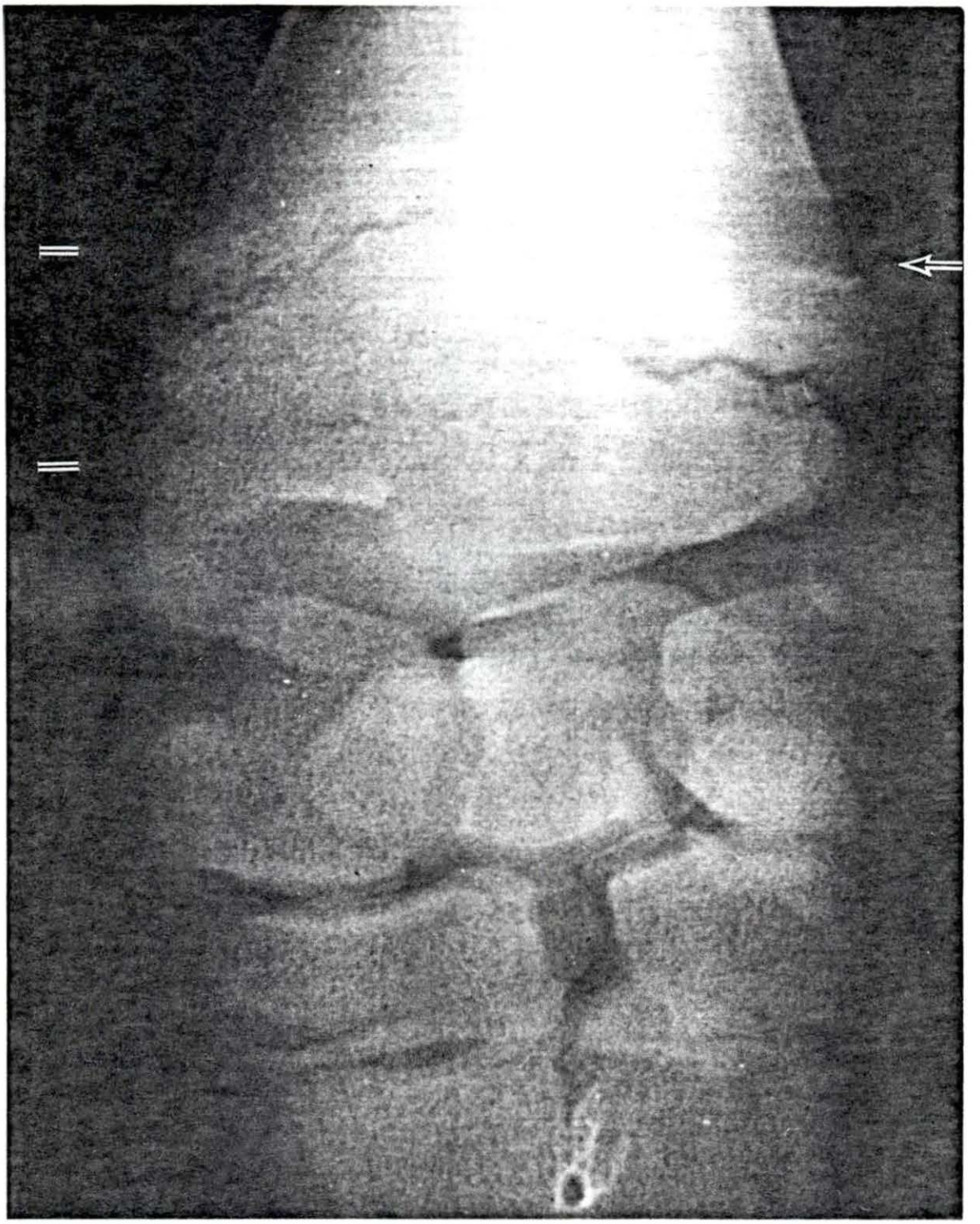


Figure 7. A photograph of a radiograph of the distal end of the radius and ulna, carpus and left metacarpus of experimental calf number 3 - a control animal. Note the lack of increased density at the distal ulnar epiphysis as compared to Figure 8

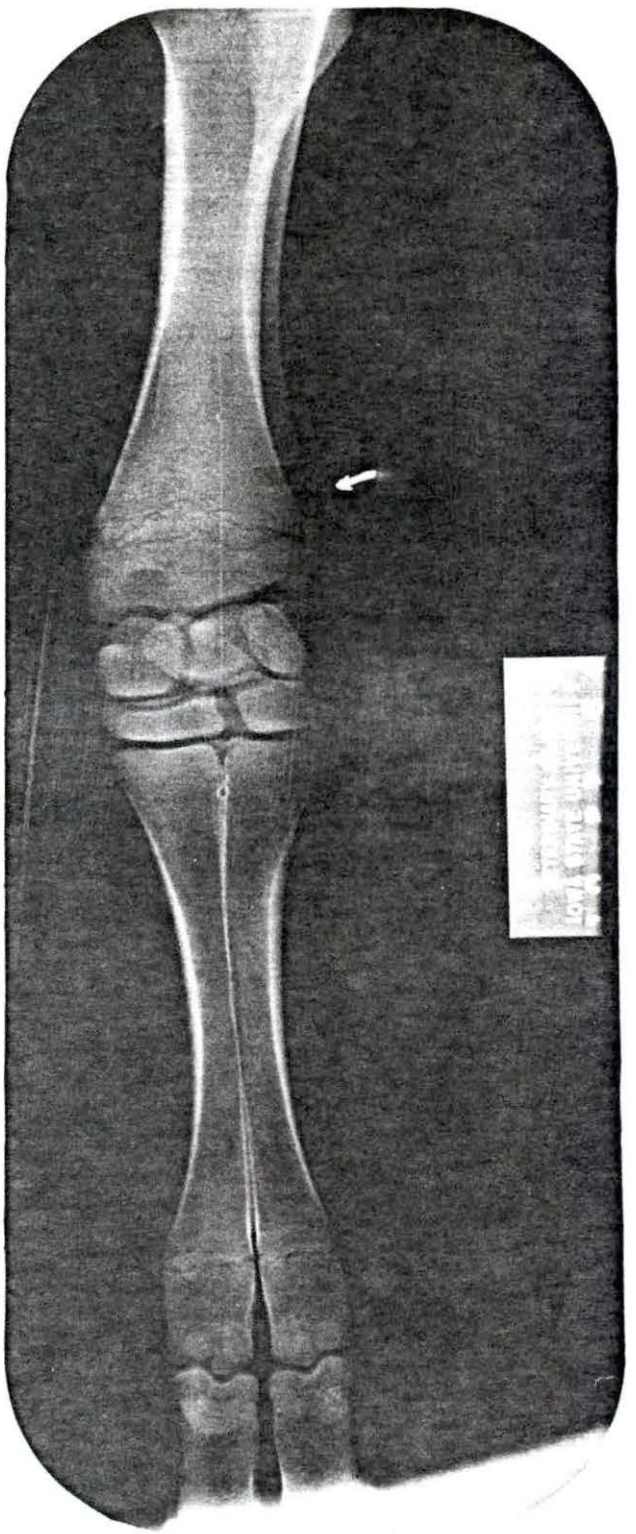


Figure 8. A photograph of a radiograph of the distal end of the left radius and ulna, carpus and metacarpus of experimental calf number 1. The arrow points to the area of increased density on the distal side of the ulnar epiphyseal plate

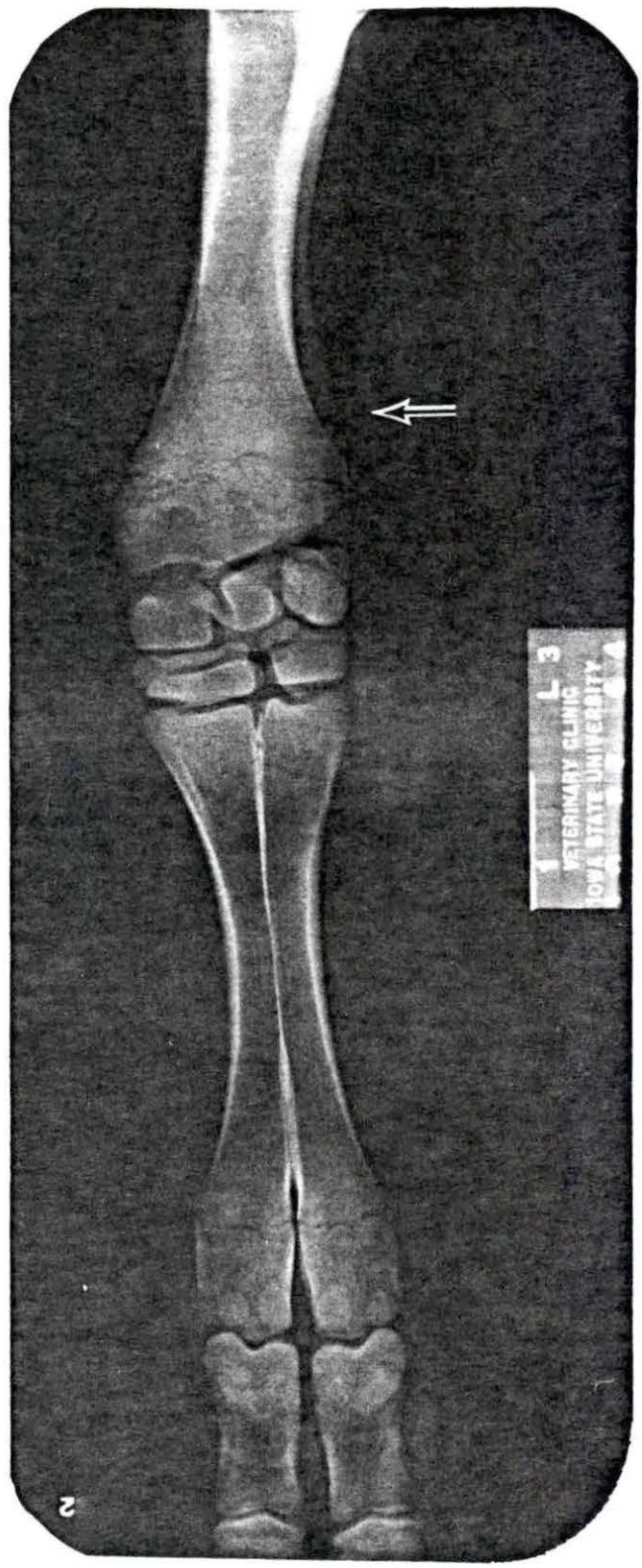
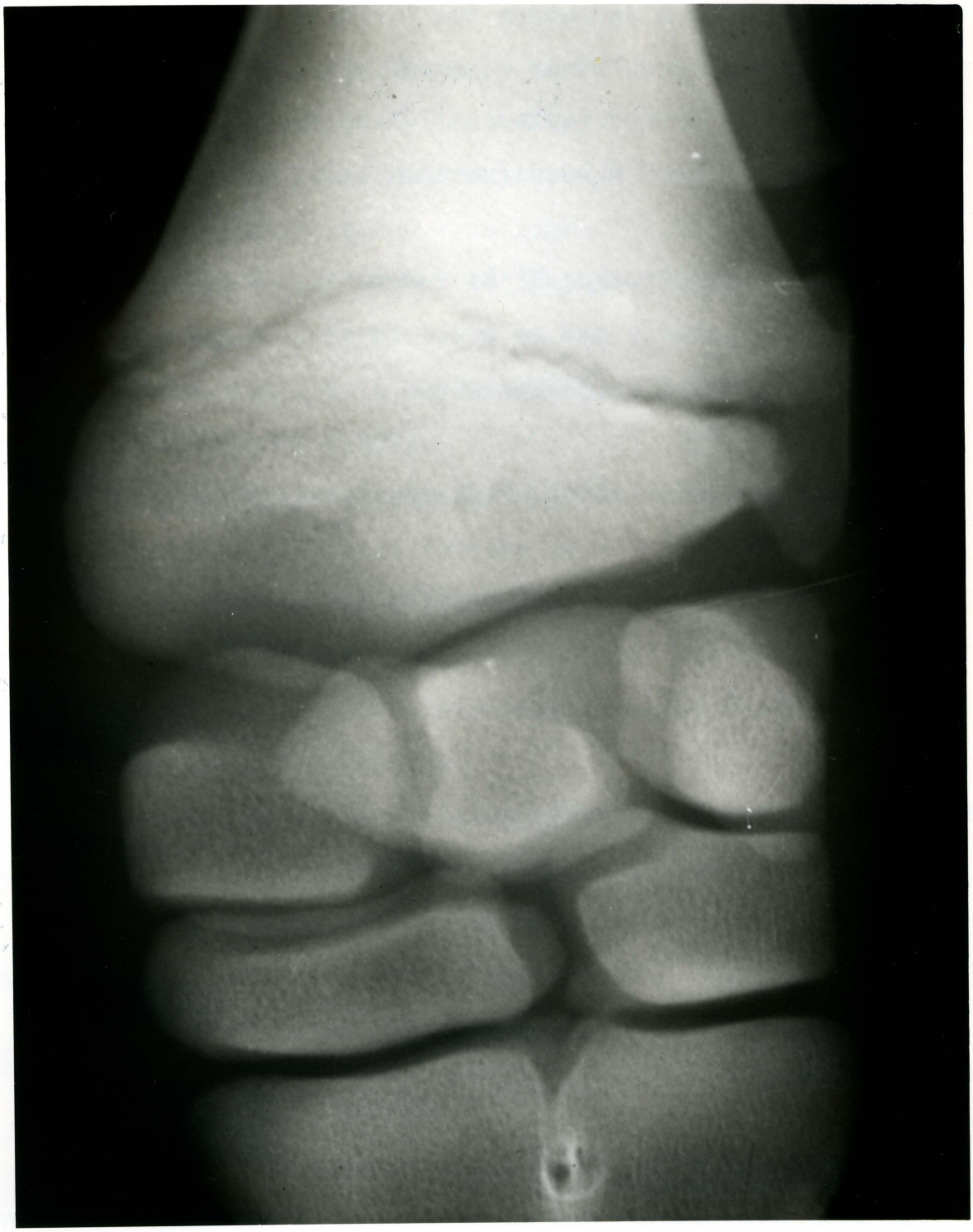

L 3
VETERINARY CLINIC
IOWA STATE UNIVERSITY
2

Figure 9. A photograph of a radiograph of the carpus of experimental calf number 18. This calf was a control animal. There is a lack of a triple epiphyseal zone and a decrease in density on the epiphyseal side of the ulnar gap when compared to calf 13 (Figure 6)



number 13 (Pathology number 64-P-865) displayed a small plaque on both the thoracic and abdominal aorta. A few scattered plaques in the thoracic aorta were observed in experimental calf number 14 (Pathology number 64-P-866).

There were no gross abnormalities detected in the hemopoietic system.

Experimental calf number 18 (Pathology number 64-P-870) had a few mahogany colored spots on the liver. A mild enteritis was observed in experimental calf number 3 (Pathology number 64-P-855).

A focal ulcerative area 10 mm in diameter was observed at the distal articulating surface of the left radius in experimental calf number 6 (Pathology number 64-P-858). The distal metaphysis of the left tibia of experimental calf number 1 (Pathology number 64-P-853) appeared white in color with a wide irregular outline.

A generalized white mottling of both kidneys was observed in experimental calf number 13 (Pathology number 64-P-865).

No gross lesions were observed in the nervous systems or endocrine systems in any of the calves.

Microscopic alterations

Enchondral ossification

Vesicular zone In the two control animals, the vesicular zone was even in outline and varied from five to seven cells in thickness. The cells were arranged in

columns of neat orderly rows. The blood vessel penetration of the hypertrophied cartilage cells was in an orderly manner.

In all animals supplemented with vitamin D_2 the vesicular zone was uneven and irregular. The hypertrophic cartilage cell layer varied from eight to twelve cells in thickness and was arranged in a haphazard fashion. The cartilage cells were active with evidence of mitosis and proliferation. The blood vessel penetration of the zone of hypertrophic cartilage and provisional calcification was irregular. At the costochondral junction of one animal, there was a marked vascularization on the diaphyseal side of the epiphyseal plate resulting in a broadening of the zone of vascular penetration. The vessels penetrated irregularly from two to ten cells into the zone of hypertrophy.

Metaphysis The metaphysis of the control animals contained one matrix trabeculae projecting distally from each two or three columns of hypertrophied cells. New bone formation was observed in the trabeculations. Osteoclasts were present but not in great numbers. Arrangement of the trabeculae in the metaphysis of the animals whose diet was supplemented with vitamin D_2 varied, but the same alterations were seen in both groups. The most severe alterations were seen in those calves on the highest supplement of vitamin D_2 (1,250,000 I.U. per day)

(Figures 10 and 11). Bone formation of cartilage trabecular spicules appeared depressed. The osteoclasts were fewer in number than those present in the metaphysis of control animals. The metaphysis contained matrix trabeculae that projected distally from each two to eight columns of hypertrophied cartilage. In one animal (experimental animal number 1, Pathology number 64-P-853) the secondary spongiosa of the fifth rib was marked by a line of necrotic cartilage approximately 20 cells wide that extended the entire width of the shaft.

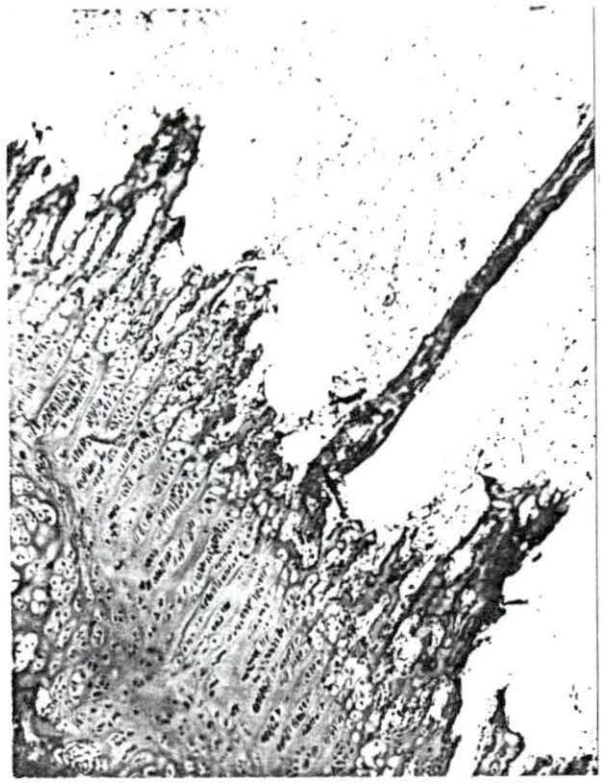
Intramembraneous ossification The circumferential periosteal surface of the control animals consisted of regularly dispersed osteoblasts on the longitudinally disposed ridges and grooves. Haversian systems were present throughout the substance of the cortex. The fibrous layer, osteogenic layer and outer circumferential lamellae of the periosteum could be differentiated.

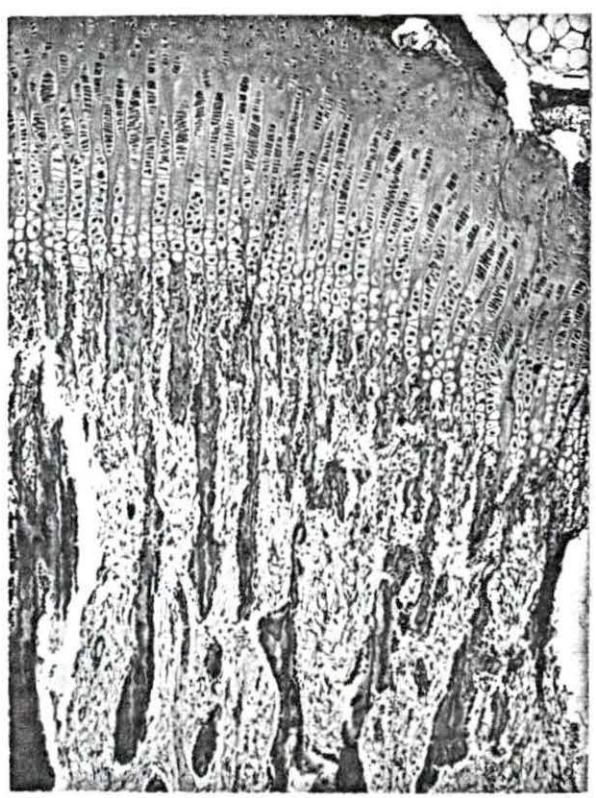
In experimental animals it was difficult to distinguish the various layers of the periosteum. There was a notable lack of osteoblasts in those animals receiving the vitamin D supplement. The Haversian systems were less numerous than those of the control group.

Articulating surface The articular surfaces of the distal end of the tibia and distal end of the metacarpus were altered in the tangential layers. In those animals receiving supplemental vitamin D, the cells of the tangential

Figure 10. The epiphyseal plate of the distal metacarpus of experimental calf number 8 (Pathology number 64-P-860). This calf received 1,250,000 I.U. of vitamin D_2 per day for 28 days. Note the lack of trabeculation x64

Figure 11. The epiphyseal plate of the distal metacarpus. From experimental calf number 18 (Pathology number 64-P-870). This calf did not receive vitamin D_2 supplement x64





layer were flattened into a narrower zone than that of the control animals. Isolated areas of erosion were present along the articulating surfaces of the tibia and metatarsus of the experimental animals. Just beneath the tangential zone in the compact cartilage of the articulating surface, vacuoles appeared at irregular intervals (Figures 12 and 13).

Teeth Differences in the teeth of the control animals and the animals receiving vitamin D_2 were not apparent as determined by examination of the enamel, dentin and pulp. The alveolar bone of the experimental animals did have an excess deposition of osteoid with an increase in osteoblastic activity in adjacent mandibular bone (Figure 14).

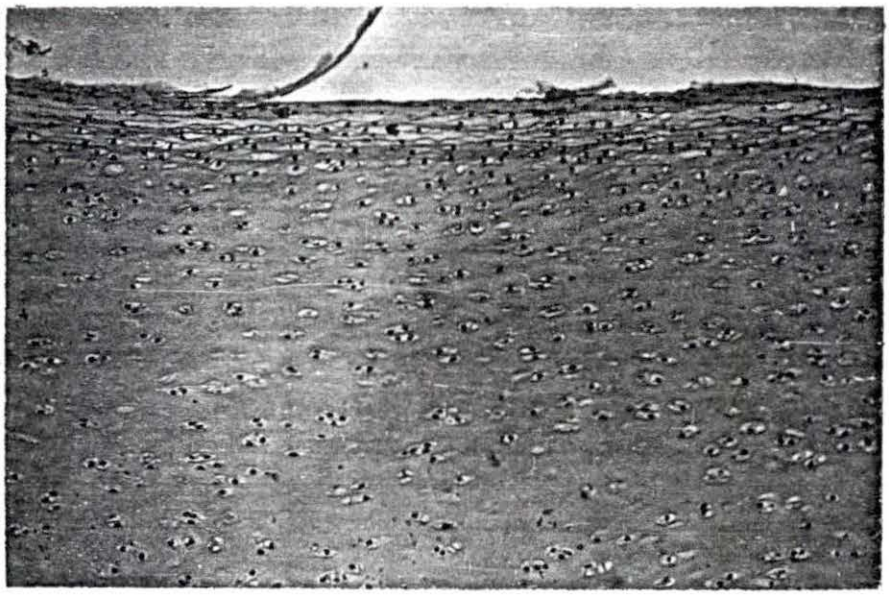
The most severe alterations were seen in those animals on experiment for 14 days, whereas changes were less severe in the more prolonged experiments (28 days and 56 days respectively).

Nonskeletal tissues Significant microscopic alterations were not observed in any of the experimental calves in the sections examined of the pituitary, adrenals, thymus, thyroids, parathyroids, heart or liver.

In experimental calves 8 (Pathology number 64-P-860) and 13 (Pathology number 64-P-865) chronic diffuse interstitial nephritis characterized by lymphocytic infiltration and plasma cells, fibroplasia of interstitial tissue, atrophy

Figure 12. The tangential layer of the articulating surface of the distal tibial articulation of experimental calf number 0 (Pathology number 64-P-856). This calf received 1,250,000 I.U. of vitamin D_2 per day for 14 days. Note the eroded articulating surface and the vaculoization of the cartilage cells in the zone of cartilage immediately below the surface x160

Figure 13. The tangential layer of the articulating surface of the distal tibial articulation of control animal, experimental calf number 3 (Pathology number 64-P-855). Note the smooth articulating surface and the lack of vaculoization in the cartilage immediately beneath the articulating surface x160



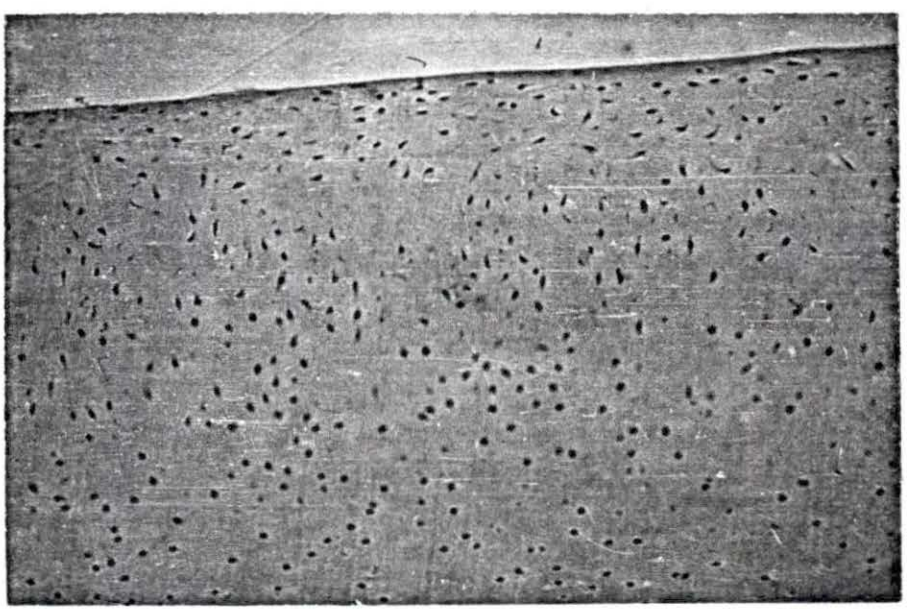
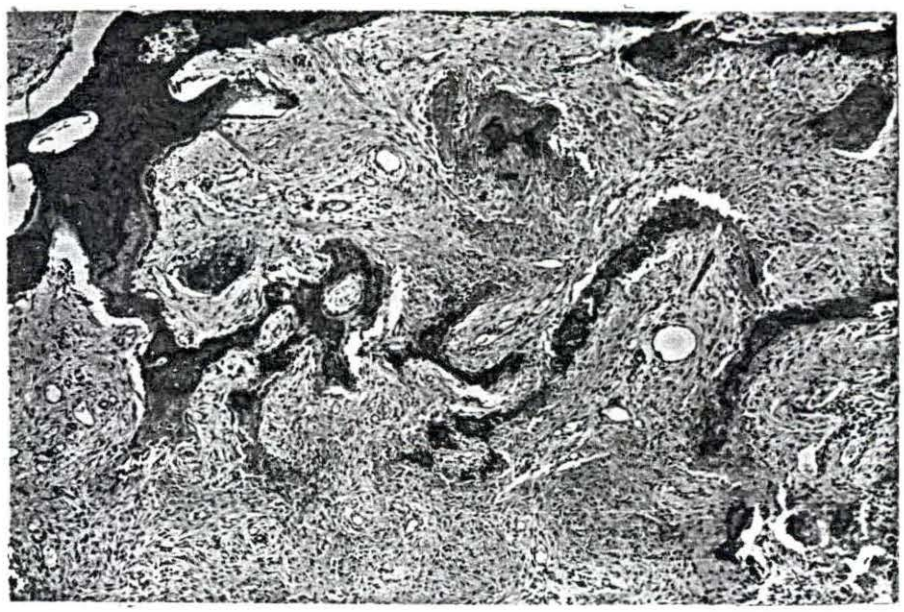
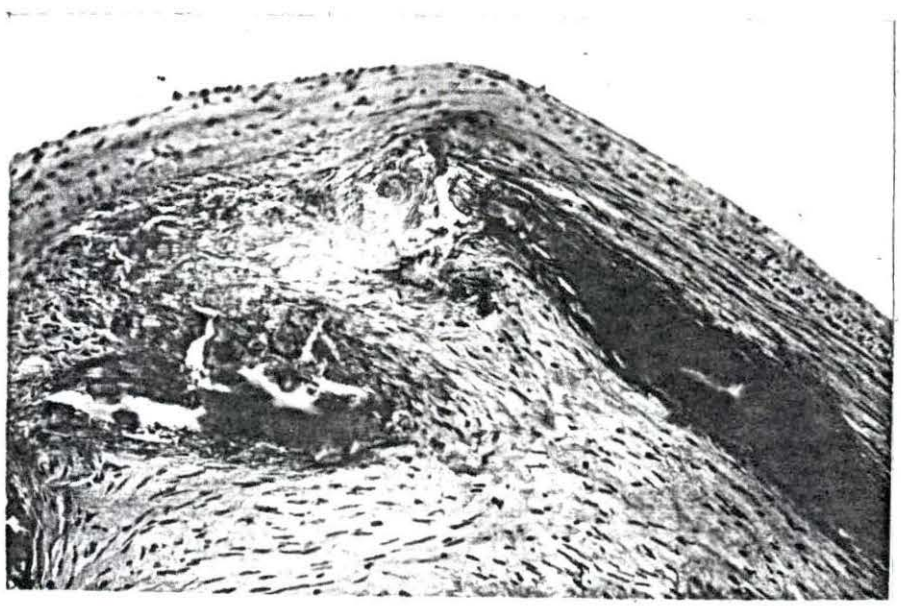


Figure 14. Note the excess osteoid of the mandibular bone adjacent to the left central incisor tooth. Experimental animal number 1 (Pathology number 64-P-853). This calf was given 1,250,000 I.U. of vitamin D_2 per day for 14 days x64

Figure 15. This figure demonstrates the mineralization of the abdominal aorta found in experimental calf number 13 (Pathology number 64-P-865) x64





of tubules, hypertrophic tubules and fibrous thickenings of Bowman's capsule was found. Distorted and shrunken glomeruli were also observed. Areas of focal nephritis characterized by multiple discrete foci were present in experimental calves number 14 (Pathology number 64-P-864) and number 16 (Pathology number 64-P-868).

Foci of bronchial pneumonia characterized by lymphocytic infiltration, emphysema and consolidation were observed in experimental calves numbers 1, 6, 14, 15 and 18 (Pathology numbers 64-P-853, 858, 866, 867 and 870). Experimental calf number 3 (Pathology number 64-P-855) had a pneumonia characterized by a lobular pattern of consolidation, catarrhal exudation of the alveoli, atelectasis, and an infiltration of mononuclear cells with cellular cuffing about the bronchioles and blood vessels.

In one calf (experimental calf number 13, Pathology number 64-P-865) a large calcified plaque was observed in the thoracic aorta (Figure 15).

Clinical Results

Group 1

In general, the clinical signs observed in the calves on supplemental D_2 did not vary markedly from those animals used as controls in this group.

The first deviation from the control animals that was noted was an increase in the amount of water consumed by

those calves on vitamin D_2. This amount was not measured, but it was observed that the water pails of those calves on supplemental D_2 had to be filled three times daily whereas it was necessary to fill the water pails of the control calves of this group once daily.

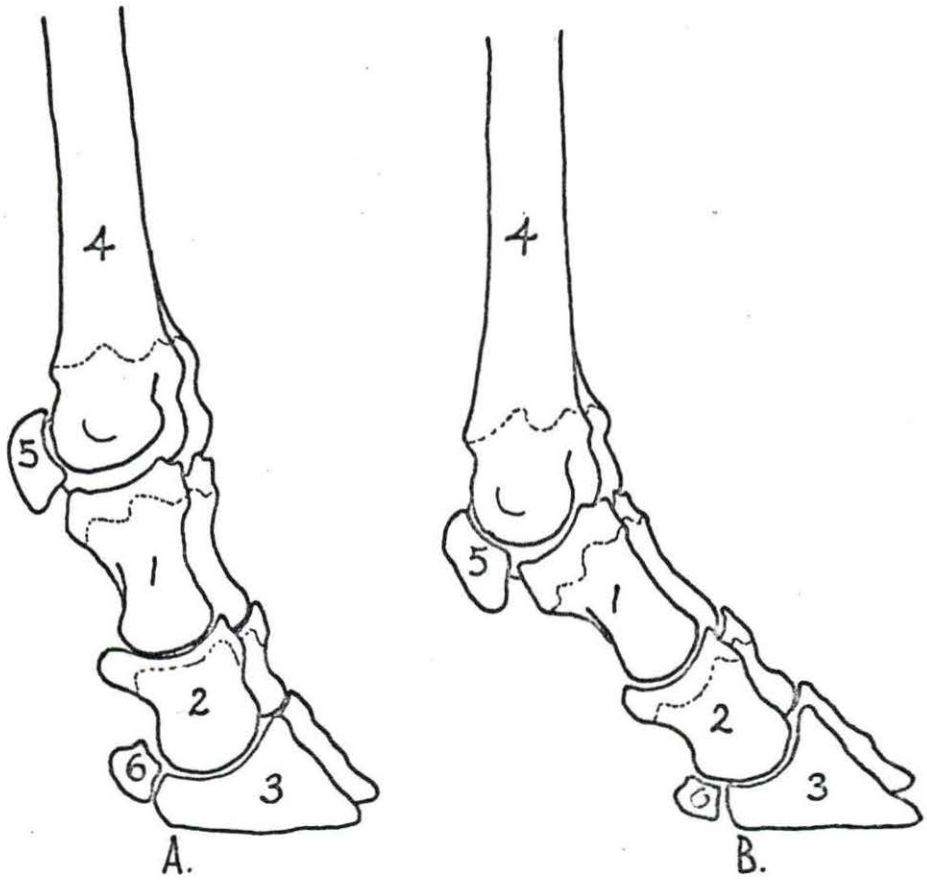
After seven days on experiment, it was observed that the pasterns of those calves on 1,250,000 I.U. of vitamin D_2 per day seemed to have dropped or become more parallel to the ground when compared with their stance upon initial examination and with the stance of the control calves (Figure 16). At the termination of the experimental period both those calves on 300,000 I.U. of vitamin D_2 per day and those calves on 1,250,000 units of vitamin D_2 were exhibiting this peculiarity.

The control pen of calves consumed 11 pounds of grain daily, the calves on 300,000 I.U. of vitamin D_2 consumed 20 pounds of grain daily and those calves on 1,250,000 I.U. of vitamin D_2 consumed 9 pounds of grain daily.

Of the control calves a weight gain did not occur in calf number 3, but calf number 6 gained 20 pounds. Of those calves on low level supplementations, calf number 2 gained 17 pounds and calf number 5 gained 12 pounds. Of the highest level, calf number 1 lost 6 pounds and calf number 0 gained 3 pounds (Figure 18).

Figure 16. A schematic illustration of the relative position of the pasturn in the controls in comparison to those calves on supplemental vitamin D_2. Drawing A is the control. Drawing B represents the pasterns of those calves on vitamin D_2

1. First phalanx
2. Second phalanx
3. Third phalanx
4. Metacarpus
5. Proximal sesamoids
6. Distal sesamoids



A schematic illustration of the relative position of the pastern in the controls in comparison to those calves on high supplemental vitamin D_2.

Group 2

Those calves on supplemental vitamin D_2 for 28 days exhibited the same peculiarity of pastern position as did those in Group 1. It did not become progressively more pronounced with the increase in the length of time the calves were on experiment.

The control calf in this group consumed 22 pounds of grain, the calves supplemented with 300,000 I.U. of vitamin D_2 consumed 48 pounds of grain and those calves supplemented with 1,250,000 I.U. of vitamin D_2 per day consumed 21 pounds of grain over the 28 day experimental period (Figure 17).

Those calves whose diets were supplemented with vitamin D_2 consumed about twice as much water as the controls.

Calf number 11 gained 20 pounds as did calf 10. Calf number 9 gained 15 pounds and calves number 7 and 8 gained 12 and 15 pounds, respectively (Figure 18).

Group 3

The calves receiving supplemental vitamin D_2 for 56 days exhibited the same position of the pastern as did Groups 1 and 2. However, it did not become more pronounced as the length of time the animals were on experiment increased.

The control pen of calves consumed 192 pounds of grain, the calves receiving 300,000 I.U. of vitamin D_2 for 56 days consumed 238 pounds of grain and the calves receiving 1,250,000 I.U. of vitamin D_2 for 56 days ingested 130 pounds

of grain (Figure 17).

In comparison to the controls, water consumption was doubled in those pens of calves in which the diet was supplemented with vitamin D_2.

Calf number 18 gained 61 pounds in the 56 days it was on experiment, calf number 17 gained 78 pounds, calf number 16 gained 95 pounds, calf number 15 gained 55 pounds, calf number 14 gained 41 pounds and calf number 13 gained 38 pounds (Figure 19).

Clinical Pathologic Results

Hematologic studies

The results of the hematologic studies are reported in Figures 20, 21, 22, 23, 24 and 25.

Blood serum chemistry

The results of the blood serum chemistry studies are reported in Figures 26, 27 and 28.

Figure 17. Total feed consumption of all calves on experiment

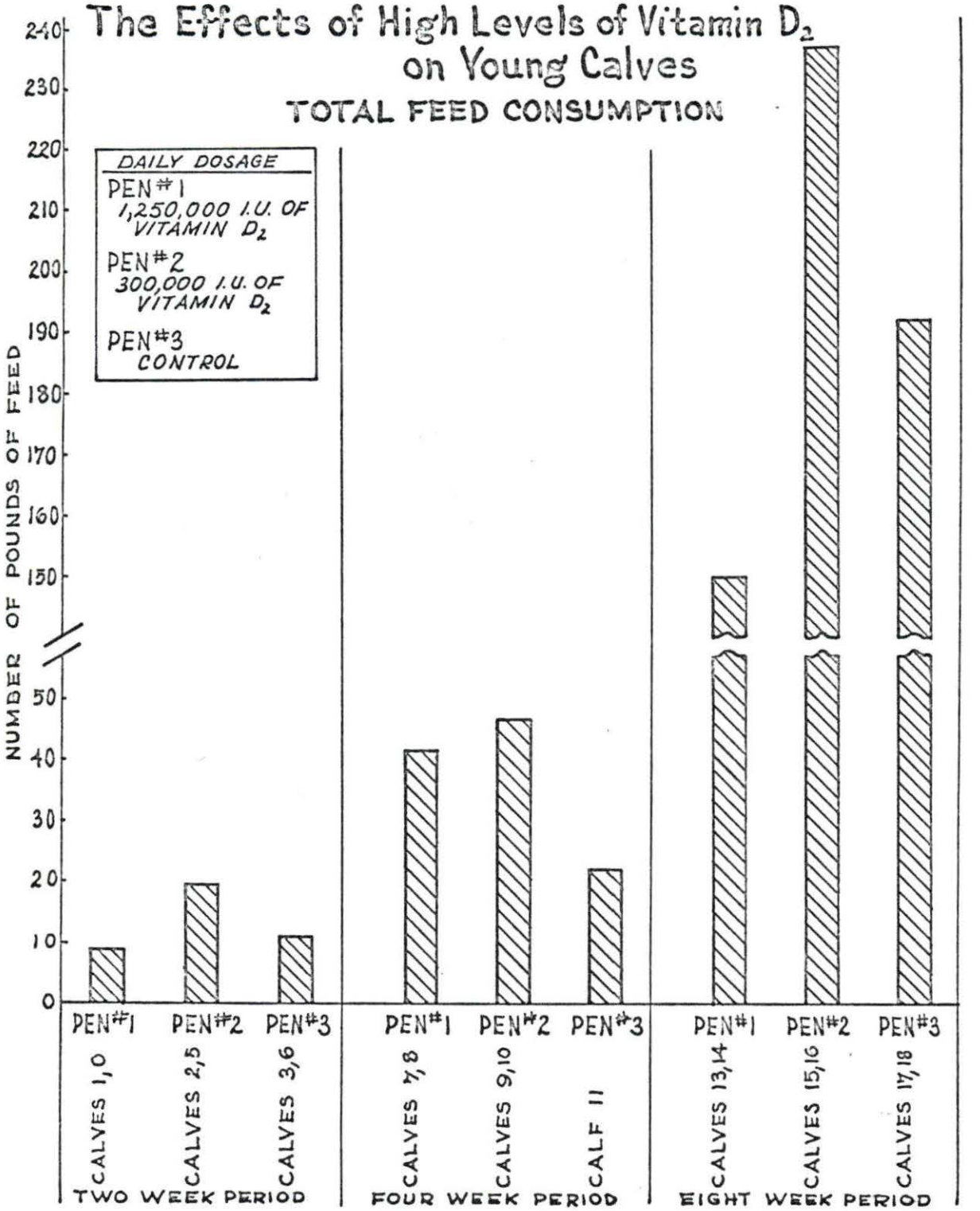

The Effects of High Levels of Vitamin D_2 on Young Calves
TOTAL FEED CONSUMPTION
DAILY DOSAGE
PEN #1 1,250,000 I.U. OF VITAMIN D_2
PEN #2 300,000 I.U. OF VITAMIN D_2
PEN #3 CONTROL
NUMBER OF POUNDS OF FEED
240
230
220
210
200
190
180
170
160
150
50
40
30
20
10
0
PEN #1
PEN #2
PEN #3
CALVES 1,0
CALVES 2,5
CALVES 3,6
TWO WEEK PERIOD
PEN #1
PEN #2
PEN #3
CALVES 7,8
CALVES 9,10
CALF 11
FOUR WEEK PERIOD
PEN #1
PEN #2
PEN #3
CALVES 13,14
CALVES 15,16
CALVES 17,18
EIGHT WEEK PERIOD

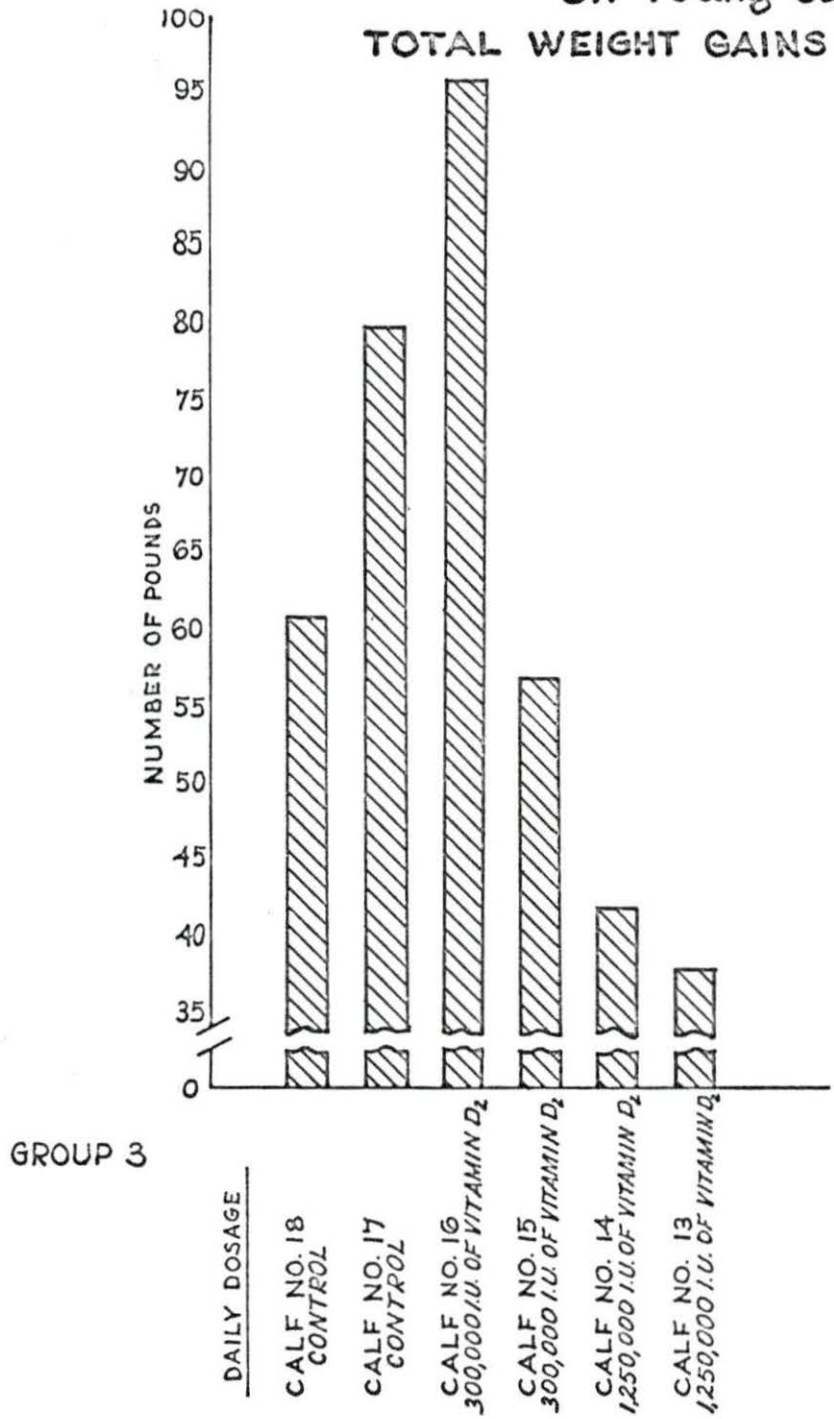
Figure 18. A graph of the weight gains at the various levels of vitamin D supplementation (Groups 1 and 2)

The Effects of High Levels of Vitamin D_2 on Young Calves

TOTAL WEIGHT GAINS

Figure 19. A graph of the weight gains at the various levels of supplementation (Group 3)

The Effects of High Levels of Vitamin D_2 on Young Calves



Group 1	Experimental day	Hemoglobin (gm. per cent)	Hematocrit (PCV)
Calf No. 3	1	8.16	31
CONTROL	7	8.16	47
	14	7.16	26
Calf No. 6	1	8.51	38
CONTROL	7	8.86	32
	14	7.16	30
Calf No. 2	1	11.15	44
300,000 I.U.	7	10.35	46
of vitamin D_2	14	9.22	35
Calf No. 5	1	10.35	42
300,000 I.U.	7	10.74	43
of vitamin D_2	14	9.96	44
Calf No. 1	1	7.49	27
1,250,000 I.U.	7	7.16	26
of vitamin D_2	14	6.84	26
Calf No. 0	1	10.35	45
1,250,000 I.U.	7	9.58	39
of vitamin D_2	14	9.96	40

Figure 20. Clinical pathologic studies of group 1 calves. Part 1 - hemoglobin and hematocrit

Group 2	Experimental day	Hemoglobin (gm. per cent)	Hematocrit (PCV)
Calf No. 11	1	12.77	49
CONTROL	7	12.37	44
	14	12.77	50
	21	11.15	55
	28	11.15	46
Calf No. 10	1	9.58	35
300,000 I.U.	7	8.86	42
of vitamin D_2	14	11.56	42
	21	8.86	37
	28	7.83	34
Calf No. 9	1	10.35	35
300,000 I.U.	7	9.22	38
of vitamin D_2	14	9.22	35
	21	8.86	37.5
	28	7.83	35
Calf No. 8	1	8.86	32
1,250,000 I.U.	7	8.51	33
of vitamin D_2	14	8.16	32
	21	8.16	38
	28	7.49	29

Figure 21. Clinical pathologic studies of group 2 calves. Part 1 - hemoglobin and hematocrit

Group 2	Experimental day	Hemoglobin (gm. per cent)	Hematocrit (PCV)
Calf No. 7	1	10.35	41
1,250,000 I.U.	7	9.96	44
of vitamin D_2	14	9.96	36
	21	9.58	40.5
	28	8.51	36

Figure 21 (Continued)

Group 3	Experimental day	Hemoglobin (gm. per cent)	Hematocrit (PCV)
Calf No. 18	1	7.16	31
CONTROL	7	11.15	41
	14	8.16	29.5
	21	8.86	34
	28	7.16	28
	35	8.86	33
	42	7.83	34
	49	9.58	38
	56	8.51	40
Calf No. 17	1	10.35	47
CONTROL	7	10.35	42
	14	9.40	37.5
	21	9.22	42
	28	9.22	47
	35	8.86	43
	42	9.58	40
	49	9.22	43
	56	9.22	44

Figure 22. Clinical pathologic studies of group 3 calves. Part 1 - hemoglobin and hematocrit

Group 3	Experimental day	Hemoglobin (gm. per cent)	Hematocrit (PCV)
Calf No. 16	1	8.51	38
300,000 I.U.	7	9.22	38
of vitamin D_2	14	8.68	37
	21	7.49	32
	28	8.86	37
	35	10.35	36
	42	10.35	49
	49	11.15	46
	56	9.96	42
Calf No. 15	1	11.15	46
300,000 I.U.	7	10.74	42.5
of vitamin D_2	14	10.74	40
	21	9.58	42
	28	10.35	45
	35	11.15	48
	42	9.96	40
	49	11.15	49
	56	10.74	46

Figure 22 (Continued)

Group 3	Experimental day	Hemoglobin (gm. per cent)	Hematocrit (PCV)
Calf No. 14	1	9.22	46
1,250,000 I.U.	7	8.86	40
of vitamin D_2	14	9.84	36
	21	8.86	38
	28	8.86	38
	35	8.51	35.5
	42	7.83	30
	49	8.16	33.5
	56	8.16	36
Calf No. 13	1	9.22	40
1,250,000 I.U.	7	9.58	40
of vitamin D_2	14	9.96	38
	21	9.58	41
	28	7.83	37
	35	8.51	33
	42	7.83	33
	49	8.16	33
	56	7.16	34

Figure 22 (Continued)

Group 1	Experimental day	Total white cell count	Lymphocyte count	Segmented neutrophil count	Band cell count	Monocyte count	Eosinophil count
Calf No. 3	1	7,250	41	21	9	1	-
CONTROL	7	8,800	48	28	12	-	-
	14	8,100	41	30	10	-	-
Calf No. 6	1	7,600	57	12	5	2	-
CONTROL	7	7,550	43	25	7	-	-
	14	11,050	49	45	15	1	-
Calf No. 2	1	7,000	40	21	9	-	-
300,000 I.U.	7	9,700	37	40	19	1	-
of vitamin D_2	14	7,600	50	22	3	1	-
Calf No. 5	1	12,050	50	42	28	-	-
300,000 I.U.	7	7,100	42	20	8	1	-
of vitamin D_2	14	9,950	57	32	10	-	-

Figure 23. Clinical pathologic studies of group 1 calves. Part 2 - total white and differential

Group 1	Experimental day	Total white cell count	Lymphocyte count	Segmented neutrophil count	Band cell count	Monocyte count	Eosinophil count
Calf No. 1	1	12,650	40	43	38	-	-
1,250,000 I.U.	7	8,500	30	42	12	-	-
of vitamin D_2	14	8,450	44	26	14	-	-
Calf No. 0	1	19,400	37	117	45	-	-
1,250,000 I.U.	7	12,500	93	17	12	3	-
of vitamin D_2	14	10,700	58	36	12	1	-

Figure 23 (Continued)

Group 2	Experimental day	Total white cell count	Lymphocyte count	Segmented neutrophil count	Band cell count	Monocyte count	Eosinophil count
Calf No. 11	1	9,250	44	33	15	-	-
CONTROL	7	8,950	57	24	7	-	-
	14	13,250	53	50	29	-	-
	21	7,750	44	27	6	-	-
	28	6,900	54	9	4	-	-
Calf No. 10	1	7,650	43	25	8	-	-
300,000 I.U.	7	6,750	46	12	9	-	-
of vitamin D_2	14	6,650	43	17	7	1	-
	21	9,900	56	36	7	-	-
	28	9,150	52	34	5	-	-
Calf No. 9	1	9,050	67	13	11	-	-
300,000 I.U.	7	7,800	54	18	5	-	1
of vitamin D_2	14	5,500	50	9	5	1	-
	21	9,400	68	21	4	-	1
	28	6,750	47	11	8	-	-

Figure 24. Clinical pathologic studies of group 2 calves. Part 2 - total white and differential

Group 2	Experimental day	Total white cell count	Lymphocyte count	Segmented neutrophil count	Band cell count	Monocyte count	Eosinophil count
Calf No. 8	1	12,750	67	40	19	1	-
1,250,000 I.U.	7	9,550	69	21	5	-	1
of vitamin D_2	14	7,400	60	11	3	-	-
	21	7,450	46	25	3	-	-
	28	9,000	48	31	11	-	-
Calf No. 7	1	9,200	57	27	7	1	1
1,250,000 I.U.	7	7,550	62	9	5	-	-
of vitamin D_2	14	6,100	45	13	3	-	-
	21	9,350	59	30	5	-	-
	28	10,550	85	14	6	-	-

Figure 24 (Continued)

Group 3	Experimental day	Total white cell count	Lymphocyte count	Segmented neutrophil count	Band cell count	Monocyte count	Eosinophil count
Calf No. 18	1	6,250	38	17	7	-	-
CONTROL	7	6,450	50	8	4	-	-
	14	7,725	52	18	8	-	-
	21	6,250	41	17	4	-	-
	28	6,900	52	11	5	-	1
	35	5,850	42	13	3	-	-
	42	7,150	54	9	8	-	-
	49	9,800	64	26	8	-	-
	56	6,550	46	14	5	-	-
Calf No. 17	1	6,750	30	29	8	-	-
CONTROL	7	5,500	40	12	3	-	-
	14	7,500	47	24	5	-	-
	21	5,800	43	12	3	-	-
	28	5,900	49	7	3	-	-

Figure 25. Clinical pathologic studies of group 3 calves. Part 2 - total white and differential

Group 3	Experimental day	Total white cell count	Lymphocyte count	Segmented neutrophil count	Band cell count	Monocyte count	Eosinophil count
Calf No. 17	35	5,650	47	8	3	-	-
CONTROL	42	5,050	27	19	5	-	-
(Continued)	49	9,050	51	28	10	-	1
	56	6,900	43	19	7	-	-
Calf No. 16	1	8,750	59	25	2	2	-
300,000 I.U.	7	6,350	34	15	3	-	1
of vitamin D_2	14	8,600	61	16	9	-	-
	21	6,000	40	17	3	-	-
	28	5,850	50	6	2	-	-
	35	5,300	42	7	4	-	-
	42	10,750	90	8	8	-	-
	49	9,000	49	31	10	-	-
	56	7,600	53	20	3	-	-

Figure 25 (Continued)

Group 3	Experimental day	Total white cell count	Lymphocyte count	Segmented neutrophil count	Band cell count	Monocyte count	Eosinophil count
Calf No. 15	1	8,150	73	8	2	-	-
300,000 I.U.	7	8,450	64	18	3	-	-
of vitamin D_2	14	12,000	80	35	12	1	-
	21	6,850	54	10	2	0	2
	28	7,900	69	6	3	0	1
	35	6,950	51	11	6	0	1
	42	8,600	54	28	4	-	-
	49	14,450	73	51	18	-	-
	56	10,150	74	16	2	-	9
Calf No. 14	1	8,100	56	16	8	-	-
1,250,000 I.U.	7	8,050	56	15	9	-	-
of vitamin D_2	14	10,000	80	6	3	-	1
	21	6,750	44	13	9	-	1
	28	7,700	66	6	5	-	-
	35	5,250	46	3	3	-	-

Figure 25 (Continued)

Group 3	Experimental day	Total white cell count	Lymphocyte count	Segmented neutrophil count	Band cell count	Monocyte count	Eosinophil count
Calf No. 14	42	7,650	49	12	16	-	-
1,250,000 I.U.	49	14,500	87	37	20	1	1
of vitamin D_2	56	10,900	83	20	5	1	-
(Continued)							
Calf No. 13	1	8,500	33	38	16	-	-
1,250,000 I.U.	7	7,600	42	26	8	-	-
of vitamin D_2	14	11,000	73	26	9	1	1
	21	8,750	44	32	10	1	-
	28	6,150	46	12	3	-	-
	35	7,150	50	14	7	-	-
	42	9,100	66	19	6	-	-
	49	10,100	54	37	10	-	-
	56	10,600	50	44	12	-	-

Figure 25 (Continued)

Group 1	Experimental day	Blood serum calcium	Blood serum phosphorus	Blood serum magnesium	Blood serum alkaline phosphatase
Calf No. 3	1	7.92	3.2	1.18	22.85
CONTROL	7	9.36	2.85	1.55	9.6
	14	8.94	3.15	2.13	5.85
Calf No. 6	1	9.03	3.0	1.76	6.55
CONTROL	7	10.22	3.0	1.41	2.37
	14	9.19	2.64	1.91	11.1
Calf No. 2	1	9.1	3.6	1.92	16.7
300,000 I.U.	7	8.72	3.45	1.45	3.75
vitamin D_2	14	8.34	3.1	1.74	5.85
Calf No. 5	1	10.1	2.18	.87	5.74
300,000 I.U.	7	10.50	3.35	1.08	3.83
vitamin D_2	14	10.50	2.7	.95	12.4
Calf No. 1	1	9.1	3.6	1.92	9.83
1,250,000 I.U.	7	8.57	3.63	1.47	2.7
vitamin D_2	14	11.3	3.7	1.22	6.35
Calf No. 0	1	9.42	3.3	1.50	18.0
1,250,000 I.U.	7	9.36	3.44	1.84	5.15
vitamin D_2	14	10.95	4.05	1.23	12.25

Figure 26. Clinical pathologic studies of group 1 calves. Part 3 - blood serum chemistry

Group 2	Experimental day	Blood serum calcium	Blood serum phosphorus	Blood serum magnesium	Blood serum alkaline phosphatase
Calf No. 11	1	10.15	3.75	1.03	4.84
CONTROL	7	8.51	3.84	1.31	5.28
	14	9.03	3.69	2.57	3.64
	21	8.78	3.31	1.97	4.69
	28	9.45	3.76	1.59	5.36
Calf No. 10	1	10.4	3.69	1.04	11.67
CONTROL	7	8.51	3.79	1.35	8.20
	14	8.7	4.86	2.57	3.46
	21	8.74	4.47	2.42	5.16
	28	8.78	3.83	1.67	5.94
Calf No. 9	1	9.94	4.43	1.06	8.56
300,000 I.U.	7	8.62	5.37	1.01	5.89
vitamin D_2	14	8.93	4.46	2.35	4.81
	21	8.05	3.98	2.27	4.36
	28	8.05	3.98	2.27	4.36
Calf No. 8	1	10.8	3.79	.7	5.76
1,250,000 I.U.	7	8.95	5.40	.53	5.94
vitamin D_2	14	9.48	5.77	1.67	2.35
	21	8.45	5.94	1.74	2.82
	28	9.40	5.38	1.67	4.31

Figure 27. Clinical pathologic studies of group 2 calves. Part 3 - blood serum chemistry

Group 2	Experimental day	Blood serum calcium	Blood serum phosphorus	Blood serum magnesium	Blood serum alkaline phosphatase
Calf No. 7	1	11.95	4.03	1.01	4.36
1,250,000 I.U.	7	8.75	5.00	1.02	6.77
vitamin D_2	14	10.07	5.15	1.59	3.35
	21	9.56	5.03	2.12	5.63
	28	9.22	5.1	1.59	6.43

Figure 27 (Continued)

Group 3	Experimental day	Blood serum calcium	Blood serum phosphorus	Blood serum magnesium	Blood serum alkaline phosphatase
Calf No. 18	1	10.85	3.85	1.67	20.05
CONTROL	7	9.67	4.07	1.57	10.85
	14	10.8	4.65	1.67	10.4
	21	13.75	4.68	1.78	9.62
	28	10.5	4.36	1.89	25.3
	35	9.62	4.82	1.46	24.85
	42	10.4	3.88	1.87	15.9
	49	9.65	4.03	1.89	20.4
	56	7.68	4.70	1.23	26.0
Calf No. 17	1	8.87	3.30	7.08	8.57
CONTROL	7	8.95	3.55	5.62	8.10
	14	8.80	3.91	5.0	10.00
	21	8.95	4.35	6.87	14.65
	28	9.26	4.31	7.81	24.95
	35	8.18	4.17	7.50	11.6
	42	9.26	4.20	8.05	20.7
	49	8.72	4.45	8.3	24.0
	56	8.80	4.85	8.25	21.4

Figure 28. Clinical pathologic studies of group 3 calves. Part 3 - blood serum chemistry

Group 3	Experimental day	Blood serum calcium	Blood serum phosphorus	Blood serum magnesium	Blood serum alkaline phosphatase
Calf No. 16	1	8.40	4.03	3.06	9.10
300,000 I.U.	7	8.40	3.78	3.06	12.50
vitamin D_2	14	8.01	4.63	2.23	13.4
	21	8.11	3.82	2.62	13.3
	28	7.88	4.43	2.75	18.4
	35	8.17	4.47	1.93	20.4
	42	7.94	4.06	2.19	11.6
	49	8.45	4.79	2.5	20.4
	56	8.33	4.83	2.44	19.70
Calf No. 15	1	11.4	3.96	2.19	10.7
300,000 I.U.	7	11.05	4.08	2.11	13.15
vitamin D_2	14	10.4	4.23	1.35	17.2
	21	7.83	4.46	1.37	19.5
	28	7.74	4.87	1.25	17.0
	35	10.35	4.26	1.96	15.4
	42	11.7	4.23	1.94	18.45
	49	10.45	3.69	2.03	8.45
	56	11.0	4.11	1.85	6.48

Figure 28 (Continued)

Group 3	Experimental day	Blood serum calcium	Blood serum phosphorus	Blood serum magnesium	Blood serum alkaline phosphatase
Calf No. 14	1	10.7	3.46	1.94	24.2
1,250,000 I.U.	7	9.51	4.35	2.23	13.9
vitamin D_2	14	9.74	4.96	2.2	10.4
	21	9.82	5.00	1.92	9.58
	28	9.38	4.45	1.92	13.8
	35	8.82	4.49	2.1	13.25
	42	9.24	3.28	2.4	23.75
	49	7.56	4.5	1.76	10.80
	56	7.74	4.33	1.48	22.25
Calf No. 13	1	11.1	3.87	2.26	19.1
1,250,000 I.U.	7	11.6	4.18	1.09	17.4
vitamin D_2	14	11.5	4.60	2.96	14.2
	21	11.5	4.63	2.96	14.0
	28	12.7	4.28	3.31	11.6
	35	12.4	4.39	3.32	16.35
	42	11.5	4.07	3.12	19.1
	49	7.92	4.5	2.87	24.3
	56	8.12	4.46	2.84	27.3

Figure 28 (Continued)

DISCUSSION

A visual examination of the radiographs of the animals on supplemental vitamin D_2 suggested a reduction in the mineral content of the cortex of the metacarpus. This could not be confirmed by photoelectric measurements, however. This visual observation was confirmed by the lack of trabeculation observed on histologic examination of the tissues. It is suggested that the method of photoelectric measurement used was not sufficiently sensitive to detect this difference.

The closure of the radiolucent line of the diaphyseal epiphyseal separation of the distal end of the tibia was approximately the same in both the control animals and those on supplemental vitamin D_2. In one control calf the line appeared to close at six weeks instead of four weeks as in the other calves. In as much as this occurred in one calf out of 18 studied, this is not considered significant and can be ascribed to individual variation. It is well to note that the more severe radiographic alterations were apparent in those calves on experiment for the shortest period of time. This suggests that a tolerance to high levels of vitamin D_2 may be developed or a cumulative effect of continued oral dosage is not apparent radiographically.

The increased radiodensity of the epiphyseal plate and the broadening of the epiphyseal zone that was visually apparent in the radiographs of those animals whose diet was

supplemented with vitamin D_2 for four weeks and eight weeks respectively, would indicate an increase in mineralization in this area. One of the normal functions of vitamin D is the proper mineralization of bone, McLean and Urist (1961, p. 117). This haphazard mineralization would indicate an abnormal activity in this area. This is in contrast to the lack of trabeculation that was observed in this area in histologic sections studied of the metaphyseal part of the epiphysis.

There were few macroscopic alterations that could be considered significant to the results of the experiment. Pneumonic lesions were observed in control calves as well as the calves receiving supplemental vitamin D_2.

Fissure of the joint articulating surfaces appeared in control as well as experimental calves. The plaques in the aorta with one exception were soft and mineral deposition could not be demonstrated by histopathologic techniques. Because mineral deposition in aortic plaques was demonstrated in one animal only (experimental calf number 13) no conclusions could be drawn from this finding.

On the basis of this experiment, the gross lesions described by Agduhr could not be confirmed, Agduhr (1927). The presence of calcified plaques in the aorta, as reported by Greig, Swan, and Cole were not observed with any consistency in these calves, Cole et al. (1957), Greig (1963), Swan (1952).

Any discussion of the histopathologic findings in hypervitaminosis D is usually prefaced by the point that elevated blood serum calcium has existed. The massive metastatic calcification reported by Quarterman in swine was accompanied by high blood serum calcium, Quarterman (1964). The extensive calcification reported by Conrad and Hansard in calves was accompanied by elevated blood serum calcium levels, Conrad and Hansard (1957). Greig reported calcification in various tissues and also a rise in serum calcium in adult cows, Greig (1963). At the levels of vitamin D_2 fed in this experiment there was not a marked rise in blood serum calcium. Neither was metastatic calcification noted as a consistent finding. Three calves (calf numbers 13, 14, and 16) displayed small plaques in the aorta. One of these (calf number 13) was a mineralized plaque. Other evidence of mineralization or calcification was lacking.

The view on the role of vitamin D in bone formation has changed in the past few years. Formerly it was thought that the vitamin promoted the deposition of calcium directly. It is now thought that its main function is in the absorption of calcium from the intestinal tract and its direct effect is in mobilization of calcium rather than in its deposition, McLean and Urist (1961, p. 117). Because of this influence on the mobilization of calcium it is reported in other species that new bone is calcified poorly or not at all in spite of the hypercalcemia found in hypervitaminosis D,

Thomas and Morgan (1958), Hass et al. (1958). The lack of trabeculation of the bone in the experimental calves would tend to substantiate this finding in the bovine. The cartilage in the zone of provisional calcification becomes necrotic and the trabeculae fail to calcify properly causing the variation in the number of trabeculae from one trabeculae to each two to three columns of hypertrophied cartilage cells in the control animals to one trabeculae for each two to eight columns of hypertrophied cartilage cells in the animals receiving vitamin D. Further, there were fewer osteoclasts found in those animals receiving vitamin D than in the metaphysis of the control animals.

Under the conditions of the experiment vitamin D also seemed to affect the intramembraneous ossification. In the control animals the layers of the periosteum were quite distinct whereas in the experimental animals these layers were not distinct and there was a lack of osteoblasts in the cortical bone of the experimental calves. The entire histopathologic pattern in regard to the bone formation in these calves was one of a lack of proper calcification of bone. This is consistent with the findings in other species, Thomas and Morgan (1958), Hass et al. (1958), Becks (1942). This has not been previously reported in the bovine, however.

The histopathologic observations of the articulating surface have not been previously reported in other species or in the bovine. It has been previously noted that the

calves on supplemental vitamin D assumed a peculiar stance. This stance could have been caused by the pain associated with the flattening of the tangential layer of the articulating surface or this lesion may also have been caused by the shifting of the articular surface due to the stance. Alteration of cartilage has not been reported in previous studies of hypervitaminosis D.

The formation of excess osteoid with an increase in osteoblastic activity of adjacent bone has been reported in the dog by Becks, Becks (1942). The formation of excess osteoid around the tooth roots of the experimental calves was the only place this occurred in the sections sampled. This then would be consistent with the observations made by Becks in the canine but not previously reported in the bovine.

The massive alterations of nonskeletal tissues reported in many species and the alterations of the kidney, heart and thymus reported by other investigators in the bovine was not observed in the calves under the conditions of this experiment, Greig (1963), Duncan and Huffman (1934), Swan (1952).

The National Research Council recommendation for the supplementation of vitamin D to the diet of calves up to 200 pounds of body weight is 300 I.U. per 100 pounds of body weight, National Research Council (1958), Bechdel (1938), Hibbs (1962). The oral amounts supplemented in this

experiment approximate 1000 times and 4000 times this amount. This figure was arbitrarially selected after reviewing the work of Bills and Wirick in a long term experiment involving large numbers of rats, Bills and Wirick (1930). This work indicated that no effects were noted at 1000 times the normal level and minimal effects of intoxication were noted at 4000 times the normal level with fatalities evident at 10,000 times the normal level, Bills and Wirick (1930).

The amount of vitamin D_2 that is produced by the action of ultraviolet light upon the skin could not be measured. The amount that was in the hay, grain, and milk supplement was not calculated. It is doubtful that the amount of vitamin D_2 supplied by these sources affected the outcome of the experiment.

Increased consumption of water has been observed by various investigators in other species in which vitamin D intoxication has been reproduced, Jones and Robson (1932), Light et al. (1931). Polydipsia was not reported in calves by Agduhr (1927) or Duncan and Huffman (1934). Greig did not mention this in attempts to produce intoxication in adult cows, Greig (1963). Drinking large quantities of water is a common mechanism of the body in an attempt to dilute irritating substances that are ingested. It does not follow therefore that polydipsia is peculiar to vitamin D intoxication. There was a marked increase in water consumption in the calves whose diets were supplemented with vitamin D_2 when

water consumption was compared to the controls in this experiment. It is a part of the clinical picture established but is not peculiar to this condition.

The position of the pasterns of all of the calves receiving supplemental vitamin D was the most pronounced and consistent clinical finding. To achieve this position it would be necessary for a complete relaxation of the flexor muscles or a lack of tone in the flexor tendons. This effect could also be observed if the extensor muscles were in a continuous state of contraction with a corresponding exhaustion of the flexor muscles. After examination of the tissue sections of the articulating surfaces of the joints another explanation might be hypothesized. The erosion and vaculoization that had taken place on the articulating surfaces of the joints would cause pain and discomfort if the animals stood in a normal position. The posture that was affected by the animals on supplemental vitamin D could be an attempt to shift the articulating surface to other areas on the tangential surface of the articulation.

Feed consumption varied with each individual group and pen. In the group on experiment for 14 days, those calves on the highest level of vitamin D_2 recorded the lowest grain consumption. Those on the lowest level of supplementation consumed the largest amount of grain (Figure 17). These results were repeated for those groups on experiment 28 days

and 56 days respectively. Under the conditions of this experiment it would seem that 1000 times the recommended supplemental amount of vitamin D_2 stimulates feed consumption whereas 4000 times the recommended amount depresses the appetite. Appetite stimulation has been observed in man by Crimm (1932), Rappaport and Reed (1933) and Reed (1934). Appetite depression was observed in the bovine by Greig (1963), Duncan and Huffman (1934), Agduhr (1927).

Weight gains were variable with each group of calves. In Group 1 one of the control calves developed clinical signs of a severe pneumonia. This was confirmed upon necropsy. This animal made no gains during the 14 day experimental period. The other calf in this group outgained all others in the experiment. Of those calves on the highest levels of vitamin D_2 calf number 1 showed a severe weight loss while calf number 0 made minimal gains. These results were not duplicated in those calves on experiment for 28 days. Although the control calf outgained the calves that were supplemented with the vitamin the differences in weight gain were not as pronounced as in the first group.

In the third group of calves which were on experiment for 56 days the weight differences between the control calves and those calves on the highest level of vitamin D_2 were marked. There was a well defined failure of those calves on the highest level of D_2 to gain weight. However, those calves on the lowest level of D_2 in one case exceeded the

gains of the control calves and in the other approximated the gains of the controls (Figure 19). These results would indicate that 4000 times the recommended level of vitamin D_2 depresses the weight gain when compared to the controls whereas at 1000 times the recommended level the gains are variable but do approximate those made by the controls. These weight gains or lack of gain are necessarily related to the amount of appetite and feed consumed by the animals.

The length of time on experiment did not increase the signs of intoxication and other than lack of weight gain, increased consumption of water and the peculiar posture; the animals supplemented with vitamin D_2 appeared to be normal, healthy animals.

At the level of oral vitamin D_2 that was administered signs reported in other species and in the bovine by Agduhr, Greig and Swan were absent or not pronounced in this experiment, Agduhr (1927), Greig (1963), Swan (1952). These findings suggest that severe or acute clinical signs are not observed when vitamin D_2 is fed at a rate of 4000 times the recommended level but that the manifestations are those of a subclinical disease.

The hemoglobin, packed cell volume, total white cell and differential studies did not reveal any marked irregularities when the calves on supplemental vitamin D_2 were compared to the controls. These results did not vary markedly from

the accepted normal ranges as described by Benjamin and by Greatorex, Benjamin (1961, p. 51), Greatorex (1954). The variations that did occur can be ascribed to the young animals violent reaction to even mild infections. Because the diet of the calves consisted primarily of milk, the anemia that was evident at various times in both the controls and the experimental calves could be expected.

Blood serum calcium did not reveal any abnormal trends in the experimental animals. All of the calves dropped in blood serum calcium levels after the first week on experiment. With diurnal variations the levels remained relatively constant in both the experimental animals and the controls. In Hibb's experiment on adult cows, feeding one million units of vitamin D_2 daily did not seem to affect the serum calcium and phosphorus. When this was increased to two million units of vitamin D_2 per day there was a rise in both serum calcium and phosphorus and a decrease in serum magnesium, Hibbs et al. (1946). Greig using injectable vitamin D and administering the material by intravenous injection at the rate of 10,000,000 units found that the serum calcium rose for seven days and then returned to normal, Greig (1963).

The oral dosage chosen for this experiment did not seem sufficient to raise the blood serum calcium in the calves. Others have reported a transient rise in blood serum calcium which returned to normal in a few days, Duncan and Huffman (1934), Conrad and Hansard (1957).

The serum phosphorus levels as reported by numerous investigators increased concurrently with the rise in serum calcium but preceded it by a short period of time. This rise was transient in nature, Duncan and Huffman (1934), Conrad and Hansard (1957), Greig (1963). In this experiment the dosage level was not high enough to produce the effects of a frank intoxication and the above results were not recorded.

Investigators have demonstrated that the blood serum magnesium levels decrease as the calcium and phosphorus levels increase, Conrad and Hansard (1957), Greig (1963). The blood serum magnesium of seven calves receiving supplemental vitamin D_2 decreased during the experimental period although the serum calcium and serum phosphous did not rise significantly.

The alkaline phosphatase levels of the control and the experimental calves were so erratic that no conclusion or discussion could be drawn from these results.

SUMMARY

1. Twelve three-day old calves were fed vitamin D_2 at the following levels: two calves were fed 1000 times the recommended daily intake, two calves were fed 4000 times the daily intake for a period of 14 days. Two calves were fed 1000 times the recommended daily intake and two were fed 4000 times the recommended intake for 28 days. Two were fed 1000 times and two 4000 times the recommended level for a period of 56 days.

2. The calves at both levels of vitamin D_2 intake demonstrated alterations when compared to the control animals of each group. These alterations consisted of:

A. Radiographic alterations.

1. Decreased mineralization of cortical bone.
2. Increased activity of the epiphyseal plate on the diaphyseal side.

B. Pathologic alterations.

1. Enchondral ossification of trabecular cartilage was depressed.
2. Intramembraneous ossification was disturbed and irregular.
3. The articulating surface of the distal end of the tibia and distal end of the metacarpus was flattened with isolated areas of erosion and generalized vacuolization below the tangential

surface.

4. There was excess deposition of osteoid on alveolar bone surrounding the central incisors.

5. In one calf a large calcified plaque was found in the thoracic aorta.

C. Clinical manifestations.

1. Polydipsia.

2. Dropped pasterns.

3. At the high level of vitamin D_2 supplementation.

a. Depressed appetite.

b. Depressed weight gains.

4. At the low level of vitamin D_2 supplementation.

a. Appetite stimulation.

b. Increased weight gains.

D. There were no significant alterations in hematology or blood serum chemistry.

3. The severity of the alterations was not compounded by the length of time the calves were fed vitamin D_2. The most severe lesions were observed in those calves on experiment for the shortest length of time.

LITERATURE CITED

Agduhr, Erik
1927 Changes in the organism caused by cod liver oil added to the food. Acta Paediatica 5: 319.

American Society of Clinical Pathologists
1948 Committee for clarification of the nomenclature of cells and diseases of the blood forming organs. American Journal of Clinical Pathology 18: 443-450.

Anning, S. T., J. Dawson, Doris E. Dolby and John T. Ingram
1948 The toxic effects of calciferol. Quarterly Journal of Medicine 17: 203-228.

Armed Forces Institute of Pathology
1960 Manual of histologic and special staining techniques. Second Edition. McGraw-Hill Book Company, New York, New York.

Ashford, Charles Amos
1930 The phosphorus distribution in blood and the calcium and phosphorus excretion during hypervitaminosis D. Biochemical Journal 24: 661-668.

Bechdel, S. I., N. W. Hilston, N. B. Guerrant, and
1938 R. A. Dutcher
The vitamin D requirement of dairy calves. Pennsylvania Agricultural Experiment Station Bulletin 364.

Becks, Hermann
1942 Dangerous effects of vitamin D overdosage on dental and paradental structures. Journal of the American Dental Association 29: 1947-1968.

Becks, H., Agnes Fay Morgan, Daniel A. Collins, and Ruth M.
1945 Freytag
Nonreversible changes due to overdosage of vitamin D in oral and other structures in the dog. (Abstract of a paper presented at the 23rd Annual Meeting of the International Association for Dental Research, May 27, 1945.) Journal of Dental Research 24: 193.

Benjamin, Maxine M.
1961 Outline of Veterinary Clinical Pathology. The Iowa State University Press, Ames, Iowa.

Bevans, Margaret and Henry K. Taylor
1947 Lesions following the use of Ertron in rheumatoid arthritis. American Journal of Pathology 23: 367-375.

Bills, Charles E.
1935 Physiology of the sterols, including vitamin D. Physiological Reviews 15: 1-97.

Bills, Charles E. and Alice M. Wirick
1930 Long time feeding experiments with activated ergosterol. Journal of Biological Chemistry 86: 117-128.

Carlson, William D.
1961 Veterinary radiology. Lea and Febiger, Philadelphia, Pennsylvania. pp. 131-139.

Cole, Clarence R., D. M. Chamberlin, J. W. Hibbs, W. D.
1957 Pounden, and C. R. Smith
Symposium on Poisoning, Part II. American College of Veterinary Pathologists. Case 11: Vitamin D (Viosterol) poisoning in a cow, from a study on post-parturient paresis. American Veterinary Medical Association Journal 130: 298-299.

Conrad, H. R. and Sam L. Hansard
1957 Effects of massive doses of vitamin D on physiological behavior of calcium in cattle. Journal of Applied Physiology 10: 98-102.

Covey, George W. and H. H. Whitlock
1946 Intoxication resulting from the administration of massive doses of vitamin D. Annals of Internal Medicine 25: 508-516.

Crimm, P. D.
1932 Vitamin therapy in pulmonary tuberculosis. II. The treatment with massive doses of activated ergosterol. American Review of Tuberculosis 26: 112-123.

Crimm, P. D. and J. W. Strayer
1934 Vitamin therapy in pulmonary tuberculosis. V. The effect of Viosterol on the diffusible and non-diffusible calcium of the blood and spinal fluid. American Journal of Medical Science 187: 557-562.

Crimm, P. D., J. W. Strayer, H. L. Watson, and G. Heimann
1933 Vitamin therapy in pulmonary tuberculosis. III. The effect of Viosterol on the absorption, retention and excretion of calcium. American Review of Tuberculosis 28: 202-216.

Danowski, T. S., A. W. Winkler, and J. P. Peters
1945 Tissue calcification and renal failure produced by massive dose vitamin D therapy of arthritis. Annals of Internal Medicine 23: 22-27.

Davies, Paget
1960 Vitamin D poisoning: A report of two cases. Annals of Internal Medicine 53: 1250-1254.

Dixon, W. E. and J. C. Hoyle
1928 The effects of irradiated ergosterol in large doses. British Medical Journal 2: 832-835.

Donegan, Charles K., Addison L. Messer and Edward S. Orgain
1949 Vitamin D intoxication due to Ertron: Report of two cases. Annals of Internal Medicine 30: 429-435.

Duguid, J. B.
1930a The toxicity of vitamin D. Lancet 219: 983-985.

Duguid, J. B.
1930b Vitamin D sclerosis in the rat's aorta. Journal of Pathology and Bacteriology 33: 697-710.

Duguid, J. B.
1934 A discussion of chronic nephritis. Royal Society of Medicine Proceedings 27: 792-796.

Duncan, C. W. and C. F. Huffman
1934 The effect of daily massive doses of Viosterol upon calcium and phosphorus metabolism and blood calcium and inorganic phosphorus in calves. Journal of Dairy Science 17: 83-91.

Eisenstein, R., R. Dedman, S. Papajiannis, and A. Hemmens
1962 Effect of hypervitaminosis D on serum factors in the rabbit. Society for Experimental Biology and Medicine Proceedings 109: 482-484.

Eisenstein, Reuben and William A. Groff
1957 Experimental hypervitaminosis D: Hypercalcemia, hypermucoproteinemia and metastatic calcification. Society for Experimental Biology and Medicine Proceedings 94: 441-447.

Fiske, Cyrus H. and Yellopragada Subbarow
1925 The colormetric determination of phosphorus. Journal of Biological Chemistry 66: 375-400.

Freeman, Smith and Chester J. Farmer
1930 Calcium partition in blood serum in vascular hypertension and in experimental hypervitaminosis D. Society for Experimental Biology and Medicine Proceedings 27: 970.

Gough, J., J. B. Duguid, and D. R. Davies
1933 The renal lesions in hypervitaminosis D: Observations on the urinary, calcium and phosphorous excretion. British Journal of Experimental Pathology 14: 137-145.

Greatorex, John C.
1954 Studies on the hematology of calves from birth to one year of age. British Veterinary Journal 110: 120-138.

Greig, W. A.
1963 Hypervitaminosis D in dairy cattle. World Veterinary Congress Proceedings 17th: 233-234.

Ham, Arthur W. and Murray D. Lewis
1934 Hypervitaminosis D rickets: The action of vitamin D. British Journal of Experimental Pathology 15: 228-334.

Harper, T. A.
1959 A rapid micro-quantitative method for estimating serum calcium. Nature 183: 232-233.

Harris, Leslie J.
1930 Hypervitaminosis D: Calcium phosphorus intake - A determining factor. Lancet 218: 236.

Harris, L. J.
1932 The mode of action of vitamin D. The parathyroid theory: Clinical hypervitaminosis. Lancet 1031-1038.

Harris, Leslie Julius and Robert Maitland Innes
1931 The mode of action of vitamin D. Studies on hypervitaminosis D. The influence of the calcium-phosphate intake. Biochemical Journal 25: 367-390.

Harris, Leslie Julius and Thomas Moore
1929 Hypervitaminosis and vitamin balance. Biochemical Journal 22: 1461-1477.

Harris, Leslie Julius and Corbett Page Stewart
1929 The effect of excessive doses of irradiated ergosterol on the calcium and phosphorus content of the blood. Biochemical Journal 23: 206-209.

Hass, George M., Richard E. Tracehaart, C. Bruce Taylor, and
1958 Marjorie Stumpe
An experimental histologic study of hypervitaminosis D. American Journal of Pathology 34: 395-431.

Hauch, J. T.
1934 Studies on the effect of high doses of irradiated and nonirradiated ergosterol on the albino rat. Journal of Nutrition 8: 163-186.

Hendricks, Jeannette B., Agnes Fay Morgan, and Ruth M. Freytag
1947 Chronic moderate hypervitaminosis D in young dogs. American Journal of Physiology 149: 319-332.

Hess, A. F., J. M. Lewis and H. Rinkin
1930 Newer aspects of the theraputics of Viosterol (irradiated ergosterol). Journal of American Medical Association 94: 1885-1889.

Hess, Alfred F., Robert F. Light, Charles N. Frey, and
1932 Joseph Gross
A study of the milk, blood and excreta of cows fed moderate and excessive amounts of irradiated yeast or ergosterol. Journal of Biological Chemistry 97: 369-377.

Hibbs, J. W.
1962 Vitamin D requirements of dairy cattle. Feed Age 12: 27-29.

Hibbs, J. W., W. E. Krass, W. D. Pounden, C. F. Monroe, and
1946 T. S. Sutton
Studies on milk fever in dairy cows. II. The effect of vitamin D on some of the blood changes in normal and milk fever cows at parturition. Journal of Dairy Science 767-781.

Hirschfelder, A. D., E. R. Serles, and V. G. Haury
1934 A simple adaptation of Kolthoff's colormetric method for the determination of magnesium in biological fluids. Journal of Biological Chemistry 104: 635.

Hojer, Axel
1926 Changes in the heart through the presence of cod liver oil in the food. Acta Pediatrica 6: 180-190.

Hoyle, J. C.
1930a Further observations on the toxic effects of irradiated ergosterol. Journal of Pharmacology and Experimental Therapeutics 40: 351-372.

Hoyle, J. C.
1930b The toxic effects of irradiated ergosterol. Journal of Pharmacology and Experimental Therapeutics 38: 271-289.

Hoyle, J. C. and H. Buckland
1929 Further observations on the effects of large doses of irradiated ergosterol. Biochemical Journal 23: 558-565.

Howard, John Eager and Richard J. Meyer
1948 Intoxication with vitamin D. Journal of Clinical Endocrinology 8: 895-910.

Hyde, Leroy and Bernard Hyde
1947 Toxicity of large doses of vitamin D (Ertron). Annals of Internal Medicine 27: 617-629.

Jones, James H., Milton Rappoport, and Horace L. Hodes
1930 The source of excess calcium in hypercalcemia induced by irradiated ergosterol. Journal of Biological Chemistry 89: 647-656.

Jones, James H. and George M. Robson
1932 The effect of large doses of irradiated ergosterol upon the ash content of the femora of young adult rats. Journal of Biochemistry 91: 43-56.

Kaufman, Paul, R. Donald Beck, and Richard D. Wiseman
1947 Vitamin D (Ertron) therapy in arthritis. Journal of the American Medical Association 134: 688-690.

Kent, Sidney P., Gordon F. Vawter, Robert M. Dowben, and
1958 Richard E. Benson
Hypervitaminosis D in monkeys. American Journal of Pathology 34: 37-59.

Kreitmair, H. and T. Moll
1928 Hypervitaminose durch grosse dosen vitamin D. Munchener Medizin Wochenchieff 75: 637-639. Translated by Paul Neubauer.

League of Nations Health Organization
1935 Memorandum on the International Standard for vitamin D and its application, No. 30. March.

Light, Robert F., Glennard Miller, and Charles N. Frey
1931 Studies on the effects of overdosage of vitamin D. II. Journal of Biological Chemistry 92: 47-52.

Mac Donald, A. M.
1958 Hypercalcemia in infants and young animals. Nutrition Society Proceedings 17: 74-77.

McLean, Franklin C. and Marshall R. Urist
1961 Bone - An introduction to the physiology of skeletal tissue. University of Chicago Press, Chicago, Illinois.

National Research Council
1958 Nutrient Requirements of Dairy Cattle. National Academy of Sciences Publication 464.

Melville, George S., Jr., Quentin L. Hartwig, Thomas
1960 Leffingwell, and Robert J. Young
Response of hemoglobin and cellular elements of blood after hypervitaminosis D in monkeys. American Journal of Veterinary Research 21: 1128-1131.

Mulligan, R. M. and F. L. Stricker
1948 Metastatic calcification produced in dogs by hypervitaminosis D and haliphagia. American Journal of Pathology 24: 451-473.

Opper, Lincoln
1941 Effect of renal damage on the toxicity of hypervitaminosis D in rats. Archives of Pathology 31: 569-577.

Pfannenstiel, W.
1928 A summary of recent work on Vigantol. Lancet 215: 845-847.

Potvliege, Paul R.
1962 Hypervitaminosis D_2 in gravid rats. Archives of Pathology 73: 371-382.

Quarterman, J., A. C. Dalgarno, Agnes Adam, B. F. Fell, and R. Boyne
1964 The distribution of vitamin D between the blood and the liver in the pig, and observations on the pathology of vitamin D toxicity. British Journal of Nutrition 18: 65-76.

Rappaport, B. Z. and C. I. Reed
1933 Viosterol of high potency in seasonal hay fever and related conditions. Journal of American Medical Association 101: 105-109.

Reed, C. I.
1934 Effect of heavy administration of Viosterol on metabolism of the rat. Society for Experimental Biology and Medicine Proceedings 32: 274-277.

Reed, C. I.
1938 On the nature of the toxic action of vitamin D. Society for Biology and Experimental Medicine Proceedings 38: 791-792.

Reed, C. I. and L. Seed
1933 The treatment of clinical tetany with ergosterol. Endocrinology 17: 136-148.

Reed, C. I., H. C. Struck, and J. E. Steck
1939 Vitamin D. University of Chicago Press, Chicago, Illinois.

Reed, C. I., E. A. Thacker, L. M. Dillman, and J. W. Welch
1933 The effects of irradiated ergosterol on the metabolism of normal dogs. Journal of Nutrition 6: 355-370.

Rosenheim, O. and T. A. Webster
1927 Rickets and cholesterol. Lancet 1: 1025-1026.

Scharfman, W. B. and S. Proff
1956 Anemia associated with vitamin D intoxication. New England Journal of Medicine 255: 1207-1212.

Schmidtmann, M. Von
1930 Gibt es im tierexperiment eine unschaliche vigantoldosis? Virchows Archiv Fur Pathologie Anatomie und Klinische Medizin 275: 408-421. Translated by Paul Neubauer.

Shelling, David H.
1931 Effect of Viosterol on serum calcium of parathyroidectomized rats. Society for Experimental Biology and Medicine Proceedings 28: 303-305.

Shelling, D. H. and D. E. Asher
1932 Johns Hopkins Hospital Bulletin 50: 318.

Shelling, D. H. and K. B. Hopper
1934 Calcium and phosphorus studies VI. Observations on the treatment of late rickets with Viosterol based on the study of 23 cases. American Journal of Diseases of Children 47: 61-90.

Shohl, Alfred T., Harry Goldblatt, and Helen Bennett Brown
1930 The pathological effects upon rats of excess irradiated ergosterol. Journal of Clinical Investigation 8: 505-531.

Spies, Tom Douglas
1930 The calcification of tubercles by means of irradiated ergosterol. American Journal of Pathology 6: 337-345.

Spies, Tom Douglas and Eugene C. Glover
1930 Renal lesions with retention of nitrogenous products produced by massive doses of irradiated ergosterol. American Journal of Pathology 6: 485-498.

Spies, T. D. and R. F. Hanzal
1934 Experimental production of hypercalcemia in human beings by means of irradiated ergosterol. Society of Experimental Biology and Medicine Proceedings 31: 747-750.

Swan, J. B.
1952 The toxic effect of massive doses of calciferol on dairy cows. The New Zealand Veterinary Journal 1: 25.

Taylor, N. B., C. B. Weld, H. D. Branion and H. D. Kay
1931 A study of the action of irradiated ergosterol and its relationship to parathyroid function. Canadian Medical Association Journal 24: 763-777.

Thomas, William C., Jr. and Gennell H. Morgan
1958 The effect of cortisone in experimental hypervitaminosis D. Endocrinology 63: 57-64.

Underhill, S. W. F.
1928 Hypervitaminosis. Lancet 215: 948.

Vanderveer, H. L.
1931 Hypervitaminosis D and arteriosclerosis. Archives of Pathology 12: 941-955.

Van Donk, Evelyn C., H. Steenbeck, and E. B. Hart
1933 The nutritive deficiency of milk with specific reference to manganese, energy and pituitary relations. American Journal of Physiology 103: 468-479.

Van Horn, H. H. and N. L. Jacobson
1963 Feeding dairy calves to 4 months of age. Iowa State University of Science and Technology Cooperative Extension Service Bulletin 263. October.

Varela, B., J. A. Collazo, J. Moreau, and W. P. Rubino
1930 Experimentelle arteriosklerose in verlaufe der hypervitaminose D bei kaninchen und ratten. Virchowis Archiv Fur Pathologic, Anatomic und Klinische Medizin 274:270-280. Translated by Paul Neubauer.

Walsh, Frank B. and John Eager Howard
1947 Conjunctival and corneal lesions in hypercalcemia. Journal of Clinical Endocrinology 7: 644-652.

Watchorn, Elsie
1930 The absorption and excretion of calcium and phosphorus by rats receiving excessive doses of irradiated ergosterol. Biochemical Journal 24: 631-640.

Williams, Gerald A., Nelson Bowser, Walter J. Henderson, and
1962 Vytavtas Uzgiris
Calcium absorption in the rat in relation to excessive vitamin D and cortisone. Society for Experimental Biology and Medicine Proceedings 110: 889-892.

Zucker, T. F., A. M. Pappenheimer, and M. Barnett
1922 Observations on cod liver oil and rickets. Society of Experimental Biology and Medicine Proceedings 19: 167-169.

ACKNOWLEDGMENTS

The counsel and guidance given by my major professor, Dr. Frank K. Ramsey, is greatly appreciated.

Special recognition is due to Dr. E. D. Roberts for the inception of this project and for his valuable assistance in evaluation of the skeletal tissues.

Dr. J. R. Duncan and Dr. W. H. Wass must be recognized for their encouragement, assistance, and suggestions that have been so helpful in the culmination of this project.

The author is especially grateful to Dr. George Christensen, Dean of the College of Veterinary Medicine, for providing financial support for this work.

The quality of the manuscript is due largely to the instruction, attention to detail and painstaking perusal on the part of Dr. F. K. Ramsey.

The special assistance of the following individuals is acknowledged and greatly appreciated:

Dr. B. W. Kingery for his personal encouragement that initiated this undertaking.

Dr. M. A. Emmerson for his guidance and suggestions in the radiographic study.

Dr. M. W. Sloss for her excellent teaching efforts in clinical hematology.

Mr. William Hilleman for his assistance in the care and handling of the experimental animals.

Mrs. P. A. Owenson, Mrs. D. Williams, Mrs. Kay Pierce, and Mrs. C. Faber for their excellent preparation of the histologic sections.

Mrs. V. Marshall for the blood chemistry studies.

Mrs. G. Shay for assisting with the manuscript.

Mrs. D. Heiden for her cooperation in the typing of the manuscript as well as the kindly encouragement and pointed suggestions that have improved the quality of the manuscript.

The use of the physical facilities of the Department of Veterinary Clinical Science and the Department of Veterinary Pathology is acknowledged and appreciated.

Dr. Larry Woghan for his aid in handling the calves.

Dr. Dennis Grummitt for his assistance in the hematology studies.

Mrs. Ellen Houser and Dr. H. P. Neubauer for their assistance in the radiographic studies.

Mrs. Ruth Loomis for her valuable assistance in composing the graphs, charts and art work.

Finally but most importantly, to Martha for just being.