HYPERVITAMINOSIS A IN THE DOG

by

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INTRODUCTION

Hypervitaminosis A has been reported by numerous workers in experimental animals and clinically in both domestic animals and man with somewhat contradictory observations. In growing animals it is characterized by specific signs involving dehydration, emaciation, roughened coat, dullness, hemorrhages and skeletal changes which include joint pain (5,12,28,53,67), multiple exostoses (12,14,19,20,42,59,67,72,73,81,90,92), spontaneous fractures (9,18,19,33,51,54,64,65,66,67,76), reduction in size of long bones, osteodystrophy (21) and osteoporosis (30,90). There are considerable differences of opinion as to which signs and lesions are specific for the toxic effect of excessive vitamin A on animals among different workers depending on the species of animals in which the observation was made. Hypervitaminosis A in the dog was reported by Maddock et al. (45) who observed degenerative vascular lesions, fatty liver as well as some skeletal changes. However they did not record exostosis and spontaneous fractures which had been reported previously in other animals.

Recently there has been a tendency to administer excessive amounts of vitamins to animals for various purposes, one of which includes overenthusiasm on the part of animal owners for vitamin supplementation.

The present experiment was designed to re-evaluate the toxic effects of excessive vitamin A on growing dogs by means of radiographical and histopathological examination.

REVIEW OF LITERATURE

Rats

The toxic effect of excessive vitamin A was first published by Takahashi et al. (78), giving excessive doses of their crude vitamin A concentrate "Biosterin" to albino rats and mice orally. The animals died after a period of a few days to several weeks with an alopecia of the head, emaciation, anemia, paralysis of the hind legs and cramp. By postmortem examination, fatty degeneration of liver, kidney and heart was found, as well as hyperemia and sometimes hemorrhages in the intestine and lungs. Takahashi et al. (78) and Matsuoka (47) found that the excess of vitamin A concentrate was also toxic when injected, usually causing death with cramp in less than one hour after the injection, while Moore and Wang (51), who injected massive doses of vitamin A either subcutaneously or by intraperitoneal injection, described that the reaction never amounted to anything more than a temporary cramping and twitching of the muscles of the hind legs. In no instance in their experiment did an animal die as a result of the treatment.

Euler and Widell (23) observed a disturbed bone growth in young rats treated with cod liver oil. Bell et al. (6) found no toxic effect in young rats on treatment with a dose which was estimated 13,500 IU of vitamin A in the form of

cod-liver oil. Baumann and Moore (5) observed limping, emaciation, loss of hair around mouth and death in the rats fed halibut liver oil concentrate equivalent to 8 mg of vitamin A daily for five to ten days.

Spontaneous fractures, which are characteristic of hypervitaminosis A, were first reported by Bomskov and Seemann (9) and at about the same time by Collazo and Rodriguez (18). The long bones of rats, fed an excess of vitamin A concentrate, became fragile and prone to fracture. After repeated dosage a disturbed bone formation resulted and multiple spontaneous fractures of the tibia and femur occurred. Davies and Moore (19) found that sometimes the broken ends of the long bones were ankylosed with the formation of large irregular calluses. Strauss (76) described that the signs, appearing after four weeks in rats given daily doses of 20,000 rat units of vitamin A, were emaciation; rough, bald pelt; conjunctivitis; anemia and dullness with postmortem findings of a fatty liver, hemosiderosis of the spleen, reduction in bone thickness and spontaneous fractures. With normal bone resorption and only slightly inhibited cartilage proliferation, the activities of the osteoblasts were greatly decreased. Further observations of the skeletal changes have been reported by Hoff and Joddeloh (36), Vedder and Rosenberg (82) and Weslaw et al. (85).

Rodahl and Moore (69) established that polar bear toxicity was due to the high content of vitamin A (18,000 IU/gm in winter months) in the liver being fed to experimental

rats. They then proceeded to produce an experimental vitamin A toxicity by feeding rats 500,000 IU of vitamin A daily in the form of halibut liver oil which produced a wide variety of toxic manifestations similar to those observed in rats fed polar bear liver. Hypervitaminosis A was also induced by Moore and Wang (51) in rats by feeding them orally doses of 25,000-50,000 IU of either crystalline vitamin A acetate or halibut liver oil. Characteristic lesions were skeletal fractures and internal or external hemorrhages. Similar observations were reported by Pavcek et al. (55) in rats fed telang livers which contain abnormally high concentrations of vitamin A. Herbst et al. (35) and Pavcek et al. (55) confirmed that the fractures were caused by overdosing with either pure vitamin A or by feeding telang liver. Walker et al. (83) reported the most characteristic lesions of hypervitaminosis A to be hemorrhages, variable in intensity and distribution, and spontaneous skeletal fractures occurring primarily in young rats. These findings agreed with those of Rodahl and Moore (69) and Moore and Wang (51).

Light et al. (43) described that hypervitaminosis A in rats was associated with a pronounced hypoprothrombinemia which could be corrected by giving vitamin K. This observation was confirmed by Walker et al. (83) who described that a secondary deficiency of vitamin K may be induced by excessive vitamin A. Contrary to these findings, Rodahl (66, 67) reported that additional supplies of vitamin K were found to have no influence on the incidence of hemorrhages or any

of the other signs in hypervitaminotic rats. He stated that it was necessary to look for other factors than vitamin K deficiency in order to explain the tendency to bleeding in hypervitaminotic rats.

Wolbach (86) made comprehensive studies of the skeletal changes produced by excessive vitamin A in rats and guinea pigs. Histological examination indicated that all features of skeletal maturation were accelerated. There was a speeding up of the metabolism of epiphyseal cartilage cells, endochondral bone formation and subperiosteal appositional growth of the cortex. The extent of changes was quantitatively related to the amount of vitamin A administered. Bone which does not require remodeling for maintenance of the normal growth pattern did not seem to respond to the administration of large amounts of vitamin A.

Rodahl (66) found that the vitamin A contents of two summer polar bear livers were 21,900 and 26,700 IU/gm respectively. Radiographic examination of the long bones of rats fed polar bear liver revealed that the bone shafts became abnormally thin and the cortical shadow was absent at both ends of the bones. Spontaneous fractures of ulna, humerus, radius, tibia and fibula developed. Microscopically, only spongy bone was observed in the long bones and the epiphysis was of normal width. He concluded that vitamin A was the toxic agent in polar bear liver, which was later confirmed by using a purified oily solution of crystalline vitamin A acetate (67,68). The bone section taken from the fractured

tibia showed marked hyperemia and great irregularity in the bone structure. There were large subperiosteal hemorrhages in the tibia and the fibula. In the metaphysis the spicules were extremely thin, and there were a large number of osteoclasts and marked hyperemia. Old hemorrhages and a large callus formation were seen in the fractures of the cortex.

Irving (37) described that the rate of formation of bone was greatly reduced, active osteoblasts became much less prominent and the number of osteoblasts were markedly decreased in the mandibles of young rats. Gorlin and Chaudhry (29) found that in hypervitaminotic rats, the lingual alveolar bone formed at a much slower rate and that a marked decrease in the number of osteoblasts occurred. The number of cartilage cells was reduced in the epiphyseal cartilage of the long bones and the width of the epiphyseal plates became markedly narrower.

Mice and Guinea Pigs

After Ypsilanti (93) failed to produce bone changes in mice by the subcutaneous injection of vitamin A concentrate, Rodahl (67) reported that the signs produced in mice by oral administration or by local application on the skin of vitamin A concentrate were identical with those observed in rats given excess of vitamin A orally. Barnicot (4) attached the fragments of crystalline vitamin A acetate to small pieces of parietal bone cut from 10-day-old mice and inserted the combination into the cerebral hemisphere of

litter mates. Advanced resorption, accompanied by numerous osteoclasts, and often leading to perforation of the bone was observed when the grafts were removed after 7 or 14 days.

Simola et al. (75) and Rodahl (67) studied vitamin A toxicity in guinea pigs and obtained identical lesions and signs as those in rats.

Rabbits and Mink

Bomskov (8) described that rabbits were resistant to overdosing with vitamin A. Rodahl (67) found that excessive vitamin A was also toxic to both young and adult rabbits. Doses between 50 and 130 IU of vitamin A per gram of body weight daily gave rise to toxic signs in adult rabbits, whereas doses between 200 and 350 IU of vitamin A per gram of body weight daily proved lethal after 10 to 30 days in young rabbits. Clinical signs and post-mortem examinations were similar to those observed in hypervitaminotic rats, mice and guinea pigs. These were loss of weight, weakness, scurffiness, alopecia, stiffness and pain in the limbs, limping and fractures. Hyperemia, enlarged adrenals, visceral hemorrhage and pneumonia were also noted, but there was no anemia.

Thomas et al. (80) described skeletal changes in rabbits due to excessive amounts of vitamin A. The normal basophilic, metachromatic and alcian blue staining properties of the matrix were lost especially in the articular and epiphyseal cartilage. The cartilage cells remained intact but were reduced in size. The changes sometimes appeared as early

as 48 hours after the initiation of daily injection of one million units of vitamin A and were usually well established by 5 days.

Helgebostad (33) observed decalcification and spontaneous fractures of the long bones in mink by giving 200 to 300 IU of vitamin A per gram of body weight daily for 6 to 8 weeks.

Birds

Rodahl (67) failed to record skeletal changes in cockerels given 208,000 IU of vitamin A daily for 18 days and Rigdon (61) in ducks fed 200,000 IU of vitamin A daily. However Rodahl (67) described that some of the renal tubules showed deposits of calcium with marked hyperemia. Wolbach and Hegstad (87,88,89) found that skeletal responses to hypervitaminosis A in both young chickens and young ducks were of the same nature as those in mammals. In growing chickens hypervitaminosis A accelerated all histological sequences concerned in bone growth in conformity with normal growth patterns.

Dogs

Maddock et al. (45) described the toxic effect of excess doses of vitamin A in dogs fed a level of 300,000 IU per Kg. of body weight daily. The epiphyseal lines of all the long bones of the experimental dogs were markedly narrower than those of controls. The cortices of long bone were less

dense and thinner. The tibia showed marked loss of density of the crest just below the epiphysis of the tuberosity and in this location the outline of the bone was fuzzy in appearance.

Histopathological findings included a marked dilatation of the lymphatics of the epicardium, marked fatty change in liver with necrotic foci and hemorrhages in various organs. Degenerative lesions of the media and mural thrombi of fused red blood corpuscles were also observed in arteries and veins of the myocardium, gall bladder, urinary bladder and one lymph node. The effects on the growth sequence of bones, including epiphyseal cartilage and remodeling processes were of the same nature as those described in the rat and guinea pig (86). In all regions of accelerated resorption of bone there was marked proliferation of periosteal cells and considerable hemorrhage, so that the appearances in such locations recalled the hemorrhages of scurvy and a deficiency in intracellular matrix formation was to be considered as a possible factor, although it was not reconcilable with the appearances at the epiphyses and the deposit of osteoid wherever required by the normal growth pattern. He explained that hemorrhage may be due to a depression of prothrombin levels resulting from the excessive vitamin A administration.

Cross sections of the femur showed exaggeration of the flattening of the posterior surface with a great increase in the number of osteoclasts, periosteal proliferation and hemorrhage. The interior of the shaft in this region showed newly deposited osteoid and formation of compact bone in

progress.

Marie and See (46) reported producing acute hydrocephalus in a 4 Kg 3 to 4 months old puppy by oral administration of 350,000 IU of vitamin A. The condition manifested itself by a very distinct tension of the fontanel and a rapid rise in plasma vitamin A level. Puppies receiving 100,000 IU of oral vitamin A for three successive days and control puppies receiving an oily solvent (vitamin A destroyed) failed to show the disorder. They concluded that in the dog, as in the human, vitamin A produced an acute transitory hydrocephalus provided there was rapid absorption of a massive dose of vitamin A and a significant increase in plasma vitamin A occurred.

Cats

Cristi (13) reported the bony changes in a group of cats, terming the condition "diffuse ankylosing osseo-periostitis" without describing the pathology. English and Seawright (22) and Seawright and English (71) presented the clinical and radiographical features of deforming cervical spondylosis of the cat. The most constant clinical signs were postural changes, cervical ankyloses, lameness and somewhat less frequently cutaneous hyperesthesia. Their pathological findings indicated that the spondylosis was a degenerative condition of bone characterized by extensive exostotic formations, particularly on the margins of the cervical and thoracic vertebrae. Because the affected cats seen by Cristi (13) had been fed a diet of raw liver and

because the condition observed by English and Seawright (22) and Seawright and English (71) was associated with predominantly raw liver diets, it was thought that the high content of vitamin A in the liver may have played some part. To test this hypothesis, a cat was given 50,000 units of vitamin A acetate orally, each day for a period of over six months and no bony changes resulted.

Seawright et al. (72) suggested that the disease could be produced by feeding young cats on a diet consisting of milk and raw liver. Seawright et al. (73) investigated further the etiology and pathology of deforming cervical spondylosis in 19 normal, newly-weaned kittens which were supplemented with vitamin A daily at the rate of 15, 30 and 150 ug per gram of body weight or were fed raw liver and milk providing average daily intakes of vitamin A of 17 and 35 ug per gram of body weight. Lesions developed in the first three diarthrodial joints of the cervical vertebrae in all except the control and the lowest vitamin A supplementary group after 24 to 41 weeks. The lesions included an extensive osseo-cartilaginous hyperplasia mainly at the margins of the joints. Marked lipid infiltration involving the tubular epithelium of the renal cortex and the reticulohistiocytic cells of the liver, lungs, spleen and hepatic lymphonode were observed in all cats in the experimental groups. They concluded that hypervitaminosis A was the cause of naturally-occurring deforming cervical spondylosis of the cat.

Clark and Seawright (15), Clark et al. (16) and Clark

(14) produced long bone lesions in kittens following vitamin A administration. Most of the long bones were considerably shorter than those of litter mate controls and some showed abnormal morphology due to an altered growth pattern in the post-dosage period. Histopathologically there was variable damage to epiphyseal growth plates and in some long bones no remnant of an epiphyseal plate remained.

Riser et al. (63) suggested that exostoses of cats receiving excessive vitamin A may be related with low calcium and high phosphorus diets. Clark et al. (17) found that the levels and relative proportions of calcium and phosphorus in the diet have little or no influence on the development of exostoses in chronic hypervitaminosis A in the cat.

Pigs

Hendricks et al. (34) found no apparent rachitogenic effect due to carotene in diet relatively low in vitamin D in pigs. Anderson et al. (2) described disorganized cartilage cell columns in the enlarged costochondral junctions, which appeared to be decalcified, with general toxic signs of hypervitaminosis A similar to those previously recorded in other experimental animals. At necropsy they observed hemorrhage in limb joints, mucosa, submucosa, subpericardium and at the cortico-medullary junction of the kidney. Slight increases in serum calcium, phosphorus and alkaline phosphatase were also recorded.

Wolke et al. (90) reported that the long bones were

decreased in length and width, with great tissue loss in the epiphysis due to lysis of chondroid matrix. Spicules of spongiosa were fewer but larger and the number of osteoblasts were significantly decreased. Intramembranous bone was thinner and had few osteoblasts and normal numbers of osteoclasts. Pryor et al. (59) produced lesions suggestive of periostitis and early exostosis formation as early as 19 days after the experiment was initiated in pigs which consumed diets supplemented with 330,600 ug of vitamin A per Kg of feed.

Dobson (21) described osteodystrophy associated with hypervitaminosis A in growing pigs which were given a single massive oral dose of 344,000 ug of a vitamin A preparation. The lesions observed were similar to those described by Wolke et al. (90).

Cattle

Hazzard et al. (32) studied chronic hypervitaminosis A in Holstein male calves and observed increased heart rate, decreased cerebrospinal fluid pressure, elevated serum transaminase and alkaline phosphatase activities, increased prothrombin time and decreased inorganic phosphorus.

Grey et al. (30) described the pathology of skull, radius and rib in hypervitaminosis A of young calves. In general, the gross and microscopic findings agreed with those lesions observed in other species of experimental animals (4,18,25,29,66,67,68). Histopathologically the essential change was a retarded osteogenesis with poor differentiation

of the mesenchymal cells into osteoblasts. As a result, the compacta was thin, osteoporotic and contained large amount of fibrous bone. The effect of excessive vitamin A on bone appeared to be retardation of bone growth due to a defect in osteoid production resulting in osteoporosis.

Human

Josephs (39) first pointed out the danger of abnormally high dosage of vitamin A in pediatric prophylactic practice and first described hypervitaminosis A in a boy 34 months of age who had received one teaspoonful of halibut liver oil corresponding to 240,000 U.S.P. units of vitamin A daily since the third month of life, and who occasionally drank the oil directly from the bottle. Toomey and Morissette (81) first observed deep, hard, tender swelling in the extremities and were the first to describe cortical thickening in tubular bones in a boy 23 months of age who had been given 250,000 to 500,000 units daily of vitamin A since the second week of life.

Dickey and Bradly (20) reported pruritus, hepatomegaly, tender swelling and cortical hyperostoses in a child 22 months of age, who was poisoned by a large amount of cod liver oil and concentrates fed daily since early infancy. These toxic signs were also observed by Rothman and Leon (70) in two children who were poisoned by excessive dosages of between 100,000 and 200,000 units daily for 13 and 21 months respectively. Fried and Grand (27) proved by radiological examination that extremely tender swellings of the extremities were due to

subperiosteal elevations of the ulna and tibia in children having received 500,000 and 180,000 units of vitamin A daily respectively as Oleum Percomorphum for many months.

Caffey (12) described the roentogenographic changes in the skeleton and the elevation of blood vitamin A in a group of hypervitaminotic children. Rineberg and Gross (62) first performed a successful surgical biopsy of a skeletal lesion and made th pathological diagnosis of "productive periostitis". Bair (3) reported that hypervitaminosis A caused skull lesions and hydrocephalus. Woodard et al. (91) described bone destruction and delayed ossification of parietal bone, however it was more likely that the lesion in the skull represented a coincidental developmental variant, namely, a persistent parietal (sagital) fontanel. Pease (56) found focal retardation and arrest of bone growth due to vitamin A intoxication in growing children.

Sulzberger and Lazar (77) presented the first report in an adult with no radiologic changes of the bones in a 44-year-old woman who had taken 600,000 units of vitamin A daily for 18 months. Since then, three additional cases of chronic hypervitaminosis A in adults have been reported (7,28,74). In a report on vitamin A toxicity in a 28-year-old woman who had been suffering from progressive crippling bone and joint pains, Gerber et al. (28) demonstrated bony changes on radiologic examination, which consisted of extensive calcification of cartilaginous, ligamentous, tendinous and subperiosteal structures.

Marie and See (46) first reported an acute poisoning

in infants following the administration of a single massive dose of vitamin A. The acute toxicities were characterized by acute hydrocephalus with spontaneous and intensive bulging of the fontanel. This condition began twelve hours after injection of the vitamin and disappeared in 24 to 48 hours. Each child received 350,000 IU of vitamin A and 300,000 IU of vitamin D_2 in one dose. In order to eliminate the role that supplementary vitamin D might have played in the disorder, 350,000 IU of vitamin A alone was administered orally to infants. Acute benign hydrocephalus was produced in three out of six infants. Therefore, vitamin A alone was believed to be responsible for this syndrome. This condition was subsequently produced in puppies by these workers.

Breslau (11) presented a comprehensive discussion of several aspects of vitamin A intoxication in both the acute and chronic phases. He made a plea for wider recognition of mild or borderline hypervitaminosis A by the practitioner. It was suggested that more effective restrictions should be imposed upon the availability to the general public of potentially harmful preparations containing inordinately large amounts of vitamin A.

MATERIALS AND METHODS

Ten mixed large breed puppies were obtained at 4 to 6 weeks of age for use in this experiment. A physical examination was performed on each puppy. Radiographs (medio-lateral view) of the extremities of each puppy were obtained to

determine if any predisposing long bone abnormalities were present prior to going on treatment. The puppies were given Vermiplex[1] for internal parasites and immunized for canine distemper with Distemperoid[2] injected twice 4 weeks apart. They were housed on concrete runs and fed Purina Puppy Chow[3] ad libitum. The experiment was started after a three week adjustment period when the puppies were about two months of age and in apparently good health.

The puppies were randomly allocated into five groups with the following treatments:

Group I (dogs 5 and 7) - Control

Group II (dogs 1 and 2) - Intramuscular injection of vitamin A at a level of 45,000 IU per pound of body weight per week.

Group III (dogs 3 and 9) - Intramuscular injection of vitamin A at a level of 90,000 IU per pound of body weight per week.

Group IV (dogs 4 and 6) - Intramuscular injection of vitamin ADE at a level of 45,000 IU of vitamin A per pound of body weight per week.

Group V (dogs 8 and 10) - Intramuscular injection of vitamin ADE at a level of 90,000 IU of vitamin A per pound of body weight per week.

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[1]Pitman-Moore, Inc., Washington Crossing, N.J. 08560.

[2]Fromm Laboratories, Inc., Grafton, Wisconsin 53024.

[3]Ralston Purina Co., Checkerboard Square, St. Louis, Mo. 63199.

The vitamin A preparation used was Vitamin A Palmitate[1] containing 100,000 IU of vitamin A palmitate per ml. The vitamin ADE preparation used was Injacom ADE[2] containing 500,000 IU of vitamin A, 75,000 IU of vitamin D_2 and 50 IU of vitamin E per ml.

The anticipated results of vitamin A toxicity were not clinically observed in 10 weeks, so the decision was made to extend the experimental treatment of the dogs in Group II plus one control (dog 7) by feeding vitamin A concentrate daily for an additional period of time to produce clinical hypervitaminosis A. The other dogs in Groups III, IV and V continued to receive the original treatments.

The oral vitamin A preparation used was Vitamin A Palmitate Synthetic[3] containing one million IU per gram. The dogs were dosed daily by stomach tube at a rate of 135,000 IU per pound of body weight. This was followed by 4 ml of corn oil[4] to rinse the vitamin A preparation down the tube. The remaining control (dog 5) was given 4 ml of corn oil alone via stomach tube daily.

The animals were weighed at weekly intervals. Radiographs were taken at frequent intervals to determine if bony

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[1]Holmes Serum Co., Inc., Chicago, Ill. 60618.

[2]Hoffman-LaRoche Inc., Nutley, N.J. 07110.

[3]National Biochemical Corporation, Cleveland, Ohio 44128.

[4]Mazola Corn Oil. Best Foods, A Div. of CPC International Inc., Englewood Cliffs, N.J. 07632.

changes were occurring in experimental animals. Two blood samples were obtained from each animal by jugular venipuncture at each collection period; one was collected in EDTA and the other as whole blood. The whole blood was centrifuged at 1,500 rpm for 4 minutes to separate serum. The samples collected were evaluated for the following; hemoglobin and packed cell volume from EDTA sample and calcium, phosphorus, alkaline phosphatase and glutamic pyruvic transaminase from the serum sample. Hemoglobin was determined by cyanmethemoglobin method, packed cell volume by microhematocrit. Serum calcium was analyzed by flame photometer method using Model-21 Coleman Flame Photometer,[1] serum inorganic phosphorus by Fiske-Subbarow Method (26) using Sigma Reagents,[2] serum glutamic pyruvic transaminase by Reitman-Frankel method (60) using DADE Reagents[3] and serum alkaline phosphatase by a modified Kind-King method (41) using Harleco Phosphazyme Set.[4]

The dogs of groups III, IV and V were sacrificed on day 97 and 98. Those of group II and a remaining control (dog 5) were sacrificed on day 162. Dog 7 died on day 121. The animals were euthanatized with barbiturate (Barb-Euthol[5])

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[1]Coleman Instruments Division, Perkin-Elmer Corp., Maywood, Ill. 60153.

[2]Sigma Chemical Co., 3500 DeKalb St., St. Louis, Mo. 63118.

[3]DADE Division, American Hospital Supply Corp., Miami, Fla. 33152.

[4]Harleco, 60th & Woodland Ave., Phila., Pa. 19143.

[5]Haver-Lockhart Lab., Shawnee, Kansas 66201.

administered intravenously. Tissues for histopathological examination were collected into 10 per cent buffered neutral formalin. Tissues collected were eye, salivary gland, esophagus, stomach, small and large intestine, lung, heart, liver, prescapular lymphnode, kidney, adrenal, bladder, prostate, epididymis, testis, ovary, uterus, skin, spinal cord, brain, humerus, radius, femur, tibia and costochondral junction of right 7th rib. The bones were cut longitudinally in the midsagittal plane, fixed in 10 per cent buffered neutral formalin and decalcified by the formic acid-sodium citrate method. Paraffin embedded sections were stained with hematoxylin and eosin. Samples of liver collected from each animal were stored in a deep freeze for analysis of vitamin A content. The vitamin A concentrate in liver was determined by Kimble method (40).

RESULTS

Body Weight

The changes in body weight of each group throughout the experiment are presented in Figure 1 and Appendix 1. The body weights shown in Figure 1 are the average weight of two dogs in each group. The dogs of groups I, II, III and IV grew well during the first 10 weeks of experiment. Group V began to lose weight at the 10th week. Group II and dog 7 steadily lost weight from day 85 until termination. This was one week after they began receiving daily oral doses of vitamin A. The control dog showed a satisfactory weight gain throughout the experiment.

Clinical Observation

The dogs were active and in good condition and no clinical abnormalities were noticeable in general appearance and behavior except group V until the body weight began to decline. Group V showed lameness and extensive swelling of the rear legs involving muscular site of injection of vitamin ADE as well as adjacent surrounding areas. The lameness and swelling lasted for 7 to 10 days, but no specific treatment was given. Group IV exhibited transient muscular swelling at injection sites only about two days. Groups II and III, in spite of the fact that they received five times the volume of injectable material, did not manifest any clinical signs at injection sites. During the first week of experiment dog 8 exhibited alopecia which on skin scraping proved to be sarcoptic mange. All of the animals were dipped in Dermaton[1] on days 6 and 16 for the treatment and prevention of mange.

At the 11th week, the experimental dogs became depressed, dull and slightly dehydrated. Bloody diarrhea, vomiting and ocular discharge were occasionally observed in groups II and V.

At the 14th week, group II dogs showed severe dehydration, roughened coat, depression, dullness, emaciation and a reluctance to walk. The eyes appeared to be dry and sunken and the gums were pale. Dog 7, which was transferred from the control group on day 73, seemed to be more severely affected

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[1]William Cooper and Nephews, Inc., Chicago, Ill. 60614.

and showed a certain degree of incoordination of the hind legs. Group II and dog 7 exhibited severe pain on manipulation at the regions of the carpal and tarsal joints while radiographs were being taken. Dog 7 was found dead on the 48th day of oral administration of vitamin A (day 121). This dog was subjected to the same post-mortem and radiographic examination as the other experimental dogs at sacrifice.

The appetite of experimental animals did not appear to be severely affected by the excessive doses of vitamin A.

Clinical Pathology

The values obtained for hemoglobin, packed cell volume, serum calcium, inorganic phosphorus, glutamic pyruvic transaminase and alkaline phosphatase are presented in Appendices 2-7. These values were within normal range for dogs of this age. Hemoglobin and packed cell volume increased and serum inorganic phosphorus and alkaline phosphatase decreased with age of the experimental animals. Serum calcium remained at the lower limit of normal range and serum glutamic pyruvic transaminase remained unchanged. No abnormalities in hematology or blood chemistries were observed until groups III, IV and V were sacrificed.

Decreased hemoglobins and packed cell volumes were noted in the blood samples drawn on day 162 from dogs 1 and 2 when compared with control. Serum calcium and inorganic phosphorus values remained unaltered, except for dog 1 which showed a slight increase in phosphorus and slight decrease in

calcium at termination. Serum glutamic pyruvic transaminase and alkaline phosphatase activities rose terminally to a slight degree in dogs 1 and 2 as compared with those of control. The blood from dogs 1 and 2 contained markedly increased number of immature red blood cells and showed slight anisocytosis and poikilocytosis. Otherwise the blood pictures showed no difference from control.

Vitamin A Concentration in Liver

The concentration of vitamin A in liver of all dogs are shown in Table 1. The concentration of vitamin A in liver did not always correlate with the amount of the vitamin A administered. The liver storage of vitamin A in dogs showed considerable individual variance. The livers of dogs receiving weekly injections of vitamin ADE contained a higher concentration of vitamin A than those of dogs receiving injections of vitamin A alone at comparable dose rates. The dogs (dogs 1 and 2) which had the highest liver concentration of vitamin A at sacrifice were those which were on daily oral administration of vitamin A for a period of 89 days.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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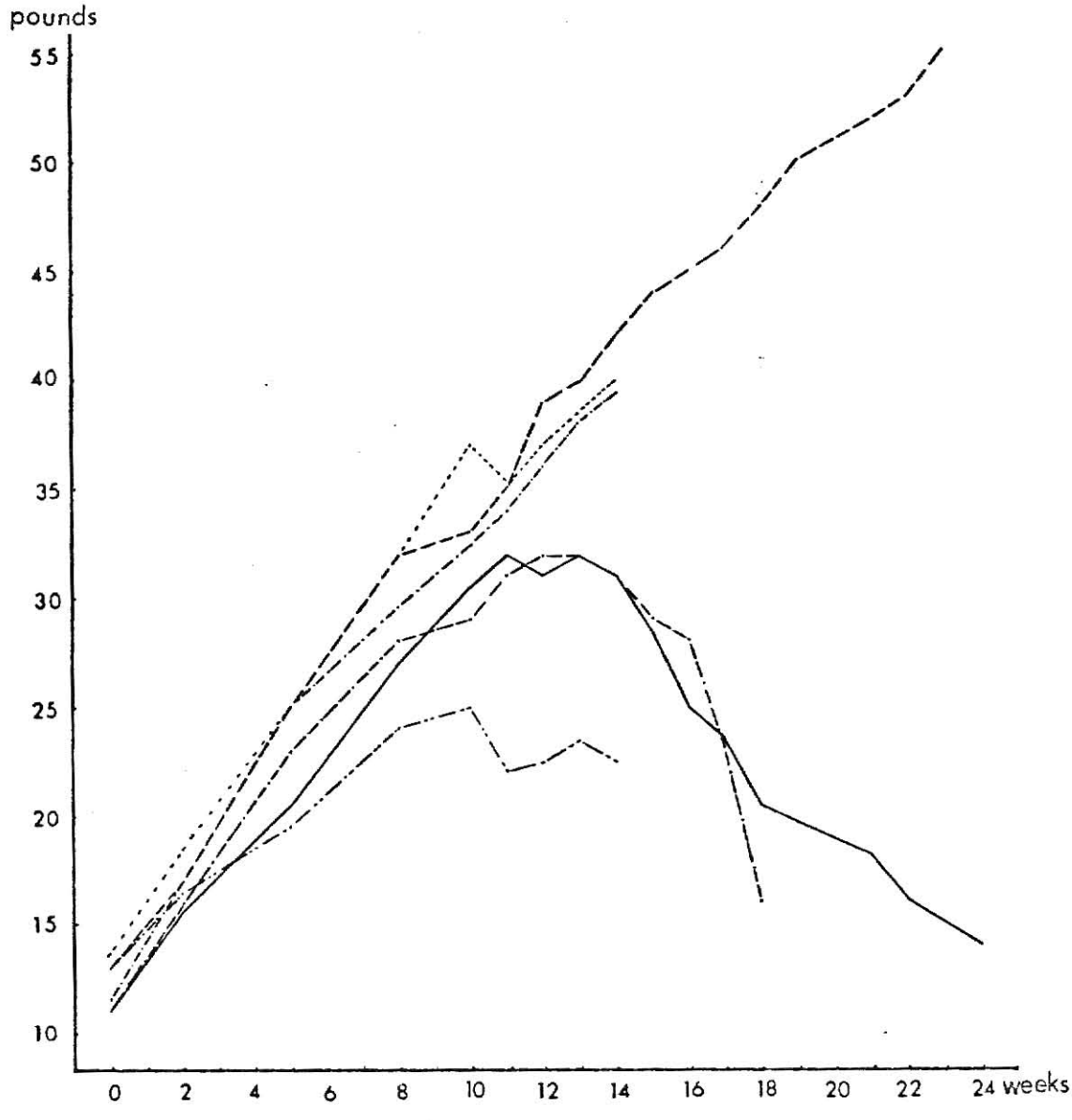

Fig. 1 - Relative Body Weight Changes of Control and Experimental Dogs.

- – – – – – Control (Dog 5)
- —·—·—·— Dog 7
- ———————— Group II (Average weight of dogs 1 and 2)
- —·—·—·—· Group III(Average weight of dogs 3 and 9)
- ····· Group IV (Average weight of dogs 4 and 6)
- —··—··—·· Group V (Average weight of dogs 8 and 10)

TABLE 1
VITAMIN A CONCENTRATION IN LIVER

Group	Dog No.	Treatment(*)	Vitamin A Concentration in Liver(IU/gm)
I	5	Control	3,105
	7	Vitamin A (1)	3,900
II	1	Vitamin A (2)	61,445
	2	" "	37,520
III	3	Vitamin A (3)	2,285
	9	" "	4,145
IV	4	Vitamin ADE (4)	4,980
	6	" "	16,030
V	8	Vitamin ADE (5)	27,390
	10	" "	

*1 - Had been control for 72 days, then received 135,000 IU of oral vitamin A/lb of body weight/day for 48 days.

*2 - IM injection of 45,000 IU of vitamin A/lb/week for 10 weeks, then oral administration of 135,000 IU/lb/day for 88 days.

*3 - IM injection of 90,000 IU/lb/week for 14 weeks.

*4 - IM injection of 45,000 IU of vitamin ADE/lb/week for 14 weeks on the basis of vitamin A level.

*5 - IM injection of 90,000 IU/lb/week for 14 weeks.

Radiographic Findings

Radiologic examination of the control dog revealed no abnormalities (Plate I, Fig. 2 & 3). The following description was based on the comparison with radiographic findings of control (dog 5). All the radiographs of extremities were taken from the medio-lateral view.

Dogs 1 and 2

The radiographs of dogs 1 and 2 showed very similar progression in changes and development of bones. On the 50th day of the experiment, the first signs of epiphysitis involving the long bones, particularly the radius and ulna were seen. This consisted of slight lipping at the metaphyseal ends of the bones. A slight developmental abnormality was observed involving the left patella of dog 1 in that the most proximal portion had a small separate ossification center which appeared to be attached only by fibrous tissue. The cortices of the bones appeared to be normal. No additional changes were noted by the 70th day.

On the 150th day, there were marked changes involving the diameters of the long bones, in particular radius, ulna, and to a lesser degree the femur and tibia. The cortices of all of the long bones had been greatly diminished in the intervening time. The epiphyseal plate had become markedly narrowed on all of the epiphyses which, when compared with the control dog, indicated there was a very definite premature closure of the epiphyses. It was noted that between the 50th and 70th

day the overall length of the right radius increased by slightly over 10 mm, however, between the 70th and 150th day, there was only 5 mm overall growth. The right radius of dog 1 grew 15 mm between the 50th and 150th day, while the right radius of the control dog grew 28 mm in the same period of time.

The fibula had become extremely demineralized and radiographically would appear to be not much bigger than a thread of string. At the distal end of the tibial crest, there had developed an osteophyte that was not visualized on any of the normal or control dogs (Plate I, Fig. 5). Also, a periosteal reaction was noted on the anterior surface of the ulna just opposite the entrance of the nutrient artery into the radius (Plate I, Fig. 4). On the 162nd day, the epiphyseal plate appeared to be more narrow and there was a very marked sclerosis involving the distal metaphysis and distal articular surface of the radius. Most certainly the growth rate was almost at a standstill at this time. The epiphyseal plate of the ulna appeared to be completely closed. There was no bowing involving the radius at this time.

Dog 7

This dog had been one of the control dogs until oral administration of vitamin A to this dog was commenced on the 73rd day. At that time no abnormalities were noted in this dog. On the 121st day, which was the 48th day of vitamin A feeding to this dog, there was a considerable reduction in diameter of the long bones. The epiphyseal plates showed

marked decrease in size very similar to the changes which took place in dogs 1 and 2. However, all the changes noted in dogs 1 and 2 were beginning to show up in this dog only to a lesser degree. There was a periosteal reaction involving the anterior surface of the right radius and the anterior distal end of the right humerus (Plate II, Fig. 6). Osteophyte was also noted at the tibial crest (Plate II, Fig. 7).

Dogs 3 and 9

Dog 3 would compare very favorably with the control dog except minimal epiphysitis changes on day 98. Dog 9 showed some signs of decreased diameter involving both right and left radius and ulna, and to a lesser degree the long bones of the rear leg (Plate II, Fig. 8). Also there were very early signs of an osteophyte forming at the distal end of the tibial crest on both right and left legs (Plate II, Fig. 9). The epiphyseal plates appeared to be definitely narrowed indicating premature closures taking place in the epiphyses. All changes were minimal in this dog. Dogs 3 and 9 were sacrificed on the 98th day.

Dogs 4, 6, 8 and 10

There was very minimal osteophyte development at the distal end of both right and left tibial crests of dog 4. In dog 8, there may have been a very minimal change in the diameter and some premature closure of the distal epiphyses of the radius and ulna with the tibia remaining normal. No other abnormalities were noted in these four dogs.

LEGENDS TO PLATE I

Fig. 2 & 3 - Radiographs of extremities of control dog, taken on day 150 of experiment at 7 months of age. Normal radiographic findings for dogs of this age.

Fig. 4 & 5 - Radiographs of extremities of dog 1, taken on day 162 of experiment at 7½ months of age. The diameters of long bones are markedly reduced and the cortices are diminished. A periosteal reaction is noted on the anterior surface of ulna. The epiphyseal plate is completely closed at this time. The fibula become markedly dimineralized. There is an osteophyte developed at the distal end of the tibial crest.

THIS BOOK CONTAINS SEVERAL DOCUMENTS THAT ARE OF POOR QUALITY DUE TO BEING A PHOTOCOPY OF A PHOTO.

THIS IS AS RECEIVED FROM CUSTOMER.

PLATE I

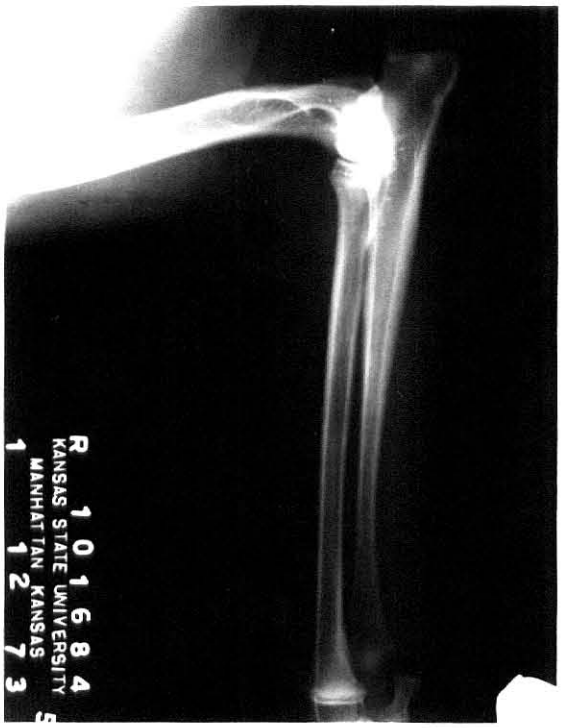

Fig. 2

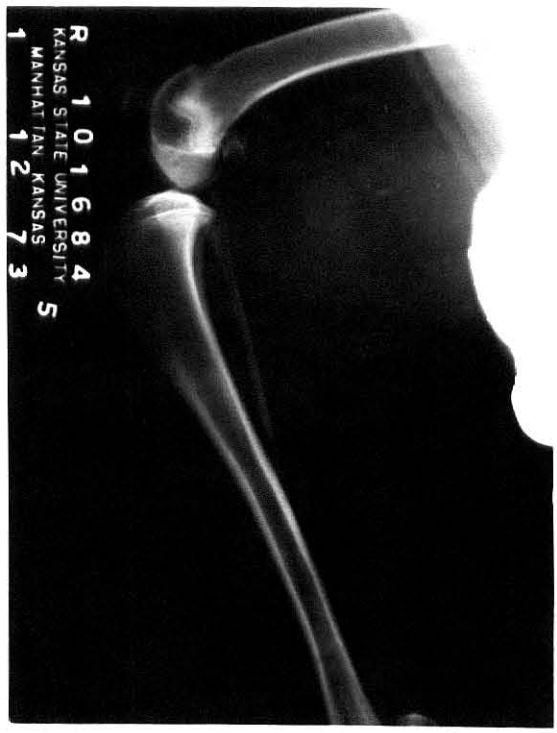

Fig. 3

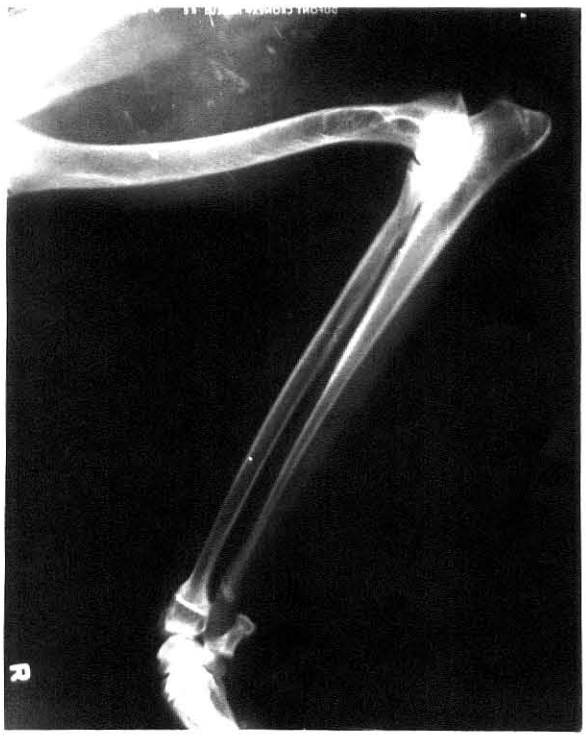

Fig. 4

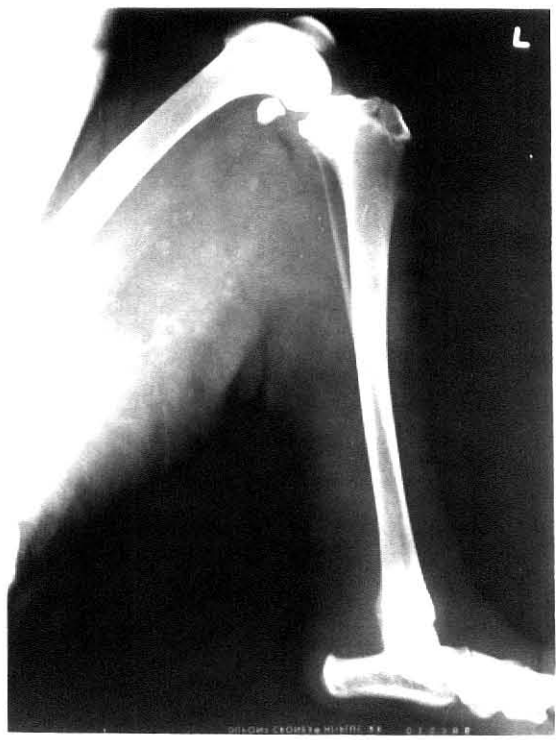

Fig. 5

LEGENDS TO PLATE II

Fig. 6 & 7 - Radiographs of extremities of dog 7, taken on 48th day of excessive vitamin A feeding at 6 months of age. The diameters of long bones are considerably reduced. The epiphyseal plates show marked decrease in thickness. There are periosteal reactions on the anterior surfaces of the radius and ulna. The fibula shows considerable decrease in density. There is also an osteophyte developed at the tibial crest.

Fig. 8 & 9 - Radiographs of extremities of dog 9, taken on day 98 of experiment at 5 months of age. The diameters of long bones are reduced. The epiphyseal plates appear to be slightly narrowed. There is an early sign of an osteophyte forming at the distal end of the tibial crest.

PLATE II

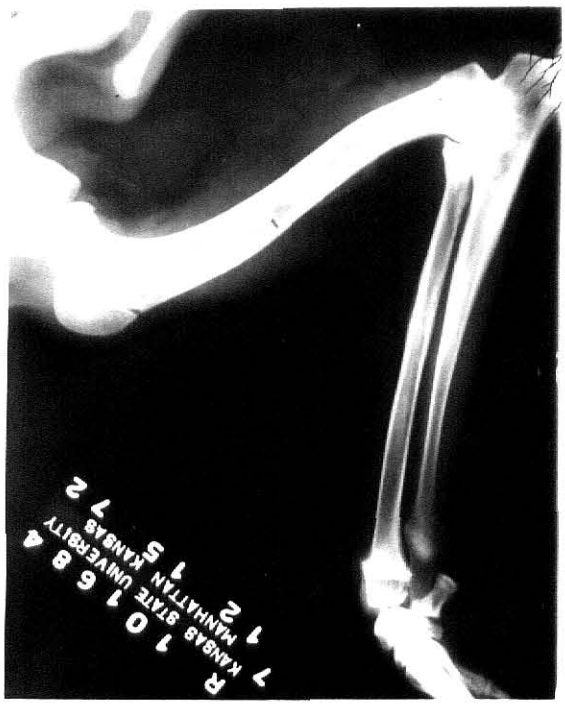

Fig. 6

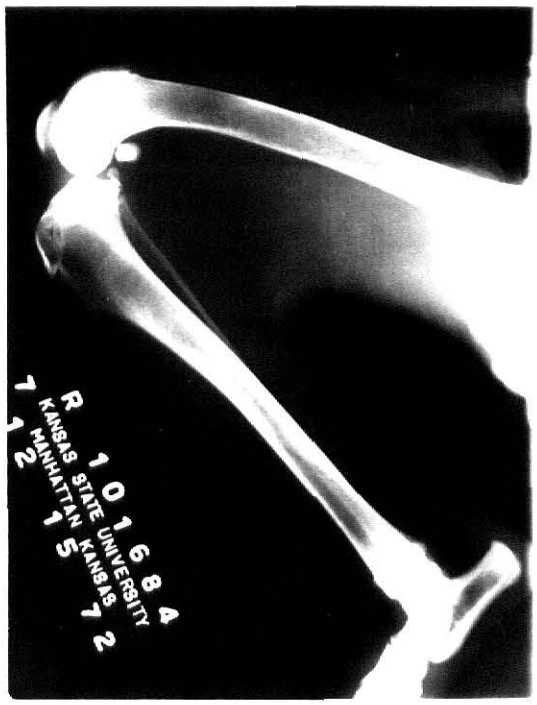

Fig. 7

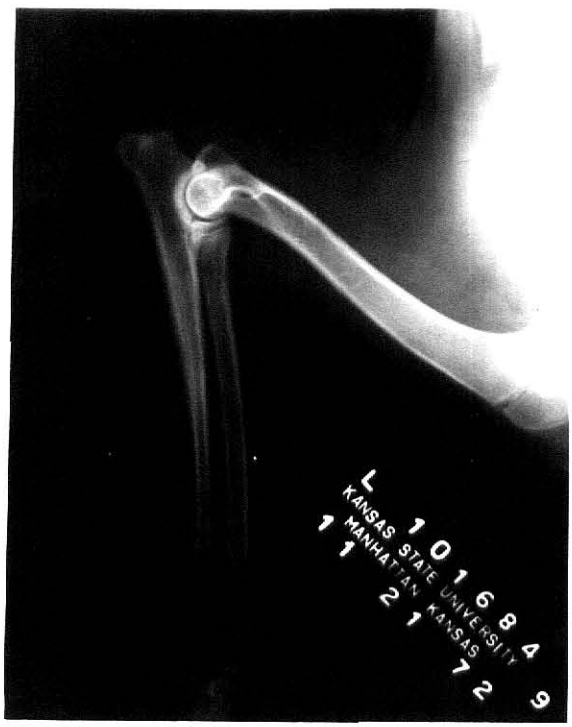

Fig. 8

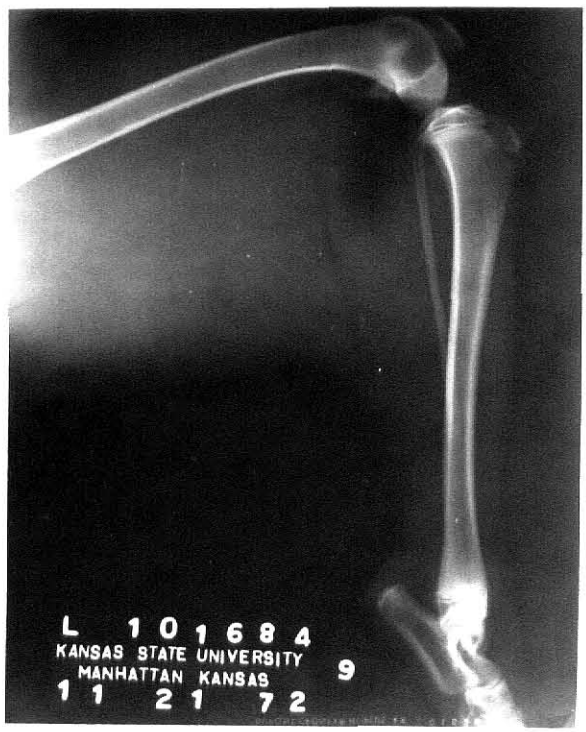

Fig. 9

Post-mortem Findings

No gross changes were found at necropsy except reduction in size of the long bones and fatty liver.

Microscopic findings

No pathological changes were observed in bone tissue from the control dog (Plate IV, Fig. 16 & 17). The microscopic changes noted in some of the experimental dogs were confined to the long bones, kidney and liver. Other tissues were normal except some hemorrhages observed in spleen, lymphnode and the mucosa of stomach and large intestine. A number of round cells were infiltrated into the mucosa of large intestine dogs 1 and 2.

Groups IV and V

The proximal end of the humerus and the distal end of the femur from the dog 10 were examined. Very minimal changes were observed in the articular cartilage. A few cartilage cells were arranged in clusters. Epiphyseal plate was decreased in thickness with irregular appearance (Plate III, Fig. 10 & 11). The number of bone spicules were reduced in the metaphysis. In several places of the epiphyseal plates, the reserve cartilage penetrated into the zone of hypertrophied cartilage (Plate III, Fig. 12) and cartilaginous matrix extended into the metaphysis. Golden-brown pigments were found in cartilage matrix and along the cortex. There were evidences of old and recent hemorrhages in cortex. The number of osteoblasts and

osteoclasts were normal.

Dog 7

Under the normal articular cartilage of the distal end of the tibia, the bones were very dense. Epiphyseal plate was reduced in thickness, but the reduction was not as marked as of dogs 1 and 2. The arrangements of cartilage cells were close to normal (Plate IV, Fig. 14 & 15). Several large tongues of epiphyseal cartilage protruded into the metaphysis (Plate III, Fig. 13). The activities of osteoblasts seemed to be considerably decreased. Marked amount of golden-brown pigment laden macrophages were distributed randomly.

Group II

The distal end of the tibia, radius and femur from dogs 1 and 2 were examined.

Tibia.--The cells of articular cartilage were pale and surrounded by a halo. There were packets of 2 to 5 cartilage cells in a cavity. Cartilaginous matrix was unevenly stained with eosin. Very dense bone formation was seen in subcartilaginous area. There was very little evidence of bone remodeling in this area. The most striking changes were found in the epiphyseal plates. The cartilaginous epiphyseal plate had almost completely disappeared, thus the bone spicules of epiphyseal and metaphyseal areas were joined and separated only by a small rim of growth plate (Plate VI, Fig. 23). Perpendicularly to the traverse band of bony plate replacing the cartilaginous epiphyseal plate, calcified cartilaginous

spicules were still present, of which osteoid was being deposited on their border. The osteoblasts appeared to be decreased in number in dog 1 and normal in dog 2. Bone spicules were fewer and extended deep into the diaphysis. Markedly calcified tissues were observed bilaterally along the cortex of the tibia, giving it a moth-eaten appearance. The osteoclastic activities seemed to be normal along the cortex which was thinner than normal. Old and recent hemorrhages and macrophages laden with golden-brown pigments which were quite similar to those seen in kidney were observed in periosteum and in the bone marrow cavity.

Femur.--In the dog 1, the cartilage of articular surface and bone trabeculi of epiphysis were normal. The cartilaginous epiphyseal plate was completely substituted by a traverse band of bony plate. These changes were almost identical to those of the tibia (Plate V, Fig. 18, 19 & 20). The osteoblastic activity seemed to be decreased in this area. Cortices were thinner than those of control, but actual osteoclasts were present. The cortical area had a number of macrophages laden with golden-brown pigments. Some focal areas of cortex showed marked fibrous tissue with capillaries and hemorrhages. The fibrous tissues were in various stages of maturation. The number of osteoclasts seemed to be normal in the cortex. The hemorrhages, fibrous tissues and the increased number of osteoblasts in certain areas and capillaries indicated the early stage of new bone formation.

The distal end of femur of the dog 2 showed somewhat different changes from those seen in the dog 1. The articular cartilage and epiphysis were normal. Epiphyseal plate was irregular and thinner. Local areas of epiphyseal plate have eosinophilic cartilage matrix, pale chondrocytes which were arranged in clusters with 3 to 5 cells in a cavity rather than in columns. In these areas the epiphyseal plate was separated from the calcified cartilage matrix and osteoid, and the number of osteoblasts were slightly reduced. The adjacent area showed small narrow epiphyseal plate with basophilic staining in clusters (Plates V & VI, Fig. 21 & 22). Epiphyseal plates of some other areas seemed to be replaced by bony plate. In the middle portion of the epiphyseal plate, the arrangements of cartilage cells were in clusters close to normal in structure. Along the cortex, marked hemorrhages, fibrous tissues and golden-brown pigments were seen. The osteoclastic and osteoblastic activities appeared to be normal.

Radius.--The distal end of right radius was studied. Identical changes with those of the tibia described above were observed except there was a marked decrease in the number of osteoblasts in the subcartilage area of the epiphyseal plate.

Costochondral junction.--Costochondral junctions of dogs 1 and 2 were widened and slightly irregular. Tongues of hypertrophied cartilage matrix protruded into the zone of provisional calcification. These hypertrophied areas contained golden-brown pigments in cartilage matrix but more markedly in

osteoid. The osteoblastic activities in this area were normal.

Group III

The distal ends of the femur and radius and the proximal end of the tibia were examined. Very minimal changes were observed in these dogs. Epiphyseal plates were decreased in thickness with slightly irregular appearance. The changes observed were similar to those described in the dogs of groups IV and V.

Kidney

No abnormalities were observed in the kidneys from the control (Plate VI, Fig. 25), groups IV and V. However, marked changes were noted in the kidneys from group II (dogs 1 and 2) and dog 7, and less marked in group III (dogs 3 and 9). Some proximal convoluted tubules were cystically dilated and occasionally contained a large amount of golden-brown pigments. Microcalculi were found in collecting tubules (Place VI, Fig. 24). Rest of the tissues were normal.

Liver

Diffuse moderate fatty changes were observed in the livers from dogs 1, 2 and 7, and less marked in dogs 3 and 9. Central veins were severely dilated and hepatocytes undergoing degeneration were distributed randomly. Most of the sinusoids were filled with red blood corpuscles. Golden-brown pigments were also found.

LEGENDS TO PLATE III

Fig. 10 - Photomicrograph of proximal epiphyseal plate of humerus from dog 10. The thickness of the plate and the number of columns of cartilaginous cells are reduced. Reserve cartilage cells are rarely seen. The spongiosa spicules are also decreased in number. H&E X60.

Fig. 11 & 12 - Photomicrographs of proximal epiphyseal plate of femur from dog 10. The plate is relatively normal in this area (Fig. 11). There is a reserve cartilaginous matrix extending into the zone of hypertrophied cartilage. H&E X60.

Fig. 13 - Photomicrograph of distal epiphyseal plate of tibia from dog 7. A large tongue of epiphyseal cartilage protrudes into the metaphysis. H&E X60.

PLATE III

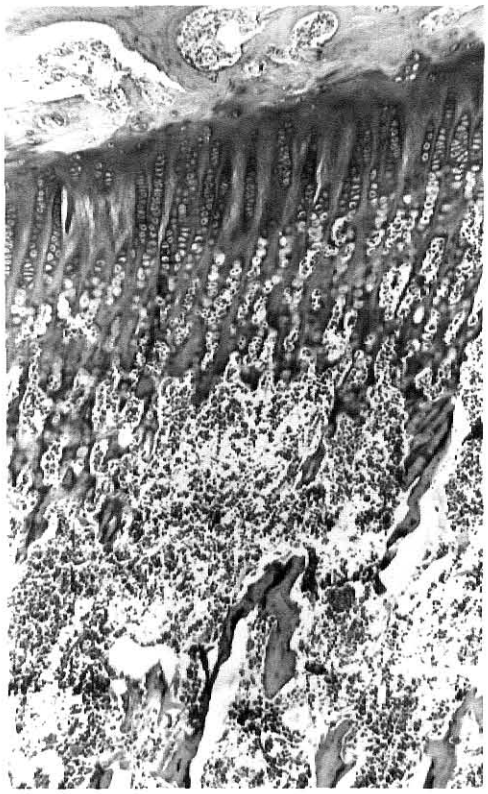

Fig. 10

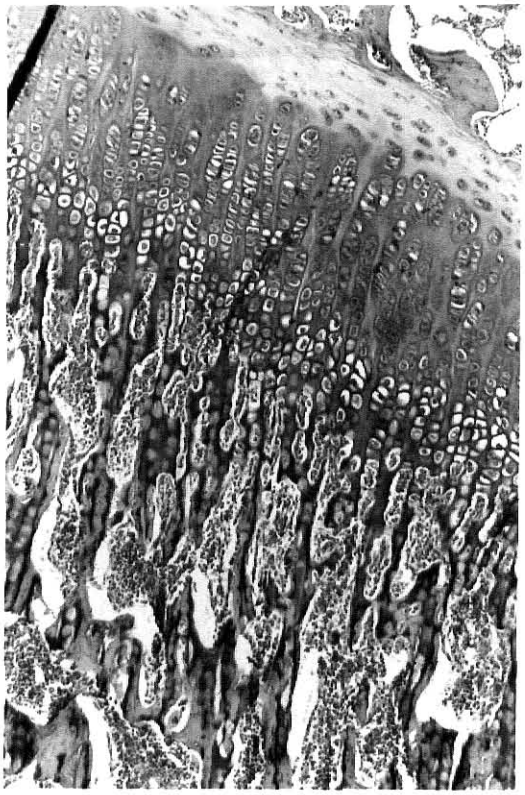

Fig. 11

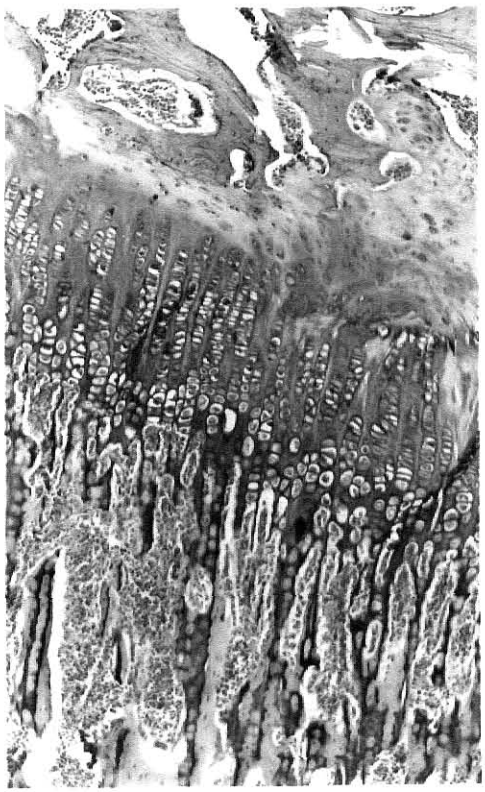

Fig. 12

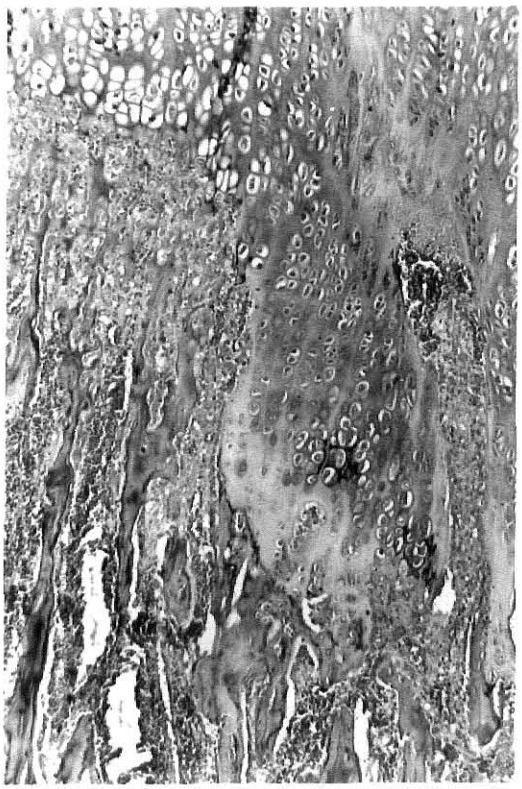

Fig. 13

LEGENDS TO PLATE IV

Fig. 14 & 15 - Photomicrographs of distal epiphyseal plate of tibia from dog 7. The growth plate is reduced in thickness and contains mainly hypertrophied chondrocytes (Fig. 14 & 15). H&E X60.

Fig. 16 & 17 - Control. Photomicrographs of distal epiphyseal plates of femur (Fig. 16) and tibia (Fig. 17). H&E X60.

PLATE IV

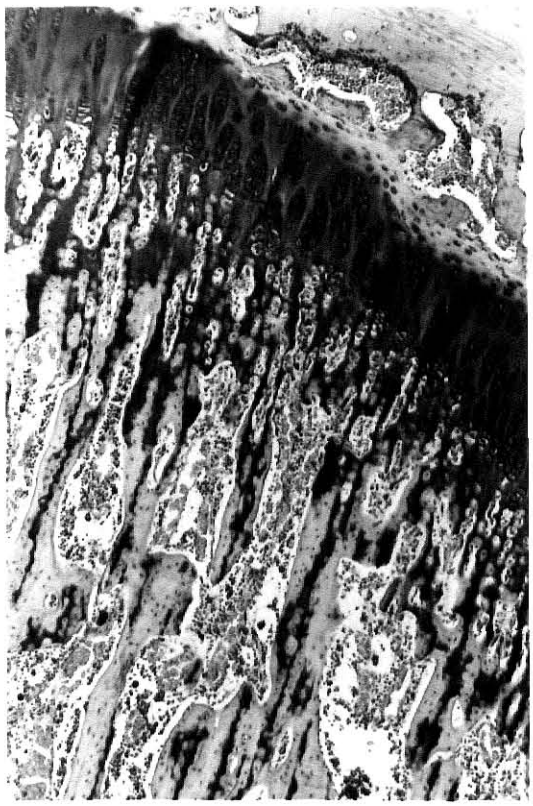

Fig. 14

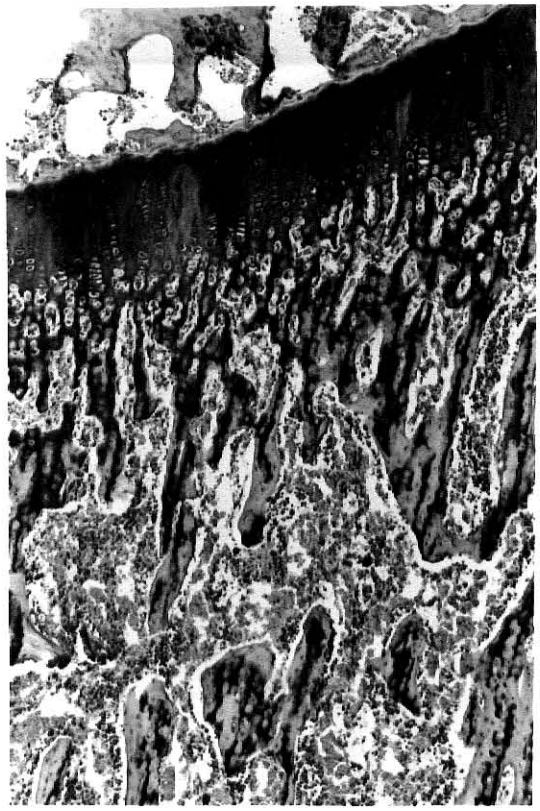

Fig. 15

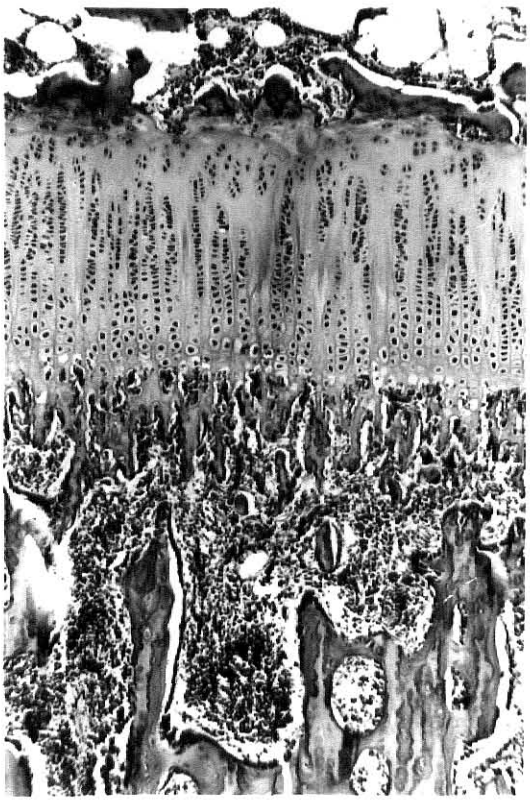

Fig. 16

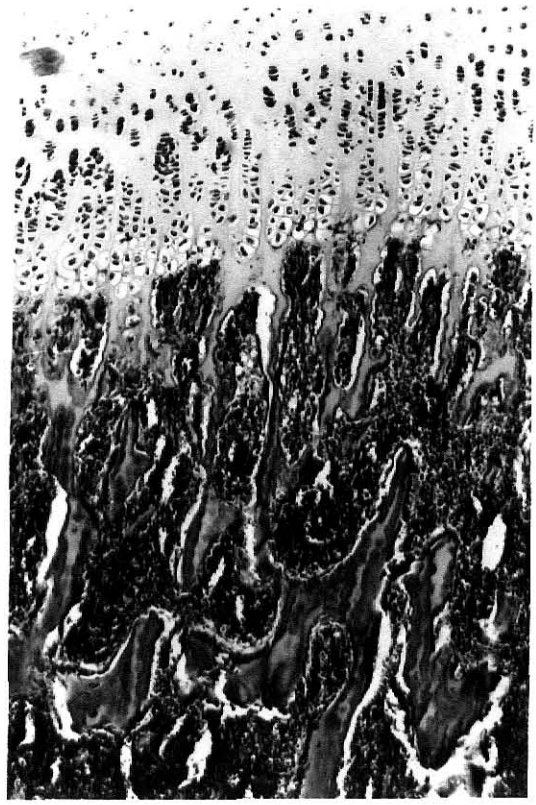

Fig. 17

LEGENDS TO PLATE V

Fig. 18, 19 & 20 - Photomicrographs of distal epiphyseal plate of femur from dog 1. There is severe loss of epiphyseal plate matrix and decreased spongiosa spicules. A traverse band of bony plate is seen along the plate. There is a zone of eosinophilic cartilaginous matrix extending into the metaphysis in Fig. 19 and 20. H&E X60.

Fig. 21 - Photomicrograph of distal epiphyseal plate of femur from dog 2. Eosinophilic epiphyseal cartilaginous matrix undergoing necrosis is seen. This is well demarcated from the adjacent area of the growth plate which contains irregular alignment of cartilage cells. H&E X60.

PLATE V

Fig. 18

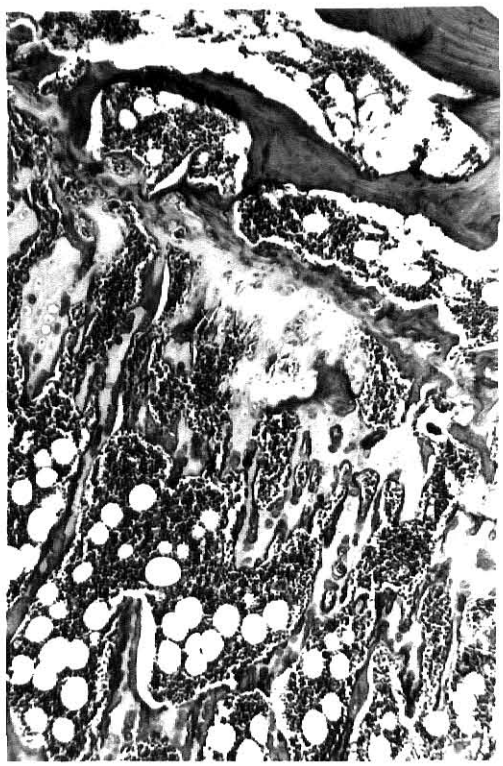

Fig. 19

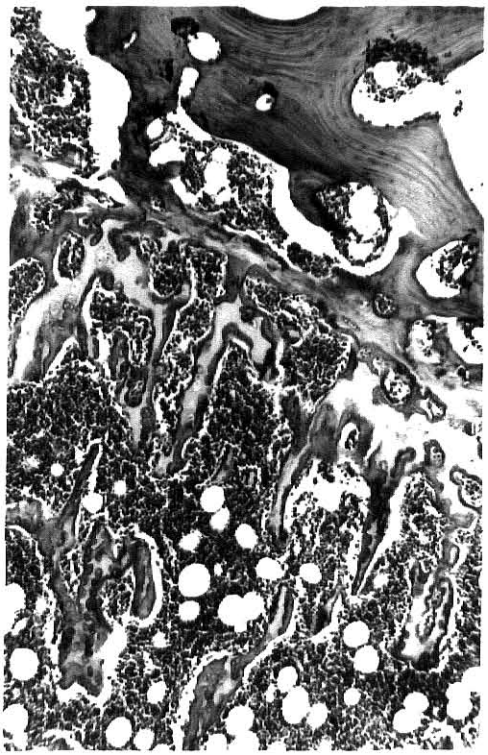

Fig. 20

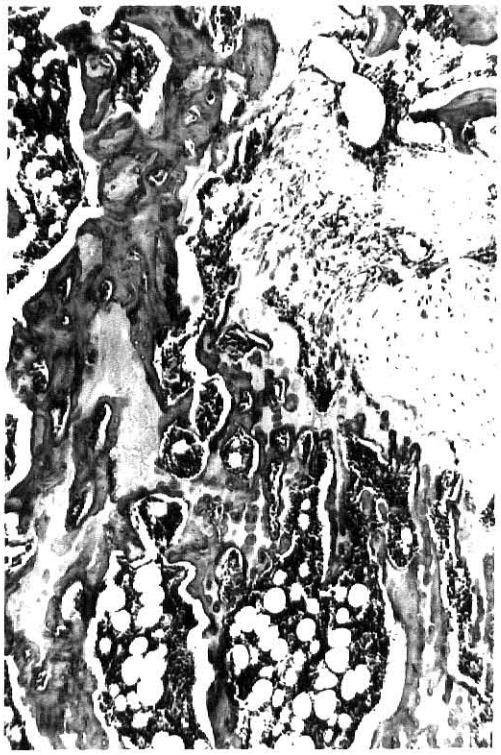

Fig. 21

LEGENDS TO PLATE VI

Fig. 22 - Photomicrograph of distal epiphyseal plate of femur from dog 2. The thickness of the growth plate is markedly decreased. The arrangements of cartilage cells are irregular. H&E X60.

Fig. 23 - Photomicrograph of distal epiphyseal plate of tibia from dog 2. The plate is completely obliterated. A eosinophilic necrotic cartilaginous matrix is seen in the metaphysis. H&E X60.

Fig. 24 - Photomicrograph of kidney from dog 1. Microcalculi are seen in collecting tubules. H&E X60.

Fig. 25 - Control. Photomicrograph of kidney from dog 5. H&E X60.

PLATE VI

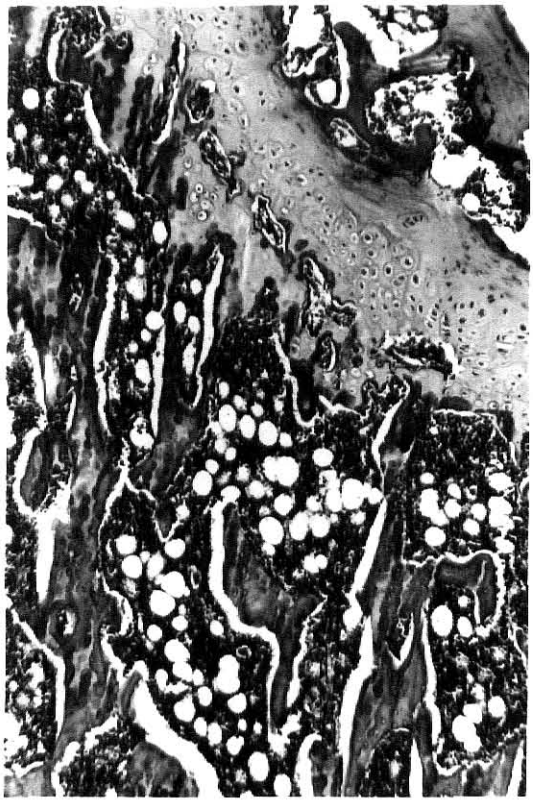

Fig. 22

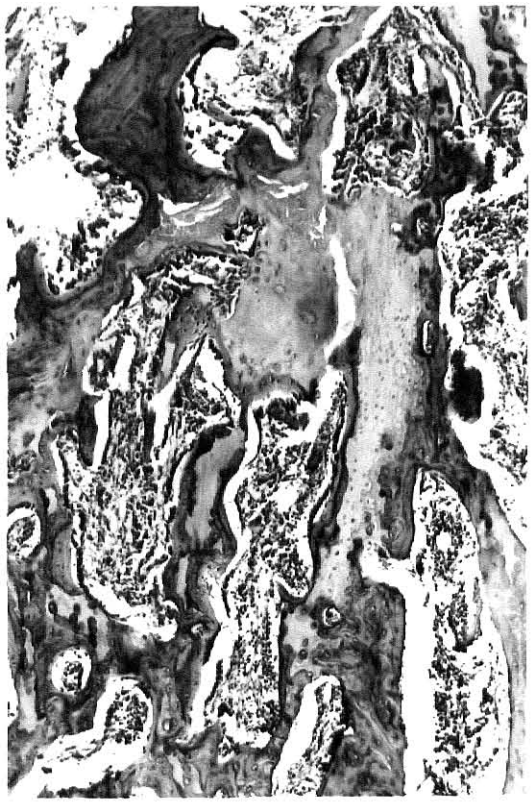

Fig. 23

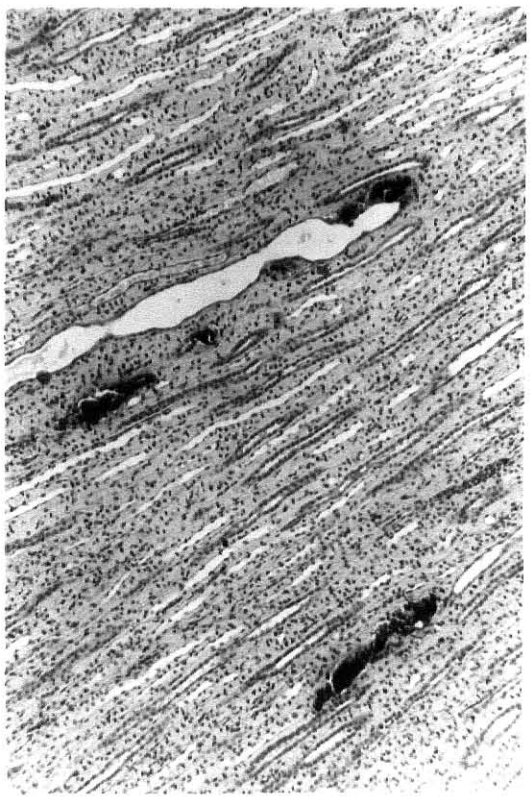

Fig. 24

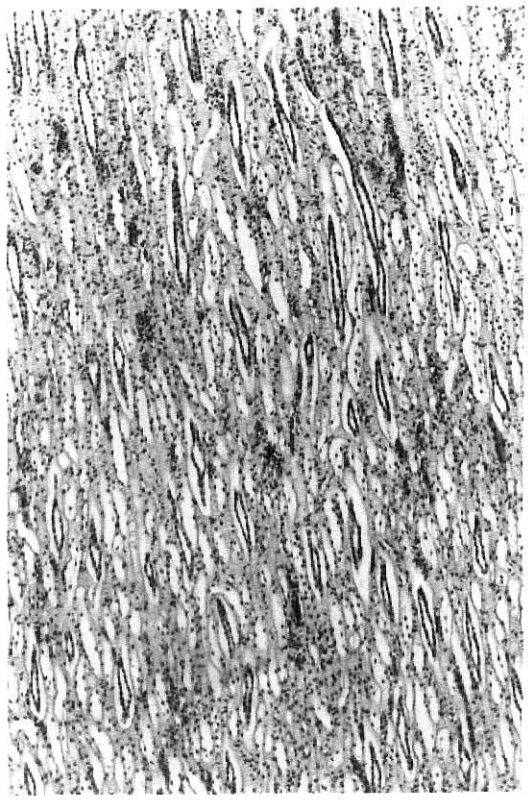

Fig. 25

DISCUSSION

Hypervitaminosis A in growing dogs due to the administration of excessive doses of vitamin A was concluded by loss of body weight, dullness, emaciation, pain of limb joints, fatty change of liver and skeletal changes. The long bones were decreased in overall length and thickness, particularly in the radius, ulna, tibia and fibula. Epiphyseal growth plates were disorganized or had partially or completely disappeared. The osteoporosis of the diaphyses resulted in a decreased amount of spongious bone. These changes were similar to those described in other animals by previous investigators (4,12,14,18,19,45,68,86,90). Spontaneous fractures previously reported in laboratory animals (9,18,19,33,51,54,64,65,66,67, 76,83) were not observed in the present study of dogs. Neither degenerative vascular lesions of the media in arteries and veins of the myocardium, gall bladder, urinary bladder and lymph node nor mural thrombi of fused red blood corpuscles described by Maddock et al. (45) were found. However, hemorrhages and hyperemia in various tissues agreed with the previous observations. Unlike the previous reports, marked lesions of microcalculi were found in the collecting tubules of kidneys (Plate VI, Fig. 24). Rodahl (67) described deposits of calcium in renal tubules of hypervitaminotic A chickens. Moore et al. (49) reported the stainable fat globules deposited in the convoluted tubules of the kidney in the hypervitaminotic cats.

Degenerative changes in cartilaginous epiphyseal plates resulting in reduction in longitudinal growth of the long bones have been reported in hypervitaminosis A in the young rat and guinea pig (67,86), dog (45), calf (30), pig (90) and cat (14, 15,16). In the present experiment, the epiphyseal plates had completely disappeared in some long bones, in particular the tibia and radius. Microscopically, changes in endochondral bones consisted of partial or complete loss in width of epiphyseal plates, disorganization of the zones of maturing and degenerating cartilage cells, fewer spongious spicules and decrease in osteoblastic activity. It has been reported that excess vitamin A caused dissolution and destruction of cartilage matrix bone in vitro (25) and in vivo (80). Reduction in bone formation through suppression of osteoblastic activity has also been described in hypervitaminosis A in some species (Wolbach (86), Rodahl (64,65,67,68), Irving (37,38), Grey et al. (30), Wolke et al. (90), Clark (14)). In the present study, the decrease in osteoblastic activity seemed to be not as great as those of other species previously reported. This may be due to a possible lessened susceptibility of dogs to the effects of high doses of vitamin A as Maddock et al. (45) presumed. The osteoclastic activity in subperiosteum appeared to be normal, which agreed with Wolke (90) who recorded no apparent increase in subperiosteal osteoclastic activity in the pig. Rodahl (67) and Wolbach (86) reported the increase in subperiosteal osteoclastic activity in the hypervitaminotic A rat and duck.

Periosteal exostoses are a radiographic manifestation of chronic hypervitaminosis A in man (12,20,42,81,92), pig (59,90), cat (14,72,73) and rat (19,67). In the present study, radiographic examination showed small but definite osteophyte development at the distal end of the tibial crest and periosteal swelling at the anterior surface of the ulna and radius. Microscopically, multiple exostotic lesions were observed along the cortices of the radius and tibia. The exostosis may be caused by forces of pull exerted on the periosteum and cortex from the muscle insertion (90). Recently, Pryor et al. (59) reported in pigs, Clark (14) in kittens, that exostotic lesions may occur before the chronic stage of the disease was reached. However, the dogs seemed to show rather late development of the lesion. It was suggested by Riser et al. (63) that the development of exostosis in cats receiving large amounts of pure vitamin A, or fed on liver rich in vitamin A, may be dependent on low calcium, high phosphorus rations, but this suggestion has been excluded by Clark et al. (17) and Clark (14). Clark et al. (17) found that the levels and relative proportion of calcium and phosphorus in the diet have little or no influence on the development of exostosis in chronic hypervitaminosis A in the cat. Nerurkar and Sahasrabudhe (52) described negative calcium and phosphorus balances in hypervitaminotic A rats.

It has been reported that inanition and resultant starvation are the probable cause of death in experimental animals receiving excessive levels of oral vitamin A (45,67). In the present study one dog died on day 48 of oral dosage of vitamin

A. This dog had been one of control dogs for 73 days and then switched to oral feeding trial. This dog appeared to be more severely affected by oral administration of excessive vitamin A as compared with the other experimental dogs receiving the same dose of vitamin A. This dog probably could not tolerate the cold weather because his natural body defenses were extremely lessened by excessive vitamin A feeding. However the definite cause of death was not determined. The experimental dogs appeared to be eating well throughout the experiment. At necropsy the stomachs of all animals contained food. Since individual food intake was not recorded, the appetite of the dogs could not be definitely evaluated.

As for the blood pictures in hypervitaminosis A, there are different opinions according to various workers. In the present study the hematology and blood chemistries observed were normal for dogs of this age until day 97 of excessive vitamin A administration. The hemoglobins and packed cell volumes observed in this experiment compare favorably with those for dogs of similar ages as reported by Andersen and Schalm (1). The serum inorganic phosphorus, alkaline phosphatase and glutamic pyruvic transaminase values observed in this study compare favorably to those reported by McKelvie (48) in dogs of the same age. The serum calcium found in this study were lower than those reported by McKelvie (48). There were no significant differences in serum calcium and inorganic phosphorus values between control and experimental dogs except one (dog 1) which showed a slight terminal

elevation of phosphorus and decreased calcium. The activities of serum glutamic pyruvic transaminase and alkaline phosphatase rose terminally to a slight degree when compared with control. Similar results have been reported in rats (19,76) and dog (45). Bomskov and Seemann (9) and Rodahl (68) found no changes in the calcium and phosphorus content of the blood in hypervitaminotic rats. Bomskov and Sievers (10), who noticed a slight hypocalcemia and hyperphosphatemia in a rabbit with a single dose of about 1,000,000 IU of vitamin A, attributed this effect to the vitamin D present in the preparation and assumed that vitamin A had no effect on the calcium and phosphorus content of the blood. Decreased serum inorganic phosphorus level, increased serum transaminase and alkaline phosphatase activities were observed in calves by Hazzard (32). Elevated serum alkaline phosphatase activity was also described in clinical cases of hypervitaminosis A of humans (53). Josephs (39) reported that hypervitaminosis A in child showed normal serum calcium and phosphorus values but increased alkaline phosphatase activities. Nerurkar and Sahasrabudhe (52) detected no changes in serum calcium and phosphorus in rats. Anderson et al. (2) reported the slight increases in serum calcium, phosphorus and alkaline phosphatase in hypervitaminotic A pigs, while Pryor et al. (59) found no difference in serum calcium, phosphorus and alkaline phosphatase values between control and hypervitaminotic A pigs.

Strauss (76) and Poumeau-Delille (57,58) reported an erythroblastic anemia appearing with hypervitaminosis A.

This was in agreement with the observation of Walker et al. (83) who also observed an increased ratio of plasma to cell count. Strauss (76), Papke (54) and Rodahl (65) described a hypochromic anemia. Anderson et al. (2) observed that the excessive vitamin A had no significant effects on hemoglobin and hematocrit. In the present study, slightly decreased blood hemoglobin and hematrocrit were found at termination in the dogs which had been fed vitamin A at the level of 135,000 IU per pound of body weight daily. Erythroblastosis, slight anisocytosis and poikilocytosis were also noted. However, the blood pictures observed in the hypervitaminotic A dogs seemed to be nonspecific to the hypervitaminosis A. Fasold and Peters (24) and Wendt (84) found a temporary rise in the total lipid and the cholesterol content of serum and fatty infiltration of the reticuloendothelial system. This implicated that vitamin A may relate with fat metabolism. Maddock et al. (45) and Nieman and Obbink (53) also pointed out the relationship of vitamin A with the fat metabolism. Maddock et al. (45) described that the dog was unusual by failing to show increased cholesterol and phospho-lipid values to excessive vitamin A administration.

Concerning the relationship between vitamins A and D, Theons (79) has asserted that vitamins A and D were antagonistic, and Gross-Selbeck (31) has reported that liberal dosing with vitamin A afforded protection against injury through excess of vitamin D. Vedder and Rosenberg (82) found that vitamin D (50,000 IU daily) gave protection against excess of

vitamin A concentrate. Strauss (76), on the other hand, described that the decalcification of the bones could not be prevented by vitamin D. Bomskov and Seemann (9) reported that excess of vitamin A resisted the ricket-curing capacity of vitamin D. Rodahl (67) described that the condition of hypervitaminosis A did not effect the development of rickets in rats kept on a rachitic diet, nor did it hinder the protective effect of vitamin D given prophylactic to rats kept on a rachitic diet. Finally excess of vitamin A did not prevent the ricket-curing effect of vitamin D in rachitic animals. However, excess of vitamin A was more injurious to rats given a rachitic diet than to rats given the usual adequate basal diet. No relationship has yet been established between vitamins A and E in hypervitaminosis A, however, Maddock et al. (44) demonstrated that the degenerative effect of hypervitaminosis A on testes of young rats could be potentiated by simultaneous supplementation with vitamin E. In the present experiment, the dogs treated with vitamins ADE seemed to be less affected than those treated with vitamin A alone. It was impossible to evaluate and compare the results because the vitamin ADE treated dogs were sacrificed much earlier than the dogs dosed with oral vitamin A. It appears therefore that the question of the interaction between these vitamins cannot be definitely settled until further investigations in this direction have been carried out.

It has been recognized that the liver is the main storage organ of vitamin A in animal body. Davies and Moore

(19) reported in the rats that the vitamin A was deposited in organs other than the liver, such as the kidney, lungs, heart and brain, this occurred to a much smaller extent than in the liver. They also pointed out that a considerable part of the vitamin A ingested lost in the immediate metabolism, probably by oxidation. However the urine of hypervitaminotic rats contained no vitamin A. The concentration of vitamin A in the liver of hypervitaminotic A animals has been reported by numerous workers. Vedder and Rosenberg (82) found an average of 5,000 IU per gram in the liver after a dosage of 100,000 IU vitamin A per day for 14 days. Baumann and Moore (5) found a liver concentration of 60,000-150,000 IU of vitamin A after 10 days with a dosage of 8 mg of vitamin A per day. Walker et al. (83) reported that young rats showed a liver value of 7,700 IU of vitamin A per gram after two weeks with an average dosage of 55,000 IU vitamin A per day. Rodahl (67) found an average of 8,000 IU of vitamin A per gram in the liver of adult rats with a dosage of 50,000-90,000 IU of vitamin A per rat per day for 6 to 48 days. Clark et al. (17) reported the liver concentration of 39,000-65,000 ug per gram in the cats fed 50 to 100 ug of vitamin A per gram of body weight per day for 21 or 31 days. In the present study the vitamin A concentration in the liver of dogs did not correlate with the level of vitamin A administered or the extent of radiographical and histopathological changes. There was a considerable individual variance in storage of vitamin A in liver. This is in agreement with Nieman and

Obbink (53) who described in the review of hypervitaminosis A on several species that the deposit of vitamin A in the liver did not parallel the total amount supplied. Seawright et al. (73) reported that there was no relationship between vitamin A intake and hepatic concentration or total storage in the liver of hypervitaminotic A cats. It has been suggested that kidney vitamin A concentration reflected the turnover rate of vitamin A in the body (49). The livers of dogs receiving weekly injections of vitamins ADE contained higher concentration of vitamin A than those of dogs receiving injections of vitamin A alone at comparable dose rates. This may be partially explained by the fact there are differences in availability from site of injection, storage in the liver or excretion from the body between the vitamin A portion of an injectable multiple vitamin preparation and an injectable vitamin A given alone at a comparable dose level. However the number of animals used was too small to evaluate relative storage of liver vitamin A. Clinically the dogs treated with vitamins ADE exhibited lameness and swelling at the injection sites, which were not observed in those treated with vitamin A alone. The vitamin ADE preparation used appeared to be unappropriate for dogs particularly when injected intramuscularly in a large dose.

It has been reported that vitamin A in the form of raw liver such as polar bear liver (50,64,66) and bovine liver (35,55) was more toxic for young rats than purified vitamin A concentrates. Seawright et al. (73) also described that

vitamin A, fed as raw liver, appeared more toxic than the pure vitamin A preparation in cats. This suggests that administration of excessive vitamin A in the form of raw liver to dogs may result in more remarkable clinical, radiological and pathological changes.

SUMMARY

Eight mixed large breed puppies, two months of age, were administered intramuscularly with vitamin A or vitamin ADE at two different levels to produce hypervitaminosis A in the dogs. The levels of vitamin A were 45,000 and 90,000 IU per pound of body weight per week for 10 weeks. Since the clinical signs of hypervitaminosis A were not observed in 10 weeks, two of the experimental dogs and one of control dogs were then fed 135,000 IU of vitamin A per pound of body weight daily via stomach tube for an additional 88 days. The other dogs continued to receive weekly injections of vitamin A or vitamin ADE at the previous level until they were sacrificed on day 97 or 98.

In general the clinical signs, radiographical and histopathological findings were in agreement with the previous reports observed in other species of animals.

The radiological findings observed in dogs in this study were retarded growth of bones resulting in diminution of the diameter and overall length, thinning of the cortices and markedly narrowed epiphyseal growth plates of the long bones. Periosteal reactions were found on the surfaces of

the radius and ulna. Osteophytes were also noted on the tibial crests.

Histopathologically the overall bone changes correlated with radiographic findings. The most striking changes were found in the epiphyseal plates, some of which had completely disappeared. The osteoblastic activities were decreased and the osteoclastic activities appeared to be normal. Markedly calcified tissues and evidences of early stage of new bone formation were found microscopically along the cortices of radius and ulna. Fatty changes were observed in the livers of the experimental dogs. Kidneys showed cystic dilatation of proximal convoluted tubules and microcalculi in collecting tubules in the dogs receiving high levels of oral vitamin A.

Appendix 1 - Body Weights of Dogs (Pounds)

Dog	Day of Experiment																
	0	15	31	52	66	73	80	85	97	105	111	115	121	133	143	150	162
5	13	17	25	32	33	35	39	40	42	44	45	46	48	50	52	53	55
7	11	16	23	28	29	31	32	32	31	29	28	23	16 (Died on day 121)				
1	11	16	21	28	32	34	33	34	34	32	29	28	25	23	21	18	15
2	11	15	20	26	29	30	29	30	28	25	21	19	16	16	15	14	13
3	12	20	31	36	40	44	47	50	52 (Sacrificed on day 98)								
9	11	14	19	23	25	24	25	26	27 (" on day 98)								
4	13	17	22	28	31	29	31	33	34 (" on day 97)								
6	14	20	28	36	43	41	43	44	46 (" on day 98)								
8	11	13	15	19	21	19	20	21	21 (" on day 97)								
10	15	20	24	29	29	25	25	26	24 (" on day 97)								

Appendix 2 - Hemoglobin Content in Blood of Dogs (gm%)

Dog	Day of Experiment: -7	4	10	18	38	53	70	97	121	145	162
5	10.9	11.5	11.6	12.1	13.1	14.8	14.6	16.0	15.8	15.8	15.5
7	10.6	10.0	10.3	11.7	12.2	14.6	14.0	15.6			
1	11.6	11.8	11.2	11.8	13.2	14.8	14.8	15.6	14.0	13.0	12.8
2	10.6	11.8	11.6	12.5	13.4	14.8	14.2	16.2	14.3	12.0	11.4
3	9.8	9.9	9.5	10.9	11.8	12.6	13.6	15.2			
9	10.3	11.2	11.5	12.0	13.6	13.8	14.2	15.6			
4	11.0	10.6	11.0	11.5	12.0	13.8	13.6	15.8			
6	9.6	10.0	10.6	10.6	11.2	11.5	13.3	14.6			
8	10.6	9.8	9.6	10.6	9.8	11.5	12.8	14.0			
10	9.6	10.0	10.6	11.2	11.4	12.0	13.4	15.0			

Appendix 3 - Packed Cell Volumes of Blood of Dogs (%)

Dog	Day of Experiment: -7	4	10	18	38	53	70	97	121	145	162
5	34	35	35	36	38	42	43	45	46	47	45
7	33	30	31	33	35	40	41	46			
1	37	36	34	35	39	45	45	45	43	42	40
2	35	35	36	38	40	44	43	45	44	45	42
3	31	28	29	33	36	39	40	45			
9	31	34	34	35	41	41	42	46			
4	34	34	33	35	37	42	41	46			
6	32	32	31	32	35	34	40	43			
8	34	30	29	31	31	36	40	43			
10	29	30	32	35	34	36	40	46			

Appendix 4 - Serum Calcium Values of Dogs (mg %)

Dog	Day of Experiment										
	-7	4	10	18	38	53	70	97	121	145	162
5	9.2	9.8	9.7	9.4	9.2	9.8	9.4	9.6	9.8	10.0	10.2
7	8.6	8.2	9.0	10.4	9.0	9.6	9.2	9.8			
1	9.6	9.6	9.6	9.8	9.6	9.4	9.4	9.4	9.2	9.2	8.8
2	9.0	9.4	9.2	9.4	9.2	9.4	9.6	9.8	9.4	9.8	9.1
3	9.0	9.6	10.0	10.2	9.6	9.2	9.2	9.4			
9	9.4	10.0	9.4	9.8	9.4	9.6	9.0	9.2			
4	9.6	9.0	9.8	9.6	9.0	9.6	9.4	9.2			
6	9.8	9.8	8.8	10.0	9.2	9.4	9.0	9.6			
8	9.4	9.0	10.2	9.8	9.2	9.8	9.8	9.0			
10	9.5	9.8	9.4	9.8	9.2	9.0	9.4	9.8			

Appendix 5 - Serum Inorganic Phosphorus Levels in Blood of Dogs (mg %)

Dog	Day of Experiment										
	-7	4	10	18	38	53	70	97	121	145	162
5	8.7	8.7	8.8	8.5	8.1	7.2	7.8	7.7	5.0	4.8	4.6
7	9.3	9.4	8.3	8.8	8.8	7.0	7.6	6.6			
1	8.7	9.8	9.0	9.3	8.0	8.8	8.0	8.0	6.2	6.4	6.8
2	9.6	9.3	9.3	9.6	7.7	8.3	7.8	7.8	5.4	5.3	5.0
3	7.9	9.4	10.0	11.4	9.2	8.8	8.5	7.9			
9	8.3	8.3	9.0	8.5	7.8	7.2	6.9	7.5			
4	8.9	8.0	9.3	8.4	8.7	7.8	8.5	6.1			
6	9.7	8.3	8.1	7.9	8.5	7.9	8.7	6.0			
8	9.0	8.3	7.9	8.3	6.3	7.8	7.7	7.5			
10	8.2	8.5	8.5	8.5	8.1	6.1	5.4	5.9			

Appendix 6 - Serum Alkaline Phosphatase Values in Blood of Dogs (Kind-King units)

Dog	Day of Experiment										
	-7	4	10	18	38	53	70	97	121	145	162
5	20	18	14	14	12	11.7	8.5	10.6	12.5	11.5	7.0
7	24	20	14	17	15.2	12.5	11.5	19.2			
1	21	18	16	15	10.5	12.0	9.7	18.0	20.0	28.0	26.5
2	21	19	15	16	11.7	10.0	9.7	16.5	21.0	30.0	23.5
3	22	17	14	22	16.5	14.5	11.5	12.0			
9	35	20	17	23	15.2	13.0	11.5	12.7			
4	24	20	23	25	17.0	13.8	15.6	16.0			
6	15	18	16	17	13.9	12.7	15.2	14.8			
8	12	15	13	12	9.8	11.2	9.2	13.4			
10	26	20	16	18	15.3	13.2	16.5	14.8			

Appendix 7 - Serum Glutamic Pyruvic Transaminase Values in the Blood of Dogs (Reitman-Frankel units)

Dog	Day of Experiment: -7	4	10	18	38	53	70	97	121	145	162
5	32	36	20	26	26	30	26	46	36	46	40
7	36	26	26	20	20	26	26	43			
1	34	50	37	32	26	30	34	34	50	68	72
2	34	40	44	36	26	26	44	51	58	72	88
3	40	32	32	32	28	20	36	48			
9	46	36	36	40	34	36	36	48			
4	18	26	32	28	20	26	36	34			
6	44	34	36	32	26	36	40	34			
8	44	30	26	32	18	30	32	56			
10	34	44	32	28	32	32	36	48			

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HYPERVITAMINOSIS A IN THE DOG

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Eight mixed large breed puppies, two months of age, were administered intramuscularly with vitamin A or vitamin ADE at two different levels to produce hypervitaminosis A in the dogs. The levels of vitamin A were 45,000 and 90,000 IU per pound of body weight per week for 10 weeks. Since the clinical signs of hypervitaminosis A were not observed in 10 weeks, two of the experimental dogs and one of control dogs were then fed 135,000 IU of vitamin A per pound of body weight daily via stomach tube for an additional 88 days. The other dogs continued to receive weekly injections of vitamin A or vitamin ADE at the previous level until they were sacrificed on day 97 or 98.

In general the clinical signs, radiographical and histopathological findings were in agreement with the previous reports observed in other species of animals.

The radiological findings observed in dogs in this study were retarded growth of bones resulting in diminution of the diameter and overall length, thinning of the cortices and markedly narrowed epiphyseal growth plates of the long bones. Periosteal reactions found on the surfaces of the radius and ulna. Osteophytes were also noted on the tibial crests.

Histopathologically the overall bone changes correlated with radiographic findings. The most striking changes were found in the epiphyseal plates, some of which had completely disappeared. The osteoblastic activities were decreased and the osteoclastic activities appeared to be normal. Markedly calcified tissues and evidences of early stage of new bone formation were found microscopically along the cortices of radius and ulna. Fatty changes were observed in the livers of the experimental dogs. Kidneys showed cystic dilatation of proximal convoluted tubules and microcalculi in collecting tubules in the dogs receiving high levels of oral vitamin A.